Three-dimensional Subcortical Atlas of the Marmoset ("SAM") monkey based on MRI and histology

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2204

Introduction

A comprehensive three-dimensional digital brain atlas of cortical and subcortical regions based on MRI and histology has a broad array of applications in anatomical, functional, and clinical studies. Despite its essential role as a research model for human brain development and neurological disorders, the marmoset monkey lacked a comprehensive, well-organized MRI-histology-based atlas of subcortical regions. In this study, we first generated a Subcortical Atlas of the Marmoset, called the "SAM," from 251 delineated subcortical regions derived from high-resolution MAP-MRI, T2W, and MTR images *ex vivo*. We then confirmed the location and borders of these segmented regions in the MRI data using matched histological sections with multiple stains obtained from the same brain specimen. This newly derived *ex vivo* 3D digital atlas is intended to provide a practical standard template for neuroanatomical, functional (fMRI), clinical, and connectional imaging studies involving subcortical targets in marmoset monkeys.

This work has been published. For more details on this study, see *Saleem et al. (2024); Cerebral Cortex 34, bhae120*

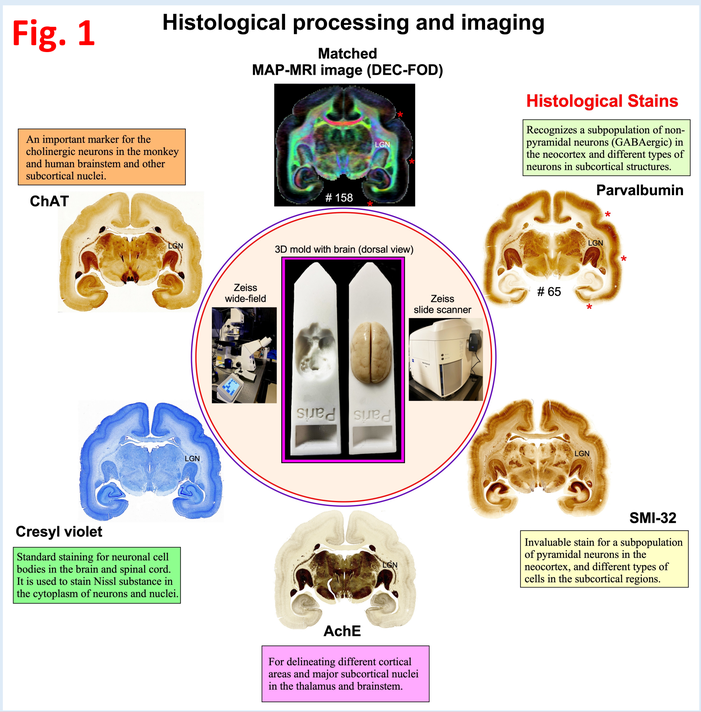
Methods

We scanned one adult perfusion-fixed marmoset brain on a 7T scanner using Mean Apparent Propagator (MAP)-MRI with 150 µm resolution. We acquired a total of 256 diffusion-weighted images with multiple b-values (bmax=10000s/mm^2), pulse duration δ=6 ms, diffusion time Δ=28 ms, and echo time (TE) 48 ms. In each voxel, we estimated the MAP and computed microstructural DTI/MAP parameters: fractional anisotropy (FA); mean, axial, and radial diffusivities (MD, AD, and RD, respectively); propagator anisotropy (PA), non-gaussianity (NG), return-to-origin probability (RTOP), return-to-axis probability (RTAP), and return-to-plane probability (RTPP), along with the fiber orientation distribution functions (fODFs). The MT ratio (MTR) was computed from images acquired with and without MT preparation (See **Figure 2** below).

Following MRI acquisition, we prepared the brain specimen for histological processing with multiple stains. An alternating series of 50 µm thick coronal sections were processed with AchE and Nissl or with antibodies against neurofilament protein (SMI-32), parvalbumin, NeuN, and ChAT (**Fig. 1**). The scanned high-resolution images of these different cell bodies and fiber-stained sections were manually registered to corresponding slices of different MRI parameters to confirm the location and architectonic boundaries of segmented subcortical regions on the 2D MRI slices.

