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Cerebral asymmetries in 12-week-old C57Bl/6J mice measured by magnetic resonance imaging

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ABSTRACT

Asymmetries of multiple components of the rodent cerebrum have been described at various levels of organization. Yet, despite its ubiquitous nature, many confusing and sometimes contradictory reports regarding structural asymmetries in the rodent brain have been published. There is a need, therefore, for a whole-brain imaging analysis technique for asymmetry studies that is both accurate, reproducible and robust. To this end, a comprehensive three-dimensional examination of differences in brain structure in an inbred mouse strain was undertaken. The goal of this study was thus to use high-resolution magnetic resonance imaging to assess structural asymmetries in the adult C57Bl/6J mouse brain. Fixed brain T2-weighted images of 20 male C57Bl/6J mice were acquired on a 7T scanner at 32 μm isotropic resolution. We used voxel-based analyses to examine structural asymmetries throughout the whole mouse brain. The striatum, medial-posterior regions of the thalamus, and motor, sensorimotor, and visual cortex were found to be asymmetrical. The most significant asymmetry was found in the hippocampus and, specifically, the dentate gyrus. In each case, the left region was larger than the right. No other regions of the mouse brain showed structural asymmetry. The results in the dentate gyrus were confirmed using stereology, revealing a correlation of $r = 0.61$ between magnetic resonance and stereological measures. Hippocampal, along with cortical asymmetry, has been discussed repeatedly in the literature, yet a clear pattern of directionality, until this point, has not been described. The findings of asymmetry in the striatum and absence of asymmetry in the rest of the brain are novel and show the advantage of using the whole-brain three-dimensional techniques developed herein for assessing asymmetry.

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Introduction

Some degree of lateral asymmetry is seen in most biological systems (Geschwind and Galaburda, 1985). In humans and many other mammals, the two hemispheres of the brain differ in their structure, function and neurochemistry (reviewed by Toga and Thompson, 2003). Since the use of inbred and genetically-altered rodents have become a leading approach in the study of human disease and structure–function relationships in the central nervous system, there have been many published reports investigating left–right asymmetries in the rodent brain (Collins, 1977; Oke et al., 1980; Diamond et al., 1981; Diamond et al., 1982; Kolb et al., 1982; Diamond et al., 1983; Lipp et al., 1984; Slomianka and West, 1987; Bulman-Fleming et al., 1992; Kaplan et al., 2003). The analysis of structural asymmetries can be useful, as in the case of some human asymmetry work, in providing the typical asymmetrical standard by which atypical asymmetries can be measured in studies using inbred mouse models of neuroanatomical disorders. In other cases, knowledge of detailed

structural brain asymmetries may provide insight into specific functional variation. To this end, asymmetries have been described at various levels of anatomical organization, from the macroscopic appearance of the cerebral hemispheres (Kolb et al., 1982) to the distribution of neurochemicals such as norepinephrine in the thalamus (Oke et al., 1980).

Several specific anatomical structures such as the amygdala (Melone et al., 1984), the hippocampus (Diamond et al., 1982; Lipp et al., 1984; Kaplan et al., 2003), the thalamus (Goldschmidt et al., 1984), and various fields of the neocortex (Diamond et al., 1981; Kolb et al., 1982; Van Eden et al., 1984) also show lateral asymmetry. Unfortunately, studies that focus on morphological asymmetries in the rodent brain are usually labor-intensive and typically focus on one or two regions of interest. The combination of these findings paint a confusing and sometimes contradictory picture of rodent cerebral asymmetry, where, in general, directionality is dictated by rodent age, gender, species and environmental factors (Diamond et al., 1983; Slomianka and West, 1987; Bulman-Fleming et al., 1992; Kaplan et al., 2003).

The most extensive rodent structural asymmetry studies have focused on the hippocampus and its components, yet a clear pattern of asymmetry has not been identified (Valdes et al., 1981; Kolb et al., 1982; Diamond et al., 1983; Rağbetli et al., 2002; Kaplan et al., 2003).

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When Kolb et al. (1982) measured the cross-sectional area of the hippocampus and the length of the granule cells of the adult male Long-Evans hooded rat, they found an absence of anatomical left–right asymmetry. However, in earlier studies, wet weights of the right and left hippocampi in the same adult rodent species showed a left-hemisphere enlargement (Valdes et al., 1981). The opposite was found from linear measures from traces of the coronal view of thionin-stained brain slices where the right hippocampus was found to be significantly thicker than the left (Diamond et al., 1983).

