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

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ARTICLE INFO

Keywords:
Auditory system
Delineation criteria
Digital brain atlas
Diffusion tensor imaging
Magnetic resonance imaging
Neuroinformatics
Reference atlas
Sprague Dawley rat

ABSTRACT

The mammalian auditory system comprises a complex network of brain regions. Interpretations and comparisons of experimental results from this system depend on appropriate anatomical identification of auditory structures. The Waxholm Space (WHS) atlas of the Sprague Dawley rat brain (Papp et al., Neuroimage 97:374–86, 2014) is an open access, three-dimensional reference atlas defined in an ex-vivo magnetic resonance imaging (MRI) and diffusion tensor imaging (DTI) volume. Version 2.0 of the atlas (Kjonigsen et al., Neuroimage 108:441–9, 2015) includes detailed delineations of the hippocampus and several major subcortical regions, but only few auditory structures. To amend this, we have delineated the complete ascending auditory system from the cochlea to the cerebral cortex. 40 new brain structure delineations have been added, and the delineations of 10 regions have been revised based on the interpretation of image features in the WHS rat brain MRI/DTI volumes. We here describe and validate the new delineations in relation to corresponding cell- and myelin-stained histological sections and previous literature. We found it possible to delineate all main regions and the majority of subregions and fibre tracts of the ascending auditory pathway, apart from the auditory cortex, for which delineations were extrapolated from a conventional two-dimensional atlas. By contrast, only parts of the descending pathways were discernible in the template. Version 3.0 of the atlas, with altogether 118 anatomical delineations, is shared via the NeuroImaging Tools and Resources Collaboratory (www.nitrc.org).

1. Introduction

The mammalian auditory system comprises the hearing organ and the complex network of brain regions involved in perception of auditory signals, i.e., recognition and interpretation of cues within a species-specific spectre of sound waves. The different regions of the auditory system and their interconnections have over the last decennia been described in several cyto- and fibroarchitectural studies in various species using histological, physiological, and biochemical methods, summarized by Malmierca (2015). Optimal planning and interpretation of such studies depend on appropriate anatomical identification and three-dimensional (3D) localization of the structures involved.

Present anatomical reference atlases of the rat brain based on selections of histological sections (Swanson, 2004; Paxinos and Watson, 2007) portray auditory regions with a variable degree of accuracy, and with little information about the anatomical criteria underlying the delineations. A new generation of murine brain atlases based on magnetic resonance imaging (MRI) provide not only anatomical delineations based on structural MRI (sMRI) and diffusion tensor imaging (DTI) data, but also share the volumetric image template that the delineations are based on (Johnson et al., 2010; Hawrylycz et al., 2011; Papp et al., 2014, 2015; Kjonigsen et al., 2015). A major advantage of these new 3D atlases is that they facilitate integration of data from diverse experiments (Amunts et al., 2014; Oh et al., 2014; Papp et al., 2014; Tiesinga et al., 2015; Majka et al., 2016; Bjerke et al., 2018). Although novel imaging methods are increasingly used to investigate the rat auditory system (Zhang et al., 2013; Gao et al., 2015; Tang et al., 2017), a detailed 3D reference atlas of the auditory system has hitherto been missing. In the second version of the Waxholm Space (WHS) atlas of the Sprague Dawley rat brain (v2.0; Kjonigsen et al., 2015), 79 brain regions, including major subcortical structures and the hippocampal formation, were delineated, but only a few auditory regions.

We have previously shown that detailed subdivisions of the hippocampal region, closely corresponding to cyto- and chemoarchitectonic
boundaries (Boccara et al., 2015) can be identified in the WHS rat brain template (Kjønigsen et al., 2015). We now extend these efforts to identify, and in detail delineate the entire rat brain auditory system in the WHS template. By comparing sMRI and DTI features with the corresponding cyto- and myeloarchitecture in histological sections and previous descriptions of morphology, we demonstrate that the main regions and the majority of the subregions and fibre tracts of the ascending auditory pathway, apart from the auditory cortex, can be delineated in the WHS template, but only part of the descending system.

We here present the revised and updated version 3 of the WHS atlas of the rat brain, along with detailed descriptions of the criteria and interpretations underlying the delineations of auditory structures. The new delineations are shared via the Neuroimaging Tools and Resources Collaboratory (NITRC, http://www.nitrc.org/; Kennedy et al., 2016).

2. Material and methods

2.1. Waxholm Space MRI/DTI template

We defined auditory structures in the Waxholm Space MRI/DTI template for the Sprague Dawley brain (v1.01; Papp et al., 2014, 2015; available from www.nitrc.org; RRID: SCR_017124), building on version 2 of the anatomical delineations accompanying the atlas (Kjønigsen et al., 2015). The WHS template comprises high-resolution contrast-enhanced MRI and DTI data acquired ex vivo from the head of an adult male Sprague Dawley rat (age 80 days, weight 397.6 g, Charles River, Wilmington, MS, USA; perfusion-fixed using a mixture of formalin and a gadolinium-based MRI contrast agent (Johnson et al., 2002)), by use of a 7 T small animal MRI system (Magnex Scientific, Yarnton, Oxford, UK) at the Duke Centre for In Vivo Microscopy (Durham, NC, USA). The template comprises $T_2^*$-weighted gradient recalled echo anatomic images with an isotropic spatial resolution of 39 μm, here referred to as structural MRI (sMRI), and colour-coded principal diffusion direction maps (DTI) with a resolution of 78 μm. Technical details are provided in Papp et al. (2014, 2015). We downloaded and used the following files from NITRC (www.nitrc.org): WHS_SD_rat_T2star_v1.01.nii.gz; WHS_SD_rat_-FA_colour_v1.01.nii.gz; WHS_SD_rat_atlas_v2.nii.gz; WHS_SD_rat_atlas_v3.label. Consistency of observations was verified by inspection of a corresponding MRI data set (G.A. Johnson, E. Calabrese, unpublished material).

2.2. Histological reference material

To aid the interpretation of sMRI and DTI contrast and colours, we used existing collections of histological material (data citation: Leergaard et al., 2018), including 50 μm thick coronal and sagittal cryosections from an adult paraformaldehyde-fixed Sprague Dawley rat brain stained with thionine or Woelcke’s myelin method (Woelcke, 1942), and a series of 120 μm thick horizontal sections from a celloidin-embedded, formaldehyde-fixed adult Wistar rat brain, stained with thionine (unpublished historical reference material produced by K.K.O.). The microscopic sections were inspected through a standard light microscope and by use of camera lucida. To facilitate more direct comparisons with the WHS template, high-resolution mosaic images of entire sections were obtained through UPlanApo 20/0.70 objectives using a slide scanner (Mirax Scan, Carl Zeiss Microimaging, Jena, Germany). Polarized light imaging (PLI) data, colour-coded to show tissue fibre orientations, and spatially registered to the Waxholm Space atlas of the rat brain (Schubert et al., 2016), were for some regions used as a supplement to the DTI data.

The microscopic images, as well as selected diagrams from stereotaxic reference atlases (Swanson, 2004; Paxinos and Watson, 2007) were spatially registered to the Waxholm Space template by affine transformations using our in-house alignment tool QuickNII (Puchades et al., 2019; available via www.nitrc.org, RRID: SCR_016854). Taking advantage of the isotropic properties of the template, MRI slices were generated in arbitrary, non-standard orientations to match the cutting angle of the histological images. Volumetric reconstructions were then created in Neuroimaging Informatics Technology Initiative (NIfTI) format to allow co-viewing of the re-sliced MRI template and the additional (originally two-dimensional) image material. In addition, the inverse transform was applied to the newly generated volumetric reconstructions for access to the images in spatial correspondence with the WHS template. The spatially registered histological images were used as supplemental anatomical information aiding the interpretation of the sMRI and DTI features (see Results), but are not integrated with the atlas.

2.3. Anatomical delineations

The sMRI/DTI template was inspected and manually delineated using the ITK-SNAP software (version 3.6.0, Yushkevich et al., 2006, www.itksnap.org). Delineations were carried out in the standard orthogonal planes (cortical, sagittal, and horizontal). In addition, selected custom-angle slices through the sMRI/DTI template were generated using QuickNII for the identification of certain boundaries and the shape and intrinsic organization of certain nuclei with axes oriented oblique to standard planes. The observed image features were interpreted in comparison with cyto- and myelin-stained histological sections, supplemented by information derived from literature and standard reference atlases (Swanson, 2004; Paxinos and Watson, 2007). Apart from the auditory cortex, all delineations are based on signal intensities (brightness) in sMRI and DTI (from fractional anisotropy maps), and diffusion orientations (colours) in DTI. With the intention to improve the readability of the anatomical descriptions and facilitate direct comparisons to the MRI/DTI template, we provide delineation criteria for each region according to direct observations in the images with regard to brightness and colour, along with our interpretation of the underlying anatomical features.

2.4. Interpretation of sMRI/DTI signals

The $T_2^*$-weighted sMRI greyscale maps reflect the differential magnetic susceptibility properties of the brain tissue (see e.g. Chavhan et al., 2009), and show high grey to white matter contrast, which highlights several cytoarchitectonic features. We observed that cell- or rather cytoplasm-rich areas tend to have higher signal intensities, and appear brighter than myelin-rich areas, which have lower signal intensities and appear darker, as in myelin-stained sections. Regions with tightly packed small cells with relatively much DNA and little cytoplasm, such as the hippocampal granular layer, appear dark in sMRI, whereas the less densely packed granule cell layer of the cochlear nuclear complex appears bright. For a comparison of contrast properties between gadolinium-enhanced $T_2^*$-weighted MRI and common histological stains (see e.g. Huang et al., 2009).

The red-green-blue (RGB) coded DTI maps reflect the prevailing orientation and magnitude of water diffusion in anisotropic tissue, i.e., tissue with some degree of parallel orientation of axons and dendrites (Beaulieu, 2002). The prevailing diffusion orientation (i.e., the orientation of the primary eigenvector in each voxel) is represented by the primary red, green, and blue colours each signifying diffusion in one of the three principal directions (red = mediolateral, green = rostrocaudal, blue = dorsoventral), while secondary colours according to the RGB model indicate intermediate orientations. The brightness of the colours represents the diffusion intensity in each voxel, as determined by fractional anisotropy (FA) computed from the three diffusion eigenvalues after tensor decomposition (Le Bihan et al., 2001). Fluid-containing spaces appear white in $T_2^*$-weighted sMRI and dark in DTI, while air-filled regions are black in both modalities.

2.5. Data availability

The new delineations are shared as standard NIfTI files (WHS_SD_rat_atlas_v3.nii.gz, with an accompanying label description file
observed in the three standard planes, aided by cyto- and myelin-stained based on sMRI signal intensities and DTI colour-coded orientations indicated below. Apart from the auditory cortex, the delineations are Boundaries of several adjacent structures were added or adjusted, as indicated below. Apart from the auditory cortex, the delineations are based on sMRI signal intensities and DTI colour-coded orientations observed in the three standard planes, aided by cyto- and myelin-stained histological sections, standard reference atlases (Swanson, 2004; Paxinos and Watson, 2007) and available publications. The criteria used for identification and delineation of boundaries are described in detail for each region. Version 3 of the WHS rat brain atlas features altogether 118 anatomical structures, of which 40 are new and 10 revised. Table 1 specifies all regions, new, revised or replaced, in the present version of the atlas.

