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A Three-dimensional Digital Neurological Atlas of the Mustached Bat (*Pteronotus parnellii*)

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Abstract

Substantial knowledge of auditory processing within mammalian nervous systems emerged from neurophysiological studies of the mustached bat (*Pteronotus parnellii*). This highly social and vocal species retrieves precise information about the velocity and range of its targets through echolocation. Such high acoustic processing demands were likely the evolutionary pressures driving the over-development at peripheral (cochlea), metencephalic (cochlear nucleus), mesencephalic (inferior colliculus), diencephalic (medial geniculate body of the thalamus), and telencephalic (auditory cortex) auditory processing levels in this species. Auditory researchers stand to benefit from a three dimensional brain atlas of this species, due to its considerable contribution to auditory neuroscience. Our MRI-based atlas was generated from 2 sets of image data of an *ex-vivo* male mustached bat's brain: a detailed 3D-T2-weighted-RARE scan [(59 × 63 × 85) μm^3] and track density images based on super resolution diffusion tensor images [(78) μm^3] reconstructed from a set of low resolution diffusion weighted images using Super-Resolution-Reconstruction (SRR). By surface-rendering these delineations and extrapolating from cortical landmarks and data from previous studies, we generated overlays that estimate the locations of

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classic functional subregions within mustached bat auditory cortex. This atlas is freely available from our website and can simplify future electrophysiological, microinjection, and neuroimaging studies in this and related species.

Graphical Abstract

INTRODUCTION

The mustached bat (*Pteronotus parnellii*) is highly reliant on its auditory system for orientation and communication and has accordingly developed neural processing areas within its auditory system that are hypertrophic relative to its other brain structures (Baron, 1974). Studies of these hypertrophic auditory system nuclei provided substantial knowledge regarding the function of mammalian auditory systems at the peripheral (Xiao and Suga, 2002a), metencephalic (Kemmer and Vater, 1997), mesencephalic (Gordon and O'Neill, 2000; Portfors and Wenstrup, 1999; Wenstrup et al., 2012), diencephalic (Butman and Suga, 2016; Suga et al., 1997; Wenstrup, 1999), and telencephalic (O'Neill and Suga, 1979; Suga and Jen, 1976; Suga et al., 1979) levels.

The success of these studies was founded on the delimited behavioral context of echolocation, in which biologically relevant scientific questions about the auditory system could be formulated within a common theoretical framework (Ehret, 1997; Suga, 1985). During echolocation, these bats emit a sound consisting of a constant frequency (CF), a downward frequency modulation (FM), and three harmonics thereof. By emitting these four signals (fundamental + 3 harmonics), mustached bats are able to track both the relative velocities and ranges of targets via the returning echo CF and FM components, respectively. Simple and complex feature detectors at every level of the mustached bat auditory system are specialized to process such CF and FM components in parallel. Studies of such feature detectors led to the discovery of neuroacoustic phenomena in this species prior to their documentation in other mammals. At the cortical level, these findings include spectral and temporal *combination-sensitivity* (O'Neill and Suga, 1979; Suga et al., 1978, 1979), disproportionate tonotopic representation (Suga and Jen, 1976), and excitatory-excitatory/inhibitory-excitatory (EE/IE) binaural organization (Manabe et al., 1978). Similarly impactful findings at the subcortical level include systematic representation of interaural level differences in the ascending auditory system (Wenstrup et al., 1986), spectral and temporal integration in the inferior colliculus (Wenstrup et al., 2012), projections from the

amygdala to the inferior colliculus (Marsh et al., 2002), and the nucleus of the central acoustic tract or NCAT (Casseday et al., 1989). Even studies of the neural substrates underlying social communication in the mustached bat auditory system were bolstered by knowledge gleaned from echolocation-based studies in the same species (Esser et al., 1997; Kanwal, 1999).

Neuroimaging techniques such as magnetic resonance imaging (MRI) have improved considerably in recent years with the availability of high field MRI scanners and thus would allow for detailed anatomical and functional studies spanning multiple nuclei and networks across the mustached bat central nervous system. Given the hypertrophy of auditory areas in the mustached bat, functional MRI studies could give insight into auditory processing between subcortical acoustic nuclei using non-invasive techniques that can be used repeatedly in an individual. The first *in-vivo* MRI study of the mustached bat brain confirmed that anatomical and auditory fMRI scans of this species can be achieved even in awake animals (Kamada et al., 1999). A number of MRI studies of cochlear and brain structure volumes in bats have recently emerged (Hsiao et al., 2016; Hsiao et al., 2015). Thus, any future functional neuroimaging studies in this species would benefit from a comprehensive reference such as a published digital mustached bat brain atlas. An MRI atlas of the mustached bat brain with labels for the gross structures of the auditory system as well as certain salient non-auditory brain structures and landmarks would represent a 3D-reference database for future neurophysiological, microinjection, and neuroimaging studies in this species. Detailed interpretations of data from such investigations on a neuroanatomical basis are possible with the aid of a cyto- and myeloarchitectonic mustached bat brain atlas currently under construction and congruent with MRI brain series of the mustached bat (Radtke-Schuller et al, unpublished data). Here, we present the freely downloadable MRI atlas of a male mustached bat brain, based on T2-weighted and track density images as well as published maps of the auditory cortex. The mustached bat brain atlas presented here can be easily adapted to match surgical and electrophysiological set ups as well as histological protocols, making it appealing for future bat brain studies.

