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Novel inter-hemispheric white matter connectivity in the BTBR mouse model of autism

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Abstract

Alterations in the volume, density, connectivity and functional activation of white matter tracts are reported in some individuals with autism and may contribute to their abnormal behaviors. The BTBR (BTBR T+tf/J) inbred strain of mouse, is used to model facets of autism because they develop low social behaviors, stereotypical and immune changes similar to those found in people with autism. Previously, it was thought a total absence of corpus callosal interhemispheric connective tissues in the BTBR mice may underlie their abnormal behaviors. However, postnatal lesions of the corpus callosum do not precipitate social behavioral problems in other strains of mice suggesting a flaw in this theory. In this study we used digital pathological methods to compare subcortical white matter connective tracts in the BTBR strain of mice with those found in the C57Bl/6 mouse and those reported in a standardized mouse brain atlas. We report, for the first time, a novel connective subcortical interhemispheric bridge of tissue in the posterior, but not anterior, cerebrum of the BTBR mouse. These novel connective tissues are comprised of myelinated fibers, with reduced myelin basic protein levels (MBP) compared to levels in the C57Bl/6 mouse. We used electrophysiological analysis and found increased corpus callosum connectivity in the posterior hemispheres of the BTBR strain compared with the anterior hemispheres. The conduction velocity was slower than that reported in normal mice. This study shows there is novel abnormal interhemispheric connectivity in the BTBR strain of mice, which may contribute to their behavioral abnormalities.

Keywords

Corpus callosum; connectivity; BTBR mice; electrophysiology; white matter

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1. Introduction

Autism is a debilitating developmental disorder which affects an estimated 1/88 children in the United States [1]. People with autism commonly display stereotypical, communicative and social behavioral deficits. The etiology of autism remains unclear although genetic, immune and environmental factors are thought to play a role [2;3]. Imaging studies in autistic individuals indicate that there are abnormalities in the volume, density and functional connectivity of white matter tissues [4] which may contribute to abnormal behaviors [5]. In addition to morphological changes in white matter tissue, there is also increasing evidence of abnormal connectivity between cerebral hemispheres and a reduction in the activity of subcortical networks [6]. Whether or not there is a causal relationship between alterations in white matter connectivity and abnormal social behaviors remains unclear.

One commonly used mouse model of autism is the inbred BTBR strain, which naturally displays stereotypical and social behavioral abnormalities [7–10]. Neuroanatomical studies of the BTBR mouse brain have previously focused only on the mid-sagittal anterior connective tissues and reported a total absence of corpus callosum connective tissues, which was subsequently implicated in precipitating abnormal behaviors [11]. Yet, a follow-up study in behaviorally normal mice found that ablating the callosal interhermispheric connective tissues did not induce aberrant social, or stereotypical behaviors [12]. Thus, it is likely that factors other than a complete lack of corpus callosal connectivity contribute to behavioral abnormalities in BTBR mice and by analogy in autistic children.

The initial studies on white matter connectivity in the BTBR strain of mice were conducted on sagittal brain tissue sections; thus, abnormalities in the connective tissues of the internal capsule would have been missed. In addition, there has not been an investigation to determine if white matter tissue in the BTBR mouse is comprised of abnormal expression of proteins such as microtubule associated protein (MAP) or myelin basic protein (MBP), both of which are implicated in autism [13]. Moreover, there are reports of increased interhemispheric electroencephaolographic (EEG) activity in autistic individuals which, we suggest, may also be found in the BTBR mouse [14]. Based on the compelling evidence that white matter deficits are found in autistic individuals, we hypothesized that there would be alterations in the inter-hemispheric connective white matter fiber structure, composition and activity in the BTBR strain of mice which may contribute to the abnormal behaviors characteristic of that strain. In order to better characterize alterations in white matter tissues in the BTBR strain of mice, we used neuropathological analysis of white matter tissues in coronal brain tissue slices, molecular biology and electrophysiology to characterize the interhemispheric connective tissues in the BTBR strain of mice. We report for the first time that BTBR mice display a hitherto unreported posterior subcortical interhemispheric connective bridge of myelinated fibers, that contain reduced MBP levels, in addition to an increased level of interhemispheric electrical connectivity compared with the anterior cerebrum.

