Age-related compartment-specific volume reductions in macaque locus coeruleus: a combined MRI, Nissl and immunofluorescence study.

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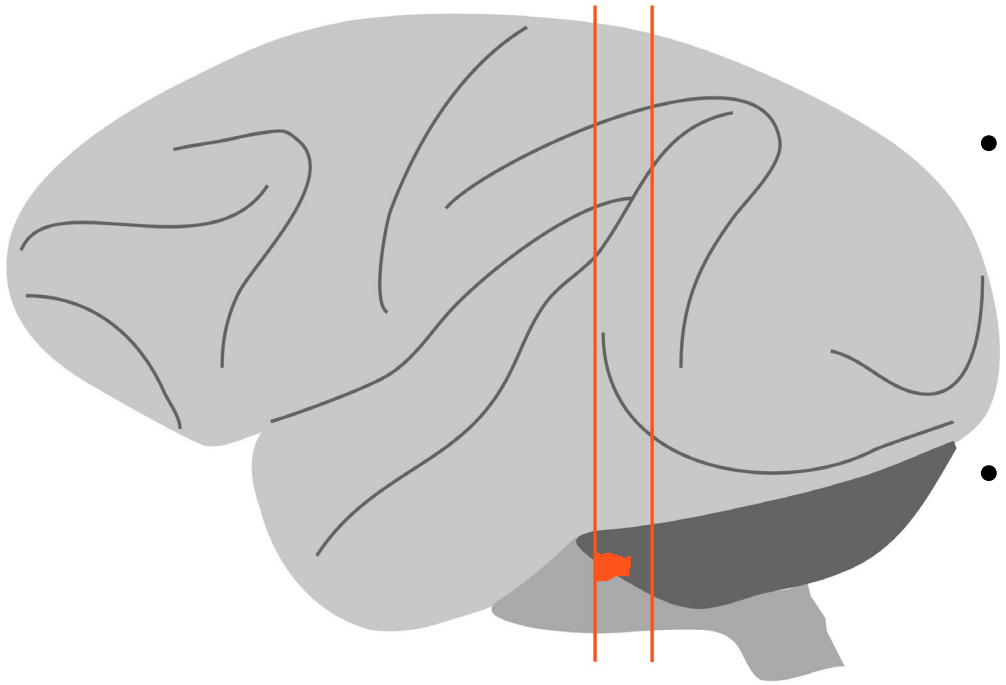
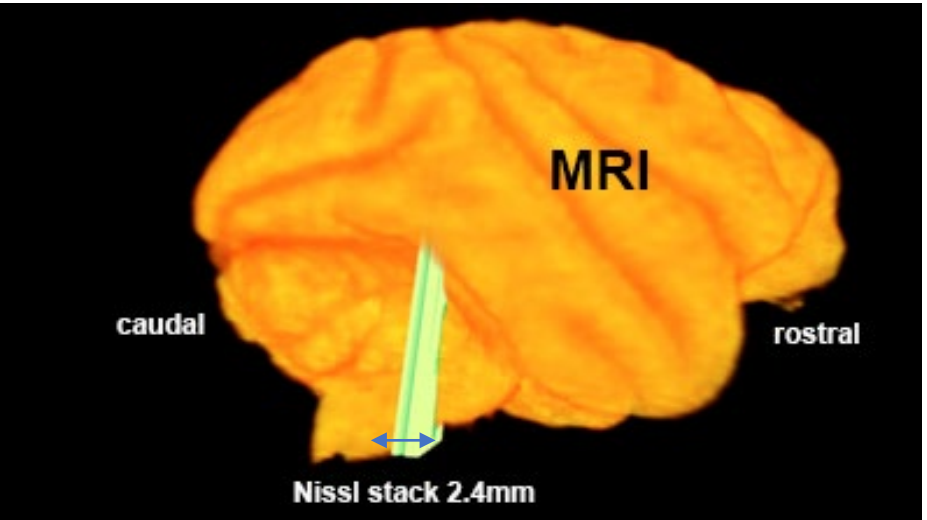
INTRODUCTION



- **The Locus Coeruleus (LC) is a brainstem nucleus with the largest group of noradrenaline producing neurons in the brain.**
- **Dysregulation of the LC system contributes to cognitive dysfunction in both normative and pathological aging brains (such as Alzheimer's disease).**
- **In this study, we describe the standardized 3D anatomy of the LC nucleus using MRI data, and brain sections stained with Nissl and immunofluorescent markers.**

Methods

- A colony of 30 cognitively assessed rhesus macaques ranging in age from 7 to 32 years (human equivalent ~21-96 years) was used for this project.
- MRI image stack .nii : 256x256x80 coronal T1-weighted radio frequency spoiled gradient recalled echo sequence (3D SPGR) whole brain images were collected *in vivo* at 0.625mmx0.625mmx1.00mm
- After perfusion, tissue was sectioned coronally at 30μm and every 4th section was labeled with standard Nissl procedures. Adjacent sections (thus sampled every 120μm) were labeled with immunomarkers for catecholaminergic (Anti-Tyrosine Hydroxylase, TH) neurons to define the LC boundaries.
- High-resolution 5x microscopy Nissl images were processed and aligned in Fiji's bioimage and Adobe Photoshop to create stack of images with appropriate dimensions.
- AMIRA software (Thermo Fisher Scientific) was used to align Nissl and MRI data using the following workflow:



