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A high-resolution 3D reconstruction of the locus coeruleus in aged macaques: a combined MRI, Nissl and anti-Tyrosine Hydroxylase (TH) immunofluorescence study

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INTRODUCTION

The Locus Coeruleus (LC) is a brainstem nucleus with the largest group of noradrenaline producing neurons. Dysregulation of LC systems contributes to cognitive dysfunctions in both healthy aged brains and brains that succumb to Alzheimer's disease. Notably, the LC is heterogeneous along the rostral-caudal and dorsal-ventral axes with respect to neuron morphology, projection targets, and vulnerability to the impact of normative brain aging and neurodegenerative disease.

In previous studies in our laboratory, we identified three distinct subnuclei in the macaque LC: a medial nucleus that was confined to the central gray area, a lateral nucleus that lies outside of the gray area laterally and blends within the mesencephalic tract (me5), and a compact area within the medial nucleus.

In this study, we describe in detail the 3D anatomy of the LC nucleus using Nissl-stained, tyrosine hydroxylase (TH)-immunoreactivity, and MRI data of one macaque. Next, we describe the neuroarchitecture of the long-range processes of TH-positive LC neurons in the midbrain. Finally, we establish a protocol using AMIRA software for counting cells along the rostral-caudal axis and within the described compartments of LC.

METHODS

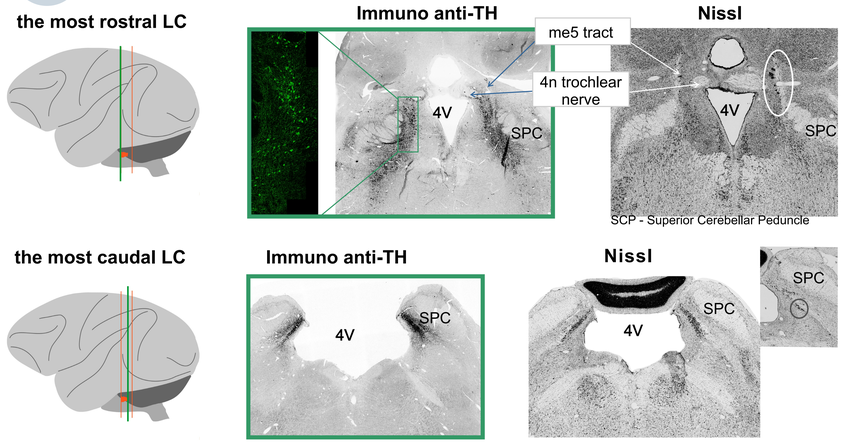
A colony of 30 cognitively assessed rhesus macaques ranging in age from 7 to 32 years (human equivalent ~21-96 years) was used for this project.
Animals underwent *in vivo* T1-weighted MRI scans (spoiled gradient echo sequence, 3D SPGR) at 0.625mmx0.625mmx1.00mm resolution.

After perfusion, tissue was sectioned coronally at 30μm and every 4th section was labeled with standard Nissl procedures. Adjacent sections (thus sampled every 8th section) were labeled with immunomarkers for catecholaminergic (Anti-Tyrosine Hydroxylase, TH) neurons to define the LC boundaries. [20]High-resolution 5x microscopy Nissl images were processed and aligned in Fiji's bioimage and Adobe Photoshop to create stacks of images with appropriate dimensions.

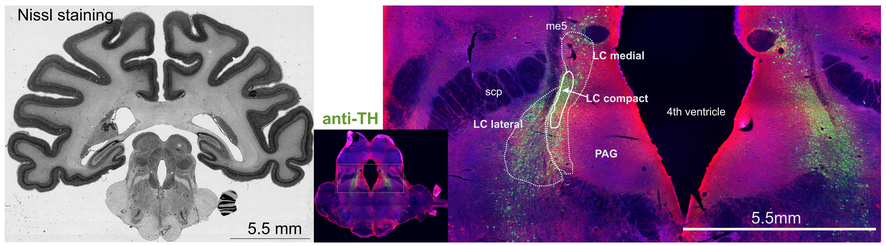
AMIRA software (Thermo Fisher Scientific) was used to align Nissl, anti-TH and MRI data using the following workflow: Segmentation of ROI brainstem from MRI stack; Segmentation of ROI brainstem from Nissl stack; Alignments of segmented Nissl stack with segmented MRI; Registration and Segmentation of LC compartments; Estimation of LC volume.

AMIRA software was also used to analyze the cell counts in each compartment. To accomplish this, LC TH-positive cells were first segmented manually to determine the range of possible cell dimensions to use for later segmentation. Next, all TH-positive neurons and processes were segmented using automatic AMIRA procedures, and the previously established cell dimensions were used to select putative cells within LC compartments. These automatic cell counts were compared with manual cell counts from within the LC nucleus to verify their accuracy.

