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Association between sociability and diffusion tensor imaging in BALB/cJ mice

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Abstract

The purpose of this study was to use high resolution diffusion tensor imaging (DTI) method to investigate the association between DTI metrics and sociability in BALB/c inbred mice. Sociability of prepubescent (30-day-old) BALB/cJ mice was operationally defined as the time that these mice spent sniffing a stimulus mouse in a social choice test. High resolution ex vivo DTI data on twelve BALB/cJ mouse brains were acquired using a 9.4 T vertical bore magnet. Regression analysis was conducted to investigate the association between DTI metrics and sociability. Significant positive regression ($p < 0.001$) between social sniffing time and fractional anisotropy (FA) was found in ten regions located in thalamic nuclei, zona incerta/substantia nigra, visual/orbital/somatosensory cortices, and entorhinal cortex. In addition, significant negative regression ($p < 0.001$) between social sniffing time and mean diffusivity (MD) was found in five areas located in sensory cortex, motor cortex, external capsule and amygdaloid region. In all regions showing significant regression with either MD or FA, the tertiary eigenvalue correlated negatively with the social sniffing time. This study demonstrates the feasibility of using DTI in detecting brain regions associated with sociability in a mouse model system.

Keywords

sociability; diffusion tensor imaging; MRI; mouse; autism

INTRODUCTION

Reduced sociability (reduced tendency to seek social interaction) is a highly disabling, treatment refractory symptom of various neuropsychiatric disorders, including autism spectrum disorders (ASD) (1). Studies employing diffusion tensor imaging (DTI), a non-invasive magnetic resonance imaging method, have identified abnormalities in brain connectivity in individuals with ASD (2,3). These abnormalities in brain connectivity may underlie reduced sociability in ASD (4–6), but much work is needed to establish the specific links between brain region connectivity phenotypes and social behaviors. Given the greater experimental control and genetic tools that mouse models offer, DTI of mouse models is potentially an important tool for elucidating the mechanistic role of structural brain phenotypes in behavioral endophenotypes. However, to our knowledge, DTI has not been used to study mouse models of reduced sociability.

Measuring water diffusion patterns using MRI offers a noninvasive way to probe microscopic structural information about tissue *in vivo*. The most common way to describe anisotropic diffusion properties of water is to use the diffusion tensor which can be estimated from a nondiffusion-weighted image plus six or more diffusion-weighted images along noncollinear directions (7). DTI has been widely used to study highly organized tissues such as the white matter in the brain and the skeletal muscle (8). DTI has also been used to study the gray matter, for instance, in stroke (9) and multiple sclerosis (10). Previous studies reported that subjects with ASD showed reduced FA in the corpus callosum (2,3). In addition, reduced FA was also found in motor/premotor areas (2), the superior/middle temporal gyri approaching the amygdala (2), and frontal cortex (11).

The BALB/cJ inbred mouse strain has been proposed as a model relevant to autism behavioral endophenotypes, because, on average, juvenile BALB/cJ mice show relatively low sociability (low tendency to seek social interaction) in comparison to the C57BL/6J and other inbred strains (6,12–16). Despite showing low sociability, genetically homogeneous BALB/cJ mice show substantial within-strain variability in both sociability and brain white matter development (6,12,17). Thus, this mouse model provides an opportunity to investigate brain regions associated with sociability, independent of any genetic alteration. Development of DTI metrics associated with social behavioral abnormalities may help in identifying specific neuro-anatomical pathway disruptions and micro-structural changes that underlie autism-relevant behavioral phenotypes. Hence, the purpose of this study was to use a high resolution DTI method for investigating the association between DTI metrics and sociability in BALB/cJ inbred mice.

