Immunohistochemical analysis of Locus Coeruleus neuronal projections and of α1, α2, and β noradrenergic receptors in the hippocampus of cognitively assessed aged and adult rhesus macaques

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Introduction

The locus coeruleus (LC)) is a noradrenaline (NA)-producing brainstem nucleus with wide projections throughout the cortex. NA acts via 3 types of NA receptors (α1, α2, β) and this signaling is critical for facilitating optimal cognitive performance. Some histological studies have suggested age-related decreases in NA fiber and varicosity density in the cortex, and autoradiographic studies have shown age- and disease-related decreases in α1 and α2 receptor densities. NA fiber density has not been investigated with density of all 3 NA receptor types or with respect to cognitive performance. To investigate this, we utilize hippocampus sections from cognitively assessed rhesus macaques labeled for NA axons, NA receptors, microglia, astrocytes and vasculature and use unbiased stereological techniques to quantify the expression of each marker.

METHODS

Behavior

Subjects: 30 rhesus macaques (16 aged, mean 24.6 years; 14 middle-aged, mean 13.9 years)

Behavioral Testing Apparatus: A modified Wisconsin General Testing Apparatus (WGTA) was used for all behavioral tasks.

Cognitive Assessment: All macaques underwent a delayed nonmatching-to-sample (DNMS; **Fig1A**), object discrimination (OD, **Fig 1B**) and delayed response (DR; **Fig1C**) task. These tasks assess object recognition memory, stimulus-reward association memory and spatial short-term memory, respectively.

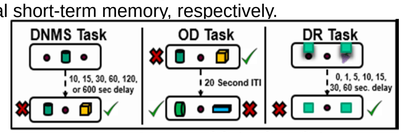

Figure 1: Schematics of the **A)** DNMS, **B)** OD, and **C)** DR tasks.

Immunohistochemistry

Sections: 30μm coronal tissue sections from 30 rhesus macaques were fixed in 4% PFA and stored long-term in OCT at -80ºC. Tissue was thawed and hemisected prior to incubation.

Antigen Retrieval: Sections were left free-floating in sodium citrate buffer and heated to 85ºC by water bath for 30 minutes.

Block Incubation: Sections were incubated in 3% NDS + 0.3% TX for 1hr at room temperature.

Primary Antibody Incubation: Sections were incubated in a Sheep anti-dopamine β hydroxylase (DBH, 1:1000) antibody with either rabbit anti-α1 adrenergic receptor (α1, 1:125), rabbit anti-α2a adrenergic receptor (α2a 1:200), or rabbit anti- β1 adrenergic receptor (β1, 1: 100) and either guinea pig anti-glial fibrillary acidic protein (GFAP,1:1000), guinea pig anti-IBA1 (1:1000) or biotinylated *Solanum tuberosum* lectin (STL, 1:200) overnight while shaking.

Secondary Antibody Incubation: Sections were incubated in their respective secondary antibodies (TH: 488; GFAP, IBA1 or STL: Cy3; α1, α2a or β1: Cy5) for 2hrs while shaking at room temperature. Sections were then incubated in DAPI (1:1000) for 15 mins.

IMAGING

Hippocampal sections were selected based on Paxinos Rhesus Monkey Atlas[1]. Images were taken at 40x on a ZEISS LSM880 inverted confocal microscope. A 2x2 tiled image of CA3 and DG comprised of individual z-stacked images were tiled together using ZEN Blue. Images were spectrally unmixed to distinguish autofluorescence from antibody signal[2]. FIJI ImageJ was used to quantify density of histological markers.

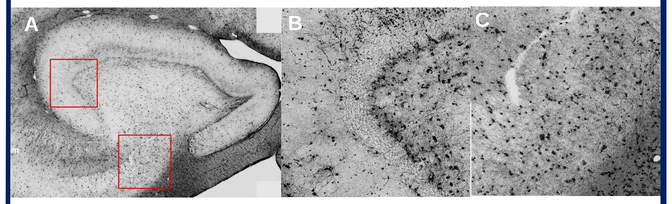

Figure 2: Hippocampal Section corresponding to Paxinos Atlas plate 88. A) An example section stained for β1 NA Receptors and imaged at 20x. Red squares indicate where 2x2 tiled images were taken of the dentate gyrus (B) and CA3 (C) regions. Image has been inverted for clarity.

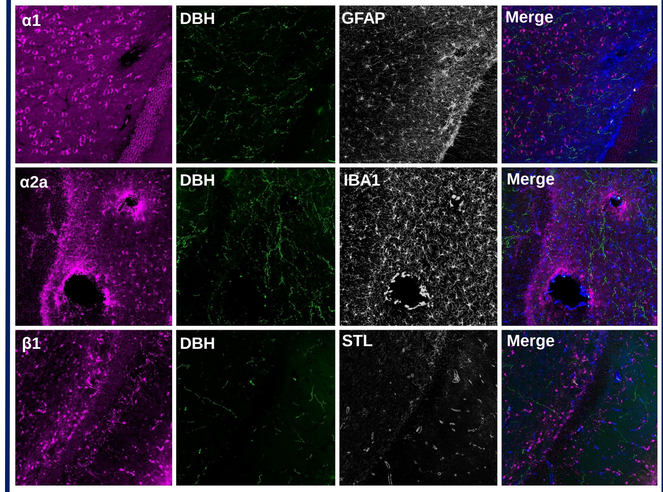

Figure 3: High magnification (40x) micrographs illustrating the 3 staining protocols used in this project. Top row: ant- α1 adrenergic receptor, anti-DBH, anti-GFAP and a merged image. Middle row: anti- α2a adrenergic receptor, anti-DBH, anti-GFAP and a merged image. Bottom row: anti- β1 adrenergic receptor, anti-DBH, STL, and a merged image.