1 Anti-TH and Nissl-stained cell bodies define LC boundaries

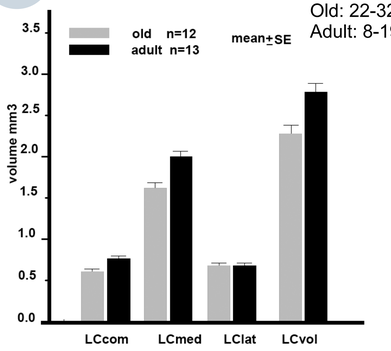


2 Identification of LC area for comparative study of LC volumes across age

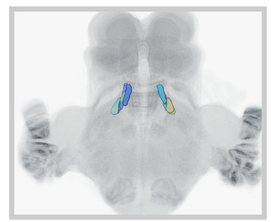


LC medial and LC lateral compartments were distinguishable based on their location with respect to the periaqueductal gray (PAG), which surrounds 4th ventricule. **LC lateral** lies outside of the gray area and blends with mesencephalic (me5) tract. Within the **LC medial subnucleus** was a region of particularly high cell density - **LC compact.**

3 A preliminary estimate of LC volume in adult and aged monkeys



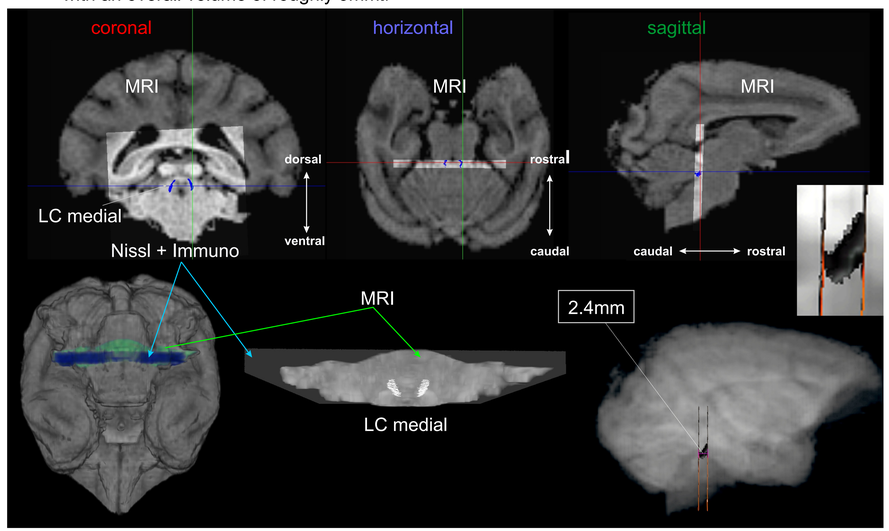
Old: 22-32 years old
Adult: 8-19 years old



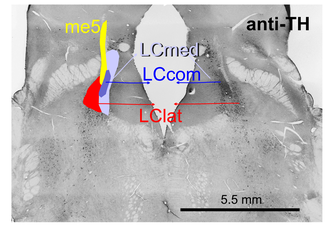
Older monkeys tended to have smaller LC volumes compared to the younger individuals. This was driven mostly by the LC medial compartment. This will need to be replicated in the full sample of 30 animals.

4 The alignment of anti-TH and Nissl-stained sections with MRI

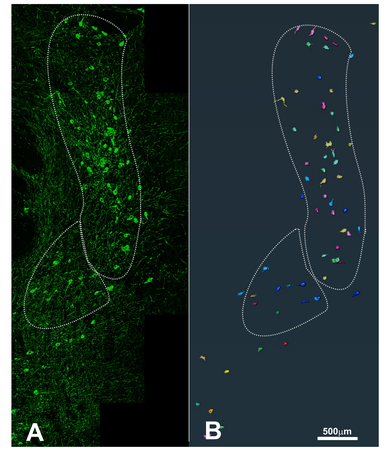
The macaque LC proper nucleus extends approximately 2.4mm along the rostro-caudal axis with an overall volume of roughly 3mm[3].

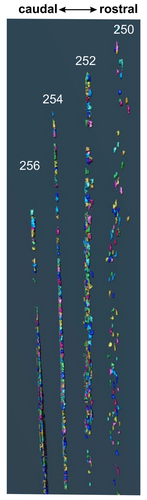


5 AMIRA software was also used to analyze the cell counts in each compartment



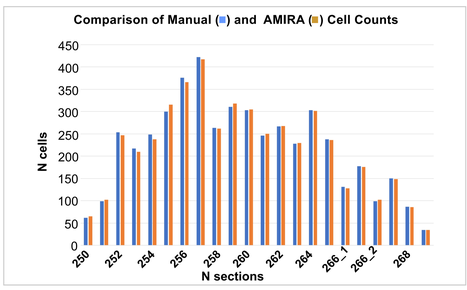
A: High-resolution images were taken at 40x on a Zeiss LSM880 inverted confocal microscope. Each LC image was comprised of individual z-stacked images tiled together using Zen Blue. **B:** The putative cells within LC compartments were obtained by AMIRA protocol during automatic cell counts. Each colored 'blob' represents the volume of a cell.

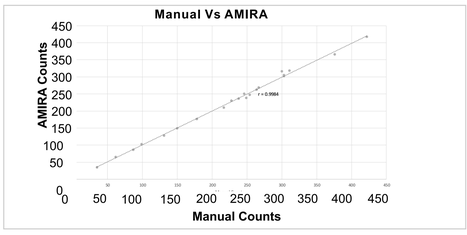




Example of the AMIRA cell body count volumes in aligned sections (saggital view). Only one side of the LC is shown.

Analysis of manual and AMIRA cell counts resulted in very high consensus between methods. Thus the AMIRA protocol cell counts will be used for cell counts in the LC compartments in adult and aged macaques.





CONCLUSIONS

This analysis pipeline will allow specific sites of vulnerability along the rostral-caudal axis of the LC to be identified for further molecular analyses aimed at understanding the mechanisms responsible for LC vulnerability and its impact on cognition in normative aging and disease.

Acknowledgments: We are grateful to Doug Cromey, M.S (Life Science North Microscope facilities). Thanks to Olivia Guswiler (Research Specialist) and Nolty Peggy Ann (Executive Assistant to the Director).
The work supported by NIH grant R01 Ag003376 and the McKnight Brain Research Foundation, P51RR000169, State of Arizona, Arizona Alzheimer's Consortium.