METHODS

Animal model

BALB/cJ ($n = 12$; 5 females and 7 males) mice were bred at the University of Pennsylvania. Litters were culled to two males and two females at 2–4 days postnatally (P2–4) to ensure adequate nutrition for all pups and to ensure that litters were balanced in numbers of males and females. Following weaning on P25, litters were divided by sex, so that two males or two females were housed in each cage. “Stimulus mice” for the social choice test were adult

(36-to-42-week-old) A/J mice that had been obtained from The Jackson Laboratory (Bar Harbor, ME, USA). These stimulus mice had been gonadectomized prior to puberty to minimize the extent to which they would elicit sexual and aggressive motivations from the test mice. Stimulus mice were housed either five males or five females to a cage. Food and water were available *ad libitum*, and the housing room was maintained on a 12-h light-dark cycle (lights on at 7:00 a.m.). All mice were housed in cages with filter cage tops, and when cage tops needed to be opened, this was done under a hood, one cage at a time. Most mice were tested for sociability on P30 (range: P29-31) because at this age most BALB/cJ mice demonstrate strikingly reduced sociability (13,14). All animals were sacrificed on P31 by trans-cardiac perfusion and the extracted brain tissues were stored in 4% paraformaldehyde for four days prior to the imaging studies. At the end of the imaging studies, six brains (three high social and three low social) were embedded in paraffin blocks which were then sectioned sagittally with 25 μ m thickness. Three sagittal sections with approximately 1 mm gap for each in were stained with Luxol fast blue for myelin and cresyl violet (Nissl) for neurons (i.e. Kluver-Barrera staining). All mice were treated in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and this study was approved by the University of Pennsylvania Institutional Animal Care and Use Committee.

Sociability test

Sociability of 29–31-day BALB/cJ test mice was measured by a social choice test using a three chambered apparatus as previously reported (12–14). Social sniffing time, within the context of a social choice test, is a widely employed, simple, operationally defined measure of sociability (18). The testing room was dimly lit (1–2 lux) during testing in order to minimize the general stress level of the mice. A Sony digital video camera with NightShot (infrared) feature for recording in low light was used to record the behavioral testing. In each of the two end chambers of the social choice apparatus, there was a clear Plexiglas cylinder with multiple holes to allow for air exchange between the inside and outside of the cylinder. In Phase 1 of the social choice test, the test mouse was placed in the center chamber and was allowed to explore all 3 chambers of the apparatus for 10 minutes with an empty cylinder (no stimulus mouse present) in each of the 2 end chambers. Then, following Phase 1, a gonadectomized A/J stimulus mouse of the same sex as the test mouse was placed in the cylinder of one end chamber (“social cylinder”) and, simultaneously, an inanimate object (black paperweight) was placed in the cylinder in the other end chamber (“nonsocial cylinder”). The time spent by the test mouse sniffing each cylinder was then recorded for 10 minutes. “Social sniffing time” was defined as time spent sniffing the social cylinder in Phase 2, and “nonsocial sniffing time” was defined as time spent sniffing the nonsocial cylinder in Phase 2. Following the social choice test, the mice were sacrificed and brains were fixed using trans-cardiac perfusion with 4% paraformaldehyde and extracted for *ex vivo* DTI studies.

Diffusion tensor imaging

High-resolution DTI scans were performed using a 9.4 T, 89 mm vertical bore magnet and a specially designed loop gap resonator probe (20 mm inner diameter). The MR pulse sequence was based on a 3D multi-echo pulsed gradient spin echo sequence (19). The

sequence parameters were TR = 1 s, TE = 52 ms, FOV = 1.8 cm × 0.75 cm × 1.1 cm, and acquisition matrix size = 128 × 52 × 78 (140 μm isotropic resolution), reconstructed image matrix size = 256 × 104 × 156 (70 μm isotropic resolution), number of acquisitions = 2, and echo train length = 6. Multiple echoes within a same TR were used to acquire data for a same phase encoding and were added to increase signal to noise ratio. The strength and duration of diffusion gradients were 13 G/cm and 9 ms, respectively, and diffusion time was 10 ms, resulting in a b-value of 882 s/mm². The diffusion-weighted images were acquired with diffusion weighting in six non-collinear directions ([−0.14 0.71 0.71], [−0.14 0.71 −0.71], [−0.71 0.71 0.14], [−0.71 −0.71 0.14], [−0.71 0.14 0.71], and [0.71 0.14 0.71]) in a total acquisition time of 15.8 hr per brain.