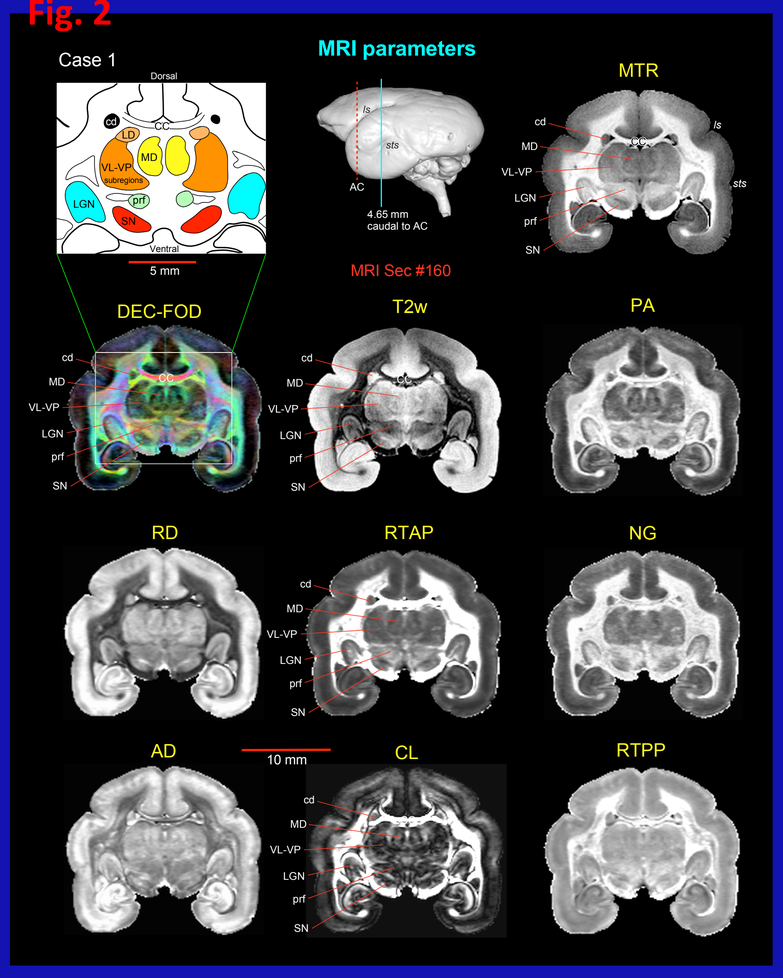
We then converted the delineated 2D subcortical/deep brain regions into a 3D volume. We adapted this new *ex vivo* 3D volume with 251 delineated regions as **"SAM"** (**Fig. 4**). This *ex vivo* atlas was then integrated into the AFNI and SUMA software packages with subcortical area labels.