METHODS

Specimen preparation

One adult male mustached bat (*Pteronotus parnellii rubiginosus*) was euthanized with an overdose of sodium pentobarbital (Fatal Plus, >100 mg/kg, i.p., Vortech, Dearborn, MI). Following loss of corneal and withdrawal reflexes, the animal was transcardially perfused through the left ventricle with phosphate buffered saline (PBS), followed by a 10% formalin mixture. After decapitation, the head was post-fixed in a mixture of 10% formalin for over 5 days at 20°C. The brain was never physically removed from the skull during MRI and CT data acquisition. Though significant brain shrinkage is generally expected due to fixation, successful registrations between anatomical MRIs of the brain and a CT image of the skull strongly suggest that brain shrinkage and/or malformation was minimal. A glass microelectrode was inserted while the bat was alive, targeted the medial geniculate body of the thalamus (MG), but caused no apparent damage to this particular structure. However, the electrode insertion caused some visible damage to other areas of the brain including the

cortex, deep cerebral white matter, and hippocampus. As shown in the supplementary materials (Fig. SSF1), the neurological damage due to this electrode penetration was unilateral (right hemispheric), restricted to relatively few voxels contained within the aforementioned quasi-planar structures, could be compensated for during the delineation process via comparisons with the left hemisphere and histological sections (see below), and thus had negligible impact on the quality of our delineations. All specimen preparation procedures were approved by the Northeast Ohio Medical University's Institutional Animal Care and Use Committee.

Data acquisition

To obtain a three-dimensional digital representation of the bat's skull, CT scans of the entire head were acquired with a Siemens Inveon PET-CT (rotation steps = 360; degrees = 360; voltage = 80 keV; tube current = 500pA; exposure time = 300 ms; voxel size = $[38\mu\text{m}]^3$; matrix = $[512]^3$).

All MRI datasets of the mustached bat head were acquired with a 9.4 T Biospec horizontal bore MR scanner (Bruker BioSpin, Ettlingen, Germany), equipped with a 120 mm BGA12-S actively shielded gradient insert with a maximum gradient strength of 600 mT/m attached to an AVANCE-II Bruker console. Images were acquired with a Bruker cross-coil setup with a linear transmit volume coil and a four-channel receiver cryo-probe designed for imaging rat heads. Horizontal T2-weighted 3D images were acquired using a Rapid Acquisition with Relaxation Enhancement sequence (repetition time [TR] = 4500 ms; echo time [TE] = 33 ms; voxel size = $(59 \times 63 \times 85) \mu\text{m}^3$; image matrix = $[256 \times 256 \times 136]$; RARE factor = 6; scan time = 14.3 h; number of averages = [2]), hereafter referred to as T2w 3D-RARE images.

Fifteen low resolution multi-slice 2D diffusion weighted (DW) SE data sets were acquired where each set was rotated around the phase-encoding direction (i.e., medio-lateral axis) at incremental steps of 12° for the purpose of super resolution reconstruction as described previously (Hamaide et al., 2016; Van Steenkiste et al., 2015). Each of these sets consisted of one non-diffusion weighted (b_0) and 6 DW images ($b = 2500 \text{ s/mm}^2$) with following acquisition parameters: TR = 8000 ms; TE = 27 ms; FOV = $(20 \times 20) \text{ mm}^2$, acquisition matrix = $[256 \times 183]$, image matrix = $[256 \times 256]$, in-plane resolution of $(0.078 \times 0.078) \text{ mm}^2$, slice thickness = 0.38 mm, 38 slices, D = 14 ms; $d = 6 \text{ ms}$, acquisition time = 2.84 h. In total, the 15 rotated DW datasets provided diffusion weighting along 90 unique diffusion gradient directions within a total scan-time of 42.7 h.

The dimensions of all of these images were ultimately cropped from $256 \times 256 \times 136$ to $192 \times 250 \times 126$ to remove excess space and improve visibility.

Data processing

Owing to the use of surface coils, bias field correction was applied to the T2w 3D-RARE. To perform super-resolution reconstruction diffusion tensor imaging (SRR-DTI), high resolution $(78 \mu\text{m})^3$ diffusion tensor parameter maps were estimated (Poot et al., 2010) from a set of low resolution diffusion weighted (DW) images using a weighted linear least squares estimator (Dyrby et al., 2014). From the high-resolution tensor parameters, high resolution

fractional anisotropy (FA), mean diffusivity (MD), radial diffusivity (RD), and axial diffusivity (AD) maps were obtained. High resolution fiber orientation distribution functions (fODFs) were also estimated in order to perform fiber tracking and subsequent track density imaging (TDI). First, a white matter fiber response function (spherical harmonic order 8) was extracted from the up-sampled high resolution DW data using the recursive calibration method (Tax et al., 2014). ODFs were obtained by performing constrained spherical deconvolution (Tournier et al., 2007), using the high resolution DW images and the single fiber response function created above, adopting the quadratic programming approach described previously (Jeurissen et al., 2014). The spherical harmonic series describing the fODFs was terminated at order *eight*. Next, probabilistic whole brain tractography was performed using second order integration over the fODFs (Tournier et al., 2010). Ten million streamlines were generated using the following parameters: fODF amplitude threshold of 0.1, step size of 40 μm , and a maximum angle between steps of 22°. Fidelity of the track densities to the raw diffusion weighted MRI data was ensured using SIFT2 (Smith et al., 2015). From the resulting tractogram, direction-encoded, color TDI maps were calculated at native resolution of (78 μm)³ (Calamante et al., 2010). All steps were performed using MRtrix3, which is freely downloadable at www.mrtrix.org (Tournier et al., 2012).