This variation in morphologic pattern can be related to hormonal and behavioural changes (Diamond et al., 1982; Rağbetli et al., 2002). However, these studies suffer from more than potential subject variation. The methodological approaches are inherently variant since there are difficulties related to defining structural anatomical boundaries. Gross morphological landmarks are variable in their presence and location which can result in rater-dependent measurements. Furthermore, the techniques are labor-intensive and only focus on a limited number of regions. They are therefore unable to provide insight into how the asymmetries influence or relate to other regions of the rodent brain. There is a need, therefore, for a whole-brain imaging analysis that is reproducible, automated and robust. One such method, a voxel-based statistical analysis, is well suited for examining structural hemispheric asymmetries. To this end, a comprehensive three-dimensional examination of differences in brain structure in an inbred mouse strain was undertaken. The goal of this study was to use high-resolution magnetic resonance imaging (MRI) to assess asymmetries in the 12-week-old adult C57Bl/6J mouse.

Since magnetic resonance (MR) images are digital, quantitative data can be readily extracted from inbred mouse strains (Chen et al., 2006). In comparison to live specimen scanning, the imaging of fixed brains can produce images with dramatically increased resolution as fine as 32 μm (this study; Natt et al., 2002; Nieman et al., 2005) or even 21 μm (Badea et al., 2006). The downside to fixed brain imaging is, on the other hand, the inability to follow the same mice over time, leading to a snapshot of asymmetry at one point in the lifespan rather than an evolving view of asymmetry that potentially changes with development.

However, fixed brain MR imaging 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 (reviewed by Chan et al., 2007). Furthermore, since brains are maintained in a natural conformation in the skull during our imaging studies, the gross anatomy of the specimens is conserved. Leaving the brains within their skulls also prevents ventricle collapse which is important for volumetric studies, especially if *ex vivo* and *in vivo* data are to be compared (Ma et al., 2008). Finally, fixed brain MR imaging allows for whole-brain coverage and fully 3D analysis, characteristics not shared in other more frequently used measurement techniques.

MRI and voxel-based analysis of fixed samples along with traditional histological methods were used in this study to identify cerebral asymmetries in the adult mouse brain. The following questions should be answered from this study: (1) Are there overall hemisphere size differences between the left and right side of the C57Bl/6J mouse brain? (2) What are the specific anatomical structures in the mouse brain that exhibit asymmetry? (3) For those structures that are deemed to be significantly asymmetrical, what are the relative differences between the two hemispheres? (4) How does volume determination of specific anatomical structures using voxel-based morphometry techniques compare to traditional stereological analysis?

Materials and methods

Mice and brain sample preparation

Male C57Bl/6J ($n=20$) 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 by Crawley, 1999). The procedure for sample preparation has been previously described (Tyszka et al., 2006; Spring et al., 2007). In summary, mice were anaesthetized 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 12–21 days post-mortem. All animal experiments were approved by the animal ethics committee of the Hospital for Sick Children (Toronto, ON).

Imaging

A multi-channel 7.0 Tesla 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 mm 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 (FSE) sequence, with TR/TE = 325/32 ms, four averages, field-of-view $14 \times 14 \times 25$ mm and matrix size = $432 \times 432 \times 780$ giving an image with $32.4 \times 32.4 \times 32.05$ μm voxels. Total imaging time was 11.3 h (Henkelman et al., 2006).

Image postprocessing

Rigid body registration of all 20 mouse brains 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., 2005), where the midline was centered on $x=0$ in world coordinate space. Each registered MR volume was then inverted through the x axis, resulting in two volumes per mouse: the regular volume and the mirror image volume. All possible pairwise 12 parameter registrations of all 40 scans (inverted and normal) were then carried out to create an unbiased linear average model of the entire dataset. All images were subsequently non-linearly aligned towards the 12 parameter average. The resulting registered volumes were resampled and averaged (Collins et al., 1994; Kovacevic et al., 2005). 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 scan was inverted, any remaining linear transformations removed, and centered to the mean of the entire set of 40 volumes (Fig. 1).

