Non-invasive DNA probe delivery enables MR detection of gene up-regulation associated with psychostimulant sensitization

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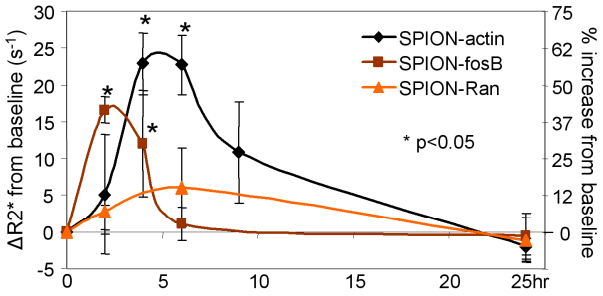
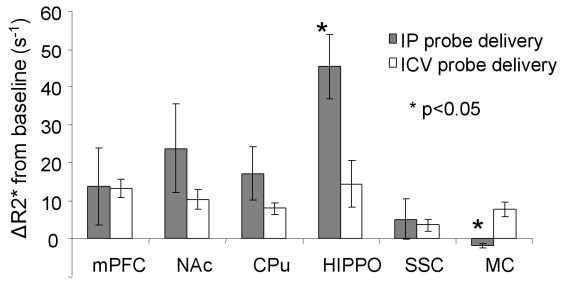
Introduction Modified gene activities (transcription alterations) are known to precede phenotypic changes that are associated with normal (developmental) and pathophysiological (disease) processes in all biological systems especially in the brain. Procedures to evaluate gene activity in the brain are not routinely performed because the techniques used rely on biopsy or autopsy samples. As the result, there remains a gap between our scientific understanding of the gene activities that take place in the brain during the evolution of diseases or disorders, as based on in vitro assessment, and what really occurs in vivo. Superparamagnetic iron oxide nanoparticles (SPION, a magnetic resonance (MR) contrast agent) labeled antisense DNA probe offers capabilities to visualize early molecular signatures using MR image predict phenotypic changes in living brains. Our laboratory has previously used this technique to differentiate drug-naïve from drug-exposed brains using MRI after intracerebroventricular (ICV) delivery of SPION-ΔfosB probe to detect mRNA of ΔfosB which is heavily implicated in drug addiction [2]. By using Gd-enhance MRI, we observed that after cortical puncture procedure, the BBB remains open for at least three weeks. This condition opens up the possibility of non-invasive probe delivery to the brain for longitudinal MR assessment of gene transcriptional changes during disease process including drug addiction.

Temporal ΔR2* changes in the striatum



SPION-ΔfosB retention profiles after chronic/withdraw/reinstated AMPH exposure



Methods Cortical punctures were performed in male C57black6 mice at least three days using sterile needle (30 gauges) prior to the study to ensure BBB bypass as described [1,2]. MR imaging was performed before and after intraperitoneal (IP) injection of SPION-DNA to these animals with BBB bypass at 2, 4, 6, 8, and 24 hours. Different SPION probes (including SPION-actin for constitutive and high expressing gene, SPION-fosB for low but inducible genes and SPION-Ran as no target control) were delivered. 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 some studies, a challenge paradigm of drug abuse was made: animals were treated with amphetamine (AMPH, 4 mg/kg, IP) every other day for a total of seven treatments (chronic) followed by two weeks of no drug (withdraw) and the same drug dose was injected to these animals on the experiment day (reinstatement). On the day of experiment, these mice were treated awake with SPION-ΔfosB (2mgFe per kg, IP). AMPH at the same dose was injected two hours later. These animals were kept in their home cages at all time to minimize gene activation by new environment. MRI R_2* maps of these mice were acquired 5 hours after AMPH. We compared ΔR_2* changes associated with AMPH-induced SPION-ΔfosB retention in the same brain regions after either IP or ICV delivery of SPION-ΔfosB probe.

Results (1) Significant ΔR2* changes in the striatum after SPION probe delivery were achieved at 2, 4, and 4, 6, 8 hours for SPION-fosB and SPION-actin probes, respectively. SPION-Ran probe did not result in significant ΔR2* changes at all time. (2) Compared to our previous results using intracerebroventricular route of delivery [2], we observed similar magnitudes of AMPH-induced ΔR2* changes within brain regions including medial pre-frontal cortex (mPFC), nucleus accumbens (NAc), Caudate Putamen (CPu) after IP or ICV delivery of SPION-ΔfosB probe, except for Hippocampus (HIPPO) and Motor Cortex (MC). Multiple MR-visible agents can be delivered and MR imaged in the same mice for up to 4 weeks after cortical puncture.

Conclusions Non-invasive DNA-based MRI probe delivery can achieve comparable detection capabilities to ICV probe delivery in detecting cerebral gene transcriptions and enable longitudinal study of gene transcriptional changes during in disease processes. [Supported by NINDS (R21NS057556), NIDA (R21DA024235, RO1DA026108), NCRR (P41RR14075) and AHA (09GRNT2060416)]

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.