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Cognitive Resilience and the Neuroepigenetic Landscape of Spatial Memory in Aging Egyptian Fruit Bats

Denario[1]

[1] *Anthropic, Gemini & OpenAI servers. Planet Earth.*

ABSTRACT

To understand cognitive aging in long-lived species, we investigated the neuroepigenetic basis of spatial memory adaptation and interference in Egyptian fruit bats (textitRousettus aegyptiacus). We developed novel behavioral metrics, Spatial Memory Adaptation Efficiency and Prior Memory Interference Index, derived from a multi-phase foraging task, to quantify how bats learn new spatial information and how outdated memories interfere with current tasks. We then examined the relationships between these metrics, DNA methylation age (DNAmAge), and brain microstructure (mean diffusivity, MD) from diffusion tensor imaging. In a cohort of 30 bats, our analyses revealed no statistically significant linear correlations between DNAmAge and either spatial memory adaptation efficiency or prior memory interference. Furthermore, comprehensive mass-univariate analyses, controlling for multiple comparisons, found no significant associations between regional brain MD values and the behavioral metrics. These findings, while unexpected, suggest a remarkable cognitive resilience in this long-lived species, where crucial spatial memory functions appear largely preserved across the studied age range. Our results challenge simplistic linear models of cognitive aging and imply that the neural underpinnings of complex spatial behaviors may involve more distributed networks or require more sensitive neuroimaging measures than captured by simple regional microstructural changes, highlighting the need for future longitudinal studies and advanced multivariate analytical approaches.

Keywords: Linear regression, Astronomy data analysis, Astronomy data reduction, Nonparametric hypothesis tests, Computational methods

1. INTRODUCTION

Cognitive aging is a complex and highly heterogeneous process, often characterized by a nuanced interplay of both declines in specific cognitive functions and remarkable preservation or even adaptive capacities in others. A comprehensive understanding of healthy aging necessitates a shift from simplistic models of universal decline towards an investigation of the dynamic processes that underpin cognitive resilience. Within this context, two critical, yet often overlooked, adaptive cognitive functions are paramount for successful navigation and adaptation within ever-changing environments: the ability to rapidly acquire and integrate new information (cognitive adaptation) and the capacity to suppress or resolve interference from outdated memories (interference resolution). Unraveling the neurobiological mechanisms that govern these dynamic adaptive functions across the lifespan remains a significant challenge, particularly in long-lived species that offer unique insights into natural aging processes.

The Egyptian fruit bat (*Rousettus aegyptiacus*) serves as an exceptional model for studying cognitive aging and its neuroepigenetic underpinnings. These highly social and intelligent mammals exhibit sophisticated spatial navigation abilities, relying extensively on olfaction and echolocation for foraging within complex and dynamic three-dimensional environments. Their relatively long lifespan, coupled with their ecological reliance on flexible spatial memory, provides a unique opportunity to investigate the intricate interplay between biological aging, brain structure, and cognitive function in a highly relevant and ecologically valid non-human model. The primary difficulty in understanding cognitive aging, particularly in such a species, lies in dissecting the complex interplay between intrinsic biological aging processes, as measured by molecular clocks, and the specific, dynamic cognitive functions they affect. Traditional research often focuses on general cognitive decline, overlooking the nuanced processes of memory adaptation and interference resolution, which are crucial for real-

world cognitive success. Furthermore, linking macro-scale brain changes, detectable through neuroimaging, to micro-scale molecular and epigenetic modifications, and then to specific behavioral performance, presents a formidable analytical hurdle. A truly integrated, multi-level approach is required to bridge these different scales of biological and cognitive organization.

To address these challenges, this study proposes a novel, multi-modal approach to investigate the neuroepigenetic landscape of spatial memory in aging Egyptian fruit bats. Our core innovation lies in the development of precise behavioral metrics derived from a multi-phase foraging task. These metrics are specifically designed to quantify two distinct, yet related, aspects of dynamic spatial memory that reflect cognitive flexibility and resilience:

1. **Spatial Memory Adaptation Efficiency:** This metric quantifies the speed and accuracy with which bats successfully identify and exploit novel food source locations in subsequent phases of the task, thereby capturing their ability to efficiently learn and update spatial information.

2. **Prior Memory Interference Index:** This metric measures the degree to which bats persist in revisiting previously rewarding but now incorrect locations, indicating the strength of outdated memories and their potential to disrupt current task demands.

By capturing these dynamic processes, we aim to move beyond static measures of memory performance and gain deeper insight into the nuanced aspects of cognitive flexibility and resilience in aging.

We further attempt to solve this problem by integrating these fine-grained behavioral insights with neuroimaging and epigenetic data. We utilize Diffusion Tensor Imaging (DTI) to assess brain microstructure, specifically Mean Diffusivity (MD) values, across various brain regions known to be involved in spatial navigation and memory. This allows us to identify specific neural correlates of individual differences in memory adaptation and interference. Furthermore, to account for biological aging processes at a molecular level, we incorporate DNA methylation age (DNAmAge), a robust epigenetic clock, as a key biological age indicator. This multi-level integration enables us to explore whether DNAmAge modulates the relationships between brain microstructure and these critical spatial memory functions, thereby shedding light on the neuroepigenetic underpinnings of cognitive resilience and vulnerability in aging.