To test the reliability of the asymmetry revealed in this study and to prove that systematic distortion in the MRI did not lead to a directional bias in the results, 6 randomly chosen subject brains were scanned in a normal and reorientated configuration of a 180 degree

rotation about the vertical axis in the MRI and the crano–caudal axis of the brain. After automatically counter-rotating the images by -180 degrees, all the new images underwent the same postprocessing procedure as described above. Relative voxel comparisons were used to confirm that MRI scanning orientation did not impact the directionality of any asymmetries discovered.

Statistical analysis

The goal of this image registration process was to map all 40 volumes (20 flipped, 20 normal) exactly into the same space. At the end of this procedure the deformation fields were analyzed to determine whether mirrored and normal volumes had to deform differently to reach this final target. The Jacobian determinants of the deformation fields – a measure of voxel expansion or contraction – were then analyzed for symmetry differences using a t test with 38 degrees of freedom (40 samples minus 2 free parameters). 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 1 mm.

In order to better visualize and interpret discovered shape changes, anatomical line drawings of select regions were created (as in Spring et al., 2007). A symmetry specific deformation field was created by averaging all non-linear deformation fields of the mirrored and normal mice separately. The final registration atlas was deformed using the inverse of these average deformation fields. An anatomical atlas containing the definition of the dentate gyrus (Dorr et al., 2008)

was then aligned towards the average normal and mirrored volumes and the outline of the dentate gyrus superimposed over the MR slice for visual assessment of shape differences.

Histology

For validation of the MRI results, stereologic volume estimation was performed on five brains that showed the most significant structural asymmetries with MRI analysis. The validation study used the most significantly asymmetrical samples to check if histological analysis of the brains that had most substantially driven the MRI asymmetry results provided similar findings. The brains were removed from the skulls, cryoprotected, sectioned at $20\ \mu\text{m}$, and stained with a NeuroTrace fluorescent Nissl stain (1:20, Invitrogen, Carlsbad, CA) and Hoechst 33258 (1:2000, Invitrogen).

Cavalieri volume estimate

Volume estimation using the Cavalieri principle on the right and left hemisphere of five mouse brains was performed with Stereo Investigator software (MicroBrightField, Williston, VT). Before Cavalieri analysis, a Nissl stained atlas was generated with contours drawn for the dentate gyrus (from -1.94 Bregma to -2.70 Bregma; Paxinos and Franklin, 2001), which was used as a guide for all analyses. Volumes were estimated by using a 1 in 6 random series of $20\text{-}\mu\text{m}$ Nissl stained sections using a $25\text{-}\mu\text{m}$ size counting grid. Between 200 and 400 grid points from 5 sections were counted for the dentate gyrus, which provided coefficient of error (CE) estimates of <0.1 . Data are presented as means \pm SEM.

