

Microtubule stabilizer ameliorates functional connectivity in a MAP6 deficient mouse: A manganese enhanced magnetic resonance imaging study

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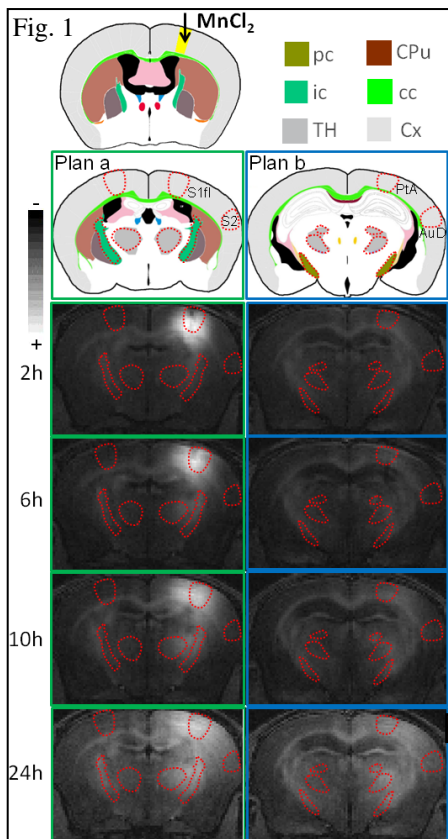
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INTRODUCTION Microtubule associated proteins-6 deficient mice (STOP/MAP6 KO) are devoid of cold stable microtubule. It has been shown that this void induces a synaptic deficit¹. The STOP/MAP6 KO mouse is considered as a model of schizophrenia. Epothilone D (Epo D), a taxol-like microtubule-stabilizing compound, improves this synaptic deficit in MAP6 KO mice². Manganese enhanced magnetic resonance imaging (MEMRI) can be used to tract neuronal connections namely “tract-tracing”³. It has been proposed that Mn-enriched vesicles are transported along microtubules and cross synapses³. In this study, we evaluate the ability of MEMRI to detect alterations in neuronal transport and its improvement by Epo D treatment in MAP6 KO mice.

MATERIELS AND METHODS *Animals:* A total of 32 male 3-6 month-old homozygous MAP6-null mice (KO, MAP6 ^{-/-}) and their wild type littermates (WT, MAP6 ^{+/+}) were used for this study. Animals arose from the same colony (BALBc/129 Sv background). All procedures were performed under isoflurane anesthesia (2%). *MEMRI* The mice (KO: n=6 and WT: n=6) undergone an intracerebral (right primary somatosensory cortex) injection of MnCl₂ (60 nL, 100 mM, 8nL/min) in physiological conditions. 2h, 6h, 10h and 24h after Mn injection, T1-weighted images (MDEFT, TR/TE= 4000/3.65ms, TI= 1000ms, pixel size: 109x109x250µm) were acquired on a 7T MRI system (Bruker) using a surface/volume cross coil configuration. *Treatment:* KO mice were treated with Epo D (intraperitoneal injection, 1 mg/ kg/week, n=10) or vehicle solution (200 µL, n=10). After 8 weeks of treatment, the same MEMRI protocol as previously described was performed. *Data analysis:* regions of interest (ROI) were manually drawn for each time point of the MRI follow-up on each hemisphere using a mouse brain atlas as a visual reference in two slices: Slice a: cortex (Cx S1fl and S2), anterior thalamus (ant TH), and internal capsule (ic); Slice b: cortex (PtA and AuD), posterior thalamus (post TH composed of VPL/VPM and Po nucleus), and cerebral peduncle (cp) (Fig. 1). The ipsilateral Cx S1fl ROI corresponds to the injection site (IS). To account for the injection variability, the signal intensities from each ROI are expressed as a fraction of the signal intensity in the IS ROI (latter named RSI, for relative signal intensity). Results are expressed as mean±Standard Deviation. Unpaired Student t-tests were performed to compare RSI between WT and KO mice and between KO mice treated with or without Epo D. Significance was: * p<0.05, **p<0.005; *** p<0.001.

RESULTS Fig. 1 shows anterior (Slice a) and posterior (Slice b) coronal MRI images at different times after MnCl₂ injection (WT mice). Thalamo-cortical and cortico-pontine structures are highlighted over time. The RSI increases linearly over time in all ROIs (data not shown) for WT and KO mice. At 24h post injection the RSI of contralateral ROIs (ic, ant TH, post TH and cp) in KO mice was smaller than that of the WT mice (Fig. 2A). After Epo D treatment, a significant increase of the RSI was observed (ic, ant TH, post TH, Cx AuD and cp) in KO mice treated with Epo D compared to vehicle (Fig 2B).

DISCUSSION MEMRI revealed difference between WT and MAP6 KO mice in structures with long range connectivity to the IS (polysynaptic structures in contralateral hemisphere⁵), while structures in short range connection to the injection site (like the monosynaptic thalamo-cortical tract⁶) do not. This result is consistent with the known ability of MEMRI to provide information about Mn transport⁷. Interestingly, Epo D treatment improved Mn transport in KO MAP6 mice, in agreement with the previously reported improvements of behaviour⁸ and synaptic function² in these KO MAP6 mice. This functional information may prove very important to better understand the functional deficits and quite uniquely an *in vivo* drug response of the KO MAP6 mice. These properties of MEMRI could be useful to study other neurodegenerative or psychiatric models.



REFERENCES ¹Andrieux et al. Genes Dev. 2002. ²Andrieux et al. Biol. Psychiatry. 2006. ³Lin and Koretsky. MRM 1997. ⁴Pautler et al. Toxicol. Appl. Pharmacol 2004. ⁵Canals et al. NeuroImage 2008. ⁶Aronoff et al. Eur J Neurosci 2010. ⁷Smith et al. NeuroImage 2007. ⁸Fournet et al. J. Neurochem. 2012.