Fig. 1 Histological processing and imaging



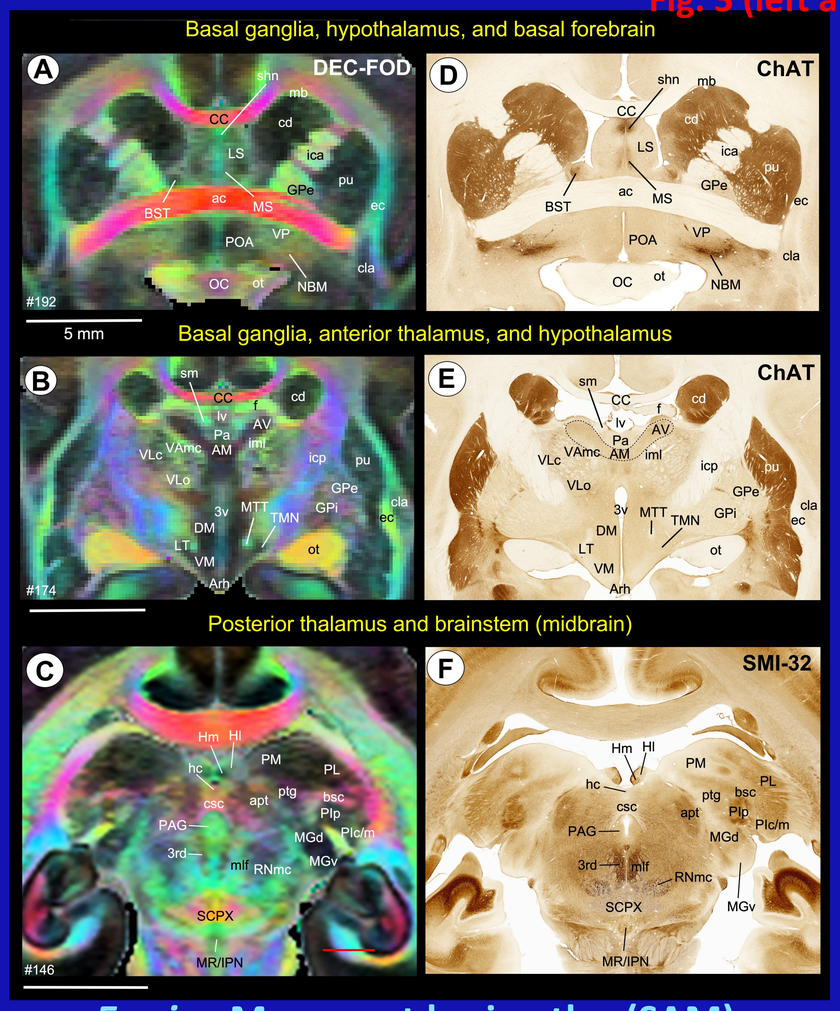
Results

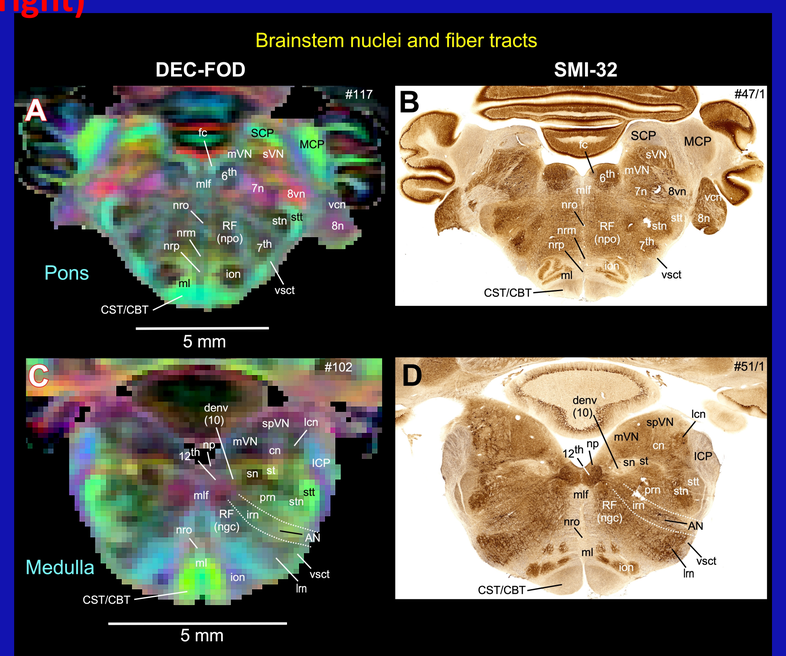
Fig. 2

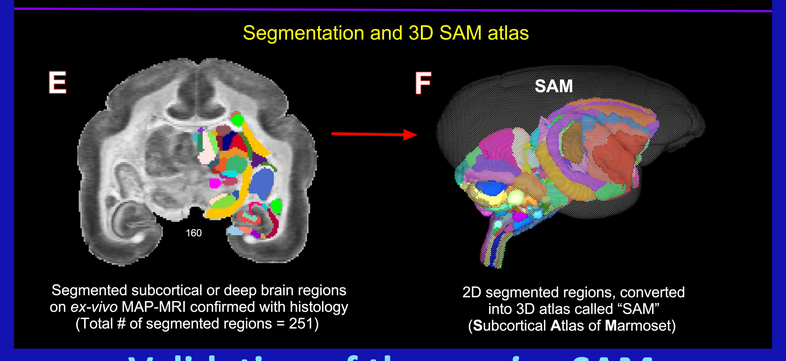


Subcortical areas for the 2D segmentation in MRI confirmed with histology

Fig. 3 (left and right)