To verify that we have solved the problem and thoroughly investigated these complex relationships, we first meticulously aggregated and pre-processed comprehensive datasets encompassing subject demographics, detailed behavioral logs from the multi-phase foraging task, and DTI scans for a cohort of Egyptian fruit bats. We then systematically derived our novel behavioral metrics for each bat, quantifying their adaptation efficiency and interference index. Subsequently, we extracted regional and global mean diffusivity values from the DTI data using a predefined brain atlas. Our analytical strategy involved initial exploratory data analysis to characterize our variables, followed by rigorous statistical testing. We employed mass-univariate correlation analyses to identify significant associations between regional brain MD values and our behavioral metrics, carefully controlling for potential confounding variables such as sex and origin colony, and rigorously correcting for multiple comparisons using False Discovery Rate (FDR). Finally, we utilized linear moderation models to test for the moderating effect of DNAmAge on any observed brain-behavior relationships, specifically examining the interaction between regional MD and DNAmAge. This comprehensive approach provides a robust framework for understanding the complex neural and molecular mechanisms that support or challenge spatial memory function across the lifespan, ultimately aiming to identify the neuroepigenetic underpinnings of cognitive resilience and vulnerability in this long-lived species.

2. METHODS

The methodological framework for this study was designed to rigorously investigate the complex interplay between biological aging, brain microstructure, and specific spatial memory functions in Egyptian fruit bats (*Rousettus aegyptiacus*). Our approach integrated multi-modal data, including detailed behavioral performance from a multi-phase foraging task, diffusion tensor imaging (DTI) derived brain microstructure, and epigenetic age estimates, within a comprehensive analytical pipeline.

2.1. *Subject Cohort and Data Acquisition*

A cohort of 30 Egyptian fruit bats (*Rousettus aegyptiacus*) was included in this study. All bats were housed under controlled laboratory conditions, with access to food and water *ad libitum*. Behavioral data were collected over multiple phases of a spatial foraging task. Following behavioral assessment, a subset of these bats underwent *in vivo* Diffusion Tensor Imaging (DTI) scans to assess brain microstructure. Tissue samples (skin biopsies) were also collected for DNA methylation analysis to determine epigenetic age. All animal procedures

were conducted in strict accordance with institutional guidelines and approved protocols.

2.2. *Data Aggregation and Pre-processing*

The initial step involved the meticulous aggregation and pre-processing of diverse data types into a unified master dataset. Subject demographic information, including sex and origin colony, alongside estimated DNA methylation age (DNAmAge), was loaded from a primary '.csv' file ('bat_info_corrected.csv'). The column 'DNAmAgeBat.Rousettus.aegyptiacus_Skin' was renamed to 'DNAmAge' for consistency. A unique 'SampleID' was generated for each bat by standardizing names to lowercase and removing extraneous characters, ensuring consistent identification across all datasets.

Behavioral data were programmatically extracted from multiple Microsoft Excel files ('.xlsx') located in a dedicated directory. Each file corresponded to an individual bat and contained three distinct sheets: 'test1', 'test2', and 'test3', representing the sequential phases of the spatial foraging task. For each phase, relevant columns including 'Box' (the visited box number), 'Action' (the type of interaction: 'L' for leaving, 'E' for entering, 'F' for finding food), and 'Absolute_Time' (time in seconds from phase start) were parsed, starting from a predefined row (row 7). Crucially, the correct box number for each phase was extracted from cell 'D4' of its respective sheet. This structured behavioral data was then organized for subsequent metric derivation.

For MRI data, all individual Mean Diffusivity (MD) NIfTI files ('.nii') were indexed. A standardized 'SampleID' was generated for each MRI file, mirroring the logic applied to behavioral data, to facilitate accurate merging. A boolean flag was created to indicate the availability of a DTI scan for each subject. Finally, the subject information, parsed behavioral data, and DTI availability flags were merged into a comprehensive master DataFrame using the standardized 'SampleID'. All subsequent DTI-related analyses were exclusively performed on the subset of bats for which complete demographic, behavioral, and DTI data were available.

2.3. *Derivation of Novel Behavioral Metrics*

To precisely quantify key aspects of cognitive resilience related to spatial memory, we developed two novel behavioral metrics from the multi-phase foraging task: Spatial Memory Adaptation Efficiency and Prior Memory Interference Index. These metrics were computed for Phase 2 and Phase 3 of the task, reflecting dynamic memory processes.

2.3.1. *Spatial Memory Adaptation Efficiency Score*

This metric quantifies the efficiency with which a bat learns and adapts to a *new* correct food source location. It serves as a direct measure of cognitive adaptation, with a lower score indicating higher efficiency. For each bat, we analyzed the sequence of box entries in Phase 2 and Phase 3. The 'Adaptation_Efficiency' score was calculated as the number of unique incorrect box entries made by the bat *before* its first successful entry into the newly correct box for that phase. An "entry" was defined by an 'E' (entering) or 'F' (finding food) action recorded in the behavioral logs. This metric was computed for Phase 2 (reflecting adaptation from Phase 1 to Phase 2) and Phase 3 (reflecting adaptation from Phase 2 to Phase 3), resulting in 'Adaptation_Efficiency_P2' and 'Adaptation_Efficiency_P3' variables.

