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Segmentation of the C57BL/6J mouse cerebellum in magnetic resonance images

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ABSTRACT

The C57BL mouse is the centerpiece of efforts to use gene-targeting technology to understand cerebellar pathology, thus creating a need for a detailed magnetic resonance imaging (MRI) atlas of the cerebellum of this strain. In this study we present a methodology for systematic delineation of the vermal and hemispheric lobules of the C57BL/6J mouse cerebellum in magnetic resonance images. We have successfully delineated 38 cerebellar and cerebellar-related structures. The higher signal-to-noise ratio achieved by group averaging facilitated the identification of anatomical structures. In addition, we have calculated average region volumes and created probabilistic maps for each structure. The segmentation method and the probabilistic maps we have created will provide a foundation for future studies of cerebellar disorders using transgenic mouse models.

Introduction

The cerebellum is involved in motor control and motor learning, especially in the coordination of body position, limb movement, and visual input (Glickstein, 2007). Cerebellar lesions in humans result in a wide variety of syndromes, generally characterized by nystagmus, intention tremor, and appendicular and truncal ataxia (Donaghy, 2009). The mouse cerebellum has become the model of choice for investigation of cerebellar disorders because of the availability of relevant mouse mutants and, more recently, the availability of gene targeting technology (Sillitoe et al., 2012). In particular, the C57BL mouse has become the focus of attempts to study the role of genes in the development and function of the cerebellum. This approach has been further facilitated by the website presenting the expression of over 20,000 genes in the mouse brain developed by Allen Brain Institute.

The anatomy of the C57BL mouse cerebellum is well characterized and several histology-based atlases have used cytoarchitectonic and chemoarchitectonic features to establish structural delineations in individual brains (Dong, 2008; Franklin and Paxinos, 2008; Hof et al., 2000; Watson and Paxinos, 2010). Histological atlases are typically based on a single specimen and therefore do not capture the anatomical variability in the cerebellum. In contrast, MRI permits the registration of multiple data sets and has the potential to assess anatomical variability (Dorr et al., 2008; Kovacevic et al., 2005; Ma et al., 2005). However, existing MRI-based atlases have typically identified five or fewer segmented cerebellar structures, limiting the level at which statistical and computational comparisons between individuals or groups can be performed. MRI also permits the examination of the cerebellar anatomy in vivo,

Abbreviations: MRI, Magnetic resonance imaging; prf, Primary fissure; pcuf, Preculminate fissure; pcn, Precentral fissure; psf, Superior fissure; ppf, Prepyramidal fissure; sf, Secondary fissure; plf, Posterolateral fissure; icf, Intercrural fissure; apmf, Ansoparamedian fissure; 1Cb, Lobule 1; 2Cb, Lobule 2; 3Cb, Lobule 3; 4/5Cb, Lobules 4/5; 6Cb, Lobule 6; 7Cb, Lobule 7; 8Cb, Lobule 8; 9Cb, Lobule 9; 10Cb, Lobule 10; Sim, Simple lobule; Crus 1, Crus 1 of the ansiform lobule; Crus 2, Crus 2 of the ansiform lobule; PM, Paramedian lobule; Cop, Copula of the pyramis; PFl, Paraflocculus; Fl, Flocculus; Lat, Lateral cerebellar nucleus; LatPC, Lateral cerebellar nucleus, parvicellular part; Med, Medial cerebellar nucleus; MedL, Medial cerebellar nucleus, lateral part; MedDL, Medial cerebellar nucleus, dorsolateral protuberance; IntA, Interposed cerebellar nucleus, anterior; IntP, Interposed cerebellar nucleus, posterior; IntPPC, Interposed cerebellar nucleus, posterior parvicellular part; IntDL, Interposed cerebellar nucleus, dorsolateral hump; DC, Dorsal cochlear nuclei; VCA, Ventral cochlear nuclei, anterior part; VCP, Ventral cochlear nuclei, posterior part; scp, Superior cerebellar peduncle; xscp, Decussation of the superior cerebellar peduncle; mcp, Middle cerebellar peduncle; icp, Inferior cerebellar peduncle; das, Dorsal acoustic stria; SMV, Superior medullary velum; vsc, Ventral spinocerebellar tract.

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thereby facilitating examination of longitudinal changes in cerebellar structure over time.

In this paper we present a detailed protocol for segmenting the cerebellum on high-resolution MRI, and we offer an atlas of the ex vivo cerebellum of the C57BL/6J mouse. In addition, we applied the segmentation to a model created from 18 brains and assessed the normal variability in the anatomical structures of the C57BL/6J cerebellum.

Materials and methods

C57BL/6J mouse brain preparation and magnetic resonance imaging

Eighteen animals (male, 12 weeks old) were perfused and fixed with 4% paraformaldehyde and 0.1% Magnevist® (gadopentetate dimeglumine, Bayer HealthCare Pharmaceuticals Inc., Wayne, NJ, USA) in phosphate buffer (PB). Brains were extracted and incubated in 0.1% Magnevist/PB for 4 days, placed in Fomblin (Solvay Solexis, Milan, Italy) and imaged on a 16.4 T (89 mm) Bruker micro-imaging system (Bruker Biospin, Karlsruhe, Germany) using a 15 mm SAW coil (M2M Imaging, USA). MRI data were acquired using a 3D gradient echo sequence with a repetition time = 50 ms, echo time = 12 ms, flip angle = 30°, 82 kHz spectral bandwidth, FOV = 2.1 × 1.5 × 0.75 cm, matrix = 700 × 350 × 250, and 8 averages, resulting in a total acquisition time of 5 h and 15 min, to produce T_1/T_2^*-weighted images at 30 μm isotropic resolution.