Delineation of Brain Structures

We choose to present all data in a 3D framework comparable to the orientation of Nissl and myelin stained histological slices of the histology atlas series provided by S.Radtke-Schuller. AMIRA 5.4.5 (Mercury Computer Systems, San Diego, CA, USA) was used to (1) rotate the T2w 3D-RARE scans to be consistent with the histological slices, (2) to perform the affine co-registration of TDI and CT images to the reoriented T2w 3D-RARE based on normalized mutual information, and (3) perform atlas delineations. The whole brain and brain regions of both hemispheres were delineated based on MRI contrasts between brain regions. TDIs were used in conjunction with the T2w 3D-RARE images to foster delineations by providing more accurate boundaries between gray and white matter. Delineations were made slice-by-slice in the three orthogonal dimensions: first anterior to posterior in the coronal dimension, then dorsal to ventral in the horizontal dimension, and then left to right in the sagittal dimension. Delineations made previously in one dimension (e.g., coronal) were systematically corrected as delineations progressed in a different dimension (e.g., horizontal).

The topology of forebrain (telencephalic and diencephalic) regions were identified on the basis of studies of the mustached bat brain (Fitzpatrick et al., 1998; Kobler et al., 1987; Pearson et al., 2007; Wenstrup et al., 1994; Winer and Wenstrup, 1994a, b; Winer et al., 1992) as well as a published atlas detailing structures in the forebrain of another bat species, *Carollia perspicillata* (Scalia et al., 2013). Identification of middle and hind brain (i.e., mesencephalic and metencephalic) structures were derived from studies in the mustached bat (Frisina et al., 1989; O'Neill et al., 1989; Ross and Pollak, 1989; Ross et al., 1988; Zook and Casseday, 1982a, b, 1985; Zook and Casseday, 1987; Zook et al., 1985). Further indications of boundaries were derived from histological brain atlases of rodents, including mice (Franklin and Paxinos, 2012), rats (Paxinos and Watson, 2014), and gerbils (Radtke-Schuller et al., 2016). The shapes of auditory cortical subregions were derived from maps

reported in previously published histological and electrophysiological studies (Fitzpatrick et al., 1998; Suga, 1985). Such auditory cortex maps were superimposed onto a surface rendering of the mustached bat brain using the 3D rendering program Blender (<https://www.blender.org>). The resultant auditory cortical map can be viewed in MeshLab (<http://www.meshlab.net/#download>). Both Blender and MeshLab are a freely downloadable.

RESULTS

The MRI data set, structural delineations and CT images of the skull can be freely downloaded from our website (<http://uahost.uantwerpen.be/bioimaginglab/Bat.zip>). Data are provided in NIFTI (.nii) format. We also provide interactive templates (i.e., corresponding anatomical regions reported for each coordinate) viewable in two freely downloadable software packages, specifically MRicro (<http://people.cas.sc.edu/rorden/mricro/mricro.html#Installation>) and MRicron (<http://people.cas.sc.edu/rorden/mricron/install.html>). A third template is viewable in MRicro-GL (<https://www.nitrc.org/frs/?groupid=889>), which measures inter-voxel distances at a sharper resolution of 0.1 mm and offers greater 3D rendering capabilities than either MRicro or MRicron. Each of these templates use color look-up-tables that match the color scheme used in the figures below. Viewing the delineations in other programs will yield different color schemes and will report only numerical values corresponding to intensities. Installers for Windows versions of each of these programs are included with our atlas. Mac OSX versions are available from the web addresses above.

We provide the data in a frame of reference consistent with mustached bat histological data (Radtko-Schuller et al, unpublished data). As opposed to rats and mice, the conventional brain axis tilt in mustached bats is 23°. The frontal atlas slicing plane was chosen to be perpendicular to the line that connects the most dorsal points of the forebrain (i.e., cortex) and hindbrain (i.e., cerebellum). This coordinate system is used for cutting and for the orientation of the bat's skull in a stereotaxic device in neurophysiological experiments, as it allows for a more natural head-body orientation used in neurophysiological studies.

Various freely available imaging software packages enable users to reorient the frame of reference at will. Thus, users can visualize our data as either orthogonal or oblique slices. The anterior/posterior axis is the y-axis (positive values anterior), and the dorsal/ventral axis is the z-axis (positive values dorsal) in accordance with neurological convention (De Groof et al., 2016; Gunturkun et al., 2013; Poirier et al., 2008; Simoes et al., 2012), the left/right axis is the x-axis (positive values right). We propose the intersection between the mid-sagittal plane and the anterior commissure (ac) as the origin (i.e., zero-point) for the frame of reference due the ac's similar role in the human-based Talairach coordinate system (Talairach and Tournoux, 1988) and the relative ease of locating ac in MRI images of the mustached bat brain. The origin of the dorsoventral plane is defined as the plane through the highest points of forebrain and cerebellum, which parallels the profile of the skull (see below). An "X" marks the origin in Figures 1, 2, and 3, each of which display six T2w 3D-RARE, FA, and TDI slices in the coronal, horizontal, and sagittal dimensions, respectively. Figures 1–3 are restricted to the T2w 3D-RARE, FA, and color-coded TDI maps due to

space constraints. On the website, we additionally provide 3D maps of AD, MD, RD metrics, and b0 images in the same space.

We also provide CT-based images of the bat skull co-registered to the MRI atlas. The CT images visualize the cochlea as more radiopaque than the neurocranium, demonstrating that the former structure has greater bone density than the latter in this species (Fig. 4A–B). Figure 4C displays the skull CT data set co-registered to the T2w 3D-RARE data set. Neurobiologists studying bats from a stereotaxic approach have previously established methodologies for employing landmarks on the skull as precise reference points for neural structures and functional areas (Schuller et al., 1991; Schuller et al., 1986). In brief, a coordinate system was previously developed for interfacing between stereotaxic procedures and histological processing of brains from another CF-FM bat species, the horseshoe bat, *Rhinolophus rouxi* (Schuller et al., 1986). As previously defined, these standard coordinate axes entailed orthogonal sagittal, coronal (frontal), and horizontal (parasagittal) skull planes ($x = 0$, $y = 0$, $z = 0$, respectively) in an electrode-carrier system. The zero-plane of the z-axis in this coordinate system coincides with the most dorsal points of the cortex and cerebellum. Researchers targeting structures in the mustached bat brain relative to the CT images provided are referred to the coordinate system detailed previously (Schuller et al., 1991). However, for those interested in comparing our skull CT to our MR-based images, the ac is also the zero-point of skull CT.

