Abstract

Autism is a highly treatable neurodevelopmental disorder, affecting about 1% of children. Based on the identification of candidate genes for autism, genetic mouse models have been developed, allowing for targeted studies of gene-to-brain interactions that may underlie neurodevelopmental changes in individuals with autism. Several genetic mouse models were found to exhibit autism-related phenotypes, including CNTNAP2 knockout (KO) mice that have been shown to recapitulate some autism-related behavior, including impaired social interaction. Here, we mapped brain circuit activation during social behavior in the Cntnap2 KO mouse model. First, we independently repeated and validated the impaired social behavior phenotype in the Cntnap2 KO mouse. We then designed a study to test our hypothesis that the loss of Cntnap2 affects selectively brain circuitry related to social processing.

1. Impaired social behavior in CNTNAP2 KO mice

A) CNTNAP2 KO showed impaired sociability (B) in the three chamber test (A), independently repeated and validated the previous published impaired social behavioral phenotype in CNTNAP2 KO mice (Hagopian et al. 2011, DOT). To investigate the brain mechanisms of the impaired social behavior in the Cntnap2 KO mice, we used serial two-photon (STP) tomography-based mapping of whole brain activity. STP tomography images the entire mouse brain in vivo, using selective two-photon excitation of endogenously fluorescent proteins, allowing the detection of cellular and subcellular level neuronal activity.

2. Experimental Design

We used a 10µm diameter, 20µm depth imaging voxel to visualize the cell counting and performed voxel-to-voxel statistical analyses between groups. We selected a median of 1000 voxels per brain from each group to minimize intersubject variability. We used a voxel-based analysis approach to detect significant differences between the CNTNAP2 KO and WT mice.

4. Anatomical ROI or evenly spaced voxel based statistics

A) The STP images were preprocessed and analyzed using a custom MATLAB script. We defined anatomical regions of interest (ROI) using the Allen Brain Atlas. We then performed a voxel-based analysis to detect significant differences in signal intensity between the CNTNAP2 KO and WT mice.

5. Socially-evoked brain activation may be altered in CNTNAP2 KO mice

A) Serial two-photon tomography-based imaging of the entire brain activity in CNTNAP2 KO and WT mice reveals a significant decrease in overall brain activity (A). Our results suggest that the loss of CNTNAP2 selectively affects brain circuitry related to social processing.

3. STP based imaging and data processing pipeline

B) The STP images were preprocessed and analyzed using a custom MATLAB script. We defined anatomical regions of interest (ROI) using the Allen Brain Atlas. We then performed a voxel-based analysis to detect significant differences in signal intensity between the CNTNAP2 KO and WT mice.

6. Decreased reward circuit activation in the KO mice

A) The STP images were preprocessed and analyzed using a custom MATLAB script. We defined anatomical regions of interest (ROI) using the Allen Brain Atlas. We then performed a voxel-based analysis to detect significant differences in signal intensity between the CNTNAP2 KO and WT mice.

8. Altered amygdala and hypothalamus activation

A) The STP images were preprocessed and analyzed using a custom MATLAB script. We defined anatomical regions of interest (ROI) using the Allen Brain Atlas. We then performed a voxel-based analysis to detect significant differences in signal intensity between the CNTNAP2 KO and WT mice.

9. Increased signal variability in CNTNAP2 KO mice

A) The STP images were preprocessed and analyzed using a custom MATLAB script. We defined anatomical regions of interest (ROI) using the Allen Brain Atlas. We then performed a voxel-based analysis to detect significant differences in signal intensity between the CNTNAP2 KO and WT mice.