

DEFORMATION BASED MORPHOMETRY AND ATLAS BASED LABEL SEGMENTATION: APPLICATION TO SERIAL MOUSE BRAIN IMAGES

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ABSTRACT

The aim of this paper is to investigate techniques that can identify and quantify longitudinal changes *in vivo* from magnetic resonance (MR) images of murine models of brain disease. Two different approaches have been compared. The first approach is a segmentation-based approach: Each subject at each time point is automatically segmented into a number of anatomical structures using atlas-based segmentation. This allows longitudinal analyses of group differences on a structure-by-structure basis. The second approach is a deformation-based approach: Longitudinal changes are quantified via registration of each subject's follow-up images to that subject's baseline image. Both approaches have been tested on two groups of mice: A transgenic model of Alzheimer's disease and a wild-type background strain, using serial imaging performed over the age range from 6-14 months. We show that both approaches are able to identify longitudinal differences. However, atlas-based segmentation suffers from the inability to detect differences across populations and across time in regions which are much smaller than the anatomical regions. In contrast to this, the deformation-based approach can detect statistically significant differences in highly localized areas.

Index Terms— *atlas-based segmentation, deformation based morphometry (DBM), longitudinal image analysis, serial mouse imaging*

1. INTRODUCTION

Mouse models are used in many biomedical research areas to study issues ranging from understanding development to drug efficacy. Murine models offer a rapid life cycle in comparison to other animals as well as a wealth of genetic information. Technology is readily available to produce transgenic (TG) mice. The human and mouse genome project has compiled a large amount of data on genes, which

makes murine models increasingly attractive as a means of enhancing understanding of disease processes.

Recent work that has used MR to study anatomical differences between mouse strains includes [1], [2] and [3]. However, the majorities of these studies have been carried out under *ex-vivo*, e.g. non-recovery conditions, and have employed techniques that are unsuitable for longitudinal investigations. The long scanning periods, often used to obtain sufficient image contrast and spatial resolution, may also prejudice recovery after long-term anaesthesia. TG animals are often physiologically fragile and this poses an additional challenge in longitudinal studies.

In this study we set out to develop and compare two different approaches to detect longitudinal changes between two groups of individuals under *in vivo* conditions. *In vivo* brain imaging by MR offers both the potential for increased sensitivity by allowing comparisons within the same individual over time and allowing for reduction in the number of animals needed since each individual can contribute data at multiple time points. We explored the use of atlas-based segmentation and deformation based morphometry (DBM) to analyse within and between group differences longitudinally. We also discuss the pros and cons of atlas-based segmentation versus DBM analysis.

2. MATERIALS

2.1 Animal handling and MR acquisition protocol

All animal experiments complied with GSK ethical and UK legal requirements. MR was carried out at 6, 9, 11 and 14 months of age. The number of mice available for scanning declined over time due to natural attrition, particularly in the TG groups. However, we did not detect any evidence that the imaging procedure had any effect on mortality. The number of mice in each group at the four time points is shown in the table below.

Time point	Wild type	Transgenic
6 months	10	16
9 months	10	13
11 months	10	9
14 months	10	4

MR images were acquired using a 4.7T (Bruker Biospec 40 cm horizontal bore magnet). The mice were anaesthetised with isoflurane and their heads were immobilised in a custom-built head holder. A multi-slice (120 slices), multi-echo (8 echoes of 10ms, 21ms, 31ms, 42ms, 52ms, 63ms, 73ms, 84ms, TR 5.84s) CPMG data set, obtained using two interleaved scans of slice thickness 0.31mm, with final voxel resolution 78x78x156 μ m, was acquired at each time-point. The field of view was 20x20x18.6mm and the matrix size was 256x256. Total imaging time was ~2 hours. Recovery from anaesthesia was uneventful and afterwards the mice were returned to their home cages.

2. METHODS

3.1 Image registration

The two methods which have been used for the analysis of longitudinal differences are based on image registration. Image registration is a key element in many morphometric applications since it enables the warping of images into a standard reference space. We have used a spline-based deformation model [4], for non-rigid registration which has been successfully used in a number of applications.

