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Sexual dimorphism revealed in the structure of the mouse brain using three-dimensional magnetic resonance imaging

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A large variety of sexual dimorphisms have been described in the brains of many vertebrate species, including humans. Naturally occurring sexual dimorphism has been implicated in the risk, progression and recovery from numerous neurological disorders, including head injury, multiple sclerosis and stroke. Genetically altered mice are a key tool in the study of structure–function relationships in the mammalian central nervous system and serve as models for human neuropsychiatric and neurological disorders. However, there are a limited number of quantitative three-dimensional analyses of the adult mouse brain structures. In order to address limitations in our knowledge of anatomical differences, a comprehensive study was undertaken using full 3D magnetic resonance imaging (MRI) to examine sexual dimorphisms in the C57BL/6J whole mouse brain. An expected difference in overall brain size between the sexes was found, where male brains were 2.5% larger in volume than female brains. Beyond the overall brain size differences in the sexes, the following significantly different regions were found: males were larger in the thalamus, primary motor cortex and posterior hippocampus, while females were larger in posterior hypothalamic area, entorhinal cortex and anterior hippocampus. Using high-definition 3D MRI on a normal inbred mouse strain, we have mapped in detail many sex-associated statistically significant differences in brain structures.

Introduction

Numerous sexually dimorphic characteristics have been identified both in the human and animal brain (Ho et al., 1980; Witelson and Kigar, 1988; Witelson, 1989, 1991; Kimura, 1992; Cowell et al., 1994; Kulynych et al., 1994; Schlaepfer et al., 1995; Murphy et al., 1996; Luders et al., 2004). Indeed, sexual differences in the nervous system have been described at virtually every anatomical level including molecular, cellular and neural system levels (reviewed in Cooke et al., 1998). Neuroimaging and morphometric studies of human subjects have shown sexual dimorphisms in brain regions such as the amygdala (Goldstein et al., 2001) and hypothalamus (Swaab and Fliers, 1985) along with differences in neuron numbers across the entire cortex, although these results lack consistency (Witelson et al., 1995; Harasty et al., 1997; Pakkenberg and Gundersen, 1997). Along with structural distinctions, sexual differences have also been reported in performance on cognitive tasks and brain physiology (Kimura, 1992). It has also been shown that naturally occurring sexual dimorphism has implications in the risk, development and recovery from numerous neurological disorders. These include head injury, multiple sclerosis, stroke and neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease (reviewed in Shulman and Bhat, 2006; Webber et al., 2005).

The incidence and progression of some psychiatric disorders have also been shown to exhibit gender differences. For example, males are known to suffer from schizophrenia more than two and half times more often than females (Castle and Murray, 1991). Males are also prone to a more severe form of the disorder, experience an earlier onset and exhibit more structural brain abnormalities. Relapses are more severe, and their response to neuroleptic medication is less favorable (Castle and Murray, 1991). Work to identify the neuroanatomical regions of significance in patients diagnosed with schizophrenia compared to healthy counterparts has shown that differences seem most common in the frontal lobes, hippocampus and temporal lobes (Green, 2001; Honea et al., 2005). These differences are heavily linked to the neurocognitive deficits which often occur with schizophrenia, particularly in areas of memory, attention, problem solving and social cognition. Neurodevelopmental disorders, such as autism which has no X- or Y-linked pattern of inheritance, are also more prevalent in males than in females (reviewed in Rutter et al., 2003). Therefore, research into sexual dimorphism has become mandatory in the understanding of a host of brain disorders with sex differences in their incidence and nature.

The use of inbred and genetically altered mice has become a leading approach in the study of structure–function relationships in the central nervous system. Furthermore, these tools have served as models for many human neuropsychiatric and neurological disorders (reviewed in Crawley and Paylor, 1997; Nieman et al.,

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2006). Furthermore, analysis into the tissue-specific expression and regulation of genes in mice has shown that hundreds of genes are sexually dimorphic in the brain (Yang et al., 2006). However, unlike in human studies, there are a limited number of quantitative 3D analyses of adult mouse brain structures (Fransen et al., 1998; Airey et al., 2001; Zygourakis and Rosen, 2003). Until now, there has not been completed a high-definition and comprehensive study that utilized full 3D analysis and a large number of individuals to examine in detail sexual differences over the whole mouse brain. The purpose of this work was to utilize 3D fixed magnetic resonance imaging (MRI) of male and female brains to study such differences.

Magnetic resonance (MR) images are digital and therefore quantitative data can be readily extracted from inbred mouse strains (Chen et al., 2006). In comparison to live specimen imaging, the imaging of fixed brains can produce images with improved resolution as fine as 32 μm (this study; Natt et al., 2002; Nieman et al., 2005) or even 21 μm (Badea et al., 2006). This technique was chosen over traditional histological methods as the ideal tool for visualizing sexual dimorphism in mouse brains for a number of other reasons as well. Firstly, it minimizes distortion artifacts produced by extreme treatment of brain tissue during fixation, embedding, sectioning and mounting and also allows for greater accuracy in determining tissue differences. Secondly, the gross anatomy of the specimens is conserved due to the maintenance of brains in a natural conformation in the skull during imaging. Finally, this technique also allows for whole brain coverage and fully 3D analysis, characteristics not shared in other more frequently used visualization techniques.