-Segmentation of ROI brainstem from MRI stack

-Segmentation of ROI brainstem from Nissl stack

-Alignment segmented Nissl stack with segmented MRI

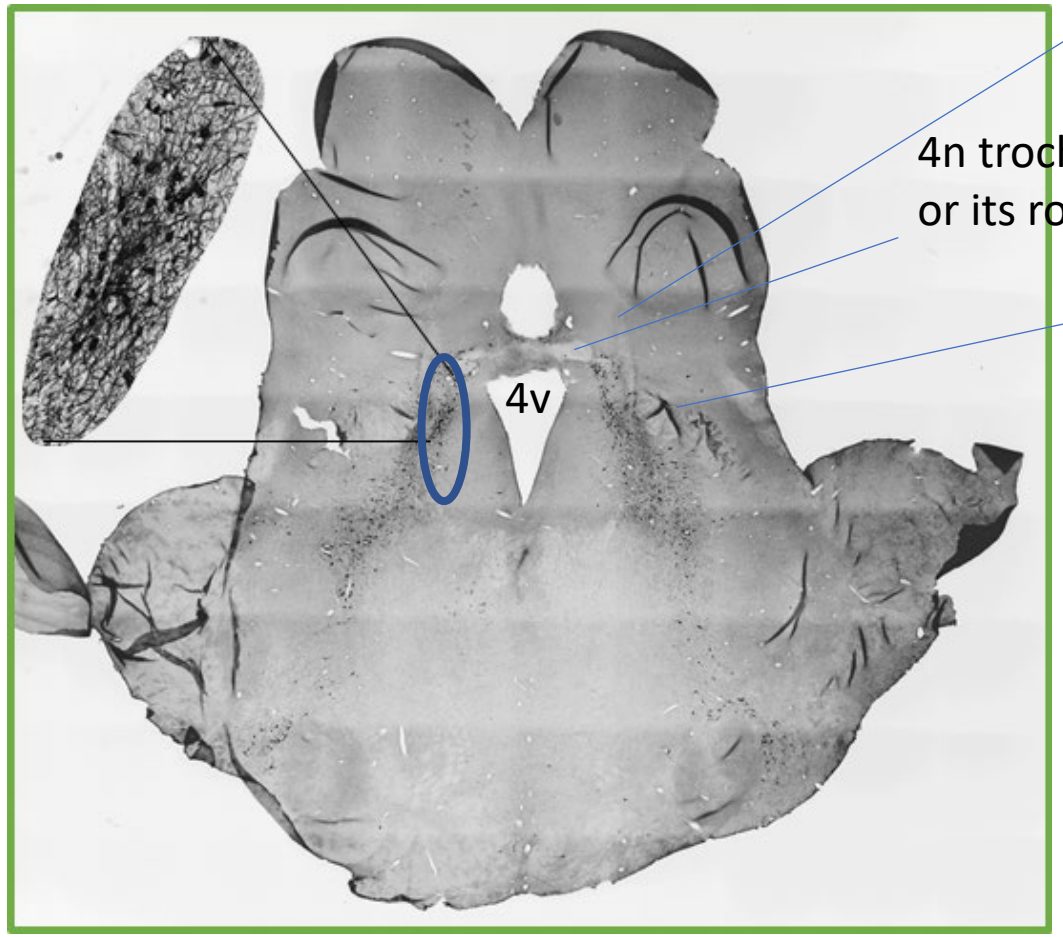
-Registration and Segmentation the LC compartment

-Estimation of the LC volume

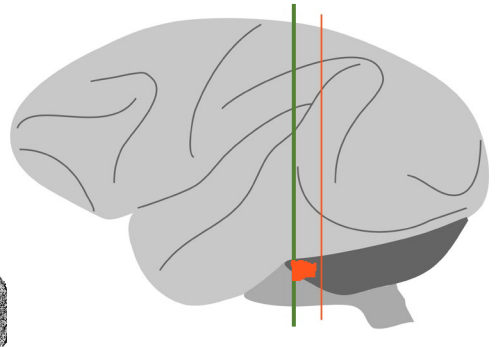
Results: The anti-TH and Nissl stain cell bodies define LC boundaries