3.1.1 Method A – Atlas based registration

One possible approach for measurements is the segmentation of the brain into anatomical structures in each animal at time point $t = 0$. All follow-up time points are then registered non-rigidly to the baseline time point $t = 0$. This enables us to compare the volumetric differences between groups and between time points on a structure-by-structure basis. We implement such an approach for the segmentation of mouse brains by first performing an affine registration of the atlas to each MR image. After affine registration, the whole brain segmentation is transformed from the atlas into each subject's MR image. After this the transformed segmentation is dilated by 5 voxels. This effectively produces a brain extraction and enables us to ignore the extra-cranial tissues during the subsequent non-rigid registration. The non-rigid registration is carried out using a multi-resolution approach starting with 2mm control point spacing. The initial control point mesh is iteratively refined during the registration and the final control point mesh has a control point spacing of 0.25mm. After we have obtained the segmentation of the baseline image we can register all subsequent follow-up time points to this baseline. Here we

can use the non-rigid registration algorithm described above again, however this time we align images from the same animal acquired at different time points. After registration, it is possible to compute the volume change between the time points via the local Jacobian determinant of the coordinate transformation at that point. Thus, local volume change (e.g. growth or atrophy) can be directly estimated from the deformation field that aligns the baseline and follow-up images. The overall volume change per structure can be estimated by integrating the volume change across all voxels of the structure Ω :

$$\Delta V = \int_{x,y,z \in \Omega} J(x,y,z)$$

where J is the Jacobian map derived from the registration.

3.1.2 Method B – Deformation Based Morphometry (DBM)

The analysis of longitudinal differences using DBM requires similar registration steps to those in the previous section. First, each baseline image is registered to the atlas using the affine and non-rigid registration steps described above. Secondly, the follow-up images from each animal are registered to the baseline image from the same animal. In contrast to the previous section, the analysis of longitudinal differences is carried out on a voxel-by-voxel basis.

To detect longitudinal difference within each animal across time we use the deformation fields obtained from the registration between the baseline and follow-up scans to compute a voxel-by-voxel map of the Jacobian determinant. These maps encode the differences in local tissue volume across time. However, these maps are not directly comparable across animals or groups of animals since these maps are defined the each animal's baseline coordinate system. Thus, we use the transformation between each subject's baseline image and the atlas to transform the voxel-wise volume change maps into a common coordinate system as defined by the atlas. Once these maps are transformed into this coordinate system they can be compared on a voxel-by-voxel basis using a t-test and the results are corrected for multiple comparisons using the False Discovery Rate (FDR) threshold [5].

4. RESULTS

4.1 Results from atlas-based segmentation

The growth curves for whole brain, cerebellum, cerebral cortex and hippocampus normalised with respect to each structure's baseline volume at 6 months are shown in Figure 1. Figure 1-(a) shows that TG mice exhibit a continual growth over the 14 month period whereas the growth for WT mice shown in Figure 1-(b) plateaus in structures such as

cerebellum, hippocampus and whole brain and reduces in volume the cerebral cortex after 11 month period. Another point worth observing is that the hippocampus growth rate is much higher in TG mice in relation to the other structures within the same group.