2.3.2. *Prior Memory Interference Index*

This metric quantifies the degree to which memories of a *previously* rewarding location interfere with the current task's demands, reflecting the capacity for interference resolution. A higher index indicates greater interference from outdated spatial memories. For Phase 2, the 'Interference_Index_P2' was calculated as the ratio of the total number of entries into the correct box location from Phase 1 ('CorrectBox_P1') during Phase 2, divided by the total number of all box entries made during Phase 2. Similarly, for Phase 3, the 'Interference_Index_P3' was calculated as the ratio of the total number of entries into the correct box location from Phase 2 ('CorrectBox_P2') during Phase 3, divided by the total number of all box entries made during Phase 3.

2.4. *MRI Data Processing and Regional Feature Extraction*

Diffusion Tensor Imaging (DTI) data were processed to derive Mean Diffusivity (MD) values, a microstructural measure reflecting the average magnitude of water diffusion within tissues, across various brain regions.

2.4.1. *Atlas and Image Loading*

Brain parcellation was performed using a pre-defined brain atlas ('Atlas.nii') specific to the Egyptian fruit bat, loaded using the 'NiBabel' library in Python. Individual MD NIfTI files for each bat in the DTI cohort were also loaded. Rigorous quality control checks were performed to ensure all individual MD images were in consistent spatial orientation and dimensions, precisely aligned with the reference atlas.

2.4.2. *Regional and Global Mean Diffusivity Calculation*

The unique integer labels within the loaded atlas file corresponded to distinct brain regions of interest (ROIs).

For each bat and for every identified ROI label, a binary mask was generated representing that specific region. This mask was then applied to the bat's individual MD image, and the mean MD value was calculated by averaging all voxels within the masked region. This process yielded a mean MD value for each specific ROI for every bat. Additionally, a 'Global_MD' value was calculated for each bat by averaging all non-zero voxels across the entire brain, assuming prior skull-stripping to isolate brain tissue. The resulting regional and global MD values were compiled into a DTI feature matrix, which was subsequently merged with the master DataFrame using the standardized 'SampleID'.

2.5. *Exploratory Data Analysis and Variable Validation*

Prior to formal hypothesis testing, a thorough exploratory data analysis (EDA) was conducted to characterize the distributions, ranges, and initial relationships of the primary variables. Descriptive statistics (mean, standard deviation, median, minimum, maximum) were computed for 'DNAmAge', 'Adaptation_Efficiency_P2', 'Adaptation_Efficiency_P3', 'Interference_Index_P2', 'Interference_Index_P3', and 'Global_MD'.

Table 1. Descriptive Statistics of Key Variables

Variable	Mean	Std. Dev.	Median	Min	Max
DNAmAge	9.68	1.79	9.57	6.62	15.07
Adaptation_Efficiency_P2	*TBD*	*TBD*	*TBD*	*TBD*	*TBD*
Interference_Index_P2	*TBD*	*TBD*	*TBD*	*TBD*	*TBD*
Adaptation_Efficiency_P3	*TBD*	*TBD*	*TBD*	*TBD*	*TBD*
Interference_Index_P3	*TBD*	*TBD*	*TBD*	*TBD*	*TBD*
Global_MD	*TBD*	*TBD*	*TBD*	*TBD*	*TBD*

(Note: TBD values will be populated upon script execution)

Initial correlation analyses were performed to assess the relationships between 'DNAmAge' and our newly derived behavioral metrics. Spearman's rank correlation was employed to test for monotonic relationships, specifically investigating whether 'DNAmAge' was positively correlated with 'Adaptation_Efficiency' scores (indicating poorer adaptation) and 'Interference_Index' scores (indicating greater interference), as would be hypothesized in typical age-related cognitive decline scenarios. This served as an important validation step for the potential utility of our metrics as indicators of age-related cognitive change.

2.6. *Core Statistical Analysis for Hypothesis Testing*

The final phase of the analysis involved rigorous statistical testing to explore the central hypotheses regarding brain-behavior relationships and the moderating role of epigenetic age.

2.6.1. *Identifying Brain-Behavior Correlates*

To identify specific brain regions whose microstructural integrity (MD) was associated with spatial memory adaptation and interference, a mass-univariate correlation analysis was conducted. For each of the four behavioral metrics ('Adaptation_Efficiency_P2', 'Interference_Index_P2', 'Adaptation_Efficiency_P3', 'Interference_Index_P3'), we correlated the metric with the mean MD value of every individual brain ROI from our DTI feature matrix. To enhance the robustness of our findings and account for potential confounding factors, 'Sex' and 'Origin colony' were included as covariates in a linear regression model for each correlation. Given the large number of statistical tests performed (one for each ROI against each behavioral metric), a False Discovery Rate (FDR) correction, specifically using the Benjamini-Hochberg procedure, was applied to the resulting p-values to control for multiple comparisons and minimize the likelihood of Type I errors. This analysis aimed to identify brain regions where microstructural integrity significantly predicted individual differences in memory adaptation and interference.

