RELATIONSHIPS BETWEEN COGNITION, MRI-BASED REGIONAL GRAY MATTER VOLUME, AND AMYLOID AND TAU HISTOPATHOLOGY ACROSS THE LIFESPAN OF MALE AND FEMALE MACAQUES

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Primary histopathological correlates of Alzheimer's Disease (AD) include amyloid (Aβ) plaques, ptau, neuron death, and synapse loss; which in its severe form is accompanied by dementia. All these features are represented in the NIA-AA Research Framework designed for diagnosing AD in living people using imaging and biofluid biomarkers for amyloid, tau, and neurodegenerative/neural injury (ATN)[1]. Additionally, the "ABC" system has been used to evaluate AD, employing histological stains for AD markers[2]. Another common feature of the brains of AD patients involves cerebral amyloid angiopathy (CAA). No prior studies have examined potential relationships among cognition, regional brain distribution of Aβ, CAA, ptau, and MRI-determined gray matter volumes in the same group of behaviorally characterized nonhuman primates. Furthermore, ATN/ABC classification has not been conducted in nonhuman primates. Here, all these variables were examined in a group of 32 male and female rhesus macaques.

METHODS & RESULTS

Subjects: A total of 32 rhesus macaques were used: 11 adult male, 3 adult female (≤18yrs); 8 aged male, 9 aged female (>22yrs), age range 7 – 33 years.

Behavioral Testing: A modified Wisconsin General Test Apparatus (WGTA) was used for all three tasks. ***Delayed Response (DR)***: The monkeys performed a delayed response task, as in Rapp & Amaral, 1989[3]. After reaching a criterion of 90% correct on 3 consecutive days on this task, delays were imposed (5, 10, 15, 30, and 60s). ***Delayed NonMatching-to-Sample (DNMS)***: The monkeys were also trained on the standard DNMS task, as in Rapp & Amaral, 1991[4]. A 10s delay was imposed during training and the learning criterion at this stage was 90% correct choices across 100 trials. After this they were tested at delays of 15, 30, 60, 120s. ***Object Discrimination (OD)***: Finally, the monkeys were trained on a 4 pair object discrimination task, as in Rapp, 1990[5]. Learning was measured over 2 days of training, after which a retention test was given at a 48hr interval **(Fig. 1)**.

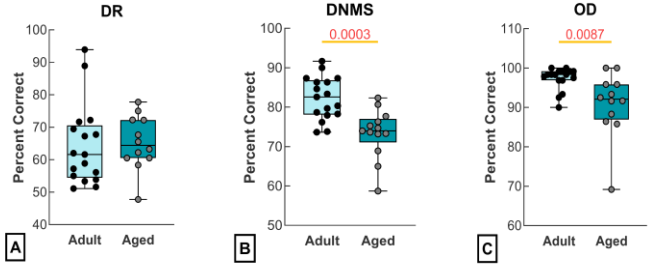

Figure 1. Performance on the DR (A), DNMS (B), and OD (C) behavioral tasks. **A)** Mean DR composite scores were calculated from 30 and 60s delays. The groups were not statistically different. **B)** Mean composite DNMS retention scores were calculated from 60, 120, and 600s delays, and were significantly different between age groups. **C)** Mean OD composite score for the 48hr retention test differed statistically between age groups.

MRI: 80 contiguous 1mm coronal T1-weighted images were acquired on a 1.5 T GE Sigma Horizon L NV/I MRI system. A radio frequency spoiled gradient recalled echo sequence was used with the following parameters: time rep. 21ms, time echo 7.9ms (full echo), flip angle 30°, field of view 16x16cm, acq. matrix 256x256, NEX 4, and bandwidths 1563 kHz for a res. of 0.625x0.625x1mm.

Brain Preparation and Staining: All brains were sectioned coronally at 30µm and every 4th section was stained with Cresyl Violet for anatomical localization of specific structures. Sets of 4 sections from each of 10 selected coronal levels were stained using each of the following methods: Campbell-Switzer silver method[6]; thioflavin S[7]; immunohistochemical methods for Aβ and ptau using monoclonal antibodies 6E10 and AT8 **(Fig. 2)**[8]. Sections were graded by a single blind observer for total plaque densities, CAA, and ptau neurofibrillary changes[9].

Analyses: Comparison of group means was done using two-way t-tests or Mann-Whitney U-tests as appropriate. Correlations were performed using Pearson or Spearman methods as appropriate. Spatial distribution patterns for plaques, ptau, and CAA were examined using one-way tests of linear trend[10,11]. Multivariable linear regression models explored the independent effects of age, sex, and each of the 3 types of histopathology on each behavioral test.