In view of the fact that structural differences have been observed in many vertebrate brains, we expect to observe mouse sexual dimorphisms that coincide with those observed in other mammal groups. For example, the density of neurons in the dentate gyrus is significantly larger in male than in female rats, as is both the volume of the CA1 region and the number of pyramidal cells (Madeira and Lieberman, 1995). Therefore, there is an expectation that the mouse brain will show sexual differentiation within this region with the male presenting with a larger hippocampal structure. Previous reports also indicate that the corpus callosum, the fiber tract that connects the two cerebral hemispheres in some vertebrates, exhibits sexual dimorphism. In one report, male mice of approximately 16 weeks of age were shown to possess a greater total corpus callosum area compared with females of the same age when differences in brain weight were taken into account (Berrebi et al., 1988).

Clearly there is a need to identify the dimorphisms between normal (non-diseased) male and female mouse brains before differences in diseased counterparts can be fully interpreted. The results of this work provide a comprehensive map of sexual differences in the mouse brain that can be readily compared to findings gathered in human studies. These comparisons will offer details and possibly an explanation about diseases and/or disorders that appear to be sex biased. This work corroborates previous work and additionally reports many novel findings, enabling a better overall understanding of sexual dimorphism in the mouse brain and augmenting previous mouse brain atlas studies (Ma et al., 2005).

Materials and methods

Mice and brain sample preparation

Male C57BL/6J ($n=20$) and female C57BL/6J mice ($n=20$) from Charles River Laboratories (Wilmington, MA) were examined at 12 weeks of age. C57BL/6J mice were chosen because they are a widely used, commercially available inbred strain. They are commonly used in a wide variety of research areas including cardiovascular biology, developmental biology, diabetes and obesity, genetics, immunology and neurobiology research. They also show intermediate values on most behavioral tasks and are reasonably reliable breeders (reviewed in Crawley, 1999). A previously described sample preparation protocol for scanning was used with slight modifications (Tyszka et al., 2006). Mice were anesthetized with a combination of Ketamine (Pfizer, Kirkland, QC) (100 mg/kg) and Rompun (Bayer Inc., Toronto, ON) (20 mg/kg) via intraperitoneal injection. Thoracic cavities were opened and animals were perfused through the left ventricle with 30 mL of phosphate-buffered saline (PBS) (pH 7.4) at room temperature (25 °C) at a rate of approximately 100 mL/h. This was followed by infusion with 30 mL of iced 4% paraformaldehyde (PFA) in PBS at the same rate. Following perfusion, the heads were removed along with the skin, lower jaw, ears and the cartilaginous nose tip. The remaining skull structures were allowed to postfix in 4% PFA at 4 °C for 12 h. Following an incubation period of 5 days in PBS and 0.01% sodium azide at 15 °C, the skulls were transferred to a PBS and 2 mM ProHance® (gadoteridol, Bracco Diagnostics Inc., Princeton, NJ) contrast agent solution for at least 7 days at 15 °C. MR imaging occurred 12 to 21 days post-mortem. All animal experiments were approved by the animal ethics committee of the Hospital for Sick Children (Toronto, ON). Standard housing conditions with cages of 7 in. W × 11 in. D × 8.5 in. H in size and 5 mice per cage were maintained.

Imaging

A multi-channel 7.0-T MRI scanner (Varian Inc., Palo Alto, CA) with a 6-cm inner bore diameter insert gradient set was used to acquire anatomical images of brains within skulls. Prior to imaging, the samples were removed from the contrast agent solution, blotted and placed into 13-mm-diameter plastic tubes filled with a proton-free susceptibility-matching fluid (Fluorinert FC-77, 3M Corp., St. Paul, MN). Three custom-built, 14-mm-diameter solenoid coils with a length of 18.3 cm and over wound ends were used to image three brains in parallel. Parameters used in the scans were optimized for grey/white matter contrast: a T2-weighted, 3D fast spin-echo sequence, with TR/TE = 325/32 ms, four averages, field-of-view 12 × 12 × 25 mm and matrix size = 432 × 432 × 780 giving an image with 32 μm isotropic voxels. Total imaging time was 11.3 h (Henkelman et al., 2006).

Image postprocessing

Rigid body registration was carried out towards a target pre-existing model based on the same mouse strain as reported previously (Collins et al., 1994; Kovacevic et al., 2004). All possible pairwise 12-parameter registrations were then carried out to create an unbiased linear average model of the entire data set. All images were subsequently non-linearly aligned towards the 12-parameter average. The resulting registered MRIs were resampled and averaged (Collins et al., 1994; Kovacevic et al., 2004). This iterative procedure was repeated for an additional five generations with ever finer deformation grid-point spacing. The final deformation field (with 60 μm grid points) for each subject was inverted, any remaining linear transformations were removed and centered to the mean of the entire population of 40 mice (Fig. 1).

