Diminishing $GABA_A$ alpha5 receptor mediated inhibition rescues hippocampal perfusion deficit in a mouse model of Down syndrome

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TARGET AUDIENCE: The presented data are of interest for scientists and clinicians involved in imaging and/or drug development with focus on neurodevelopmental disorders.

PURPOSE: Increased GABA-mediated inhibition has been proposed[1] as a mechanism underlying long-term potentiation (LTP) and cognition deficits in the Ts65Dn (TS) mouse model[2] of Down Syndrome (DS). We have recently shown that the selective $GABA_A$ α5 negative allosteric modulator (NAM) RO4938581 (F. Hoffmann-La Roche) rescues synaptic plasticity and improves spatial learning in TS mice[3]. In search for biomarkers with translational potential in clinics, in the present study we have used arterial spin labeling (ASL) MRI and single-voxel ^{1}H MRS to assess regional perfusion and hippocampal metabolites of TS and control (WT) mice at baseline and of TS mice following chronic treatment with RO4938581.

METHODS: **MRI/MRS** was carried out on a Bruker BioSpec 9.4T system equipped with a 72 mm bird-cage resonator for excitation and a surface coil for reception. Study in Ts65Dn (B6EiC3Sn a/A Ts(1716)65Dn) comprised assessment of regional brain perfusion and hippocampal metabolites before (baseline), following **chronic treamtent** with RO4938581 (RO, daily i.p., 10mg/kg) and again after a drug-free period (Fig.1). For imaging mice were sedated with medetomidine (s.c. 0.6mg/kg/h following a priming dose of 0.3mg/kg). **FMRI** was obtained by means of perfusion imaging based on continuous ASL with centred-RARE readout (TR/TE = 3000ms/5.4ms, RARE factor = 32, FOV = 2cm x 2cm, 128 x 64 matrix, 0.6mm slice thickness, 8 slices, 2 averages, 3s labeling, 0.4s post labeling delay). For subsequent registration to an anatomical template with associated atlas defining 30+ regions of interest (ROIs), T_2-weighted images were acquired. Perfusion values for each ROI were normalized to plane-wise brain-mean perfusion to derive region-specific values independent of inter-individual differences of the animals' global hemodynamic status. ROI-wise differences between the groups were tested for significant effects using ANOVA and post-hoc Welch's t-test. **1H MR spectra** were acquired using PRESS single voxel (TR 2 s, TE 10 ms, spectral width 4 kHz, VAPOR water suppression interleaved with outer volume suppression, 2048 complex points, 512 averages, acquisition time 17 min). A 1.3x2x1.7mm^3 voxel was assessed in the left lateral hippocampus.

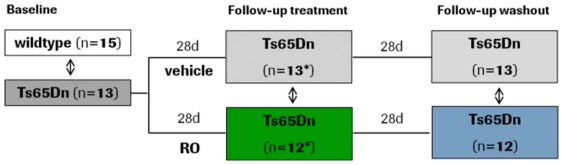

Fig. 1: Study design. *After baseline acquisition Ts65Dn mice were equally distributed to RO and vehicle group and supplemented by additional mice from same cohort.

RESULTS AND DISCUSSION: Ts65Dn mice at baseline showed perfusion deficits in several hippocampal (Hpc) subregions such as CA1, subiculum (Sub) and dentate gyrus (DG), as well as in the nucleus accumbens (N.acc.) and enhanced perfusion in the thalamus and substantia nigra (SN) (Fig.1 and 2). 4w chronic treatment with RO4938581 rescued perfusion deficits in TS mice, specifically in the hippocampal subregions Sub (Fig. 3), CA1 and DG (data not shown). We did not observe any treatment effect in other regions which presented an altered perfusion at baseline (Fig. 3). As exemplified for the subiculum, perfusion values in the hippocampus of Ts65Dn mice return to baseline values after a subsequent 4 week drug washout period, further supporting a treatment-specific effect at the follow-up time point.

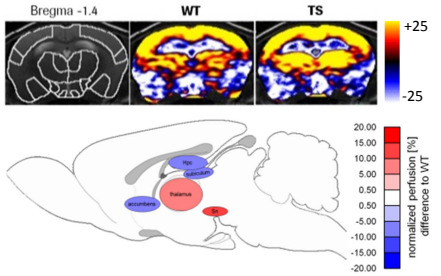

Fig. 2: Perfusion maps (%, normalized to total brain perfusion) of WT and TS mice of a single slice (upper panel) indicating an increased perfusion in the thalamus. Lower panel shows the perfusion phenotype (TS>WT) on schematic sagittal brain section.