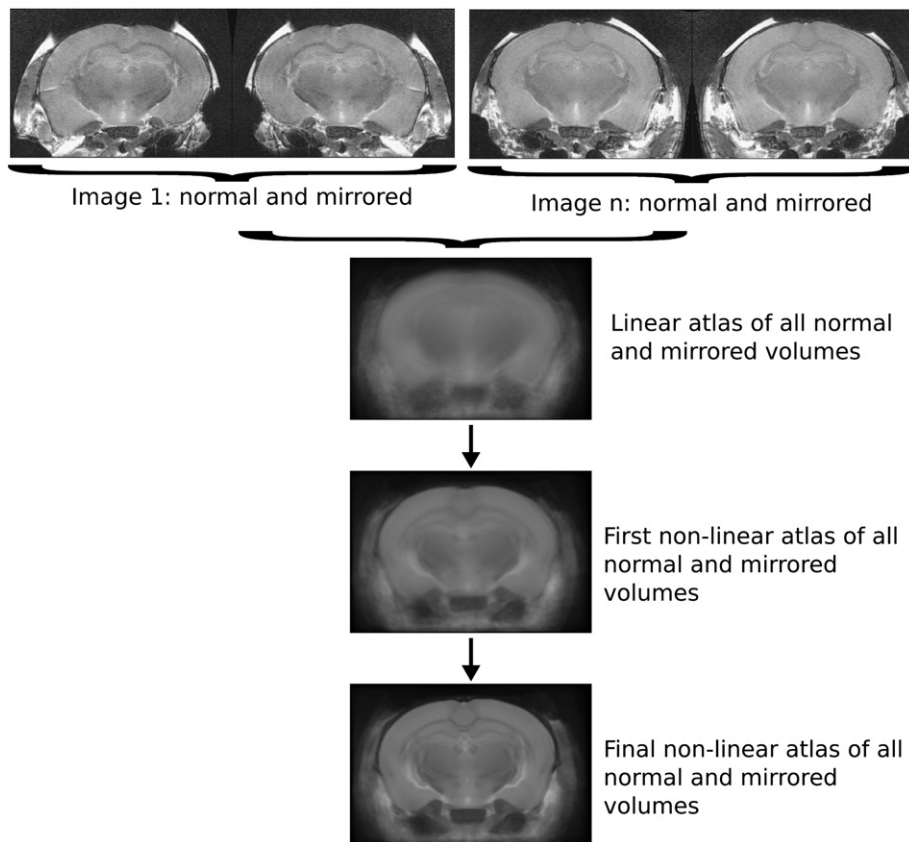


Fig. 1. Methodological illustration of the analysis process. All scans underwent rigid body alignment towards a pre-existing model (Collins et al., 1994). Each image was automatically inverted across its centre along the x axis, creating two images for each mouse, one normal and one mirrored. All 40 images (20 normal, 20 inverted) were then used to create an unbiased linear atlas of all normal and mirrored volumes. All images were then non-linearly aligned towards the 12 parameter registration average. The resulting registered volumes were resampled and averaged. This iterative process was repeated for an additional five generations with ever finer deformation grid-point spacing to produce the final non-linear atlas of all normal and mirrored volumes wherein the anatomies of all 40 images are identical (Kovacevic et al., 2005).

Results

Differences in overall brain size

The purpose of this study was to examine left–right differences in the whole mouse brain using full 3D MRI. Our analysis identified several regions of structural asymmetry in the male mouse brain. 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 and compared with the left hemisphere found to be 2.8% larger ($p = 0.0009$) than the right hemisphere. All subsequent results reported in this paper have been corrected for these overall differences in hemisphere size.

Per-voxel Jacobian statistical results

A Jacobian statistical analysis was utilized to identify regions within the mouse brain that showed structural asymmetry. Representative single horizontal slices taken from per-voxel data sets are shown to indicate regions of asymmetrical per-voxel expansion and illustrate relative voxel size differences between the left and right brain (Fig. 2). Significant enlargements are shown in the striatum, the medial–posterior regions of the thalamus, and motor, sensorimotor,

and visual cortex. Asymmetry is also found in the hippocampus region as highlighted by the Jacobian statistical results. In each of the above cases, the left hemisphere was larger than the right. Corresponding deformation graphs are included to show the expansion of specific asymmetrical voxels and highlight the varying degree of asymmetry in different neuroanatomical regions (Fig. 2a). These findings correspond to a $15.5 \pm 2.5\%$ ($q < 0.0008$) difference between the left and right highlighted region in the cortex, a $9.2 \pm 1.3\%$ ($q < 0.0001$) difference between the highlighted hippocampus regions, a $7.0 \pm 1.6\%$ ($q < 0.01$) difference between the asymmetrical region of the striatum and $2.8 \pm 1.5\%$ ($q < 0.0008$) asymmetry within the thalamus. In analysis of the whole mouse brain, no other significant asymmetrical differences were found.

Shape and volume differences in the dentate gyrus

Since the hippocampus of the mouse brain showed the most significant asymmetry when comparing normal and mirrored MR images, a more detailed analysis of this region was undertaken. Deformation in this region, as detected by Jacobian statistical maps, is clearly illustrated when the dentate gyrus is outlined and the traces are compared for visual assessment of shape differences (Fig. 3). Both an overall difference in size is apparent as is a more superior location of the lateral aspects of the dentate gyrus.

