Published in final edited form as:

Brain Res Rev. 2009 December 11; 62(1): 1–18. doi:10.1016/j.brainresrev.2009.09.001.

A combined histological and MRI brain atlas of the common marmoset monkey, *Callithrix jacchus*

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Abstract

The common marmoset, *Callithrix jacchus*, is of growing importance for research in neuroscience and related fields. In the present work, we describe a combined histological and magnetic resonance imaging (MRI) atlas constructed from the brains of two adult female marmosets. Histological sections were processed from Nissl staining and digitized to produce an atlas in a large format that facilitates visualization of structures with significant detail. Naming of identifiable brain structures was performed utilizing current terminology. The histological sections and a simplified schematic atlas are available online at http://udn.nichd.nih.gov/brainatlas_home.html.

Keywords

primate neuroanatomy; Callitrichidae; female; forebrain; thalamus; midbrain; brainstem; magnetic resonance imaging

1. Introduction

The common marmoset, *Callithrix jacchus*, is of growing importance for research in neuroscience and related fields, including pharmacology, ethology, genetics, endocrinology, and reproductive biology. Their relatively small body size (adults range from 14 to 18 cm and weigh 250–500 g), and social organization in small family groups lend themselves to being kept in manageable captive housing facilities. Their lissencephalic brain is smaller than that of the rhesus macaque (Figure 1), making brain preparation and experimentation less cumbersome than with larger primate brains. Yet, their brains share a number of anatomical features found in other primates, with a well-defined lateral fissure, temporal lobes, and internal structures that are very similar to that of other primates, including humans.

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For readers less familiar with the marmoset and research based on this primate, we offer the following section as an overview. Marmosets and tamarins (family Callitrichidae) are small New World primates. Most live in small family groups, in which the father and older offspring play a major role in caring for the infants, which are typically born as twins. Marmosets have high reproductive capacity, reaching breeding age at around 18 months, and females can produce two litters per year. The common marmoset, *Callithrix jacchus*, is probably the best known, and the most widely used in research, spanning a wide range of fields. These include behavioral pharmacology (e.g., Cagni, et al., 2009), reproductive physiology (e.g., Haig, 1999; Saltzman and Abbott, 2009), genetics (e.g., Datson, et al., 2007; Raveendran, et al., 2008), transgenics (e.g., Sasaki, et al., 2009; Schatten and Mitalipov, 2009), comparative psychology (e.g., Hook and Rogers, 2008), and social behavior (e.g., Barbosa, et al., 2009; Clara, et al., 2008; Zahed, et al., 2008).

Notably, a draft sequence of the marmoset genome has recently been released (<http://www.hgsc.bcm.tmc.edu/project-species-p-Marmoset.hgsc?pageLocation=Marmoset>). Perhaps of greatest relevance to the marmoset brain atlas we are presenting here, there is an extensive literature on the use of common marmosets in neuroscience research. Some examples of this rich literature include chemoarchitecture of calcium-binding proteins (Bourne, et al., 2007), architecture of frontal cortex (Burman, et al., 2009), sex differences in brain anatomy (Reinius, et al., 2008), auditory physiology (Wang, 2007; Wang, et al., 2008), cortical neurotransmitters (Walker, et al., 2009), parietal cortex anatomy and physiology (Qi, et al., 2002; Burish, et al., 2008), visual cortex anatomy (Rosa, et al., 2009), early developmental manipulations and gene expression in the brain (Law, et al., 2009; Pryce, 2008), and marmosets as models for studying human neuromuscular and autoimmune diseases (Eslamboli, 2005; Hohjoh, et al., 2009; 't Hart and Massacesi, 2009).

Several neuroanatomical overviews of the marmoset brain have been published (Osman Hill, 1957; Hershkovitz, 1977; Prado-Reis and Erhart, 1979a,b; Saavedra and Mazzuchelli, 1969). Descriptions of marmoset cerebral cortex have also been published (Brodmann, 1909; Peden and von Bonin, 1947). Until very recently, only one comprehensive brain atlas had been published, and is now out of print and difficult to obtain (Stephan, Baron, and Schwerdtfeger, 1980). In 2008, a new stereotaxic atlas became available (Palazzi and Bordier, 2008). This atlas presents brain sections stained for acetylcholine esterase (AChE). Although the plates in the printed atlas are quite small to show much detail, the accompanying Compact Disc permits enlarging the figures in considerable detail. AChE staining leaves out much of the cellular detail that permits a researcher to reliably identifying a structure under the microscope, and distinguishing cellular masses (nuclei) with the clarity that Nissl material permits. Most recently, motivated by the creation of a public internet database for histological brain sections (<http://brainmaps.org>, Miluka, et al., 2007), Tokuno, et al. (2009) constructed a database of histological sections of the marmoset brain that were converted to digital images and which are available online (<http://marmoset-brain.org:2008>).