The diffusion-weighted raw images were combined to estimate the effective diffusion tensors for individual voxels using weighted multivariate linear regression (20). Three eigenvalues and eigenvectors were estimated from diagonalization of the diffusion tensor. The size and shape of a diffusion tensor can be measured using the eigenvalues and their combinations, which are rotationally invariant quantities. Several scalar indices have been proposed to characterize diffusion anisotropy, and we used the most commonly used scalar indices, mean diffusivity (MD = (λ₁ + λ₂ + λ₃)/3) and fractional anisotropy (FA) (7):

$$FA = \sqrt{3 \left[\left((\lambda_1 - \langle \lambda \rangle)^2 + (\lambda_2 - \langle \lambda \rangle)^2 + (\lambda_3 - \langle \lambda \rangle)^2 \right) \right]} / \sqrt{2 (\lambda_1^2 + \lambda_2^2 + \lambda_3^2)} \quad [1]$$

where λ₁, λ₂, and λ₃ are the eigenvalues sorted in descending order and ⟨λ⟩ is the average of the eigenvalues. These two parameters can be sensitive to overall change of the diffusivities in three eigenvector directions as well as changes in the ratio.

BALB/cJ brain template

Fractional anisotropy maps of all twelve BALB/cJ mice were used to generate an averaged brain template of the FA map for BALB/cJ strain, based on the method reported by Kazemi et al. (21). Briefly, a template was created through a two-step process; affine coregistration for global alignment and non-linear normalization for local alignment. This procedure was performed using Statistical Parametric Mapping (SPM5) software (UCL, UK). In the first step, one of the FA maps was selected as an arbitrary selected reference image. All the other images were aligned to the selected reference image using a 12-parameter affine transformation. The second step is to iteratively perform nonlinear coregistration, averaging the inverse transformation parameters, and generating a template unbiased to the initially selected reference image (21). Two iterations were used to generate a template FA image.

Data analysis

FA maps of individual mouse brains were co-registered to the FA template. The same transformation parameters were used to transform the MD maps to match with the template image. A regression analysis was performed between the FA values in each voxel and the social sniffing time of the animals using the social choice test described above. SPM (UCL, UK) was used for data analysis. Initially, T-tests were used to identify all voxels with regression coefficients significantly (p < 0.005) different from zero and cluster size > 200 voxels. Subsequently, only the clusters with significance level of p < 0.001 (corrected for the

entire brain) were selected. For each cluster, correlation between the mean FA and the sniffing time was evaluated. The same analysis was repeated with MD. In addition to MD and FA, individual eigenvalues of each cluster was also measured to provide a fuller description of DTI measurement in each cluster which may be located in either the gray or the white matter. The result of the voxel based analysis was compared with that of manually drawn regions of interest (ROI) analysis for the corpus callosum. The corpus callosum ROI was drawn on the mid sagittal FA map to include the corpus callosal region with FA > 0.35.

RESULTS

The social choice test and DTI scan were performed on 5 female and 7 male mice. There was no significant ($p=0.9$, two-tailed t-test) difference in the social sniffing time between female (57.6 ± 58.8 s) and male (61.9 ± 52.6 s) mice (Fig. 1). Hence, the comparison between DTI metrics and the social sniffing time was performed with all mice regardless of sex. Signal-to-noise ratio in the frontal cortex region was 190 ± 22 for b0 image and 102 ± 10 for diffusion weighted images.

Voxel-wise regression analysis of FA and the social sniffing time found ten clusters with significant positive regression ($p < 0.001$). There was no cluster with significant negative regression. Fig. 2 shows the clusters with positive regression overlaid on the cross-sectional images of the template FA map. The clusters were located in various gray matter regions, including mediomedial area of secondary visual cortex, somatosensory cortex, lateral orbital cortex, zona incerta/subthalamus, thalamic nucleus, dorsal endopiriform claustrum, and entorhinal cortex. Fig. 3 shows representative plots of MD and FA versus social sniffing time for a cluster in prethalamic/thalamic nucleus (shown in Fig. 2g). A weak association ($R^2 = 0.06$, $p = 0.443$) was found between the average MD values of the cluster and the social sniffing time (Fig. 3a). In contrast, there was a strong association ($R^2 = 0.88$, $p < 0.001$) between the FA and the social sniffing time (Fig. 3b). The stronger association of sniffing time with FA than MD can be explained by the observation that the tertiary eigenvalues (λ_3) had a strong negative regression ($R^2 = 0.69$, $p < 0.001$) with the social sniffing time, whereas the primary eigenvalues (λ_1) increased slightly with the social sniffing time (Fig. 3c). A summary of DTI measurements and regression with social sniffing time in the ten clusters shown in Fig. 2, is presented in Table 1. Similar to the example shown in Fig. 3, all ten clusters showed strong associations ($R^2 = 0.49 - 0.88$) between FA and the social sniffing time, but relatively weak associations ($R^2 = 0 - 0.54$) between the MD and the social sniffing time. Among the three eigenvalues, only the tertiary eigenvalues had stronger association with the social sniffing time than the other two eigenvalues.