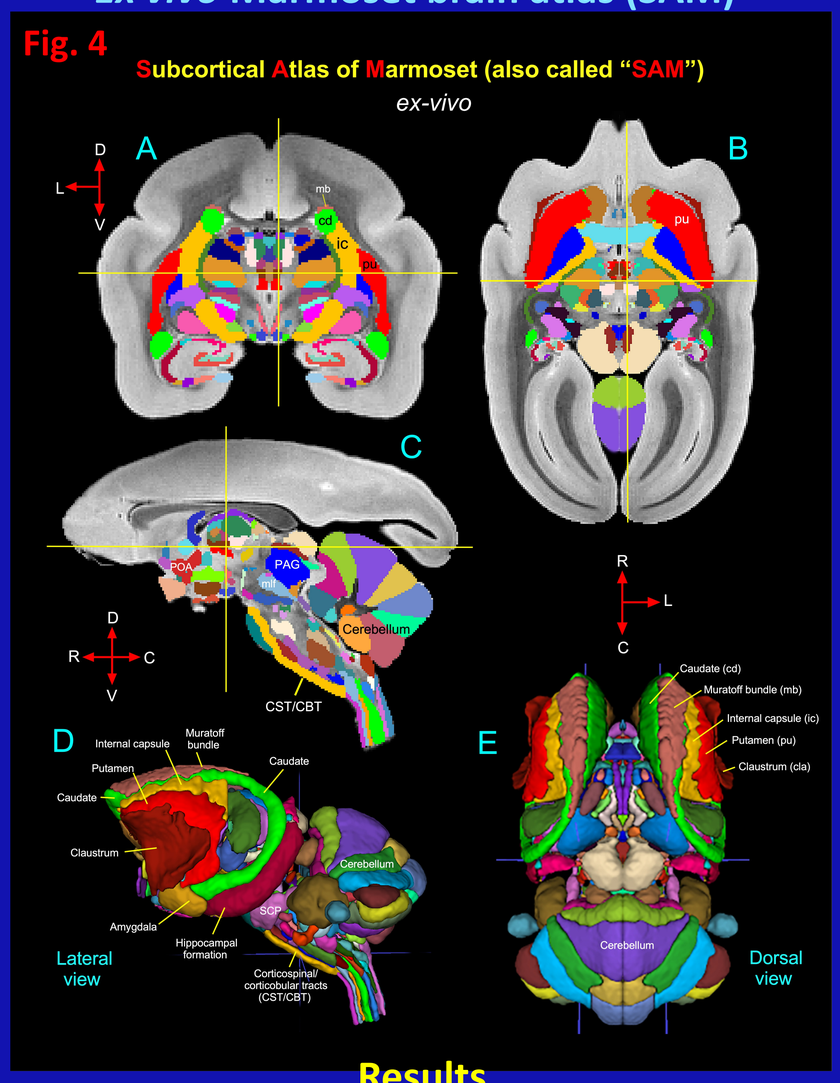






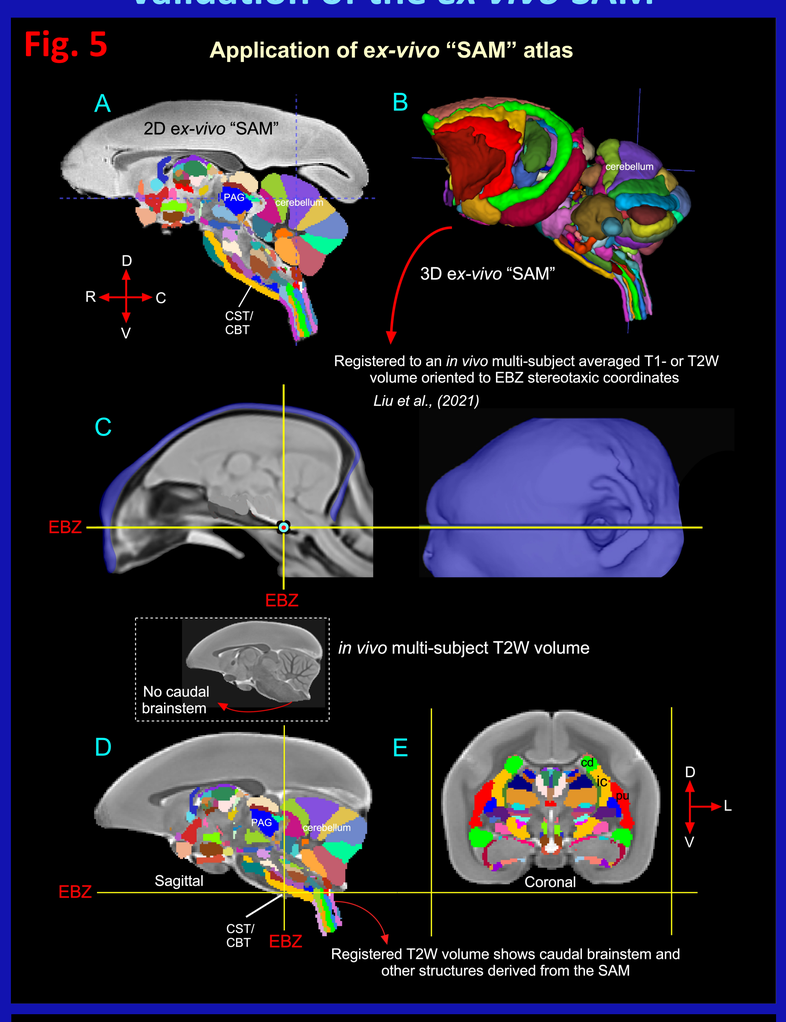
Ex vivo Marmoset brain atlas (SAM)