the most rostral LC

Immuno anti-TH



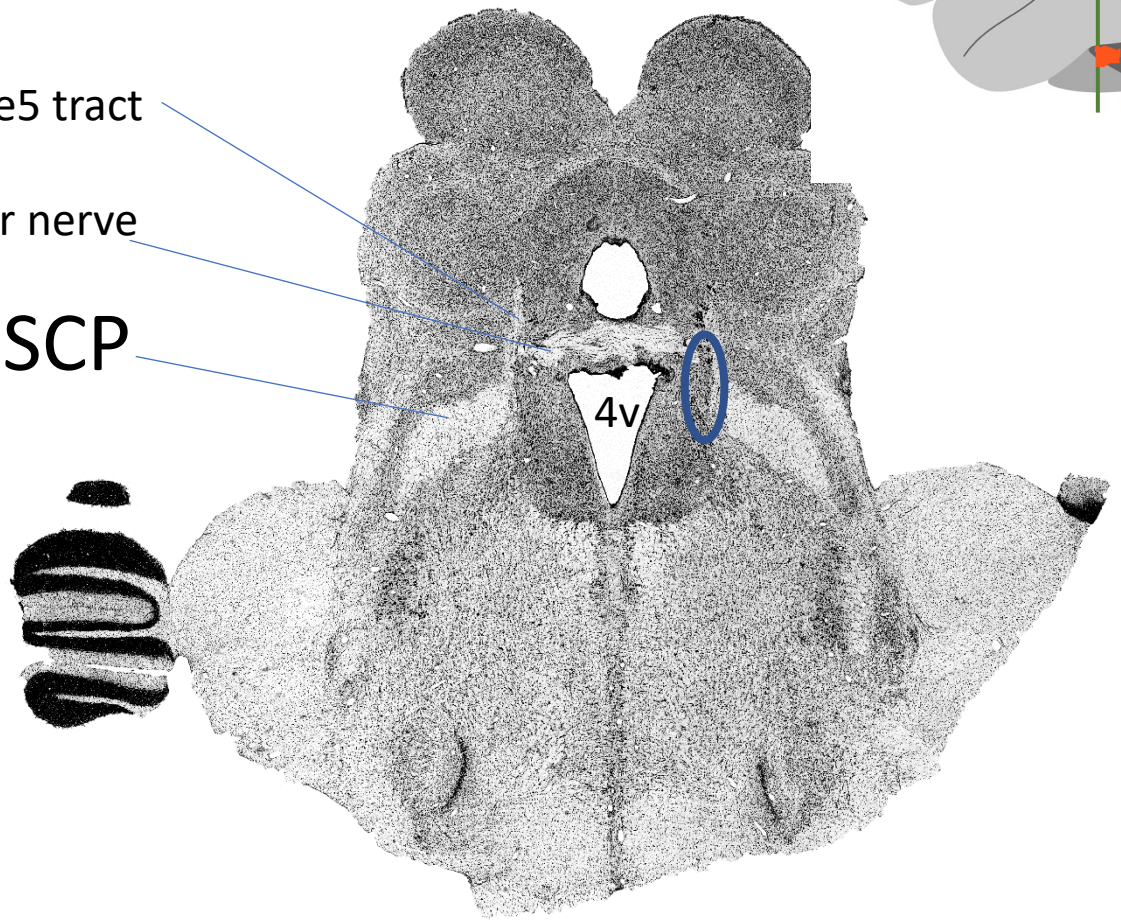
Nissl



Me5 tract

4n trochlear nerve
or its root

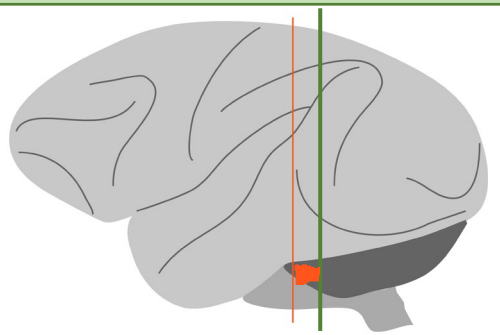
SCP



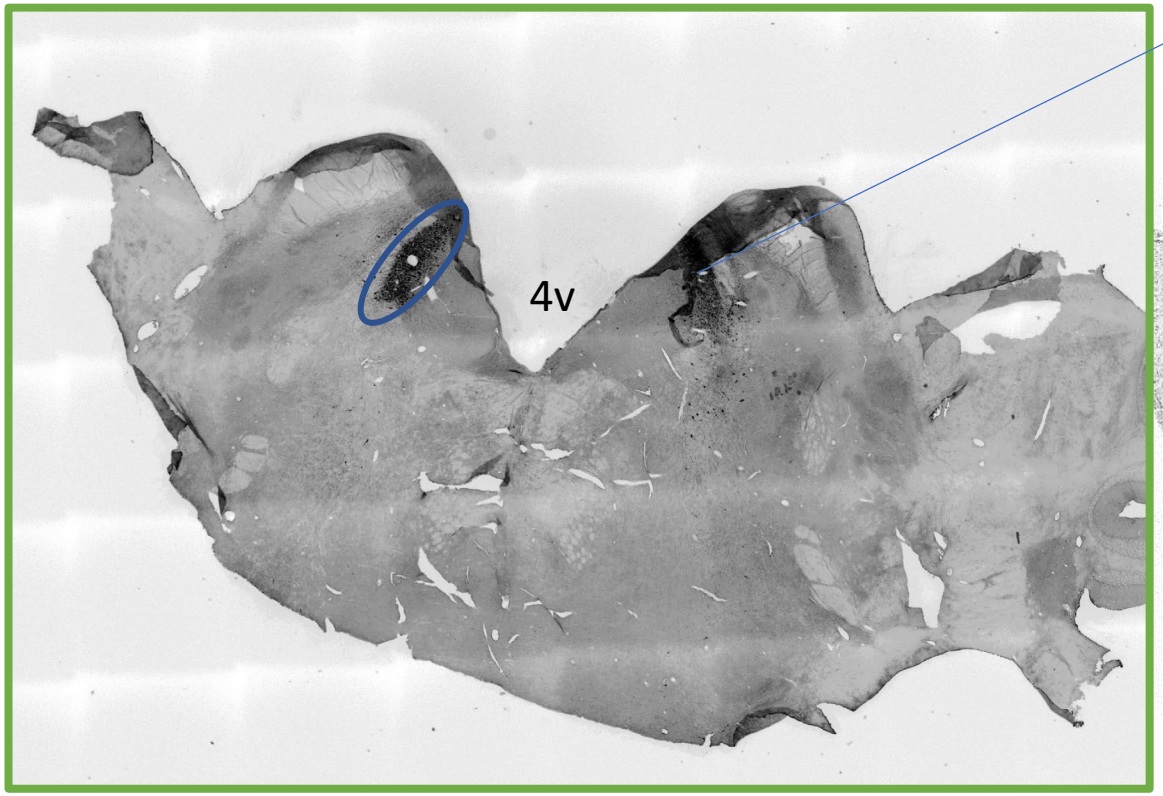
SCP –superior cerebellar peduncle