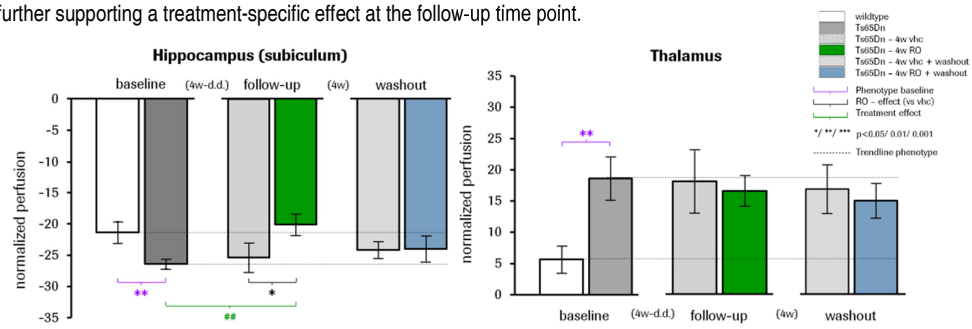

Fig. 3: Complete reversal of perfusion deficits in the hippocampus (CA1 region) after chronic treatment with RO4938581. Perfusion differences in the thalamus between TS and WT are not affected by chronic treatment. Similarly we did not observe any treatment effect in the N.acc or SN (data not shown).

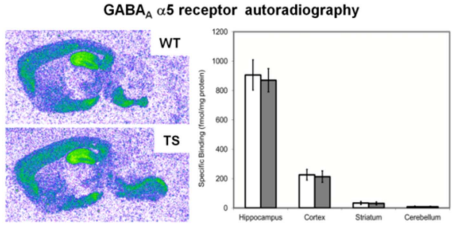

Fig. 4: In vitro autoradiography of sagittal brain sections of WT and TS mice using the $GABA_A$ α5 subtype specific radioligand [^{3}H]-L655,708 (left panel). Right panel shows quantification of specific [^{3}H]-L655,708 binding sites (@ 2nM) for WT (white bars) and TS (grey bars) mice.

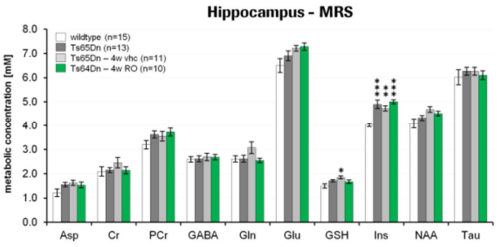

Fig. 5: Selected hippocampal metabolites in TS and WT at baseline and after treatment assessed by MRS. Significantly different levels of ml present at baseline are not affected by $GABA_A$ α5 NAM treatment. Metabolite quantification was carried out using LCModel®.

RO4938581 effects on perfusion were also observed in striatum, insular and prefrontal cortex, regions which do show $GABA_A$ α5 receptor expression, although to a much lesser degree than the hippocampus. Autoradiography analysis of [^{3}H]-L655,708 binding sites (a radioligand specific for the $GABA_A$ α5 receptor subtype) in WT and TS brains suggested similar expression levels of α5-subunit containing $GABA_A$ receptors in both strains. In addition, consistently with previous studies, we detected elevated myo-inositol (mI) levels using ^{1}H MRS. Elevation of this metabolite has been linked to the trisomic expression of the brain mI transporter both in TS mice and DS individuals[4,5]. 4 week chronic treatment with $GABA_A$ α5 NAM did not affect mI levels.

CONCLUSION: Using ASL-fMRI we revealed a regional perfusion phenotype in the TS mouse model of DS extending previous MRI studies in TS mice which focused on structural alterations only[6,7]. The results of the treatment study demonstrate that reducing GABAergic inhibition with RO4938581 can reverse hippocampal perfusion deficits of TS65Dn mice. This is in line with recently observed effects of RO4938581 to rescue deficits in memory, hippocampal synaptic plasticity, and adult neurogenesis[3] and further supports the potential therapeutic use of selective $GABA_A$ α5 NAMs to treat cognitive dysfunction in DS along with a potential pharmacodynamic imaging marker.

REFERENCES: **[1]** Fernandez F, et al., (2007) Nat Neurosci 10:411–413; **[2]** Reeves RH, et al., (1995) Nat Genet 11:177–184; **[3]** Martínez-Cué C, et al., (2013). J Neurosci 33(9):3953-66. **[4]** Huang W, et al., (2000). Neuroreport.11(3):445-8. **[5]** Lamar M, et al., (2011). Neuroimage 57(1):63-8. **[6]** Bearer EL, et al. (2007) NeuroImage 37;230–242. **[7]** Chen Y, et al., (2009) Neurobiology of Aging 30;1453–1465.