Study axonal transport rate and neuronal turnover rate of the olfactory system using novel MRI anatomical contrast agent GdDOTA-CTB

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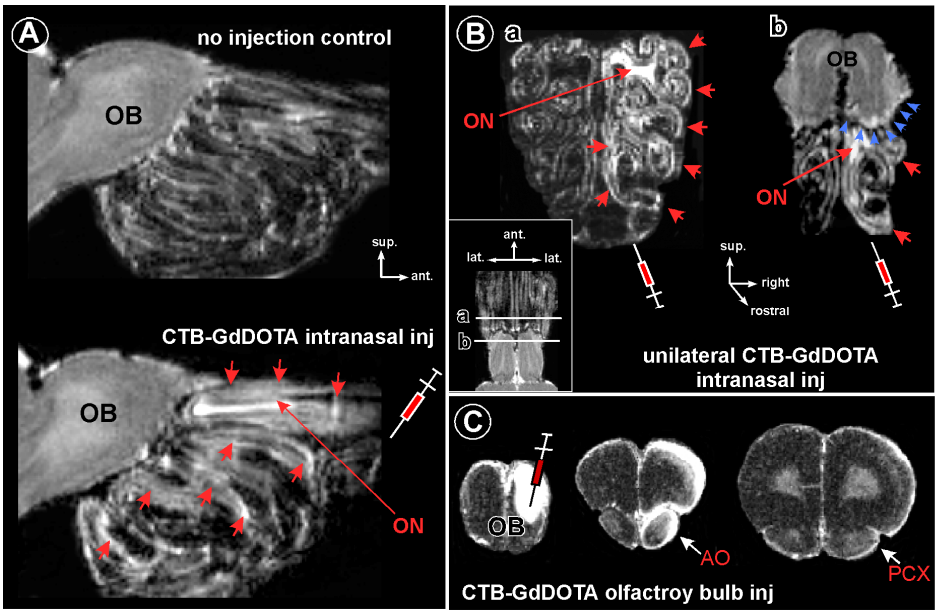
Introduction: Previously, we reported the successful development of a novel MRI contrast agent, GdDOTA-CTB, that conjugates conventional neuroanatomical tracer cholera toxin subunit B (CTB) with gadolinium chelates, to allow *in vivo* visualization of brain circuitry (ref 1). We found that the neuronal transport of GdDOTA-CTB is target-specific, monosynaptic, stable for several weeks, and very reproducible. In addition, its slow transport rate (~ 1 mm/day) suggests GdDOTA-CTB is probably mediated by the slow axonal transport mechanisms. Because of its virtues of excellent stability and relatively slow clearance rate from neurons after being initially uptake, GdDOTA-CTB is an excellent tool to reliably detect the connected anatomical circuitry and allow longitudinally monitor possible alternation in the monosynaptically connected brain circuitry. **Materials and Methods:** GdDOTA-CTB bioconjugate was made at the Imaging Probe Development Center (NHLBI, NIH) using commercial available products (ref 2). CTB and $GdCl^3$ were purchased from Sigma-Aldrich (St. Luis, MO), *S*-2-(4-isothiocyanatobenzyl) 1,4,7,10 tetraazacyclododecane-tetraacetic acid (BnDOTA) was obtained from Macrocyclics (Dallas, TX). MALDI-TOF mass spectrometry was performed on a Shimadzu Biotech Axima-CFP Plus using sinapic acid as a matrix. Analytical HPLC was performed on an Agilent 1200 Series instrument. The optimal ratio of GdDOTA to CTB is 1:1.3 to 1:3. Five adult Sprague-Dawley rats received unilateral injection of GdDOTA-CTB in the olfactory bulb or one of the nostrils. Olfactory bulb injections surgeries were carried out under aseptic conditions during isoflourine anesthesia. Animals' heads were fixed in a stereotaxic frame, a craniotomy was performed, and the GdDOTA-CTB was slowly injected using microsyringe into the olfactory bulb (20%, 0.5 µl, AP +6.5, ML -2.0, DV +4.5±0.1 mm). For the nostril injection, 15-20 µl of GdDOTA-CTB (10-20%) was slowly delivered using a soft-tip micropipette. Animals were imaged at baseline prior to injection, immediately (~2 hr) post injection, on days 2, and continue every 7 days, until 1 month post-injection. The MRI imaging was performed on 7T scanner using a Bruker pharmascan 70/16 (Bruker Biospin, Ettlingen, Germany) with a H-resonance-frequency of 300 MHz. A 15-mm-diameter receive-only surface coil and a birdcage transmit coil were used to acquire 2D and 3D T1 weighted and inversion recovery MR images. During MRI acquisition, animals were anesthetized with 1–2% isofluorane using a nose cone. Their heads were fixed in place with a bite bar and ear bars, in order to minimize motion artifact. Rectal temperature was maintained at 37±1°C with a heated water pad. A multi-slice spin-echo T1 weighted (T1-W) or T1 inversion recovery (T1-IR) sequences was acquired to obtain MRI image from S1 with in-plane resolution of 156 x 156 um pixel size, by using MSME and RARE pulse sequence was applied to acquire T1-weighted spin-echo 2D or 3D images with the following parameters: for 2D, TE/TR=7.9/500 ms, matrix size=165x165, FOV=25.6x25.6, slice thickness=1 mm. For 3D, TE/TR=10.5/950 ms, matrix size=165x165x144, FOV=25.6x25.6x18 mm. In addition, a modified driven equilibrium Fourier transform (MDEFT) pulse sequence with 150 µm isotropic resolution was applied to enhance and differentiate the gray versus white matter contrast of the brain using the following parameters: TR=3000 ms, echo TR/TE=10/7.2 ms, TI=950 ms, number of segments=4, averages=4, matrix size =165x165x50, FOV=25.6x25.6x6.4 mm. Image reconstruction and data analysis were performed using ParaVision (Bruker Medical GmbH), Amide (The free software Foundation, Inc, Boston, MA), and ImageJ (NIH).
Results: (1) Unilateral injection of GdDOTA-CTB into the nostril cavity result in strong signal enhancements in the olfactory epithelium exclusively ipsilateral to the injection, as early as 12 hours (the second MRI time point) following injection. **(2)** By day 2, robust enhancement was clearly detected throughout the olfactory epithelium and along the olfactory tract ipsilateral to the injection (Fig.A). Weaker enhancement was also found in the outer layer of the inferior olfactory bulb (i.e. glomeruli layer). Some individual glomerul in the specific region of the olfactory bulb can be easily identified based on the MRI enhancement patterns (Fig.B). **(3)** By 14 days, signal decay was found in the epithelium, which is at least 3 weeks earlier than the normal clearance rate of GdDOTA-CTB (ref 1). **(4)** The injection of GdDOTA-CTB into one nostril did not enhance signal in the contralateral nostril pathway, consistent with the known anatomical evidence (refs 3-4). Together, these results suggest that GdDOTA-CTB can be used to trace peripheral olfactory pathways, in addition to central connections. **(5)** Following unilateral injection of the olfactory bulb, MR signal enhancement was found on day 7 in other regions of the olfactory bulb, and in part of the ipsilateral anterior olfactory nucleus (Fig.C). Weaker enhancement could also be detected in the ipsilateral projection of the central olfactory pathway to pyriform cortex (Fig.C). **(6)** The location and pattern of GdDOTA-CTB transport is consistent with that in known olfactory pathways using monosynaptic conventional tracers (refs 5-6).