Magnetic resonance imaging (MRI) has established itself as the most widely used modality for imaging the whole brain in vivo. MRI excels in neuroimaging because of its intrinsic soft-tissue contrast, high resolution, and ability to follow in vivo processes longitudinally. The marmoset has been increasingly studied with MRI (Figure 2). However, to date, there are no studies relating the soft tissue contrast obtained with MRI with the cytoarchitectonic information provided by histology. The goal of the present work is to present a combined histological and MRI-based atlas of the marmoset brain that may serve as a reference for studies in contemporary neuroscience research, including electrophysiology, gene expression studies (e.g., through the use of immunocytochemistry) and functional magnetic resonance imaging. We also concluded, through our own experience and careful reading of existing stereotaxic atlases, that distortions created by histological preparation and inter-brain variability in the

dimensions and spatial location of internal brain structures made reliance on the positional information found in a stereotaxic atlas problematic. Our goal here is to present an atlas with good quality histology, in a large format that facilitates visualizing detailed structure, and using terminology that reflects current conventions.

2. Results

The complete set of Nissl images upon which the atlas is based may be found at http://udn.nichd.nih.gov/brainatlas_home.html. For the present atlas, each Nissl image was cropped to present only one hemisphere, and structures were labeled with Adobe Photoshop Elements 2.0. Each plate is identified by an 'A' or 'P' number to designate anterior or posterior coordinates respectively, as one would expect to find in a stereotaxic atlas. These designations are meant only as plate identifiers, and not as accurate stereotaxic levels. Structures were identified by reference to existing atlases, in particular, the marmoset atlas by Stephan, et al. (1980). However, their use of different nomenclature, together with several cell clusters in our female's brain that appeared to have no equivalent in the male brain as used in the atlas by Stephan, et al., made identification of a number of structures problematic. We took a conservative approach, and wherever doubt existed about the identity of a particular cell cluster, no identification was made.

The brain atlas is presented both as a series of Nissl histology plates (Figures 3–31), and a series of MRI images (Figures 32–60) matched as closely as possible to the Nissl plates. Each Nissl plate shows a 40 micron-thick section. The distance between sections is 480 microns. The MRI images are 66 micron-thick. To improve the signal-to-noise ratio of the MRI while preserving gray-to-white matter contrast, the fixed brain was soaked for one week in a 10% formalin solution doped with 5 mM gadopentetate dimeglumine (see Experimental Procedure below). This MRI contrast agent reduces both T1 and T2 relaxation time constants of the fixed tissue, allowing the MRI to be acquired in a shorter period of time. A T2-weighted sequence was chosen to enhance the MRI contrast between gray and white matter under the conditions of a shorter repetition time, and excellent contrast between gray and white matter structures were obtained. While similar contrast could have been obtained using a T1-weighted sequence, as it is commonly used for in vivo MRI, a much longer acquisition time would have been necessary. Even though the contrast in the MRI images was different than the contrast in either the corresponding Weil or Nissl histological plates, the spatial resolution of 66 microns was sufficient to allow clear delineation of all areas identified in the histological sections. The images of the sections have been cropped to highlight the detailed histology of deep brain structures. While this excludes the cortex, cortical maps now tend to be made on a variety of evidence from anatomical, neurochemical, and functional studies, rather than from histology alone (Rosa and Tweeddale, 2005). Examination of the histology underlying the atlas indicates that the basic neuroanatomical organization described in earlier publications of the marmoset brain is present in our atlas brain. The Nissl images used for the atlas are also available online (<http://udn.nichd.nih.gov>), showing the complete section in each instance, along with matching Weil-stained sections for myelinated fibers. Eventually, the MR images, along with higher-resolution Nissl and Weil images, will be available online.

