Changes in noradrenergic receptor density in the hippocampus across the lifespan of the rhesus macaque

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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), object discrimination (OD) and delayed response (DR) task **(Figure 1).** These tasks assess object recognition memory, stimulus-reward association memory and spatial short-term memory, respectively.

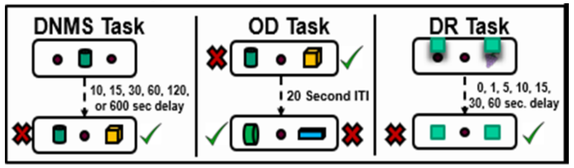

Figure 1: Schematics of the DNMS, OD, and DR tasks.

Immunohistochemistry

Sections: 30μm coronal sections from the 30 rhesus macaques were fixed in 4% PFA and stored at -80ºC. Tissue was thawed, hemisected and underwent antigen retrieval and a blocking procedure prior to incubation.

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

Secondary Antibody Incubation: Sections were incubated in their respective secondary antibodies (DBH: 488; GFAP, IBA1 or STL: Cy3; α1, α2a or β1: Cy5) for 2hrs. Sections were then incubated in DAPI 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.

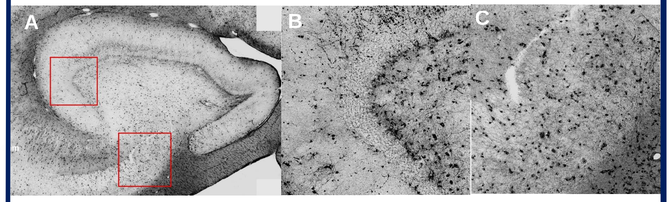

Figure 2: Hippocampal Section corresponding to Paxinos Atlas plate 80. A) An example section stained for β1 NA Receptors and imaged at 20x. Red squares indicate where 2x2 tiled images were taken of the dentate gyrus (B) and CA3 (C) regions. Image has been inverted for clarity.

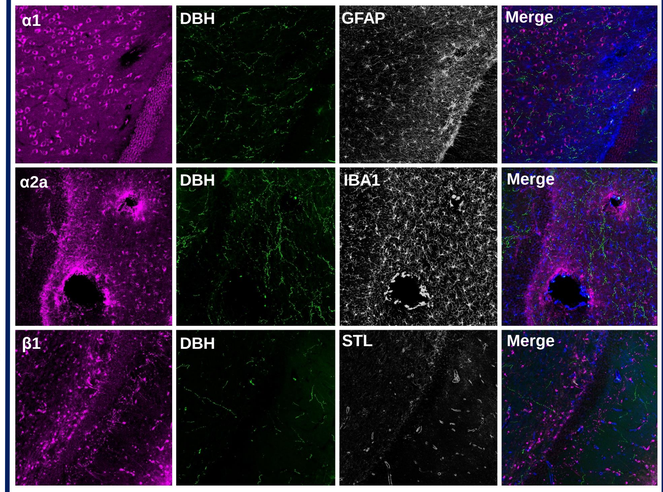

Figure 3: High magnification (40x) micrographs illustrating the 3 staining protocols used in this project. Top row: ant- α1 adrenergic receptor, anti-DBH, anti-GFAP and a merged image. Middle row: anti- α2a adrenergic receptor, anti-DBH, anti-GFAP and a merged image. Bottom row: anti- β1 adrenergic receptor,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

