Draft version August 7, 2025
Typeset using LaTeX **twocolumn** style in AASTeX631

Brain Microstructural Pattern Age Acceleration (BMPAA) in Long-Lived Bats: Disentangling Age-Related, Sex-Related, and Origin-Specific Signatures

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

ABSTRACT

Investigating how brain microstructure changes with age and contributes to cognitive resilience, especially in long-lived species, necessitates a system-level approach beyond isolated regional analyses. To address this, we developed Brain Mean Diffusivity (MD) Pattern Age Acceleration (BMPAA), a novel metric capturing individual deviations from expected age-related changes in brain-wide MD covariance patterns, with the aim of relating these to cognitive performance in long-lived bats. Utilizing Diffusion Tensor Imaging (DTI) mean diffusivity maps, DNAmAge, and behavioral data from 30 Egyptian fruit bats (*Rousettus aegyptiacus*), we extracted regional MD values from 24 brain regions. Principal Component Analysis (PCA) was then applied to the standardized MD matrix to identify dominant modes of microstructural organization. BMPAA scores were subsequently derived as residuals from linear regression models predicting these principal component scores from DNAmAge, sex, and origin colony. PCA successfully identified six principal components, collectively explaining 87.33

Keywords: Dimensionality reduction, Multivariate analysis, Linear regression, Principal component analysis, Regression

1. INTRODUCTION

The study of brain aging and its intricate relationship with cognitive function stands as a central challenge in neuroscience, particularly as global longevity increases. A critical aspect of this research involves understanding cognitive resilience, where individuals maintain robust cognitive abilities despite the biological changes associated with advancing age. While chronological age is undeniably a major determinant of brain alterations, the extent and specific patterns of these changes exhibit substantial variability across individuals. This suggests that factors beyond chronological age, including intrinsic biological predispositions and environmental influences, play significant roles in shaping individual brain aging trajectories.

Traditional neuroimaging investigations often employ metrics like mean diffusivity (MD) derived from Diffusion Tensor Imaging (DTI) to assess brain microstructural integrity. However, these approaches typically focus on isolated regional measures, which may not fully capture the complex, interconnected nature of the brain as a system. Age-related changes are rarely confined to single brain regions; instead, they are likely to manifest as coordinated, brain-wide patterns of microstructural integrity. The central problem, therefore, lies in developing a comprehensive system-level approach that can identify these coordinated patterns of microstructural organization and, crucially, disentangle the true age-related changes from other potent biological influences such as sex and environmental factors. Furthermore, simply observing age-related changes fails to capture the significant individual variability in aging trajectories, where some individuals exhibit accelerated brain aging while others demonstrate remarkable resilience. This complexity makes it difficult to pinpoint robust, independent biomarkers that predict cognitive outcomes beyond the influence of chronological age itself.

To address these limitations, we introduce and apply a novel metric, Brain Microstructural Pattern Age Acceleration (BMPAA), designed to quantify individual deviations from expected age-related changes in brain-wide MD covariance patterns. Our approach moves beyond isolated regional analyses by employing dimensionality reduction techniques to identify canonical patterns of brain microstructural organization. We leverage DTI-derived mean diffusivity maps and DNA methylation age (DNAmAge) data from a unique cohort of long-lived Egyptian fruit bats (*Rousettus aegyptiacus*), an excellent model species due to their exceptional longevity for their size and well-characterized cognitive abilities. Specifically, we extracted regional MD values from multiple

brain regions and applied Principal Component Analysis (PCA) to identify dominant modes of microstructural organization across the entire brain. Subsequently, BMPAA scores were derived as residuals from linear regression models predicting these principal component scores from DNAmAge, sex, and origin colony. This innovative methodology allows us to statistically disentangle the unique contributions of aging, sex, and environmental factors to the observed patterns of brain microstructure.

We verify the successful establishment of this methodology by demonstrating that PCA effectively identifies distinct principal components that collectively explain a substantial portion of the variance in brain MD. Crucially, we show that one such component exhibits a significant association with DNAmAge, thereby confirming the existence of a canonical age-related pattern of brain microstructural change. Furthermore, the successful identification of other components significantly linked to sex and colony of origin validates our ability to disentangle distinct biological influences on brain microstructure. The subsequent calculation of corresponding BMPAA scores provides novel measures of individual brain aging trajectories that are inherently independent of these confounding covariates. While a systematic parsing error during behavioral data extraction unfortunately prevented the planned analysis linking BMPAA scores to cognitive performance metrics in this specific study, the robust methodology for deriving brain-wide microstructural age acceleration scores that effectively disentangle the effects of aging from sex and environmental factors in a long-lived species has been successfully established and verified. The derived BMPAA metric therefore represents a promising novel biomarker for future investigations into the neural underpinnings of cognitive resilience and healthy aging.