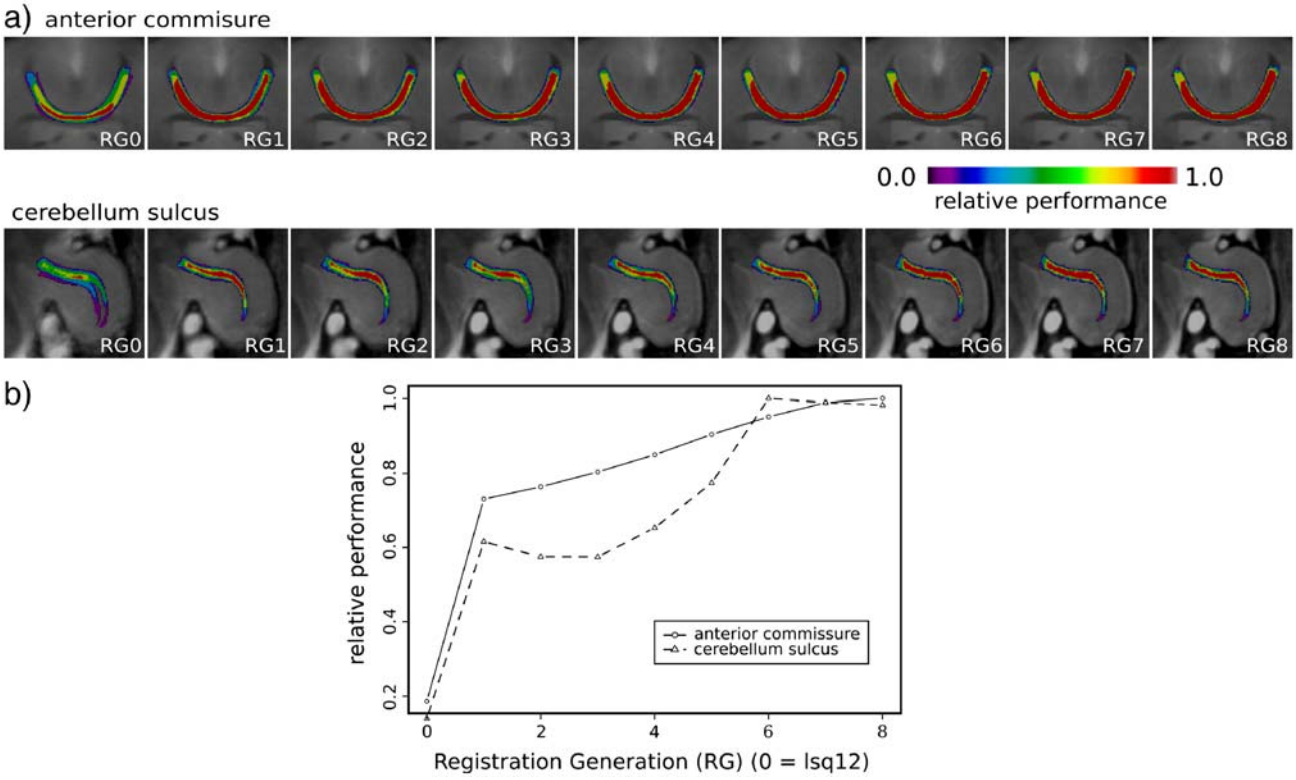

Fig. 1. The creation of a model independent atlas. The anterior commissure and the white matter of one cerebellar sulcus were manually segmented on five MRI scans (a). The segmentations were then propagated through the non-linear generations, with the probability map of the two structures shown for each generation. The graph captures the trend numerically, the relative performance being the number of voxels with perfect overlap following the five segmented scans divided by the maximum across the 8 generations (b). Improvements can be seen until generation 6 (the final registration used for this study) leveling off in generations 7 and 8.

Statistical analysis

The goal of this image registration process was to map all 40 MRIs exactly into the same space. At the end of this procedure, the deformation fields were analyzed to determine whether male and female mice had to deform differently to reach this final target. Three types of statistical analyses were performed to evaluate this question. The first analysis asked whether there was a global change in size related to gender. The scale factor was therefore extracted from the 12-parameter (3 scales, 3 translations, 3 rotations, 3 shears) registration matrix of each mouse. A *t*-test with 38 degrees of freedom was used to determine if there was a significant size effect by gender.

The second test consisted of an analysis of differences of displacement and was measured using Hotelling's T^2 statistic (Thompson et al., 1997; Collins et al., 1998; Cao and Worsley, 1999; Chung et al., 2001). The result was an F map of $F_{(3,37)}$ degrees (where 3 designates the *x*, *y* and *z* coordinates) of freedom describing whether there was a by gender effect of movement at each voxel. Multiple comparisons were corrected for using a stringent 1% false discovery rate (FDR) (Genovese et al., 2002). To reduce random noise and assure normality under the central limit theorem, deformation maps were blurred prior to analysis with a Gaussian kernel with a full width at half maximum (FWHM) of 0.5 mm.

The third type of analysis involved assessing volume change at each voxel by computing the Jacobian determinant of each deformation field (Chung et al., 2001). Effects of gender were determined using a *t*-test with 38 degrees of freedom, multiple comparisons accounted for using the same 1% false discovery rate. The deformation maps were blurred with a Gaussian kernel of FWHM = 1.0 mm before analysis.

In order to better visualize and interpret discovered shape changes, anatomical line drawings of select regions were created. A gender-specific deformation field was created by averaging all non-linear deformation fields of the male and female mice, respectively. The final registration atlas was deformed using the inverse of these average deformation fields. Identical slices of specific regions of interest were then extracted, and key anatomical structures were outlined using vector drawing software (Inkscape, http://www.inkscape.org). The two tracings were then superimposed over the MR slice for visual assessment of the shape differences.