Voxel-wise regression analysis of MD and the social sniffing time resulted in five clusters with significant negative regression ($p < 0.001$). There was no cluster with significant positive regression. Fig. 4 shows the clusters with negative regression, overlaid on the cross-sectional images of the template FA map. The clusters were located in gray matter regions, such as frontal and temporal association cortices, secondary motor cortex, auditory, visual cortex, and amygdaloid, and a white matter region, external capsule. Fig. 5 shows representative plots of DTI parameters and the social sniffing time for the cluster in right frontal association cortex/secondary motor cortex, as shown in Fig. 4b. A strong association

($R^2 = 0.9$, $p < 0.001$) was found between the average MD values of the cluster and the social sniffing time (Fig. 5a). In contrast, there was no such association between the FA and the social sniffing time (Fig. 5b). This trend (negative regression with MD, but no significant regression with FA) may be explained by the fact that the three eigenvalues also had negative regression ($R^2 = 0.73$, 0.75 and 0.72 for λ_1 , λ_2 , and λ_3 , respectively, $p < 0.001$ for all three) with the social sniffing time (Fig. 5c). A summary of DTI measurements and regression with social sniffing time in the five clusters shown in Fig. 4 is presented in Table 2. Similar to the example shown in Fig. 5, all five clusters showed strong associations ($R^2 = 0.64 - 0.90$) between MD and the social sniffing time, but relatively weak associations ($R^2 = 0 - 0.21$) between FA and the social sniffing time.

Figure 6 presents scatter plots of ROI measures of the corpus callosum and the social sniffing time. There was no significant correlation between the mid-sagittal area of the corpus callosum and the social sniffing time ($R^2 = 0.04$, $p = 0.542$) (Fig. 6a). The mean FA from the corpus callosum ROI had a weak positive correlation ($R^2 = 0.30$) with the social sniffing time, however, it was not statistically significant ($p = 0.066$). A separate analysis of the DTI metrics in the subsections of the corpus callosum ROI (genu, body and splenium) were also compared with the social sniffing time. There was no significant correlation between FA and social sniffing time in all three subsections of the corpus callosum ($r = 0.14$, 0.33 , and 0.10 with $p = 0.67$, 0.33 , and 0.77 for genu, body and splenium, respectively). The correlations of social sniffing time with the MD were also weak ($r = 0.32$, 0.14 , and 0.13 with $p = 0.31$, 0.68 , and 0.70 for genu, body and splenium, respectively).

Figure 7 shows Kluver-Barrera stained sections of two mice; one with high sociability (sniffing time = 115 s) (Fig. 7a–c) and another with low sociability (sniffing time = 8 s) (Fig. 7d–f). Compared with the highly social mouse, the low social mouse has darker blue stain in the frontal cortex regions as shown in Fig. 7d and 7e (block arrow). Another distinctive feature of the low social mouse was that cells have large vesicular nuclei as shown in Fig. 7e and 7f (thick arrow).

DISCUSSION

In this study, the association between DTI metrics and a measure of sociability in inbred BALB/cJ mice was investigated. We observed a strong association between the social sniffing time and FA with positive regression in ten clusters and association between the social sniffing time and MD with negative regression in five clusters. A closer look at the association between eigenvalues and social sniffing time suggests that negative regression of MD with social sniffing time was accompanied by negative regression with all three eigenvalues, whereas positive regression with FA was mainly due to negative regression with the tertiary eigenvalue only. In all regions with significant regression of either MD or FA, the tertiary eigenvalues had negative regression with social sniffing time. This observation implies reduced sociability can be associated with higher tertiary eigenvalue of diffusion tensor, which is in line with increased radial diffusivity reported by Alexander et al. (3) and Fletcher et al. (22). Higher tertiary eigenvalues have been observed when there is disruption in fibrous tissue structure, for instance, by demyelination (23) or tumor infiltration (24).

