

# Assessing *in vivo* Axonal Transport Rates from Deep Brain Structures in Mouse Models of Human Disease

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**Background:** Axonal transport is a cellular process responsible for the transport of proteins, organelles and other cell constituents to and from the neuronal soma. As a result, it is important for the normal function and viability of the neuron. *In vitro* perturbations in axonal transport rates have been demonstrated in diabetes and many neurodegenerative diseases. However, a detailed *in vivo* investigation of these observations has not been possible because axonal transport measurement techniques have been highly invasive, required the sacrifice of the animal and was limited to superficial structures of the central nervous system. Our methodology demonstrates that *in vivo* axonal transport rates from deeper brain structures and can be measured quickly by utilizing intracerebrally injected contrast agents in conjunctions with MEMRI protocols without requiring sacrificing the animal.

**Methods: Animal Models:** Experiments were carried out using the Tg2576 mouse model of Alzheimer's disease and C57/BL6 non-diabetic and diabetic mice. Decreased axonal transport rates have been reported in both. Prior to imaging, 20nL of 20mM manganese chloride (MnCl<sub>2</sub>) was injected stereotaxically into the right dorsal striatum of the mouse brain. All animals were handled in compliance with institutional and national regulations and policies. Animal protocols were approved by Institutional Animal Subjects Committee at Baylor College of Medicine.

**Imaging Protocol:** All images were obtained using a 9.4T, Bruker Avance BioSpec Spectrometer with a 21cm horizontal bore (Bruker BioSpin, Billerica, MA) and a 35mm resonator. Mice were anesthetized using 5% isoflurane with oxygen and placed into the animal holder with a water phantom, where they were kept at 2% isoflurane for the rest of the imaging time. One hour post-injection, mice were imaged using Rapid Acquisition with Refocused Echoes (RARE) 3D protocol with TE=11.30ms, TR=400ms, FOV=25mm, matrix size=128x128x128, number of cycles=8 with each cycle taking approximately 17 minutes and 55 seconds using Paravision software (Bruker BioSpin, Billerica, MA). During imaging, body temperature was maintained at 37.0°C using an animal heating system (SA Instruments, Stony Brook, NY).

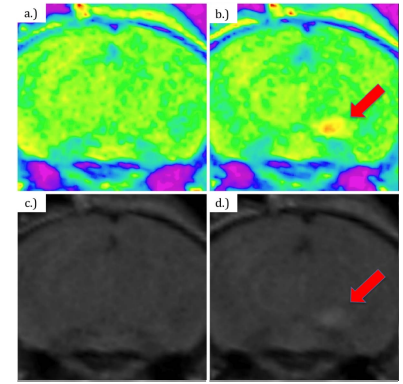


Figure 1: Images shows the first (a,c) and last (b,d) scans with identical slices containing the SN. Increase in SI can be seen in the SN as indicated by red arrow.

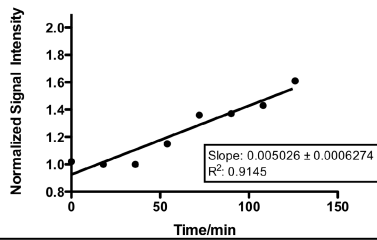


Figure 2: Sample data set obtained in axonal transport measurement. Shows increase in SI in the SN demonstrating axonal transport from striatum

**Data Analysis:** Obtained images were analyzed using Paravision software. Regions of interest (ROI) within the substantia nigra (SN) and the water phantom consisting of one pixel and 9 pixels respectively were selected and copied across each of the 8 cycles. Signal intensities (SI) of SN and water phantom ROIs were measured. SI values obtained for SN were then normalized to those from the water phantom. The correlation between normalized signal intensity in the SN and time were assessed using Prism (GraphPad Software, San Diego, CA).

**Results:** Linear regression analysis shows that there is high correlation between signal intensity as shown in Fig 2 with a R<sup>2</sup> value of 0.9145. Slopes of these graphs are indicative of the axonal transport rates being measured. Comparing the axonal transport rates in 16-18 month Tg2576 mice (n=2) vs. age matched WT controls (n=2) in Fig 3, we observed that there is a trend towards decreased axonal transport rates in the Tg2576 mice. The C57/BL6 Diabetic (n=1) vs. non-diabetic (n=4) mice also demonstrated a trend towards decreased axonal transport in the striatonigral circuitry.

**Discussion:** Our preliminary data indicate that there is a trend towards decreased axonal transport rates through the striatonigral circuitry. These data are consistent with what has been reported in the olfactory system in the Tg2576 model, which utilized 2D MRI datasets. Trends towards decreased *in vivo* axonal transport rates in diabetic mice are also consistent with results that have shown *in vitro*, as well as non-MRI *in vivo* experiments. We demonstrate that utilizing a 3D protocol allows us to easily measure axonal transport rates from deep brain structures in two mouse models of human disease.

## References:

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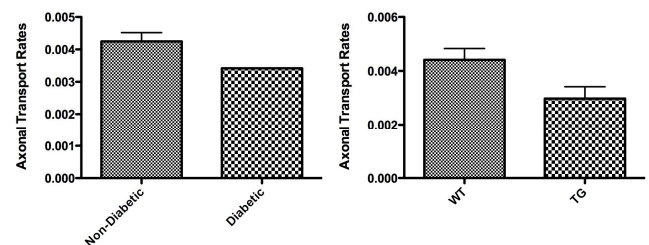


Figure 3: Comparison of axonal transport rates between the different groups tested and shows trends towards decreased axonal transport rates in both diabetic and Tg2576 groups.