3. Results

We have bilaterally delineated 38 structures in the rat ascending auditory system from the cochlea to the auditory cortex (Table 1, Fig. 1). Boundaries of several adjacent structures were added or adjusted, as indicated below. Apart from the auditory cortex, the delineations are based on sMRI signal intensities and DTI colour-coded orientations observed in the three standard planes, aided by cyto- and myelin-stained

### Table 1
Overview of delineated structures.

<table>
<thead>
<tr>
<th>Chpt.#</th>
<th>Auditory structures</th>
<th>Abbreviation</th>
<th>Label #</th>
<th>Status</th>
<th>Comments with label #</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.3.</td>
<td>Cochlea</td>
<td>Co</td>
<td>120</td>
<td>New</td>
<td>replacing inner ear (44)</td>
</tr>
<tr>
<td>3.4.</td>
<td>Cochlear nerve</td>
<td>cn</td>
<td>121</td>
<td>New</td>
<td>partly replacing brainstem (47)</td>
</tr>
<tr>
<td>3.3.</td>
<td>Spiral ganglion</td>
<td>SG</td>
<td>162</td>
<td>New</td>
<td>not previously delineated</td>
</tr>
<tr>
<td>3.5.1.</td>
<td>Ventral cochlear nucleus, anterior part</td>
<td>AVCN</td>
<td>158</td>
<td>New</td>
<td>partly replacing brainstem (47)</td>
</tr>
<tr>
<td>3.5.2.</td>
<td>Ventral cochlear nucleus, posterior part</td>
<td>PVCN</td>
<td>159</td>
<td>New</td>
<td>partly replacing brainstem (47)</td>
</tr>
<tr>
<td>3.5.3.</td>
<td>Ventral cochlear nucleus, cap area</td>
<td>Cap</td>
<td>160</td>
<td>New</td>
<td>partly replacing brainstem (47)</td>
</tr>
<tr>
<td>3.5.4.</td>
<td>Ventral cochlear nucleus, granule cell layer</td>
<td>GGL</td>
<td>123</td>
<td>New</td>
<td>partly replacing brainstem (47)</td>
</tr>
<tr>
<td>3.5.5.</td>
<td>Dorsal cochlear nucleus, molecular layer</td>
<td>DCNM</td>
<td>126</td>
<td>New</td>
<td>partly replacing brainstem (47)</td>
</tr>
<tr>
<td>3.5.5.</td>
<td>Dorsal cochlear nucleus, fusiform and granule cell layer</td>
<td>DCNFG</td>
<td>127</td>
<td>New</td>
<td>partly replacing brainstem (47)</td>
</tr>
<tr>
<td>3.5.5.</td>
<td>Dorsal cochlear nucleus, deep core</td>
<td>DCND</td>
<td>128</td>
<td>New</td>
<td>partly replacing brainstem (47)</td>
</tr>
<tr>
<td>3.6.1.</td>
<td>Trapezoid body</td>
<td>tz</td>
<td>130</td>
<td>New</td>
<td>partly replacing brainstem (47)</td>
</tr>
<tr>
<td>3.6.2.</td>
<td>Acoustic striae</td>
<td>as</td>
<td>129</td>
<td>New</td>
<td>partly replacing brainstem (47)</td>
</tr>
<tr>
<td>3.7.1.</td>
<td>Lateral superior olive</td>
<td>LSO</td>
<td>134</td>
<td>New</td>
<td>partly replacing brainstem (47)</td>
</tr>
<tr>
<td>3.7.2.</td>
<td>Medial superior olive</td>
<td>MSO</td>
<td>133</td>
<td>New</td>
<td>partly replacing brainstem (47)</td>
</tr>
<tr>
<td>3.7.3.</td>
<td>Superior parasympathetic nucleus</td>
<td>SPN</td>
<td>132</td>
<td>New</td>
<td>partly replacing brainstem (47)</td>
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<tr>
<td>3.7.4.</td>
<td>Nucleus of the trapezoid body</td>
<td>NTB</td>
<td>131</td>
<td>New</td>
<td>partly replacing brainstem (47)</td>
</tr>
<tr>
<td>3.7.5.</td>
<td>Periolivary region</td>
<td>POR</td>
<td>135</td>
<td>New</td>
<td>partly replacing brainstem (47)</td>
</tr>
<tr>
<td>3.7.5.</td>
<td>Ventral periolivary nucleus</td>
<td>VPO</td>
<td>136</td>
<td>New</td>
<td>partly replacing brainstem (47)</td>
</tr>
<tr>
<td>3.8.</td>
<td>Lateral lemniscus</td>
<td>II</td>
<td>141</td>
<td>New</td>
<td>partly replacing brainstem (47)</td>
</tr>
<tr>
<td>3.8.1.</td>
<td>Lateral lemniscus, ventral nucleus</td>
<td>VLL</td>
<td>137</td>
<td>New</td>
<td>partly replacing brainstem (47)</td>
</tr>
<tr>
<td>3.8.1.</td>
<td>Lateral lemniscus, intermediate nucleus</td>
<td>ILL</td>
<td>138</td>
<td>New</td>
<td>partly replacing brainstem (47)</td>
</tr>
<tr>
<td>3.8.1.</td>
<td>Lateral lemniscus, dorsal nucleus</td>
<td>DLL</td>
<td>139</td>
<td>New</td>
<td>partly replacing brainstem (47)</td>
</tr>
<tr>
<td>3.8.2.</td>
<td>Lateral lemniscus, commissure</td>
<td>cll</td>
<td>140</td>
<td>New</td>
<td>partly replacing brainstem (47)</td>
</tr>
<tr>
<td>3.8.3.</td>
<td>Nucleus sagulum</td>
<td>Sag</td>
<td>163</td>
<td>New</td>
<td>partly replacing brainstem (47)</td>
</tr>
<tr>
<td>3.9.1.</td>
<td>Inferior colliculus, external cortex</td>
<td>ECIC</td>
<td>145</td>
<td>New</td>
<td>replacing inferior colliculus (49)</td>
</tr>
<tr>
<td>3.9.2.</td>
<td>Inferior colliculus, dorsal cortex</td>
<td>DCIC</td>
<td>142</td>
<td>New</td>
<td>replacing inferior colliculus (49)</td>
</tr>
<tr>
<td>3.9.3.</td>
<td>Inferior colliculus, central nucleus</td>
<td>CNIC</td>
<td>143</td>
<td>New</td>
<td>replacing inferior colliculus (49)</td>
</tr>
<tr>
<td>3.9.4.</td>
<td>Inferior colliculus, commissure</td>
<td>cic</td>
<td>69</td>
<td>Revised</td>
<td></td>
</tr>
<tr>
<td>3.9.5.</td>
<td>Inferior colliculus, brachium</td>
<td>bic</td>
<td>146</td>
<td>New</td>
<td>replacing inferior colliculus (49)</td>
</tr>
<tr>
<td>3.10.1.</td>
<td>Medial geniculate body, marginal zone</td>
<td>MGMZ</td>
<td>150</td>
<td>New</td>
<td>partly replacing thalamus (39)</td>
</tr>
<tr>
<td>3.10.2.</td>
<td>Medial geniculate body, medial division</td>
<td>MGM</td>
<td>147</td>
<td>New</td>
<td>partly replacing thalamus (39)</td>
</tr>
<tr>
<td>3.10.3.</td>
<td>Medial geniculate body, ventral division</td>
<td>MGV</td>
<td>149</td>
<td>New</td>
<td>partly replacing thalamus (39)</td>
</tr>
<tr>
<td>3.10.3.</td>
<td>Medial geniculate body, dorsal division</td>
<td>MGD</td>
<td>148</td>
<td>New</td>
<td>partly replacing thalamus (39)</td>
</tr>
<tr>
<td>3.11.</td>
<td>Auditory radiation</td>
<td>ar</td>
<td>157</td>
<td>New</td>
<td>partly replacing brainstem (47)</td>
</tr>
<tr>
<td>3.11.</td>
<td>Reticular thalamic nucleus, auditory segment</td>
<td>RTa</td>
<td>164</td>
<td>New</td>
<td>partly replacing brainstem (47)</td>
</tr>
<tr>
<td>3.12.</td>
<td>Auditory cortex, primary</td>
<td>AuI</td>
<td>151</td>
<td>New</td>
<td>partly replacing neocortex (92)</td>
</tr>
<tr>
<td>3.12.</td>
<td>Auditory cortex, dorsal secondary area</td>
<td>AuD</td>
<td>152</td>
<td>New</td>
<td>partly replacing neocortex (92)</td>
</tr>
<tr>
<td>NA</td>
<td>Inner ear</td>
<td>IE</td>
<td>44</td>
<td>Replaced</td>
<td>fully replaced by cochlea and vestibular apparatus</td>
</tr>
</tbody>
</table>

List of newly created, revised or replaced anatomical delineations of auditory and non-auditory structures presented in version 3 of the Waxholm Space atlas of the Sprague Dawley rat brain. Structures not listed are unchanged from version 2 of the atlas (Kjonigsen et al., 2015). Chapter numbers refer to the corresponding sections in the Results section. Label numbers are identical to label IDs in the atlas delineation files (.nii.gz and .label) shared via www.nitrc.org.
for delineation of the auditory pathway. We found both sMRI and DTI to correlate better with myeloarchitecture than cytoarchitecture, when viewed at the optimal zoom level corresponding to low magnification light microscopy (2.5 x objective, 10 x ocular). Fibre tracts, however, could not be traced when organized in widely dispersed thin fascicles as in parts of the acoustic striae, the commissure of the lateral lemniscus and the olivocochlear bundle. Generally, the relatively higher spatial resolution of the sMRI allows distinction of borders better than with DTI, as in the superior olivary complex, but there are exceptions, as the inferior colliculus, where the delineation depended on DTI. Intranuclear parts of fibre tracts were not delineated, apart from the cochlear nerve root (see 3.4). The left and right hemispheres of the brain were delineated individually thus avoiding inaccuracies in the delineation due to the small right/left asymmetry which in part may be ascribed to a minor deviation in the angle of the general, the atlas on which the present atlas is based, is largely similar, but with differences concerning the naming of several nomenclatures exist for the auditory system. The nomenclatures used in The Rat Brain in Stereotaxic Coordinates (Paxinos and Watson, 2007), the recent review by Malmierca (2015), and the Terminologia Anatomica (Federative Committee on Anatomical Terminology, 1998) are largely similar, but with differences concerning the naming of some structures, notably the vestibular and cochlear nerves, the medial geniculate body, and the auditory cortex with subdivisions. For the present atlas we adopted names that are similar across sources, and selected the remaining names as shown in Table 1, except for: acp, auditory corticopeduncular tract; aib, auditory forebrain bundle; cas, commissural acoustic stria; cc, corpus callosum; das, dorsal acoustic stria; ias, intermediate acoustic stria; PL, perilemniscal region; ReIC, recess of the inferior colliculus. Scale bar: 1 mm, valid for B and C.

### 3.2. Nomenclature

Several nomenclatures exist for the auditory system. The nomenclatures used in The Rat Brain in Stereotaxic Coordinates (Paxinos and Watson, 2007), the recent review by Malmierca (2015), and the Terminologia Anatomica (Federative Committee on Anatomical Terminology, 1998) are largely similar, but with differences concerning the naming of some structures, notably the vestibular and cochlear nerves, the medial geniculate body, and the auditory cortex with subdivisions. For the present atlas we adopted names that are similar across sources, and selected the remaining names as shown in Table 1.

