# The Use of MEMRI to Assess *In Vivo* Axonal Transport Rates in the Murine Central Nervous System (CNS)

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## Introduction

It would be extremely useful to have a non-invasive method of determining *in vivo* alterations in axonal transport. Currently, methods used are invasive and only apply to easily discriminated, linear axonal tracts. We assessed manganese transport by less invasive means in the complex, highly interconnected murine olfactory system. Our assessment included manipulating temperature and microtubule stability, two aspects known to affect axonal transport. Here, results demonstrate a novel application of MEMRI to detect *in vivo* alterations in axonal transport.

## Materials & Methods

Eight week old C57/Bl6 inbred mice were obtained in house and anesthetized with ketamine/xylazine (0.75mg/ml)/(0.5 mg/ml) in phosphate buffered saline, 0.1 ml per 10g body weight. 10 minutes after administration of the anesthesia, a manganese lavage of 4 µl of 0.75 mg/ml $MnCl_2$ was administered and animals were imaged 1 hr later. For imaging, animals were induced on 5% isoflurane and maintained with 2% isoflurane in 100% $O_2$. Images were acquired utilizing a 9.4T, Bruker Avance Biospec Spectrometer, 21 cm bore horizontal scanner with 35 mm volume resonator (Bruker BioSpin, Billerica, MA). The imaging parameters to acquire olfactory multi-spin/multi-echo MEMRI images were as follows: TR = 500 ms; TE = 10.2 ms; FOV = 3.0 cm; slice thickness = 1 mm; matrix = 128 x 128; NEX = 2; number of cycles = 15; each cycle ~2 min. Core temperature was maintained at 37°C during scanning. For the temperature experiments the animals were maintained at 37°C for 40 min (Fig 2A), cooled to 29°C for 40 min (Fig 2B) and then re-warmed to 37°C for another 40 min (Fig 2C) (1). Colchicine (Sigma C9754) (1 mg/kg) & vehicle controls (.9% normal saline) were administered by lavage 24 hr prior to $Mn^{2+}$ administration. Data was acquired using Paravision (Bruker BioSpin) and then analyzed using linear regression and two-tailed t-tests with Prism (Graphpad Software, Inc). Region of interest (ROI) was placed on an axial slice 1.5 mm from the posterior of the olfactory bulb. The ROI measured 0.12 X 0.12 mm and was vertically centered on the dorsal olfactory neuronal layer. This ROI was copied for each cycle and each value normalized to the unaffected muscle of the same slice (Fig 1).

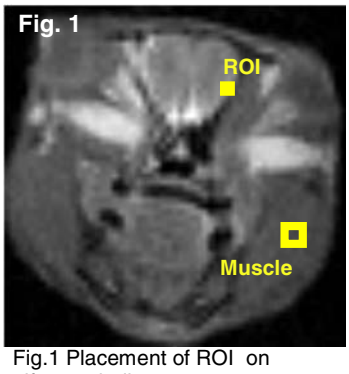


Fig.1 Placement of ROI on olfactory bulb

## Results

Figure 2 shows a representative dataset of signal intensity over time due to $Mn^{2+}$ transport and the responses to alternations in temperature. 2A) the change in SI (ΔSI), over time (x-axis), increases (left y-axis) at physiological temperature (right y-axis). 2B) ΔSI then flattens at the abnormally low temperature of 29-31°C. 2C) the ΔSI again increases upon return to normal temperature. Quantification of the slope, ΔSI/Time (min), shows a 100% decrease between normal and severely reduced temperature groups (x-axis) (Figure 3). We also used colchicine to verify that $Mn^{2+}$ is transported along microtubules. Colchicine administered 24 hrs before $Mn^{2+}$ administration completely stopped the movement of $Mn^{2+}$ (Fig. 4).

**Fig. 2**

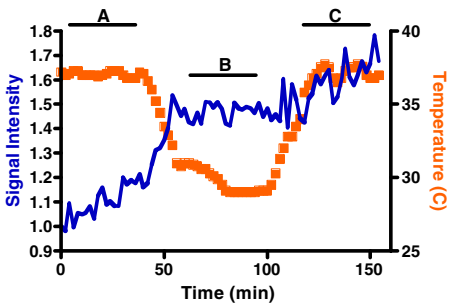


**Fig. 3**

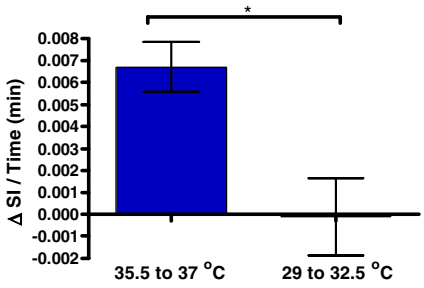


**Fig. 4**

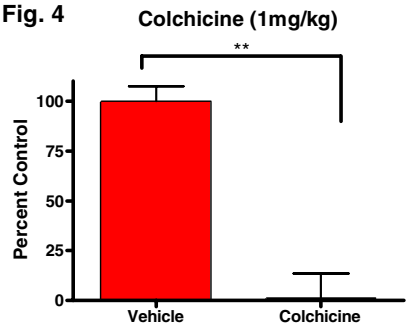


Fig. 2 Temperature (right y-axis) dependence of $Mn^{2+}$ transport (left y-axis/x-axis). A=37°C, 40 min; B=29-31°C, 40 min; C=36-37°C 40 min.
Fig. 3 Change in SI/Time (min) is temperature dependent. *p<0.005 n=9
Fig. 4 Colchicine blocks $Mn^{2+}$. **p=.0015 $n_{vehicle}$=3; $n_{colchicine}$=4

## Discussion

Previous methods for measuring axonal transport take the distance from the $Mn^{2+}$ injection site divided by the time from injection to produce the rate (3). This technique is limited to straight, well-defined axonal tracts, like the optic nerve. The complex interconnections of the other neuronal systems within the brain complicate the interpretation of data acquired in areas that do not conform to these criteria. The present study establishes that there is a robust signal increase over time through the olfactory neuronal layer, a complex, highly interconnected area of the brain. This signal increase was affected by 2 factors known to affect axonal transport: a decrease in temperature and a microtubule disruptor. We interpret this data to mean that the signal increase is due to axonal transport of manganese. This is a potential new tool for characterizing transgenic mice and assessing neurological disorders.

## References

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