An Investigation into Young and Aged Rat Brain Volume Differences by Optimized Voxel-Based Morphometry

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Introduction

Voxel-based-morphometry (VBM) has proven to be an effective and reliable tool for analysing between-group tissue composition differences in human brains (Ashburner & Friston, 2000). However, a standardized approach to using optimized VBM has yet to be developed for the rodent brain. Indeed, this is the first occasion a (modified) version of VBM has been applied to study age-induced volume changes in the rat brain. Here, we report the development of an optimized VBM protocol for the rat brain. We have applied this protocol in a study of aged versus young animals.

As an unbiased whole brain analysis technique, VBM comprises a local voxel-wise comparison of grey and white matter between two (or more) groups of subjects (Good et al., 2001). Comparing grey or white matter images at a voxelwise level requires that they first be transformed into a standard stereotactic space. Non-linear registration is used to transform the original brain images to a standard template image. Optimized VBM then averages the output to produce a study-specific template file, on which a variety of statistical tests (such as group comparisons) are carried out.

Method

Here we describe the modifications required for an optimized VBM analysis of the rat brain, by way of investigating age-induced changes in rat brain volume. Structural MRI images of 12 rats (5 young, 7 aged) were obtained using a Bruker 7 Tesla animal scanner (Bruker BioSpin, Ettlingen, Germany) then compared using FSL (FMRIB Software Library) 4.0 tools typically reserved for human brain analysis.

First, structural images were skull-stripped manually, since FSL's own brain extraction tool failed to satisfactorily remove the brains' surrounding tissue. Next, the images were aligned to the same stereotactic space through a generic template image constructed from all the original data. The resulting images were then averaged to create a study-specific template, to which the native images were re-aligned. The revised segmented images were modulated to adjust for local change, thus preserving the total amount of grey matter signal in the normalized segments. The images were then smoothed using a more lenient range of smoothing kernels than would normally be applied in a human study (sigma = 0.1, 0.8mm). Finally, permutation-based non-parametric testing was carried out, forming clusters and testing for significance at $p < 0.1$.

Results and Conclusions

Our study is exceptional for several reasons. Firstly, our investigation showed decreases in rat brain volume with age. Our optimized VBM analysis produced a statistical map which revealed areas of grey matter concentration which differed significantly between our two rat groups. Despite our limited sample size, age-induced grey matter volume decreases were found in areas CA1 and CA2 of the hippocampus, primary and secondary motor cortices, primary visual cortex, and cingulate cortex. Secondly, we have successfully shown that VBM can be tailored for use with volumetric analyses in non-human experiments. In summary, this unique study is one of the first of its kind to describe volume changes that occur with age in the rodent brain, using analysis tools designed exclusively for examining human MRI images.

References:

1. Ashburner, J. and Friston, K.J. (2000) Voxel-Based Morphometry: The Methods. *Neuroimage* **11:** 805 821.
2. Good, C.D., Johnsrude, I.S., Ashburner, J., Henson, R.N.A., Friston, K.J. and Frackowiak, R.S.J. (2001) A Voxel-Based Morphometric Study of Ageing in 465 Normal Adult Brains. *Neuroimage* **14:** 21-36.
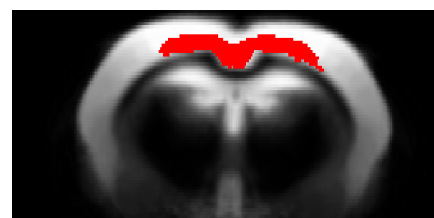3. FMRIB Software Library tools freely available at http://www.fmrib.ox.ac.uk/fsl

Acknowledgments

Images

(a): Slice 20/54 of grey matter template overlayed with statistical map of significant concentration differences

(b): Slice 27/54 of grey matter template overlayed with statistical map of significant concentration differences

(c): Slice 34/54 of grey matter template overlayed with statistical map of significant concentration differences