Serial two-photon tomography-based whole-brain activity mapping in CNTNAP2 KO mouse model of autism

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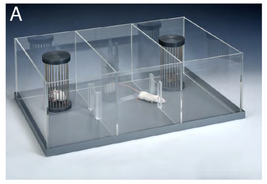
Abstract

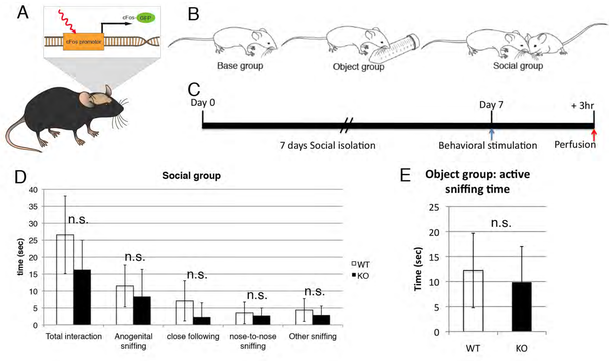
Autism is a highly heritable neurodevelopmental disorder, affecting about 1% of children. Based on the identification of candidate genes for autism, genetic mouse models have been developed, allowing for targeted studies of gene-to-brain interactions that may underlie neurodevelopmental changes in individuals 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 behaviors, 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 mice (Peñagarikano et al., 2011). 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 at cellular resolution with near complete 3D reconstruction (Ragan et al., 2012). We used immediate early gene c-fos, a molecular marker of neuronal activation, to visualize brain activation by STP tomography in transgenic c-fos-GFP mice crossed with Cntnap2 KO mice. The c-fos-GFP datasets were processed by computational methods that detect the activated GFP-positive neurons, warp their distribution to a reference brain registered to the Allen Mouse Brain Atlas, and identify activated brain regions by statistical tests in the c-fos-GFP+Cntnap2 KO and c-fos-GFP+Cntnap2 wild type (WT) littermates. We compared three groups of male mice in each genotype: 1) a home-cage baseline group (no experimental manipulation), 2) an object group (a novel object added to the home cage for 90 seconds), and 3) a social group (an ovariectomized Cntnap2 heterozygote female added to the home cage for 90 seconds). Preliminary results suggest that brain activation patterns are altered in the KO compared to the WT mice during social behavior. Moreover, the KO mice showed higher signal variability than the WT mice, especially during the social behavioral stimulation. Our current hypothesis is that the loss of Cntnap2 affects selectively brain circuitry related to social processing.

1. Impaired social behavior in CNTNAP2 KO mice



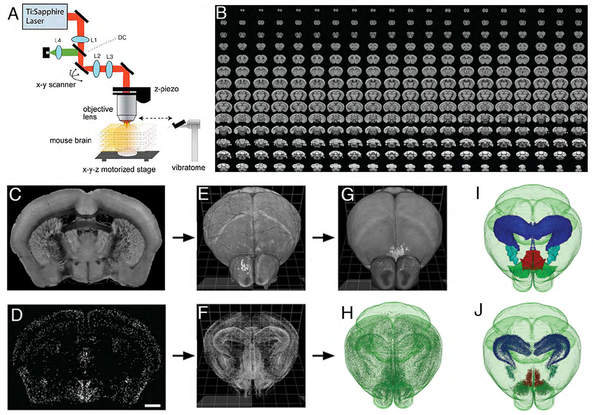
(A-B) CNTNAP2 KO showed impaired sociability (B) in the three chamber test (A), independently repeated and validated the previous published impaired social behavioral phenotype in CNTNAP2 KO mice (Peñagarikano et al., 2011, Cell)

2. Experimental Design



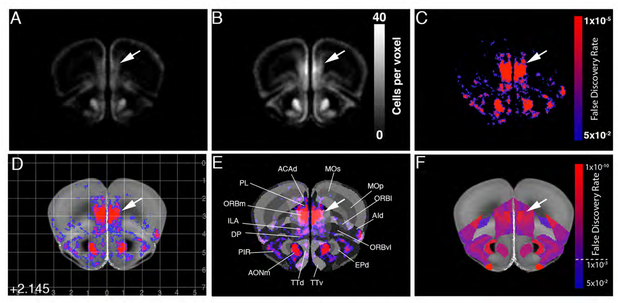
(A) c-fos-GFP mice were crossed with CNTNAP2 KO mice to report neuronal activity. c-fos-GFP(+)/CNTNAP2 KO and c-fos-GFP(+)/CNTNAP2 WT littermates were compared in three different behavior groups. (B) Behavior groups to study task specific brain activation. (C) Behavioral protocol; either an inanimate novel object (Object) or an ovariectomized conspecific CNTNAP2 hetero female (Social) was given to the target mice in the home cage. Mice in the base group had no stimulation. N = 7 – 8 per group. (D-E) c-fos-GFP(+)/CNTNAP2 KO and c-fos-GFP(+)/CNTNAP2 WT littermates did not show significant behavioral differences during either social (D) or object (E) stimulation. Error bar = standard deviation. n.s. = no significant difference