### 3.3. Cochlea (co)

The osseous labyrinth, with the cochlea, vestibule and semicircular canals were delineated in sMRI on the basis of the brighter signal in the fluid-containing interior of the labyrinth relative to the surrounding dark temporal bone (Fig. 2A, D). The vestibule and semicircular canals were collectively labelled as the vestibular apparatus (Fig. 1B; see also 3.14). In DTI, the interior of the labyrinth appears dark with the edge of the bone marked by brighter signals (Fig. 2C, F). As shown in Fig. 1, the...
The cochlea is oriented with its base in the coronal plane and its apex pointing rostrally. The cochlear duct flanked by the vestibular and tympanic scalae could be identified in sMRI, but because of its tiny details (relative to the size of sMRI voxels), the membraneous labyrinth was not delineated separately (Fig. 2B, E). The border of the cochlea with the vestibular part of the labyrinth was identified in sMRI slices at the basalmost end of the cochlear duct (dotted line in Fig. 2D–F). In the 3D reconstruction shown in Fig. 3A, where the vestibular part is omitted, the borderline (marked by arrows) cuts through the oval window, save for the blunt basal end of the scala tympani with the contours of the round window. The spiral ganglion (Fig. 2A,B,D,E and 3B–D) appears distinct particularly in sMRI, but its connections to the cochlear nerve could only be identified at the very distal end of the nerve (Fig. 2A–C, arrowhead). The osseous labyrinth could only be completely delineated on the right side, as the left side partly lacks signal (Fig. 1B). The brighter DTI signal at the edge of the osseous labyrinth allowed an extension of the version 2 delineation on the left side, although still incomplete.

3.4. Cochlear nerve (cn)

In the rat cochlear nerve, the glial-Schwann cell border, which could not be distinguished with certainty in MRI and DTI, is situated deep within the modiolus, with scattered neurons (root neurons) occurring all the way out to the border (Merchan et al., 1988, their Figs. 1 and 2; Osen et al., 1991, their Fig. 1). As such, the entire “nerve” should in principle have been referred to as a nerve root. We here, however, restrict the term nerve root to the central end situated between the antero- and posterior cochlear nuclei (see 3.5). As an exception to our general rule not to delineate intranuclear fiber tracts (see 3.1), we have here delineated the root and the nerve as one continuous structure labelled the cochlear nerve (cn). The thinner, distalmost intramodiolar part of the nerve, arising from the apical and middle coils of the cochlea, is oriented rostrocaudally, and hence seen lengthwise in sagittal slices (Fig. 2A–C). The thicker proximal part, including fibers also from the basal coil, is on the contrary cut lengthwise in coronal slices, where it is seen to course medially and dorsally from the caudal base of the cochlea to the ventral cochlear nucleus, which it enters from ventral (Fig. 2D–F, 3). In DTI, the cochlear nerve displays alternating green, red and blue signals (Fig. 2C,F, 4F,I), like a rainbow, particularly distally, reflecting the spiralling of its fibre fascicles (cat: Arnesen and Osen, 1978; rat: Osen et al., 1991, their Fig. 1). The cochlear and vestibular nerves, together constituting the eighth cranial nerve, are intimately connected at their entrance into the brainstem, with the cochlear nerve situated caudal to the vestibular nerve (Fig. 4G–I). Within the nerve root, the cochlear nerve fibres bifurcate in
ascending and descending branches (Fig. 3E; Ramón y Cajal, 1911; Harrison and Irving, 1966a, their Fig. 1; Feldman and Harrison, 1969, their Fig. 2; see also 3.5). As seen in horizontal slices, the cochlear nerve root (Fig. 2G–I) spans the entire mediolateral extent of the ventral cochlear nucleus, completely separating the anteroventral and posteroventral cochlear nuclei. As the cochlear nerve root thins out dorsally, it keeps its contact with the medially situated vestibular nerve root (Fig. 2E, H, 3F). At the site of contact, the coronal and horizontal sMRI slices lack the thin darker band, presumably made up of trapezoid fibres, that otherwise separates the ventral cochlear nuclear complex from the medially situated vestibular nerve root (Fig. 2D, G). In DTI, the cochlear nerve root tends to appear blue in accordance with the ventrodorsal course of the main fibres (Fig. 2I). It is indistinctly delimited, however, both in sMRI and DTI, possibly due to the bifurcation and crossing of the fibres and the intermingled clusters of globular bushy cells (Osen et al., 1991, their Fig. 1). Our interpretation and delineation of the cochlear nerve was therefore aided by inspection of myelin-stained histological sections.

3.5. Cochlear nuclear complex (CNC)

The cochlear nuclear complex consists of a ventral and a dorsal nucleus. The ventral cochlear nuclear complex is flattened parallel to the lateral surface of the medulla (Fig. 1), with the dorsal end tilted about 35° medially (Fig. 2D–F) and the rostral end tilted about 20° laterally (Fig. 2G–I) with respect to the sagittal plane. The dorsal cochlear nucleus curves around the inferior cerebellar peduncle on the floor of the lateral recess of the fourth ventricle (Figs. 1, 4A–C). A 3D surface model of the cochlear nuclear complex and its main subdivisions is provided in Fig. 3 together with a diagram of the bifurcation of the cochlear nerve fibres and the tonotopic organization of their ascending and descending branches. This fibre pattern is to a large extent reflected in the sMRI and DTI features observed in the different subregions of the cochlear nuclear complex (see 3.5.1–3.5.5). As appears from Fig. 3E, the ascending branches form caudorostrally oriented parallel arrays in the anteroventral cochlear nucleus (rat: Harrison and Irving, 1966a). The descending branches, on the other hand, converge caudally and dorsally through the posteroverentral cochlear nucleus (rat: Harrison and Irving, 1966b) and into the dorsal cochlear nucleus where they become caudorostrally oriented, in parallel with the ascending branches in the anteroventral cochlear nucleus (cat: Osen, 1970; mouse: Muniak et al., 2013). Both in the ventral and dorsal cochlear nuclei, the fibres contact a number of different cell types, which in turn give rise to specific projections to higher auditory centres (review Cant and Benson, 2003). With the exception of the superficial granule cell layer (see 3.5.4), the cytologically defined areas of the rat ventral cochlear nucleus (Harrison and Irving, 1965, 1966a; 1966b) do not lend themselves for delineation in sMRI or DTI. These cells include the rostrally located spherical cells, the caudally situated octopus cells, and various cell types occurring both within and anterior and posterior to the nerve root, i.e., the globular cells, the T-stellate (multipolar) cells and the D-stellate (multipolar) cells (cat: Osen, 1969; for review, see, Cant and Benson, 2003). Most cells of the ventral cochlear nucleus project centrally through the trapezoid body (see 3.6.1), while the caudally located octopus cells and the scattered large D-stellate cells project dorsally through the acoustic striae (see 3.6.2) as do the fusiform (pyramidal) and giant cells of the dorsal cochlear nucleus (see 3.5.5) (Smith et al., 2005; Kecskes et al., 2013). Some cells also contribute to the reciprocal connection between the dorsal and ventral cochlear nuclei through the lateral tuberculoventral tract (see 3.5.3, 3.5.5). For overview of the component cell types of the mammalian cochlear nuclear complex and their central projections, see Osen (1969), Cant and Benson (2003), Malmierca (2015), and Oertel et al. (2018a, b).
3.5.1. Ventral cochlear nucleus, anterior part (AVCN)

The anteroventral cochlear nucleus is characterized in sagittal sMRI by slightly darker, caudorostrally oriented, parallel fascicles of ascending branches of the cochlear nerve fibres (Fig. 4D,G, arrowheads), as schematically shown in Fig. 3E, and also described in silver-stained sagittal sections by Harrison and Irving (1965, their Fig. 3). In DTI, the anteroventral cochlear nucleus is characterized by a mixture of colours, but with a predominance of green, apparently reflecting the ascending branches (Fig. 4F, I). Ventrally and particularly caudally there are many red voxels (Figs. 2F and 4I) that might relate to second order fibres projecting centrally through the trapezoid body. The difference between the dorsal and ventral parts in this regard may be related to the oblique orientation of the anteroventral cochlear nucleus, and its dorsal position with respect to the trapezoid body (Fig. 2E). In coronal myelin-stained sections, it is clearly seen that fibres projecting from the dorsal part course nearly vertically, while those from the ventral part course horizontally toward the trapezoid body. It should be noted that the red colour is most apparent close to the nerve root where there are many globular cells with remarkably thick, medially coursing axons (Harrison and Warr, 1962, their Figs. 2 and 4). Because of its oblique orientation with respect to all standard planes, a more complete view of the anteroventral cochlear nucleus and its topographic relation to the dorsal cochlear nucleus can be obtained in oblique sMRI and DTI slices (not shown). A 3D model of the anteroventral cochlear nucleus in lateral and medial views is provided in Fig. 3D,F.

3.5.2. Ventral cochlear nucleus, posterior part (PVCN)

The posteroventral cochlear nucleus has a more upright position than the anteroventral cochlear nucleus with its caudodorsal end tucked up underneath the dorsal cochlear nucleus (Figs. 1A and 3D,F). In sMRI, it exhibits a heterogeneous texture with medium signal intensities (Fig. 4A, D). In DTI maps, the rostral two thirds (near the cochlear nerve root) appear red, while the caudodorsal third exhibits an even brighter blue signal (Fig. 4F). The blue signal most probably represents the octopus cell area where the closely packed descending branches of the cochlear nerve fibres in the AVCN are marked by arrowheads. Borderlines are transposed from the segmentation onto sMRI and DTI as dashed lines. BS, brainstem; Cb, cerebellum; icp, inferior cerebellar peduncle; mcp, middle cerebellar peduncle; nVII, facial nerve; SG, spiral ganglion; sp5, spinal trigeminal tract; Sp5, spinal trigeminal nucleus; vn, vestibular nerve; tz, trapezoid body; 4 V, fourth ventricle (lateral recess). Scale bar: 1 mm.
Fig. 5. The trapezoid body and the superior olivary complex. (A,B,D) are from coronal slice no 376, with segmentation in (A), DTI in (B) and sMRI in (D). For orientation, level and DTI colour codes, see insets. The periolivary region (POR) has been delineated as one continuous field enveloping the lateral superior olive (LSO), the medial superior olive (MSO), the nucleus of the trapezoid body (NTB), the superior paraolivary nucleus (SPN), and the ventral nucleus of the periolivary region (VPO). Borderlines from A are transposed onto B and D as dashed lines. (C,E,F) are from a corresponding cell-myelin-stained histological section. (G) is a 3D model of the superior olivary complex seen from caudodorsal, with POR made transparent. BS, brainstem; nVII, facial nerve; py, pyramidal tract; rs, rubrospinal tract; tz, trapezoid body. Scale bars: 1 mm in A, also valid for B, D and G; 0.2 mm in E, also valid for F.