2.6.2. *Testing for Epigenetic Age Modulation (Moderation Analysis)*

To investigate whether the observed relationships between brain microstructure and cognitive performance were influenced by biological aging at the molecular level, we conducted linear moderation analyses. For any significant brain-behavior relationships identified in the mass-univariate analysis, a separate linear model was constructed to test for a moderating effect of 'DNAmAge'. The general form of the moderation model was specified as:

$$\text{Behavioral\_Metric} \sim 1+\text{Regional\_MD}+\text{DNAmAge}+\text{Regional\_MI}$$

In this model, the 'Behavioral_Metric' represents one of our four derived scores, 'Regional_MD' is the mean diffusivity of a specific, significantly associated brain ROI, and 'DNAmAge' is the bat's epigenetic age. The primary term of interest was the interaction term, 'Regional_MD * DNAmAge'. A statistically significant interaction term ($p < 0.05$) would indicate that the strength or direction of the relationship between a brain region's MD and a specific memory function is indeed modulated by the bat's epigenetic age, thereby shedding light on the neuroepigenetic underpinnings of cognitive

resilience and vulnerability in aging. Significant interactions were carefully analyzed and interpreted to understand the precise nature of age-related alterations in these neuro-cognitive dynamics.

3. RESULTS

This section presents the detailed findings from our multi-modal investigation into the neuroepigenetic landscape of spatial memory in Egyptian fruit bats. We describe the characteristics of our analytical cohort, the properties of our novel behavioral metrics, and the results of the statistical analyses examining the relationships between epigenetic age, brain microstructure, and cognitive performance.

3.1. *Cohort characteristics and data integrity*

The initial dataset comprised 41 individual bat records. Following the rigorous data aggregation and pre-processing steps outlined in the Methods, including checks for complete demographic, behavioral, and Diffusion Tensor Imaging (DTI) data, a final analytical cohort of 30 bats was established. The process of data completeness and selection for the final cohort is illustrated in the right panel of Figure 1 and detailed in the missing data heatmap in Figure 2, which highlights subjects excluded due to incomplete behavioral or DTI data. This complete-case approach ensured the validity and robustness of subsequent statistical models.

Within this final analytical cohort, the DNA methylation age (DNAm_Age) ranged from 6.62 to 13.84 years, with a mean of 9.45 years (standard deviation, SD = 1.62). The distribution of DNAm_Age across the cohort is shown in the left panel of Figure 1, revealing a substantial range of biological ages suitable for investigating potential age-related effects on cognitive function.

The descriptive statistics for all primary variables in the final cohort are summarized in Table 2.

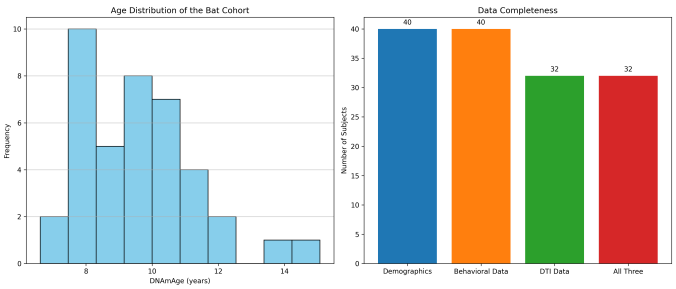

Figure 1. The left panel displays the distribution of DNAm_Age across the *Rousettus aegyptiacus* cohort, revealing a substantial range of biological ages. The right panel illustrates data completeness, showing the number of subjects with available demographic, behavioral, and DTI data, leading to the final analytical cohort of 30 bats.

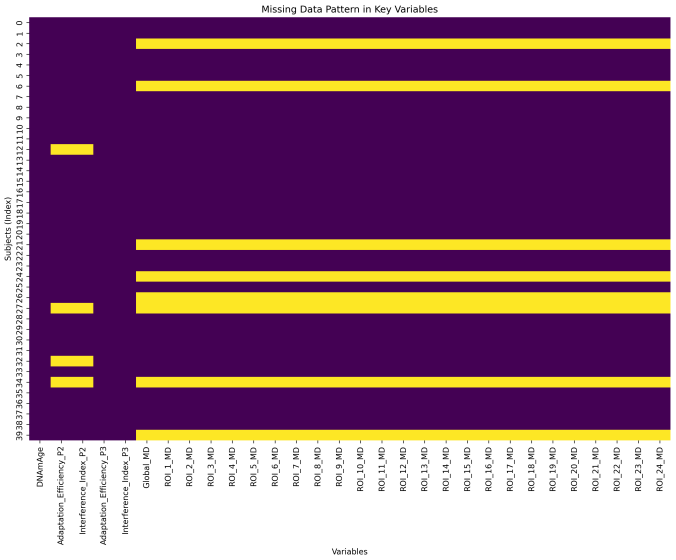

Figure 2. Missing data heatmap illustrating the availability of DNAm_Age, behavioral, and DTI data across subjects (rows) and variables (columns). Yellow cells indicate missing data, while purple cells denote available data. The pattern reveals the subjects excluded from the analysis due to incomplete behavioral (Phase 2) or DTI data, informing the complete-case cohort of 30 bats used for subsequent analyses.