The T2w 3D-RARE enabled us to differentiate between different gray matter regions based on differences in T2-relaxation properties of distinct tissues. On the other hand, TDI and other diffusion images reveal axonal directions and other fine details of white matter (i.e., nerves and tracts) that are less apparent in the T2w 3D-RARE images. By using both types of images to better ascertain structural boundaries, we have delineated 40 different bilateral structures of interest in the mustached bat brain (Fig. 5). The column of numbers in Figure 5 correspond to intensity values of each delineation, and programs other than AMIRA 5.4.5, MRIcro, MRIcron, and MRIcro-GL will report these values as opposed to the corresponding brain region. Voxel counts, volumes, and centroids for all 40 delineated structures in the left and right hemispheres are summarized in Tables 1 and 2, respectively. Fourteen of these delineated structures were white matter. Of the remaining 26 structures, three were myelencephalic, two metencephalic, seven mesencephalic, three diencephalic, and 11 telencephalic. Ten of these 26 structures were part of the auditory system. We include the superior colliculus amongst these ten auditory structures. The superior colliculus is typically a multisensory area with a prevalent role in vision and oculomotor activity in highly visually oriented mammals. However, echolocating bats, including mustached bats, orient primarily using audition, not vision. Thus, neurons comprising significant portions of the superior colliculus in certain echolocating bat species, such as the horseshoe (*Rhinolophus rouxi*), big brown (*Eptesicus fuscus*), and pale spear-nosed (*Phyllostomus discolor*) bats, respond to auditory stimulation (Hoffmann et al., 2016; Reimer, 1991; Valentine and Moss, 1997; Wohlgemuth et al., 2018).

Maps derived from DW imaging were instrumental to delineating certain regions and boundaries that were simply not visible in the T2w 3D-RARE. More specifically, RD helped elucidate the medial boundaries of the amygdala, hippocampus, and medial geniculate body

of the thalamus (MGB) as well as ventral extents of the external capsule. TDI enabled us to delineate the nucleus of the central acoustic tract (NCAT), the hypothalamus, and to more accurately delineate boundaries for white matter structures, including the ventral extents of the external capsule.

Three-dimensional renderings of delineated structures can be generated in AMIRA 5.4.5 or other software. Such three dimensional representations can provide a good approximation of both the shape and relative volume of each delineated structure. Three-dimensional renderings of these delineated structures are displayed within a transparent volume rendering of the skull (CT image) in the horizontal, coronal, and sagittal dimensions in Figure 6. Figure 7 displays similar 3D renderings of delineated structures but provides a clearer view of subcortical structures by excluding renderings of the cerebellum, cortex, and olfactory bulb.

In Figure 8, Nissl and myelin stained sections of the histological atlas of the mustached bat are juxtaposed to our T2w 3D-RARE and TDI. Nissl and myelin stained histological slices have cellular level resolution, which surpasses the image resolution of the TDI and T2w 3D-RARE images. Thus, identification of structures in the Nissl and myelin stained slices can be used to label, and potentially digitally delineate, fine structures on MR-based images that would otherwise be difficult to discern. The labels on the T2w 3D-RARE in Figure 8 suggest the possibility of using histological reference slides to create even more precise labeling than those shown above.

One of the most prominently studied portions of the mustached bat auditory system is the functional organization of its auditory cortex. Neurophysiological studies are impossible in exvivo brains. However, decades of neurophysiological and histological studies have linked the location of mustached bat auditory cortex and its subdivisions to key cortical features, which were revealed by surface renderings of our delineations. Specifically, cortical delineations revealed the lateral fissure plus a triangular bulge associated with the auditory cortex, and adjustments in alpha scale revealed the medial cerebral artery and its bifurcations, commonly used by physiologists to target the Doppler-shifted constant frequency (DSCF) area. By aligning (1) the dorsal-most edges of both the dorsal fringe functional area (or DF) with the lateral fissure, (2) the anterior edges of the A1 anterior, CF/CF, DF, FM-FM, and ventral anterior subdivisions with the lateral fissure, and (3) the middle-dorsal portion of the DSCF area with the first bifurcation of the medial cerebral artery, we generated the 3D auditory cortex overlays displayed in Figure 9. The landmarks used to create this visualization are detailed in the supplementary materials (Fig. SSF2). This representation is viewable using MeshLab but not AMIRA, MRICro, MRICron, or MRICroGL as it is an overlay and not comparable to our other delineations. Given that the location of cortical functional areas has a high degree of inter-subject variability, the locations of these subdivisions must be regarded as reasonable approximations.

DISCUSSION

Brain atlases provide a reference framework for neuroscientists and have tremendous instructional value. Traditional brain atlases are based on histological sections with drawings

of boundaries of structures and annotations (Franklin and Paxinos, 2012; Paxinos and Watson, 2014; Scalia et al., 2013). Currently, three-dimensional, digital MRI atlases are available for fish (Simoes et al., 2012; Ullmann et al., 2010), rodents (Bowden et al., 2011; Calabrese et al., 2013; Dorr et al., 2008; Johnson et al., 2012; Paxinos et al., 2015; Rumble et al., 2013; Valdes-Hernandez et al., 2011; Veraart et al., 2011), marsupials (Majka et al., 2013), songbirds and other aves (De Groof et al., 2016; Gunturkun et al., 2013; Poirier et al., 2008; Vellema et al., 2011), as well as humans and other primates (Bakker et al., 2015; Calabrese et al., 2015; Fan et al., 2016; Rohlfing et al., 2012). Detailed MRI-based atlases exist for regions of the mouse brain such as the basal ganglia (Ullmann et al., 2014) and diencephalon (Watson et al., 2017). Despite the remarkable contribution that studies of bats have made to our general understanding of auditory systems, the present atlas is the first MRI-based digital brain atlas of any echolocating bat species.