Fig. 6. The lateral lemniscus with nuclei. (A-C) are from coronal slice no. 420 presented as a triplet with sMRI to the left, segmentation in the middle, and DTI to the right. For orientation, level, and DTI colour codes see insets. The slice cuts through the long, dorsoventral axis of the lateral lemniscus (ll) with the ventral (VLL), intermediate (ILL) and dorsal (DLL) nuclei and the commissure (cll) of the lateral lemniscus, and the adjacent nucleus sagulum (Sag) of the descending auditory system. The borderlines in B are transposed onto A and C as dashed lines, together with the contours of the perilemniscal region (PL) of the descending auditory system and the neighbouring rubrospinal tract (rs) which have not been delineated. BS, brainstem; Cx, cerebral cortex; ECIC, inferior colliculus, external cortex; mcp, middle cerebellar peduncle; PN, pontine nuclei; py, pyramidal tract; sp5, spinal trigeminal tract; tfp, transverse fibres of the pons. Scale bar: 1 mm.
Fig. 7. The inferior colliculus in the coronal plane. Fields from coronal slices through the inferior colliculus (IC), no 435 (A–C) from its rostral part, and no 420 (D–F) from its rostrocaudal middle, presented as triplets with MRI to the left, segmentation in the middle, and DTI to the right. For slice orientation, levels and DTI colour codes, see insets. Dashed lines in sMRI and DTI correspond to the borderlines in the segmentation, while the dotted line indicates the intranuclear, wedge-like extension of the lateral lemniscus that has not been delineated. In (A–C), the larger part of the IC is made up of the external cortex (ECIC). In (D–F) the central nucleus (CNIC) appears enveloped by the ECIC and the dorsal cortex (DCIC). Note that in D–F the delineation of cerebellum is too narrow, here it should completely separate the 4th ventricle from the recessus of the IC, as indicated by a yellow dashed line. aq, aqueduct; bic, inferior colliculus, brachium; BS, brainstem; Cb, cerebellum; cic, inferior colliculus, commissure; Cx, cerebral cortex; PAG, periaqueductal grey; PrS, presubiculum; ReIC, recess of the inferior colliculus; 4V, 4th ventricle. Scale bar: 1 mm.

Fig. 8. The inferior colliculus in the sagittal plane. Fields from sagittal slices through the inferior colliculus (IC), no 284 (A–C) immediately medial to the dorsolateral border of the external cortex (ECIC) with the dorsal cortex (DCIC), and slice no 332 (D–F) from the lateralmost part of the ECIC with the brachium of the inferior colliculus (bic). For orientations, levels, and DTI colour codes, see insets. Dashed lines in sMRI (A,D) and DTI (C,F) correspond to the borderlines in the segmentation (B,E). In (A–C), the central nucleus (CNIC) is flanked dorsally by the DCIC while elsewhere it is surrounded by ECIC. At this mid-collicular sagittal level, the commissural fibres (red in DTI) are largely regarded as intranuclear and are therefore not delineated. In (D–F), the ECIC is covered by the brachium of the inferior colliculus (bic) which joins the medial geniculate body. afb, auditory forebrain bundle; Cb, cerebellum; Cx, cerebral cortex; DG, dentate gyrus; MGB, medial geniculate body, dorsal division; MGM, medial geniculate body, medial division; MGV, medial geniculate body, ventral division; SC, superior colliculus; Sub, subarachnoidal space. Scale bar: 1 mm.
which in the rat occur both anterior and posterior to the nerve root (Harrison and Irving, 1965, 1966b, their region II). An updated diagram of the cytoarchitecture of the cochlear nuclear complex in rat is lacking, but in guinea pig (Hackney et al., 1990, their Fig. 16), in contrast to cat (Osen, 1969), the entire posteroverentral cochlear nucleus except the caudal octopus cell area is rich in globular cells.

3.5.3. Ventral cochlear nucleus, cap area (cap)

The cap area constitutes a small-cell field dorsally and laterally in the ventral cochlear nucleus between the diverging ascending and descending branches of the cochlear nerve fibres, subjacent to the granule cell layer (Fig. 3C and D). It is relatively larger in cat (Osen, 1969) and human (Moore and Osen, 1979) than in rat, being classified by Doucet et al. (2009) as “a nexus of regulatory pathways within the auditory system”. The cap is supplied by collaterals of low spontaneous inner hair cell afferents (Liberman, 1993), and is connected reciprocally with the olivocochlear system (Osen et al., 1984; Ye et al., 2000). It contributes to the commissural acoustic stria (see 3.6.2; Smith et al., 2005; Doucet et al., 2009), and projects to the inferior colliculus and the medial division of the medial geniculate body (Malmierca et al., 2002).
The cap area also receives input from the somatosensory nuclei (rat: Weinberg and Rustioni, 1987; cat: Itoh et al., 1987), and the auditory cortex (Feliciano et al., 1995).

In sagittal sMRI, the cap area features as a homogeneous, medium intensity (grey) field that lacks the darker ascending and descending branches of the cochlear nerve fibres (Fig. 4D,G). It is, however, pierced by dorsoventrally oriented dark bands (Fig. 4D, arrows), apparently representing the reciprocal tubulocochlear tract (cat: Osen, 1988, her Fig. 4), which is composed of collaterals of T-stellate (multipolar) cells in the ventral cochlear nucleus and of tubulocochlear (vertical) cells in the dorsal cochlear nucleus (see 3.5.5; mouse: Wickers and Oertel, 1988, their Fig. 5; rat: Doucet and Ryugo, 1997; Munjak and Ryugo, 2014). In DTI, the cap area appears darker than the subjacent anteroventral cochlear nucleus, while lateral to the posterior part of the ventral cochlear nucleus it attains a pink colour (Fig. 4F) possibly reflecting the collaterals coursing laterally towards it from the subjacent branches of cochlear nerve fibres (Liberman, 1993).

3.5.4. Ventral cochlear nucleus, granule cell layer (GCL)

The granule cells form a superficial layer over the ventral cochlear nucleus and an incomplete lamina between the ventral and dorsal cochlear nuclei, while in the dorsal cochlear nucleus granule cells are located deep to the molecular layer together with the fusiform cells (Figs. 3 and 4; Mugnaini et al., 1980). All granule cells project unmyelinated, parallel fibres to the molecular layer of the dorsal cochlear nucleus. The granule cells are linked to the outer hair cells both by being the main target of afferents from these cells (Brown et al., 1988a) and by receiving collaterals of the olivocochlear efferent fibres to them (Osen et al., 1984; Brown et al., 1988b; Baasch et al., 2015). The absence of the granule cell granule cell system in cetacea (Osen and Jansen, 1965) and human (Moore and Osen, 1979) substantiates a different target of outer hair cell afferents in these species, probably in the compensating cap area. The granule cell layer appears homogeneously light grey in sMRI (Fig. 4A, D, G). The signal intensity is higher than in the granule cell layer of the cerebellum and the granular layer of the hippocampus, probably due to less dense packing of granule cell somata (see 2.4). In DTI, the granule cell layer is hardly discernible against the black background as the thickness of the layer approaches the voxel size, but it appears to be dark.

3.5.5. Dorsal cochlear nucleus (DCN)

The dorsal cochlear nucleus has a layered organization reminiscent of the cerebellum with a superficial molecular layer, a combined fusiform and granule cell layer, and a deep core. The lamination is visible both in sMRI and DTI, but with indistinct borders between layers (Fig. 4A–C). The relatively broad and indistinctly limited fusiform/granule cell layer is consistent with previous studies in albino rats (Mugnaini et al., 1980; Godfrey et al., 2016). In sMRI (Fig. 4A), the signal intensity decreases gradually from the light grey molecular layer, which is dominated by unmyelinated parallel granule cell axons, through the fusiform/granule cell layer, and the deep core to the almost black acoustic striae adjacent to the inferior cerebellar peduncle. In DTI (Fig. 4C,F), the superficial molecular layer appears dark while the fusiform-granule cell layer and particularly the deep core appear grey, in conformity with the caudorostral orientation of the descending branches of the cochlear nerve fibres (Fig. 3E; Munjak et al., 2013). The acoustic striae exhibit a bright red signal in agreement with the orientation of the fibres parallel to the dorsoventrally slanting long axis of the nucleus (Fig. 4C). Besides input from the descending branches of the cochlear nerve fibres and the granule cell layer ipsilaterally, the dorsal nucleus receives input from multipolar (T-stellate and D-stellate) cells of the ventral cochlear nucleus bilaterally (Doucet and Ryugo, 1997; Smith et al., 2005). The fusiform (pyramidal) cells and the deep core giant cells project fibres to the contralateral dorsal nucleus of the lateral lemniscus, the inferior colliculus, and the medial division of the medial geniculate body (cat: Osen, 1972; rat: Malmierca et al., 2002; Kelly et al., 2009), through the dorsal acoustic stria which can be traced both in sMRI and DTI as far as the genu of the facial nerve where it is lost to view (Fig. 1; see 3.6.2). The tubulocochlear (vertical) cells of the deep core (Wickers and Oertel, 1988; Osen et al., 1990; Munjak and Ryugo, 2014) contribute to the tubulocochlear tract, which in sMRI (Fig. 4D) can be seen passing through the cap area (see 3.5.3). A 3D reconstruction of the dorsal cochlear nucleus is seen from lateral in Fig. 3A–D and from ventromedial in Fig. 3F.

3.6. Central pathways from the cochlear nuclear complex

As mentioned above in the description of its subdivisions, the cochlear nuclear complex projects fibres centrally either along the ventrally located trapezoid body or along the dorsally located dorsal, intermediate or commissural acoustic striae. In contrast to the massive trapezoid body, the tiny striae are traceable in sMRI and DTI only in their first part where they are adjoined (see 3.6.2). The separate continuations of the striae are indicated by dashed lines in Fig. 1A according to Smith et al. (2005).

3.6.1. Trapezoid body (tb)

The trapezoid body is situated ventrally and superficially in the medulla, immediately caudal to the pons (Fig. 1). It originates from the ventral cochlear nucleus, with the exception of the superficial granule cell layer and the caudal octopus cell area. In sMRI, it appears dark with slightly higher signal intensities laterally where it crosses the vestibular nerve root (Fig. 2D–G, 4D), perhaps due to relatively more glial cytoplasm between the interlacing myelinated fibre fascicles (Feldman and Harrison, 1969, their Fig. 1). In DTI, the lateral part of the trapezoid body appears blue due to the dorsoventral orientation of the fibres, while the remaining part is bright red in accordance with their mediolateral orientation (Figs. 2F, 4F and 5B). In both sMRI and DTI, the trapezoid body is seen to have a laterocaudal extension (the descending bundle of Lorente de Nó, 1933) into the deep core of the dorsal cochlear nucleus (Osen, 1988, her Fig. 4), probably representing the ventral route of the commissural acoustic stria (see 3.6.2). Medial to the facial nerve root and the rubrospinal tract (rs in Fig. 5B, bright green in DTI, not delineated), the trapezoid body encompasses the superior olivary complex (Fig. 5). Although the trapezoid body supplies the nuclei, it has for technical reasons been delineated as surrounding rather than merging with the superior olivary complex (Fig. 1). Rostralateral to the superior olivary complex, the trapezoid fibres continue into the lateral lemniscus after a sharp rostroventral bend.