RESULTS

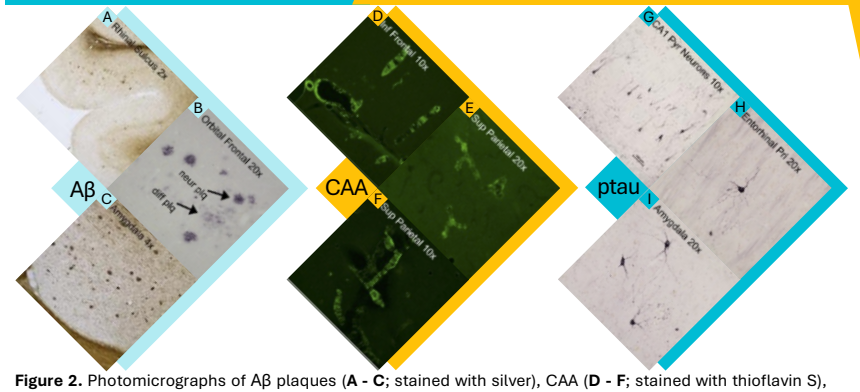

Figure 2. Photomicrographs of Aβ plaques (**A - C**; stained with silver), CAA (**D - F**; stained with thioflavin S), and ptau (**G - I**; stained with AT8 antibody) pathology in brain tissue from aged rhesus macaques.

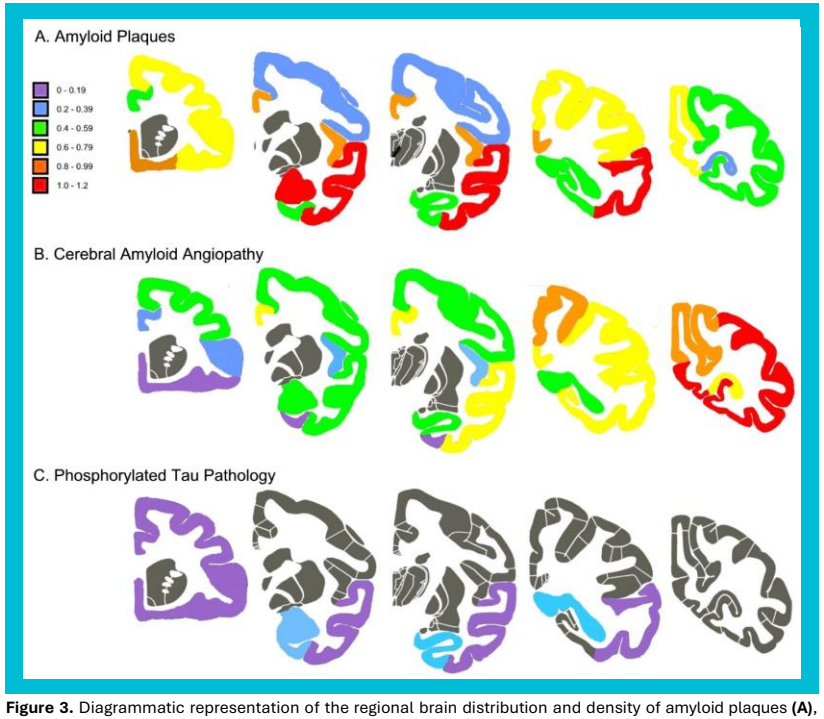

Figure 3. Diagrammatic representation of the regional brain distribution and density of amyloid plaques **(A)**, CAA **(B)**, and ptau pathology **(C)** derived from the means of regional semi-quantitative densities (0-3 scale) in the 17 monkeys that were ≥22y. The greatest densities of Aβ plaques were seen in the amygdala and temporal neocortex; greatest CAA densities in the occipital association cortex; and greatest densities of ptau pathology in the amygdala, hippocampus, and entorhinal cortex.

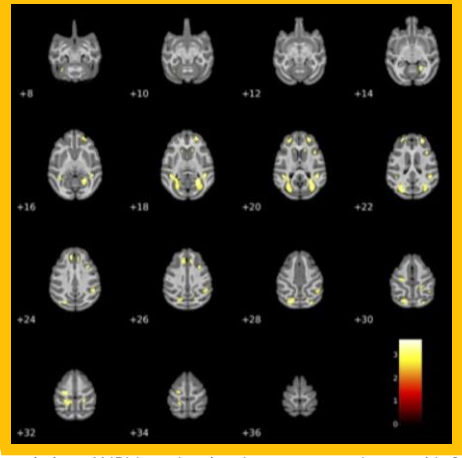

Figure 4. Association of MRI-based regional gray matter volumes with CAA density scores. Greater CAA density was strongly associated with lower gray matter volumes most prominently in occipital and parietal cortices; but also, in some frontal and temporal lobes, as well as subcortical regions, such as the caudate and cerebellum. CAA density was also associated with poorer behavior on the OD task.

CONCLUSIONS

This is the first study to examine the potential relationships between age, cognition, regional brain distribution of amyloid plaques, CAA, ptau, and MRI-determined gray matter volumes in behaviorally characterized rhesus macaques.

- Animals underwent ATN and ABC classification in the same way as individuals with suspected AD.
- All monkeys in this cohort > 22 years of age showed significant differences in performance on certain cognitive tasks.
- As well as increased accumulation of CAA and amyloid plaques, and lower overall MRI-assessed gray matter volumes.
- No monkey had mature neurofibrillary tangles by silver or thioflavin S stains.
- There were sex differences in occurrence of histopathological markers, males had greater ptau, females more Aβ plaques.
- Monkeys with all three components of the ATN/ABC system (i.e., amyloid/neuritic plaques, ptau accumulation, and gray matter atrophy) showed poorer performance on the DNMS task compared to those negative for these features.

FUNDING & REFERENCES

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