2. Experimental Procedures

2.1. Animals

Male and female eight week old C57Bl/6 and BTBR mice were purchased from The Jackson Laboratory. All mice were housed in clear plexiglas cages with stainless steel wire lids in a temperature (21–23°C) controlled room, maintained in a sterile, pathogen-free environment on a 12:12 hour light:dark cycle (lights on at 7:00am). All procedures were approved by the Institutional Animal Care and Use Committee at the Wadsworth Center. In addition, all animal procedures were in accordance with the Guide for the Care and Use of Laboratory

Animals of the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council (National Academy Press, Washington, DC 1996), and Dept of Health, Education and Welfare (DHEW) Publ. No. 0309-05377-3, "Guide for the Care and Use of Laboratory Animals." For neuroanatomical, pathological and electrophysiological analysis all experiments were conducted in a blinded fashion.

2.2. Brain tissue dissections

Mice were sacrificed by CO_2 asphyxiation followed by decapitation and then brains were immediately removed and rapidly frozen at −80° C. Frozen forebrains were cryo-micro-dissected on a rostral-caudal gradient into a series of 500 μm thick sections which were maintained on dry ice. For analysis of gross white matter abnormalities, photomicrographs were obtained of 20 serial sections per mouse, with an 8 megapixel camera further detailed. For protein analysis, brain tissue micro-punches were cryo-microdissected from frozen sections using a steel 0.25 mm bore steel needle at ~ Bregma −0.5mm, beneath the cingulated cortex on the left and right hemispheres, and within the midline fibers which cross the cerebral hemispheres. Tissue punches did not contain subcingulate Probst bundles. The tissue punches were homogenized in 200 μl of NP40 lysis buffer containing protease inhibitors, frozen at −80° C and retained for biochemical analysis. The bicinchoninic acid (BCA) assay was used to determine protein levels in brain tissues. Western blotting was used to detect MBP and MAP isoforms according to their molecular weight and ELISA assays were used to calculate total MBP and MAP expression.

2.3. Neuroanatomical analysis

Digital photomicrographs of serial brain tissue sections from the BTBR and C57Bl/6 mice were imported into Image J (NIH) and converted to high resolution images (500 dpi), rendered grayscale stored, and scaled to equal dimensions. The mouse brain atlas was used to verify the levels at which sections were obtained [15]. Sections were then coded and two blinded reviewers graded the photomicrographs on the basis of the presence or absence of specific features including the presence (1) or absence (0) of lateral ventricles; the presence (1) or absence (0) of a connective inter-hemispheric bridge and the presence (1) or absence (0) of a grossly enlarged third ventricle (Figure 1). We used the digital tracing options in Image J software to calculate the mean area of the third ventricle reported in pixels per area in tissue sections from the BTBR and C57Bl/6 mice at ~Bregma −0.3 mm. We also used the measurement functions in Image J to calculate the distance between the cingulum, in tissue sections at Bregma −1.2 mm to −3.1 mm. Data were exported from Image J into SPSS for statistical analysis.

2.4. Total MBP and MAP expression

100 μl of the brain tissue homogenate solution containing 20 μg of protein per sample was added to a 96 well plate. The plate was centrifuged for 8 minutes at room temperature (RT) (2,000 rpm). In a chemical fume hood 100 μl of 8 % paraformaldehyde solution (in PBS) was added per well. A vinyl plate seal was then added to each plate, and the fixed tissues centrifuged for 5 minutes at RT (2,000 rpm). Paraformaldehyde was aspirated from each well, and the plate washed in PBS. 200 μl of a peroxidase blocking solution (0.1% H_2O_2) was added to the plate and incubated for 20 mins. The plate was washed in PBS and 200 μl of a protein blocking solution added to each well, and incubated for 1 hour. After removal of the blocking solution, the plate was incubated with 100 μl of the primary antibody (mouse anti MBP 1:1000 Millipore, or mouse anti β-actin 1:10,000 Sigma) for 1 hour. The plate was then washed with PBS and incubated with 100 μl of the anti-mouse biotinylated antibody (1:1000 Pierce) for 25 mins. The plate was then washed with PBS and incubated with 100 μl of horseradish peroxidase (HRP)-strepatavidin solution (1:1000 Sigma) for 25 minutes. The plate was then washed in PBS and 50 μl of the TMB substrate solution was added to each

well. After 15 minutes 50 μl of the acidic stop solution (Pierce) was added to each well. The optical density was then read using a Perkin-Elmer spectrophotometer at 450 nm. For ELISA assays n= 9 C57Bl/6 and n= 14 BTBR mice were used, and duplicate samples were prepared from each mouse with the with mean values directly entered into SPSS for statistical analysis.

