Evaluation of functional connectivety in a cold-microtubule deficient mice using manganese enhance magnetic resonance imaging in vivo

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INTRODUCTION Stable tubule only polypeptide deficient mice (STOP KO) are devoid of cold stable microtubule. It has been shown that this void induce a synaptic deficit[1]. Manganese enhanced magnetic resonance imaging (MEMRI) can be used to tract neuronal connections namely "tract-tracing"[2]. It has been proposed that Mn-enriched vesicles are transported along microtubules and cross synapses[3]. In this study, we evaluate whether MEMRI can detect alterations in Mn transport in STOP KO mice.

MATERIELS AND METHODS ***Animals*****:** Male 3-6 month-old homozygous STOP-null mice (KO, STOP -/-, n=10) and their wild type littermates (WT, STOP +/+, n=10) were used for this study. Animals arose from the same colony (BALBc/129 Sv background). All procedures were performed under isoflurane anesthesia (2%). ***Histology***: 4 KO and 4 WT animals were sacrificed and perfused with PFA 4%. Brains were quickly removed, dehydrated in sucrose 20%, frozen in isopentane and stored at -80°C. Free floating thin brain cryosections (25 µm) were obtained. Gold labelling was performed to highlight neuronal tracts. The contours of the internal capsule (ic) and cerebral peduncle (cp) were manually drawn using image J software and their areas were determined and averaged across both hemispheres. ***Mn injections*****:** The remaining mice (KO: n=6 and WT: n=6) undergone an intracerebral (right primary somatosensory cortex) injection of $MnCl_2$ (60nL, 100mM, 8nL/min) in physiological conditions. ***In vivo MRI:*** 2h, 6h, 10h and 24h post-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. ***Data analysis*****:** Five regions of interest (ROI) were manually drawn on each hemisphere using a mouse brain atlas as a visual reference: cortex, anterior and posterior thalamus (at, pt respectively), ic and cp. The ipsilateral cortex ROI corresponds to the injection site. To compensate for the injection instability, the signal intensities in the at, pt, ic, and cp ROIs are expressed as a fraction of the signal intensity in the ipsilateral cortex ROI (Fig 2A). Results are expressed as mean±Standard Deviation. Unpaired Student t-tests were performed to compare KO and WT data. Significance was: * $p<0.05$, *** $p<0.001$

RESULTS Gold staining reveals that ic and cp tracts are smaller in KO than in WT mice in both hemispheres, as observed on the histological snapshots (Fig 2A). For each animal type (WT or KO), tract areas were comparable between hemispheres and were thus averaged. KO mice had a 51.5% and 41.8% reduction in, respectively, their ic and cp tract areas compared to WT (Fig. 2B). Fig. 1A shows MRI images at different times after $MnCl_2$ injection. Different structures are highlighted over time (e.g. compare the data obtained at 10h and 2h). The relative signal intensities for at (ispilateral), ic (contralateral), and cp (ispilateral) are represented in Fig 1B. The relative signal increases linearly over time in at (ipsilateral) for WT and KO mice. The same is observed in the pt nuclei (ispilateral, data not shown). At 24h post injection, a significant decrease of the relative signal intensity from MEMRI images by 24.5% and 31.9% was found, respectively, in ic (contralateral) and cp (ipsilateral) of KO mice compared to WT. In the five contralateral ROI (cortex, at, pt, ic, cp), the relative signal intensity in KO mice was smaller than that of the WT mice.

DISCUSSION These data indicate that there is a bilateral reduction in tract areas while there is an asymetric reduction in Mn transport. These data thus suggest that a reduction in tract areas does not necessarily yield a comparable reduction in Mn transport. This observation is consistent with data reviewed by Damoiseaux et al[4] who indicate that structural and functional connectivity are not always correlated. Interestingly, MEMRI revealed difference between WT and KO in two structures with long range connectivity to the injection site (ic and cp[5]), while structures in short range connection to the injection site (like the thalamo-cortical tract[6]) do not. This result is consistent with the known ability of MEMRI to provide information about Mn transport[7]. This functional information may prove very important to better understand the functional deficits of the KO STOP mice and more generally neurodegenerative or psychiatric mice models.

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