A) T1-W images in the sagittal plane, acquired two days after unilateral nostril injections. GdDOTA-CTB strongly enhanced the entire peripheral olfactory projection to its central target olfactory bulb. Prominent signal increases were also detected in the olfactory epithelium (red arrowheads), the olfactory nerve tract (ON, long red arrows). Note that injection of GdDOTA-CTB in one nostril did not enhance signal in the contralateral nostril pathway. B) T1-W images in the coronal plane at a different level (a and b, as shown in the box), acquired 7 days after a right nostril injection. In addition to the olfactory epithelium (red arrowheads) and olfactory nerve tract (red arrows), in the outer glomeruli layer of the olfactory bulb, some individual glomeruli were also enhanced (blue arrowheads). C) T1-IR images showing signal enhancement in the central olfactory pathway 7 days following olfactory bulb injection. MR levels were enhanced in the anterior olfactory nucleus (AON) and pyriform cortex (PCX).

Conclusions: (1) GdDOTA-CTB reliably reveals the mono-synaptically connected anatomical circuitry in the olfactory system, in both peripheral and central neural pathways. **(2)** The transport rate in the peripheral pathway appears faster than central pathway. **(3)** In the peripheral pathway, signal decay was detected earlier than its normal clearance rate, likely reflecting the naturally occurred neuronal turnover in olfactory epithelium. **(4)** This promising new MRI agent opens possibility for further olfactory neurogenesis studies. We have started investigating the rate of axonal transport and clearance in the olfactory pathways of diseased animal models.

References: (1) Wu et al., ISMRM, Stockholm (2010); (2) Vasalatiy et al., American Chemical Society meeting (2009) Washington, DC, USA. (3) Imai and Sakano, Neuron (2008):465-467. (4) Kikuta et al., J Neurosci (2008):11989-11997. (5) Smithson et al., Neuroscience (1989):277-287. (6) Smithson et al., Brain Res Bull (1992):209-220.