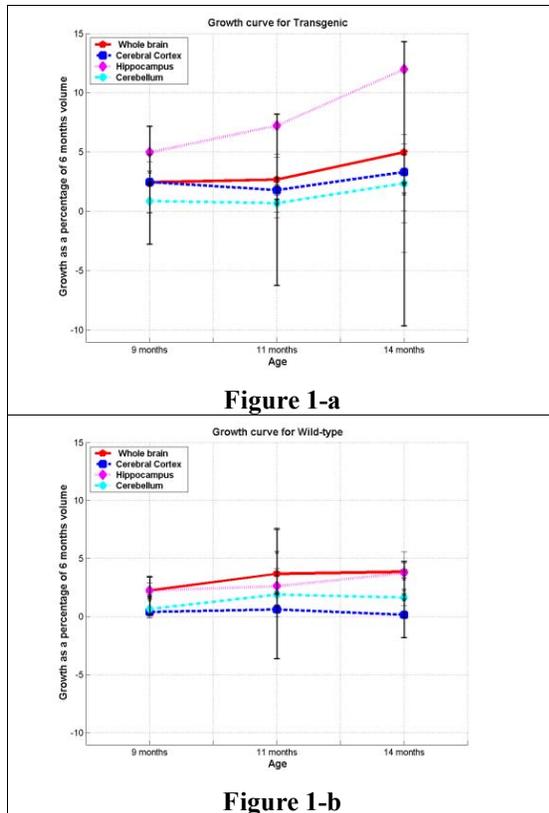


Figure 1 Average Growth curves for TG and WT mice. The TG group exhibits continual growth whereas the WT group plateaus after the 11-month period. The hippocampus of the TG group grows faster in comparison to other regions within the same group.

4.2 Results from deformation-based morphometry

Figure 2 illustrates longitudinal changes in a coronal slice of the brain from baseline for TG and WT that were found to be significant at the 5% level (corrected for FDR). Red color denotes significant increase and blue denotes significant reduction in volume with respect to the baseline. The TG group shows larger areas of growth in comparison with WT. Moreover, the growth is prominent around the hippocampus, corpus callosum and thalamic region in the TG group. The continual growth around the same regions in TG from 6 months to 9 and 11 months can be seen (see Figure 2 green arrows), but at 14 months this appears to be less in comparison to the previous time points. This observation may be attributed to the sample size, which is lower than

previous time points due to natural attrition. In the WT case as shown in Figure 2 (see blue arrows), anatomical regions such as cerebral cortex show a reduction in volume whereas regions such as corpus callosum indicate a growth in this group. This observation of change in volume is different to our conclusion from label propagation analysis, where we showed the WT brains to plateau or reduce after 11 months

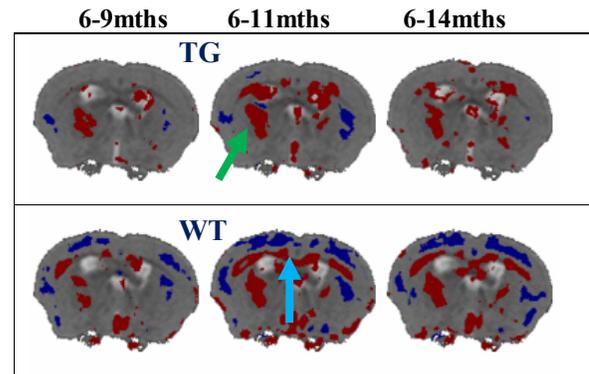


Figure 2 Longitudinal changes of TG and WT group. Red and blue indicate statistically significant growth and reduction from baseline respectively. The hippocampus, ventricles show growth in the TG group (green arrows). In the case of WT, the change appears to be growth and reduction from baseline. The cerebral cortex shows reduction in volume whereas the corpus callosum shows an increase in volume (see blue arrows).

Figure 3 shows significant clusters identified and placed within a biological context using label definitions from atlas-based segmentation. The diagram shows statistically significant growth (red) and reduction (blue) areas for the WT group from baseline to 11 months. The label definitions for cerebral cortex (green) and hippocampus (yellow) are shown for slices 58 and 60. The results from these slices may be interpreted as volume reduction in hippocampus and cerebral cortex regions from baseline to 11 months in the WT group.

5. DISCUSSION AND CONCLUSIONS

Image analysis techniques were developed to detect longitudinal changes between two groups. The methods were explored and tested using serial MR data acquired from two groups of mice: a TG model of AD and the WT background strain (C57Bl6) from which the model derives. This allowed for longitudinal analyses to be studied.