2.5. Total MBP and MAP expression

Brain tissue homogenates were diluted 1:1 with a buffer containing 10% (w/v) sodium dodecyl sulphate (SDS), 30% (v/v) glycerol, 2% (v/v) 2-ME, 0.25% (w/v) bromophenol blue, and Tris Buffered Saline with Tween (TBS-T) (pH 7.2). 20 μg of protein per sample was loaded per well into Pierce precise protein gels. SDS-PAGE was performed with 4-20% agarose gels for 65 mins at 120 mV after which the gels were washed in transfer buffer and electroblotted using a semi-dry transfer apparatus onto Immobilon-P transfer membranes (Millipore Corporation, MA). Nonspecific sites on the membrane were blocked by incubating with 5% fish gelatin in TBS-T overnight at 4°C. The membranes were then incubated overnight with antibodies to myelin basic protein (MBP) (mouse 1:500 Millipore), α-tubulin (mouse 1:1000 Sigma), microtubule associated protein (MAP1a) (mouse 1:1000 Millipore) or β-Actin (mouse 1:20,000 Sigma) at 4 °C. The membranes were then washed with TBS-T, incubated with biotinylated goat-anti-mouse Ig G (1:10,000: Pierce Biotechnology Inc., Rockford, IL) for 2 hrs at RT, washed with TBS-T and incubated with streptavidin conjugated with HRP (1:5,000 in blocking buffer; Pierce Biotechonology) for 1 hr. The blot was developed with Super Signal/ECL chemiluminescent substrate (Pierce Biotechnology) and photographed with a LAS-1000plus (Fuji). Image J was used to quantify the density of each band per lane and the raw OD data entered directly into SPSS for statistical analysis. For gels n=4 BTBR and n = 4 C57Bl/6 mice were used per gel and gels were repeated in duplicate.

2.6. Electrophysiological analysis of BTBR hemispheric connectivity

Mice were anesthetized with isoflurane and mounted in a stereotaxic frame (Kopf, Tujunga, CA). Stainless steel screw electrodes (0.29-mm diameter; PlasticsOne, Roanoke, VA) were implanted bilaterally over frontal areas (1.5 mm lateral of midline, 1.7 mm anterior of Bregma) and bilaterally over parietal cortex (2 mm lateral of midline, 2 mm posterior of Bregma). The reference electrode was placed above the cerebellum (5 mm posterior of Bregma) (Fig 3 a). Each screw was connected to a gold-plated socket placed in a pedestal, secured to the skull via dental acrylic, and covered with a dust cap (PlasticsOne, Roanoke, VA). After surgery, mice received an analgesic and were checked daily for illness and weight loss. After recovery, the dust cap was removed and the pedestal was connected to a tethered cable which carried the wires from the head-mounted sockets to bipolar amplifiers (A-M Systems, Carlsborg, WA).

The electrophysiological data was collected for an average of 4 minutes per subject. The data was preprocessed by removal of line noise (60 Hz Notch) and artifacts. We estimated the frequency spectrum of each channel for 1–20 Hz, using Wavelet Transforms (3 cycles and 0.5 expansion factor). We then estimated the cross-spectral density (CSD) (the frequency domain analysis of the cross-covariance function) of the left hemisphere versus right hemisphere signals in order to investigate the frequency dependent cross-covariance in the left versus right frontal and left versus right parietal signals. The CSD was calculated for one second moving windows (no overlap) for 1–20 Hz. Further, to examine the presence of underlying source signals in the anterior and posterior brain we used Independent Component Analysis (Temporal Decorrelation) and recovered independent signals to determine the signal processing speed between the hemispheres [16].