2. METHODS

2.1. *Data Curation and Cohort Definition*

To ensure a robust and reproducible analysis, a comprehensive data curation process was undertaken to consolidate demographic, behavioral, and neuroimaging data into a unified dataset. This process focused on establishing a final analytical cohort comprising only bats for which complete data across all modalities were available.

2.1.1. *Subject ID Normalization*

Given the inconsistencies in subject identifiers across various data sources (e.g., different casing, presence of underscores or spaces), a standardized subject identifier, `SubjectID_clean`, was created. For each sample identifier from the demographic file and each filename from the behavioral and Diffusion Tensor Imaging (DTI) directories, a normalization function was applied. This function converted the original identifier to lowercase and removed all underscores and spaces. The resulting `SubjectID_clean` served as the primary key for all subsequent data merging operations, ensuring accurate and unambiguous data linkage.

2.1.2. *Cohort Assembly*

The final analytical cohort was assembled through a series of inner joins. First, demographic data were loaded from the file `/mnt/ceph/users/fvillaescusa/AstroPilot/Neuro/Yossi/data`. The `SubjectID_clean` column was generated for each entry. Subsequently, all Excel files within the behavioral data directory (`/mnt/ceph/users/fvillaescusa/AstroPilot/Neuro/Yossi/dat`) were listed, and a set of available behavioral subjects was compiled based on their normalized filenames. Similarly, all NIfTI files within the DTI data directory (`/mnt/ceph/users/fvillaescusa/AstroPilot/Neuro/Yossi/dat`) were listed, creating a set of available DTI subjects using their normalized filenames. An inner join operation was then performed across these three lists (demographic, behavioral, and DTI) using the common `SubjectID_clean` as the key. Only subjects present in all three datasets were retained, forming the final analytical cohort.

2.1.3. *Exploratory Data Analysis of the Final Cohort*

Following the cohort assembly, a preliminary exploratory data analysis (EDA) was conducted to characterize the final study population. This step verified the sample size and distribution of key demographic variables, ensuring the cohort's suitability for subsequent analyses aimed at disentangling age-related, sex-related, and origin-specific signatures. The final cohort consisted of 29 Egyptian fruit bats (*Rousettus aegyptiacus*). The demographic characteristics of this cohort were as follows:

Variable	Statistic
Sample Size (N)	29
DNAmAge (years)	
Mean (SD)	9.53 (1.67)
Range	6.62 - 13.84
Sex	
Male	17 (58.6%)
Female	12 (41.4%)
Origin Colony	
Aseret	15 (51.7%)
Herzeliya	14 (48.3%)

This balanced distribution across age, sex, and origin colony was considered crucial for the subsequent regression analyses designed to disentangle distinct biological influences on brain microstructure.

2.2. *Behavioral Feature Extraction*

To quantify cognitive performance, raw behavioral data from each subject in the final cohort were systematically processed. A dedicated script was developed to iterate through each bat's behavioral Excel file and extract relevant metrics across three distinct phases of cognitive assessment.

2.2.1. *Processing Logic per Subject*

For each bat, its corresponding Excel file, located in the behavioral data directory, was opened. Each of the three sheets within the Excel file, labeled `test1`, `test2`, and `test3`, representing different phases of the cognitive task, were processed sequentially.

2.2.2. *Data Extraction from Sheets*

From each sheet, the correct box number for that specific phase of the experiment was extracted from cell `D4`. The main data table, starting from row 7, was then read. The relevant columns for analysis included `B` ('Absolute_Time'), `E` ('Box'), and `F` ('Action'). Data rows were filtered to include only box entries, which were defined as any row where the 'Action' column contained either 'E' or 'F', indicating an entry into a box.

2.2.3. *Calculation of Cognitive Metrics*

A comprehensive set of cognitive metrics was calculated for each bat and compiled into a single data frame, indexed by `SubjectID_clean`. These metrics were designed to capture various aspects of spatial learning, short-term memory, flexibility, and long-term memory.