The observation of reduced FA and higher diffusivity of a mouse model in the present study is consistent with recent DTI studies in patients with ASD, although there is some discrepancy in their anatomical locations (2,3,25). Barnea-Goraly and colleagues reported that reduced FA was observed in the white matter adjacent to the ventromedial prefrontal cortices, anterior cingulate gyri, temporoparietal junctions and genu of the corpus callosum (2). These authors suggested that the abnormal increase in the white matter volume in early childhood of autistic patients may be due to aberrations in axonal density or from myelin abnormalities (2). A recent study on high functioning autistic patients also found a decrease in FA values in the posterior mid body of the corpus callosum, the left, and the right anterior corona radiata near the genu of the corpus callosum (25). Lee and colleagues reported a significant correlation between low FA, high mean diffusivity, increased radial diffusivity and reduced corpus callosum size in patients with high functioning autism (26). However, we did not see the association between sociability and DTI parameters in the corpus callosum, probably because of large intra-animal variability in the corpus callosum of the BALB/cJ mice (17,27). The high radial diffusivity also corresponded with reduced processing speeds as measured by the IQ tests (3). Radial diffusivity is usually defined as average of secondary and tertiary eigenvalues. Higher radial diffusivity associated with ASD or reduced processing speed appears to be consistent with our finding of higher tertiary eigenvalues associated with reduced social sniffing time in BALB/cJ mice.

While the trend of FA in our study is similar to the observations from human studies as mentioned above, the specific brain regions associated with reduced sociability, as defined by social sniffing time in mice, do not necessarily match with those in DTI studies of human social behaviors. Particularly our results suggest a number of regions in the cortical and subcortical gray matter are associated with reduced sociability. One of the major characteristics of the mouse brain that differ from the human brain is highly aligned columnar structure of the gray matter (28). The cerebral cortex of the mouse brain has homogeneous radial directionality. DTI can clearly depict this distinct feature of the mouse gray matter because mice have nonconvoluted brains, i.e. lissencephalics. Such homogeneous columnar structure of the cortex also helps to detect any abnormalities, for instance using DTI. In contrast, the human brain has highly convoluted brain structure, i.e. gyrencephalics. Columnar structure in the gray matter also becomes less distinct within the first year of life, possibly due to dramatic increase in dendritic growth (29,30). Thus, DTI of the human brain may be less sensitive to any change in the gray matter, compared to that of the mouse brain. Our finding of association of the gray matter with the sociability appears to be in line with a recent report on the apparent perturbation in the fundamental organization of minicolumns in the autistic brain (31). While it is not a trivial to perform high-resolution DTI studies with patients, BALB/cJ mouse model can be a suitable model to look at similar changes in the gray matter regions associated with reduced sociability as demonstrated in this study.

Further evidence of cortical involvement in autism can be found from previous neuroimaging studies using techniques other than DTI (32–35). Neuropathologic studies found abnormalities in the size and density of neurons in amygdala and entorhinal cortex (32) and increased frontal cortical neuronal density (33). We also observed differences at the cellular level between low and high social mice, in frontal cortical regions with Kluver-

Barrera stain, although it warrants further study to investigate how these differences are related with the DTI observations. A functional imaging study by Zilbovicius and colleagues (34) showed delayed maturation of the frontal lobes. Reduced activity was also found in cortical regions including temporal gyrus and amygdala in autistic children during face processing tasks (35). In the current study, we also observed the association with sociability in these brain regions, such as amygdala, entorhinal, frontal and temporal cortices. For instance, we observed that the MD of the amygdala increased as social sniffing time decreased. The amygdala is active during the implicit processing of expressions and is implicated in normal social and emotional behavior (35). Thus, dysfunction of the amygdala may partially explain some of the social deficits of these mice with reduced social sniffing time. In contrast, we found that the FA of the entorhinal cortex decreased as social sniffing time decreased. Decrease in FA was mainly due to increase in the tertiary eigenvalues implying some change in the way that the neuronal fibers are organized. The entorhinal cortex regulates interactions between the hippocampus and the cortex. Thus, disruption in the entorhinal cortex can contribute to reduced sociability (36).