3. STP based imaging and data processing pipeline



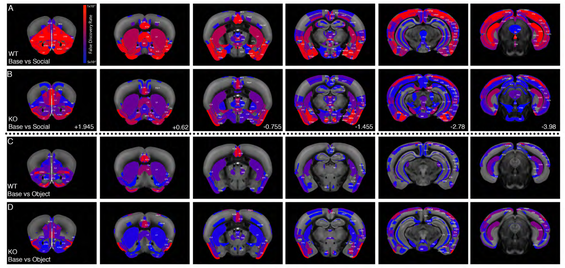
(A) Schema of serial two-photon (STP) microscopy. (B) A sample 280-serial section dataset of a c-fos-GFP mouse brain imaged by STP tomography. (C-H) Registration of convolutional neural network (CN)-detected c-fos-GFP+ cells in the RSTP brain. (C) A coronal section shows the autofluorescence signal, which is used for warping of the 3D reconstructed sample brain (E) onto the RSTP brain (G). (D) 2,177 c-fos-GFP+ cells were detected in the same coronal section; scale bar = 1 mm. (F) 360,183 c-fos-GFP+ cells were detected in the whole brain, reconstructed in 3D and (H) registered onto the RSTP brain using the warping parameters established in the (E-G) step. (I-J) Region of interest (ROI)-based segmentation of the whole-brain c-fos-GFP+ cell count. (I) Examples of ABA ROI segmentation and (J) the corresponding c-fos-GFP+ cell counts: hippocampus—dark blue; 33,508 cells; medial amygdalar nucleus—light blue; 3,035 cells; nucleus accumbens—green; 13,627 cells; and infralimbic cortical area—red; 4,665 cells.

4. Anatomical ROI or evenly spaced voxel based stastics



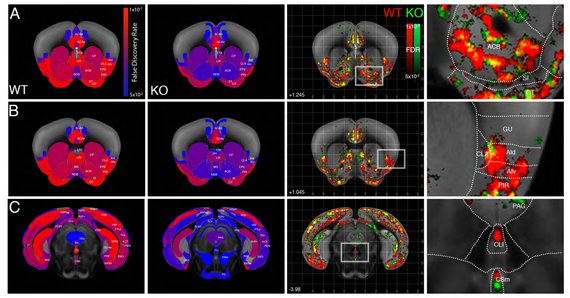
We used a sphere (100μm diameter, 20μm interval) to voxelize the cell counting and performed voxel to voxel statistics between groups. (A-F) Examples of statistical result (C) from voxel-based c-fos-GFP+ cell counts in the object (A) and social (B) groups comparison (dataset from another independent study). The brightness of the signal is based on the number of cells per voxel, as shown in the heat map index in B. The arrow points to an example of a large hotspot of activation in the medial orbital cortex. (D) The voxel statistical output is overlaid on the RSTP brain and (E) overlaid by the ABA ROI labels to annotate the anatomical location of the voxel hotspots. Abbreviations for the ROIs are available in http://atlas.brain-map.org/

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



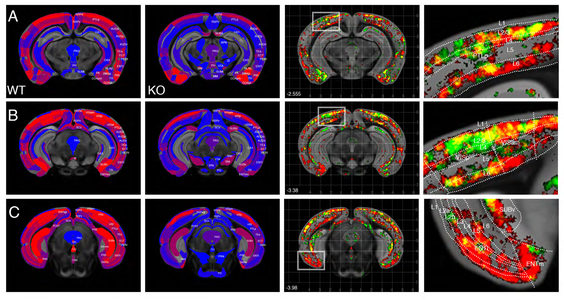
(A-D) Anatomical ROI-based statistical analysis. Examples of ABA ROIs with significant c-fos-GFP induction evoked by social behavior (A-B) or object exploration (C-D), derived from the comparison against the base group. The ABA anatomical labels are superimposed on the RSTP brain and the statistical significance is color-coded according to the heat map index shown in the first panel. Social group (A-B) showed strong and specific activation in medial prefrontal cortex (mPFC), amygdala, hypothalamus, association thalamus, and neuro-modulatory regions, compared to the object group (C-D). In the social groups, KO group (B) showed reduced activation in mPFC, ACB, and CLI but had increased activation in SNr and PVT, compared to WT group (A).