Fig. 4



Validation of the *ex vivo* SAM

Fig. 5

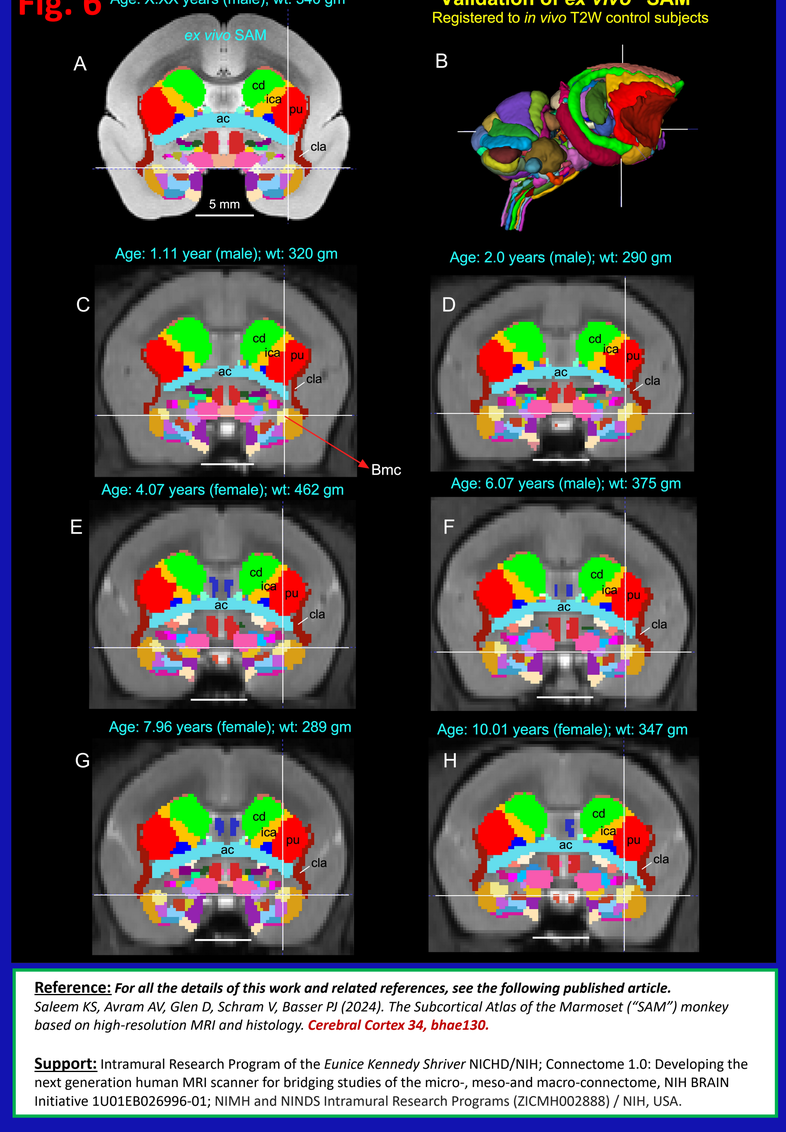


Results

Using a combined *ex-vivo* MAP-MRI with direction encoded color (DEC) map derived from the fiber orientation distribution (FOD) functions and histology, we identified and segmented 211 gray matter subregions in the deep brain structures, including the basal ganglia, thalamus, hypothalamus, brainstem (midbrain, pons, and medulla), amygdala, bed nucleus of stria terminalis, and the basal forebrain. In addition, we also distinguished and segmented 40 fiber tracts of different sizes and orientations associated with the basal ganglia, thalamus, brainstem, and cerebellum. The examples in **Figure 3** illustrate the subcortical gray and white matter regions in MAP-MRI (DEC-FOD) that are segmented with reference to matched histological sections for the 3D atlas. This newly segmented volume is called *ex vivo* "SAM," or the Subcortical Atlas of the Marmoset. The SAM atlas in **Figure 4** shows the segmented subcortical regions on the 2D coronal, axial, and sagittal MRI and in 3D. This new digital atlas provides a practical standard template for neuroanatomical, functional (fMRI), clinical, and connectional imaging studies. The *ex vivo* digital template atlas is available as volume and surfaces in standard NIFTI and GIFTI formats.

We also estimated, confirmed, and validated the atlas-based areal boundaries of subcortical areas by registering this *ex vivo* atlas template to *in vivo* T1- or T2W MRI datasets of different age groups (single vs. multisubject population-based marmoset control adults) using a novel pipeline developed within AFNI and SUMA (**Figs. 5, 6**). These results demonstrate that affine and nonlinear warpings are sufficient to distinguish and provide atlas-based estimates of areal boundaries of marmoset subjects *in vivo*.

Fig. 6 Age: X.XX years (male); wt: 340 gm



Summary and Conclusion