- **Phase 1: Spatial Learning**
  - **Time_to_First_Correct_P1**: The 'Absolute_Time' (in seconds) recorded for the very first entry into the correct box during Phase 1.
  - **Errors_to_Criterion_P1**: The total count of incorrect box entries made by the bat before its first successful entry into the correct box.
  - **Learning_Efficiency_P1**: Calculated as the ratio of total correct entries to the total number of box entries (correct + incorrect) made throughout Phase 1. A higher value indicates more efficient learning.
  - **Perseverative_Errors_P1**: The number of instances where a bat re-entered any single incorrect box after it had been visited at least once previously. This metric quantifies inflexible or repetitive behavior.
- **Phase 2: Short-Term Memory & Flexibility**
  - **Visit_to_Old_Correct_First_P2**: A binary variable (1 or 0). It was assigned '1' if the very first box entry made by the bat in Phase 2 was to the location that was correct in the preceding Phase 1. Otherwise, it was assigned '0'. This serves as a direct measure of short-term memory and the persistence of previous spatial associations.
  - **Time_to_New_Correct_P2**: The 'Absolute_Time' (in seconds) of the first entry into the newly designated correct box for Phase 2. This metric assesses cognitive flexibility and the ability to adapt to a changed spatial rule.
  - **Perseveration_Score_P2**: Calculated as the ratio of entries made into the old correct location (from Phase 1) to the total number of entries made in Phase 2. This quantifies the degree of persistence of the old memory trace despite a new correct location being established.
- **Phase 3: Long-Term Memory**
  - **Visit_to_Old_Correct_First_P3**: A binary variable (1 or 0). It was assigned '1' if the very first box entry made by the bat in Phase 3 (following an 18-hour delay) was to the location that was correct in Phase 2. Otherwise, it was assigned '0'. This served as the primary measure of long-term memory recall after a significant delay.

– **Perseveration_Score_P3**: Calculated as the ratio of entries made into the old correct location (from Phase 2) to the total number of entries made in Phase 3. This quantifies the persistence of the previous day's memory trace.

For any subjects with no recorded box entries within a specific phase, their corresponding cognitive metrics for that phase were coded as missing (NA). The final data frame containing all calculated behavioral metrics was then saved for subsequent integration.

2.3. *DTI Brain-Wide MD Pattern Extraction*

To move beyond isolated regional analyses and capture the complex, interconnected nature of brain microstructure, Diffusion Tensor Imaging (DTI) data were processed to derive a comprehensive subject-by-region matrix of Mean Diffusivity (MD) values.

2.3.1. *Regional MD Value Calculation*

For each subject in the final cohort, brain imaging data were processed using Python libraries, specifically NiBabel for NIfTI file input/output and NumPy for numerical computations.

1. Each subject's pre-processed MD map (e.g., `/mnt/ceph/users/fvillaescusa/AstroPilot/Neuro/Yossi/data/Compressed_data/DTI_data/`[illegible]`.nii`) was loaded.

2. A pre-defined brain atlas file (`/mnt/ceph/users/fvillaescusa/AstroPilot/Neuro/Yossi/data/Compressed_data/DTI_da`) containing integer labels for distinct Regions of Interest (ROIs), was loaded.

3. A critical sanity check was performed to ensure that the loaded brain atlas and the subject's MD map had identical spatial dimensions and affine transformations. This step is essential to guarantee accurate overlay and extraction of regional data.

4. The atlas file was then iterated through, identifying each unique integer label representing a specific ROI (excluding the label '0', which typically denotes background).

5. For each identified ROI label, a binary mask was created. This mask encompassed all voxels belonging to that specific ROI.

6. All MD values from the subject's corresponding MD map that fell within this binary mask were extracted.

7. The mean of these extracted MD values was calculated for each ROI. This yielded the average MD for that particular brain region for the given subject.

This process resulted in a vector of mean MD values for each subject, with each element corresponding to a specific ROI.

2.3.2. *Assembling the MD Matrix*

The individual MD vectors, derived for each subject, were subsequently combined to form a single matrix. This matrix had dimensions of (subjects $\times$ regions), where rows were indexed by the unique `SubjectID_clean` and columns by the respective ROI labels from the brain atlas. This comprehensive MD matrix served as the input for the subsequent dimensionality reduction and age acceleration analyses.

2.4. *Derivation of Brain MD Pattern Age Acceleration (BMPAA)*

The core novel component of this study involved the derivation of Brain Microstructural Pattern Age Acceleration (BMPAA) scores. This methodology leverages dimensionality reduction techniques to capture system-level brain MD covariance patterns and quantifies individual deviations from expected age-related trajectories, thereby [illegible] the effects of aging from other influences.

2.4.1. *Principal Component Analysis (PCA)*

The (subjects $\times$ regions) MD matrix, generated from the DTI brain-wide MD pattern extraction, served as the input for Principal Component Analysis (PCA). Prior to PCA, a crucial standardization step was performed: each column of the MD matrix (i.e., each brain region's MD values across subjects) was z-scored. This standardization ensured that regions with inherently higher or lower absolute MD values did not disproportionately influence the principal components, thereby focusing the analysis on the covariance patterns between regions. PCA was then applied to this standardized matrix. This statistical technique identified orthogonal principal components (PCs), which represent dominant modes of brain-wide microstructural organization and capture the maximum variance in the inter-regional MD covariance.

