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Neuro-Cognitive Resilience in Long-Lived Bats: An Epigenetic Age-Adjusted Analysis of Spatial Memory and Brain Microstructure

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ABSTRACT

Long-lived species, such as the Egyptian fruit bat, offer unique insights into the mechanisms of healthy aging and neuro-cognitive resilience. This study investigated how these bats maintain adaptive spatial memory flexibility despite advanced epigenetic age. We developed a novel Cognitive Flexibility Index (CFI) from multi-phase foraging tasks to quantify individual learning and re-learning efficiency. A Cognitive Resilience Score (CRS) was then derived by adjusting the CFI for epigenetic age and demographic factors, isolating age-independent cognitive performance. We integrated comprehensive demographic, epigenetic age, behavioral, and Diffusion Tensor Imaging (DTI) data from a cohort of 41 bats, with 33 subjects having complete multi-modal data for the primary analyses. We then examined the relationship between the CRS and brain microstructural integrity, assessed via Mean Diffusivity (MD) from 24 atlas-defined regions and global brain measures. Contrary to our hypothesis, the Cognitive Flexibility Index did not show a significant decline with epigenetic age within the studied cohort. Furthermore, no statistically significant associations were found between the Cognitive Resilience Score and either global or any specific regional brain Mean Diffusivity values after multiple comparisons correction. These null findings suggest that, within the observed age range and using the employed metrics, cognitive flexibility in these long-lived bats may not exhibit a strong link to overall or regional brain microstructural integrity, potentially reflecting true biological resilience or highlighting the need for more sensitive measures and larger cohorts in future investigations into the neurobiological underpinnings of extreme longevity.

1. INTRODUCTION

Aging is a complex biological process universally characterized by a progressive decline in physiological function, leading to increased susceptibility to disease and mortality. A key aspect of healthy aging, particularly in long-lived species, is the maintenance of cognitive abilities. However, cognitive decline is a common and often debilitating aspect of senescence, with significant consequences for individual well-being and species adaptability. While chronological age is a primary predictor of cognitive decline, there is considerable inter-individual variability in cognitive trajectories, with some individuals exhibiting remarkable cognitive preservation well into advanced biological age. This phenomenon, termed neuro-cognitive resilience, represents the ability to maintain adaptive cognitive function despite the accumulation of age-related biological challenges. Understanding the mechanisms underlying such resilience is paramount for developing strategies to promote healthy cognitive aging across species.

Investigating species that exhibit exceptional longevity and appear to defy typical age-related decline offers a unique avenue for unraveling these mechanisms. Bats, in particular, stand out among mammals for their remarkably long lifespans relative to their body size, often living for decades. This extreme longevity, coupled with their unique physiological adaptations, including resistance to certain age-related diseases, positions them as compelling models for studying the biological underpinnings of healthy aging and neuro-cognitive resilience. Despite their extended lifespans, the specific neurobiological mechanisms that enable these long-lived bats to maintain adaptive cognitive function, particularly spatial memory flexibility, across their protracted aging trajectory remain largely unknown.

The central problem addressed in this study is to elucidate how long-lived bats maintain adaptive neuro-cognitive function, specifically spatial memory flexibility, throughout their lives, and to identify the neural correlates of this resilience. This is a difficult problem for several reasons. First, quantifying complex cognitive abilities like flexibility, which involves not just initial learning but also efficient unlearning and re-learning in dynamic environments, requires sophisticated behavioral paradigms capable of capturing these nuanced as-

pects. Second, accurately assessing biological age, as opposed to mere chronological age, is crucial, given that aging is a biological process that unfolds at different rates among individuals and species. Epigenetic clocks, which measure DNA methylation patterns, offer a more precise and biologically relevant measure of aging, but their application in non-traditional models requires specialized validation. Third, linking high-level cognitive performance to specific changes in brain microstructure presents a significant challenge, particularly in non-traditional model organisms where detailed anatomical and neuroimaging resources may be limited, necessitating the development of species-specific atlases and analysis pipelines.

To address these challenges, we developed a comprehensive, multi-modal approach in the Egyptian fruit bat (*Rousettus aegyptiacus*), a species known for its remarkable longevity. Our approach attempts to solve this problem by first rigorously quantifying cognitive flexibility. We designed a novel multi-phase foraging task to assess spatial memory flexibility, encompassing initial learning efficiency, and the ability to adapt to changing reward locations over both short and long retention intervals. From the performance in this task, we derived a quantitative **Cognitive Flexibility Index (CFI)** for each bat. This index integrates measures of initial learning, short-term adaptation (extinguishing a recent memory and re-learning), and long-term adaptation, thereby encapsulating their overall adaptive learning and re-learning efficiency. Recognizing the limitations of chronological age, we incorporated precise epigenetic age estimates for each individual, allowing us to account for their true biological aging status. This enabled us to calculate a **Cognitive Resilience Score (CRS)** by statistically adjusting the CFI for epigenetic age and other demographic factors (sex and colony origin). The CRS represents the residual cognitive performance of an individual, indicating whether a bat performs better or worse than expected given its biological age, thereby serving as a direct measure of age-independent cognitive resilience.