3. Discussion

While there is, at present, no detailed description of internal differences in the brains of male and female marmosets, most primate brain atlases employ the brain of a male of the species. Given that the female brain is at least as interesting from a research standpoint as the male brain, we chose to use the brain of a female for our atlas. We chose not to produce a stereotaxic atlas for several reasons. In our experience, the coordinates presented in atlases, at least for the marmoset and the squirrel monkey, with which we have extensive experience, are not very

accurate. This may be because of variability in the location and shape of brain structures across different individual monkeys, or because of spatial distortions introduced during fixation, histological sectioning and staining. As Gergen and MacLean (1962, p. 5) state in their squirrel monkey atlas, ‘...one would have a 50% chance of coming within 0.5 mm of a point described in the present atlas.’ This is not to say that the use of the stereotaxic frame and an atlas developed for stereotaxic research doesn’t have continuing value in neuroscience research. Rather, our focus has been to provide a reference tool useful for those doing immunocytochemical and other types of anatomical studies for which stereotaxic coordinates are of little value. The authors agree with the statement by C. N. Woolsey that an atlas based on the actual brain histology is preferable to line drawings, ‘...since line drawings necessarily force one to make arbitrary decisions concerning boundaries between structures when, without experimental verification, the boundaries may be uncertain or, in fact, not at all sharply definable’ (p. vi, in Emmers and Akert, 1963).

Some nuclei identified in Stephan, et al (1980) were not clearly identified in our brain sections, and, as consequence, fewer structures in our atlas are identified. It is unclear whether this is due to the fact that we used a female brain whereas the earlier atlas used a male brain, or, more likely, to the possibility that we were more conservative in labeling only structures that were clearly delineated in our histological material. Our use of English equivalents for nomenclature is a departure from most of the older brain atlases, although English terminology is used in more recent atlases (e.g., Paxinos, et al., 2000). The history of anatomical nomenclature suggests that attempts to standardize the naming of anatomical structures using Latin terms, as laid out in the BNA (Basle Anatomical Nomenclature, e.g., Jamieson, 1916), has not been without controversy. The original list of terms was derived from human anatomical treatises, so it is not surprising that attempts to transfer these terms to the brain anatomy of other species will not meet universal acceptance. We chose the English terms used for the rhesus macaque brain illustrated at the <http://brainmaps.org> website because of the agreement in internal positioning of what appear to be the equivalent structures in the marmoset brain used in our atlas (Table 1).

4. Experimental Procedure

Subjects

Experiments were performed in two adult female common marmosets, two years in age, 500 g body weight, from our breeding colony. All procedures followed the guidelines of the NIH Animal Care and Use Committee.

Histology

One of the female marmosets was deeply anesthetized with sodium pentobarbital (100mg/kg) and perfused through the heart with PBS followed by 10% formalin. The brain was then sent to Neuroscience Associates of Knoxville, TN, who prepared it for histological analysis. The brain was cut in the coronal (frontal) plane at 40 microns, every sixth section stained for Nissl granules with thionine and the adjacent section stained for myelinated fibers with the Weil technique. Only Nissl-stained sections are used in the present atlas. The actual distance between the Nissl-stained sections was 240 microns. We selected every other section to produce an atlas with sections spaced every 480 microns. The mounted sections were photographed at the NIH (Medical Arts and Photography Branch). The equipment used was a Nikon Multiphot optical bench with Zeiss Luminar 100 mm lens, and scanned with a Better Light 6100 scan back driven by Better Light Viewfinder 5.3 software. The final images were saved as arrays of 6000×8000 pixels in Adobe Photoshop 6.0. Some additional re-touching (brightness and contrast) was done with Adobe Photoshop Elements 2.0.

Magnetic Resonance Imaging

MRI images were made in the fixed brain of another female marmoset. The marmoset was sacrificed under deep anesthesia (sodium pentobarbital, 100 mg/kg, iv) via perfusion fixation through the ascending aorta with 500 mL of ice-cold 4.0% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The brain was removed and further fixed by immersion in the paraformaldehyde solution for 2 hours at 4°C. It was then stored in 10% neutral buffered formalin doped with 5 mM gadopentetate dimeglumine (Magnevist, Berlex Laboratories, Wayne, NJ, USA) contrast agent for one week. MRI was performed on a 7T/30 cm USR/AVIII MRI scanner (Bruker-Biospin, Inc., Ettlingen, Germany) equipped with a 15 cm gradient set capable of 450 mT/m strength (Resonance Research, Inc., Billerica, MA, USA). The brain was imaged in a 3.5 cm inner diameter transmit/receive birdcage coil (Bruker-Biospin) with a T₂-weighted, 3D multi spin-echo sequence (TE = 13, Number of Echoes = 2, TR = 350 ms, FOV = 34 mm × 26 mm × 22 mm, Matrix = 512 × 395 × 335) in about 13 hours. The displayed coronal sections from this data set are 66 µm thick with an in-plane resolution of 66 µm.