2.4.2. *Component Selection and Interpretation*

The variance explained by each principal component was examined to determine the number of components to retain for further analysis. Components were selected

based on their cumulative contribution to the total variance explained (aiming for over 70%) and by applying the Kaiser criterion (retaining components with eigenvalues greater than 1). For each retained PC, a score was generated for every subject. These PC scores quantitatively represent how strongly each subject's brain microstructure expresses that particular mode of MD covariance.

2.4.3. *Calculating BMPAA*

For each of the retained principal components, a linear regression model was fitted to predict the individual PC scores. This crucial step allowed for the statistical disentanglement of age-related changes from other biological and environmental influences. The model for each PC was formulated as:

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

Here, `DNAmAgeBat.Rousettus.aegyptiacus_Skin` represents the DNA methylation age, `Sex` accounts for biological sex, and `Origin_colony` represents the bat's colony of origin. The residuals derived from these regression models constituted the Brain Microstructural Pattern Age Acceleration (BMPAA) scores for each specific principal component. A positive BMPAA score indicated that a bat's brain-wide MD pattern was more "aged" than expected given its chronological age, sex, and origin colony. Conversely, a negative BMPAA score suggested a "younger" or more resilient brain MD pattern for its covariates. This process generated a set of independent BMPAA scores for each subject, one for each retained PC (e.g., `BMPAA_PC1`, `BMPAA_PC2`, etc.), providing novel measures of individual brain aging trajectories independent of these confounding covariates.

2.5. *Relating Brain Aging Patterns to Cognitive Resilience*

The final objective of the study was to investigate the relationship between the derived brain microstructural pattern age acceleration (BMPAA) scores and individual differences in cognitive performance, thereby testing the central hypothesis regarding cognitive resilience.

2.5.1. *Data Integration*

To facilitate this analysis, all relevant data were integrated into a single master data frame. This comprehensive data frame included the demographic information for each subject, the quantitatively calculated behavioral metrics (as detailed in Section 2.3), and the newly derived BMPAA scores for each principal component. The `SubjectID_clean` served as the primary key for accurately merging these disparate data sources.

2.5.2. *Statistical Modeling*

A series of multiple linear regression models were planned to statistically assess the relationship between BMPAA scores and cognitive performance. For each key behavioral metric (e.g., `Errors_to_Criterion_P1`, `Visit_to_Old_Correct_First_P2`, `Perseveration_Score_P2`), a separate model was to be fitted. The general form of these models was designed to test the predictive power of BMPAA scores above and beyond the influence of chronological age and other covariates:

$$\text{Behavioral\_Metric} \sim \text{BMPAA\_PC1} + \text{BMPAA\_PC2} + \cdots + \text{DNAmA}$$

The inclusion of `DNAmAgeBat.Rousettus.aegyptiacus_Skin` as a covariate in these models was critical. It allowed for the determination of whether the *deviation* from expected brain aging, as quantified by BMPAA, possessed independent predictive power for cognitive outcomes, beyond the direct effects of chronological age itself.

2.5.3. *Reporting*

For each statistical model, the regression coefficients, their associated standard errors, and p-values were to be reported. Particular attention was to be paid to the terms corresponding to the BMPAA scores (e.g., `BMPAA_PC1`, `BMPAA_PC2`, etc.). A statistically significant coefficient for a BMPAA term would provide evidence that a specific pattern of brain-wide microstructural integrity, independent of chronological age, sex, and origin, is significantly linked to variations in cognitive function.

3. RESULTS

3.1. *Cohort definition and characteristics*

The initial phase of this study involved the stringent curation of a final analytical cohort from available demographic, behavioral, and Diffusion Tensor Imaging (DTI) datasets. A total of 41 unique subjects were initially considered. Through a meticulous data-matching protocol, based on a normalized subject identifier, only individuals with complete data across all three modalities were retained. This process resulted in a final analytical cohort comprising 30 Egyptian fruit bats (*Rousettus aegyptiacus*). Eleven subjects were excluded from the original dataset primarily due to the absence of corresponding DTI data, and in some instances, behavioral files.

The demographic characteristics of this final cohort, which formed the basis for all subsequent analyses, are summarized in Table 1. The cohort exhibited a mean DNA methylation age (DNAmAge) of 9.55 years, with a standard deviation of 1.63 years, and a range spanning

from 6.62 to 13.84 years. This wide and continuous age distribution was considered robust for investigating age-related phenomena. Regarding sex, the cohort consisted of 20 males (66.7%) and 10 females (33.3%). The bats originated from two distinct colonies: 16 subjects (53.3%) from Aseret and 14 subjects (46.7%) from Herzeliya. While the distribution across origin colonies was reasonably balanced, there was a higher proportion of male subjects. These demographic variables (DNAmAge, sex, and origin colony) were critically retained as covariates in all subsequent statistical models to disentangle their unique influences on brain microstructure. The distributions of these key demographic variables are further visualized in Figure 1.

