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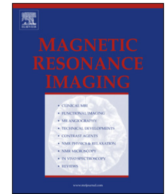

Voxel-based morphometry with templates and validation in a mouse model of Huntington's disease

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ABSTRACT

Despite widespread application to human imaging, voxel-based morphometry (VBM), where images are compared following grey matter (GM) segmentation, is seldom used in mice. Here VBM is performed for the R6/2 model of Huntington's disease, a progressive neurological disorder. This article discusses issues in translating the methods to mice and shows that its statistical basis is sound in mice as it is in human studies. Whole brain images from live transgenic and control mice are segmented into GM maps after processing and compared to produce statistical parametric maps of likely differences. To assess whether false positives were likely to occur, a large cohort of ex vivo magnetic resonance brain images were sampled with permutation testing. Differences were seen particularly in the striatum and cortex, in line with studies performed ex vivo and as seen in human patients. In validation, the rate of false positives is as expected and these have no discernible distribution through the brain. The study shows that VBM successfully detects differences in the Huntington's disease mouse brain. The method is rapid compared to manual delineation and reliable. The templates created here for the mouse brain are freely released for other users in addition to an open-source software toolbox for performing mouse VBM.
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1. Introduction

Huntington's disease (HD) is an inherited neurological condition characterised by chorea, progressive cognitive deficits and behavioural changes [1]. No cure has yet been found and the condition is invariably fatal. The discovery that the disease results from a single defective gene has led to the development of several transgenic animal models in mice [2–4] of which the most widely used in HD research is the R6/2 mouse which contains a fragment of the gene responsible for the disease in humans [5].

MRI studies were first used simply to identify in vivo the characteristic features already known from post mortem studies of established HD, such as the enlarged lateral ventricles seen with atrophy of the caudate nucleus [6]. The development of sophisticated automated image analysis algorithms, however, has led to a wide number of studies conducted to identify pathology across the brain in established HD but more importantly in preclinical patients, before symptoms are apparent [7–12]. The most useful animal models will recapitulate features seen in human patients, and this includes an MRI phenotype. In the R6/2 mouse, we have previously demonstrated patterns of atrophy of structures known to be affected in humans [13,14].

R6/2 mice with a CAG repeat length of 250 (as used in the present study) have brain pathology characterised by abnormal aggregates of protein [15] and changes in synaptic plasticity [16] by three weeks of age. They show progressively impaired motor [17] and cognitive [18] function from around 6 weeks of age. The mice stop growing around 10-12 weeks of age and then start to lose weight. They typically die prematurely at around 24 weeks of age [17]. These mice have also been shown to have disintegrated circardian rhythms [19] and cardiac dysfunction [20].

Voxel-based morphometry (VBM) describes the automated process of analysing morphological differences between images of brains by performing voxel-wise statistics [21]. Images are registered into the same stereotactic space and segmented into images of tissue classes, with four commonly represented: grey matter (GM), white matter (WM) and cerebrospinal fluid (CSF) and 'everything else'.

Typically the GM segmented image will form the basis of statistical tests used to assess hypotheses concerning the data in a statistical parametric map (SPM) which is then thresholded to identify localised regions where (e.g.) a null hypothesis can be rejected at a particular level.

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Since its introduction in 1995 [22], the technique has seen rapidly growing usage: a simple keyword search for the technique on the PubMed repository (http://ncbi.nlm.nih.gov) reveals 260 papers from 2000-2005, 1,152 from 2006 to 2010 and 454 papers in 2011 alone. The technique can be widely applied to studies in neurology, psychiatry and psychology to identify structural differences in healthy as well as abnormal brains.

Despite this, the method has seen little application to the mouse brain. Since VBM is still the most common analysis technique used for human brain morphometry, it is surprising that more groups performing translational research with mouse brain imaging are not employing it. We speculate that the reasons for its lack of use are the perceived complexity of the method coupled with the lack of software tailored to non-human brains. After considerable development for human use [23–26] the major software packages used, SPM (Wellcome Trust Centre for Neuroimaging, University College London, UK) and FSL (FMRIB, University of Oxford), are pre-configured with default settings that do not work for non-human brains. Simplified interfaces that have led to the huge success of these packages in human studies, used in the majority of papers cited above, are obstructive for other species as they conceal many important scaling parameters. In an effort to boost the usage of VBM in the mouse brain, we have released a software toolbox for SPM (SPMMouse) including our templates so that mouse brain analysis can be performed as readily as human studies.

In this study, we apply VBM as described to in vivo images of the R6/2 transgenic mouse brain with wildtype (WT) controls. To our knowledge this is the first application of VBM as described to in vivo data from a HD mouse model.