Results

Differences in overall brain size

The purpose of this study was to examine sexual dimorphisms in the whole mouse brain using full 3D MRI. Our analysis identified several anticipated differences between the male and female mouse brain. It also revealed novel findings that demonstrate many additional sex-specific differences between C57BL/6J mice at 12 weeks of age. However, before subtle

differences in neuroanatomical regions using 3D registration and statistical comparisons of prominent structures could be undertaken, the overall brain size of the mice was determined. An expected overall brain size difference was detected where male brains were 2.5% larger in volume than female brains. The difference was strongest along the *Y* axis (anterior to posterior, 1.5%) and weakest along the *X* axis (0.03% difference). This difference was removed when weight was covaried. There was, however, no significant relationship between weight and brain size within each gender. Thus, within this study, brain size is determined by animal sex and within each sex is independent of body weight.

Per-voxel Hotelling and Jacobian statistical results

The 3D images allowed for the clear identification of many other sexual differences throughout the whole brain. Representative single coronal and sagittal slices taken from per-voxel data sets are shown for the adult male and female groups in Fig. 2. Per-voxel differences in neuroanatomical tissues were detected with the use of a Hotelling statistical map that compares registration displacements with a random Gaussian vector field. Significant differences are shown in the corpus callosum at the fornix, the hippocampus and the cerebellum when global size differences between the sexes are removed. The most dramatic structural differences can be seen at the posterior hypothalamic area extending to the mammillothalamic tract, the medial lemniscus and the dorsal third ventricle of the mouse brain. These differences appear mostly left/right symmetrical.

Using a Jacobian statistical map that illustrates the expansion of tissue based on gender, it becomes apparent that some structures, such as the fornix, corpus callosum and posterior hippocampus, are relatively larger in males while the hypothalamic area, fimbria and anterior hippocampus are relatively larger in the female brain (Fig. 3). A comprehensive summary of the major region-specific sex differences is shown in Table 1.

Shape differences in neuroanatomical structures

Global shifts and shape differences in structures of the male and female brains were next identified. When comparing Hotelling and Jacobian statistical maps, a downward shift of a region in a male brain with respect to the same region in the female brain will give a large displacement corresponding to a statistically significant area represented in a Hotelling's map (for example, Fig. 2b, *ii*). This displacement downward will result in a decreasing volume below as shown on the Jacobian map (Fig. 3b, *ii*) and an additional increasing volume above (Fig. 3a, *ii*). These displacements are clearly illustrated when specific structures are outlined and the traces compared for visual assessment of shape differences (Figs. 2–4, Table 1). A number of differences between traces become evident when examining a coronal view of the entire brain (Fig. 4). Again, the most dramatic changes can be seen in the posterior hypothalamic area extending to the mammillary bodies and thalamus where movement of 150 to 180 μm is evident. Other differences that were identified when analyzing these traces include the lateral ventricles and medial lemniscus.

Sex differences in the order of up to 120 μm are seen in the cerebellum along with dimorphisms in the cerebellar folia of the mouse brain that are also visible when the folds are manually traced (Fig. 5a). A corresponding Hotelling map was overlaid on the same slice showing the statistically significant differences between the sexes (Fig. 5b). This provides an illustration of the correspondence between the line drawings and the Hotelling data. However, cases where the line drawings show differences depending on gender but which do not appear significant according to the statistical map are the result of large variance among subjects. These results illustrate why differences in the line

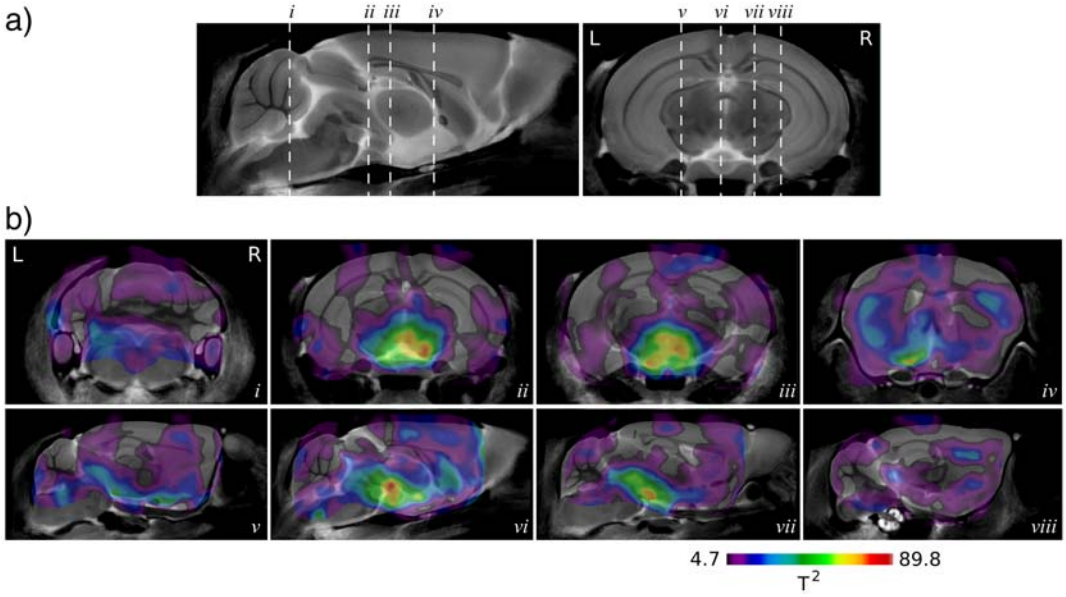

Fig. 2. Differences in neuroanatomical tissues with the use of a Hotelling statistical map. Slashed lines corresponding to slices *i* through *viii* (a) indicate the position of coronal and sagittal views of the average mouse brain with the Hotelling statistical parametric map overlaid (b). All colored regions are statistically significant and have a less than 1% chance of being a false positive. Significant differences in the hypothalamic area, the corpus callosum at the fornix, the hippocampus and the cerebellum can be seen. L and R represent the left and right side of the brain, respectively.