Model creation

Images were placed in the Waxholm stereotaxic coordinate space (Johnson et al., 2010) and a symmetric model was created using a recursive non-linear hierarchical fitting strategy similar to that employed by Fonov et al. (2011). The final fitting step used a nonlinear transformation with a step size of 30 μm. Interpolation resulted in a model with 15 μm^3 isotropic voxels.

Segmentation

The major anatomical features of the cerebellum were primarily identified on the coronal slices of the model by a single expert anatomist (CW). Anatomical features were mapped on the basis of differences in signal intensity and/or their location in reference to cerebellar fissures, with constant reference to the histological atlas of Franklin and Paxinos (2008). Structures were then partitioned using a vector-based segmentation via a Cintiq tablet (Wacom Company, Ltd). The complete data set was then exported to Amira (Visage Imaging, Inc.) where structural boundaries were checked in the other two orthogonal planes by JFPU and CW. The nomenclature and abbreviations used here were taken from Franklin and Paxinos (2008) and the color palette for cerebellar structures is based on that used in the BrainNavigator (Elsevier Inc.) system. Finally, smoothed three-dimensional surface reconstructions were created in Amira.

Construction of probabilistic maps

A probabilistic model was created using the same method that was previously employed to create the average ICBM152 model of the human brain (Collins et al., 1995). The segmented structures from the average model were nonlinearly transformed back to native space and a lower order nonlinear native space to model space transform was then applied. In our case this was a grid transform with a step size of four times the voxel size of the model (60 μm). We then assessed the probability of a particular voxel in Waxholm space being occupied by the structure of interest using the number of brains from the 18 datasets in which this was the case. A probability of 1 (100%) for a given voxel signifies that all individual segmentations in the 18 brains overlap at that voxel.

Results

The cerebellum was segmented into major regions and subregions and the average volume for each structure was computed (Table 1). While the boundaries of the major cerebellar regions and subregions were originally defined on the basis of histological features and histology-based mouse brain atlases served as the foundation for defining the borders, the level of contrast and resolution achieved in the model permitted identification of the majority of the cerebellar structures without the need for a direct histological confirmation in our set of brains.

Identification of anatomical boundaries

We have segmented the cerebellum according to the scheme originally developed by Bolk (1906), and subsequently refined and expanded by Larsell (1952, 1970). The Bolk/Larsell scheme has since been adopted by all major modern atlases of the mouse brain (Dong, 2008; Franklin and Paxinos, 2008; Sidman et al., 1971; Watson and Paxinos, 2010). The mouse cerebellum consists of a central vermis and two lateral hemispheres. The vermis is delineated, at least in part, from the hemispheres by paramedian sulci. The vermis consists of a series of ten lobules (1Cb to 10Cb) separated by a series of named fissures. Each of the ten lobules has a traditional name which was current before the development of the Bolk/Larsell scheme: lobule 1 is the lingula; lobules 2 and 3 are the central lobule; lobules 4 and 5 are the culmen; lobule 6 is the declive; lobule 7 is the folium and tuber; lobule 8 is the pyramis; lobule 9 is the uvula; and lobule 10 is the nodulus. In the mouse, the anterior lobe vermal lobules (lobules 1 to 5) do not contribute to the cerebellar hemispheres, but each of lobules 6 to 10 has a substantial extension into the hemisphere: lobule 6 extends into the simple lobule and crus 1 of the ansiform lobule; lobule 7 extends into crus 2 of the ansiform lobule and the paramedian lobule; lobule 8 extends into the copula of the pyramis; lobule 9 extends into the flocculonodular lobe; and lobule 10 extends into the flocculus. While lobules 6–8 can be seen to be directly continuous with their lateral extensions, lobules 9 and 10 are more difficult to visualize. The reason is that the flocculus and paraflocculus are displaced rostrally with an attenuated connection to their parent lobules.

Fissures

The key to cerebellar segmentation is the identification of the fissures. The fissures separate the major vermal lobules and the parts of the cerebellar hemispheres (Fig. 1). When attempting to identify the fissures it is useful to refer to a mid-sagittal section of the cerebellum (Fig. 1A). Note that a fissure separating two lobules must have a molecular layer on each side, while the mid-space between any two fissures should be a strip of white matter.

The primary fissure (prf) extends coronally between lobules 5 and 6 to separate the vermis into rostral and caudal lobes. Located in the anterior lobe and positioned rostral to the primary fissure is the preculminate fissure (pcuf), which separates lobules 4 and 3, and the precentral fissure (pcn), which separates lobules 3 and 2. Caudal to the primary fissure is the posterior superior fissure (psf), which is a shallow fissure that separates lobules 6 and 7, the prepyramidal fissure (ppf), which separates lobules 7 and 8, and the secondary fissure (sf), which is a deep fissure found between lobules 8 and 9. Finally, the posterolateral fissure (plf) separates lobule 10 (the nodule) from lobule 9.

Two fissures, psf and ppf, extend the entire width of the cerebellum to subdivide also the cerebellar hemispheres. The psf creates a boundary between the simple lobule and crus 1 of the ansiform lobule