Results: The anti-TH and Nissl stain cell bodies define LC boundaries

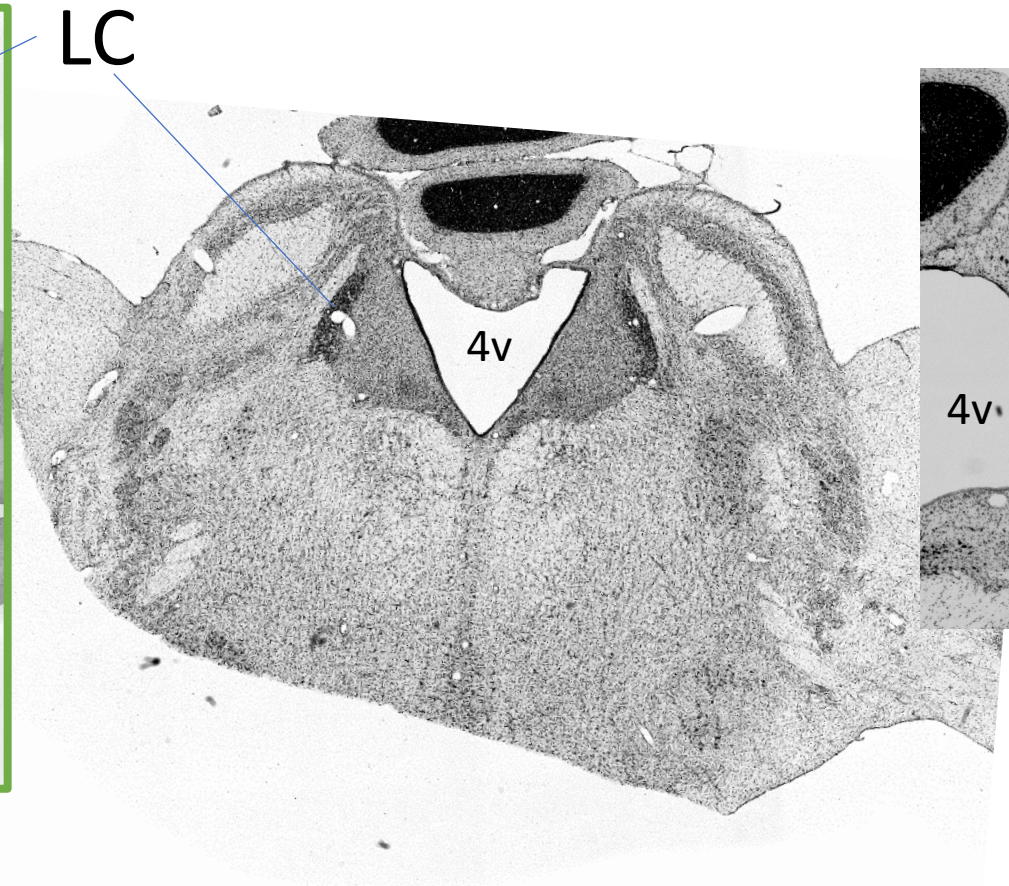
the most caudal LC

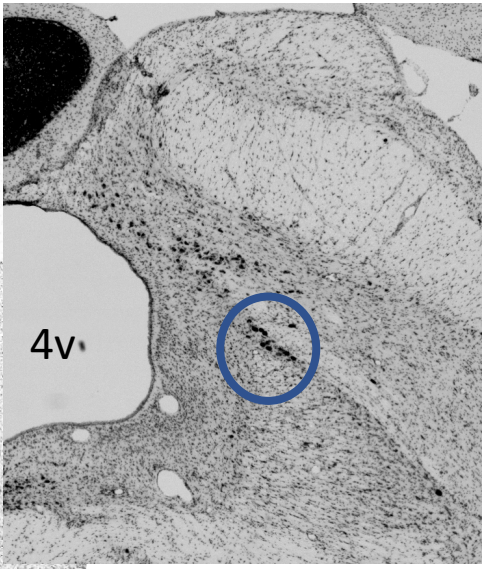


Immuno anti-TH



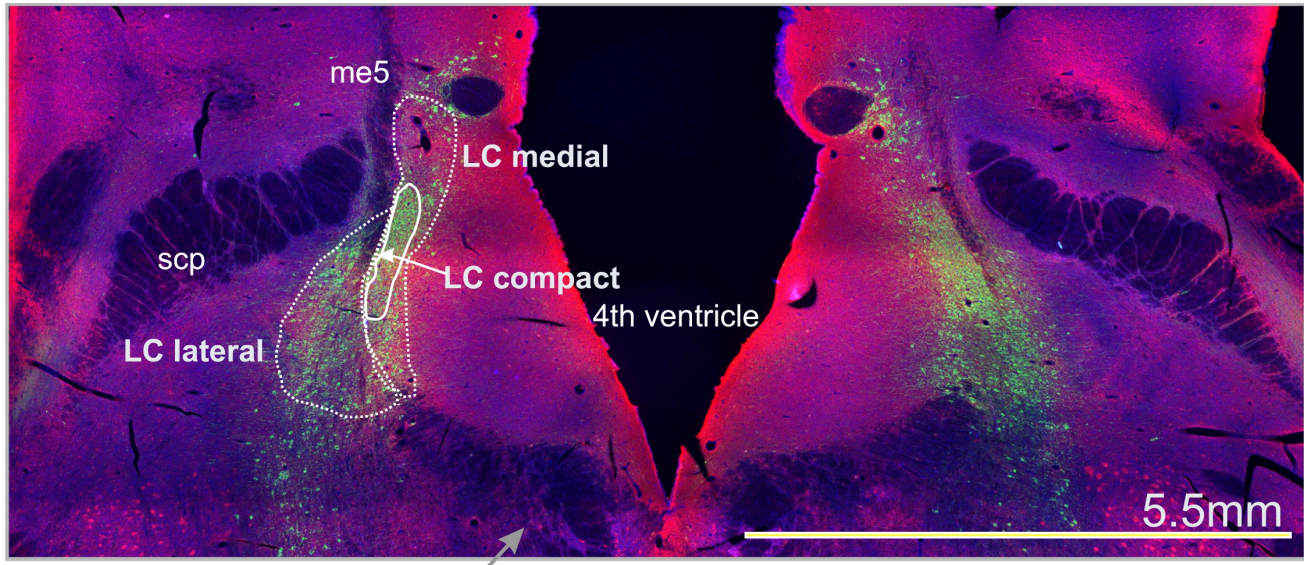
Nissl