Our results also show that the area associated with reduced sociability was not limited to one or two focal areas in the brain, but was observed in many regions throughout the brain. This is also consistent with the observations from recent DTI studies of patients with ASD wherein wide spread regions across the brain were associated with ASD (2,25). Barnea-Goraly and colleagues reported that the areas with reduced FA in patients with ASD include the corpus callosum, the bilateral ventromedial frontal regions, left temporal and occipital regions, and right temporal, occipital, and frontal regions (2). A recent study by Keller et al (25) reported reduced FA in an area near the corpus callosum, but also in the right posterior limb of the internal capsule. This area contains a number of white matter tracts, including the posterior thalamic radiation, the superior longitudinal fasciculus, the inferior longitudinal fasciculus, and the inferior fronto-occipital fasciculus. These results suggest a complex nature of structural brain changes associated with ASD. Reduced sociability displayed by BALB/cJ appears to have a similar complexity in terms of distribution of the regions associated with the impairment in social behaviors.

Although there is no single rodent model that can fully encompass the entire spectrum of phenotypes across the very heterogeneous set of human disorders that we commonly refer to as ASD, brain structural and behavioral endophenotypes relevant to ASD can be studied experimentally in mice for the purposes of testing hypotheses about brain-behavior relationships (18). Some inbred strains of mice including the BTBR (18,37) and BALB/cJ (38) strains have been reported to exhibit behavioral phenotypes relevant to ASD, such as reduced sociability (reduced tendency to seek social interaction). Mouse models can also be used for longitudinal DTI studies which are challenging and expensive in ASD patients and would take several years to complete. On the other hand, mouse studies have the advantages of allowing greater experimental control (e.g. experimental control over genes and environment), allowing follow-up studies of underlying cellular and molecular mechanisms, and providing the opportunity to longitudinally study prepubescent to postpubescent development in a relatively short time.

One of drawbacks of our study is the potential impact of brain fixation and handling on the DTI measures. A concerted effort was made to carefully handle all brain samples consistently. We also kept the fixation time same (4 days) for all the brains to minimize any confounding effect from fixation. Sun et al. (39) reported that formalin fixation of the mouse brain does not change diffusion anisotropy, but reduces the difference in diffusion trace compared to that measured in vivo. This finding implies that our result with FA would be similar to what can be found in vivo. In contrast, our findings with MD might be underestimated. In conclusion, the results presented in this study demonstrate the feasibility of using DTI for studying structural brain changes relevant to social behaviors in the BALB/cJ mouse model. DTI.

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Abbreviations used

DTI	diffusion tensor imaging
ASD	autism spectrum disorders
FA	fractional anisotropy
MD	mean diffusivity
DEC	directionally encoded color
SPM	statistical parametric mapping

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Figure 1.

Comparison of mean social sniffing time between female ($n = 5$) and male ($n = 7$) BALB/cJ mice. The error bars represent standard deviations. The difference between the mean values was not statistically significant ($p=0.90$, t-test).

Figure 2.

Clusters with significant ($p < 0.001$, corrected for entire brain volume) positive regression of the social sniffing time with FA. Each cluster is shown on the background of the axial, coronal, and sagittal sections of the FA template of BALB/cJ. The clusters are located in mediomedial area of secondary visual cortex (a, b), somatosensory cortex (c), lateral orbital cortex (d), zona incerta/substantia nigra (e, f), thalamus (g, h), dorsal endopiriform claustrum (i), entorhinal cortex (j).

Figure 3. Scatter plots of the social sniffing time and DTI metrics; MD (a), FA (b), and eigenvalues (c). Circles are the mean values from the cluster located in thalamus shown in Fig.2g. The error bars represent the standard deviation of the values in the cluster of each brain. The dotted lines are the regression lines for the mean values and the social sniffing time. Eigenvalues (c) are shown with three different symbols; circles, triangles, and crosses, for the primary, secondary, and tertiary eigenvalues, respectively.

