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Structural covariance networks in the mouse brain

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ABSTRACT

The presence of networks of correlation between regional gray matter volume as measured across subjects in a group of individuals has been consistently described in several human studies, an approach termed structural covariance MRI (scMRI). Complementary to prevalent brain mapping modalities like functional and diffusion-weighted imaging, the approach can provide precious insights into the mutual influence of trophic and plastic processes in health and pathological states. To investigate whether analogous scMRI networks are present in lower mammal species amenable to genetic and experimental manipulation such as the laboratory mouse, we employed high resolution morphoanatomical MRI in a large cohort of genetically-homogeneous wild-type mice (C57Bl6/J) and mapped scMRI networks using a seed-based approach. We show that the mouse brain exhibits robust homotopic scMRI networks in both primary and associative cortices, a finding corroborated by independent component analyses of cortical volumes. Subcortical structures also showed highly symmetric inter-hemispheric correlations, with evidence of distributed antero-posterior networks in diencephalic regions of the thalamus and hypothalamus. Hierarchical cluster analysis revealed six identifiable clusters of cortical and sub-cortical regions corresponding to previously described neuroanatomical systems. Our work documents the presence of homotopic cortical and subcortical scMRI networks in the mouse brain, thus supporting the use of this species to investigate the elusive biological and neuroanatomical underpinnings of scMRI network development and its derangement in neuropathological states. The identification of scMRI networks in genetically homogeneous inbred mice is consistent with the emerging view of a key role of environmental factors in shaping these correlational networks.

1. Introduction

Correlation analyses of magnetic resonance imaging (MRI) data have produced evidence of integrated structural and functional networks of brain regions, thus providing information on brain organization beyond the segregated local properties classically revealed by univariate methods (Bullmore and Sporns, 2009). Complementary to networks mapped with resting state functional MRI and white matter pathways reconstructed with diffusion weighted imaging, large scale networks of structural covariance measured with MRI (scMRI) represent an additional valuable source of information about inter-regional connectivity (Alexander-Bloch et al., 2013b). Specifically, this approach permits to study the extent to which inter-individual differences in regional structures are coherently organized within networks of gray matter volumes or cortical thickness that emerge across a population of individuals (Alexander-Bloch et al., 2013a; Evans, 2013).

Anatomical covariance mapping with MRI has provided valuable insight into the structural organization of the brain. Recent scMRI studies have substantially expanded and corroborated early post-mortem evidence of anatomical covariance between regions of the visual and motor systems (Andrews et al., 1997; White et al., 1997) by highlighting robust correlations between inter-hemispheric homotopic regional gray matter volume in motor, somatosensory and associative cortical regions of the human brain (Mechelli et al., 2005; Zielinski et al., 2010). Similarly, limbic cortical and non-cortical regions have been shown to be part of more distributed covariance network that encompass wide portion of prefrontal and temporal regions (Bernhardt et al., 2013).

Anatomical covariance mapping has also offered initial insights into the abnormal structural organization of networks in brain disorders. For example, reduced extension of the right anterior insular network has been reported in patient diagnosed with autism spectrum disorder (Zielinski et al., 2012b). Analogously, basal ganglia, parietal and fronto-temporal scMRI networks exhibit reduced gray matter content in schizophrenic patients compared to healthy controls (Xu et al., 2009), and decreased inter-hemispheric correlations between postcentral gyrus and parietal lobule have been observed in patients diagnosed with Alzheimer's disease (He et al., 2008).

Despite the increasing interest in scMRI and its emerging use to investigate the trophic development of gray matter, fundamental questions regarding the origin and significance of these correlative networks

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remain unanswered. For example, recent evidence has linked genetic polymorphisms with the development of specific functional and anatomical networks (Pezawas et al., 2005), however, the genetic determinants underlying the emergence of these networks remain poorly understood. Moreover, although correlations between cortical gray matter thickness and structural connectivity have been described (Lerch et al., 2006), with recent estimates suggesting that white matter MRI connectivity explains approximately 35–40% of the thickness correlations across the cerebral cortex (Gong et al., 2012), whether anatomical covariance requires intact axonal connectivity, or can develop in the face of altered connectional substrates like in the case of congenital callosal alterations or white matter abnormalities (Sforazzini et al., 2014a; Tyszka et al., 2011), remains to be determined. Finally, although both genetic and environmental factors have been identified to play a role in shaping these networks (Rimol et al., 2010; Schmitt et al., 2009; Schmitt et al., 2008), the relative contribution of these components is poorly understood and it is not clear to what extent covariance is a causal result of genetic influence, development and aging, or experience-related plasticity (Evans, 2013).