2.7. Statistical analysis

All data from the neuropathological analysis were entered into SPSSv18 and decoded after data collection had been completed. ANOVA was used to determine statistical significance for the main effects of strain (BTBR vs control) and sex on white matter and protein analysis. Bonferroni post-hoc correction was used to determine individual group differences in parameters. Significance was set at $p<0.05$ for all analyses.

3. Results

3.1. White matter and ventricular pathology in the BTBR mice

There was a lack of callosal connective tissues in the anterior portion of the brain between Bregma +0.86 and −0.3 mm (Figure 1). However, posterior to Bregma −0.3 mm there was a connective bridge of tissues, a novel corpus callosum structure between the left and right hemispheres in all of the BTBR mice. This unique structure was not present in any of the C57Bl/6 mice (Table 1). In addition, at Bregma −0.3 mm we noted that the third ventricle was grossly enlarged in comparison with the third ventricle found in the C57Bl/6 at this level (Bregma −0.3 to −1 mm). Quantification of the area of the enlarged 3rd ventricle in the BTBR mice compared with the 3rd ventricle area at a corresponding level in the C57Bl/6 mice revealed it was ~650% larger than that found in the control strain. In addition, the lateral ventricles in the BTBR mice were noticeably reduced, if not absent, as compared with the C57Bl/6 mice. Furthermore, as other groups have noted, the left and right hippocampus were grossly displaced in the BTBR mice as compared with the C57Bl/6 mice.

3.2 Composition of connective tissues

We next determined whether the novel posterior interhemispheric callosal tissue in the BTBR mice was composed of similar white matter associated proteins as found in corpus callosal tissue of the C57Bl/6 mice. Firstly, we noted that all of the aforementioned proteins were expressed in the novel white matter tissues of the BTBR mice (Figure 2). Secondly, using ELISA analysis we found there was a significant reduction in MBP levels in the BTBR mice ($p= 0.024$, $F = 5.798$) compared with the C57Bl/6 mice. In addition using western-blot techniques, we confirmed this reduction, and found a significant reduction in the density of MBP in the BTBR mice compared with the C57Bl/6 mice ($p= 0.044$, $F= 7.182$). In addition we immunostained fibers from these tissues with MBP and GFAP and include digital photomicrographs of the aberrant interhemispheric myelinated fibers as a supplemental figure (Supplemental figure 1).

3.3. Interhemispheric electrical signals

The interhemispheric synchronization was quantified by averaging the cross spectral density across the entire data. Synchronization between the hemispheres is commonly believed to indicate activity across the corpus callosum [17]. We assessed functional connectivity between the left and right hemispheres by analyzing the interhemispheric synchronization of the electrophysiology activity in the anterior and posterior cortex. The frequency bandwidth analyzed was 1–20 Hz, but we only detected interhemispheric synchrony in the 0–4 and 7–10 Hz range. Firstly, we noted a difference in the synchronized left and right hemispheric activity detected within the 7–10 Hz frequency bandwidth between the parietal and anterior segments of the cortex (Figure 3a). The lack of synchronization across anterior electrodes is not due to decreased sensitivity of these electrodes as amplitude spectra were similar across all electrodes (Figure 3a).

There was a noticeable increase in activity at 8 Hz in particular at the posterior electrodes as compared with the anterior electrodes where there was little or no 8 Hz activity (Figure 3b). The 8 Hz frequency band in particular is associated with subcortical and therefore callosal

connectivity, whereas the 0–7 frequencies are more commonly associated with subcortical and likely thalamic connectivity. Thus the electrophysiological data showing connectivity between the posterior but not anterior hemispheres of the BTBR mice supports our neuroanatomical findings. In addition, two prominent independent source signals were found in the posterior brain in all the BTBR mice. The posterior sources were found to display a correlation delay of ~120 msec, which is slower than that reported in normal mice (~8 msec) [18].