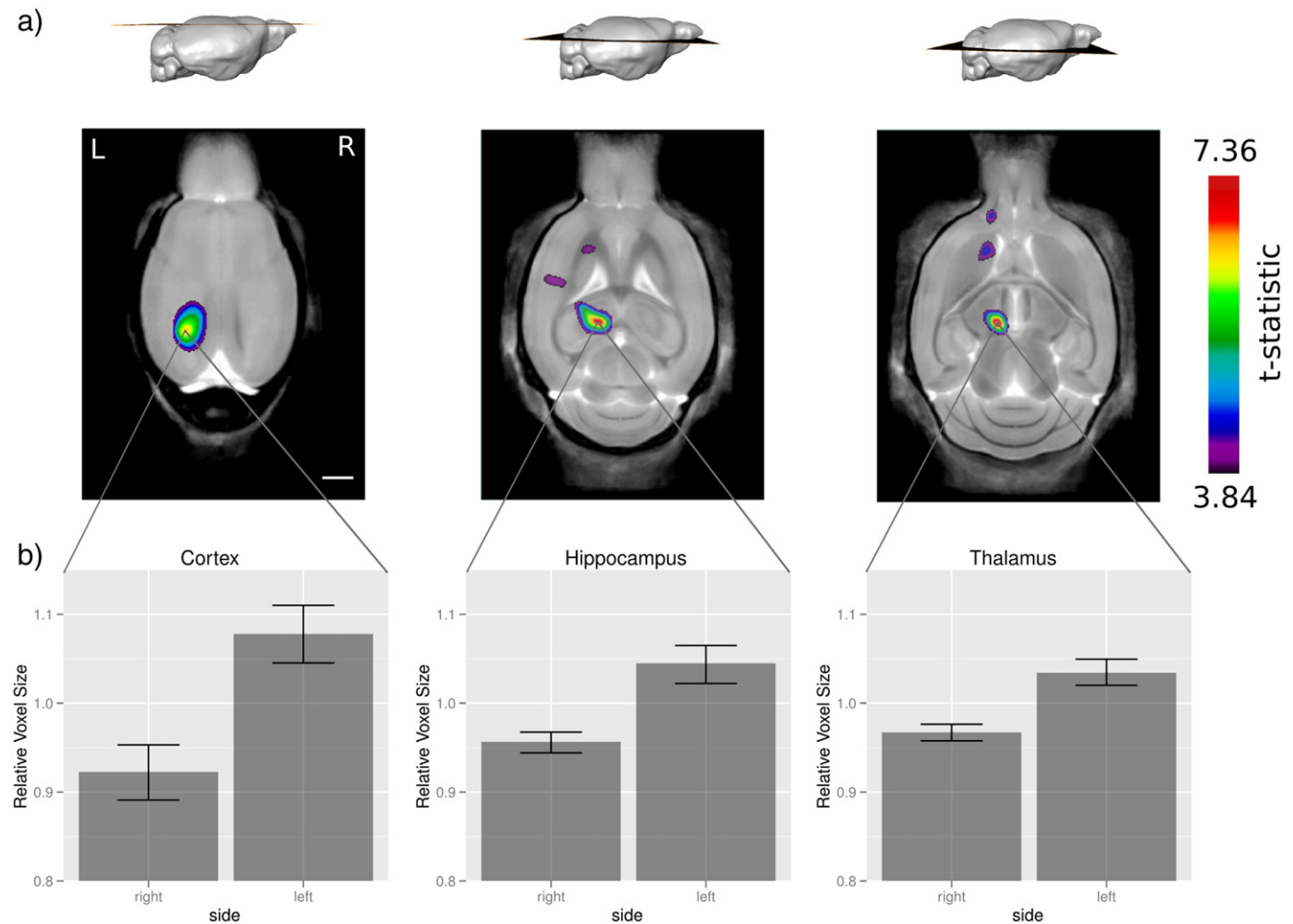


Fig. 2. (a) Asymmetrical per-voxel expansion of neuroanatomical structures is illustrated with a Jacobian statistical map. Slices correspond to positions indicated by the three-dimensional sagittal brain above each anatomical slice. All colored regions are statistically significant and have a less than 1% chance of being a false positive. Significant differences in the hippocampus, thalamus, and motor, sensorimotor, and visual cortex can be seen. (b) Corresponding 95% bootstrapped confidence interval plots are included to show the expansion at specific asymmetrical voxels to show relative differences at that voxel between the left and right side of the brain. L and R represent the left and right side of the brain, respectively. Scale bar is 1 mm.