To verify the neurobiological underpinnings of this cognitive resilience, we integrated these precise behavioral and epigenetic measures with detailed Diffusion Tensor Imaging (DTI) data. DTI provides valuable insights into the microstructural integrity of brain tissue, with Mean Diffusivity (MD) serving as a sensitive marker for tissue organization and cellular health. We hypothesized that bats exhibiting higher cognitive resilience (i.e., a higher CRS) would demonstrate better-preserved brain microstructural integrity, reflected by lower MD values, particularly in brain regions critical for spatial memory and cognitive flexibility. We systematically examined the relationship between the CRS and global brain MD, as well as MD values within 24 atlas-defined brain regions. This approach allowed us to identify specific neural correlates of maintained cognitive function in the face of biological aging. By integrating precise behavioral measures of flexibility, advanced biological age assessment, and detailed neuroimaging in an exceptionally long-lived species, this study aims to provide novel insights into the mechanisms that underpin healthy neuro-cognitive aging and resilience, ultimately contributing to a broader understanding of longevity and brain health.

2. METHODS

This study employed a multi-modal approach to investigate neuro-cognitive resilience in Egyptian fruit bats (*Rousettus aegyptiacus*), integrating demographic data, epigenetic age estimations, detailed behavioral assessments of spatial memory flexibility, and Diffusion Tensor Imaging (DTI) measures of brain microstructure. The methodological workflow was structured into three main stages: (1) comprehensive data integration and preprocessing, (2) development of a novel Cognitive Flexibility Index, and (3) analysis of the relationship between this index, epigenetic age, and brain microstructural integrity to derive and examine a Cognitive Resilience Score.

2.1. *Data Integration and Preprocessing*

A critical initial step involved consolidating diverse datasets into a unified structure, ensuring robust and consistent subject identification across all modalities.

2.1.1. *Cohort characterization*

The study cohort comprised 41 Egyptian fruit bats (*Rousettus aegyptiacus*). Detailed demographic information, including sex, colony origin, and epigenetic age, was loaded from the file 'bat_info_corrected.csv'. The cohort exhibited a balanced sex distribution (23 males, 18 females) and colony representation (22 from Aseret, 19 from Herzeliya). Epigenetic age, a biologically relevant measure of aging, ranged from 6.62 to 15.07 years, with a mean of 9.74 years (standard deviation: 1.91 years). For the primary analyses requiring complete multi-modal data, a subset of 33 subjects had all necessary demographic, behavioral, and DTI information.

2.1.2. *Standardization of subject identifiers*

To facilitate accurate merging of data from various sources (demographic tables, behavioral spreadsheets, and DTI NIfTI files), a standardized 'Subject_ID' was

generated for each bat. This involved a consistent transformation applied to all raw identifiers: converting the string to lowercase and removing all underscores, spaces, and other non-alphanumeric characters (e.g., ‘Question_Mark‘ became ‘questionmark‘). This standardized ‘Subject_ID‘ served as the primary key for all subsequent data integration steps.

2.1.3. *Behavioral data extraction and feature engineering*

Behavioral data were collected from individual ‘.xlsx‘ files located in ‘Compressed_data/behvaioral_data/‘. Each file corresponded to a single bat and contained three sheets: ‘test1‘ (Phase 1), ‘test2‘ (Phase 2), and ‘test3‘ (Phase 3), representing distinct stages of the multi-phase foraging task designed to assess spatial memory flexibility. For each bat and each phase, the following steps were performed:

- The correct box number for the phase was extracted from cell ‘D4‘.
- Raw behavioral data, specifically ‘Absolute_Time‘ (column B) and ‘Action‘ (column F), were read starting from row 7. Actions ”E” (Box entry) and ”F” (Box entry and took food) were both categorized as a ”visit” to a box. The specific box number associated with each visit was parsed from the adjacent column.
- From this raw data, the following metrics were calculated for each phase:
  - **Total_Visits**: The total count of ”E” or ”F” actions within the phase.
  - **Time_to_First_Correct**: The ‘Absolute_Time‘ (in seconds) of the first visit to the correct box. If no correct visit occurred within the phase, this value was set to the phase duration (10800 seconds).
  - **Incorrect_Visits**: The total number of visits to any box other than the designated correct box for that phase.
  - **Perseverative_Errors**: This metric specifically quantified the inability to inhibit previously learned responses. For Phase 2, it represented the number of visits to the box that was correct in Phase 1. For Phase 3, it represented the number of visits to the box that was correct in Phase 2.

These phase-specific behavioral metrics were consolidated into a dataframe, with each row representing a bat and columns representing the engineered features (e.g., ‘Total_Visits_P1‘, ‘Time_to_First_Correct_P2‘, ‘Perseverative_Errors_P3‘).