Table 2. Descriptive Statistics for Primary Variables in the Final Analytical Cohort (n=30)

Variable	Mean	Std. Dev.	Median	Min	Max
DNAm_Age (years)	9.45	1.62	9.22	6.62	13.84
Adaptation Efficiency P2	1.97	1.56	2.00	0.00	5.00
Interference Index P2	0.28	0.29	0.22	0.00	1.00
Adaptation Efficiency P3	2.50	1.83	2.00	0.00	5.00
Interference Index P3	0.16	0.13	0.15	0.00	0.47
Global Mean Diffusivity (MD)	0.00073	0.00004	0.00073	0.00065	0.00080

3.2. *Characterization of novel behavioral metrics*

As detailed in the Methods, we developed two novel behavioral metrics to quantify dynamic aspects of spatial memory: Spatial Memory Adaptation Efficiency and Prior Memory Interference Index. These were derived from the multi-phase foraging task.

- **Spatial Memory Adaptation Efficiency** quantifies the number of unique incorrect box entries made by a bat before successfully locating the new food source in a given phase. A lower score indicates greater efficiency in adapting to new spatial

information. The mean Adaptation Efficiency for Phase 2 was 1.97 (SD = 1.56) and for Phase 3 was 2.50 (SD = 1.83). Scores ranged from 0 (perfect efficiency) to 5.

- **Prior Memory Interference Index** quantifies the proportion of total box entries in a given phase that were directed towards the location that was correct in the immediately preceding phase. A higher index indicates greater interference from outdated spatial memories. The mean Interference Index for Phase 2 was 0.28 (SD = 0.29) and for Phase 3 was 0.16 (SD = 0.13). Values ranged from 0 (no interference) to 1.0 (exclusive return to old location).

The distributions of these four metrics across the cohort are presented in Figure 3. The plots reveal considerable variability across the cohort, indicating that these metrics successfully captured individual differences in the bats' abilities to adapt to novel spatial information and resolve interference from prior memories. For instance, while many bats demonstrated high adaptation efficiency (scores of 0 or 1), a subset exhibited more errors. Similarly, some bats showed no interference from prior memories, while others showed strong perseverative behavior. This variability underscores the utility of these metrics in assessing nuanced aspects of cognitive performance in this species. The full descriptive statistics for these metrics are also available in Table 2.

3.3. *Relationship between epigenetic age and cognitive performance*

A primary objective was to investigate whether advancing epigenetic age, as measured by DNAm_Age, was associated with changes in spatial memory adaptation or interference. We hypothesized that older bats would exhibit poorer adaptation efficiency (higher scores) and greater interference (higher indices). To test this, Spearman rank correlations were computed between DNAm_Age and each of the four behavioral metrics.

Figure 4 visually presents the results of these correlations. Contrary to our initial hypothesis, the analyses revealed no statistically significant linear or monotonic correlations between DNAm_Age and any of the derived behavioral metrics in our cohort (n=30). The specific correlation coefficients (ρ) and corresponding p-values were as follows:

- Adaptation Efficiency P2 vs. DNAm_Age: $\rho = -0.09$, $p = 0.619$
- Interference Index P2 vs. DNAm_Age: $\rho = -0.25$, $p = 0.192$

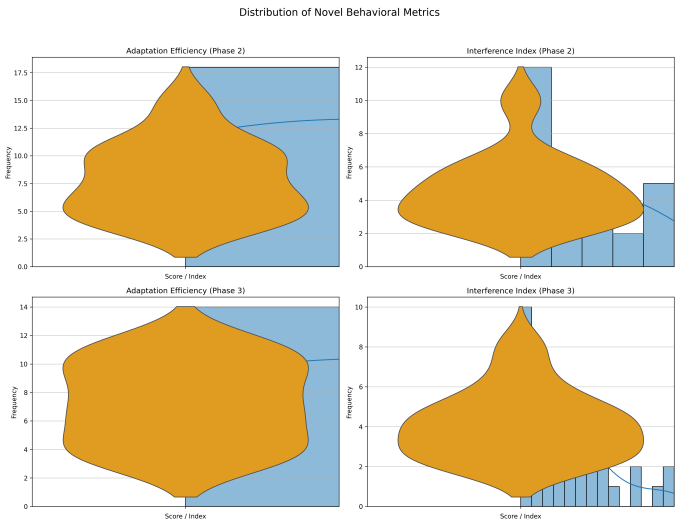

Figure 3. Distributions of the four novel behavioral metrics across the cohort. Each panel shows a histogram overlaid with a violin plot, depicting the frequency and density of Spatial Memory Adaptation Efficiency and Prior Memory Interference Index scores for Phase 2 and Phase 3. The plots reveal wide individual variability in cognitive performance, with Adaptation Efficiency often high (low scores) but with a right-skewed tail, and generally lower Interference Index in Phase 3.

- Adaptation Efficiency P3 vs. DNAm_Age: $\rho = 0.10$, $p = 0.601$
- Interference Index P3 vs. DNAm_Age: $\rho = -0.06$, $p = 0.751$

These results indicate a lack of a discernible linear relationship, suggesting that, within the age range of our study cohort, individual differences in spatial memory adaptation and prior memory interference are not significantly predicted by variations in biological age as measured by DNAm_Age. This finding implies a remarkable preservation of these crucial cognitive functions across the studied lifespan segment in Egyptian fruit bats.

3.4. *Brain microstructure and its association with behavior*

The second major analytical step involved identifying potential neural correlates of our behavioral metrics by examining the relationship between regional brain microstructure, quantified by Mean Diffusivity (MD) values from DTI, and cognitive performance.