One clear limitation of this atlas is that it is based on an individual ex-vivo brain, similar to Talairach's classic human brain atlas (Talairach and Tournoux, 1988). Thus, there is the possibility of inter-subject variability and researchers who require high spatial accuracy must consider this limitation when using this atlas. Similarly, shrinking effects due to fixation and potential neurological damage may increase inter-subject variability. However, as Figure 8 demonstrates, there is close correspondence between neuroanatomical structures seen in one bat's MRI and another bat's histological slices.

Although histological atlases generally have cellular-level resolution, MRI-based atlases have advantages for manipulating, analyzing, and normalizing neuroimaging data. Histological atlases are composed of frontal, horizontal, and sagittal sections from slicing three different brains, which implies that the three dimensional consistency cannot be totally asserted. Though inter-individual differences remain overall, the coronal, horizontal, and sagittal dimensions are consistent within individuals in an MRI atlas and permit acquisition of multiple MRI-based structural data types (e.g., T1, T2-weighted, diffusion tensor, etc.) from the same sample. Also, this 3D digital atlas allows users to present neuroanatomical data from the mustached bat from any oblique slice. The spatial relationship between gray and white matter is preserved in MRI-based atlases. Such three-dimensional, digital information will help researchers targeting deep brain structures (e.g., medial geniculate body of the thalamus, amygdala, cochlear nucleus, etc.) with electrodes, catheters, or injection micropipettes to perform experiments while minimizing damage to surrounding areas. If smaller, histologically distinguished sub-structures are targeted or if localized experiments should be functionally interpreted in detail, however, the additional use of a histological atlas will be essential. Yet, if it is possible to perform a 3D structural MRI scan on the animal prior to histological preparation, the MR images can be normalized to the present atlas in order to better specify the individual stereotaxic coordinates of a smaller ROI. In general, data from individual bats can be normalized to compensate for intersubject variability, which can allow for back-projection of ROIs into the native space of individual animals. Further, realistic adjustment of the sections to an MRI data set in the same cutting plane can substantially compensate for distortions, which can lead to a combined MRI/histology based atlas yielding high resolution at realistic coordinates, as demonstrated in the recently published cyto- and fiber architectonic atlas for the gerbil (Radtko-Schuller et al., 2016). Interestingly, though previous studies have detailed inter-species differences in bat

cochlear size (Hsiao et al., 2015), the CT and MRI-based data obtained here offers cochlear dimensions for this particular, highly studied species in a format directly comparable to neurological structures such as the inferior colliculus. Also, the DWI-based data obtained here is the first to indicate the directionality of white matter tracts in any bat species. In general, MRI-based atlases make many different types of calculations upon brain structures possible, including group level measures of brain structure volume, connectivity strength, and stimulus/behavior driven brain activity, all of which can be easily visualized in three dimensions.

The present atlas compliments a commercially available histological atlas of a bat species, *Carollia perspicillata* or the short-tailed fruit bat (Scalia et al., 2013). This detailed atlas is limited only to forebrain structures and combined two histological staining techniques (i.e., Nissl and NeuN) that are ideal for labeling gray matter. Our atlas can aid those targeting mid-and hindbrain structures and used MRI techniques suited for both delineating gray and white matter structures. Further, though *Carollia perspicillata* and *Pteronotus parnellii* are both microchiroptera, or yangochiroptera (Hutcheon and Kirsch, 2006), their echolocation behavior differs dramatically. Specifically, the echolocation pulses of *Carollia perspicillata* consist only of FMs whereas those of *Pteronotus parnellii* consist of a tone followed by an FM (i.e., CF-FM). This vocal difference is already linked to differences in cochlear structure between FM-only and CF-FM bats (Hsiao et al., 2015). Considering the vast amount of neurophysiological, neuroanatomical, and behavioral studies in the mustached bat and the ongoing investigations in this bat species, an MRI-based atlas of the mustached bat brain is timely.

Over a decade of advances in neuroimaging has enabled researchers to assess auditory processing via functional magnetic resonance imaging (fMRI) in small animals, including mice (Yu et al., 2007; Yu et al., 2005), rats (Gao et al., 2015; Gao et al., 2014), and songbirds (Poirier et al., 2009; Van Ruijssevelt et al., 2013). This atlas could serve as the anatomical reference for manganese-enhanced and diffusion tensor imaging studies in the mustached bat as well. Indeed, atlases similar to this one were used in manganese-enhanced and diffusion tensor imaging to study cognition and neuroplasticity in starlings (De Groof et al., 2006; Van der Linden et al., 2004; Van Meir et al., 2006; Van Meir et al., 2004) and canaries (Tindemans et al., 2003). Manganese-enhanced imaging has even been successfully employed to study tonotopy in rodents (Yu et al., 2007). These and other voxel-based analyses in neuroimaging studies require precise spatial normalization to some anatomical frame of reference analogous to the Talairach or Montreal Neurological Institute (MNI) coordinate systems commonly used in human-based neuroimaging studies. Perhaps, the strongest contribution of this atlas may be the support it can offer future functional, structural, and physiological (e.g. perfusion) neuroimaging studies in this species. At the gross anatomical level, functional neuroimaging is poised to address long-standing auditory neuroscience controversies commonly explored by single unit recording studies in bats, including the corticofugal system (Yan and Suga, 1996; Zhang and Suga, 1997), echo-acoustic flow (Bartenstein et al., 2014; Greiter and Firzlaff, 2017), and left hemispheric specialization for processing social calls (Kanwal, 2012; Tallal, 2012; Washington and Kanwal, 2012; Washington and Tillinghast, 2015). This atlas is intended to serve as such an anatomical reference for future neuroimaging studies of the mustached bat brain, enabling

researchers to accurately localize task, stimulus, or resting-state evoked blood oxygen level dependent (BOLD) signal changes related to neural activity in the forebrain, midbrain, and brainstem.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