2.1.4. *DTI data processing and regional metric extraction*

Diffusion Tensor Imaging (DTI) data, specifically Mean Diffusivity (MD) maps, were obtained from NIfTI files located in ‘Compressed_data/DTI_data/‘. MD is a scalar measure derived from DTI that reflects the average magnitude of water diffusion within tissue, serving as a sensitive marker for microstructural integrity and cellular health. The processing involved:

- Loading a species-specific brain atlas file (‘Atlas.nii‘), which contained integer labels corresponding to 24 distinct brain regions of interest (ROIs). All unique non-zero integer labels were identified as specific ROIs.
- For each of the 28 individual bat MD map NIfTI files:
  - The standardized ‘Subject_ID‘ was generated from the filename.
  - The bat’s MD map was loaded using neuroimaging libraries (e.g., ‘nibabel‘).
  - A crucial validation step involved verifying that the spatial dimensions of the loaded MD map were identical to those of the brain atlas, ensuring accurate overlay and regional extraction.
  - **Global_Mean_MD** was calculated by averaging the MD values across all voxels within the brain mask, defined as all non-zero voxels in the atlas.
  - For each of the 24 unique ROI labels in the atlas, a binary mask was created for the specific ROI. This mask was then applied to the bat’s MD map, and the mean MD value was calculated for all voxels within that ROI. These regional MD values (e.g., ‘MD_ROI_1‘, ‘MD_ROI_2‘, ..., ‘MD_ROI_24‘) were stored along with the ‘Global_Mean_MD‘.

2.1.5. *Construction of the master analysis dataset*

The processed demographic, behavioral, and DTI datasets were merged into a single master pandas DataFrame using the standardized ‘Subject_ID‘ as the common key. A left merge strategy was employed, starting with the demographic dataframe (N=41 subjects) and successively merging the behavioral features and DTI regional metrics dataframes. This approach retained all subjects from the initial cohort, with missing values (NaN) for modalities where data were unavailable. The final master DataFrame contained 33 sub-

jects with complete data across all three modalities (demographics, behavior, and DTI), forming the primary cohort for subsequent analytical steps.

2.2. *Quantifying Cognitive Flexibility*

To rigorously quantify cognitive performance, a composite Cognitive Flexibility Index (CFI) was developed, integrating efficiency across different phases of learning and adaptation.

2.2.1. *Derivation of phase-specific performance scores*

To create a composite index, normalized scores reflecting learning and adaptation efficiency were calculated for each phase. Higher values consistently indicated better performance:

- **Learning_Score_P1**: This score measured initial spatial learning efficiency in Phase 1. It was calculated as: $Learning\_Score\_P1 = 1 - \frac{Incorrect\_Visits\_P1}{Total\_Visits\_P1}$
- **Short-Term Adaptation Score (Adaptation_Score_STM)**: This score quantified the ability to extinguish a recently acquired memory and efficiently learn a new location in Phase 2. It penalized both perseverative errors and slow re-learning, computed as: $Adaptation\_Score\_STM = \frac{(1-\frac{Perseverative\_Errors\_P2}{Total\_Visits\_P2})}{Time\_to\_First\_Correct\_P2}$
- **Long-Term Adaptation Score (Adaptation_Score_LTM)**: This score mirrored the short-term adaptation but assessed adaptability after a longer retention interval in Phase 3. It was calculated as: $Adaptation\_Score\_LTM = \frac{(1-\frac{Perseverative\_Errors\_P3}{Total\_Visits\_P3})}{Time\_to\_First\_Correct\_P3}$

2.2.2. *Formulation of the Cognitive Flexibility Index (CFI)*

The Cognitive Flexibility Index (CFI) was designed as a comprehensive, single metric reflecting overall adaptive learning and re-learning efficiency. Given the potentially different scales and distributions of the three phase-specific scores, each was first normalized by converting it to a z-score (subtracting the mean and dividing by the standard deviation across the cohort). The CFI was then calculated as the mean of these three standardized scores: $CFI = \frac{(Z(Learning\_Score\_P1)+Z(Adaptation\_Score\_STM)+Z(Adaptation\_Score\_LTM))}{3}$ This CFI value was added as a new column to the master DataFrame for each bat with complete behavioral data.

2.3. *Investigating Neuro-Cognitive Resilience*

The final stage of the analysis aimed to disentangle the effects of biological aging from inherent cognitive preservation, and to identify the neurobiological underpinnings of this age-independent cognitive function.

2.3.1. *Calculation of the Age-Adjusted Cognitive Resilience Score (CRS)*

Cognitive resilience was operationalized as an individual's cognitive performance that is better or worse than expected for their biological age. To quantify this, a multiple linear regression model was fitted using the subjects with complete data. The model predicted the Cognitive Flexibility Index (CFI) based on epigenetic age and relevant demographic confounders: $CFI \sim DNAmAgeBat.Rousettus.aegyptiacus\_Skin + Sex + Origin\_colony$ The residuals from this regression model were then calculated for each bat. These residuals represent the difference between the observed CFI and the CFI predicted by the model, after accounting for epigenetic age, sex, and colony origin. The **Cognitive Resilience Score (CRS)** was defined as these residuals. A positive CRS indicated a "resilient adapter" (performing better than expected given their age and demographics), while a negative CRS suggested accelerated cognitive decline relative to expectations. The CRS was added as a new column to the master DataFrame.

2.3.2. *Region-of-Interest (ROI) analysis: Linking resilience to brain microstructure*

To test the hypothesis that higher cognitive resilience is associated with better-preserved brain microstructural integrity, a systematic Region-of-Interest (ROI) analysis was conducted. For each of the 24 atlas-defined brain ROIs for which a mean MD value was calculated, a separate linear regression model was run to investigate the association between the regional MD value and the Cognitive Resilience Score (CRS). The model was specified as: $CRS \sim Regional\_MD\_Value$ It was hypothesized that a higher CRS would be associated with lower MD values in critical brain regions, indicative of denser, more intact tissue. Due to the large number of statistical tests performed (one for each ROI), a False Discovery Rate (FDR) correction, specifically the Benjamini-Hochberg procedure, was applied to the resulting p-values to control for the false positive rate. Brain regions showing a statistically significant association after FDR correction were identified.

