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Waxholm Space atlas of the rat brain hippocampal region: Three-dimensional delineations based on magnetic resonance and diffusion tensor imaging

Lisa J. Kjonigsen [a], Sveinung Lillehaug [a], Jan G. Bjaalie [a], Menno P. Witter [b], Trygve B. Leergaard [a,*]

[a] Institute of Basic Medical Sciences, University of Oslo, Oslo, Norway
[b] Kavli Institute for Systems Neuroscience and Centre for Neural Computation, Norwegian University of Science and Technology, Trondheim, Norway

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ABSTRACT

Atlases of the rat brain are widely used as reference for orientation, planning of experiments, and as tools for assigning location to experimental data. Improved quality and use of magnetic resonance imaging (MRI) and other tomographical imaging techniques in rats have allowed the development of new three-dimensional (3-D) volumetric brain atlas templates. The rat hippocampal region is a commonly used model for basic research on memory and learning, and for preclinical investigations of brain disease. The region features a complex anatomical organization with multiple subdivisions that can be identified on the basis of specific cytoarchitectonic or chemoarchitectonic criteria. We here investigate the extent to which it is possible to identify boundaries of divisions of the hippocampal region on the basis of high-resolution MRI contrast. We present the boundaries of 13 divisions, identified and delineated based on multiple types of image contrast observed in the recently published Waxholm Space MRI/DTI template for the Sprague Dawley rat brain (Papp et al., Neuroimage 97:374–386, 2014). The new detailed delineations of the hippocampal formation and parahippocampal region (Waxholm Space atlas of the Sprague Dawley rat brain, v2.0) are shared via the INCF Software Center (http://software.incf.org/), where also the MRI/DTI reference template is available. The present update of the Waxholm Space atlas of the rat brain is intended to facilitate interpretation, analysis, and integration of experimental data from this anatomically complex region.

Introduction

The hippocampal formation and adjacent parahippocampal areas, together constituting the hippocampal region, are intensively investigated in the rat brain in the context of understanding memory and learning processes (Eichenbaum et al., 2007) and neurological diseases such as Alzheimer's disease and epilepsy (Braak and Braak, 1991; Schwarcz and Witter, 2002). Due to the anatomical complexity of this region, accurate atlas resources are important for planning experiments, conducting analyses, reporting findings, and comparing results across investigations. The hippocampal region is subdivided by interpretation of cyto-, chemo-, and myeloarhitectonic patterns observed in histological materials. Several parcellation schemes are available in the form of atlas diagrams (Swanson, 2004; Paxinos and Watson, 2007), or textual descriptions (e.g. Witter and Amaral, 2004; Andersen et al., 2007; Bota and Swanson, 2010). The currently most detailed and up-to-date accounts of hippocampal parcellation in the rat comprise an interactive web-based resource linking delineated histological images with up-to-date criteria for subdividing the hippocampal region (Kjonigsen et al., 2011), and a comprehensive description of boundaries observed in three standard sectional planes (Boccara et al., 2014).

Improved imaging technologies have given rise to a new generation of three-dimensional (3-D) digital rodent brain atlases, highly relevant for orientation in the hippocampal region. These volumetric brain atlases, based on high resolution magnetic resonance imaging (MRI) templates (Johnson et al., 2010; Bowden et al., 2011; Hawrylycz et al., 2011; Veraart et al., 2011; Papp et al., 2014) provide anatomical reference spaces that are suitable both for 3-D tomographical and two-dimensional (2-D) experimental data, and have the advantage that they can be resliced in arbitrary planes. But, currently available 3-D rat brain atlases lack hippocampal subdivisions, the underlying MRI templates have considerably lower spatial resolution than histological images, and it is unknown whether hippocampal subregions can be identified on the basis of MRI contrast. It was recently demonstrated that combined use of high-resolution structural and diffusion MRI in the Waxholm Space template of the Sprague Dawley rat brain allows delineation of a large number of brain regions (Papp et al., 2014).