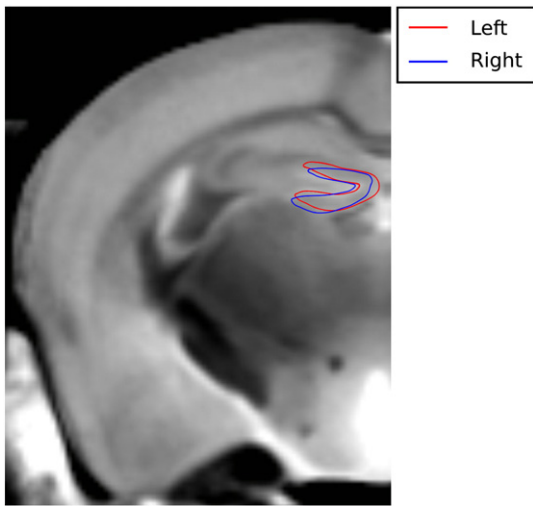


Fig. 3. Asymmetries of the mouse dentate gyrus as seen with traces of left (red) and right (blue) anatomical structures. Deformation in the dentate gyrus, as indicated by Jacobian statistical maps (Fig. 2), is clearly illustrated when the left and right dentate gyrus is outlined from the 20-mouse average image and the traces overlaid and compared for visual assessment of shape differences. Both an overall difference in size is apparent as well as a more superior location of the lateral aspects of the dentate gyrus.

To confirm structural asymmetrical differences of the dentate gyrus, histology and stereological analysis was undertaken using the five brains that showed the most significant structural asymmetries with MRI analysis. Stereological data correspond to MR results with the left dentate gyrus measuring $0.094 \pm 0.002 \text{ mm}^3$ and the right dentate gyrus measuring $0.085 \pm 0.004 \text{ mm}^3$, $p = 0.036$ using a one-tailed t test. MR based volume measures correlated with the stereological data with a correlation coefficient of 0.61, $p = 0.03$. A comparison between MR and stereological dentate gyrus volume estimates (after normalizing for mean volume) is shown in Fig. 4a. A correlation graph that compares normalized stereology and MRI volume estimates of the dentate gyrus for the five brains also confirms significant similarity between histology and MRI and is shown in Fig. 4b.

Methodological reliability

To test the reliability of the asymmetry revealed in this study and prove that systematic distortion in the MRI did not lead to a leftward bias in the results, six randomly chosen subject brains were scanned in a normal and rotated configuration (180 degree rotation about the vertical axis). After automatically re-rotating the images by -180 degrees about the vertical axis, they underwent the same postprocessing procedure as the original 20 experimental brains used for this study. Relative voxel comparison of a voxel within the dentate gyrus