3.6.2. Acoustic striae (as)

Three separate pathways project dorsally from the cochlear nuclear complex: the dorsal acoustic stria (das), the commissural acoustic stria (cas), and the intermediate acoustic stria (ias). In sMRI and DTI, only the first common path of the three striae could be distinguished and delineated as a common narrow band, black in sMRI and red in DTI, deep to the dorsal cochlear nucleus on the dorsolateral aspect of the inferior cerebellar peduncle (Fig. 4A–C). The dorsal and commissural striae together were further traced and delineated underneath the floor of the fourth ventricle until the genu of the facial nerve root where they become fasciculated and are lost to view in sMRI and DTI. The intermediate acoustic stria, instead, bends ventrally immediately medial to the inferior cerebellar peduncle as seen in myelin-stained sections (not illustrated). The striae further cross through the reticular formation as widely dispersed fascicles, which were not traceable in MRI and DTI, and therefore not delineated. Their main course, however, has been indicated in Fig. 1 (dashed lines), as described by Smith et al. (2005), with the dorsal stria heading for the contralateral laterallemniscus and the inferior colliculus (and the medial geniculate body, Malmierca et al., 2002), the commissural stria reaching the opposite cochlear nuclear complex through a dorsal and ventral route, and the intermediate acoustic stria supplying the superior paralivary nucleus on both sides and heading for the contralateral ventral nucleus of the lateral lemniscus.
3.7. Superior olivary complex (SOC)

The superior olivary complex is embedded in the trapezoid body and comprises four main nuclei, the lateral superior olive (LSO), the medial superior olive (MSO), the nucleus of the trapezoid body (NTB), and the superior paraolivary nucleus (SPN), surrounded by a region of more scattered neurons, delineated as one continuous enveloping perioliolivary region (POR) with a ventral perioliolivary nucleus (VPO) (Fig. S; Osen et al., 1984; Ottersen et al., 1995, their Fig. 7). In sMRI, the nuclei appear brighter than the surrounding perioliolivary region (Fig. 5D), as in myelin-stained sections (Fig. 5C,E,F), while in DTI the strong red signals from the mediolaterally coursing trapezoid fibres tend to overshadow nuclear boundaries (Fig. 5B). The delineations were therefore based largely on the sMRI images. In horizontal sMRI as well as in 3D reconstructions (Fig. 5G), the nuclei appear as longitudinally arranged columns with their rostral end located slightly dorsal and lateral to their caudal end, in accordance with the overall direction of the ascending auditory pathway. The four main nuclei are primarily part of the ascending auditory pathway, whereas the ventral perioliolivary nucleus and certain cells in the lateral superior olive belong to the descending medial and lateral olivocochlear system, respectively (White and Warr, 1983; Warr et al., 1986). Fibres descending to the cochlea through the olivocochlear bundle course dorsally through the reticular formation as scattered fascicles that are not traceable in sMRI and DTI until they collect into a distinct bundle underneath the genu of the facial nerve root (rat: Baashar et al., 2015; Osen et al., 1984; cat: Warr et al., 1986, their Fig. 3). The olivocochlear bundle was therefore not delineated.

3.7.1. Lateral superior olive (LSO)

The lateral superior olive is situated caudolaterally in the complex (Fig. 5G), well positioned to receive ipsilateral input directly from spherical cells of the anteroventral cochlear nucleus (rat: Harrison and Irving, 1966a; cat: Smith et al., 1993). In sMRI images, the lateral superior olive appears brighter and more homogeneous than its surroundings, being most distinctly S-shaped in coronal slices from its rostrocaudal middle (Fig. 5D). In DTI, it appears dominated by green signals of a significantly lower intensity than the bright green signals of the rostrocaudally directed rubrospinal tract that is situated immediately lateral to it (rs in Fig. 5B, not delineated). The fibre orientation patterns reflected in DTI and PLI are consistent with the existence of rostrocaudally oriented isofrequency laminae composed of flattened dendritic arbours and anisotropic efferent fibre plexa oriented orthogonal to the curved mediolateral frequency axis of the nucleus (Rietzel and Friauf, 1998, their Fig. 3), as visible also in cell-melanin-stained sections (Fig. 5E). The DTI purple colour ventrally in the lateral limb may signify the horizontal orientation of the laminae in this site, if not fascicles of trapezoid fibres passing through the limb parallel to the laminae as indicated in PLI (not illustrated). The ventral hilus is blue in DTI (Fig. 5B) in accordance with the vertical orientation of the dense bundle of afferents seen in myelin-stained sections (Fig. 5E).

3.7.2. Medial superior olive (MSO)

The medial superior olive is situated ventrally in the mediolateral middle of the complex. It is composed largely of densely packed, mediolaterally oriented bipolar cells (Smith, 1995, his Fig. 1), which receive bilateral input from the spherical cells of the ventral cochlear nucleus, the lateral dendrites from the ipsilateral side, and the medial dendrites from the contralateral side (rat: Harrison and Irving, 1966a; cat: Smith et al., 1993). In coronal sMRI slices, the medial superior olive appears as a short, dorsoventrally oriented rod with very bright signal, flanked medially and laterally by a zone of lower signal intensity (Fig. 5D). In DTI, the rod appears black with red voxels scattered in the adjacent areas (Fig. 5B). The centre evidently represents the densely packed perikarya, while the adjacent zones reflect the plexus of the medially and laterally extending dendrites and their trapezoid afferents. By tradition, only the central part with the perikarya is defined as the nucleus proper. In 3D reconstruction (Fig. 5G), the medial superior olive appears as a slightly curved, evenly thick rod reaching from the caudal to the rostral end of the complex with the rostral end situated more dorsal and lateral than the caudal end.

3.7.3. Superior paraolivary nucleus (SPN)

The superior paraolivary nucleus, being typical for rodents, is situated dorsally in the complex between the lateral superior olive and the nucleus of the trapezoid body, well positioned to receive bilateral input from octopus cell axons in the intermediate acoustic stria (see 3.6.2) as this crosses the midline in the reticular formation dorsal to the superior olivary complex (Smith et al., 2005). The superior paraolivary nucleus is indistinct both in sMRI and DTI, but slightly more distinct in myelin staining (Fig. 5C). In sMRI, it appears with slightly higher signal intensity and as a more homogeneous structure than its surroundings (Fig. 5D). In DTI, it is dominated by dark blue and green voxels possibly reflecting its anisotropic organization with sagittally oriented isofrequency laminae (Fig. 5B; Saldana and Berrebi, 2008). In 3D reconstructions (Fig. 5G), it forms a column largely at the same rostrocaudal level as the lateral superior olive, but reaching slightly more caudally and rostrally.

3.7.4. Nucleus of the trapezoid body (NTB)

The nucleus of the trapezoid body is situated dorsomedially in the superior olivary complex. It is composed of glycinergic cells interposed in rows between the thick fascicles of trapezoid fibres immediately dorsolateral to the pyramidal tract (Ottersen et al., 1995, their Fig. 7). Being optimally located for their task, these cells convert excitatory signals from the globular cells of the contralateral ventral cochlear nucleus (Harrison and Irving, 1966a; Smith et al., 1991) to inhibitory signals directed to the remaining nuclei of the ipsilateral superior olivary complex, notably the lateral superior olive and the superior paraolivary nucleus (Banks and Smith, 1992), as well as the ipsilateral ventral and intermediate nuclei of the lateral lemniscus (Kelly et al., 2009). In both sMRI and DTI, the nucleus appears indistinctly delimited. In sMRI (Fig. 5D), it can be distinguished from its surroundings as a mixture of voxels of contrasting intensities varying from bright to dark probably reflecting the rows of cells between the fibre fascicles seen in cell-myelin staining (Fig. 5F). In DTI (Fig. 5B), it exhibits various shades of dark purple, the bluish tinge probably reflecting the dorsal bend of the trapezoid fibres, while the darker voxels might indicate the cell rows.

3.7.5. Periolivary region (POR)

Between and around the main nuclei of the superior olivary complex there are scattered neurons collectively delineated as the periolivary region (Fig. 5). Various periolivary nuclei have been described by others, but we were only able to distinguish the ventral periolivary nucleus (see 3.7.6). In DTI, the periolivary region exhibits a mixture of bright red and green signals (Fig. 5B) probably representing crossing trapezoid fibres of various origins. Rostrally, at the level of the caudal end of the pons, it is directly contiguous with the ventral nucleus of the lateral lemniscus that appears darker in DTI. The border with the ventral nucleus of the lateral lemniscus was delineated in horizontal DTI slices, aided by inspection of thick horizontal thionine-stained sections.

3.7.6. Ventral periolivary nucleus (VPO)

The ventral periolivary nucleus is located ventral to the medial and lateral superior olive. It contains large AChE-positive cells that receive input from the inferior colliculus (Faye-Lund, 1986; Caicedo and Herbert, 1993) and the auditory cortex (Feliciano et al., 1995), and project fibres dorsally through the olivocochlear bundle to the outer hair cells (White and Warr, 1983; Warr et al., 1986), with collateral to the granule cell regions and cap area of the cochlear nuclei (Osen et al., 1984; Baashar et al., 2015). Other cells project to the cochlear nuclear complex via the trapezoid body (Sherriff and Henderson, 1994). We were not able to subdivide the ventral periolivary nucleus or to trace the olivocochlear bundle as it courses dorsally in fascicles through the reticular formation.
In sMRI (Fig. 5D), the ventral periolivary nucleus appears as an indistinctly delimited band of medium signal intensity, in general slightly brighter than the periolivary region. Its ventral border coincides approximately with the delineated border of the periolivary region. In DTI, the ventral periolivary nucleus is not distinguishable from the rest of the periolivary region (Fig. 5B). Our delineation of the ventral periolivary nucleus does not cover the more rostrally located olivocochlear neurons registered by Faye-Lund (1986) and others.

3.8. Lateral lemniscus (ll)

The lateral lemniscus, which encloses three elongated nuclei, continues dorsally and rostrally from the trapezoid body and the superior periolivary region, linking the cochlear nuclei and the superior olivary complex to the inferior colliculus (Fig. 1). It contains both ascending and descending fibres. The ascending fibres, which are in the majority, originate from the cochlear nuclei, mainly contralaterally, and the superior olivary complex, mainly ipsilaterally, terminating largely in the lemniscal nuclei and the central nucleus of the inferior colliculus (Kelly et al., 2009), with some fibres also heading for the medial geniculate body (Malmierca et al., 2002). Descending fibres originate in the auditory cortex and the inferior colliculus, terminating in the periolivary region and the superior olivary and cochlear nuclear complexes (Faye-Lund, 1986; Feliciano et al., 1995; Saldana, 2015). All three lemniscal nuclei contribute fibres to the lemniscus with targets in the ipsilateral central nucleus of the inferior colliculus (Kelly et al., 2009). The dorsal nucleus in addition projects to its counterpart and the central nucleus of the inferior colliculus on the opposite side via the commissure of the lateral lemniscus (see 3.8.2.). In sMRI (Fig. 6A), the peripheral fibrous sheath exhibits medium signal intensities, while in DTI (Fig. 6C), it is blue in accordance with the vertical orientation of the fibres. Ventrally, where the trapezoid fibres make a sharp rostrodorsal turn into the lemniscus, the sheath features lighter blue signals and nuances of green. At this ventral level, the lemniscus exhibits a C-shaped cross section, surrounding the ventral nucleus of the lateral lemniscus only rostrally and laterally. More dorsally, the cross section becomes larger and tube-like with the nuclei closer to the centre.