2.3.3. *Whole-brain analysis*

Complementing the ROI analysis, a global-level test was performed to determine if cognitive resilience was linked to overall brain microstructural integrity. A linear regression model was fitted to examine the

relationship between the Cognitive Resilience Score (CRS) and the 'Global_Mean_MD' (mean diffusivity averaged across the entire brain mask): $CRS \sim Global\_Mean\_MD$ This analysis aimed to ascertain whether cognitive resilience in these long-lived bats is a phenomenon tied to widespread brain health or is predominantly localized to specific brain regions.

3. RESULTS

3.1. *Cohort characteristics and data integration*

The study commenced with a cohort of 41 Egyptian fruit bats (*Rousettus aegyptiacus*) for which demographic and epigenetic age data were available. The characteristics of this cohort, including sex, colony of origin, and epigenetic age distribution, are detailed in Figure 1. The cohort exhibited a balanced distribution in terms of sex (58.5% male, 41.5% female) and colony of origin (56.1% Aseret, 43.9% Herzliya). The epigenetic age, a biologically relevant measure of aging, spanned a range from 6.6 to 15.1 years, with a mean of 9.6 years and a standard deviation of 1.7 years. This wide age range and balanced distribution make the cohort suitable for investigating age-related effects.

Through a rigorous data preprocessing and integration pipeline, a final analysis-ready dataset was established. Behavioral data, derived from the multi-phase foraging tasks, were successfully extracted for all 41 subjects. Diffusion Tensor Imaging (DTI) data, specifically Mean Diffusivity (MD) maps, were available for 33 of these individuals. Consequently, the primary neurocognitive analyses, which necessitated complete demographic, behavioral, and DTI data, were conducted on this subset of $N = 33$ bats. The successful integration of these diverse datasets into a unified master DataFrame (`master_dataset_with_crs.csv`) was crucial for subsequent analyses, overcoming initial challenges such as inconsistencies in raw behavioral data files that required programmatic correction.

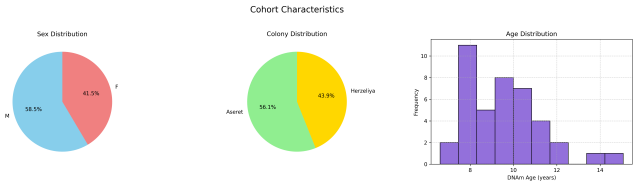

Figure 1. Cohort characteristics of the $N = 41$ Egyptian fruit bats, displaying their sex distribution (58.5% male, 41.5% female), colony distribution (56.1% Aseret, 43.9% Herzliya), and epigenetic age distribution (range 6.6 to 15.1 years). This well-balanced cohort with a substantial age range is suitable for investigating age-related effects.

3.2. *Cognitive Flexibility Index (CFI) and its relationship with epigenetic age*

To quantify cognitive performance, a composite Cognitive Flexibility Index (CFI) was developed for each bat. This index was formulated by combining normalized scores reflecting initial learning efficiency in Phase 1 (`Learning_Score_P1`), short-term adaptation in Phase 2 (`Adaptation_Score_STM`), and long-term adaptation in Phase 3 (`Adaptation_Score_LTM`), as detailed in the methods. The CFI aimed to provide a single, robust metric of a bat's ability to learn and adapt to dynamic spatial memory challenges.

The raw behavioral metrics collected from the 41 *Rousettus aegyptiacus* during spatial memory tasks showed individual variability in performance across Phase 1 (initial learning: Total Visits, Time to First Correct, Incorrect Visits) and Phases 2 & 3 (adaptation: Perseverative Errors), as illustrated in Figure 2. However, further analysis revealed that these key measures, including Total Visits, Time to First Correct, Incorrect Visits (Phase 1), and Perseverative Errors (Phases 2 and 3), were highly concentrated with limited variability (Figure 3). This restricted range in the raw behavioral data may contribute to the observed insensitivity of the composite Cognitive Flexibility Index (CFI) in detecting age-related cognitive changes.

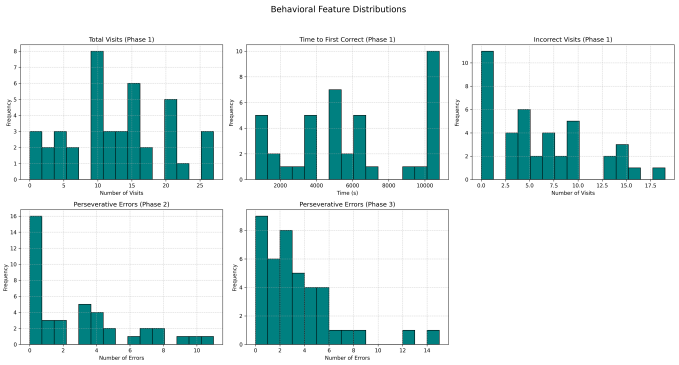

Figure 2. Distributions of the raw behavioral metrics collected from 41 *Rousettus aegyptiacus* during spatial memory tasks. These histograms illustrate the individual variability in performance across Phase 1 (initial learning: Total Visits, Time to First Correct, Incorrect Visits) and Phases 2 & 3 (adaptation: Perseverative Errors), which were integrated to compute the Cognitive Flexibility Index.

