Neurofunctional and neurochemical endophenotypes in mouse models of autism spectrum disorder investigated by fMRI and MRS

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PURPOSE: Autism spectrum disorder (ASD) is a neuropsychiatric disorder characterised by stereotyped behaviours and impairments in communication and social interactions. Despite its neurodevelopmental origin and high incidence (>1 in 100 children), the aetiology and pathology of ASD are still largely unknown. Substantial heterogeneity of clinical manifestations of ASD has been reported as a major obstacle in further uncovering disease aetiology and specific biomarkers and therefore animal models that mimic specific facets of the disease, i.e. endophenotypes, have been developed. These animal models of ASD have been assessed mainly by behavioural testing and/or ex vivo structural MRI[1]. Here we leveraged neurofunctional and neurochemical appraisals with the goal of bridging the gap between genetic/molecular findings and ASD-related behavioural phenotypes. A potentially translational approach was taken with in vivo fMRI and MRS that were carried out in five distinct mouse models of idiopathic ASD ranging from inbred strains and environmental challenges to specific gene mutations and copy number variants.

METHODS: In vivo MRI/MRS and behavioural studies were carried out in five ASD mouse lines encompassing inbred BTBR mice, prenatal valproic acid challenge (VPA) model, SHANK3 knockout (KO), NLGN3 knockin (KI), and 15q11-13 duplication mice. Cohorts of n=12-20 animals per model and corresponding wild type littermates (C57Bl/6 for BTBR mice) were assessed in two independent replicates. **MRI/MRS** was carried out in anaesthetised animals on a Bruker BioSpec 9.4T/20cm system equipped with a 72 mm resonator for excitation and a head surface coil for reception. Regional neural activity was assessed with perfusion-based fMRI by means of continuous ASL with centred-RARE readout, TR/TE=3000/5.4ms, RARE factor=32, FOV=(2cm)^2, 128 x 64 matrix, 0.6mm slice thickness, 16 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) and quantification, T_2-weighted images and T_1 maps were acquired. Perfusion values for each ROI were normalized to plane-wise brain-mean perfusion in order to derive region-specific values independent of inter-individual differences of the animals' global hemodynamic status. ROI-wise differences between ASD model and corresponding wild type were tested for significance using ANOVA and post-hoc Welch's t-test. **^{1}H-MR spectra** were acquired using PRESS single voxel spectroscopy (TR/TE=2000/10ms, spectral width 4 kHz, VAPOR water suppression, outer volume suppression, 2048 data points, 512 averages, 17 min acquisition time). A 4.5uL voxel placed in the prefrontal cortex was assessed. Absolute metabolite quantification was carried out using LCModel with water referencing. **Behavioural testing** was performed in the same animals following neuroimaging in order to correlate alterations in neural activity and neurochemistry with ASD-related behavioural deficits.

RESULTS AND DISCUSSION: Figure 1 shows a summary of neural activity patterns observed in five mouse models of ASD compared to controls. These mouse models were characterized by significantly altered activity in neural networks involved in motivation, cognition, anxiety, reward and socio-emotional processiong (e.g. cortex, hippocampus, amygdala hypothalamus, VTA), repetitive behaviour (e.g. striatum) as well as sensory processing (e.g. somatosensory cortex, colliculi). Each model harbours alterations encompassing several brain regions and neural circuitries involved in core ASD-related behavioural deficits[2,3], thus supporting their face validity. However, the phenotypical overlap between the investigated animal models of ASD is rather modest. Yet, this finding reflects the heterogeneity and complexity of ASD observed in patients.

Figure 2 reports the levels of the neurotransmitters glutamate (Glu) and GABA, as determined by MRS in mPFC in the different mouse models of ASD (shown as percentage of controls). Significantly altered concentration of Glu was observed in all the models, whereas GABA levels remained unchanged, except for BTBR mice. This finding supports the notion of an excitatatory/inhibitory dysbalance that has been postulated as an underlying substrate of ASD in humans[4].

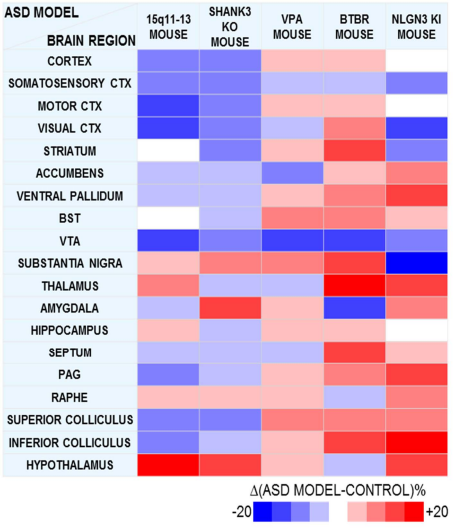

***Fig.1**: Basal neural activity in ASD mouse models as investigated by perfusion-based fMRI. Data are depicted as a percentage of perfusion difference to control.*

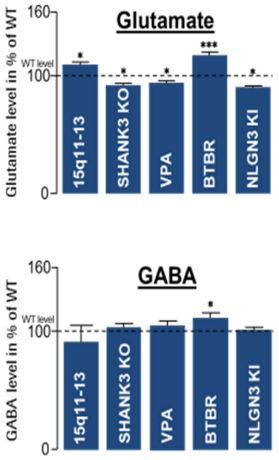

***Fig.2**: Baseline levels of glutamate and GABA in mPFC of mouse models of ASD. Neurotransmitter levels are represented as percentage of controls.*

CONCLUSION: Using ASL-fMRI and MRS we have unveiled prominent functional and neurochemical alterations in the brains of five mouse models of ASD that mimic different putative underlying causes of ASD in humans, and thus have significantly extended previous MRI studies focusing on structural alterations[1]. Excitatory neurotransmitter levels and neural activity were found to be significantly altered in all the models. By correlating these endophenotypes with behavioural deficits we have started establishing putative mechanistic links between underlying genetics and behavioral outputs.

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