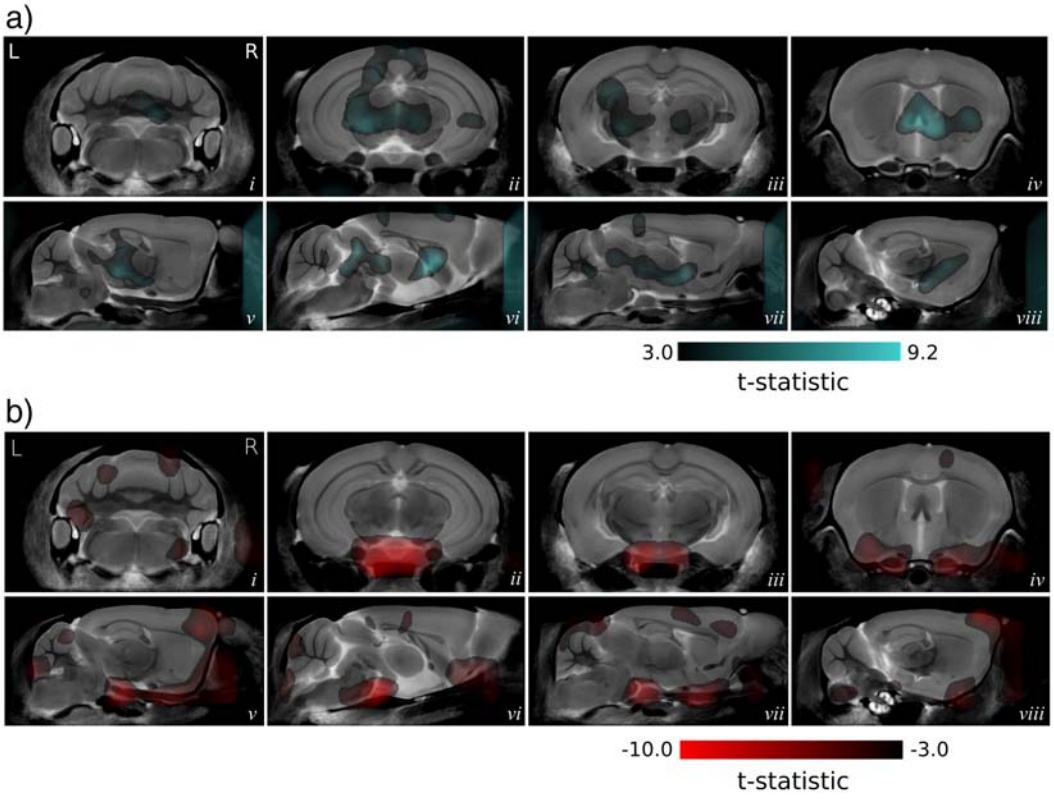

Fig. 3. Per-voxel expansion of neuroanatomical structures in male and female mice is illustrated with a Jacobian statistical map where aqua indicates that males are larger than females at that voxel (a) while red means that female are larger at that voxel (b). All colored regions are statistically significant (1% FDR). Slices correspond to positions *i* through *viii*, indicated in Fig. 2. L and R represent the left and right side of the brain, respectively.

drawings between sexes do not always precisely match the statistical map data.

On the other hand, sometimes very small displacements of only 30 to 50 μm are identified to be statistically significant at a *P* value of less than 10^{-3}. These are identifiable because of the high quality of the MRI, the accuracy of the registrations and the large number ($n=40$) of genetically identical individuals in the study.

When examining the corpus callosum and fornix from a sagittal view (Fig. 6a), the shape of this structure is clearly different in the male and female brain as the structures do not align when traced and compared (Fig. 6b). When the corpus callosum and fornix are explicitly aligned to where the fornix branches off, it is evident that there is a shape difference at the posterior region of the corpus callosum, known as the splenium (Fig. 6c). A size difference is also

Table 1
Region-specific sex differences in the mouse brain

Selected regions of interest	Reference (this study)	Approximate distortion/movement (μm)	$F_{(3, 37)}$	*P* value	Larger in
Posterior hypothalamic area extending to mammillothalamic tract, medical lemniscus, dorsal third ventricle, and dorsal tuberomammillary nuclei	Figs. 2, 3b and 4	150–180	25–75	5.2×10^{-9}–8.8×10^{-16}	Females
Thalamus	Figs. 3 and 4	30–100	20–60	7.1×10^{-8}–2.8×10^{-14}	Males
Posterior hippocampus	Figs. 2 and 3a	50	10	5.8×10^{-5}	Males
Corpus callosum	(Figs. 2, 3a and 6)	60	20	7.1×10^{-8}	Males
Anterior hippocampus	Data not shown	30	5	0.005	Female
Fimbria	(Figs. 2, 3b and 6)	60	10	5.8×10^{-5}	Female
Cerebellum	(Figs. 2, 3a, b and 5)	<120	<20	$<7.1\times10^{-8}$	Male/female
Straitum	(Figs. 2, 3a and 8)	60–130	3–28	0.007–1.3×10^{-9}	Male/female
Olfactory bulb	Data not shown	110–160	No data	No data	Males

