Journal of Neuroscience Methods 267 (2016) 62–73

Contents lists available at ScienceDirect

Journal of Neuroscience Methods

journal homepage: www.elsevier.com/locate/jneumeth

Semi-automated registration-based anatomical labelling, voxel based morphometry and cortical thickness mapping of the mouse brain

Marco Pagani[a,b,*,1], Mario Damiano[a,1], Alberto Galbusera[a], Sotirios A. Tsaftaris[c,d,2], Alessandro Gozzi[a,*,2]

[a] Functional Neuroimaging Laboratory, Center for Neuroscience and Cognitive Systems, Istituto Italiano di Tecnologia, Rovereto (TN), Italy
[b] Centro Interdipartimentale Mente/Cervello (CIMeC)—University of Trento, Rovereto (TN), Italy
[c] Institute for Digital Communications, School of Engineering, University of Edinburgh, Thomas Bayes Road, EH9 3FG, Edinburgh, UK
[d] IMT Institute for Advanced Studies, Lucca (LU), Italy

HIGHLIGHTS

- We describe registration-based methods for mouse brain morphoanatomical imaging.
- Detailed workflows for anatomical labelling, voxel based morphometry and cortical thickness are reported.
- The same preprocessing can be applied to map multiple complementary anatomical readouts.
- The present work may help to promote the use of rodent morphoanatomical imaging.

ARTICLE INFO

Article history:
Received 5 May 2015
Received in revised form 7 April 2016
Accepted 8 April 2016
Available online 11 April 2016

Keywords:
Voxel based morphometry
Cortical thickness
Anatomical labelling
Mouse brain
MRI

ABSTRACT

Background: Morphoanatomical MRI methods have recently begun to be applied in the mouse. However, substantial differences in the anatomical organisation of human and rodent brain prevent a straightforward extension of clinical neuroimaging tools to mouse brain imaging. As a result, the vast majority of the published approaches rely on tailored routines that address single morphoanatomical readouts and typically lack a sufficiently-detailed description of the complex workflow required to process images and quantify structural alterations.
New method: Here we provide a detailed description of semi-automated registration-based procedures for voxel based morphometry, cortical thickness estimation and automated anatomical labelling of the mouse brain. The approach relies on the sequential use of advanced image processing tools offered by ANTs, a flexible open source toolkit freely available to the scientific community.
Results: To illustrate our procedures, we described their application to quantify morphological alterations in socially-impaired BTBR mice with respect to normosocial C57BL/6J controls, a comparison recently described by us and other research groups. We show that the approach can reliably detect both focal and large-scale grey matter alterations using complementary readouts.
Comparison with existing methods: No detailed operational workflows for mouse imaging are available for direct comparison with our methods. However, empirical assessment of the mapped inter-strain differences is in good agreement with the findings of other groups using analogous approaches.
Conclusion: The detailed operational workflows described here are expected to help the implementation of rodent morphoanatomical methods by non-expert users, and ultimately promote the use of these tools across the preclinical neuroimaging community.

1. Introduction

A deep understanding of the genetic, physiological and anatomical underpinnings of brain disease is essential for the development of improved therapies. A milestone towards this goal is the generation of genetically modified mouse lines that recapitulate targeted genetic mutations in experimentally controlled studies. Genetically modified mouse lines permit to relate genetic mutations

* Corresponding authors at: Functional Neuroimaging Laboratory, Center for Neuroscience and Cognitive Systems, Istituto Italiano di Tecnologia, Corso Bettini, 31 – 38068 Rovereto (TN), Italy.
E-mail addresses: marco.pagani@iit.it (M. Pagani), alessandro.gozzi@iit.it (A. Gozzi).
[1] The two authors equally contributed to this work.
[2] The two authors share senior authorship.

http://dx.doi.org/10.1016/j.jneumeth.2016.04.007
0165-0270/

to clinically relevant endophenotypes without the complexity of genetic heterogeneity and the uncontrolled impact of gene–gene and gene–environment interactions in adult human populations (Nestler and Hyman, 2010).

Magnetic resonance imaging (MRI) methods offer a privileged point of view to study genetically altered mouse models of neuropsychiatric disorders in many respects. First, the use of comparable imaging readouts in men and mice permits a cross-species comparison of brain endophenotypes of translational relevance, thus enhancing the transfer of information from and to the clinic. At the same time, MRI readouts can also be employed to assess the extent to which mouse models of central nervous system pathology replicate neuroimaging findings observed in clinical populations, informing preclinical researchers on the translational validity of these models. Moreover, high resolution morphometric MRI, achievable at ultra-high field strength or in ex vivo formalin-fixed samples (Lerch et al., 2012; Tucci et al., 2014) can be employed to obtain a fine-grain assessment of structural brain alterations that could serve as a convenient surrogate for labour intensive manual morphometric measurements in ex vivo brain slice preparations, with the additional advantage of being non-invasive and multi-dimensional.

