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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 neural dysfunctions observed in aging and Alzheimer's disease. We used AMIRA software to reconstruct the LC from tyrosine hydroxylase (TH)-immunofluorescence and Nisslstained serial sections aligned with previously collected MRI data. Using this method, we established the 3D structure of the LC nucleus and its subnuclei: LC lateral, that lies outside of the periaqueductal gray area laterally; LC medial, that is confined to the periaqueductal gray region and LC compact that lies within the medial nucleus.*

Here we present further analysis and description of these subregions and emphasize details of one of the LC subnuclei: LC compact.

METHODS

A colony of 30 cognitively assessed rhesus macaques ranging in age from 8 to 33 years (human equivalent ~24-99 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 $30\mu m$ and every 4th section was labeled with standard Nissl procedures. Every other adjacent section (thus sampled every 8th section) was labeled with immunomarkers for catecholaminergic (anti-TH) neurons to define the LC boundaries. Vasculature was labeled using the Solanum Tuberosum Lectin (STL) conjugated to streptavidin.

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 (see 1 below).



6-3D reconstruction of LC nuclei in Nissl+Immuno Stack

resolution confocal stack of the LC with Nissl+Immuno

Detailed examination of the locus coeruleus subnucleus - LC compact - in rhesus macaques

*Irina SINAKEVITCH¹, Kelsey E. McDERMOTT¹, Carol A BARNES^{1,2} Evelyn F. McKnight Brain Institute, Tucson, AZ, USA

Anti-TH and Nissl-stained cell bodies define LC boundaries z0 (0) (C) (a) z2(270) dfh z7(750) (g) z19 (

Identification of LC subcompartments along with their volumes



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(a1) shows the complete 3D reconstruction of the LC (medial LC is light blue, lateral LC is dark blue). (a2) shows the 3D reconstruction of the lateral LC (LClat). (a3) shows the 3D reconstruction of the medial LC (LCmed). (a4) shows the 3D reconstruction of the compact LC (Lccom). Scale bar: 1mm

A preliminary estimate of LC volume and anti-TH-stained cells in a subset of the macaques (n=8)

Monkey name, age	LC med vol mm3	LC lat vol mm3	LC total vol mm3	LC com vol mm3	LC com cell#	LC med cell#	LC lat cell#	LC total cell#
_awrence By6mo m	5.58	2.61	8.19	1.76	25817	34255	4904	39159
Buzz I3y7mo m	5.08	1.71	6.79	1.31	17480	28323	3868	32191
Arnauti I6y1mo m	4.98	2.82	7.8	1.65	21541	29434	6238	35672
Gronde I7y7mo m	5.34	2.45	7.79	1.60	29456	36000	4641	40641
Narouz 22y9mo m	4.25	1.83	6.08	1.41	19262	27469	4945	32414
Horton 26y 4mo m	4.54	2.15	6.69	1.24	18967	26766	5171	31937
Mountolive 28y7mo m	4.09	2.19	6.28	1.44	17719	28391	6654	35045
Joan 81y8mo f	4.36	1.90	6.26	1.68	23961	29937	5014	34951

² Department of Psychology, Neurology and Neuroscience, University of Arizona, Tucson, AZ

RESULTS



The green lines in (a) correspond to the stral-caudal level from which images e selected for the micrographs nown in **b**, **c**, **d**, **f**, **and h**. The Nisslained sections in e, g, and i are acent to the corresponding images of nti-TH-stained sections in d, f, and h. The image of anti-TH labelled brainstem ctions in (b) and (c) are the most stral and the most caudal brainstem aions, respectively. The numbers in the per right-hand corner of each panel flect the section number. The anti-TH stained cells in (**b**)are scattered (*i*) and aried in number from 5 to 30 cells per C. The next section, (d), is in the ostral region of the LC. In the adjacent ssl-stained section (e), large cell odies are apparent (black arrows) alongside the me5 tract, which serves as an additional marker indicating the ostral part of the LC. These cells are so indicated by black arrows in (g and). In the middle part of the LC nucleus f), in addition to scattered TH-positive eurons near the 4n-trochlear nerve white arrows; details in *ii*), a group of ensely packed neurons (detail in *iii*) ppear at the level of me5 (*). Some LC ell bodies reside outside of PAG as hown in detail in *(iv)*. The rostral region f LC is shown in **(h)**. All cells in this egion are densely packed. scpuperior cerebellar peduncle; 4V-4th entricle; PAG-periaqueductal gray, ne5-mesencephalic nerve. Scale bar: 5mm (a), 1mm (b-i), 100µm *(i-iv)*



(a1) Example cells from the rostral LC med where anti-TH (green) stained neurons are scattered, as well as STL labeled vasculature (magenta). (a2) In contrast to LCmed, the LC compact is characterized by much more densely packed anti-TH-stained neurons and by dense vascular beds (STL, magenta). Scale bar: 200µm (b) A 3D reconstruction of the LCmed is illustrated above the most caudal Nissl-stained section of the brainstem. In our eight macaques the rostro-caudal extent of the LC ranged from 2.10-2.55 mm in length and contained 70-85 sections through the brainstem from individual animals. (c) Schematic illustration of the LCcom, which is the part of the LC nucleus with the highest density of anti-TH-stained neurons. Three regions were identified within the LCcom, as stral (r), middle (m), and caudal (c) parts. The rostral LCcom begins with a small area of densely packed cells near the me5 tract. The middle LCcom extends across the entire dorso-ventral length of the LC. The caudal LCcom occupies a small area of densely packed cells. The extent of the LCcom ranges in length rostro-caudally from 1.44 to 1.95mm and contains 48-65 sections from individual animals

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Reference: *Sinakevitch et al. Program No. 574.08. 2022 Neuroscience Meeting Planner. San Diego, CA: Society for Neuroscience, 2022.



The LC compact is the area of the LC with

CONCLUSIONS

• The LC compact is the area of the LC with the highest neuronal density. It is located within the LC medial nucleus. LC compact extends rostro-caudally from 1.44-1.95 mm within the LC medial and it has TH-positive neurons with similar structure and cell diameters ranging from 29-43 μ m.

• In rhesus macaques the LC compact has three subregions: rostral, middle, and caudal. The rostral LC compact begins with a small area of cells with high density near the enlarged mesencephalic nerve (me5) in the PAG. The middle LC compact extends through almost all the LC medial along the dorso-ventral axis, and the caudal LC compact is a small area with the highest density of cells.

• The fact that the LC compact closely follows the me5 tract raises the question of whether this structure may interact with the me5 tract. These detailed characterizations of LC compact might be used to further examine the specificity of the impact of age on this LC subnucleus.