6. Decreased reward circuit activation in the KO mice



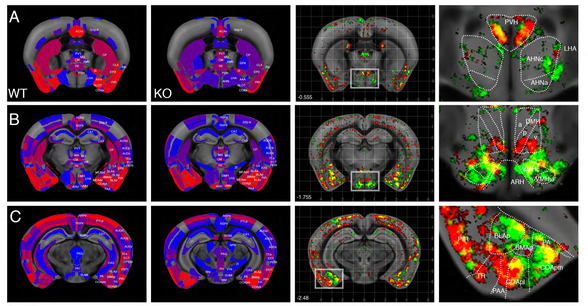
Nucleus of accumbens (ACB, row A), agranular insular cortex (AI, row B) and central linear raphé (CLI, row C) are parts of striato-pallido-thalamo-cortical circuitries to modulate reward response. The KO mice showed decreased activation in these reward circuit components, suggesting social stimulation perceived less rewarding in the KO mice. 1st and 2nd column: Significantly activated anatomical ROI result from the social and the base comparison in the WT and the KO mice, respectively. 3rd column: Significantly activated voxel hotspot from the social and the base comparison in the WT (red) and the KO (green) mice, overlaid on the RSTP brain. 4th column: zoom-in view of the boxed region in the 3rd column.

7. Reduced cortical activation in the KO mice



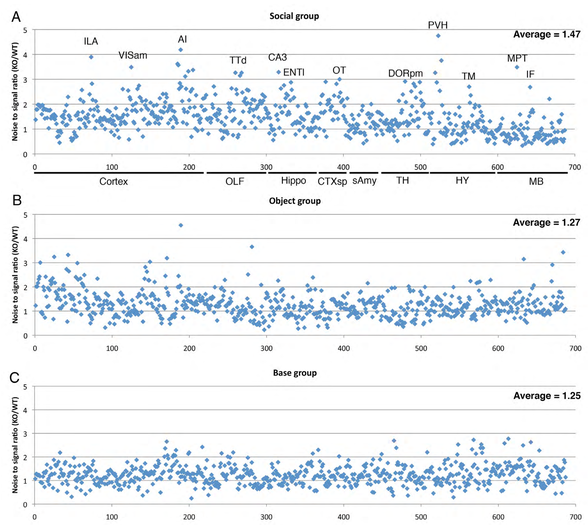
Many cortical regions were less activated in the KO mice such as posterior parietal association area (PTLp, row A), anteromedial visual cortex (VISam, row B), and lateral entorhinal cortex (ENTl). Note that deep cortical layers (layer 5 and 6) were less activated in the KO mice more selectively, compared to other layers (row A and B). This result suggests that the KO mice may have impairment in the long-range functional connection from the cortex to other sub-cortical structures. Figure layout is the same as Figure 5.

8. Altered amygdala and hypothalamus activation



Row A: Paraventricular hypothalamic nucleus (PVH), regulating hormonal response such as oxytocin, showed less activation in the KO mice. In contrast, anterior hypothalamic nucleus (AHN) had aberrant activation in the KO mice. Row B: Note, hyper activation in the ventral medial hypothalamus but almost no activation in the posterior dorsomedial hypothalamus in the KO mice. Row C: note, posterior basolateral amygdala (BLAp) showed higher activation in the KO mice. Figure layout is the same as Figure 5.

9. Increased signal variability in CNTNAP2 KO mice



To measure signal variability, standard deviation was divided (normalized) by mean c-fos-GFP count in each ROI to generate noise to signal ratio (NSR). Then, NSR from the KO mice was divided by one from the WT mice in the same ROI to generate NSR ratio between genotype. ROIs (x axis) were organized according to Allen Brain Atlas structural hierarchy. Approximate regional clustering is annotated in (A). Overall, KO groups showed higher NSR ratio compared to the WT groups. Particularly, the social group showed high signal variability in selected area in the KO mice (ROIs with NSR > 2.5 were annotated in (A)).

Summary and Conclusion

- CNTNAP2 KO mice showed altered activation in many social brain circuits compared to CNATNAP2 WT mice.
1. Reduced activation in CLI, ACB, and AI suggests dysfunction in brain reward circuit in the CNTNAP2 KO mice.
2. Preferential reduction in the deep layers of the cortex suggests impaired cortical-to-subcortical region signal transmission in the KO mice.
3. Altered amygdala and hypothalamic response implies changed social stimulation signal integration to generate behavioral response.
- CNTNAP2 KO mice showed less reliable (noisy) brain activation upon social exposure.
- Taken together, this study provides comprehensive functional brain mapping to reveal circuit abnormalities in processing social stimulation, caused by CNTNAP2 synaptic gene knockout in mice.

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