Complex interplay between structural and functional brain connectivity in acallosal BTBR T+tf/J mice

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Introduction

Recent investigations of the relationship between functional and structural connectivity in healthy brain support the notion that correlations in spontaneous brain activity reflect patterns of anatomical connectivity (*1, 2*). However, studies examining congenital or surgical alteration of the corpus callosum (i.e. the main direct structural connection between the two cerebral hemispheres) have produced conflicting results which somewhat challenge this view. For example, preserved inter-hemispheric connectivity in individuals with callosal agenesis has been recently described (3), in contrast with previous reports in humans (4, 5) and surgical callostomy studies in non human primates (6). These results highlight a complex (and still elusive) interplay between structural connections and functional networks that is apparent in acute or congenital neurological states characterized by large white matter topological alterations. In order to better elucidate this relationship, here we used DTI tractography and resting-state fMRI (rsfMRI) to probe structural and functional brain connectivity in BTBR *T+tf/J* mice, a congenital acallosal mouse line widely used in the preclinical community to mimic autism-like symptoms owing to its robust communication and social deficits (*7*). Normo-callosal C57Bl6/J mice were used as comparators owing to their recently characterized rsfMRI connectional signature (*8*).

Methods

All experiments were carried out in accordance with Italian regulations governing animal welfare and protection. **Animals:** MRI experiments were performed on adult male *BTBR T+tf/J* (BTBR, n=10) mice and control C57Bl/6 (B6) mice (n=10). **Animal preparation:** The procedure employed for rsfMRI has been recently described (*8, 9*). Briefly, mice were anaesthetized with isoflurane, intubated, and artificially ventilated, and blood gases and arterial pressure monitored continuously. rsfMRI timeseries were acquired under controlled halothane anaesthesia (0.7%). **rsfMRI:** All experiments were performed using a 7.0 Tesla MRI scanner using a single-shot EPI sequence with TR/TE 1000/15 ms, matrix 100 × 87, field of view 2.3 × 2 cm^2, 16 coronal slices, slice thickness 0.75 mm an NT=360 (*8*) **DTI:** We recently described a preliminary tractographic investigation of the BTBR brain (*10*). High resolution DTI images were acquired on ex-vivo PFA fixed brain inside the intact skull as recently described using 81 encoding directions and a spatial resolution of 0.13 x 0.13 x 0.26 mm^3. Cross-correlation connectivity matrices were computed using deterministic tractography and counting streamlines originating and ending in a parcellated atlas of the mouse brain (*8*). **rsfMRI data analysis:** A detailed description of rsfMRI pre-processing employed in this study has been recently published (*8*). Seed map correlations and cross-correlation matrices in a parcellated atlas of the mouse brain were generated as previously described (*8*). Group maps were thresholded at $|Z| > 2$, and cluster-corrected at $p < 0.01$.