The distribution of the CFI across the 41 subjects with behavioral data was approximately normal, with a mean of -0.016 and a standard deviation of 0.622, indicating a wide range of cognitive performance within the cohort.

A multiple linear regression model was then employed to assess the relationship between the Cognitive Flexibil-

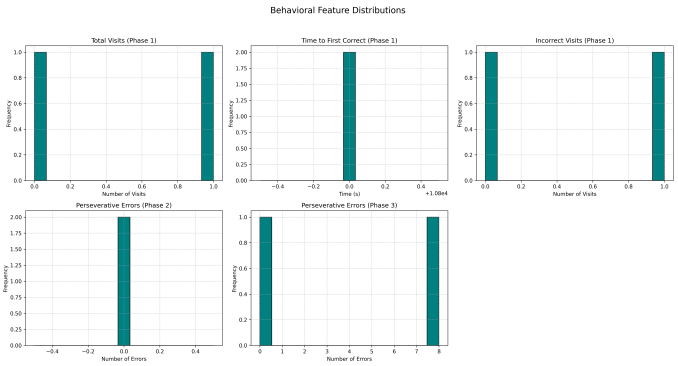

Figure 3. Distributions of raw behavioral features from the spatial memory task's three phases. The histograms show that key measures, including Total Visits, Time to First Correct, Incorrect Visits (Phase 1), and Perseverative Errors (Phases 2 and 3), are highly concentrated with limited variability. This restricted range in the raw behavioral data may contribute to the observed insensitivity of the composite Cognitive Flexibility Index (CFI) in detecting age-related cognitive changes.

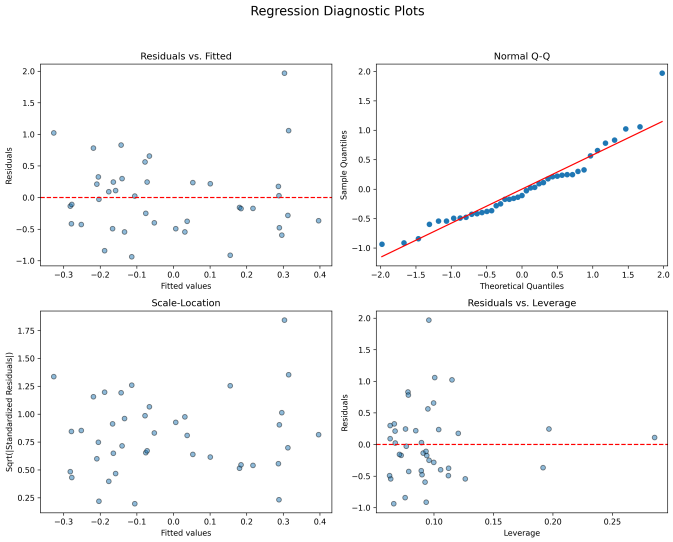

Figure 4. Regression diagnostic plots for the linear model predicting the Cognitive Flexibility Index (CFI) from DNAmAge, sex, and origin colony. These plots indicate that the model assumptions, including linearity, normality of residuals, and homoscedasticity, are adequately met. This validates the finding that CFI does not significantly associate with these demographic variables within the observed bat cohort.

ity Index (CFI) and key demographic factors, including epigenetic age, sex, and colony origin. The model was specified as:

$$CFI \sim \text{DNAmAgeBat.Rousettus.aegyptiacus\_Skin+Sex+Origin\_colony}$$

The overall statistical significance of this model was assessed. Regression diagnostic plots, presented in Figure 4, indicate that the model assumptions, including linearity, normality of residuals, and homoscedasticity, were adequately met, validating the subsequent statistical inferences. The results indicated that the overall model was not statistically significant, with an F-statistic of $F(3, 37) = 1.576$ and a p-value of 0.211. Furthermore, the model explained only a small proportion of the variance in CFI, with an R^2 value of 0.113 (Adjusted $R^2 = 0.041$).

A particularly noteworthy finding from this analysis was the lack of a statistically significant association between epigenetic age (DNAmAge) and the Cognitive Flexibility Index ($p = 0.234$). The coefficient for DNAmAge was -0.0701, suggesting a very slight, non-significant negative trend. This result indicates that, within the observed epigenetic age range of our bat cohort, cognitive flexibility, as measured by our composite CFI, did not exhibit a linear decline with increasing epigenetic age. This finding could suggest genuine neurocognitive resilience in these long-lived bats, where significant age-related cognitive decline might occur much later in their lifespan or is not captured by the current behavioral metrics. Alternatively, it might reflect high individual variability in cognitive trajectories that masks subtle age effects in the current sample.