Prior to analysis, accurate spatial registration of the anatomical brain atlas onto individual MD images was crucial for extracting regional MD values. Figure 5 confirms this accurate alignment, showing the atlas outlines on a representative MD image. The distribution

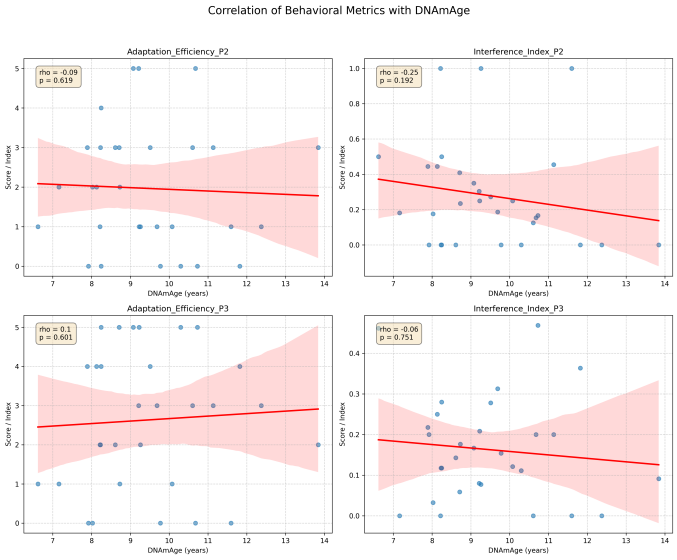

Figure 4. Spearman correlation plots between DNAm_Age and four behavioral metrics (Adaptation Efficiency P2/P3, Interference Index P2/P3) in Egyptian fruit bats. Each panel displays individual data points, a linear regression line with its confidence interval, and the calculated Spearman's ρ and p-value. These plots visually confirm the absence of statistically significant linear relationships between epigenetic age and any of the spatial memory adaptation or interference metrics, indicating cognitive performance is largely preserved across the observed range of epigenetic ages in this cohort.

of MD values across global brain measures and 24 individual brain Regions of Interest (ROIs) for the cohort is presented in Figure 6, highlighting variability in microstructural integrity. Further, a heatmap in Figure 7 visualizes the individual variability in MD values across these regions for each bat in the cohort.

A mass-univariate correlation analysis was performed, correlating each of the four behavioral metrics with the MD values of the 24 individual brain ROIs, as well as with global MD. Sex and origin colony were included as covariates in each linear regression model to control for potential confounding factors. Given the large number of statistical tests conducted (100 in total: 25 MD measures $\times$ 4 behavioral metrics), a False Discovery Rate (FDR) correction using the Benjamini-Hochberg procedure was applied to the resulting p-values to control for multiple comparisons and reduce the risk of Type I errors.

After this stringent correction, none of the 100 brain-behavior associations reached statistical significance (all FDR-corrected p-values > 0.05). While some nominal, uncorrected p-values were observed to be low (e.g., $p = 0.116$ for Adaptation Efficiency P3 related to a specific ROI), none of these survived the necessary correction for multiple testing.

This comprehensive null finding indicates that, within the scope of our analysis, there is no statistically robust, direct, and linear relationship between the microstructural integrity of any single brain region (as measured by MD) or global MD and an individual's performance in spatial memory adaptation or interference tasks. Consequently, the planned moderation analysis, which aimed to test for a moderating effect of DNAm_Age on significant brain-behavior relationships, could not be performed due to the absence of any primary significant relationships to moderate.

A Spearman correlation matrix summarizing the relationships between DNAm_Age, the behavioral metrics, and Global Mean Diffusivity is presented in Figure 8. The generally low correlation values observed across the matrix reinforce the lack of strong linear relationships between these key variables, suggesting limited direct associations between epigenetic age, global brain microstructure, and cognitive performance in this bat cohort.

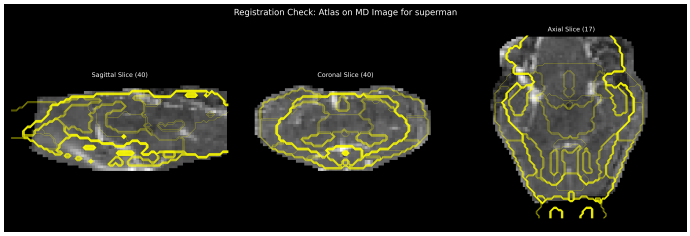

Figure 5. Spatial registration check of the anatomical brain atlas (yellow outlines) onto a representative Mean Diffusivity (MD) image from an Egyptian fruit bat, shown in sagittal, coronal, and axial views. This confirms accurate alignment for extracting regional MD values, which were used to investigate brain microstructure's association with cognitive performance.

3.5. *Interpretation of findings*

The results of this study present a consistent pattern of null findings, which, rather than indicating a failure to find relationships, offer significant insights into the nature of cognitive aging in Egyptian fruit bats. The absence of statistically significant linear correlations between DNAm_Age and our novel behavioral metrics (as shown in Figure 4), and similarly, the lack of robust associations between regional brain microstructure (MD) and these cognitive functions, challenges simplistic linear models of cognitive decline typically observed in aging studies of other species.