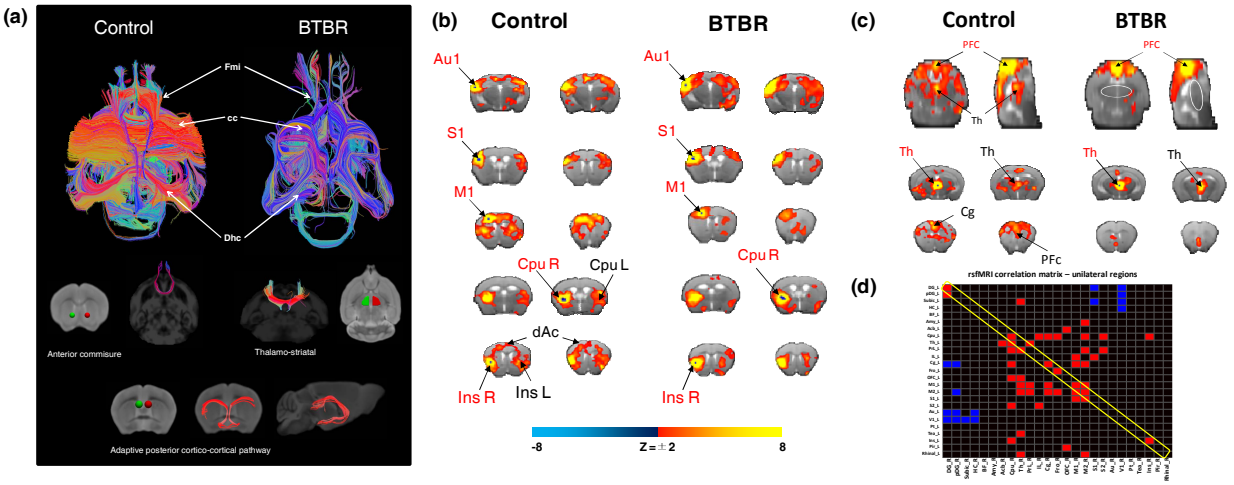

Figure 1 (a) *Diffusion tensor tractography (DTT) showed complete callosal agenesis and lack of hippocampal commissure in BTBR mice (right) together with a major antero-posterior rearrangement of white matter (left).Conserved sub-cortical tracts, together with a novel adaptive cortico-cortical inter-hemispheric path were observed.**(b)** Seed-based rsfMRI mapping (seeds in red) revealed impaired frontal inter-hemispheric connectivity (S1, M1, Cpu) but conserved posterior-cortical inter-hemispheric correlations (Au1). Connectivity with the insular cortex highlighted lack of dorsal-prefrontal recruitment in BTBR subjects, a signature of a disrupted salience network (8).**(c)** Impaired long-range fronto-thalamic and hippocampal connectivity was also apparent in BTBR mice **(d)**.Univariate rsfMRI correlation analyses (corrected at FDR<0.05) corroborated these results, and highlighted significantly increased intra-hemispheric connectivity in perihippocampal areas of BTBR (red Control > BTBR; blue, Control < BTBR)[Au1: auditory cortex; S1: primary somatosensory cortex; M1: primary motor cortex; Cpu: caudate putamen, Ins: insular cortex ,dAc: dorsal anterior cingulate; PFC: prefrontal cortex; Th: thalamus, Cg: cingulate cortex]. Abbreviations in **(d)** are described in (8).*

Results & Discussion

Figure 1 summarises the main findings of this study. DTT corroborated the complete lack of inter-hemispheric connections in the corpus callosum and dorsal hippocampal commissure of *BTBR* mice, together with a rostro-caudal reorganisation of WM tracts in these animals, as previously reported (*10*). Intact inter-hemispheric fascicles were observed in the anterior commissure and in ventro-medial thalamic regions in *BTBR* subjects. Seed region analysis of rsfMRI BOLD signals highlighted impaired inter-hemispheric connectivity in frontal-cortical but not posterior cortical regions. Univariate analyses of DTT correlation matrices highlighted the presence of an adaptive cortico-cortical inter-hemispheric tract innervating these areas, which could explain the presence of intact connectivity in these regions. Major inter-hemispheric deficits were also observed in the striatum, insula and across hippocampal areas of BTBR. These finding are interesting as they suggest that callosal alterations can produce sub-cortical inter-hemispheric desynchronization. Similarly, major long-range rsfMRI connectional deficits were observed between fronto-thalamic and fronto-hippocampal regions. This finding is of interest in the light of translational use of the models to mimic autism, as these impairments are sometimes regarded as a clinical hallmark of the disorder (*11*). Increases intra-hemispheric cortical connectivity was observed in BTBR with respect to normo-callosal control mice. Together, these data suggest that callosal agenesis can produce a complex reorganization of the connectional architecture of the brain, with effects that are prominent in subcortical regions and in fronto-thalamic areas. Compensatory processes can restore cortico-cortical connectivity via generation of adaptational tracts.

References [1] Honey et al., PNAS 106, 2035 (2009) **[2]** Sui, et al., NeuroImage **[3]** Tyszka, et al,. J Nsci 15, 154 (2011) **[4]** Johnston et al., J Nsci 28, 6453 (2008) **[5]** Quigley et al., J Neuroradiol 24, 208 (2003) **[6]** O'Reilly et al., PNAS 110, 13982 (2013) **[7]** Scattoni, et al., PLoS ONE 3, (2008) **[8]** Sforazzini et al., NeuroImage In press, (2013) **[9]** Yang et al., Submitted (2013) **[10]** Dodero et al., PLoS ONE 8, e76655 (2013) **[11]** Anagnostou et al., Molecular Autism 2, 4 (2011).