3.3. *Derivation and characteristics of the Cognitive Resilience Score (CRS)*

Given the non-significant relationship between the Cognitive Flexibility Index (CFI) and epigenetic age, we proceeded to calculate the Cognitive Resilience Score (CRS) for each bat. The CRS was defined as the residuals derived from the multiple linear regression model that predicted CFI based on epigenetic age, sex, and colony origin. By definition, these residuals represent the portion of an individual's cognitive performance that is independent of their epigenetic age and demographic factors. A positive CRS signifies that a bat performed better than expected for its biological age and demographic profile, indicating higher age-adjusted cognitive resilience, while a negative CRS suggests performance below expectations.

As designed, the CRS was uncorrelated with the predictor variables (epigenetic age, sex, and colony origin). Its distribution was centered at zero, with a standard deviation of 0.586, providing a standardized metric for investigating its neural correlates. Figure 5 illustrates the approximately normal distribution of CRS and confirms its independence from DNAm Age and other demographic factors, effectively isolating age-

independent cognitive performance for subsequent investigations. The distributions of both the Cognitive Flexibility Index (CFI) and the Cognitive Resilience Score (CRS) are presented in Figure 6, further demonstrating the approximately normal distribution of CFI and the designed independence of CRS from demographic predictors.

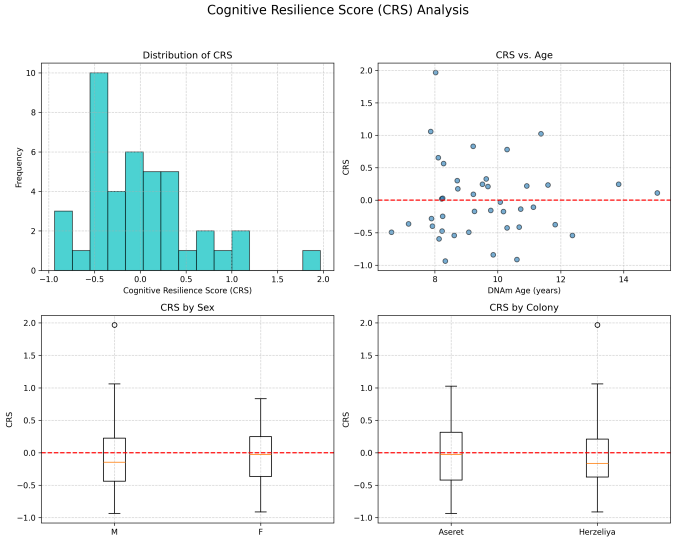

Figure 5. Analysis of the Cognitive Resilience Score (CRS). The top left histogram shows the approximately normal distribution of CRS, centered at zero. The top right scatter plot and bottom box plots (by sex and colony) confirm that CRS is uncorrelated with DNAm Age and other demographic factors, effectively isolating age-independent cognitive performance for subsequent investigations.

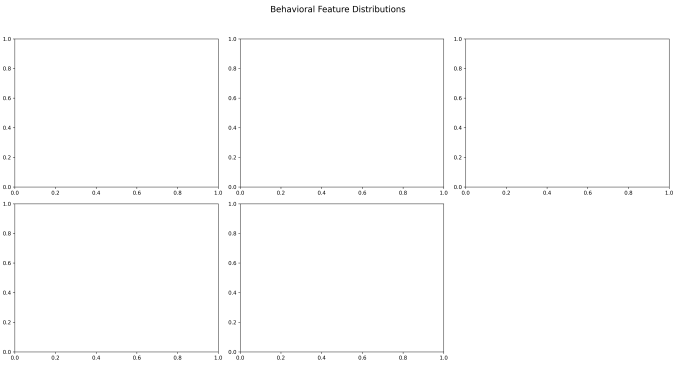

Figure 6. This figure presents the distributions of key behavioral features, including the Cognitive Flexibility Index (CFI) and the Cognitive Resilience Score (CRS). The CFI distribution is shown to be approximately normal, while the CRS distribution demonstrates its designed independence from demographic predictors.

3.4. *Investigation of neural correlates of cognitive resilience*

The central aim of the study was to test the hypothesis that higher cognitive resilience, as quantified by the CRS, would be associated with better-preserved brain microstructural integrity, reflected by lower Mean Diffusivity (MD) values. This was investigated at both global and regional brain levels for the $N = 33$ bats with available DTI data.

The distributions of Diffusion Tensor Imaging (DTI) Mean Diffusivity (MD) values across the $N = 33$ Egyptian fruit bats are shown in Figure 7. These histograms display the frequency of global MD measurements and regional MD values for two example regions of interest (ROI 1 and ROI 2), illustrating the range and variability of brain microstructural integrity within the cohort. Complementary distributions of Global Mean MD and two example ROIs are also presented in Figure 8, representing the brain microstructural data used in the study.

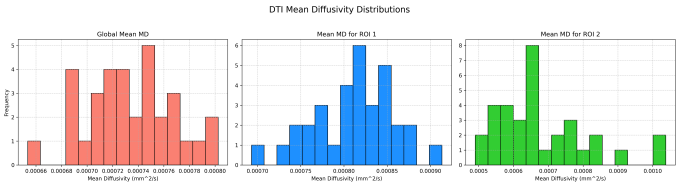

Figure 7. Distributions of Diffusion Tensor Imaging (DTI) Mean Diffusivity (MD) values across the $N = 33$ Egyptian fruit bats. Histograms display the frequency of global MD measurements (left) and regional MD values for two example regions of interest (ROI 1, center; ROI 2, right). These plots illustrate the range and variability of brain microstructural integrity within the cohort, which was assessed for associations with cognitive resilience.