The investigation of networks of anatomical covariance in laboratory mice – where a wide repertoire of genetic, molecular and cellular manipulations can be readily implemented – could complement human research on the emergence of gray matter covariance networks, and generate novel hypothesis about the etiopathological origin of aberrant scMRI findings in human brain diseases (Alexander-Bloch et al., 2013a). In the present work, we used high resolution structural imaging and voxel-based morphometry (Dodero et al., 2013; Sannino et al., 2014) to probe the presence of cortical and subcortical networks of anatomical covariance in the mouse brain. To this end, scMRI mapping was carried out in a large cohort ($N = 53$) of genetically-homogeneous inbred C57Bl6/J mice, thus permitting to assess the emergence of these networks under controlled genetic and environmental conditions, an essential prerequisite for the implementation of scMRI approaches in transgenic models. Our result demonstrates the presence of robust homotopic scMRI gray matter networks in cortical and sub-cortical regions of the mouse brain, paving the way to the application of interventional approaches to study the physiological and pathological effectors of this phenomenon.

2. Materials and methods

2.1. Ethical statement

All research involving animals was carried out in accordance with the European directive 86/609/EEC governing animal welfare and protection, which is acknowledged by the Italian Legislative Decree 116–27 January 1992, and following the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. Animal research protocols were also reviewed and consented by the Animal Care Committee of the Istituto Italiano di Tecnologia (permit date 07-2012).

2.2. Sample preparation and image data acquisition

High-resolution morphoanatomical T2-weighted MR imaging of C57Bl6/J male mouse brains ($n = 53$) was performed in paraformaldehyde (4% PFA; 100 ml) fixed specimens, a procedure employed to obtain high-resolution images with negligible confounding contributions from physiological or motion artifacts (Cahill et al., 2012). Standard sample preparation and MRI acquisition have been recently described (Dodero et al., 2013; Sforazzini et al., 2014a) and are reported below to provide a comprehensive description of all the experimental procedures involved. Briefly, male B6 mice were deeply anesthetized with an intraperitoneal Avertin injection (375 mg/Kg) 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, Italy) at a concentration of 10 mM and 5 mM, respectively, to shorten longitudinal relaxation times.

A four-channel 7.0 Tesla MRI scanner (Bruker Biospin, Milan, Italy) 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: FLASH 3D sequence with TR = 17 ms, TE = 10 ms, $\alpha = 30°$, matrix size of 260 × 160 × 180, field of view of 1.83 × 1.26 × 1.26 cm, voxel size of 90 μm^3 (isotropic).

2.3. Image data preprocessing and VBM

VBM of gray matter was performed using ANTs (Avants et al., 2010), a flexible open source toolkit widely adopted for mice and human studies. Nonlinear registration-based VBM procedure on the mouse brain has been thoroughly described in a previous methodological study and it is only briefly reported herein (Pagani et al. under review). Each high-resolution T2W image was corrected for intensity non-uniformity and skull stripped to remove extra brain tissue. A study based template was created by aligning pre-processed images to a common reference space using affine and diffeomorphic registrations. After registering individual images to the study based template, spatially normalized images were segmented to calculate tissue probability maps. The separation of the different tissues is improved by initializing the process with the probability maps of the study based template previously segmented. The Jacobian determinants of the deformation field were extracted and applied to modulate the gray matter probability maps calculated during the segmentation. This procedure permits the analysis of gray matter probability maps in terms of local volumetric variation instead of tissue density. Jacobian determinants were also normalized by the total intracranial volume (range 390–531 mm^3) to account for inter-subject variability in total brain volume (Bassett et al., 2008; Zielinski et al., 2012a). The resulting modulated gray matter probability maps were then smoothed using a Gaussian kernel with a sigma of three voxel width.

2.4. Gray matter variance map

Ninety-nine neuroanatomical (68 cortical and 31 extracortical) volumes from previously published parcellated reference neuroanatomical atlases of the mouse brain (Dorr et al., 2008; Ullmann et al., 2013) were registered to each image. This procedure standardizes the location and size of each brain region, thus avoiding operator-dependent bias related to manual anatomical recognition and improves replicability of findings. We used this method also to identify VOIs for agglomerative hierarchical clustering and for seed-based correlation mapping (described below). The variance of gray matter volumes in each neuroanatomical volume was then calculated across subjects, yielding a region-by-region map of the gray matter variability of our inbred mice.

2.5. Agglomerative hierarchical clustering of the correlation matrix

Agglomerative hierarchical clustering is a bottom-up data driven approach that aims to find clusters based on a similarity measure and has the advantage of requiring no a priory information on the number of cluster to be computed. We used the R package 'gplots' (http://cran.r-project.org/web/packages/gplots/index.html) to calculate the correlation matrix of the mean gray matter volumes of major neuroanatomical volumes of interest (VOIs) and to perform the agglomerative hierarchical cluster analysis adopting Euclidean distance as similarity measure (Schmitt et al., 2008). Color coding to highlight the diverging nature of the correlation matrix was obtained using the R package 'RColorBrewer' (http://cran.r-project.org/web/packages/RColorBrewer/index.html). A dendrogram was also displayed both to visualize the degree of similarity between the VOIs – where similar vectors of correlation are

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