RESULTS: BEHAVIOR

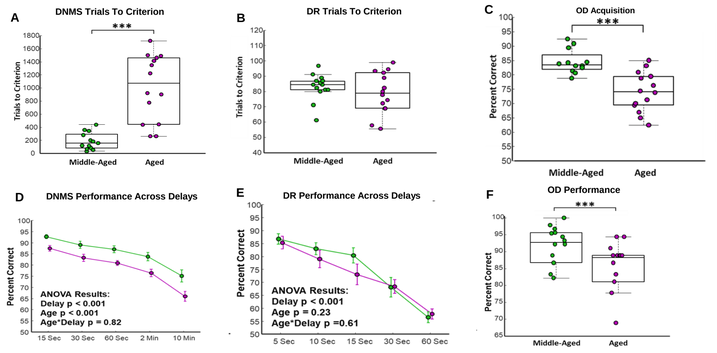

Figure 4: Trials to reach criterion (90% correct over 5 consecutive trials) by age group for the **A)** DNMS and **B)** DR task. Criterion performance for the **C)** OD task was determined by the percentage correct on the first two training sessions an animal underwent. Behavioral performance was also 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

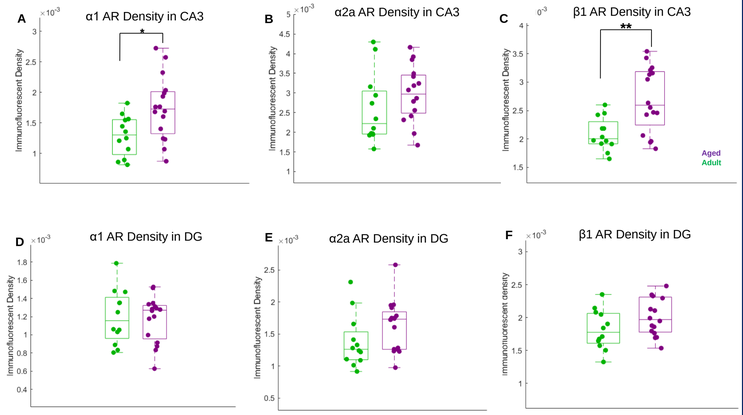

Figure 5: Comparisons of receptor densities in adult and aged animals in CA3 (top row) and DG (bottom row). **A)** Higher density of α1 NAR in old animals was seen in CA3 (p=0.014), **B)** Higher density of α2a NAR in CA3 of aged animals (p=0.03) **C)** Higher β1 NAR density in CA3 of aged animals (p = 0.0023). Aged animals also had higher densities of microglia and vasculature. In DG, α1 NAR density did not differ between age groups(**D),** α2a NAR did not differ between groups **(E)** and there were no age-related differences in β1 **(F)** densities. In the DG, older animals had higher densities of vasculature as measure by STL+ area. * p<0.05, ** p<0.01.

RESULTS: NORADRENERGIC AXON DENSITY

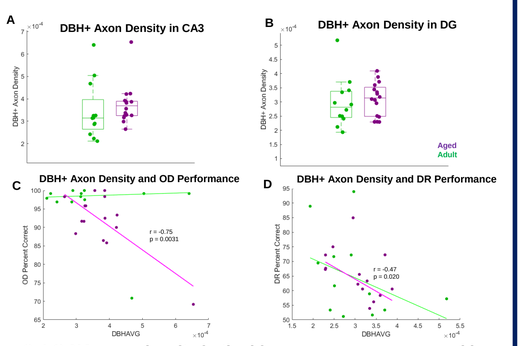

Figure 6: LC-NA axon density in the hippocampus, as measured by DBH. **A)** No difference in DBH+ density was seen in the CA3 subfield of the hippocampus between adult and aged macaques. **B)** No difference in DBH+ density was seen in the DG. In aged animals, higher DBH+ density was associated with worse **C)** OD retention and **D)** DR performance.

SUMMARY AND CONCLUSIONS

- There was no difference in dopamine-β hydroxylase-positive axon density between age groups in any hippocampus region
- In the CA3 subfield of the hippocampus, the densities of the α1 and β1 receptor subtypes are increased.
- In the DG subfield, receptor densities did not differ between adult and aged monkeys.
- Older animals with higher DBH+ density performed worse on OD and DR tasks, potentially suggesting that more noradrenaline release sites coupled with increased receptor densities may in some cases result in worse cognition.
- Future *in vivo* studies will have to be conducted to determine the homeostatic balance of the NA system necessary to optimize behavior.

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.