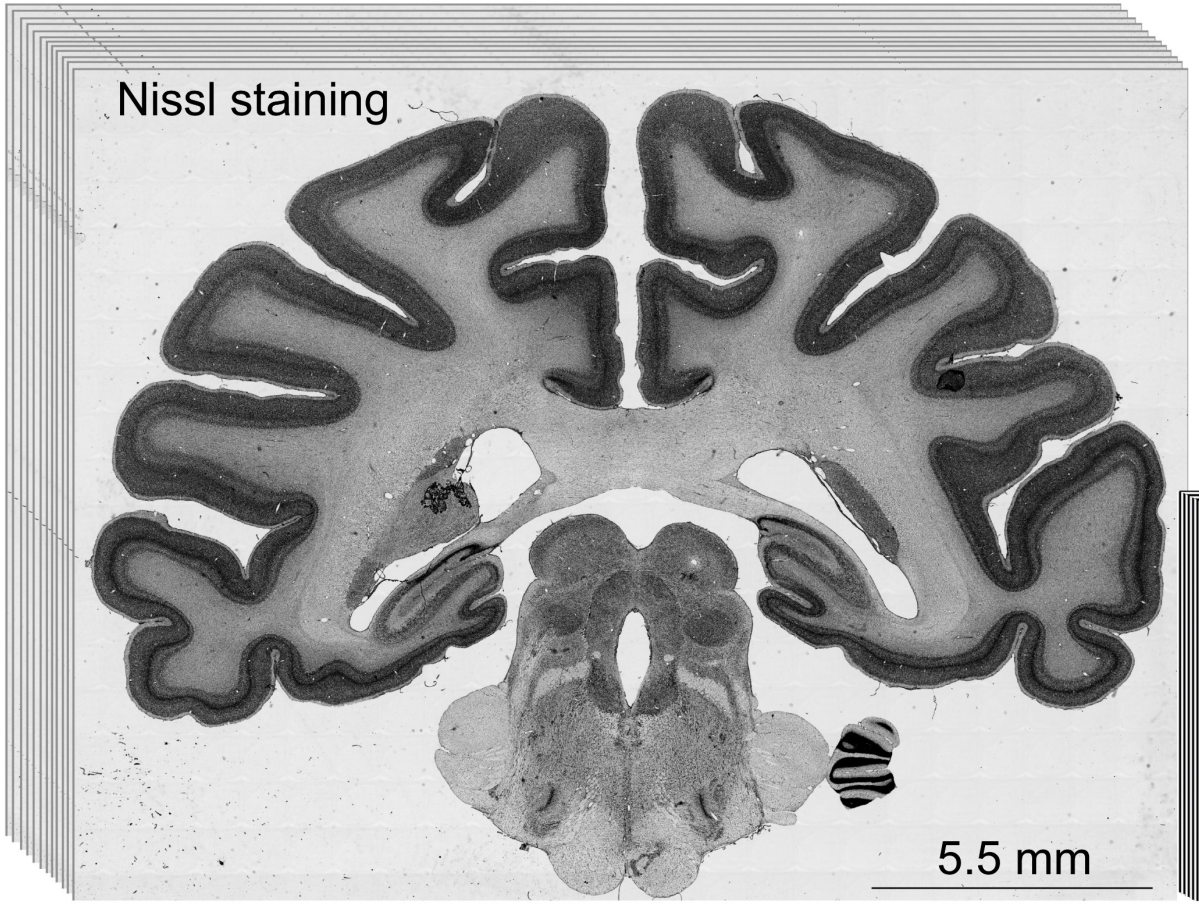


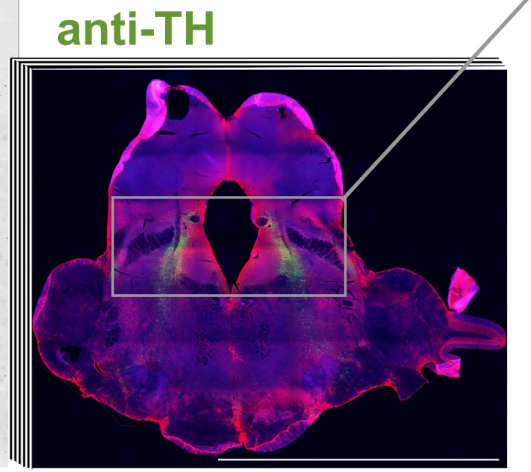


Results: Identification of the LC area for comparative study of LC volumes across age

- **LC medial and LC lateral** compartments were distinguishable based on their place in the gray area that bordered the 4th ventricle. **LC lateral** is outside of the gray area and is mixed with mesencephalic 5 (me5) tract.
- Within the **LC medial subnucleus** was a region **of particularly high cell density - LC compact.**