4. Discussion

Alterations in white matter connectivity are reported in individuals with developmental disorders such as autism, and are suggested to underlie some of the abnormal behavioral changes found in humans and the BTBR mouse. Previously, by virtue of analysis of only the posterior sagittal portion of the corpus callosal connective tissues it was reported that the strain of mice was totally lacking in the corpus callosum. However, our analysis of white matter subcortical tissues, in a series of coronal tissue sections from the BTBR mice, has revealed for the first time posterior bilateral connectivity. The posterior connective white matter tissues are comprised of similar white matter proteins as found in corpus callosal tissues from the control strain of mouse. This connective tissue is unlike one found in the control strain of mice or that reported in the mouse stereotaxic atlas [15]; therefore we suggest it is a unique and hitherto unreported anatomical feature particular to the BTBR strain of mice.

We next determined whether the protein composition of the posterior connective white matter tissues in the BTBR mouse was similar to that found in the control strain. We specifically focused on MAP and MBP because there are associations with abnormal expression of both proteins in autistic individuals in the literature [13;19]. We quantified both the total levels of these proteins, and their low molecular weight isoforms because both could arguably contribute to alterations in white matter connectivity and function in the BTBR mice. We found no difference in the expression of low molecular weight isoforms of either MAP or MBP in the BTBR male or female mice compared with controls. This means that the composition of these proteins which are essential for normal axonal function is not altered in the BTBR mice. We did however note a reduction in the total levels of MBP protein, as normalized to β-actin within the novel connective white matter tissues as compared with white matter tissues obtained from a corresponding level in the control strain. The change in total protein level, rather than specific isoforms suggests that it is the expression rather than composition of MBP that is affected in the BTBR mice.

We used electrophysiological analysis to determine whether there was a functional connection between the activity between the left and right hemispheres at the posterior and anterior portions of the corpus callosum. Synchronized activity between left and right hemispheres is generally thought to require an intact functional corpus callosum [17]. In accordance with our neuroanatomical study which found a lack of connective tissues in the anterior but not posterior portion of the cerebrum, we found increased activity particularly in the 8 Hz frequency bandwidth, in the posterior hemispheres as compared with the anterior hemispheres. Our findings of novel interhemispheric connective tissues may help explain some contradictory findings regarding the relationship between acallosity and abnormal social behaviors in mice.

The initial report of a 100% absence of corpus callosum interhemispheric connective tissues in the BTBR mice led to the suggestion that the complete absence of interhemispheric cortical connection underscored the aberrant social and stereotypical behaviors found in the strain. However, when Yang *et al.* (2009) attempted to model social behavioral deficits by

lesioning the corpus callosum in mice, they found that a surgical lesion on postnatal day 7 had no effect on juvenile play, adult social approaches or repetitive self-grooming, and thus concluded that the lack of corpus callosum could not account for the unusual behaviors found in the BTBR strain of mice [12]. Our study shows that not only are there interhemispheric connective tissues in the BTBR mice, but that they are functionally active. Thus, our study supports that of Yang *et al.* who concluded that subtle disruptions in white matter connectivity more likely contribute to aberrant behaviors in the BTBR strain of mice. Moreover, reductions in MBP protein levels in the absence of changes in the density of the axonal related proteins MAP/β-actin strongly support the reduced conduction velocity we found in the BTBR strain compared with values reported in the literature. It should be noted that the axons within the novel inter-hemispheric structure may also provide connectivity indirectly via the subthalamic structures that they also project to, which may contribute to the latency, or correlation delay found in Interhemispheric synchronization in the BTBR mice. Overall we suggest that the abnormal conduction between the posterior hemispheres may be insufficient to compensate for a lack of corpus callosum connectivity in the anterior hemisphere. The question as to how the unusual connective tissue arises in the BTBR mouse still remains however.

It is worth noting that the third ventricle was grossly enlarged, by over 600% in the BTBR mice compared with the control strain. An enlarged third ventricle could have been formed developmentally to compensate for the reduction in lateral ventricles in the strain [20]. More importantly, we hypothesize that formation of the grossly enlarged third ventricle during development may have produced too large of a void, preventing the normal migration of oligodendrocytes across the midline and the formation of normal connective corpus callosal fibers. If this were the case then we should find that the lack of corpus callosal fiber formation in the anterior portion of the brain during embryonic development [21] is preceded by the formation of the grossly enlarged third ventricle, followed by the formation of a compensatory bridge of posterior connective tissues. Addressing this hypothesis is important not only because it may yield a new insight regarding the formation of abnormal connective tissues, but also because it is reliant on developmentally characterizing the formation of abnormal white matter connective tissues in the BTBR strain of mice. In the BALB/cj strain in which abnormal callosal fibers are also reported, there is a correlation between white matter alterations observed using diffusion tensor imaging (DTI) and social behavioral deficits from post natal day 30 through day 70 [22]. We suggest that by using neuroanatomical techniques at earlier time points we can pin-point the timing at which the BTBR white matter development starts to go awry and thus use molecular interventions, such as growth factors, to encourage axonal crossing and myelin formation and by extension remediate abnormal behaviors.