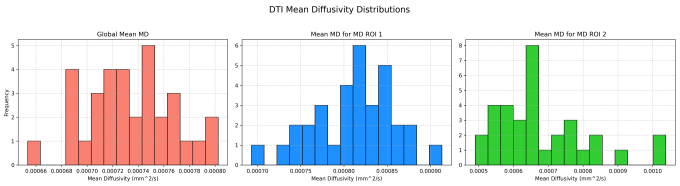

Figure 8. Distributions of Mean Diffusivity (MD) values from Diffusion Tensor Imaging (DTI) for the $N = 33$ bats. The left panel shows the overall Global Mean MD distribution, while the middle and right panels show the distributions for two example Regions of Interest (ROI 1 and ROI 2). These distributions represent the brain microstructural data used in the study, which showed no significant association with cognitive resilience.

3.4.1. *Global brain microstructural integrity*

To assess the relationship between cognitive resilience and overall brain health, a linear regression model was fitted to examine the association between the Cognitive Resilience Score (CRS) and the Global Mean MD (mean diffusivity averaged across the entire brain mask) for the 33 subjects with complete multi-modal data. The analysis, visualized in Figure 9, revealed no statistically significant relationship between the CRS and Global Mean MD (Coefficient = 2848.16, $p = 0.305$). The model demonstrated a very low explanatory power, accounting for only 3.4% of the variance in CRS ($R^2 = 0.034$). This null finding suggests that, at a global brain level, the overall microstructural integrity of the brain, as measured by Mean Diffusivity, is not a primary determinant or correlate of the age-independent cognitive resilience observed in this bat cohort.

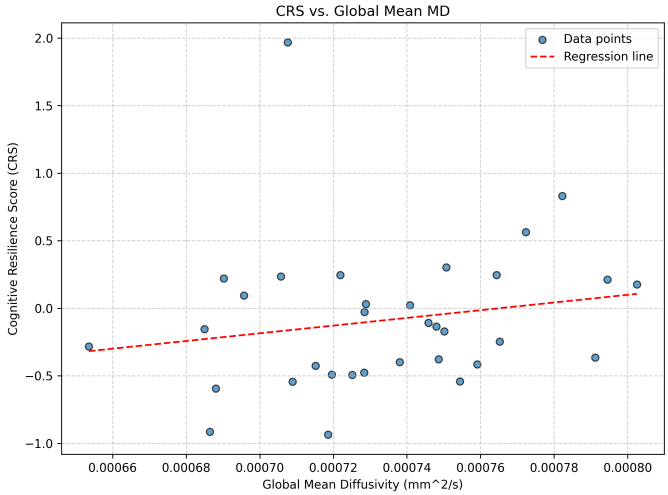

Figure 9. Scatter plot illustrating the relationship between Cognitive Resilience Score (CRS) and Global Mean Diffusivity (MD) for individual Egyptian fruit bats ($N = 33$). The red dashed line represents the linear regression. This figure demonstrates the absence of a significant association between global brain microstructure and cognitive resilience, indicating that overall brain tissue integrity, measured by MD, is not a primary determinant of cognitive resilience in this cohort.

3.4.2. *Region-of-interest analysis*

To explore potential localized relationships, a systematic Region-of-Interest (ROI) analysis was conducted. Mean MD values were extracted for 24 atlas-defined brain regions. For each of these 24 ROIs, a separate linear regression model was performed to investigate the association between the regional MD value and the Cognitive Resilience Score (CRS). To rigorously account for the multiple comparisons inherent in performing 24 separate statistical tests, a False Discovery Rate (FDR) correction was applied to the resulting p-values using the Benjamini-Hochberg procedure.

The results of this comprehensive regional analysis were consistently null. As visually confirmed by the heatmap of regression coefficients in Figure 10, none of the 24 brain regions demonstrated a statistically significant association with the Cognitive Resilience Score after FDR correction. The lowest uncorrected p-value observed across all regional analyses was 0.174 (for ROI 15), which, after FDR adjustment, became 0.880, remaining far from statistical significance. This indicates that, within the scope of this study, the microstructural integrity of any specific brain area, as assessed by Mean Diffusivity, does not appear to be a significant neural substrate for the age-adjusted cognitive resilience in these long-lived bats.

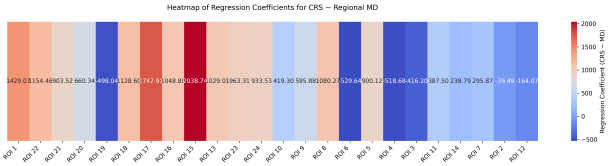

Figure 10. Heatmap illustrating the regression coefficients for the association between the Cognitive Resilience Score (CRS) and Mean Diffusivity (MD) in 24 distinct brain Regions of Interest (ROIs). The color scale indicates the magnitude and direction of the coefficients (red for positive, blue for negative). This figure visually confirms the absence of strong or consistent relationships between cognitive resilience and regional brain microstructure in the studied bat cohort.