Approximate distortions or movement of structures when comparing male and female brains are indicated in μm. Male/female indicates that either male or female mouse brain shows an increase in size of this structure depending on position within this structure.

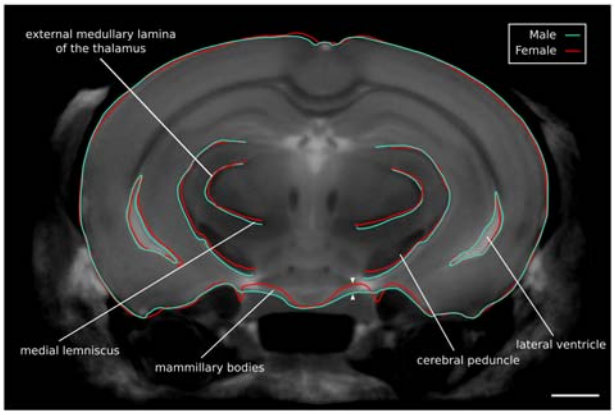

Fig. 4. Differences in brain structures between male and female C57BL/6J mice. Coronal view of averaged male and female mouse brain with prominent structures outlined in aqua (male structures) and red (female structures). Image is a representation of the non-linear average of 20 male brains. Scale bar is 1 mm. △▽ indicates a displacement of 150 to 180 μm.

apparent, as the male splenium appears larger than that of the female (Figs. 3a, *vii* and 6c). Statistical analysis of this region shows, in general, that an approximate movement in the order of 60 μm is evident when comparing the male and female regions of the corpus callosum (Table 1).

Robust differences between males and females also are observed in specific hippocampal structures. Examining the hippocampus from a horizontal view shows some differences in the lateral ventricle and granule cell layer with movement of as much as 50 μm in some areas of the structure (Fig. 7). Finally, when the striatum of the male and female brain is compared, significant shape differences in the lateral globus pallidus and caudate putamen become evident (Fig. 8).

Discussion

The 3D MR acquisition of male and female C57BL/6J mice yielded very high resolution 3D neuroanatomical images with excellent signal-to-noise ratio (SNR). This work analyzed a large number ($n=40$) of mice belonging to a well-known inbred strain and was able to provide images with superb resolution acquired in efficient scan times (Ma et al., 2005). Furthermore, variance within the inbred mouse group is very small compared to human studies that are unable to control for genetic heterogeneity and numerous other variable factors. Therefore, any differences identified between the mice groups in this study can be confidently linked to gender. The analysis of a large number of individuals in this study provided the power to detect relatively small differences in anatomy between sexes. Previous work considering the differences in forebrain structures such as the hippocampus, amygdala, striatum and lateral and third ventricles between male and female mice with MRI found several sexual dimorphisms within the lateral ventricles and amygdala using 3 mice for each group with a resolution of 50 μm (Koshibu et al., 2004, 2005). Increasing the sample size to 40 produced data with increased significance and allowed for the ability to make farther reaching conclusions. Furthermore, while previous studies have measured global volumes, our work analyzed the acquired data on a per-voxel level allowing for the screening of the entire brain and not just specific structures.