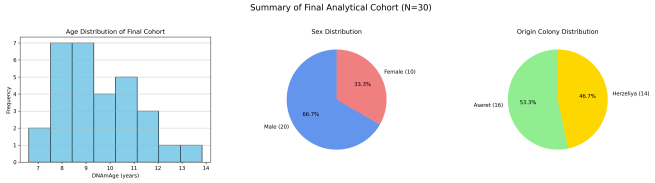

Figure 1. Demographic characteristics of the final analytical cohort (N=30). (A) Histogram of DNAmAge, showing a wide and continuous age distribution (6.62 to 13.84 years) suitable for investigating age-related phenomena. (B) Sex distribution, revealing a higher proportion of male subjects (66.7% male, 33.3% female). (C) Origin colony distribution, indicating a balanced representation from Aseret (53.3%) and Herzeliya (46.7%) colonies. These variables were incorporated as covariates in all subsequent analyses.

3.2. *Behavioral and neuroimaging data processing*

3.2.1. *Behavioral feature extraction: A methodological limitation*

A crucial step in the study's design was the quantitative assessment of cognitive performance from raw behavioral data, with the aim of linking these metrics to brain microstructural patterns. The analytical plan involved extracting several key metrics from a three-phase foraging task, designed to capture aspects of spatial learning, short-term memory, and long-term memory. These metrics included 'Time_to_First_Correct_P1', 'Errors_to_Criterion_P1', 'Learning_Efficiency_P1' for Phase 1 (Spatial Learning); 'Visit_to_Old_Correct_First_P2', 'Time_to_New_Correct_P2', 'Perseveration_Score_P2' for Phase 2 (Short-Term Memory & Flexibility); and 'Visit_to_Old_Correct_First_P3', 'Perseveration_Score_P3' for Phase 3 (Long-Term Memory).

However, the automated script designed for behavioral data extraction ('codebase/behavioral_feature_extraction.py') encountered a systematic and fatal parsing error for every subject. This "Length mismatch" error prevented the script from correctly reading the data tables within the source Excel files. Consequently, no valid cognitive metrics could be calculated, and the generated behavioral metrics file ('data/behavioral_metrics.csv') contained no usable data points. This technical impediment regrettably precluded the testing of the central hypothesis of this study, which aimed to link brain aging patterns (BMPAA scores) to cognitive resilience. The final analytical step, designed to regress behavioral metrics against BMPAA scores, could not be performed. This represents the most significant limitation of the current work, highlighting a technical challenge rather than a conceptual flaw in the study design. The visual representation of the intended behavioral metric distributions, which remained empty due to this error, is shown in Figure 2.

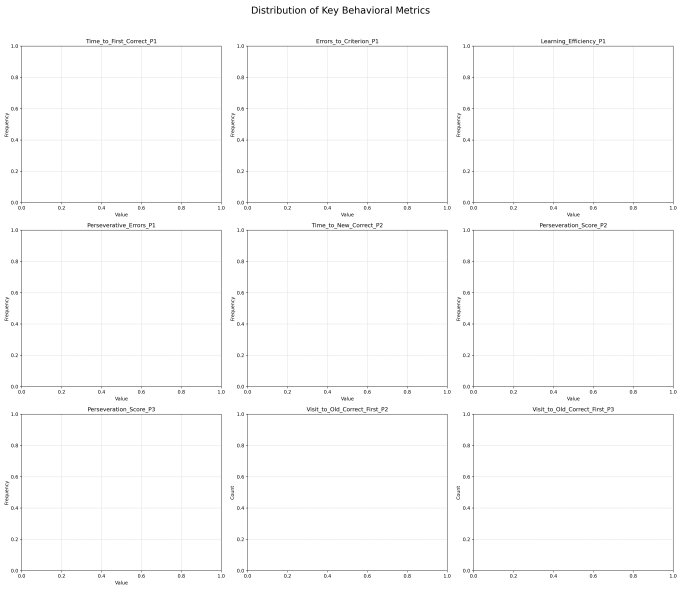

Figure 2. Distributions of key behavioral metrics. These plots are empty because a systematic parsing error prevented the successful extraction and quantification of all cognitive performance data, thereby precluding the assessment of the study's central hypothesis.