Figure 4.

Clusters with significant ($p < 0.001$, corrected for entire brain volume) negative regression of the social sniffing time with MD. Each cluster is shown on the background of the axial, coronal, and sagittal sections of the FA template of BALB/cJ. The clusters are located in visual/auditory/somatosensory cortex (a), frontal association cortex/secondary motor cortex (b, c), external capsule (d), amygdaloid (e).

Figure 5.

Scatter plots of the social sniffing time and DTI metrics; MD (a), FA (b), and eigenvalues (c). Circles are the mean values from the cluster located in right frontal association cortex/secondary motor cortex shown in Fig.4b. The error bars represent the standard deviation of the values in the cluster of each brain. The dotted lines are the regression lines for the mean values and the social sniffing time. Eigenvalues (c) are shown with three different symbols; circles, triangles, and crosses, for the primary, secondary, and tertiary eigenvalues, respectively.

Figure 6.

Scatter plots of social sniffing time and measures of the corpus callosum; mid-sagittal area (a) and mean FA (b). The error bars in (b) represent the standard deviation of FA in the corpus callosum. Solid lines are the linear regression lines of which slopes are not significantly different from zero ($R^2 = 0.04$ ($p = 0.542$) and $R^2 = 0.30$ ($p = 0.066$) for (a) and (b), respectively).

Figure 7.

Sagittal histology sections of a mouse with high sociability (sniffing time = 115 s) (a–c) and one with low sociability (sniffing time = 8 s) (d–e). They were stained using Kluver-Barrera method in which Luxol fast blue stains myelin and Cresyl violet (Nissl) stains neurons. The images in (b) and (e) correspond to the black boxes in (a) and (d), respectively. Likewise, the images in (c) and (f) are from the boxes in (b) and (e), respectively. The red block arrow in (e) indicates a region with darker staining of Luxol fast blue which could be due to abnormal myelination or increased collagen in extracellular space that is often stained by Luxol fast blue. The thin red arrow in (f) indicates a cell with a large vesicular nucleus. The nucleoli seem densely concentrated and appear as dark dots.

Table 1

DTI parameters in the regions with positive regression between FA and social sniffing time.