We here extend this work to investigate the extent to which subdivisions of the hippocampal region, as defined in our recent accounts (Kjonigsen et al., 2011; Boccara et al., 2014), can be identified in the

* Corresponding author at: Institute of Basic Medical Sciences, University of Oslo, P.O. Box 1105 Blindern, N-0317 Oslo, Norway. Fax: +47 22851278.
E-mail address: t.b.leergaard@medisin.uio.no (T.B. Leergaard).

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Waxholm Space MRI template. We present a 3-D atlas of 13 divisions of the hippocampal region with boundaries identified and delineated by use of multiple features observed in structural MRI and diffusion tensor imaging (DTI) data, and interpreted in relation to cyto- and chemoarchitectonic features in stained histological sections. These delineations represent the first major update of the existing Waxholm Space atlas of the Sprague Dawley rat brain (Papp et al., 2014), and are shared via the INCF software center (http://software.incf.org/).

Methods

Waxholm Space template for the rat brain

The present atlas of the hippocampal region was defined in the Waxholm Space MRI/DTI template for the Sprague Dawley brain (version 1.01; Papp et al., 2015), available from the INCF Software Center (http://software.incf.org/software/waxholm-space-atlas-of-the-sprague-dawley-rat-brain). This template comprises microscopic resolution, contrast enhanced MRI/DTI data acquired ex vivo from an adult male Sprague Dawley rat head (age 80 days, weight 397.6 g, Charles River, Wilmington, MA, USA) by use of a 7 *T* small animal MRI system (Magnex Scientific, Yarnton, Oxford, UK) at the Duke Center for In Vivo Microscopy (Durham, NC, USA). The template includes T_2*-weighted gradient recalled echo anatomic images with a Nyquist isotropic spatial resolution of 39 μm, and diffusion tensor datasets acquired with a Nyquist isotropic spatial resolution of 78 μm. Technical details are provided in Papp et al. (2014). The high resolution Waxholm Space MRI/DTI template for the Sprague Dawley rat brain (v1.01; Papp et al., 2015; NIfTII format) was downloaded from the INCF Software Center (http://software.incf.org/software/waxholm-space-atlas-of-the-sprague-dawley-rat-brain). The present delineations were defined using T_2*-weighted MRI, DTI, and fractional anisotropy (FA) maps from the following downloadable files: T2*-weighted MRI template v1.01; Color FA map v1.01; FA map v1.01. The first version of the atlas (Sprague Dawley Atlas v1.01; ITK-SNAP label v1-v1.01) was used as a starting point for the delineations.

Image delineation

Divisions of the hippocampal formation and parahippocampal region were manually delineated using ITK-SNAP software (version 2.4, Yushkevich et al., 2006, www.iktsnap.org). MRI data were viewed in ITK-SNAP using the default 16-bit grayscale color map (black to white); where dark areas in the image correspond to low signal intensity and bright areas correspond to high signal intensity. Neuroanatomical boundaries were delineated on the basis of image contrast observed in T_2*-weighted images and diffusion tensor images (DTI). In T_2*-weighted images, below referred to as structural MRI (sMRI) maps, white matter regions tend to have low signal intensities and appear dark, while gray matter regions generally have higher signal intensities and appear brighter. The DTI color maps show the orientation and magnitude of diffusion occurring at each voxel, with the red, green, and blue (RGB) components of each voxel defined by the primary eigenvector of the diffusion weighted images, and the brightness determined using fractional anisotropy (FA) values computed from the three diffusion eigenvalues after tensor decomposition (for further details, see Papp et al., 2014). FA values are also visualized in grayscale FA maps in which voxel brightness is determined by the degree of anisotropic diffusion. Our objective was to delineate the same structures as previously defined in the histology-based Rat Hippocampus Atlas (Kjonigsen et al., 2011; Boccara et al., 2014). Boundaries were manually delineated on the basis of observable features in the volumetric data, aided by comparison to cyto- and chemoarchitectonic features visible in histological section images from Long Evans rats, available from the online Rat Hippocampus Atlas (Kjonigsen et al., 2011; Boccara et al., 2014), as well as cyto- and myeloarchitectonic features observed in a collection of coronal, sagittal, and horizontal sections from normal adult Sprague Dawley rat brains (T.B. Leergaard and J.G. Bjaalie, unpublished work; see, also Leergaard et al., 2010; White et al., 2013). The left and right hemispheres of the brain were delineated separately. The delineated divisions were color-coded in agreement with recent reports (van Strien et al., 2009; Kjonigsen et al., 2011).