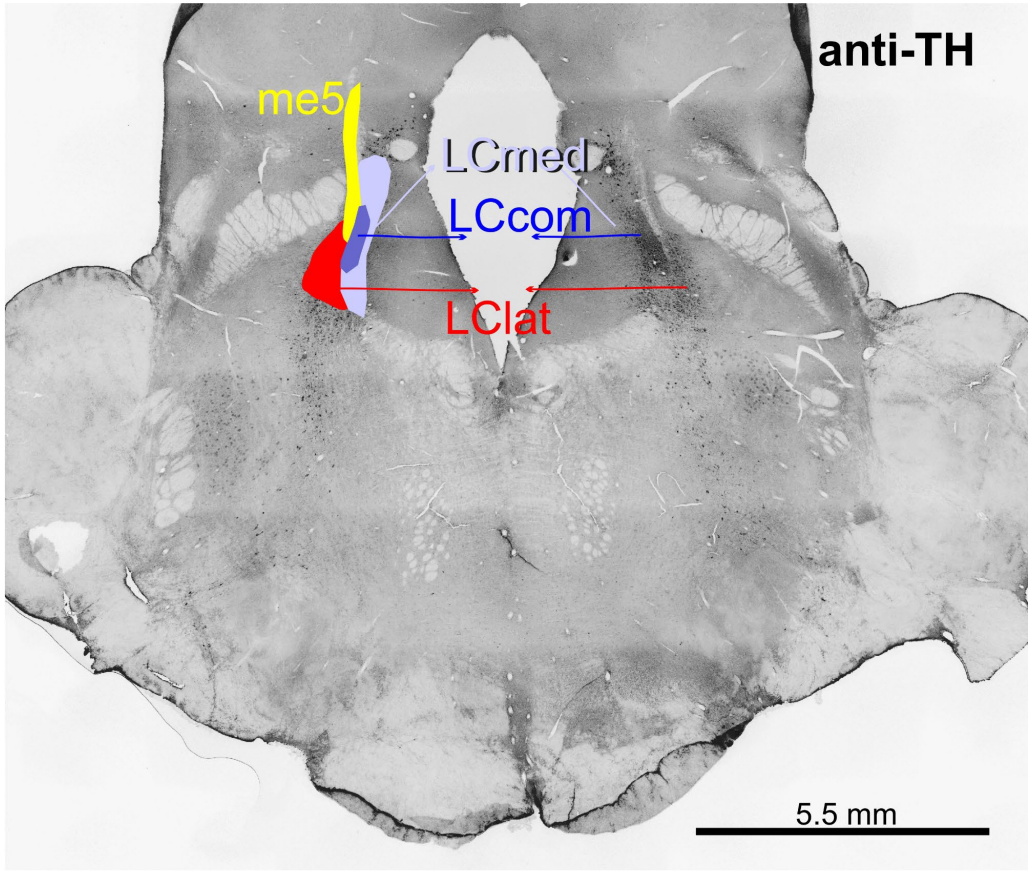


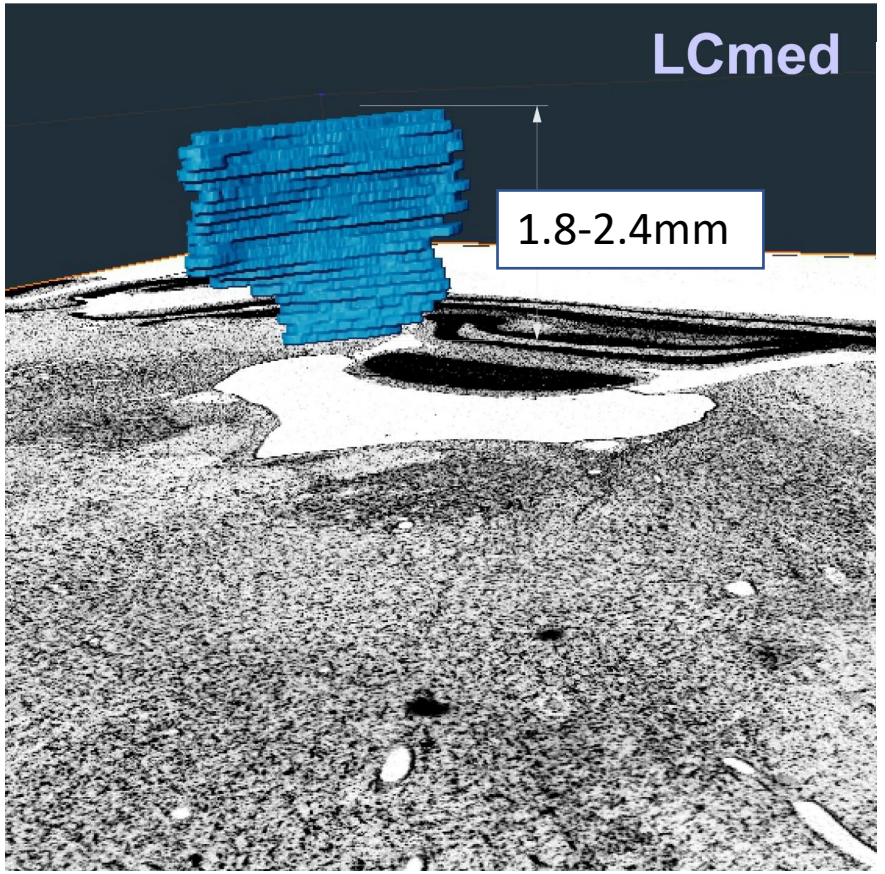


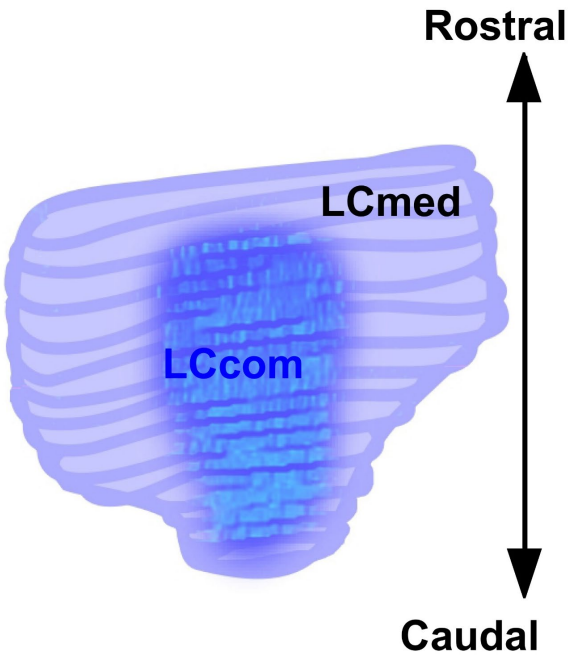


Results: 3D view and structure of the LC medial and LC compact

Within the LC medial subnucleus – LC compact region of particularly high anti-TH and Nissl stains cell body density