Nomenclature

The nomenclature and abbreviations used in our atlas have come, almost exclusively, from brainmaps.org (<http://brainmaps.org/index.php?action=viewslides&datid=1>) (Table 1). The coronal sections of a male rhesus macaque posted there meet the criteria of good quality histology, and identification of sufficient structures to be useful.

Acknowledgments

The authors wish to thank Ms. Julie Mackel and Ms. Deborah Bernhards for technical assistance. This research was supported by the Intramural Research Program of the NIH, NICHD and NINDS.

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Figure 1.
Adult marmoset brain, with mm ruler for size reference

Figure 2.
Cumulative number of MRI studies of marmosets

A 3.5

Figures 3–31.
Nissl atlas (A 12.0-P 2.0)

Figures 32–60.
MRI atlas (A 12.0-P 2.0)

Table 1

List of Abbreviations

AAA	anterior amygdaloid area
ABL	basolateral nucleus of the amygdala
ABM	basomedial nucleus of the amygdala
ACC	nucleus accumbens
ACE	central nucleus of the amygdala
AD	anterodorsal nucleus of the thalamus
AHA	amygdalo-hippocampal area
ALA	lateral nucleus of the amygdala
AME	medial nucleus of the amygdala
AV	anteroventral nucleus of the thalamus
BNM	basal nucleus of Meynert
CA	caudate nucleus
CG	cingulate gyrus
CGr	central gray
CI	claustrum
CL	central lateral nucleus of the thalamus
CM	centre median nucleus of the thalamus
CNF	cuneiform nucleus
CR	central lineal nucleus of the raphe
DBH	horizontal nucleus of the diagonal band
DBV	vertical nucleus of the diagonal band
DG	dentate gyrus
DR	dorsal raphe
ENT	entorhinal cortex
EW	Edinger-Westphal nucleus
FC	frontal cortex*
GPe	external division of the globus pallidus
GPI	internal division of the globus pallidus
GR	gyrus rectus*
Gu	Gudden's nucleus*
HF	hippocampal formation
HI	lateral habenular nucleus
Hm	medial habenular nucleus
ICC	central nucleus of the inferior colliculus
III	oculomotor nucleus
INF	infundibular nucleus
Ins	insular cortex
IO	inferior olive complex
ISC	islands of Calleja
ITG	inferior temporal gyrus
IV	trochlear nucleus
LC	locus coeruleus
LD	lateral dorsal nucleus of the thalamus
LG	lateral geniculate complex*
LLD	dorsal nucleus of the lateral lemniscus
LLV	ventral nucleus of the lateral lemniscus
LP	lateral posterior nucleus of the thalamus
MD	mediodorsal nucleus of the thalamus
MG	medial geniculate complex
ML	lateral mammillary nucleus
MM	medial mammillary nucleus
MS	supramammillary nucleus
NC	central nucleus of the brainstem
OAN	anterior olfactory nucleus
OLT	olfactory tubercle
Pa	paraventricular nuclei of the thalamus
PAG	periaqueductal gray
PAH	paraventricular nucleus of the hypothalamus
PB	parabrachial nucleus
Pf	parafascicular nucleus of the thalamus
Pla	anterior nucleus of the pulvinar
Pli	inferior nucleus of the pulvinar
Plm	medial nucleus of the pulvinar
POA	preoptic area
PPN	peripeduncular nucleus
PRP	prepiriform cortex
PSB	presubiculum
PTA	anterior pretectal nucleus
Pu	putamen
R	reticular nucleus of the thalamus
RN	red nucleus
Rs	retrosplenial cortex
RTP	reticulotegmental pontine nucleus
SB	subiculum

SC	superior colliculus
SLN	lateral septal nucleus
SNe	pars compacta of the substantia nigra
SNM	medial septal nucleus
SNr	pars reticulata of the substantia nigra
SO	superior olive complex*
SOM	medial superior olivary nucleus
SON	supraoptic nucleus
STG	superior temporal gyrus
V	trigeminal nuclear complex
VA	ventral anterior nucleus of the thalamus
VI	abducens nucleus
VII	facial nucleus
VL	ventral lateral nucleus of the thalamus
VLp	ventral lateral posterior nucleus of the thalamus
VMH	ventral medial nucleus of the hypothalamus
VPI	ventral posterior inferior nucleus of the thalamus
VPM	ventral posterior medial nucleus of the thalamus
VR	ventral raphe
VS	vestibular nuclei
ZI	zona incerta

All abbreviations and terminology are from brainmaps.org, except for terms with an*