Structural MRI based imaging methods – such as voxel based morphometry (VBM) of grey matter (GM), cortical thickness mapping and anatomical labelling – have been widely employed to study brain morphology in human populations (Mueller et al., 2012). The application of analogous readouts to map genetically determined brain alterations in transgenic mouse lines has been recently proposed, an effort collectively referred to as MRI phenotyping (Borg and Chereul, 2008; Johnson et al., 2007; Lerch et al., 2011a). Recent improvements in MRI sequences and hardware, together with the development of fixation protocols for ex vivo imaging of stained brain specimens (Lerch et al., 2012), have made it possible the acquisition of artefact-free and high resolution – with a voxel size less than 80 μm – mouse brain volumes even at relatively low magnetic field strengths. These efforts have resulted in the publication of several examples or the application of morphoanatomical imaging to transgenic mouse models (Lerch et al., 2008; Sawiak et al., 2009; Xie et al., 2010; Yushkevich et al., 2006).

The development of standardised preprocessing and analytical pipelines for human imaging data, and their implementation in popular software toolkits such as such as FMRIB Software Library (FSL) (Jenkinson et al., 2012), Statistical Parametric Mapping (SPM) (Friston et al., 1994) and Advanced Normalization Tools (ANTs) (Avants et al., 2009), have been instrumental to the widespread use of MRI in human brain research. However, substantial differences in the dimensions and anatomical organisation of the human and rodent brain prevent a straightforward extension of these tools to morphoanatomical mouse brain mapping. As a result, several research groups have developed tailored procedures for the preprocessing and analyses of morphoanatomical brain MRI readouts in mouse models (Badea et al., 2012; Borg and Chereul, 2008; Delatour et al., 2006; Johnson et al., 2007; Lee et al., 2010; Lerch et al., 2011a; Nieman et al., 2005; Sawiak et al., 2009; Sawiak et al., 2013). However, the vast majority of the published approaches typically address single morphoanatomical readouts (e.g. VBM *or* anatomical labelling *or* cortical thickness), and lack a detailed description of the complex workflow and computational parameters required to process, analyse and quantify structural MRI alterations, thus complicating the implementation of these procedures by non-expert users.

To begin to address these issues, here we provide a detailed methodological description of a semi-automated operational workflow for VBM, cortical thickness estimation and automated anatomical mapping of the mouse brain. To simplify and streamline operations, we based image processing mainly on ANTs (Avants et al., 2009), a flexible and powerful open source toolkit freely available to the scientific community. Importantly, our approach has been recently applied by our research group to map fine-grain brain anatomy alterations in different mutant mouse lines (Dodero et al., 2013; Lassi et al., 2015; Minervini et al., 2014; Sannino et al., 2014; Tucci et al., 2014) and to describe large-scale networks of anatomical covariance between grey matter regions in wild-type mice (Pagani et al., 2016), with excellent agreement between MRI and manual morphometric measurements (Sannino et al., 2014), exhibiting corresponding morphoanatomical features in mice and reference clinical populations (Cutuli et al., 2016; Tucci et al., 2014). Below, we provide a detailed description of our procedural workflow and show its capabilities by describing its application to quantify morphological alterations in socially-impaired BTBR T+Itpr3tf/J mice with respect to normo social C57BL/6J controls (Dodero et al., 2013; Squillace et al., 2014), a comparison that has been recently described by our research group (Dodero et al., 2013) and others (Ellegood et al., 2013), thus permitting an empirical cross-laboratory assessment of the validity of our findings.

2. Materials and methods

2.1. Ethical statement

All in vivo studies were conducted in accordance with the Italian law – D.L. no. 116, 1992, Ministero della Sanità, Roma – and following the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The animal research protocol was approved by the Animal Care Committee of the Istituto Italiano di Tecnologia (Permit Date 07-2012). All surgical procedures were performed under deep anaesthesia.

2.2. Sample preparation and MR acquisition

High-resolution morphoanatomical T2-weighted MR imaging of mouse brains was performed in paraformaldehyde (4% PFA; 100 ml, Sigma, Milan) fixed specimens, a procedure employed to obtain high-resolution images with negligible confounding contributions from physiological or motion artefacts (Cahill et al., 2012). Sample preparation and MRI acquisition of BTBR T+Itpr3tf/J (BTBR) and C57BL/6J (B6) mice has been recently described in previous work (Dodero et al., 2013; Sforazzini et al., 2014a,b) and is briefly summarised here. Male BTBR ($N = 9$, 15–26 weeks old) and age-matched control B6 ($N = 9$) mice were deeply anaesthetized with an intraperitoneal Avertin injection (375 mg/kg, Sigma, Milan) and their brains were perfused in situ via cardiac perfusion. The perfusion was performed with phosphate buffered saline followed by paraformaldehyde (4% PFA; 100 ml). Both perfusion solutions were added with a Gadolinium chelate (Prohance, Bracco, Milan) at a concentration of 10 and 5 mM, respectively, to shorten longitudinal relaxation times (Lerch et al., 2012).

A multi-channel 7.0 Tesla MRI scanner (Bruker Biospin, Milan) was used to acquire anatomical images of the brain, using a 72 mm birdcage transmit coil, a custom-built saddle-shaped solenoid coil for signal reception, and the following imaging parameters: 3D RARE spin-echo sequence, TR = 550 ms, TE = 33 ms, RARE factor = 8, echo spacing 11 ms, matrix size of 192 × 170 × 170 and voxel size of 0.09 mm (isotropic), with a total acquisition time of 4 h and 25 min.

2.3. Image preprocessing and analysis

A detailed description of the image processing workflow employed to create a study based template, to estimate cortical thickness, and to perform automated anatomical labelling and VBM is reported below for structural images acquired at 7 Tesla. We