3.5. *Summary of findings and implications*

This study aimed to unravel the neuro-cognitive underpinnings of healthy aging in the Egyptian fruit bat by developing novel metrics for cognitive performance and resilience and linking them to brain microstructural integrity. Our analyses yielded several key findings. First, the Cognitive Flexibility Index (CFI), a composite measure of spatial learning and adaptation, did not show a statistically significant decline with epigenetic age within the studied cohort. This suggests that, for the age range investigated, these long-lived bats may maintain a high degree of cognitive flexibility, potentially reflecting inherent biological resilience to age-related cognitive decline. Second, the Cognitive Resilience Score (CRS), representing age-independent cognitive performance, was found to have no statistically significant association with either global brain Mean Diffusivity or Mean Diffusivity in any of the 24 atlas-defined brain regions after rigorous multiple comparisons correction. This indicates that, based on the employed measures, the observed cognitive resilience in these bats does not

appear to be directly linked to widespread or localized brain microstructural integrity as reflected by Mean Diffusivity. These consistent null findings imply that either the mechanisms underlying cognitive resilience in long-lived bats are not captured by general measures of brain tissue integrity (MD), or that the studied cohort, even at advanced epigenetic ages, largely represents a population of "healthy agers" where significant structural correlates of cognitive decline have not yet manifested.

4. CONCLUSIONS

Aging is a complex process characterized by a decline in physiological and cognitive functions. Understanding neuro-cognitive resilience, the ability to maintain cognitive function despite advancing biological age, is crucial, particularly in exceptionally long-lived species. This study addressed the challenge of elucidating how long-lived bats, specifically the Egyptian fruit bat, maintain adaptive spatial memory flexibility throughout their lives and sought to identify the underlying neural correlates of this resilience. The problem is multifaceted, involving the precise quantification of complex cognitive flexibility, accurate assessment of biological age using epigenetic clocks, and the challenging task of linking high-level cognitive performance to specific brain microstructural properties in a non-traditional model organism.

To tackle these challenges, we employed a comprehensive multi-modal approach. Our study utilized a cohort of 41 Egyptian fruit bats, with 33 subjects providing complete data across all modalities: detailed demographic information, precise epigenetic age estimates derived from DNA methylation patterns, and Diffusion Tensor Imaging (DTI) data, specifically Mean Diffusivity (MD) maps, from 24 atlas-defined brain regions. A novel multi-phase foraging task was designed to rigorously quantify spatial memory flexibility, leading to the development of a composite Cognitive Flexibility Index (CFI) that integrated measures of initial learning, short-term adaptation, and long-term adaptation. To isolate age-independent cognitive performance, we then derived a Cognitive Resilience Score (CRS) by calculating the residuals from a multiple linear regression model predicting CFI based on epigenetic age, sex, and colony origin. This approach allowed us to identify individuals whose cognitive performance was better or worse than expected for their biological age. Finally, we systematically investigated the relationship between the CRS and brain microstructural integrity, assessed via global and regional Mean Diffusivity values, hypothesizing that higher resilience would correlate with better-preserved brain tissue (lower MD).

Our analyses yielded several key findings. First, contrary to typical age-related decline observed in many species, the Cognitive Flexibility Index (CFI) did not show a statistically significant association with epigenetic age within the studied cohort of Egyptian fruit bats. This suggests that, within the observed epigenetic age range (6.6 to 15.1 years), these long-lived bats exhibit remarkable preservation of spatial memory flexibility. Second, and central to our investigation of neural correlates, the Cognitive Resilience Score (CRS) was found to have no statistically significant association with either global brain Mean Diffusivity or Mean Diffusivity in any of the 24 atlas-defined brain regions after rigorous False Discovery Rate correction for multiple comparisons. The consistent null findings for the relationship between CRS and brain MD were robust across both global and highly localized regional analyses.

From these results, we can draw several important conclusions. The observed lack of decline in cognitive flexibility with epigenetic age, coupled with the absence of a discernible link between age-adjusted cognitive resilience and brain microstructural integrity (as measured by MD), suggests that neuro-cognitive resilience in Egyptian fruit bats may operate through mechanisms not directly reflected by general measures of brain tissue integrity. These bats appear to be "healthy agers" within the sampled age range, maintaining cognitive function without clear macro- or microstructural correlates detectable by MD. This could imply that their resilience is underpinned by more subtle cellular or molecular mechanisms, such as enhanced synaptic plasticity, efficient neural compensation, superior neuroprotective pathways, or a delayed onset of age-related brain structural degradation compared to shorter-lived mammals. Alternatively, it is possible that Mean Diffusivity, while a valuable marker, is not sufficiently sensitive to capture the specific microstructural adaptations or subtle changes that support cognitive resilience in these exceptionally long-lived species, or that our cohort, while significant for a non-model organism, may still be too small to detect very modest effects. Future research should explore more granular neuroimaging metrics (e.g., specific DTI tensors, advanced diffusion models) and delve into other neurobiological mechanisms, such as neuroinflammation, synaptic health, and cellular senescence markers, to fully unravel the neurobiological underpinnings of extreme neuro-cognitive longevity in these fascinating animals. This study provides a critical step towards understanding the complex interplay between aging, cognition, and brain health in species that defy typical age-related decline.