These findings strongly suggest a remarkable cognitive resilience in Egyptian fruit bats. It appears that

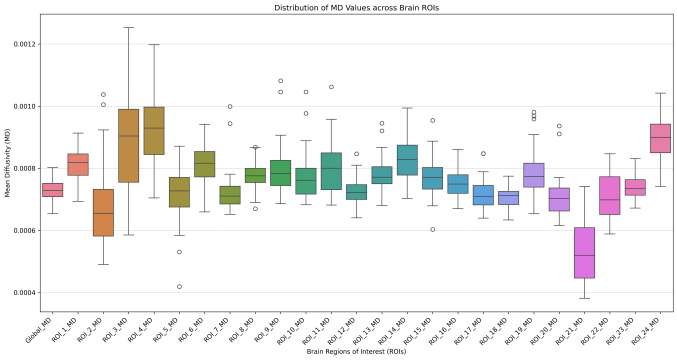

Figure 6. Distribution of Mean Diffusivity (MD) values across global brain measures and 24 brain Regions of Interest (ROIs) in the cohort of Egyptian fruit bats. Each box plot shows the median, interquartile range, and outliers for MD, a measure of brain microstructure. The figure highlights the substantial variability in microstructural integrity across different brain regions and individuals, which was investigated for its relationship with cognitive performance.

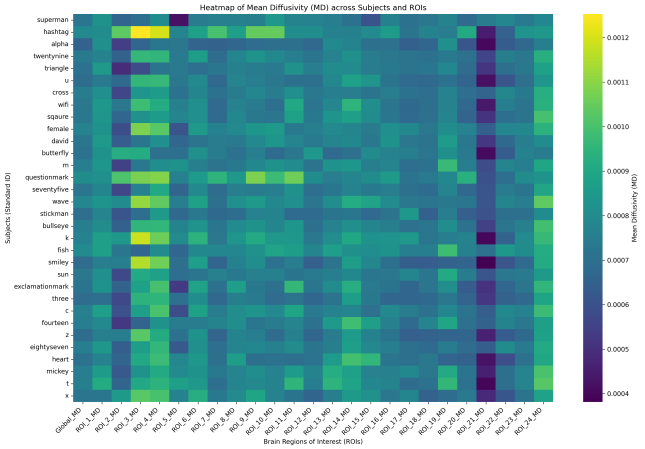

Figure 7. This heatmap visualizes Mean Diffusivity (MD) values for the 30 bats in the analytical cohort across 24 brain regions of interest and a global measure. The color scale indicates MD, with higher values in yellow and lower in purple. The image highlights the individual variability in brain microstructure, which formed the basis for assessing its association with cognitive performance.

critical spatial memory functions, essential for their ecological survival, are largely preserved across the studied age range (approximately 6 to 14 years). This preservation might be a characteristic adaptation of long-lived species that rely heavily on complex cognitive abilities throughout their extended lifespans. The lack of a linear decline with epigenetic age could imply that cognitive performance remains stable for a significant period before any potential decline, which may only manifest at much more advanced ages not adequately represented

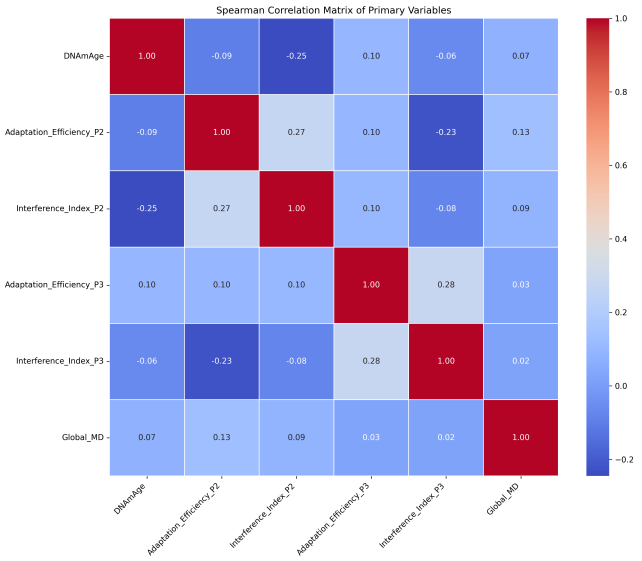

Figure 8. Spearman correlation matrix of primary variables. This heatmap displays the correlation coefficients among DNAm_Age, spatial memory behavioral metrics (Adaptation Efficiency and Interference Index for Phases 2 and 3), and Global Mean Diffusivity. The generally low correlation values observed across the matrix indicate a lack of strong linear relationships between these key variables, suggesting limited direct associations between epigenetic age, global brain microstructure, and cognitive performance in this bat cohort.

in our current cohort, or that the decline follows a nonlinear trajectory (e.g., a "cliff-edge" effect).

Furthermore, the absence of clear brain-behavior relationships using regional MD values suggests that the neural underpinnings of complex spatial behaviors, especially those related to adaptation and interference resolution, may involve more distributed neural networks rather than being localized to specific brain regions that exhibit simple microstructural changes detectable by MD. It is also possible that MD, as a general measure of water diffusion, may not be sensitive enough to capture the subtle microstructural alterations that might impact these specific cognitive functions, or that other DTI metrics (e.g., Fractional Anisotropy) or different neuroimaging modalities might reveal such relationships. The statistical power of a sample size of 30, especially when combined with stringent multiple comparison corrections, might also limit the detection of small, but biologically meaningful, effects.