Using combined multimodal MRI and histology, we segmented 251 subcortical or deep brain regions and generated a 3D digital template atlas of the marmoset called the "**SAM**" (or **S**ubcortical **A**tlas of the **M**armoset) *ex vivo*.

The strengths of this work compared to others are that,

- It uses many high-resolution microstructural DTI/MAP and other MRI parameters with fODFs/DEC.
- This study utilizes high-resolution MRI (150 µm) and corresponding histology sections with 5 different stains from the same brain (i.e., MRI-histology correlation) to segment subcortical regions.
- This integrated "***multimodal***" approach yields a more objective and reproducible delineation of nuclei and their boundaries in the deep brain structures, which include the basal ganglia, thalamus, hypothalamus, limbic regions (amygdala), basal forebrain, and the brainstem (midbrain, pons, and medulla).
- Finally, we estimated and confirmed the atlas-based areal boundaries of subcortical regions by registering this *ex vivo* atlas template to *in vivo* T1-or T2W MRI datasets of different age groups (single vs. multisubject population-based marmoset control adults) using a novel pipeline developed within AFNI and SUMA software packages.

Tracing and validating these important deep brain structures in 3D (atlas) will improve neurosurgical planning, anatomical tract tracer injections, navigation of deep brain stimulation probes, functional MRI and brain connectivity studies, and our understanding of brain structure-function relationships.

Reference: ***For all the details of this work and related references, see the following published article.***
Saleem KS, Avram AV, Glen D, Schram V, Basser PJ (2024). The Subcortical Atlas of the Marmoset ("SAM") monkey based on high-resolution MRI and histology. ***Cerebral Cortex 34, bhae130.***

Support: Intramural Research Program of the *Eunice Kennedy Shriver* NICHD/NIH; Connectome 1.0: Developing the next generation human MRI scanner for bridging studies of the micro-, meso-and macro-connectome, NIH BRAIN Initiative 1U01EB026996-01; NIMH and NINDS Intramural Research Programs (ZICMH002888) / NIH, USA.