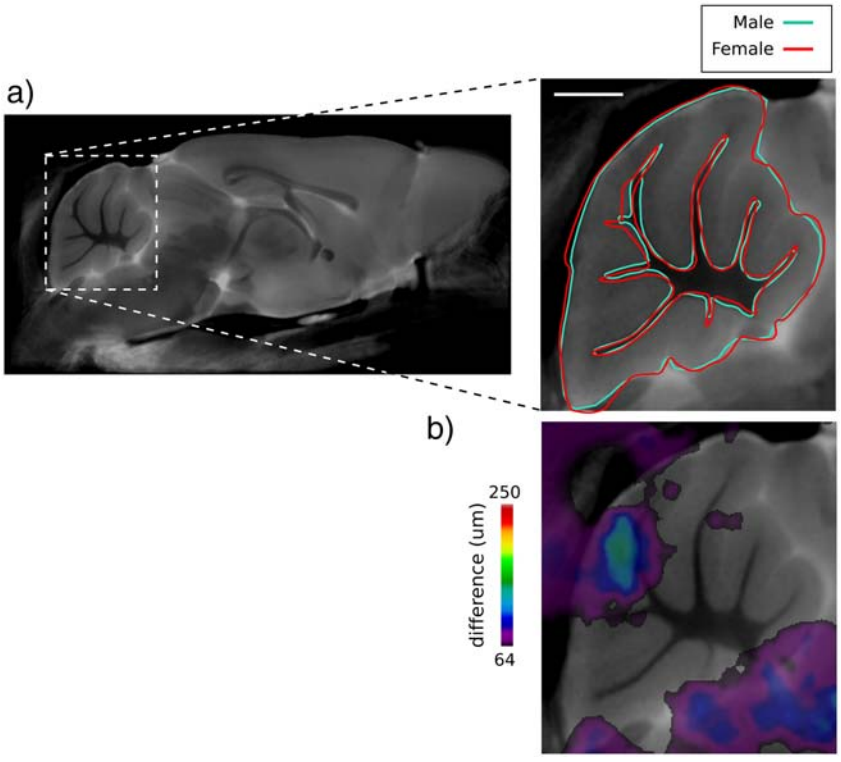

Fig. 5. Gender-associated shape differences in the cerebellar folia of the mouse brain. Note the many sexual dimorphisms in the convolutions of the cerebellum between the male (aqua) and female (red) (a). A corresponding Hotelling map is overlaid on the same slice (b). Only statistically significant differences in regions between the sexes are colored. These colored regions correspond to differences seen in the anatomical traces. Scale bar is 1 mm.

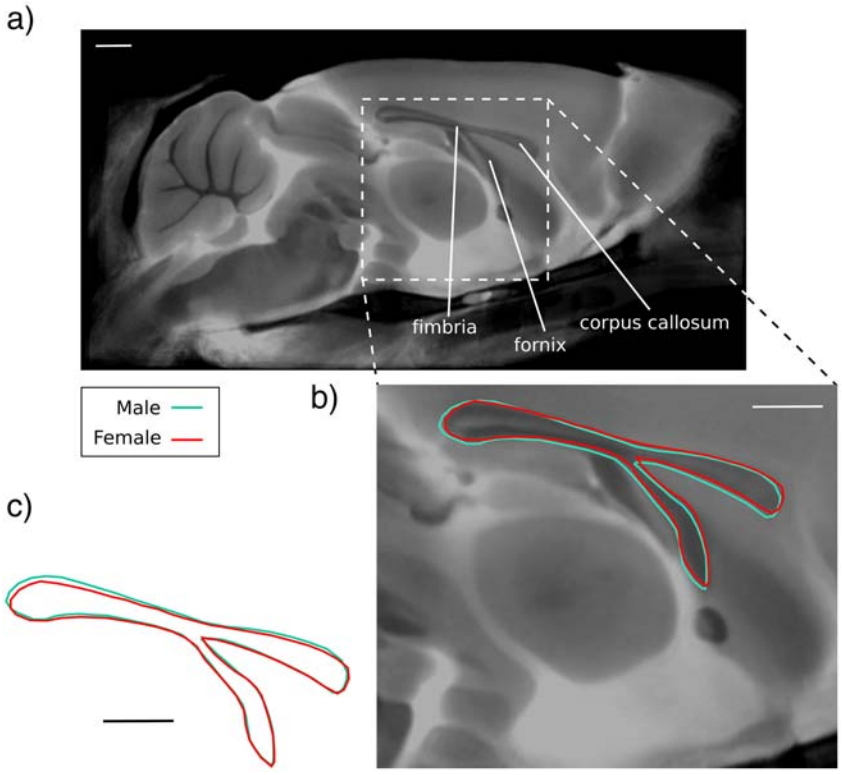

Fig. 6. Global shift and shape change of the fimbria, fornix and corpus callosum structures based on gender. A sagittal view of the average mouse brain shows these structures clearly (a). Taking the same slice from both the average male and average female mouse brain and tracing the same structure shows significant global shift in location (b). When explicitly aligning this structure at the region where the fornix branches off, it becomes apparent that there is a small shape change in this structure when comparing it in the male versus the female brain (c). Scale bar is 1 mm.

This study demonstrated the well-known difference in overall size between the male and female mouse brain. An expected overall brain size difference was detected with male brains being 2.5% larger in volume than female brains. These results are in concordance with human studies that indicate that males have an approximately 9–10% larger cerebral volume than females (Dekaban and Sadowsky, 1978; Giedd et al., 1997). Along with replicating data that indicate that normal males have larger cerebrums than their female counterparts, this work showed additional region-specific differences in adult brain volumes relative to cerebrum size. That is, the sexual dimorphisms seen were not diffusely spread across the brain.

Due to the larger overall brain size in males, it was necessary to control for sex differences for examination of regional size or shape differences. After correcting for the overall brain size differences between males and females, our results revealed a surprisingly large number of significant differences in brain anatomy between the sexes. Shape distortions were detected for

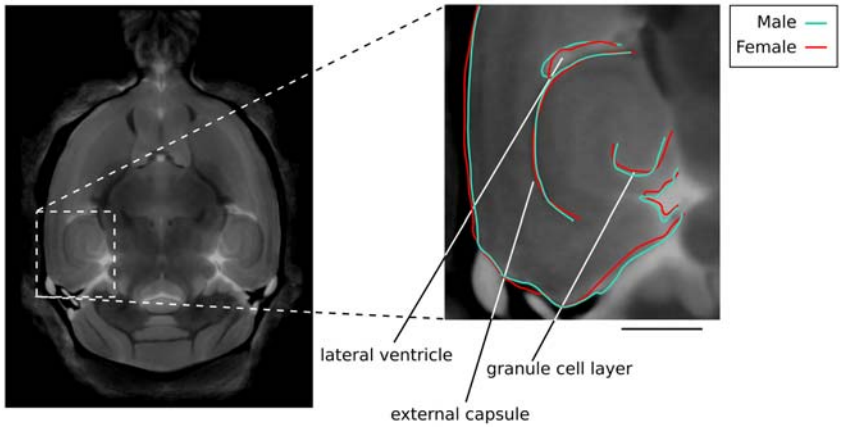

Fig. 7. Males and females show shape differences in the posterior hippocampus. It is evident that various structures within the hippocampus region including the lateral ventricle, the external capsule and the granule cell layer differ in the male and female brain. These small differences correspond to Hotelling map data overlaying a sagittal view of the hippocampus seen in Fig. 2b, *vi*. Scale bar is 1 mm.