3.8.1. Nuclei of the lateral lemniscus (VLL, ILL, DLL)

The ventral (VLL), intermediate (ILL) and dorsal nucleus (DLL) of the lateral lemniscus appear indistinctly delimited towards the fibrous sheet both in sMRI and DTI, apparently because of intermingling of cells and fibres. Given the C-shaped distal part of the fibre sheet, the ventral nucleus is directly contiguous caudally and medially with the superior periolivary region and the reticular formation (Fig. 1B and C; see 3.7.5 and 3.8). In sMRI (Fig. 6A), the nuclei appear somewhat lighter than the fibre sheath, while in DTI (Fig. 6C), the opposite is true with the nuclei featuring a mixture of black and dark blue, red and green voxels against the brighter blue sheath. At the site where the ventral nucleus is in direct contact with the reticular formation, both DTI and myelin-stained histological data are indicative of fibres coursing between the two (not illustrated). These fibres could possibly belong to the intermediate acoustic stria, which terminates in the ventral nucleus of the lateral lemniscus (see 3.6.2). The level of the dorsal nucleus is recognizable by its relationship with the commissure of the lateral lemniscus, but the borders between the three nuclei appear indistinct, and have therefore been extrapolated from earlier studies (Paxinos and Watson, 2007; Kelly et al., 2009).

3.8.2. Commissure of the lateral lemniscus (cil)

In sMRI, the commissure of the lateral lemniscus, which projects to the contralateral dorsal nucleus of the lateral lemniscus, and the central nucleus of the inferior colliculus (Kelly et al., 2009), can be traced as scattered dark fascicles (Fig. 6A, between arrows) extending medially from the dorsal nucleus. In DTI (Fig. 6C), the fibres appear bright red when organized in coarse fascicles, but they are lost to view in the superior cerebellar peduncle and hence cannot be traced over the midline. The fascicles are delineated as one compact structure that is truncated medially where they are lost to view (Fig. 6B).

3.8.3. Nucleus sagulum (Sag)

The nucleus sagulum fills the gap between the pia and the lateral lemniscus at the level of the dorsal lemniscal nucleus (Fig. 6). It receives direct input from the auditory cortex and projects fibres to the medial and dorsal divisions of the medial geniculate body (rat: Feliciano et al., 1995; Saldana, 2015; cat: Beneyto et al., 1998). Thin fibres are in myelin-stained sections seen to course between the sagulum and the inferior colliculus, consistent with previous observations (Caicedo and Herbert, 1993; Beneyto et al., 1998). In sMRI (Fig. 6A), the nucleus sagulum exhibits a medium grey homogeneous texture, while in DTI (Fig. 6C) it differs from its surroundings by being dominated by green voxels. The perilemniscal region (PL), which likewise is part of the descending auditory system (Feliciano et al., 1995; Saldana, 2015), is situated medial to the lateral lemniscus, both dorsal and ventral to the rubrospinal tract (rs). It is not delineated, but indicated by dashed lines in Figs. 1A and Fig. 6A–C.

3.9. Inferior colliculus (IC)

As illustrated in Fig. 1, the egg-shaped inferior colliculus constitutes the largest subcortical relay centre of the auditory pathway. It consists of three main subregions: the central nucleus (CNIC), the external cortex (ECIC), and the dorsal cortex of the inferior colliculus (DCIC), surrounded by a thin fibre capsule which is regarded as part of the subjacent cortices (Figs. 7–9). The central nucleus is part of the tonotopically organized auditory core projection, while the cortices contribute to the auditory belt projection which integrates auditory, visual and somatosensory stimuli. Regarding the pattern of ascending projections, we refer (with reservations for species differences and uncertainties regarding subdivision homologies and boundaries) to the detailed study of Calford and Atkin (1983) in cat, supplied by the less detailed study of LeDoux et al. (1985) in rat (see also review by Malmierca, 2015). The main afferent and efferent pathways of the inferior colliculus, i.e., the lateral lemniscus and the brachium of the inferior colliculus, respectively, are both connected to its ventral base, while the connection between the two sides takes place through the commissure of the inferior colliculus, which is located dorsoventrally (Fig. 9). At caudal levels (not illustrated), the dorsal cortex of the two sides are also directly contiguous around the recess of the inferior colliculus. In sMRI, the darker capsule appears distinct, while the interior seems uniformly grey, apart from the horizontally oriented extensions of the commissural fibre fascicles in the rostral part of the external cortex, and the vertically positioned wedge-like extension of the lateral lemniscus within the lateralmost part of the central nucleus (Figs. 7 and 8), neither of which have been delineated. Within the wedge (indicated in Fig. 7 by dotted lines), the lemniscal fibres gradually turn dorso-medially as an integral part of the isofrequency laminae of the central nucleus (Fig. 1A). The sharp lateral border of the wedge coincides with the lateral borderline of the central nucleus, while its pointed end indicates the mediolateral position of the border between the external and dorsal cortex (Fig. 7E). These sMRI features were in agreement with observations from myelin-stained sections. Otherwise, the identification and delineation of subdivision boundaries were based on DTI, aided by cytoarchitectural subdivision schemes derived from earlier Golgi studies in rat (Faye-Lund and Osen, 1985). In Golgi material, the central nucleus differs from the cortices by the presence of certain neurons with flattened dendritic arbours oriented in parallel with the isofrequency laminae (Malmierca et al., 1993). In tracer studies in rat, the terminal field of both descending fibres from the auditory cortex and the intrinsic fibres from the inferior colliculus on both sides follow the same laminar pattern, but in these cases the terminal laminae extend throughout the entire inferior colliculus (Saldana and Merchan, 1992, their Fig. 12; Saldana et al., 1996), continuing into the
dorsal cortex and the rostral portion of the external cortex with the same orientation as in the central nucleus, while in the lateral part of the external cortex they become almost vertically oriented after a sharp, dorsally open bend at the border of the central nucleus. In rat, in contrast to cat (Osen, 1972), the lemniscal fibres from the ventral and dorsal cochlear nuclei may contribute to the isofrequency laminae in the lateral part of the external cortex as well as in the central nucleus (Lotus et al., 2008; not illustrated in Fig. 1A).

3.9.1. Inferior colliculus, external cortex (ECIC)

The external cortex of the inferior colliculus envelopes the central nucleus laterally, rostrally, caudally and ventrally (Figs. 1 and 9). It is part of the multisensory belt projection, with input from various sources and projection to among others the medial and dorsal divisions of the medial geniculate body (cat: Calford and Aitkin, 1983), and the superior olivary complex (Faye-Lund, 1986; Caicedo and Herbert, 1993). In DTI, the superficial fibre capsule appears green laterally where fibres may be oriented rostrocaudally (Fig. 7F), while it tends to be red on the caudal surface of the inferior colliculus, where fibres may take a mediolateral course. Laterally and caudally, the deeper layers of the external cortex feature a predominantly blue colour (Fig. 7F). This was to be expected from the nearly vertical orientation of the isofrequency laminae (see 3.9).

In myelin-stained and PLI sections, however, the majority of vertically oriented fibres are directed across the isofrequency laminae from dorsomedial to ventrolateral as if heading for the lateral lemniscus and/or the brachium. In DTI, the deep layers of the lateral external cortex become greener rostrally, apparently reflecting a larger contingent of rostrocaudally oriented fibres approaching the brachium (Fig. 7C). Ventrally, the external cortex is penetrated by the beginning of the lemniscal wedge. Medial to the wedge, the lateral cortex exhibits red signals apparently corresponding to both the superficial capsule and some deeper mediolaterally oriented fibres (Fig. 7F). These signals should not be confused with the intensely red signals from the pericolumnar tegmentum and the trochebral nerve that courses laterally immediately caudal to the base of the inferior colliculus. Rostral to the central nucleus, the external cortex is closely related to the commissure of the inferior colliculus, which may explain the larger contingent of red voxels in DTI (Figs. 7C and 8C). This is also in agreement with myelin-stained and PLI sections, in which the rostral part is dominated by mediolaterally oriented fibre fascicles clearly related to the commissure (not illustrated). A large proportion of the commissural fibres continues laterally and bends ventrally into the lateral part of the external cortex.

3.9.2. Inferior colliculus, dorsal cortex (DCIC)

The dorsal cortex of the inferior colliculus covers the dorsal end of the central nucleus (Figs. 1 and 9). It receives input from the primary auditory cortex (Faye-Lund, 1985; Saldana et al., 1996), largely through the auditory forebrain bundle (see 3.13), and projects fibres to the dorsal division of the medial geniculate body (cat: Calford and Aitkin, 1983), both fibre categories passing through the brachium of the inferior colliculus. In DTI (Figs. 7F and 8C), the dorsal cortex is characterized by bright green signals dorsal to the central nucleus both in the superficial fibre capsule and the deeper tissue, with the addition of a small amount of bright red voxels, both in the capsule and below. The red voxels probably reflect the restricted number of fibres crossing the midline at this rostrocaudal level (Fig. 7F).

One unproven explanation for the green signals is that they reflect rostrocaudally oriented afferent and efferent fibres relative to the brachium. In DTI, fibres were directed medially towards the subjacent central nucleus was arbitrarily set at the ventral border of the continuously green area, while the lateral border with the external cortex was guided by the top point of the wedge of the lemniscal fibres (Fig. 7E). The dorsal cortex also extends somewhat ventrally along the medial and caudal aspects of the central nucleus, with a blue tinge in DTI (Figs. 7F and 8C). The border with the caudal part of the external cortex was arbitrarily set more dorsally than in the subdivision scheme of Faye-Lund and Osen (1985). The colour shift from green dorsally to blue caudally fits observations in myelin-stained sections where the dorsal cortex exhibits a considerable amount of fibres coursing parallel to the surface in the parasagittal plane, i.e., with rostrocaudal orientation at dorsal levels, shifting to dorsoventral at caudal levels. The reciprocal connection with the sagulum (see 3.8.3; Caicedo and Herbert, 1993; Beneyto et al., 1998) may also contribute to such fibres.

3.9.3. Inferior colliculus, central nucleus (CNIC)

The central nucleus of the inferior colliculus is flattened rostrocaudally, being slightly curved like an erythrocyte with the concavity pointing rostrally and slightly mediadorsally (Fig. 9). As part of the tonotopically organized auditory core projection, it receives input from both lower and higher auditory regions and projects fibres to the ventral division of the medial geniculate body with minor projections to the medial division (rat: LeDoux et al., 1985; cat: Calford and Aitkin, 1983). In DTI, it exhibits a mixture of red and green voxels, with a gradual shift from a majority of red voxels ventrolaterally, to a majority of green voxels dorsomedially (Fig. 7F). It is tempting to speculate that the red voxels signify the dorsomedially slanting lemniscal afferents in the isofrequency laminae (Faye-Lund and Osen, 1985; Malmierca et al., 1993; Saldana et al., 1996; Lotus et al., 2008). Unexpectedly, red voxels also dominate within the lateral wedge of lemniscal fibres. Here the expected blue DTI signals from the ventrodorsally oriented fibres must be disrupted by the fibres bending medially into the isofrequency laminae, which seem to start already within the wedge. The wedge appears blue only in the very rostrolateral part of the nucleus where the lemniscal fibres may be heading for the dorsalmost laminae (Fig. 7C). The source of the green voxels is not clear, but it might be pertinent that the laminae are connected with their counterparts on the opposite side through the commissure (Saldana and Merchán, 1992), and with the higher auditory centres through the brachium, both pathways being located rostral to the central nucleus (Saldana et al., 1996). This could entail a rostrocaudal orientation of the related fibres in the central nucleus. The gradual relative increase of green voxels in the dorsomedial direction would match the stronger corticocollicular input to dorsal levels including the dorsal cortex (Saldana et al., 1996). Fibres entering or leaving the central nucleus from rostral might also be responsible for the blurring of the rostral border with the external cortex which has been set somewhat arbitrarily, aided by the dendritic anisotropy of the central nucleus in Golgi material (Faye-Lund and Osen, 1985; Malmierca et al., 1993) and the scarcity of fibre fasciculation in myelin-stained and PLI sections (not illustrated). In the delicate anisotropic web of fibres and flattened dendritic arbours in the central nucleus there may be little room for fasciculated fibres.