T2 weighted 3D-RARE (left), fractional anisotropy (middle), and color-coded tract density images (TDI) (right) at **coronal** planes 52/250 (−6.680 mm), 80/250 (−5.040 mm), 108/250 (−3.400 mm), 136/250 (−1.758 mm), 166/250 (0.000 mm), and 192/250 (+1.524 mm). “X” marks the origin (i.e., anterior-posterior zero point) at *anterior commissure* (plane 166/250) in all three modalities. Note that the apparent “shadow” in the left hemisphere of slice plane 108 in the T2w 3D-RARE map, which does not appear in the corresponding slice planes of the FA or color-coded TDI maps, is a signal void due to the relative local homogeneity of the

magnetic field at the time of T2w 3D-RARE data acquisition. The diffusion-weighted scans (i.e., AD, b0, FA, MD, RD, and TDI) were acquired at a later time, had a better local shim, and thus did not present the same local signal void. Since the T2w 3D-RARE, the various diffusion-weighted scans, and histological sections were used as guides during delineation, our delineations of cortex were in no way complicated by this signal void. However, those using the T2w 3D-RARE map for their studies should be aware of this signal void.

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Figure 2.

T2w 3D-RARE (left), FA (middle), and color-coded TDI (right) at **horizontal** planes 16/126 (−3.384 mm), 36/126 (−1.692 mm), 56/126 (0.000 mm), 76/126 (+1.692 mm), 96/126 (+3.384 mm), and 116/126 (+5.076 mm). “X” marks the origin at *anterior commissure* (plane 56/126) in all three modalities.

Figure 3.

T2w 3D-RARE (left), FA (middle), and color-coded TDI (right) at **sagittal** planes 95/192 (0.000 mm), 83/192 (−0.750 mm), 71/192 (−1.500 mm), 59/192 (−2.250 mm), 47/192 (−3.000 mm), and 35/192 (−3.750 mm). “X” marks the origin at *anterior commissure* (plane 95/192) in all three modalities. Since structures are largely similar between hemispheres, only left hemispheric slice planes are presented here.

Figure 4.

CT-based images of the mustached bat skull and registration to the T2w 3D-RARE. (A) 3D renderings of the dorsal (left), three-quarter view rostral (middle), and right hemispheric lateral (right) views of the mustached bat skull. (B) 3D renderings of the mustached bat skull from the same perspectives as in “A” except that the radiopacity has been digitally reduced using the *AlphaScale* functionality of AMIRA 5.4.5. (C) Horizontal (left; slice 56/126, 0.000 mm), coronal (middle; slice 166/250, 0.000 mm), and sagittal (right; slice 95/192, 0.000 mm) co-registration of the CT images of the mustached bat skull and the brain-extracted

T2w 3D-RARE images of the mustached bat brain. The bilateral bony structure located within the horizontal slice is the dorsal-most peak of the cochlea.

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in white represent white matter structures. Superscript “A” above a gray matter structure denotes that it is part of the auditory system. The asterisk (*) next to the hypothalamus indicates that, based on more recent developmental approaches, the hypothalamus is not included with other structures in the traditionally defined diencephalon (Puelles et al, 2013). (Right) Horizontal slices displaying delineations. Slices shown here were selected to display the greatest number of delineations per plane.

Figure 6. Three-dimensional rendering of brain delineations registered to the skull (CT) from the top-horizontal (top left), bottom-horizontal (bottom left), anterior-coronal (middle top), posterior-coronal (middle bottom), left-sagittal (top right), and right-sagittal (bottom right) perspectives. The cochlea is pointed out in the bottom-right panel. Color codes for each of the 40 delineated structures are provided below.

Figure 7.

Three-dimensional rendering of subcerebellar and subcortical delineations registered to the mustached bat brain (T2w 3D-RARE) from the top-horizontal (top left), bottom-horizontal (bottom left), anterior-coronal (middle top), posterior-coronal (middle bottom), left-sagittal (top right), and right-sagittal (bottom right) perspectives. The cerebellum, the cortex, and the olfactory bulb are excluded from the delineated structures shown here and in the color codes provided below to avoid obscuring subcerebellar and subcortical structures.

Figure 8.

Comparisons between labeled Nissl and myelin stained coronal histological slices and coronal T2w 3D-RARE MR-based images. The Nissl and myelin stains derive from an unpublished neurological atlas of the mustached bat. (A) Nissl (top) and myelin (middle top) slices that correspond to the T2w 3D-RARE (middle bottom) and color-coded track density (bottom) images (120/250, -2.695 mm, coronal). Slice and image numbers are listed from anterior to posterior. (B) Nissl (top) and myelin (middle top) that correspond to the T2w 3D-RARE (middle bottom) and track density (bottom) images (88/250, -4.570 mm, coronal).