To establish whether the statistical basis of this method is sound, we took a larger cohort of brains from an open-access library of ex vivo mouse brain images. We randomly permuted labels of these datasets as WT or transgenic to see how often the method produced false positive results compared to predictions by chance. Additionally, we kept track of where these false positive results were discovered so that we could identify whether type I errors in this technique are biased towards particular brain regions. A larger pool of datasets was used for this so that we were more likely to identify subtle effects.

We have included in the methods section below a brief discussion of the purpose of each step in the VBM pipeline and our rationale for selecting the parameters we have used so that readers unfamiliar with the VBM process can understand the choices that we have made.

2. Methods

2.1. Image acquisition

2.2. Animals (in vivo)

Six WT control mice (aged 14±1 weeks), and six R6/2 transgenic mice with 250 abnormal CAG repeats (age 15±1 weeks) were imaged for this study. There was no significant difference in age between groups and at this age a phenotype is clearly established. To put this age into context more than 95% of this line of R6/2 mice will die as a consequence of having this gene by 20 weeks of age [17].

Animals were anaesthetised with isoflurane (1–2% in 1l/min O_2). Respiration rate was monitored using a respiratory pillow to control anaesthetic depth (SA Instruments Inc., Stony Brook, NY, USA). Core body temperature was measured with a rectal probe and maintained in the normal range using a flowing water heated blanket.

All procedures were approved by a local ethical review committee and were performed in accordance with the UK Animals (Scientific Procedures) Act 1987.

2.2.1. Animals (ex vivo)

For the statistical validation, ex vivo images were taken from the Cambridge HD public library [27]. The brain images used were from mice aged 18 weeks of mixed sexes with 42 WT brains and 42 R6/2 mice with 250 CAG repeats used and the animals originate from the same colony as the in vivo mice used here.

2.2.2. Imaging parameters

Images were acquired at 4.7 T in vivo using a rapid-acquisition with relaxation enhancement (RARE) sequence (TR/TE_{eff} 3500/32ms, ETL 16 FOV 25.6×19.2×10.0mm^3, matrix 256×192×100, spatial resolution 100μm in 1h33m). Images were acquired and reconstructed using ParaVision 4.0 with a Bruker BioSpec 47/40 system (Bruker Inc., Ettlingen, Germany). An actively decoupled quadrature-mode mouse brain surface coil (model T9788) was used for signal reception and a 72-mm birdcage coil (model T5346) was used for transmission, both supplied by Bruker.

The ex vivo protocol is fully described elsewhere [27], a RARE sequence is also used though higher resolution (70 μm isotropic) and 4 averages were used for greater signal to noise ratio.

2.3. Pre-processing

The steps involved in VBM are illustrated in Fig. 1 and discussed in more detail below.

Before voxelwise statistics can be calculated, images have to be registered into the same stereotactic space and segmented to give tissue probabilities. Affine registration refers to a global geometric transformation applied identically to each part of the image which preserves parallel lines. Non-linear registration provides a finer match between images by allowing local transformations that adjust different parts of the image in different ways. Segmentation based on intensities is unreliable without taking account of coil inhomogeneity [28], and registration is usually addressed with first an affine step followed by a finer non-linear step. These are considered in more detail below.

2.3.1. Affine registration

For human studies, Tailarach or MNI coordinates are often used for the analysis and presentation of results, see e.g. [29]. The SPM software is supplied with templates and atlases in MNI space. For rodent studies, the most common coordinates used are those of the Franklin and Paxinos atlas, based on the bregma point of the skull [30]. For the mouse brain, we calculated the distribution of affine parameters from a large number of ex vivo scans [13] and this is included in the SPMMouse package. Taking advantage of this prior knowledge has been shown to improve the quality of affine registration [31]. In addition, the matrices encoded in these headers are aligned with the coordinates from the Franklin and Paxinos atlas for easy reference to stereotactic space when reading and reporting results from the software. The mouse brain templates included in SPMMouse for initial alignment and/or registration were derived as a minimum-deformation atlas [32] in our previously published VBM study. The mean and standard deviation of the rigid transformation invariant parameters (i.e. scales and shearing values) in the polar decomposition form used by SPM were calculated and stored as a mean and covariance matrix for use in SPM.

The mean and standard deviation of the affine parameters of the brains to the atlas is 1.00±0.01, 1.00±0.02, 1.00±0.02 for scaling in left-right (x), anterior-posterior (y), inferior-superior (z) directions and 0.00±0.02, 0.01±0.04, 0.02±0.01, for shearing matrix values xy, xz and yz. In comparison with the human brain priors for MNI templates in SPM based on 227 scans, these values are about a quarter as large (the human standard deviations are 3–4% for each axis).

2.3.2. Non-linear registration and segmentation

The registration step used routinely in SPM uses approximately 1,000 parameters. These are enough to correct for overall brain

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