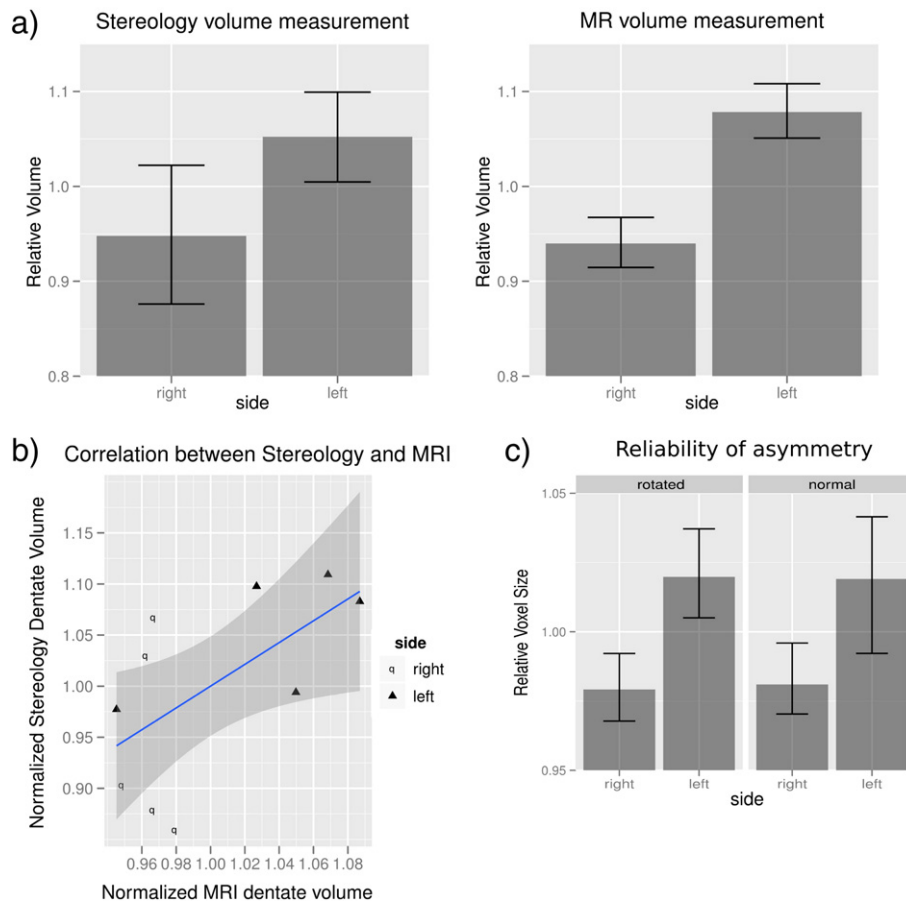


Fig. 4. (a) Volume estimation of the dentate gyrus from MRI and stereology is compared in these 95% bootstrapped confidence interval plots, estimated from the same five mice. The asymmetry found is clearly similar. (b) A strong correlation between MRI and stereology results is further illustrated in this graph comparing left and right side normalized volume estimates for the same five brains. Ten values are shown, acquired from the left and right side of the brain using both methodologies. (c) The reliability of asymmetry findings was tested by using six randomly chosen subject brains scanned in a normal and rotated configuration (180 degree rotation about the vertical axis) in the MRI. Relative voxel size for one voxel in the dentate gyrus was compared in both normal and rotated images. Reorientation of brains during scanning did not impact the directionality or magnitude of the results.

was used to confirm that MRI scanning orientation did not impact the directionality of any asymmetries discovered. The results of this analysis show that even when the brains were reoriented for scanning, the directionality and magnitude of the asymmetry results remains. This is illustrated in Fig. 4c.

Discussion

This work analyzed a large number ($n = 20$) of mice belonging to a well-known inbred strain and was able to provide images with superb resolution along with excellent signal-to-noise ratio (SNR) acquired in efficient scan times. Variance within the inbred mouse strain is very small compared to human studies that are unable to control for genetic heterogeneity and various environmental factors. Therefore, any differences identified between the mice groups in this study can be linked to structural asymmetry. The analysis of a large number of subjects in this study provided the power to detect relatively small differences in anatomy between cerebral hemispheres. Furthermore, while previous studies have measured global volumes or numerical density of neurons, our work analyzed the acquired data on a per-voxel level allowing for the screening of the entire brain and not just specific structures. However, there are limitations to this technique. For instance, the data acquired and analyzed provides very little information regarding possible structure–function relationships in those areas deemed asymmetrical. In such cases, further research into the cellular basis of these asymmetries and functional assays would have to be carried out.

The methods used to assess asymmetry are similar to the ones used in previous investigations of hemispheric asymmetries from human MR scans. Watkins et al. (2001), for example, linearly aligned all brains in their study towards Talairach space, then flipped each brain around the x axis, and compared classified tissue maps for laterality. A similar approach was taken by Dorsaint-Pierre and colleagues (2006) in examining language lateralization. The general approach of comparing grey matter voxel density maps between normal and mirrored images was validated by Luders et al. (2004) by comparing these automated methods to manual region of interest based investigations. Our methods are similar in spirit to these papers outlined above; the key difference is that, rather than relying on tissue classification and density maps, we used the simpler topology of the mouse brain to our advantage and employed non-linear registration to align all normal and mirrored images together and then compared the Jacobian determinants of the deformation fields for asymmetries. The use of fixed brain samples with an overnight 3D FSE T2-weighted acquisition provided high-resolution data which allowed for the identification of even subtle asymmetries using the computational analysis. The main results should, however, also be obtainable using live mouse MRI.

Up until now, there has been a lack of structural asymmetry research that has revealed a clear pattern of asymmetry in the rodent brain. We believe that our voxel-based technique provides a robust and automated three-dimensional method to assess asymmetry accurately and in the whole brain. Our work identified that the hippocampus, regions of the cortex, striatum and thalamus each had significant left–right asymmetry in the C57Bl/6J mouse brain. No other parts of the brain showed structural asymmetry through this analysis. In all areas where asymmetry was detected, the left structure was larger than the right. The asymmetrical nature of the most significant region, the hippocampus, was verified using histology with Nissyl staining and stereological analysis in the dentate gyrus. The goal of the histology within this study was to provide validation for the MRI results and not provide a direct comparison between stereology and MRI for every part of the brain. As such, the use of five histological sample brains should be appropriate for providing reassurance in regards to MRI results.