3.9.4. Inferior colliculus, commissure (cic)

The commissure of the inferior colliculus is situated dorsally and rostrally at the border with the superior colliculus (Fig. 9). It appears distinct both in sMRI and DTI (Fig. 7A, C), being dark grey in sMRI and bright red in DTI. Fascicles of commissural fibres can be traced laterally through the rostral part of the lateral cortex, bending ventrally into the lateral part. The intracollicular parts of the fascicles have not been delineated. The tectal longitudinal column (Saldana et al., 2007) within the commissure could not be identified.

3.9.5. Inferior colliculus, brachium (bic)

The brachium of the inferior colliculus can be seen in sMRI as a gradual thickening of the superficial dark fibre capsule, eventually forming a gutter-like fibre tract covering the rostroventral extension of the external cortex laterally, and ending up on the medial side of the medial geniculate body (Fig. 8D–F, 9). At the level of the medial geniculate body, the brachium splits into fascicles that gradually enter the medial division of the medial geniculate body, as apparent also in myelin-stained sections. The brachium conveys fibres from the inferior colliculus to all parts of the medial geniculate body, as well as recurrent fibres to the inferior colliculus from the medial division of the medial geniculate body.
The ventral and dorsal divisions make up the larger part of the medial geniculate body. The ventral division is tonotopically organized with afferent projections largely from the central nucleus of the inferior colliculus and efferent projections to the primary auditory cortex. The dorsal division, like the medial division, is part of the auditory belt projection with input largely from the cortical regions of the inferior colliculus and projection to the non-primary auditory cortex (Calford and Aitkin, 1983; reviewed by Malmierca, 2015). The border between the two divisions is indistinct both in MRI and DTI and has been set according to Clerici and Coleman (1990, 1998; Fig. 1) as slanting slightly from ventromedial to dorsolateral (Fig. 10B). In sMRI, the dorsal and ventral divisions exhibit continuous shades of grey (Fig. 10A, D). According to Clerici and Coleman (1990), the ventral division is richer in myelin, with myelinated fibres constituting the so-called midgeniculate bundle at the subdivision boundary. In sMRI, some scattered dark spots consistent with myelinated fibres were found in the ventral part, but a midgeniculate bundle was not discernible. In DTI (Fig. 10C, F), both divisions show a mixture of green, blue and red voxels, with the relative number of green voxels increasing rostrally, possibly representing rostrocaudally oriented fibres connecting to higher order centres through the rostrally emerging auditory radiation (see 3.11). The subnuclei of the two divisions, as described by Clerici and Coleman (1990), could not be distinguished.

3.11. Auditory radiation (ar) and the reticular thalamic nucleus, auditory segment (RTa)

As evident from anterograde and retrograde tracer studies in rat (see e.g. Saldana et al., 1996) and the mouse brain connectivity database shared through the Allen Brain Atlas data portal (Oh et al., 2014; http://connectivity.brain-map.org/, experiment 120491896), the medial geniculate body is reciprocally connected with the auditory cortex through the auditory radiation (ar). The radiation appears dark in sMRI (Fig. 10D), green in DTI (Fig. 10F), and can be traced from the rostro-lateral edge of the medial geniculate body, through the superior thalamic radiation to the auditory segment of the reticular thalamic nucleus which it penetrates before being lost to view within the caudal part of the internal capsule (Fig. 10E). In sMRI, the reticular thalamic nucleus is hard to distinguish (Fig. 10D), particularly the auditory segment, which was arbitrarily delineated by extrapolation from the coordinates of electrophysiological rat studies (Shosaku and Sumitomo, 1983), according to which it is situated in the caudal 1 mm of the nucleus ventral to the visual segment, reaching slightly caudal to the rostral pole of the dorsal lateral geniculate body, and 0.7–0.8 mm away from the rostral tip of the medial geniculate body.

3.12. Auditory cortex (Au1, AuD, AuV)

The auditory cortex is situated in the temporal region of the cerebral cortex, occupying the lateralmost part of the brain (Fig. 1). Several alternative parcellation schemes have been proposed on the basis of cytoarchitectonic criteria, differences in myelination and neurotransmitter densities, and connectivity (reviewed by Malmierca, 2015, who concluded that the issue was still unresolved). In sMRI and DTI, the temporal cortex presents no obvious criteria for segmentation. To nevertheless include the auditory cortex in the present atlas, we here chose to base our delineation on the rat brain atlas of Paxinos and Watson (2007). In this parcellation, the auditory cortex consists of three parallel, horizontally oriented areas, with the primary auditory cortex (Au1) in

(Senatorov and Hu, 2002). Immediately caudal to the level of the medial geniculate body, the brachium is joined by the auditory forebrain bundle (Clerici and Coleman, 1990), which branches off from the auditory corticopeduncular tract (see 3.13) and leads corticothalamic fibres to the inferior colliculus (afb in Figs. 1A, 8-D; Saldana et al., 1996). In DTI, the brachium is dominated by green voxels, reflecting the rostrorocaudal component of the fibre orientation (Fig. 8F). The rostroventral extension of the lateral cortex deep to the brachium apparently corresponds to the nucleus of the brachium of the inferior colliculus defined by others (see e.g. Paxinos and Watson, 2007), but we found no sMRI or DTI criteria to substantiate such a distinction.

3.10. Medial geniculate body (MGB)

The medial geniculate body is situated ventrorostral to the inferior colliculus (Figs. 1 and 9). As the obligatory thalamic relay in the ascending auditory pathway to the neocortex and other subcortical structures, it is intercalated between the brachium of the inferior colliculus that joins it from medial, and the auditory radiation that protrudes from its rostrolateral edge (Fig. 10). It is slightly elongated rostrocaudally with flattened medial and rostral borders and a convex lateral and caudal surface. The caudal half protrudes on the lateral side of the brainstem, while the rostral half is covered laterally by the lateral geniculate body (Fig. 10E). It is composed of a marginal zone (MGMZ), and a medial (MGM), ventral (MGV) and dorsal division (MGD) (Fig. 10B-E; Clerici and Coleman, 1990, 1998; Winer et al., 1999). The ventral division is part of the tonotopically organized auditory core projection, the others of the multisensory belt projection. According to Clerici and Coleman (1990), the subdivisions are distinguishable in rat by their myeloarchitecture, with the medial division containing most myelin and the dorsal division the least. In sMRI (Fig. 10A, D), the medial geniculate body, in harmony with myelin-stained sections, is surrounded by a dark brous capsule, the external capsule (Fig. 10E). In DTI, all details are overshadowed by green signals (Fig. 10C, F), probably in part reflecting caudorostrally orientated fibres reciprocally interconnecting each of the three divisions with the auditory cortex (Clerici and Coleman, 1990).

3.10.1. Medial geniculate body, marginal zone (MGMZ)

In sMRI (Fig. 10A, D), the medial geniculate body, in harmony with myelin-stained sections, is surrounded by a dark fibrous capsule, the marginal zone, laterally, dorsally and ventrally. In DTI, the blue colour of the vertical parts of the zone caudally and laterally and the red colour of its horizontal parts dorsally and ventrally indicate a circumferential orientation of fibres (Fig. 10C, F).

3.10.2. Medial geniculate body, medial division (MGM)

The medial division makes up the medial side of the medial geniculate body. Our delineation probably also includes the suprageniculate nucleus (Clerici and Coleman, 1990, 1998). Being part of the multisensory auditory belt projection, the medial division is the main target of the ascending projection from the external cortex of the inferior colliculus (Calford and Aitkin, 1983) to which it is reciprocally connected (Senatorov and Hu, 2002). It projects fibres to the non-primary auditory cortex, but as the adjacent thalamic regions, it also has reciprocal connections with many subcortical structures such as the caudate putamen and the amygdala (LeDoux et al., 1985; Ottersen and Ben-Ari, 1979). In thionine-stained sections, the medial division is characterized by particularly large neurons. In sMRI, it is dominated by dark fibre fascicles that appear crosscut in coronal slices (Fig. 1A), while in horizontal slices (Fig. 10D), the fascicles appear to bend into it from the brachium of the inferior colliculus, from which it is only indistinctly delimited in agreement with findings in myelin-stained sections. The border with the ventral and dorsal divisions appears jagged probably reflecting fascicles of brachial fibres traversing the borderline. In DTI (Fig. 10C, F), the medial division exhibits massive green signals like the brachium. The medial division shares many of its multisensory connections (Linke, 1999) with the multimodal posterior intralaminar nucleus (Doron and Ledoux, 2000, their Fig. 2), which is located immediately ventromedial to it. This nucleus has not been delineated.
the middle flanked by the secondary auditory cortex dorsal (AuD) and the secondary auditory cortex ventral (AuV). The three areas were topographically redrawn by combining information from Paxinos and Watson (2007) atlas diagrams spatially registered to the sMRI template, and measurements of distances from the rhinal fissure and the earlier delineated perirhinal regions (Kjonigsen et al., 2015). The external part of the auditory radiation, in the internal capsule between the reticular thalamic nucleus and the neocortex, could not be distinguished in sMRI and DTI, but its approximate position is indicated by stippling in Fig. 1A, guided by previous tracer studies in rat (Saldana et al., 1996, their Fig. 3).

3.13. Auditory corticopeduncular tract (acp)

The auditory corticopeduncular tract is the auditory part of the descending corticofugal pathways (see 3.14.4) and has been delineated as part of the latter (Fig. 10B). As indicated in Fig. 1A, it originates in the auditory cortex, but takes off from the main auditory radiation to descend in the caudodorsal part of the internal capsule/cerebral peduncle, bypassing the thalamic reticular nucleus and the medial geniculate body (Saldana et al., 1996, their Fig. 3). Ventral to the medial geniculate body, the fibres en route to the inferior colliculus take off from the cerebral peduncle as the auditory forebrain bundle (afb; Clerici and Coleman, 1990, their Figs. 1 and 7) which reaches the inferior colliculus through the brachium of the inferior colliculus (Fig. 8D–F, 10A–C; see, also 3.9.5). According to Feliciano et al. (1995) and Saldana (2015), the remaining corticofugal auditory fibres continue caudally in the cerebral peduncle, some joining the lateral lemniscus at the pontine level and reaching the perilenmisal regions, the nucleus sagulum and also the inferior colliculus. Others continue distally to supply among others the superior olivary complex and the cochlear nuclei. We here did not delineate the auditory corticopeduncular tract as a separate structure, but rather expanded the earlier delineation of the collective structure named the descending corticofugal pathways to include the tract with the auditory forebrain bundle as a side branch of the cerebral peduncle (Fig. 10B). Besides this, we delineated the sagulum and indicated the existence of the perilenmisal region (PL) by dashed lines in Fig. 1A, both belonging to the descending auditory pathway.

3.14. Non-auditory structures

Delineations of 12 non-auditory structures adjacent to the auditory regions were added (vestibular apparatus, vestibular nerve, 4th ventricle), adjusted (facial nerve, descending corticofugal pathways, granule cell layer of the cerebellum, ventricular system, periventricular grey, spinal trigeminal tract), or partly replaced and revised (brainstem, thalamus, neocortex) as part of the present atlas version, as indicated in Table 1. Only the delineations of new or substantially revised structures are commented below. The vestibular apparatus, comprising the vestibule and semicircular canals, is mentioned above along with the cochlea (3.3). For the remaining structures, adjustments concern minor corrections or partial replacements of large collective structures by new subdivisions, and are not further commented.