Cluster		Average $\pm$ Standard deviation (R^2 with sniffing time, p value for regression)				
Location	No. of voxels	MD ($\mu m^2/ms$)	FA	λ_1 ($\mu m^2/ms$)	λ_2 ($\mu m^2/ms$)	λ_3 ($\mu m^2/ms$)
Secondary visual cortex	217	0.69 $\pm$ 0.12 (0.67, 0.001)	0.24 $\pm$ 0.05 (0.49, 0.012)	1.09 $\pm$ 0.14 (0.58, 0.004)	0.54 $\pm$ 0.15 (0.47, 0.014)	0.45 $\pm$ 0.11 (0.65, 0.001)
Secondary visual cortex	293	0.71 $\pm$ 0.05 (0.59, 0.004)	0.20 $\pm$ 0.03 (0.80, 0.000)	1.02 $\pm$ 0.06 (0.06, 0.448)	0.63 $\pm$ 0.06 (0.46, 0.015)	0.48 $\pm$ 0.05 (0.84, 0.000)
Somatosensory cortex	1316	0.71 $\pm$ 0.03 (0.54, 0.007)	0.26 $\pm$ 0.03 (0.73, 0.000)	1.10 $\pm$ 0.06 (0.00, 0.983)	0.61 $\pm$ 0.05 (0.21, 0.130)	0.43 $\pm$ 0.04 (0.79, 0.000)
Lateral orbital cortex	376	0.67 $\pm$ 0.03 (0.00, 0.883)	0.21 $\pm$ 0.03 (0.79, 0.000)	0.98 $\pm$ 0.05 (0.12, 0.260)	0.59 $\pm$ 0.05 (0.00, 0.985)	0.45 $\pm$ 0.04 (0.39, 0.029)
Right zona incerta / substantia nigra	545	0.58 $\pm$ 0.03 (0.02, 0.682)	0.30 $\pm$ 0.03 (0.74, 0.000)	0.89 $\pm$ 0.05 (0.36, 0.039)	0.52 $\pm$ 0.04 (0.22, 0.122)	0.32 $\pm$ 0.03 (0.63, 0.002)
Left zona incerta / substantia nigra	205	0.60 $\pm$ 0.02 (0.01, 0.706)	0.35 $\pm$ 0.03 (0.83, 0.000)	0.97 $\pm$ 0.05 (0.27, 0.083)	0.54 $\pm$ 0.03 (0.31, 0.062)	0.29 $\pm$ 0.03 (0.60, 0.003)
Left thalamus	1074	0.66 $\pm$ 0.07 (0.06, 0.443)	0.23 $\pm$ 0.04 (0.88, 0.000)	0.96 $\pm$ 0.09 (0.03, 0.573)	0.60 $\pm$ 0.09 (0.05, 0.499)	0.43 $\pm$ 0.05 (0.69, 0.000)
Right thalamus	292	0.58 $\pm$ 0.04 (0.08, 0.382)	0.35 $\pm$ 0.04 (0.68, 0.001)	0.93 $\pm$ 0.05 (0.04, 0.531)	0.51 $\pm$ 0.06 (0.06, 0.428)	0.29 $\pm$ 0.03 (0.55, 0.006)
Dorsal endopiriform claustrum	493	0.70 $\pm$ 0.05 (0.09, 0.353)	0.25 $\pm$ 0.04 (0.77, 0.000)	1.05 $\pm$ 0.11 (0.04, 0.527)	0.60 $\pm$ 0.04 (0.28, 0.078)	0.44 $\pm$ 0.05 (0.52, 0.009)
Entorhinal cortex	540	0.59 $\pm$ 0.03 (0.00, 0.899)	0.45 $\pm$ 0.05 (0.76, 0.000)	1.05 $\pm$ 0.06 (0.08, 0.386)	0.49 $\pm$ 0.05 (0.00, 0.998)	0.23 $\pm$ 0.03 (0.37, 0.036)

Table 2
DTI parameters in the regions with negative regression between MD and social sniffing time.

Cluster		Average $\pm$ Standard deviation (R^2 with sniffing time, p value for regression)				
Location	No. of voxels	MD ($\mu\text{m}^2/\text{ms}$)	FA	λ_1 ($\mu\text{m}^2/\text{ms}$)	λ_2 ($\mu\text{m}^2/\text{ms}$)	λ_3 ($\mu\text{m}^2/\text{ms}$)
Visual / auditory / somatosensory cortex	3665	0.69 ± 0.05 (0.79, 0.000)	0.20 ± 0.02 (0.21, 0.130)	1.02 ± 0.07 (0.60, 0.003)	0.57 ± 0.06 (0.51, 0.009)	0.47 ± 0.05 (0.75, 0.000)
Right frontal association cortex / secondary motor cortex	1795	0.68 ± 0.06 (0.90, 0.000)	0.24 ± 0.03 (0.00, 0.890)	1.07 ± 0.11 (0.73, 0.000)	0.54 ± 0.05 (0.75, 0.000)	0.42 ± 0.05 (0.72, 0.000)
Left frontal association cortex / secondary motor cortex	1520	0.71 ± 0.05 (0.75, 0.000)	0.24 ± 0.02 (0.16, 0.190)	1.12 ± 0.08 (0.62, 0.002)	0.57 ± 0.04 (0.54, 0.006)	0.45 ± 0.04 (0.78, 0.000)
External capsule	829	0.66 ± 0.04 (0.82, 0.000)	0.22 ± 0.03 (0.02, 0.66)	0.94 ± 0.06 (0.49, 0.011)	0.62 ± 0.04 (0.55, 0.006)	0.44 ± 0.04 (0.37, 0.035)
Amygdaloid	640	0.73 ± 0.08 (0.64, 0.002)	0.22 ± 0.04 (0.11, 0.292)	1.11 ± 0.17 (0.64, 0.002)	0.61 ± 0.06 (0.26, 0.093)	0.48 ± 0.05 (0.40, 0.026)