Our work identified the hippocampus, a limbic structure that regulates several brain activities including memory, learning and

regulation of the neuroendocrine axis (Seifert, 1983), as the most asymmetrical region in the adult C57Bl/6J mouse brain. Hippocampal asymmetries have been discussed frequently in the literature (Diamond et al., 1982; Lipp et al., 1984; Kaplan et al., 2003; Lister et al., 2006), though the direction of asymmetry appears to vary by strain, age, and type of environment (Tang et al., 2003). Our voxel-based analysis indicated that the hippocampus was larger in the left hemisphere than the right. Studies in human subjects show that, in most cases, the hippocampus is larger in the right hemisphere than the left (Bilir et al., 1998; Free et al., 1995; Hasboun et al., 1996; Pegues et al., 2003; Watson et al., 1992). In other studies, no asymmetry has been reported (Bhatia et al., 1993; Reiman et al., 1998; Strakowski et al., 1999). Inter-study differences appear to be dependent on the methodological approach used including measurement techniques and the definition of anatomical boundaries. The functional implications of the rightward structural dominance in the human hippocampus is yet unknown.

Specific analysis of the mouse dentate gyrus using stereological techniques confirmed our MRI analysis results and showed significant similarity between stereology and MRI (see Figs. 4a and b). Deformation in this region, as indicated by Jacobian statistical maps, was clearly illustrated when the dentate gyrus was outlined and the traces compared for visual assessment of shape differences. Both an overall difference in size was apparent as was a more superior location of the lateral aspects of the dentate gyrus.

The use of a voxel-based morphometry techniques to study the structure and volume of mouse brain regions provides a number of benefits when comparing to traditional stereological methods. For instance, the technique allows for investigation of a large number of study samples within the desired tissue region without preplanned sectioning or concern for specimen damage. Furthermore, the same subject or region of interest can be analyzed in multiple planes which would be impossible when histological sectioning is required. However, as in the case of stereological analyses of histology sections, volumetric analyses using voxel-based methodology require the experimenter to define anatomical boundaries. Additionally, voxel-wise volumetric analyses are limited by image resolution for fine morphological asymmetry studies.

The hippocampal asymmetry reported in this study could be a reflection of either additional functional capacity in one hemisphere and/or alternative functional capacities when comparing hemispheres, such as the known preference for spatial memory to be situated in the right hemisphere (Shinohara et al., 2007) and, in humans, verbal memory being preferentially associated with the left hippocampus (Milner, 1972). Investigation into the type and nature of cells which contribute to the size and shape distinction between the two hemispheres may provide insight into a possible link between asymmetry and functionality.

The asymmetrical nature of the rodent cortex has also been described previously (Diamond et al., 1981; Dowling et al., 1982; Sherman and Galaburda, 1984; Zilles et al., 1996). The asymmetry reported in these studies showed that the cerebral cortex of the male rodent was thicker on the right side than the left (Diamond et al., 1981; Dowling et al., 1982; Diamond et al., 1983). However, in some cases, asymmetries in thickness, length, width and weight can be misleading since they do not account for the full measure of cortical volume. For example, a small difference in thickness can be counter-balanced by a change in surface extent. For this reason, subsequent research focused on total neocortical volume of the right and left hemispheres of rodents (Sherman and Galaburda, 1984). These studies found, again, that the neocortex of the male rodent was asymmetrical with the right side being larger than the left. However, in most cases, an asymmetry between the two whole hemispheres of the rodent subjects was not examined, or was noted yet not used, to adjust findings in subsequent subregion studies (Dowling et al., 1982; Kolb et al., 1982; Diamond et al., 1983; Zilles et al., 1996). Our study

used three-dimensional voxel-based analysis to study the whole brain and found asymmetry in the motor, sensorimotor, and visual portions of the cortex, but only after correcting for overall differences in hemisphere size.

Although asymmetry in other rodent brain regions has been identified, the majority of previous rodent asymmetry studies have focused on the hippocampus and neocortex. Some preliminary studies identified mast cell number variation in the thalamus of the rodent brain, finding a greater number in the left hemisphere (Goldschmidt et al., 1984). Yet the identification of specific morphological asymmetries in the region had, until this point, not been explored. Furthermore, the findings of structural asymmetry in the rodent striatum and absence of asymmetry in the rest of the brain are novel to our study and show one of the advantages of using whole-brain three-dimensional techniques for assessing structural asymmetry.

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