In summary, by virtue of a cohesive comparative neuroanatomical, biochemical and electrophysiological study we report, for the first time, posterior but not anterior interhemispheric connectivity in the BTBR mice, which is associated with the formation of a grossly enlarged third ventricle.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Research Highlights

- BTBR mice display novel interhemispheric posterior connective tissues but are lacking anterior corpus callosum.
- White matter connective tissues in the BTBR strain contain reduced MBP levels than the control strain.
- BTBR mice have greater posterior interhemispheric connectivity with slower conduction velocity.
- BTBR mice have grossly enlarged 3rd ventricles and reduced lateral ventricles compared with the control strain.

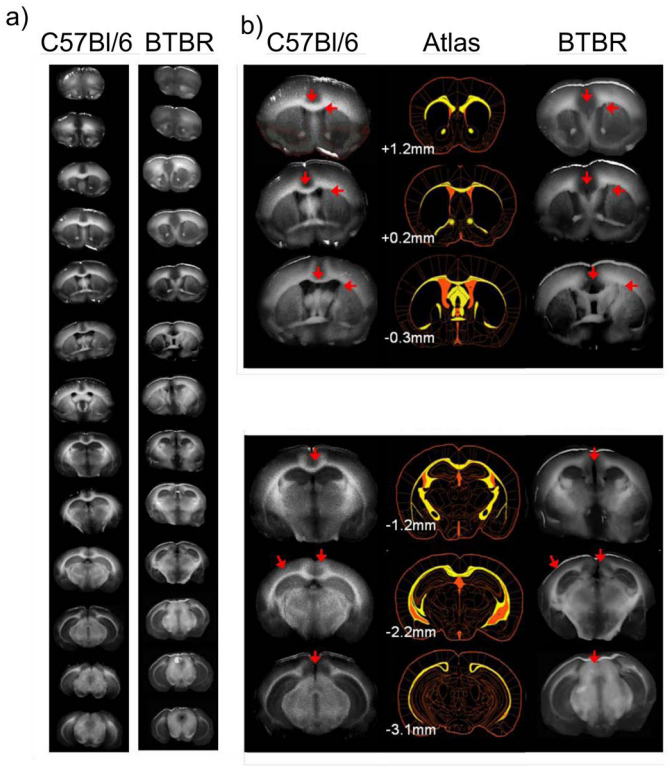

Figure 1.
Gross White Matter Alterations.
a) The panel on the left shows serial rostra-caudal 500 μm thick unstained tissue sections from BTBR and C57Bl/6 strains of mice. Inset at higher magnification we have highlighted gross white matter abnormalities found in the BTBR mice as compared with the C57Bl/6 mice at different levels. We also generated a digitized anatomical map of white matter structures found at a corresponding level in the standardized mouse brain based on Paxinos *et al*. At ~Bregma + 1.2 mm there was a noticeable lack of anterior corpus callosal connective tissues in the BTBR strain of mice compared with the corresponding level in the C57Bl/6 mice or atlas of the normal mouse brain as indicated with the red arrows. The external capsule and anterior commissure are present at this level in BTBR mice. At ~Bregma + 0.2 mm as indicated by the red arrows, the external capsule subcortical white matter fibers are present in the BTBR mice, but the corpus callosal connective tissue is absent. In addition the lateral ventricles which are prominent in the C57Bl/6 mice and the mouse atlas at the same level, are absent in the BTBR mice. At ~Bregma −0.3 mm we noted the presence of novel white matter connective tissues between the left and right hemispheres in the BTBR mice, underneath which is a grossly enlarged third ventricle and on either side an absence of the lateral ventricles. In the C57Bl/6 there is a comparatively normal corpus callosal structure at this level, which is almost identical to that reported in the standard mouse brain atlas. In addition we highlight the prominent lateral ventricles found in the C57Bl/6 mice with a red arrow. c) There were also gross distortions in the structure of white matter tissues in the posterior subcortical structures. As other groups have noted we observed lateral displacement of the hippocampus highlighted in the red arrows at Bregma levels −1.2 mm to −3.1 mm [20].