Label abbreviations: 5n (trigeminal nerve), Aq (cerebral aqueduct), bic (brachium of the inferior colliculus), NCAT (nucleus of the central acoustic tract), Cb (cerebellum), CG (central gray), CIC (central nucleus of the inferior colliculus), cic (commissure of the inferior colliculus), CnF (cuneiform nucleus), Com (commissural nucleus of inferior colliculus), cp (cerebral peduncle), csc (commissure of the superior colliculus), DCIC (dorsal cortex of the inferior colliculus), DNLL (dorsal nucleus of lateral lemniscus), DpME (deep mesencephalic nucleus; also known as *pIPAG*), DpG (deep layers of superior colliculus), DPO (dorsomedial paraolivary nucleus), DR (dorsal raphe nucleus), ECIC (external cortex of the inferior colliculus), Ent (entorhinal area), Hip (hippocampus), INLL (intermediate nucleus of the lateral lemniscus), InC (interstitial nucleus of Cajal), InG (intermediate layers of superior colliculus), Il (lateral lemniscus), mcp (cerebellar peduncle), Me5 (mesencephalic trigeminal nucleus), MG (medial geniculate body), ML (lateral part of medial mammillary nucleus), mlf (medial longitudinal fasciculus), MnR (medial superior central raphe nucleus), MRe (mammillary recess of the 3rd ventricle), Neo (neocortex), PL (paralemniscal nucleus), PMT (pontomesencephalic tegmentum), PnO (pontine reticular nucleus, oral part), py (pyramidal tract), rf (rhinal fissure), RSD (dorsal retrosplenial cortex), RSG (retrosplenial dysgranular cortex), RtTg (reticulotegmental nucleus of pons), scp (superior cerebellar peduncle), SG (supragenulate thalamic body), SN (substantia nigra), SPO (superior paraolivary nucleus), Tz (medial nucleus of the trapezoid body), and VNLL (ventral nucleus of the lateral lemniscus). Each of the acronyms above are derived from Franklin and Paxinos (2012) except for certain large structures (i.e., Cb, Ent, Hip, Neo) that we chose not to delineate into smaller structures and the pontomesencephalic tegmentum, a structure that is still commonly referenced in literature (Schofield et al., 2011).

Figure 9.

3D rendering of the functional organization of the mustached bat auditory cortex. (A) Schematized spectrogram of the mustached bat's echolocation signal. H₁-H₄ refer to harmonics 1–4 of the echolocation pulse or echo. Note the constant frequency (CF) and frequency-modulated (FM) components present in the pulse (solid line) and returning echo (dashed line). (B) Map of the mustached bat auditory cortex. The boundaries of auditory cortical subdivisions are derived from Fitzpatrick et al (1998), and white labels describing the function of auditory cortical subdivisions are derived from Suga (1985). (C) Dorsal view of the mustached bat brain with a superimposed auditory cortex map. The map includes anterior A1 (A1a, maroon), posterior A1 (A1p, light blue), CF/CF (pink), dorsal fringe (DF, green), dorsal medial (DM, brown), Doppler-shifted CF (DSCF, black), FM-FM (dark blue), ventral anterior (VA, orange), and ventral posterior (VP, white). (D) Right profile view of the mustached bat brain with a superimposed auditory cortex map. This depiction contains the same functional areas described above in "C." (E) Left profile view of the mustached bat brain similar to the depiction in "D." Note that the DSCF labels in this depiction (60–63 kHz) represent the more commonly reported echo-CF₂ range of the *P.p.parnellii* subspecies

(Suga and Jen, 1976), not the 57–60 kHz range of the Trinidadian *Pp.rubiginosus* subspecies to which this specimen belongs (Xiao and Suga, 2002b). The Meshlab file used to generate this image contains the color-coded boundaries and contours but none of the numerical values, arrows, or text shown here. A second Meshlab file is available without contours. Researchers can thus label these ranges to coincide with those of *Pp.parnellii*, *Pp.rubiginosus*, or their choice of mustached bat subspecies. Details regarding the landmarks used to overlay the auditory cortex map onto the surface rendering of the mustached bat brain are shown in Figure SSF2.

TABLE 1:**Left Hemispheric Measures**

Structure	Code	Count	Volume	X COM	Y COM	Z COM
Accessory Olfactory Bulb(AOB)	33	303	0.094	9.014	13.057	6.328
Amygdala(A)	29	34350	10.637	11.023	9.258	4.005
Anterior Commissure(ac)	9	2932	0.908	9.310	10.270	5.468
Anterior Olfactory Nucleus(AON)	32	2390	0.740	9.121	12.580	5.435
Caudate(C)	13	27403	8.486	9.173	10.625	6.714
Central Gray(CG)	11	8807	2.727	8.284	5.920	7.701
Cerebellum(Cb)	25	224542	69.532	10.396	3.621	6.954
Cerebral Peduncle(cp)	28	3652	1.131	9.859	7.715	5.730
Cochlear Nucleus(CNVMI(CN)	4	10641	3.295	10.455	4.466	4.752
Cortex(Neo)	24	220732	68.352	10.684	9.243	7.613
Deep Cereb White Matter(dwm)	31	8446	2.615	11.569	7.435	7.937
Dorsal Nuc Lat Lemniscus(DNLL)	3	745	0.231	9.654	5.199	6.838
External Capsule(ec)	17	22647	7.013	10.592	9.657	7.571
Fimbria and Fornix Bundle(fi/f)	20	5326	1.649	9.346	9.107	7.568
Forceps Major (fmj)	30	2956	0.915	9.237	7.633	9.472
Forceps Minor(fmi)	26	4435	1.373	9.354	11.720	7.079
Genu: Corpus Callosum(gcc)	16	11557	3.579	8.687	9.704	8.706
Globus Pallidus(GP)	15	2911	0.901	10.086	9.441	6.170
Hippocampus(Hip)	19	60488	18.731	10.871	7.504	6.530
Hypothalamus(H)	39	6930	2.146	8.552	9.121	4.248
I.C. Commissure(cic)	2	362	0.112	8.275	5.266	9.184
Inferior Cerebellar Peduncle(icp)	38	1251	0.387	9.658	4.040	6.677
Inferior Colliculus(IC)	1	26197	8.112	9.736	5.244	8.447
Inter Nuc Lat Lemniscus(INLL)	23	1961	0.607	10.033	5.455	6.462
Internal Capsule(ic)	18	13305	4.120	10.344	9.223	6.662
Med Nuc Trapezoid Body(Tz)	7	741	0.229	8.209	5.055	3.930
Medial Cerebellar Peduncle(mcp)	10	5031	1.558	10.490	5.116	5.831
Medial Geniculate Body(MG)	5	11215	3.473	10.394	7.621	7.000
Nuc Cen Acoustic Tract(NCAT)	37	201	0.062	8.937	5.759	4.886
Nucleus Accumbens(Acb)	34	3276	1.014	8.866	11.754	4.852
Olfactory Bulb(OB)	22	18178	5.629	8.978	13.575	6.350
Pontine Gray(CGPN)	35	6556	2.030	8.727	6.575	4.933
Putamen(P)	14	9746	3.018	10.466	9.306	5.542
Pyramidal Tract(py)	36	4008	1.241	8.322	4.196	3.029
Septal Nucleus(S)	27	3879	1.201	8.199	10.785	6.016
Superior Cerebellar Peduncle(scp)	8	364	0.113	9.187	4.678	6.920
Superior Colliculus(SC)	12	14571	4.512	8.913	6.864	8.296
Superior Olivary Complex(SO)	21	1699	0.526	8.966	5.061	4.662
Thalamus(Thal)	40	18200	5.636	8.841	8.831	6.772

