# A novel MR contrast probe that reports amphetamine-induced cerebral gene transcription in vivo

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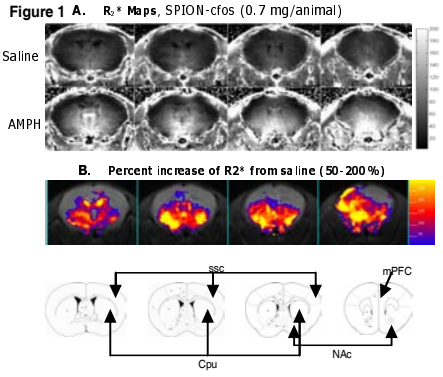
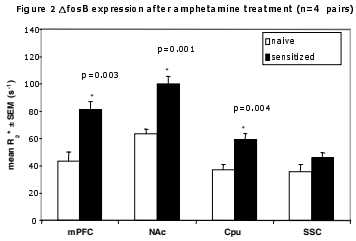
**Introduction** Altered expression of endogenous genes in the brain often accompanies acute or chronic neurotoxin exposure. Chronic exposure to amphetamine up regulates ΔFosB protein, which has been proposed to be a major biomarker of drug addiction[1]. In this study, we utilized a novel MR contrast probe to detect different effects on cerebral gene transcripts in live mouse brains after an acute or chronic exposure to amphetamine. We infused mice intracerebroventricularly (ICV) with an iron-based MR $T_2$ susceptibility agent (SPION)-labeled phosphorothioate-modified oligodeoxynucleotide (sODN) prior to amphetamine or saline (vehicle) injection and performed R2* acquisition afterwards[2]. Three types of mRNA targeting probes were included in this study: (1) in the acute exposure: cfos mRNA targeting probe (SPION-cfos) and (2) in chronic exposures: fosB or ΔfosB mRNA targeting probe (SPION-fosB or SPION-ΔfosB). We also included a non-targeting probe with randomized sequence (SPION-Ran) as the control. We compared R2* values from regions of interest (ROI) in the mouse forebrain (1) between the amphetamine and saline treated mice in the acute exposure study and (2) between the amphetamine naïve and pre-treated mice in the chronic exposure study.

**Methods** For pretreatment of the animals, male C57black6 mice were injected intraperitoneally (IP) with amphetamine (4mg/kg) or saline every other day for two weeks followed by a period of withdraw for the next two weeks. On the day of the experiment, mice were anesthetized using katamine (100 mg/kg, IP) and xylazine (16 mg/kg, IP) and ICV infused with the contrast probe (SPION =190 pmol/kg) via ICV route (LR:-1; AP:-0.2; DV:-3 mm, bregma). Four hours later, mice were IP injected either amphetamine (4mg/kg) or saline (10 ml/kg). We acquired MR images in live animals (with pure $O_2$ plus 2% halothane [800 ml/min flow rate]) using a 9.4Tesla magnet three hours following the amphetamine challenge. We acquired $R_2$* maps using serial GEFI sequences (TR/TE=500/3, 4, 6, 8, 10ms, FOV=1.5cm, 128×128, α=30). Region of interest was placed in the brain regions which are related the dopaminergic pathway (e.g. medial pre-frontal cortex, nuclear accumbens, caudate putamen) and a control region (somatosensory cortex) contralateral to the infusion hemisphere.




**Results** Acute exposure: R2* maps are shown to demonstrate the regional elevation of R2*. No elevation in R2* was seen in animals that received SPION-Ran, with or without acute amphetamine administration (not shown). Animals that received SPION-cfos and amphetamine exhibited robust elevation of R2* in the forebrain (Fig 1A). Maps of percent change of R2* of amphetamine mouse brain to saline mouse brain is shown in Fig 1B. ROI analysis in Fig 1C shows that R2* elevation occur in nucleus accumbens (NAc); less robust in the striatum (CPu) and cingulate and prelimbic/infralimbic cortices (collectively the medial pre-frontal cortex, mPFC), and little elevation in somatosensory cortices (SSC). These results are consistent with distinct patterns of Fos expression in the forebrains of C57black6 mice associated with acute amphetamine stimulation3. Chronic exposure: Figure 2 summarizes the ROI analysis comparing the R2* elevation of pre-treated mouse brains to the naïve mouse brains after a single dose of amphetamine on the day of experiment. While we did not observe difference between the naïve and pre-treated animals on the R2* elevations from the retention of SPION-fosB, we observed significant elevation of R2* in the pre-treated mice from the retention of SPION-ΔfosB.

**Conclusions** Brain regions exhibiting elevated R2*, as detected by MRI, are consistent with reports by other investigators using $^{35}$S-riboprobes for detection of elevated mRNA levels after a similar stimulation paradigms on postmortem rat brains[4-6]. These data imply that the SPION-labeled sODN can detect biomarkers for amphetamine addiction.

1. Natl Acad Sci U S A. 89(13), pp. 5764-8. 2. Liu Ch et al., J.Mol.Imaging, in press. 3. Brain Res. 1025(1-2) 59-66. 4 J Neurosci 18(24):10579-93. 5. Brain Res Mol Brain Res 122(2): 151-7. 6. Eur J Neruosci 13(10): 1977-83.