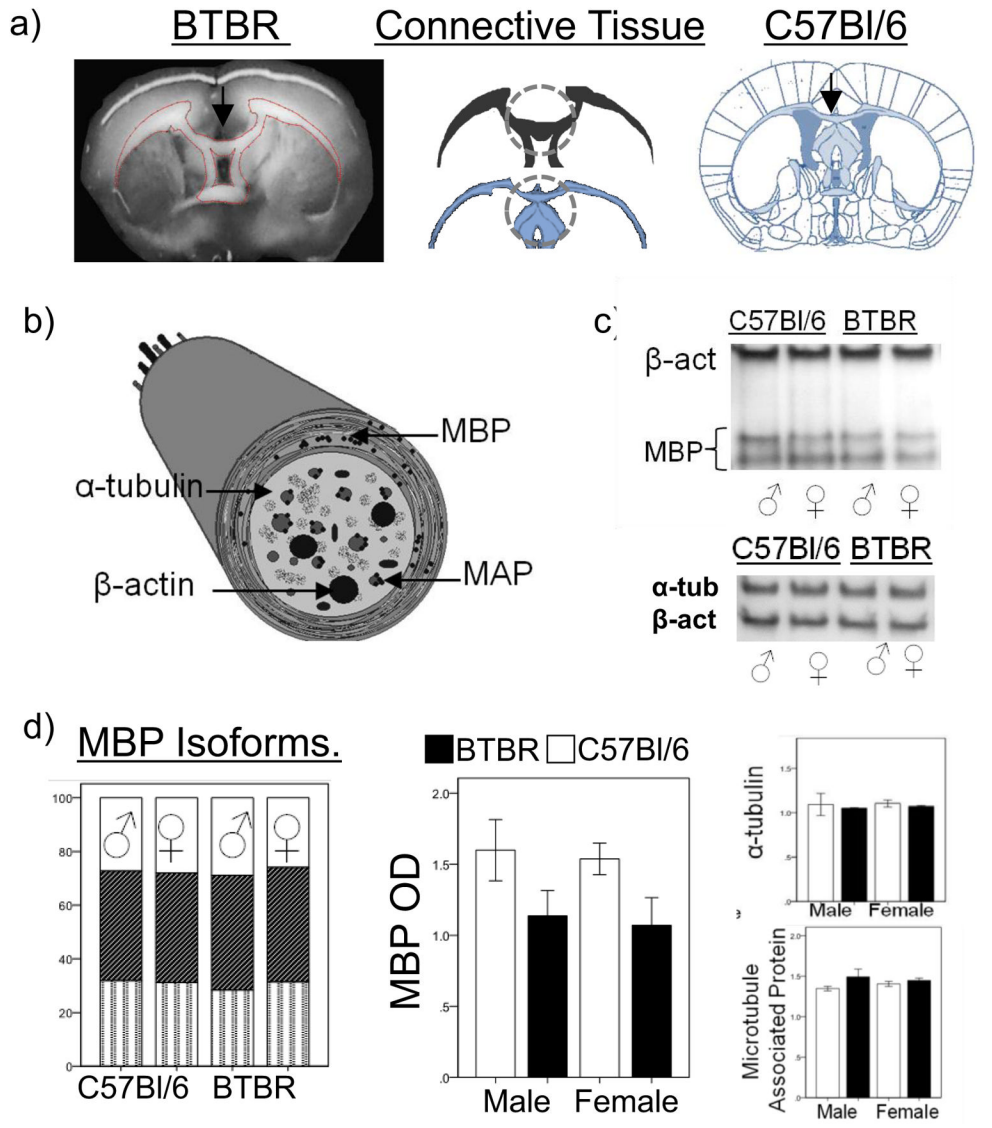

Figure 2.