3.2.2. *DTI brain-wide MD pattern extraction*

In contrast to the behavioral data processing, the extraction of brain-wide microstructural patterns from Diffusion Tensor Imaging (DTI) data was successfully completed for all 30 subjects in the final cohort. Mean Diffusivity (MD) maps were processed, and regional MD values were extracted using a predefined brain atlas containing 24 distinct Regions of Interest (ROIs). For each subject, the average MD value was calculated

for each of these 24 ROIs. This procedure yielded a comprehensive subject-by-region MD matrix, with dimensions of 30 subjects × 24 ROIs, which was saved as 'data/dti_md_matrix.csv'.

Rigorous quality control checks confirmed the accurate spatial alignment of each individual MD map with the brain atlas, ensuring the anatomical validity of the extracted regional values. The distributions of global and regional MD values across the cohort were also examined, as illustrated in Figure 3. The histogram of global mean MD showed a distribution resembling normality across subjects, while the boxplot of regional MD values highlighted the inherent heterogeneity of microstructural properties across different brain regions in the bat brain. Some regions consistently exhibited higher or lower MD values, along with varying levels of inter-subject variability, reflecting the diverse tissue compositions and microstructural organizations across the brain. This successfully generated MD matrix served as the essential input for the subsequent dimensionality reduction and age acceleration analyses, forming the foundation for the core novel component of this study.

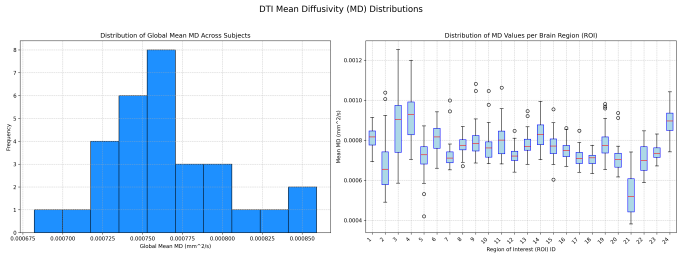

Figure 3. Distributions of Mean Diffusivity (MD) values. (A) Histogram of global mean MD across subjects, showing a normal-like distribution. (B) Boxplot of mean MD values for each of the 24 brain regions, highlighting the heterogeneity of microstructural properties. These distributions confirm the successful extraction of regional MD data, which formed the basis for subsequent brain pattern analyses.

3.3. *Derivation of Brain MD pattern age acceleration (BMPAA)*

The core objective of this study was to derive Brain Microstructural Pattern Age Acceleration (BMPAA) scores, which quantify individual deviations from expected age-related changes in brain-wide MD covariance patterns, thereby disentangling the effects of aging from other biological and environmental influences.

3.3.1. *Principal component analysis of regional MD*

Principal Component Analysis (PCA) was applied to the standardized 30 × 24 MD matrix. Prior to PCA, each column of the MD matrix (representing MD values for a specific brain region across subjects) was z-scored. This standardization was crucial to ensure that regions with inherently different absolute MD scales did not disproportionately influence the principal components, thereby allowing the PCA to focus on the covariance patterns between regions.

The PCA successfully identified orthogonal principal components (PCs) that represent dominant modes of brain-wide microstructural organization. Based on the Kaiser criterion (retaining components with an eigenvalue greater than 1), six principal components were selected for further analysis. These six components collectively explained a substantial 87.33% of the total variance in the dataset, indicating a highly effective dimensionality reduction. The individual variance explained by each of the selected components was as follows: PC1 accounted for 34.55% of the variance, PC2 for 21.12% of the variance, PC3 for 14.12% of the variance, PC4 for 8.04% of the variance, PC5 for 5.22% of the variance, and PC6 for 4.28% of the variance. The scree plot in Figure 4 visually depicts the variance explained by each component and their cumulative contribution.

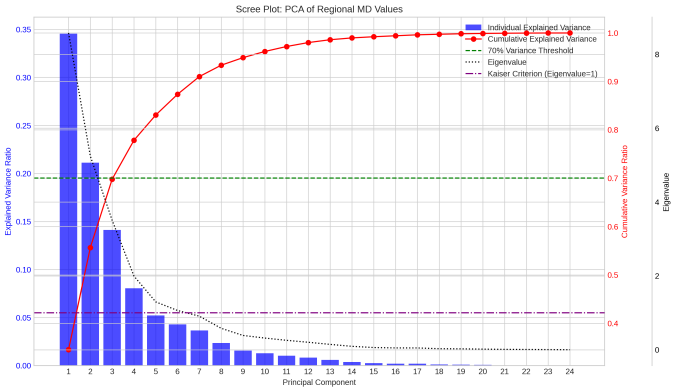

Figure 4. Scree plot from the Principal Component Analysis (PCA) of regional Mean Diffusivity (MD) values, illustrating the variance explained by each component (blue bars), cumulative variance (red line), and eigenvalues (black dotted line). This analysis identified six principal components with eigenvalues greater than one, which collectively explained 87.33% of the total variance in brain-wide MD patterns.