Waxhom Space atlas v2.0

The delineations presented here replace the delineations of the hippocampal formation and parahippocampal region provided in the first release (v1.0/v1.01) of the atlas (Papp et al., 2015). In addition to the updated hippocampal delineations, we have adjusted the delineation of regions bordering on the hippocampus and hippocampus proper. The new delineations are included in the downloadable file “Sprague Dawley Atlas v2.0”. This file includes 79 anatomical structures, of which 13 are the new or revised delineations of the hippocampal formation and parahippocampal region, and 66 structures are delineations from v. 1.01, as described in Papp et al. (2015). Two structures (‘neocortex’ and ‘corpus callosum and associated subcortical white matter’) were adjusted to match the updated outer boundaries of the hippocampal formation and parahippocampal region. The anatomical delineations provided in the present report and corresponding label descriptions are made available through the INCF Software Center (http://software.incf.org/software/waxholm-space-atlas-of-the-sprague-dawley-rat-brain) in formats compatible with ITK-SNAP and the Mouse BIRN Atlasing Toolkit (MBAT, Lee et al., 2010). Volumetric images are provided as standard Neuroimaging Informatics Technology Initiative (NIfTI) files.

Results

We here present a volumetric atlas of the rat hippocampal region defined in a Waxholm Space structural and diffusion weighted MRI template of the Sprague Dawley rat brain (Papp et al., 2014). We have delineated 13 divisions in the hippocampal formation and parahippocampal region on the basis of architectural features observed in sMRI and DTI/FA images, compared to cyto- and chemoarchitectonically defined regions (Kjonigsen et al., 2011; Boccara et al., 2014).

Nomenclature

The hippocampal region comprises the hippocampal formation and the parahippocampal region (Cappaert et al., 2014). The hippocampal formation is a C-shaped structure, positioned posteriorly in the hemisphere of the rat brain (Figs. 1A, A′), bordering on the septal complex dorsally and the amygdaloid complex ventrally. It has the characteristic three layered organization of the allocortex, with a superficial, neuron sparse plexiform layer, a middle, densely packed principal cell layer, and an inner, fibrous polymorph layer (Figs. 1D–F). The hippocampal formation encompasses four main subfields, distributed from proximal to distal along the transverse axis of the hippocampus (Fig. 1A′), with the dentate gyrus as the most medial and proximal portion, laterally flanked by the cornu ammonis (CA) with its three subfields (CA1, CA2, CA3), and the subiculum. The fourth subfield is the fasciola cinereum, which extends medially as a longitudinal continuation of the hippocampal formation (Stephan, 1975). The parahippocampal region (Fig. 1B) includes several interconnected six-layered cortical areas (Figs. 1D–F) that are all reciprocally connected with the hippocampal formation (Cappaert et al., 2014). The parahipocampal areas are the presubiculum, parasubiculum, entorhinal cortex (with a medial and lateral part), perirhinal cortex (including areas 35 and 36), and postrhinal cortex. We employ boundary definitions as outlined in The Rat Hippocampal Atlas (Kjonigsen et al., 2011, www.rbwb.org), that have recently been further detailed for the three main planes