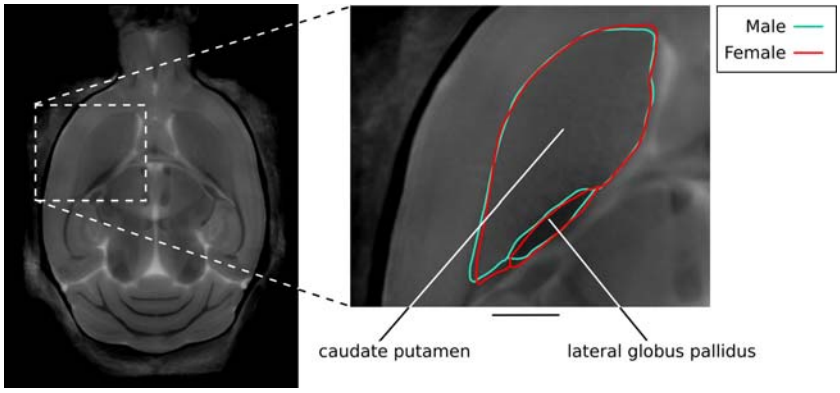

Fig. 8. Comparison of striatum brain structure. Distinction of lateral globus pallidus and caudate putamen between male and female striatum brain structures is evident. Scale bar is 1 mm.

the posterior hypothalamic area, the corpus callosum at the fornix, hippocampus, cerebellum and overall brain structure. Many of the regions that we identified have been implicated in various common human diseases in which disease susceptibility and progression is sex biased (Green, 2001; Honea et al., 2005).

MRI studies of shape dimorphisms in the basal ganglia between schizophrenic and comparison subjects revealed significant differences in the caudate, putamen and globus pallidus when total volume differences were included as covariates (Mamah et al., 2006). The correlation between a particular shape and disease incidence is not always conclusive but may provide important information about the functional cause of a disease or be an indicator in diagnosis. Interestingly, the shape of the lateral globus pallidus, caudate and putamen are significantly distinct between male and female mice. Since subtle differences in morphological alterations in the brain appear very important in distinguishing schizophrenic or other sex-biased disorders such as Alzheimer's disease (Wang et al., 2006) from control subjects, any animal or human studies that attempt to investigate the importance of these shape distinctions must first take sexual dimorphisms into account.

The corpus callosum, the fiber tract that connects the two cerebral hemispheres in some vertebrates, was another region in the mouse brain to show sexual dimorphism. The results agreed with previous studies that showed that the male rodent brain possessed a greater total corpus callosum area compared with females of the same age when differences in brain size were taken into account (Berrebi et al., 1988). However, human subjects do not display the same simple dimorphisms of the corpus callosum. Dubb et al. (2003) showed that specific regions of the corpus callosum, such as the genu, were larger in the human male, while the opposite was true of other regions like the splenium. Our study was able to provide more detailed information about the differences in the mouse brain using statistical analysis. Specifically, it provided details about the differences in shape and size between the male and female structures instead of reporting just on size dimorphisms.

The observation that there are sexual dimorphisms in the hypothalamus, or specifically that the female mouse brain has a larger posterior hypothalamic region compared to a male counterpart, is not in itself surprising. Specific regions within the human hypothalamus, such as the sexually dimorphic nucleus of the preoptic area, have long been known to be larger in males than females (Swaab and Fliers, 1985). However, other regions such as the anteroventral periventricular nucleus of the rat hypothalamus are larger in females than in males, a development that occurs peripubertally (Davis et al., 1996). The hypothalamic region of the brain has been shown to contain the control systems that are critically involved in many physiological, endocrine and behavioral processes. Structural and functional differences in the hypothalamus and other limbic structures are presumed to cause functional sex differences in reproduction, gender and sexual orientation and in the incidence of neurological and psychiatric diseases (Swaab and Hofman, 1995). The role of the human hypothalamus in the neuroendocrine response to illness has only recently begun to be explored (Fliers et al., 2006). Knowledge regarding the sexual dimorphisms of the mouse hypothalamus may provide an important basis to study disorders and diseases that are linked to its malfunction.

Overall, this study successfully provides a basis for further research into sex-based differences of neurological disorders and disease so that they can be better understood. The following can be generalized from this work:

1. even after linear effects have been removed, there are many small but statistically significant differences in the male and female mouse brain;
2. larger differences, such as those seen in the posterior hypothalamic area extending to the mammillothalamic tract, reach displacement magnitudes of 150 to 180 μm; and
3. there is a left/right symmetry to most of these differences in the brain.

Acknowledgments

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