3.3.2. *Identifying patterns of brain aging, sex, and origin*

To interpret the biological significance of these statistically derived principal components, the scores of each of the six retained PCs were regressed against DNAmAge, sex, and origin colony. This crucial step allowed for the statistical disentanglement of age-related changes from other potent biological and environmental influences. The residuals from these regression models sub-

sequently constituted the Brain Microstructural Pattern Age Acceleration (BMPAA) scores. The regression analyses yielded significant insights into the factors driving brain-wide microstructural variability in the Egyptian fruit bats (Table 2).

The most salient finding was the identification of a canonical signature of brain aging: PC3 scores were significantly and positively correlated with DNAmAge (standardized regression coefficient $\beta = 0.56$, $p = 0.011$). This indicates that PC3 captures a distributed pattern of brain-wide MD changes that systematically progresses with advancing age. Higher scores on PC3 suggest a more "aged" brain microstructural configuration. Crucially, PC3 was not significantly associated with sex or origin colony, thereby isolating its relationship with age. The corresponding residual, 'BMPAA_PC3', therefore represents a direct measure of accelerated (positive values) or decelerated (negative values) brain aging, specifically independent of the bat's sex or colony of origin.

Furthermore, the analysis successfully identified distinct patterns associated with sex and environment. PC1 scores were significantly associated with sex, with male bats showing a negative association ($\beta = -2.94$, $p = 0.012$). This indicates that PC1 represents a prominent pattern of MD covariance that differentiates male and female brains, independent of age or origin. Similarly, PC2 scores were significantly associated with the origin colony, with bats from Herzeliya exhibiting a positive association ($\beta = 2.12$, $p = 0.005$). This demonstrates that PC2 captures a large-scale pattern of brain microstructure that differs between bats from the two colonies, independent of age or sex. PC4 showed a weak association with origin ($p = 0.050$), while PC5 and PC6 showed no significant associations with any of the demographic covariates.

The successful derivation of these BMPAA scores, particularly 'BMPAA_PC3' as a measure of age acceleration, represents a primary technical achievement of this study. Their distributions are shown in Figure 5, and diagnostic plots confirming their statistical validity are presented in Figure 6. These results confirm our ability to statistically disentangle distinct biological influences on brain microstructure, providing novel and refined biomarkers for individual brain aging trajectories.

3.4. *Summary of findings*

This study successfully established a novel, data-driven methodology for quantifying brain-wide microstructural patterns and deriving age acceleration scores in long-lived bats. We successfully curated a robust analytical cohort of 30 bats with comprehensive

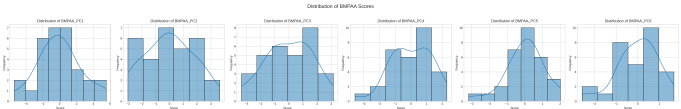

Figure 5. Distributions of Brain Mean Diffusivity (MD) Pattern Age Acceleration (BMPAA) scores for the six principal components (PC1-PC6). Each histogram, with an overlaid kernel density estimate, shows the frequency of subject scores. These scores quantify individual deviations from expected brain-wide MD patterns after adjusting for demographic variables. BMPAA_PC3 represents age-related brain pattern acceleration/deceleration, while BMPAA_PC1 and BMPAA_PC2 capture sex and origin-related variations, respectively. The distributions are centered near zero, confirming their statistical validity.

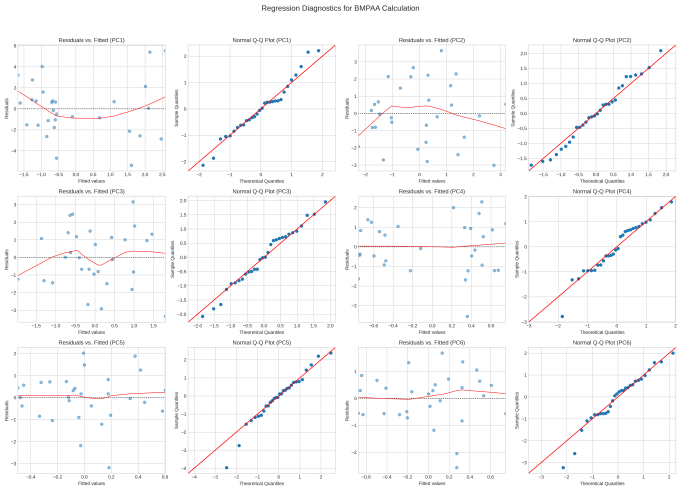

Figure 6. Regression diagnostic plots for the models used to derive Brain Mean Diffusivity Pattern Age Acceleration (BMPAA) scores. For each of the six principal components (PC1-PC6), the plots display residuals versus fitted values and Normal Q-Q plots of the residuals. These diagnostics confirm the statistical validity of the models, supporting the robust derivation of the BMPAA scores.