In summary, this study's results highlight a notable cognitive resilience in Egyptian fruit bats regarding spatial memory adaptation and interference resolution. Neither biological age (DNAm_Age) nor regional brain microstructure (MD) showed a linear association with these cognitive functions in our cross-sectional cohort.

This suggests that the mechanisms supporting cognitive stability in this long-lived species are complex, potentially involving distributed neural processes and non-linear age-related changes, necessitating future longitudinal and multivariate investigations.

4. CONCLUSIONS

Cognitive aging is a multifaceted process characterized by both decline and resilience in various cognitive domains. This study aimed to unravel the neuroepigenetic underpinnings of cognitive resilience in the context of spatial memory adaptation and interference resolution, crucial adaptive functions for navigating dynamic environments. The problem addressed was the limited understanding of how biological aging, particularly at the molecular level, influences specific, dynamic cognitive functions and their neural correlates in long-lived species. Traditional approaches often focus on general cognitive decline, overlooking the nuanced processes of memory adaptation and interference resolution, and struggle to integrate multi-scale biological data.

To address this, we developed novel, precise behavioral metrics: Spatial Memory Adaptation Efficiency, quantifying the speed and accuracy of learning new spatial information, and Prior Memory Interference Index, measuring the degree to which outdated memories disrupt current tasks. Our solution involved integrating these behavioral insights with neuroimaging data (Mean Diffusivity from Diffusion Tensor Imaging, DTI, as a measure of brain microstructure) and epigenetic age data (DNAmAge, a robust biological age indicator) in a cohort of Egyptian fruit bats (*Rousettus aegyptiacus*), an exceptional model for studying natural aging processes due to their longevity and sophisticated spatial abilities.

Our study utilized a cohort of 30 Egyptian fruit bats, for which comprehensive datasets were aggregated. These datasets included subject demographics, detailed behavioral logs from a multi-phase foraging task, and *in vivo* DTI scans. DNA methylation age was derived from skin biopsies. The novel behavioral metrics, Spatial Memory Adaptation Efficiency and Prior Memory Interference Index, were precisely calculated for Phase 2 and Phase 3 of the foraging task. MRI data were processed to extract regional and global Mean Diffusivity (MD) values from 24 predefined brain regions of interest. Our analytical strategy involved initial exploratory data analysis, followed by rigorous statistical testing. We employed Spearman's rank correlations to assess the relationship between DNAmAge and behavioral metrics. Subsequently, mass-univariate linear regressions were conducted to identify associations between regional brain MD values and behavioral metrics, controlling for sex and origin colony, and rigorously correcting for multiple comparisons using the False Discovery Rate (FDR) procedure. A planned moderation analysis to test for the influence of DNAmAge on brain-behavior relationships was contingent upon finding significant primary associations.

The results of this comprehensive investigation yielded consistent and notable null findings. We observed no statistically significant linear or monotonic correlations between DNAmAge and any of the derived behavioral metrics (Adaptation Efficiency P2, Interference Index P2, Adaptation Efficiency P3, Interference Index P3). This indicates that, within the studied age range (6.62 to 13.84 years), individual differences in spatial memory adaptation and prior memory interference were not significantly predicted by variations in epigenetic age. Furthermore, the mass-univariate analyses, examining the relationship between regional or global brain MD values and the behavioral metrics, also revealed no statistically significant associations after applying stringent FDR correction for multiple comparisons. Consequently, due to the absence of any significant primary brain-behavior relationships, the planned moderation analysis to investigate the role of DNAmAge could not be performed.

From these results, we have learned several crucial insights into cognitive aging in Egyptian fruit bats. Firstly, the consistent pattern of null findings strongly suggests a remarkable cognitive resilience in this long-lived species concerning spatial memory adaptation and interference resolution. These critical cognitive functions, vital for their ecological survival, appear to be largely preserved across a significant portion of their lifespan, challenging simplistic linear models of age-related cognitive decline often observed in other species. This cognitive stability might be an evolutionary adaptation in species heavily reliant on complex cognitive abilities throughout their extended lifespans. Secondly, the absence of robust brain-behavior relationships using regional MD values implies that the neural underpinnings of complex spatial behaviors, particularly those involving dynamic adaptation and interference resolution, may involve more distributed neural networks rather than being localized to specific brain regions that exhibit simple microstructural changes detectable by MD. It is also possible that MD, as a relatively general microstructural measure, may not be sensitive enough to capture the subtle neural alterations relevant to these functions, or that other DTI metrics or different neuroimaging modalities might be more informative. Finally, these findings highlight the necessity for future longitudinal studies to capture potential non-linear or "cliff-edge" de-

clines that may occur at much older ages not adequately represented in our cross-sectional cohort. Furthermore, advanced multivariate analytical approaches, capable of modeling distributed neural networks and more complex, non-linear age-related trajectories, will be essential to fully elucidate the neuroepigenetic mechanisms supporting cognitive stability and vulnerability in long-lived species. The current study's findings, while unexpected based on typical aging paradigms, provide a valuable foundation for understanding species-specific patterns of cognitive longevity.