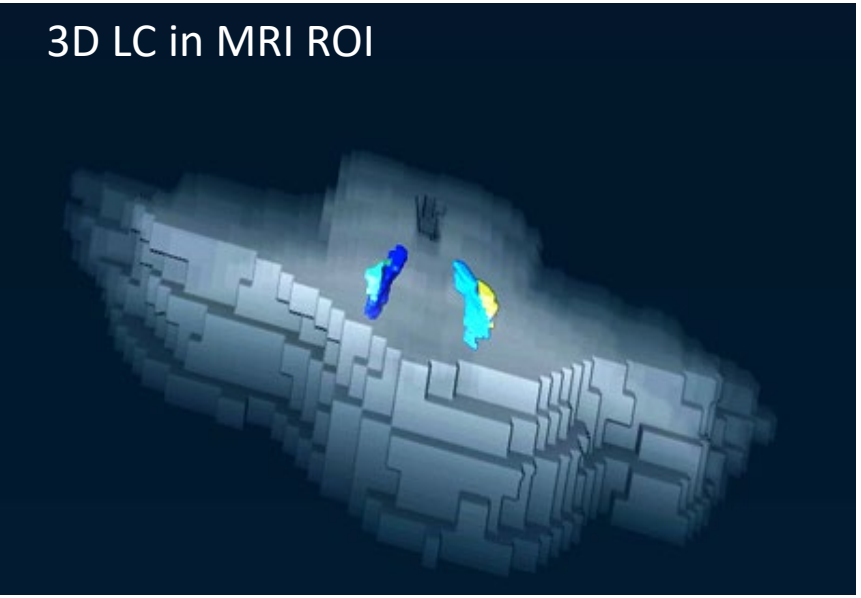


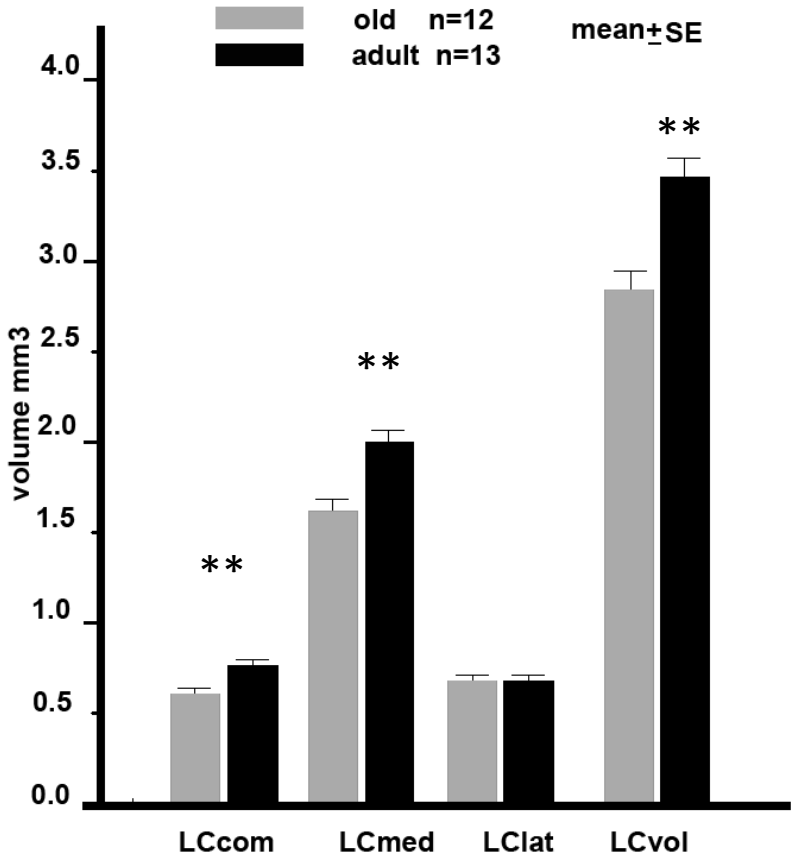


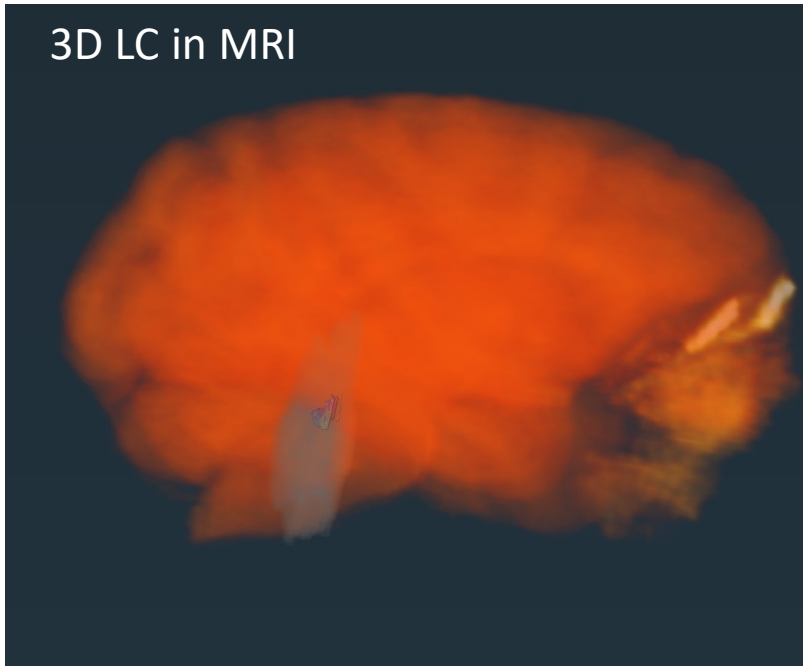


Results: A preliminary estimate of LC volume in adult and aged monkeys

Old: 22-32 years old
Adult: 8-19 years old







** At the 0.05 level, the means are significantly different (one-way ANOVA)

Conclusions

- Preliminary data from 25 animals indicate that the macaque LC extends approximately 2400 mm along the rostro caudal axis.
- We observed a core LC nucleus with high TH+ cell densities that became more scattered in more rostral brain sections.
- Overall volumes that comprised both the lateral and medial LC nucleus varied between 1.5 and 3.5 mm^3, and the older monkeys tended to have smaller LC medial nucleus volumes compared to the younger individuals.
- For the lateral nucleus there were no differences in the volume of the LC.
- This analysis pipeline will allow specific sites of vulnerability along the rostral-caudal axis of the LC to be identified.
- Furthermore, the data presented here indicate that the distinct compartments of the LC are somewhat independently impacted by brain aging.

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