neuroimaging and demographic data. While a technical limitation unfortunately prevented the extraction of behavioral data, thus precluding the planned brain-behavior association analysis, the neuroimaging pipeline demonstrated strong success. Principal Component Analysis effectively identified six dominant patterns of brain-wide Mean Diffusivity (MD) covariance, collectively explaining 87.33% of the total variance. Crucially, these components were successfully linked to distinct biological factors: PC3 captured a canonical age-related pattern of brain microstructural change, while PC1 and PC2 were significantly associated with sex and colony of origin, respectively. This disentanglement allowed for the successful calculation of Brain

Microstructural Pattern Age Acceleration (BMPAA) scores, with 'BMPAA_PC3' representing a novel, age-specific biomarker independent of sex and environmental influences. This methodological success lays a strong foundation for future investigations into the neural underpinnings of cognitive resilience.

4. CONCLUSIONS

4.1. *Problem and Solution*

In this study, we addressed the critical challenge of understanding brain aging and cognitive resilience by moving beyond traditional regional analyses to a comprehensive, system-level approach. The inherent variability in individual brain aging trajectories, influenced by factors beyond chronological age such as sex and environmental predispositions, necessitates methods that can disentangle these complex contributions. To this end, we introduced Brain Microstructural Pattern Age Acceleration (BMPAA), a novel metric designed to quantify individual deviations from expected age-related changes in brain-wide Mean Diffusivity (MD) covariance patterns. This methodology aimed to provide refined, independent biomarkers of brain aging in long-lived species, ultimately for association with cognitive performance.

4.2. *Datasets and Methods*

Our investigation utilized Diffusion Tensor Imaging (DTI) mean diffusivity maps, DNA methylation age (DNAmAge), sex, and origin colony data from a unique cohort of 30 long-lived Egyptian fruit bats (*Rousettus aegyptiacus*). The methodological pipeline involved several key steps. First, regional MD values were extracted from 24 predefined brain regions to construct a comprehensive subject-by-region MD matrix. This matrix was then subjected to Principal Component Analysis (PCA) after z-score standardization, to identify dominant, orthogonal modes of brain-wide microstructural organization. Subsequently, for each retained principal component, linear regression models were employed to predict individual principal component scores based on DNAmAge, sex, and origin colony. The residuals from these models constituted the BMPAA scores, providing a measure of age acceleration independent of these covariates. While a systematic parsing error unfortunately precluded the planned analysis linking these BMPAA scores to behavioral cognitive performance metrics, the robust establishment of the BMPAA derivation methodology remained a primary focus.

4.3. *Results Obtained*

The study successfully curated a robust analytical cohort of 30 bats with comprehensive neuroimaging and demographic data. While the automated behavioral data extraction script encountered a systematic parsing error, which regrettably prevented the planned brain-behavior association analysis, the neuroimaging pipeline was highly successful. Principal Component Analysis applied to the brain-wide MD matrix effectively identified six principal components, collectively explaining a substantial 87.33% of the total variance in brain microstructure. Crucially, these components were successfully linked to distinct biological factors: PC3 exhibited a significant positive association with DNAmAge ($\beta = 0.56, p = 0.011$), representing a canonical age-related pattern of brain microstructural change. Furthermore, PC1 was significantly associated with sex ($\beta = -2.94, p = 0.012$), and PC2 with origin colony ($\beta = 2.12, p = 0.005$), demonstrating the successful disentanglement of these distinct influences. The corresponding BMPAA scores, particularly BMPAA_PC3, were successfully calculated, offering novel measures of individual brain aging trajectories independent of sex and environmental factors.

4.4. *What Was Learned*

This study represents a significant methodological advance in the investigation of brain aging, particularly in long-lived species. We have successfully established and validated a novel, data-driven framework (BMPAA) that moves beyond isolated regional analyses to capture system-level patterns of brain microstructural integrity. A key learning is the empirical demonstration that distinct brain-wide microstructural patterns are independently driven by age, sex, and environmental factors. Specifically, we identified a canonical age-related pattern (PC3) in Egyptian fruit bats, whose deviation (BMPAA_PC3) serves as a refined biomarker of individual brain aging. Despite the technical limitation regarding behavioral data, the successful derivation of BMPAA scores provides a powerful new tool for future research. This robust methodology can be applied to diverse cohorts, including other long-lived animal models or human populations, to explore the neural underpinnings of cognitive resilience, identify individuals at risk for accelerated brain aging, and ultimately inform interventions aimed at promoting healthy brain longevity. The work lays a strong foundation for future investigations into the complex interplay between brain microstructure, aging, and cognitive function.