White Matter Protein Composition.

a) The photomicrograph shows the novel connective tissues present in the posterior portion of the BTBR brain, and the corpus callosum structures found at the corresponding level in the standardized mouse brain based on Paxinos and Franklin (2004). The inset figure illustrates where the micropunches for protein analysis using ELISAs and Western Blotting were taken. b) Components of myelinated fibers are shown, myelin basic protein (MBP) microtubule associated protein (MAP), a-tubulin and b-actin. c) Representative western blots showing the density of MBP isoforms (20 and 24 Kd) relative to β-actin (42 Kd) in the BTBR and C57Bl/6 mice, in addition to α-tubulin (55 Kd) relative to β-actin (42 Kd). d) We quantified the density of MBP isoforms relative to total protein density in the BTBR and C57Bl/6 mice as shown in the bar graph. There was no difference in the relative density of MBP isoforms between the strains. There was however a reduction in total MBP levels using both ELISA and western blot assays in the BTBR strain compared with the C57Bl/6 mice. There was no difference in α-tubulin or MAP expression between the BTBR and C57Bl/6 mice.

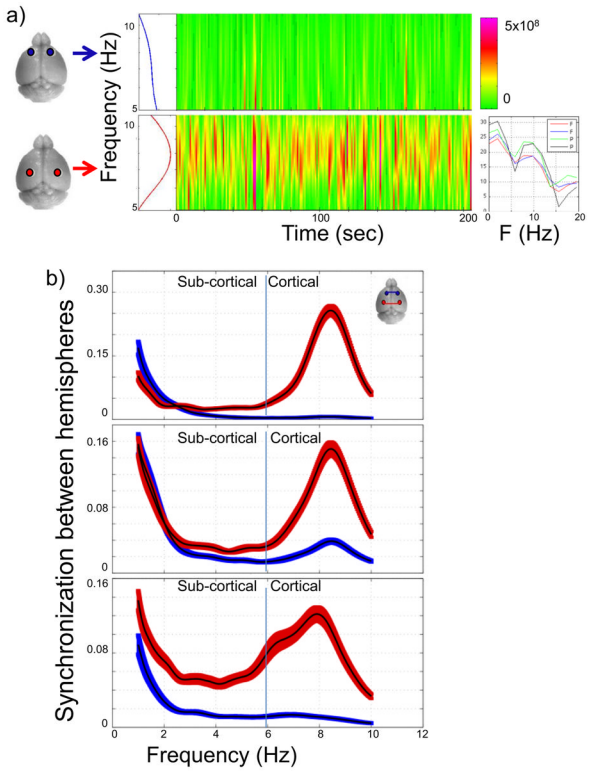

Figure 3.

Interhemispheric Connectivity.

The regions at which the steel electrodes were placed are shown on the left, the corresponding interhemispheric cross-spectral density from subdural EEG signals from BTBR mice, is shown on the right hand side. As can be seen there is a greater level of bilateral connectivity particularly in the 7–8 Hz frequency band between the posterior hemispheres as compared with the anterior hemispheres. The spectra inset shows that all four of the electrodes were functioning normally and that a lack of connectivity between the anterior hemispheres was not due to a lack of sensitivity of the electrodes or due to lack of activity in the 7–8 Hz frequency band. b) The line graphs show the degree of synchronization between the hemispheres from three different BTBR mice. The blue line designates the anterior hemispheric synchronization whereas the red line designates the posterior hemispheric synchronization. There is a greater level of synchronization particularly at the 8 Hz frequency in the posterior hemispheres as compared with the anterior hemispheres in the BTBR mice.

Table 1

The cumulative scores for the presence (+1) or absence (0) of the novel corpus callosal tract, the enlarged third ventricle and lateral ventricles in all BTBR mice (n=18) and C57Bl/6 mice (n=16) are shown.

Neuro-anatomical Features	BTBR ♂	BTBR ♀	C57Bl/6 ♂	C57Bl/6 ♀
Novel CC tracts.	8	10	0	0
Enlarged third ventricle.	8	10	0	0
Reduced lateral ventricles.	8	10	0	0