Kinesin mutations induce defects in Mn2+ transport in the important memory circuit from hippocampus to basal forebrain

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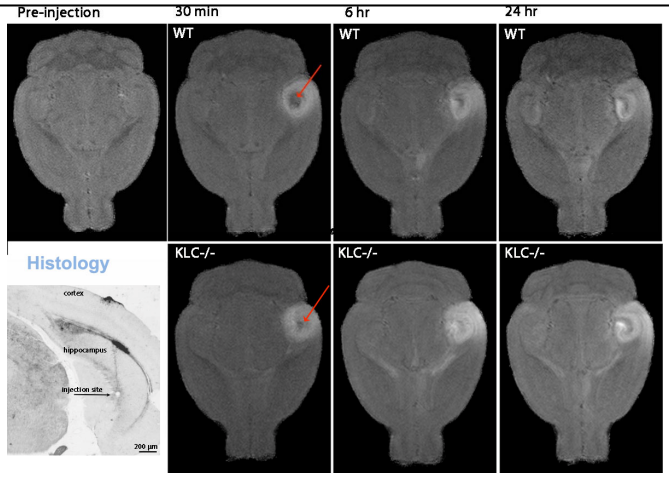
Introduction

Axonal transport delivers cargo along axonal projects. Introduction of tracers into transport systems has commonly been used to study connections between distant brain regions. Manganese, which gives a hyper-intense signal in T1-weighted MRI, enters neurons and is distributed along their projections, probably by endogenous axonal transport machinery. Thus Mn^{2+} serves both as a tracer of axonal projections and as a marker for normal and abnormal transport dynamics [1, 2]. Conventional kinesin is a cellular motor thought to mediate a portion of axonal transport. To determine whether transport defects can be detected by MEMRI, we imaged transgenic mice mutant in one of the kinesin 1 subunits, KLC1, after injection of Mn^{2+} into the eye. We measured intensity changes along the optic nerve over time in time-lapse T1-weighted MR images. We reported that KLC1 knock out reduces the rate of Mn^{2+} transport in the optic track and that a 30% reduction in Mn^{2+} induced intensity changes could be detected in the optic nerve by this methodology [3]. We now test whether transport is delayed in deeper tracts in the CNS, in particular the projections from the hippocampus to basal forebrain, a circuit involved in Alzheimer's disease where we have reported increased Mn^{2+} accumulations in Down's syndrome mice[4].

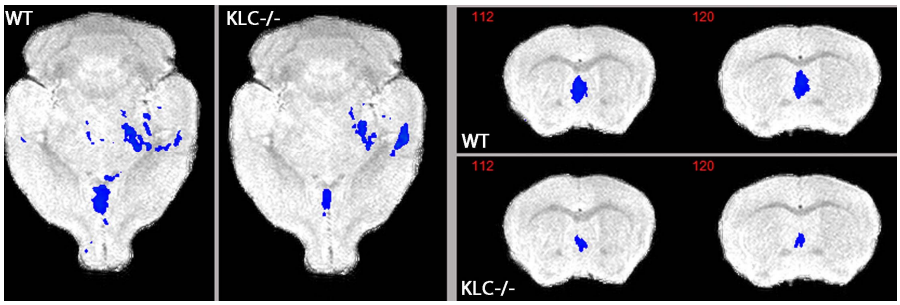
Fig. 1. Atlas at each time point. WT (top), KLC (lower)



Materials and Methods

After collection of a pre-injection image, MnCl2 (600 mM, 3-5 nL) was injected into the right hippocampus (coordinates x −3.2 mm (midline), y −4.1 mm (Bregma), z 3.4 mm (down)) of 9 KLC1-KO or 10 WT littermate mice (10-13 mo old). Immediately after injection, mice were anesthetized with 0.8% isofluorane and MR images acquired at 11.7T (Bruker BioSpin Inc.) using a 35mm linear birdcage RF coil with a 3D RARE imaging sequence, a RARE factor of 4, 4 averages, TR/TEeff=300 ms/21 ms; matrix size of 256,160,128; FOV 23 mm, 14.4 mm, 11.5 mm; yielding 90 µm isotropic voxels with 102 min scan time. Full brain images were begun 30 minutes and repeated at 6 hr and 24 hr post injection. After MRI, mice were sacrificed, perfusion fixed, embedded in gelatin and sectioned in parallel for microscopy correlation. After brain stripping using Multitracer and Tracewalker, all 3D MR images from 4 time points across 5 KLC KO and 6 WT littermates were align-warped using LONI Pipeline software into a single atlas. Subsets of images were isolated from the atlas and intensity patterns compared statistically using SPM5.

Fig. 2. Statistical parametric mapping of hippocampal-forebrain trajectories in wildtype vs KLC KO at 6 hr>30 min post injection.



Results and Discussion

Mn^{2+} enhanced intensity appeared as a halo around the injection site in the 30 min post-injection images and the needle track identifiable in brain sections, which precisely located the injection site (Figure 1). Analysis across all 11 mice demonstrated that injections were consistent with respect to location in CA3 of the hippocampus, within 0.5mm, with similar intensity and dimension of the halo. At 6 hr, 3D SPM comparisons of intensity changes between pre-injection and 6 hr 3D datasets revealed decreased accumulation along hippocampal projections in the KLC KO brain compared to WT littermates (Fig. 2). These differences were sustained 24 hr. Noticeably more Mn^{2+} signal remained at the injection site in the KLC KO than WT.

Conclusion

This study shows that transport dynamic defects measured in the optic nerve an be indicative of similar defects in the central nervous system. In addition, that a loss of a microtubule motor subunit, kinesin light chain 1, depresses the rate of distal accumulation of Mn^{2+} further supports a role for microtubule-based motility, probably via the vesicular transport system, in redistribution of Mn^{2+} along neuronal projections. Supported by NINDS NS062184.

1. Lin, Y.J. and A.P. Koretsky, Magn Reson Med, 1997. **38**(3): p. 378-88.
2. Pautler, R.G., R. Mongeau, and R.E. Jacobs, Magn Reson Med, 2003. **50**(1): p. 33-9.
3. Bearer, E.L., et al., Neuroimage, 2007. **37 Suppl 1**: p. S37-46.
4. Bearer, E.L., X. Zhang, and R.E. Jacobs, NeuroImage, 2007. **37**(1): p. 230-242.