BEHAVIORAL RESULTS

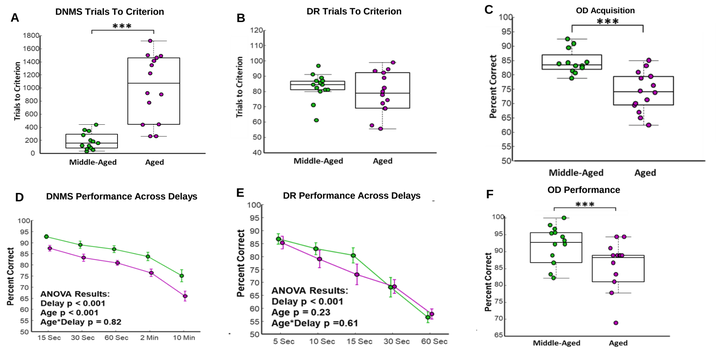

Figure 4: Trials to reach criterion (90% correct over 5 consecutive trials) by age group for the **A)** DNMS and **B)** DR task. Criterion performance for the **C)** OD task was determined by the percentage correct on the first two training sessions an animal underwent. Behavioral performance was also measured by percent of correct trials (across delays, where applicable) for the **D)** DNMS, **E)** DR, and **F)** OD task. ** = p < 0.01; *** = p < 0.001.

RESULTS: NORADRENERGIC RECEPTOR DENSITY

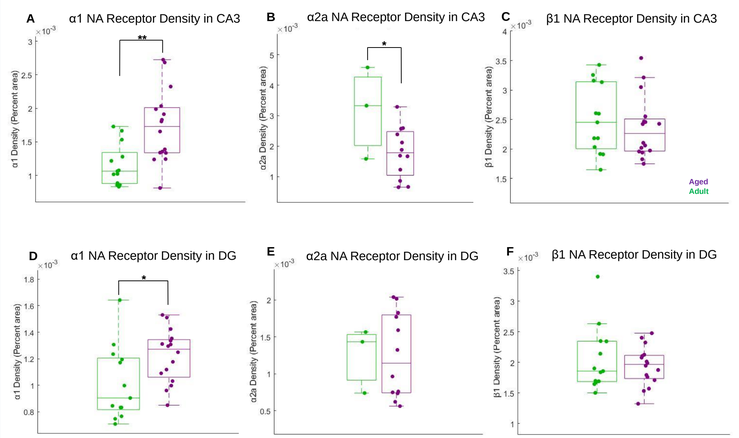

Figure 5: Comparisons of receptor densities in adult and aged animals in CA3 (top row) and DG (bottom row). **A)** Higher density of α1 NAR in old animals was seen in CA3 (p=0.002), **B)** Lower density of α2a NAR in CA3 of aged animals (p=0.049) **C)** No differences in β1 NAR density in CA3. In DG, α1 NAR density was significantly higher in aged animals (p=0.027) (**D),** with no differences between adult and aged animals in α2a NAR **(E)** or β1 **(F)** densities. * p<0.05, ** p<0.01

RESULTS: NORADRENERGIC AXON DENSITY

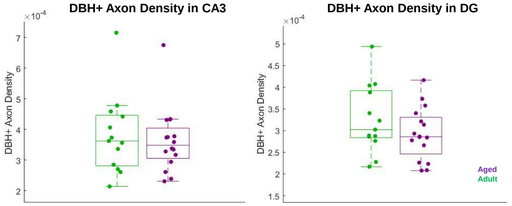

Figure 6: LC-NA axon density in the hippocampus, as measured by DBH. A) No difference in DBH+ density was seen in the CA3 subfield of the hippocampus between adult and aged macaques. B) No difference in DBH+ density was seen in the DG

SUMMARY AND CONCLUSIONS

There was no difference in dopamine-β hydroxylase-positive axon density in the hippocampus between adult and aged macaque monkeys. In the CA3 subfield of the hippocampus, the density of the α1 NA receptor is increased and the α2a NA receptor is reduced in aged compared to adult monkeys. In the DG subfield, aged monkeys had higher densities of the α1 NA receptor. We also saw no differences in glia or vascular densities between aged and adult monkeys.

No anatomical variables were associated with performance on our memory tasks.

While data collection is ongoing, these preliminary results indicate that LC projections to the hippocampus remain stable with age while local NA receptor densities are altered in the CA3 and DG subfields. There is no association with behavior detected at this point, which suggests that changes in NA densities in the hippocampus may not impact memory in old age.

ACKNOWLEDGMENTS & REFERENCES

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[1]Paxinos, G. et al., (2000). The Rhesus Monkey Brain in Stereotaxic Coordinates. Academic Press, San Diego, CA.

[2]Pyon, W. S., Gray, D. T., & Barnes, C. A. (2019). An Alternative to Dye-Based Approaches to Remove Background Autofluorescence From Primate Brain Tissue. Frontiers in neuroanatomy, 13, 73.