3.14.1. Vestibular nerve (vn)

The vestibular nerve is intimately connected to the rostral side of the cochlear nerve upon entering the medulla medial to the anteroventral cochlear nucleus (Figs. 2 and 4). Ventral to the latter, the vestibular nerve appears red in DTI, reflecting the lateromedial course of the fibres (Fig. 4). After its entrance into the brainstem, the vestibular nerve continues dorsocaudally through the trapezoid body between the ventral cochlear nucleus and the spinal tract of the trigeminal nerve to the region of the vestibular nuclei (Fig. 2E, H), in DTI exhibiting various shades of blue, lilac and red in harmony with its curved path (Fig. 2F, I). In sMRI, the otherwise dark vestibular nerve appears lighter and spotted grey in the region where it interweaves with the trapezoid body (Fig. 2D, G).

3.14.2. 4th ventricle (4v)

In the first version of the atlas (Papp et al., 2014), the 4th ventricle was delineated as part of the ventricular system on the basis of sMRI signals varying from white to light grey due to the presence of the contrast agent. For the present version of the atlas, the 4th ventricle was redefined as a separate structure. It is situated ventral to the cerebellum, and dorsal to the brainstem and the periventricular grey. Rostrally it connects to the third ventricle via the cerebral aqueduct, and caudally it continues as the central canal of the spinal cord. The ventricle is covered dorsally by the tela choroidea, which has a caudal opening to the subarachnoidal space. The lateral recess with the choroidal plexus extends laterally between the cerebellar folliculus dorsally and the dorsal and ventral cochlear nuclei ventrally, having a wide lateral opening to the subarachnoidal space (Figs. 2E and 4B, E, H).

3.14.3. Facial nerve (nVII)

The facial nerve emerges laterally from the medulla and exits the skull ventral to the vestibular and cochlear nerve. We extended the original delineation of the facial nerve on the basis of its bright red appearance in DTI to include its trajectory from the genu through the trapezoid body and out of the brain stem (Fig. 5B). On the right side, it has been traced laterally through the subarachnoidal space (Fig. 4D–I) and the geniculate ganglion to its exit from the temporal bone (not illustrated).

3.14.4. Descending corticofugal pathways

The delineation of the descending corticofugal pathways (a collective white matter structure comprising successively the: internal capsule, ic; cerebral peduncle, cp; longitudinal fibres of the pons, lfp; pyramidal tract, py) was corrected both at the level of the medial geniculate body, where the auditory cerebral peduncle (acp), composed of descending auditory fibres, was added with the auditory forebrain bundle (afb) of Clerici and Coleman (1990) as a side branch (Fig. 10B, see 3.13), and at the level of the trapezoid body, where the cross-section of the pyramids was corrected (Fig. 5A).

4. Discussion

We have added 40 new structure delineations to version 2 of the Waxholm Space atlas of the Sprague Dawley rat brain and revised the delineation of 10 structures on the basis of observations made in the sMRI/DTI template, aided by microscopic studies of myelo- and cytoarchitecture, and earlier publications describing histological boundaries, neuronal morphologies (light microscopic level), and axonal connections. The new version 3 of the atlas now includes delineations of 38 auditory structures, and a total of 118 structures. We provide comprehensive descriptions of delineation criteria, and demonstrate that it is possible to identify the majority of the regions and subregions of the ascending auditory system, and some parts of the descending system, but not auditory areas in the cerebral cortex. We have, to our knowledge, provided the first MRI-based anatomical description and the first comprehensive 3D view of the near-complete auditory system in rat, which hopefully can aid interpretation of morphological features based on spatial coordinates (Papp et al., 2014, 2015), and thus facilitate further investigation of this morphologically complex system.

The present work expands and refines the anatomical delineations of earlier atlas versions with a detailed regional analysis of the auditory system. All changes relative to the previous version of the atlas are accounted for in Table 1. As also reported for the hippocampal region (Kjonigsen et al., 2015), we found an overall good correspondence between sMRI/DTI features and histologically observed boundaries. We have to a larger extent than before utilized additional histological data spatially registered to the Waxholm Space template. Myelin-stained histological material, and to some extent also PLI data (Schubert et al., 2016), that demonstrate finer details of fibroarchitecture were particularly useful for interpreting the MRI. These analyses also made it possible to interpret several interesting features reflected in the DTI, including the
“rainbow” image of the spiralling cochlear nerve fibres, and the very bright signals from the sharp turning of trapezoid fibres into the lateral lemniscus. Compared to conventional analysis of anatomical boundaries in two-dimensional (2D) histological material, the delineation software we used provides the opportunity to view the 3D brain structures in several planes simultaneously, to rapidly toggle between different modalities (sMRI, DTI and segmentations), and to directly compare the MRI to histological features. These functionalities enabled us to recognize subdivisions within complicated structures such as the cochlear nuclear complex, and indistinctly marked borderlines like the one between the periolivary region and the ventral lemniscal nucleus. The possibility to cut the template in non-standard planes of orientation was useful for understanding the organization of obliquely oriented structures as the cochlear nuclear complex. This was only utilized to a limited degree in the present study, and could for future endeavours be exploited more extensively to improve or add further delineations.

The resolution limitations of the sMRI and particularly the DTI data represent a particular challenge when it comes to the tiny structures within the auditory pathways in rat. Structures like the granule cell layer and cap area of the ventral cochlear nucleus were hardly visible, while certain fibre tracts like the acoustic striae and the olivocochlear bundle, which partly occur scattered in thin fascicles, were more or less untraceable. Some boundaries were difficult to set, partly because of the large voxel size, but also because of a mixture of vague secondary colour signals from complex areas (e.g. subdivisions of the cochlear nuclear and superior olivary complexes), and reduced or erroneous diffusion signals related to partial volume effects and crossing fibre orientations (e.g., in the cochlear nerve root, the crossing of the vestibular nerve and the trapezoid body, and the laminical wedge in the central nucleus of the inferior colliculus), which are not reliably resolved by the diffusion tensor model (Beliveau, 2002; Le Bihan et al., 2006).

The Waxholm Space rat brain template was acquired in situ within the cranium from a perfusion-fixed specimen, using an active contrast-enhancing staining protocol (Papp et al., 2014), developed to yield high-resolution, and high-contrast data with intact brain morphology (Badea et al., 2007; Benveniste et al., 2007). Several earlier studies have shown that tissue fixation does not introduce major morphological differences (Badea et al., 2007; Benveniste et al., 2007; Oguz et al., 2013) relative to the live brain, and that the relevant diffusion orientations seen in the DTI data are preserved, although the diffusion parameters are changed relative to in vivo acquisitions (Sun et al., 2003, 2005; D’Arceuil and de Crespigny, 2007). The sMRI and DTI based delineation criteria provided here should therefore in principle be possible to apply to in vivo MRI acquisitions from rats, likely also in mice and other mammals. The delineation criteria presented here may represent a useful starting point for further efforts to compare the appearance of auditory structures in MRI/DTI across species, e.g. using available atlas volumes for the mouse (Johnson et al., 2010; Hawrylycz et al., 2011) and bat brain (Washington et al., 2018).

Despite some imaging artefacts located close to the base of the skull, it was possible to recognize the intracranial course of the cranial nerves, and in the case of the vestibulocochlear nerve also of the receptor organ, i.e., the osseous and membranous labyrinth. Because of the small size of the latter, only the former was delineated here. The facial nerve could be traced all the way through the facial canal, to its exit through the stylo-mastoid foramen. Not all structure boundaries were consistently visible in sMRI or DTI, but by combining observations from multiple modalities, including histological material and raw experimental data it was possible to completely delineate most structures. It should be noted that the sMRI and DTI template also reveals several interesting anatomical features, such as intranuclear parts of fibre tracts that for technical reasons were not delineated. This deficiency is in part compensated for by including hodological aspects in the main text above.

Both sMRI and DTI have low sensitivity for finer cytoarchitectural features and lack the spatial resolution and image contrast to identify classic cytoarchitectonic boundaries of cerebrocortical areas. We were therefore not able to identify auditory regions of the cerebral cortex. The geometrically defined representations of the auditory cortex provided in the present atlas are useful as indications, but further work is needed to reconcile information from the different and partly inconsistent descriptions of the auditory cortex, reviewed by Malmierca (2015). Certain structures that are hodologically defined by their axonal connections, such as the auditory fibres in the internal capsule, are not possible to distinguish without access to tract tracing data or possibly improved DTI data that allow tractography of fibre orientations. The present work does not account for strain differences, but in agreement with earlier observations from the hippocampal region (Kjonigsen et al., 2015), we found that the morphology of the auditory system in the Sprague Dawley rat brain template were generally consistent with observations in histological material from Wistar and Long Evans rats.

Interpretations based on the present atlas should consider the methodological limitations outlined above, and also note that the atlas is based on a canonical template based on one animal, rather than a population average, and thus does not account for inter-individual or ontogenetic variations in brain architecture. While the atlas at large is comparable to other rat brain atlases (Swanson, 2004; Paxinos and Watson, 2007), it also features some differences with respect to nomenclature and delineation criteria used. Comparisons across atlases can however be achieved by use of spatial (stereotaxic or Waxholm Space) coordinates.

A fundamental contribution of the Waxholm Space rat brain atlas is that the atlas delineations are publicly shared together with the underlying template and descriptions of delineation criteria. As a result, the atlas is accessible for further, community-driven development, and integration with different neuroinformatics tools and analytic pipelines. The atlas has been applied as a spatial template for integration of experimental rat brain data in the EU Human Brain Project (HBP; www.humanbrainproject.eu), in context of building a large-scale infrastructure for accumulating, integrating, and sharing heterogeneous murine and human brain research data (Bjerke et al., 2018). The HBP has also established tools for spatially registering diverse types of 2D rat image data to the 3D atlas template (Papp et al., 2016; Puchades et al., 2019), and analytic workflows for extraction and counting of features of interest in regions defined by use of a 3D atlas, such as the Waxholm Space rat brain atlas (see, www.humanbrainproject.eu).

Further efforts are underway to refine and improve Waxholm Space rat brain atlas delineations in other brain regions, including the thalamus and basal ganglia (Imad et al., 2017) using a range of image data registered to the template (Schubert et al., 2016). As additional delineations become available, careful tracking of how the atlas has changed across versions will be important. As long as anatomical delineations are defined within the same atlas template, it is feasible to maintain multiple, alternative parcellations optimized for different analytic purposes, or reflecting different anatomical traditions.

Acknowledgements

We thank Ana L. B. Oliveira, Lisbeth M. L. Manurung, and Muhammed Zoabi for their important contributions to the early stage delineations. We thank Gergely Csics and Dmitri Darine for expert technical assistance and Jan G. Bjaalie for valuable discussions. Histo- logical section images were acquired at the NORBRAIN Slidescanning Facility at the Institute of Basic Medical Sciences, University of Oslo. This work was partly funded by the European Union’s Horizon 2020 Framework Programme for Research and Innovation under the Specific Grant Agreement No. 720270 (Human Brain Project SGA1) and 785907 (Human Brain Project SGA2), and The Research Council of Norway under Grant Agreement No. 269774 (INCF), with additional support through the SCORE Research Exchange program of the International Federation of Medical Students’ Association.