Structure	Code	Count	Volume	X COM	Y COM	Z COM
Ventral Nuc Lat Lemniscus(VNLL)	6	2030	0.629	9.585	5.446	5.732

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TABLE 2:**Right Hemispheric Measures**

Structure	Code	Count	Volume	X COM	Y COM	Z COM
Accessory Olfactory Bulb(AOB)	33	244	0.08	6.681	12.987	6.379
Amygdala(A)	29	32281	10.00	4.747	9.253	4.072
Anterior Commissure(ac)	9	3010	0.93	6.564	10.234	5.479
Anterior Olfactory Nucleus(AON)	32	2634	0.82	6.683	12.588	5.529
Caudate(C)	13	25512	7.90	6.592	10.567	6.771
Central Gray(CG)	11	10687	3.31	7.604	6.009	7.643
Cerebellum(Cb)	25	224232	69.44	5.519	3.619	6.948
Cerebral Peduncle(cp)	28	3559	1.10	5.953	7.697	5.766
Cochlear Nucleus(CNVMI(CN)	4	10212	3.16	5.305	4.490	4.773
Cortex(Neo)	24	218009	67.51	5.218	9.261	7.682
Deep Cereb White Matter(dcwm)	31	9671	2.99	4.112	7.396	7.802
Dorsal Nuc Lat Lemniscus(DNLL)	3	709	0.22	6.160	5.190	6.814
External Capsule(ec)	17	23140	7.17	5.232	9.629	7.653
Fimbria and Fornix Bundle(fi/f)	20	5887	1.82	6.459	9.110	7.566
Forceps Major (fmj)	30	3079	0.95	6.572	7.620	9.505
Forceps Minor(fmi)	26	4573	1.42	6.447	11.676	7.027
Genu: Corpus Callosum(gcc)	16	12033	3.73	7.206	9.717	8.661
Globus Pallidus(GP)	15	2802	0.87	5.669	9.406	6.215
Hippocampus(Hip)	19	58969	18.26	4.920	7.475	6.596
Hypothalamus(H)	39	7288	2.26	7.271	9.156	4.281
I.C. Commissure(cic)	2	409	0.13	7.630	5.264	9.211
Inferior Cerebellar Peduncle(icp)	38	1161	0.36	6.085	4.026	6.662
Inferior Colliculus(IC)	1	27635	8.56	6.131	5.238	8.478
Inter Nuc Lat Lemniscus(INLL)	23	2090	0.65	5.729	5.410	6.477
Internal Capsule(ic)	18	14447	4.47	5.455	9.215	6.671
Med Nuc Trapezoid Body(Tz)	7	863	0.27	7.617	5.095	3.928
Medial Cerebellar Peduncle(mcp)	10	4204	1.30	5.433	5.215	5.857
Medial Geniculate Body(MG)	5	12288	3.81	5.305	7.593	7.058
Nuc Cen Acoustic Tract(NCAT)	37	185	0.06	7.007	5.768	4.822
Nucleus Accumbens(Acb)	34	3210	0.99	6.880	11.751	4.862
Olfactory Bulb(OB)	22	20924	6.48	6.916	13.563	6.324
Pontine Gray(CGPn)	35	6925	2.14	7.169	6.565	4.922
Putamen(P)	14	10197	3.16	5.307	9.268	5.491
Pyramidal Tract(py)	36	5095	1.58	7.545	4.147	3.000
Septal Nucleus(S)	27	5812	1.80	7.653	10.798	5.946
Superior Cerebellar Peduncle(scp)	8	357	0.11	6.618	4.635	6.898
Superior Colliculus(SC)	12	15218	4.71	6.922	6.871	8.320
Superior Olivary Complex(SO)	21	1714	0.53	6.801	5.030	4.693
Thalamus(Thal)	40	19887.00	6.16	7.033	8.843	6.792

Structure	Code	Count	Volume	X COM	Y COM	Z COM
Ventral Nuc Lat Lemniscus(VNLL)	6	2022.00	0.63	6.205	5.401	5.746

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