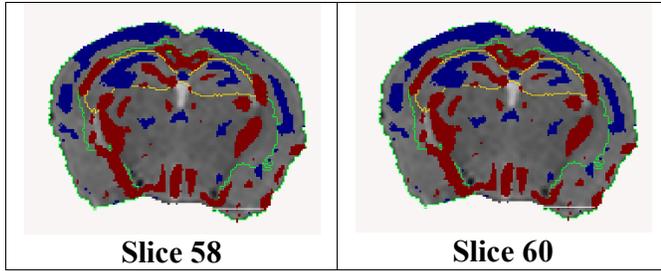


Figure 3 The significant clusters are identified and placed within a biological context using label definitions. The diagram shows statistically significant growth (red) and reduction (blue) areas for the WT group from base line to 11 months. The label definitions for cerebral cortex (green) and hippocampus (yellow) are shown for slice 58, 60. The results from these slices may be interpreted as volume reduction in hippocampus and cerebral cortex regions.

The research focused on identifying and testing methods that are likely to be suitable to detect subtle changes introduced by genetic manipulation. A key objective was to allow use of in vivo data, so paving the way to viable longitudinal studies, which offer advantages both by virtue of using each animal as its own control and have the potential to greatly reduce the numbers of individuals that must be studied to achieve significant results. The atlas based segmentation and deformation based morphometry methods were used to study the longitudinal differences between the two groups.

The growth curves in Figure 1 revealed different growth patterns for the two strains and also provided evidence for differential growth over time between different brain structures within a strain. In this case the TG model is intended to mimic the amyloid accumulation seen in human Alzheimer's disease, and so accelerated growth of the hippocampus in the TG animals is plausibly consistent with histological evidence of amyloid deposition and associated inflammation[6]. A disadvantage of basing the detection of change on anatomical labels is that the actual change in any individual case may not be uniformly distributed across the selected anatomical structures, and this can lead to a reduction in the magnitude of averaged detected differences. However, labels do have the advantage of firmly anchoring the analysis in a coherent anatomical framework.

The DBM analysis on the other hand, detects clusters which are significantly different between groups of subjects. This approach allows the cluster of voxels that show most change or difference to be identified and this is likely to achieve greater sensitivity, although at the cost of requiring a separate step to define the structural location of the detected

effect. A reassuring aspect of the current study is that the DBM analysis produced similar findings to the label propagation analysis, for example hippocampus areas were found to grow and cortical areas in the cerebral cortex were shown to reduce in volume in the WT group. The clusters of voxels identified automatically often paralleled the previously identified anatomical structures, although the boundaries for the clusters differed in detail.

Deciding which method to apply to analyse longitudinal data will largely depend on the questions addressed. However, given non-rigid registration uses a voxel-by-voxel approach, it seems logical not to constrain the analysis to predefined regions of voxels prior to testing for significant differences. However, having identified the locations of difference or change, the option to efficiently and authoritatively place the results in an anatomical context can greatly aid biological interpretation.

In conclusion, the two methods suggested gave equivalent results, with the DBM method offering more sensitivity but less anatomical specificity. Combined strategy of DBM and atlas based label propagation is a good way to analyse serial data, where significant clusters are first identified and then anchored with labels in a common reference to enhance the biological understanding.

6. REFERENCES

- [1] Ali, A. A., A. M. Dale, et al. (2005). "Automated segmentation of neuroanatomical structures in multispectral MR microscopy of the mouse brain" *Neuroimage*, 27: 425-435.
- [2] Kovacevic, N., J. T. Henderson, et al. (2005). "A Three-dimensional MRI Atlas of the Mouse Brain with Estimates of the Average and Variability,." *Cerebral Cortex* 15: 639-645.
- [3] Nieman, B. J., N. A. Bock, et al. (2005). "Magnetic resonance imaging for detection and analysis of mouse phenotype,." *NMR in Biomedicine* 18: 447-468.
- [4] Rueckert, D., L. I. Sonoda, et al. (1999). "Non-rigid Registration Using Free-Form Deformations: Applications to Breast MR Images." *IEEE Transactions On Medical Imaging* 18: 712-721.
- [5] Genovese, C. R., N. A. Lazar, et al. (2002). "Thresholding of statistical maps in functional neuro-imaging Using the False Discovery Rate." *Neuroimage* 15: 870-878
- [6] Howlett, D. R., J. C. Richardson, et al. (2004). "Cognitive correlates of A-Beta deposition in male and female mice bearing amyloid precursor protein and presenilin-1 mutant transgenes,." *Brain Research*, 1017: 130-136