Supplemental Material

DNA-based MRI probes for Specific Detection of Chronic Exposure to Amphetamine in Living Brains

Running title: Differential MRI on fosB and ΔfosB mRNA in vivo

We showed here that AMPH treatment produced locomotor behavior modification to what was reported in the literature(Giordano et al., 2006).

Locomotor Assessment. We measured horizontal locomotion and fine motor activity using an automated recording device (San Diego Instrument, San Diego, CA) located in the same room in which the animals were individually housed (described in (Kachroo et al., 2005). The system has eight chambers, each of which is composed of frames equipped with five infrared photocell beams (5cm apart) for one polypropylene cage (15 x 25 cm). Animals were tested in their own home cages. The photocell beams traverse each cage in a plane above the floor. We recorded the frequency of locomotion (ambulation) as the number of sequential breaks in two adjacent beams, and measured fine motor activities (activities such as grooming or other stereotyped motions) by counting the number of sequential breaks in a single beam. Recordings were made every minute for 60 minutes, starting one minute after the injection was

administered. The total distance traveled in one hour is the frequency times 5 cm per minute times 60 minutes per hour.

Results: We conducted a gross comparison of the total locomotor activities, and compared changes in locomotion between AMPH-treated and saline-treated animals in various exposure paradigms (SAL vs. AMPH groups in acute exposure, SAL7/W/S vs. A7/W/A and SAL7/W/S vs A7/W/S [placebo] in chrionic exposure groups). Using one-way ANOVA followed by Newman-Keuls Multiple Comparison test, we found that AMPH induced a significant main effect ($p < 0.001$), with an exception between the SAL7/W/S and A7/W/S groups ($p > 0.05$). The average rate of locomotion (in meters per hour) was 57 ± 6 and 105 ± 6 for A1 and A7/W/A, respectively (Figure S).

Summary and Limitations of the MRI Method:

When **delivered** by ICV route, sODN-cfos (Fig S2), a charged molecule labeled with fluorescein isothiocyanate (FITC), **attaches** first to the ventricular wall and then gradually diffuses into the parenchyma, lateral ventricle and beyond (Cui et al., 1999), where we now know it is **distributed** (labeled with FITC or SPION) via the Virchow-Robins space (Liu et al.,

2007a), and **binds to intracellular mRNA** (Liu et al., 1994), with a sequence dependent manner (Liu et al., 2007a). Others have reported the same in mice (Ogawa et al., 1995). We have also exploited these properties of sODNs to report gene transcription in live mouse brains, and described the development of this novel technique for MRI in a recent review (Liu et al., 2007e). The conjugation of sODN to SPION (SPION-sODN) facilitates cellular uptake of this brain probe by live brains. The MR probe is shown to bind to mRNA, mostly in the **perinuclear region** using in situ RT-PCR (Liu et al., 2007a). At high magnification, FITC-sODN appeared as punctuate formations on nucleus where mRNA is transported as punctuate formations to the cytoplasm (Vargas et al., 2005). In addition, when sODN is replaced with a randomized sequence, it has a shorter window of retention in the brain. The control (MR probe without sODN, non-targeting probe) shows no uptake by the brain (null elevation in R2* values) (Liu et al., 2007b), except in a few cells with macrophage or astrocyte morphological features, as others have also reported (Muldoon et al., 2005; Muldoon et al., 2004; Sun et al., 2005).

Although live brain MR detection with SPION-ΔfosB probes enables differentiation between animals that experience acute v.s. chronic AMPH exposure, it is important to note that in vivo

MR detection of altered cerebral gene expression is not without limitations and difficulties in this nanotechnology that we pioneered.

Firstly, although high-field in vivo MRI of small subjects like mouse brains provides superb image signal-to-noise, the high detection power can be compromised (biased) by a greater vulnerability to image artifacts near the air-bone interface and probe and/or blood accumulation near the surgical site. The presence of such artifacts precludes the ability to assess gene expression profiles in these brain regions. Possible solutions include MRI studies in larger animals such as rats and non-human primates, which suffer less from tissue interfaces as a consequence of the larger brain size, or to image clinically available field strengths like 1.5 and 3 Tesla, where there is less vulnerability to susceptibility artifacts.

Secondly, the advantages of in vivo detection must be weighed against the complexity and heterogeneity of probe delivery, retention, and clearance; this issue is unlike histological methods that apply dyes or probes directly to brain sections after sacrifice with subsequent washing to purge excess free probes. A less invasive SPION-sODN delivery route such as intraperitoneal or eye drop delivery can reduce surgery-related artifacts (Liu et al., 2008a). In vivo MR assessment of SPION-sODN retention profiles can capture the dynamics of probe

delivery and clearance. With careful experimental design, it is possible to visualize gene expression profiles in live brains, as we have demonstrated here and in previous work (Liu et al., 2007c; Liu et al., 2007b; Liu et al., 2007a; Liu et al., 2008a).

Finally, T2*-weighted imaging is a sensitive MRI method, but quantification of probe concentration currently is not optimal. In the same way that functional MRI depends upon a heterogeneous distribution of magnetized blood vessels, SPION probes in brain tissue are heterogeneously distributed and compartmentalized within brain cells. While we expect a monotonic relationship between probe concentration and R2*, this relationship may not be linear as in the case for fMRI (Boxerman et al., 1995). We simply have assumed that R2* signal enhancement represents elevated probe retention in cells that express the specific mRNA targets. Therefore, there are rooms for improvement.

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