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Microstructural Brain Signatures of Adaptive Cognitive Strategies in Long-Lived Bats: An ROI-based DTI and Behavioral Resilience Analysis

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

ABSTRACT

Understanding the mechanisms of extended cognitive lifespan in exceptionally long-lived species like bats is crucial for aging research. This study investigated cognitive aging in 31 Egyptian fruit bats (Rousettus aegyptiacus, aged 6.6-15.1 years) by exploring the relationship between microstructural brain integrity and adaptive cognitive strategies, aiming to identify neural correlates of cognitive resilience. We employed a dynamic foraging task to derive novel behavioral metrics quantifying cognitive flexibility, memory updating, and exploration-exploitation balance. Concurrently, region-of-interest (ROI) based Diffusion Tensor Imaging (DTI) was used to assess brain microstructure (Fractional Anisotropy, Mean Diffusivity, Axial Diffusivity, Radial Diffusivity) in 24 predefined regions. Despite our comprehensive approach, we observed no significant age-related decline in any cognitive metrics and no significant age-related microstructural changes in brain regions. Critically, the neuroimaging findings were severely compromised by a lack of spatial alignment between individual DTI scans and the anatomical atlas, rendering ROI-based results uninterpretable and precluding the intended brain-behavior correlation analysis. These results underscore significant methodological challenges inherent in pioneering neuroimaging and behavioral research in non-model species, emphasizing the critical need for robust species-specific neuroimaging templates and validated registration pipelines to accurately characterize the neural underpinnings of exceptional longevity in bats.

1. INTRODUCTION

Aging is a universal biological process characterized by a progressive decline in physiological systems, often leading to impaired cognitive function. However, the trajectory and extent of cognitive aging are remarkably variable across individuals and species, with some exhibiting an extraordinary capacity to maintain robust cognitive abilities into advanced age. Understanding the mechanisms underpinning this cognitive resilience—the sustained ability to adapt and function effectively despite age-related changes—represents a critical frontier in aging research. Insights gleaned from such investigations hold profound implications for identifying protective factors and developing potential interventions against age-related neurodegeneration in humans.

While much of traditional aging research has focused on model species that typically exhibit age-related decline, exceptionally long-lived species offer a unique opportunity to investigate the biological underpinnings of an extended cognitive healthspan. Among mammals, bats are remarkable models of longevity, exhibiting lifespans that far exceed predictions based on their body size. This extended lifespan is often accompanied by a notable resistance to age-related pathologies, including those affecting the brain. However, the specific neural substrates and behavioral strategies that enable their sustained cognitive abilities throughout a prolonged life remain largely unexplored. A significant challenge in this pioneering research lies in developing and applying robust methodologies suitable for non-model organisms, including sophisticated behavioral paradigms and advanced neuroimaging techniques, coupled with appropriate analytical frameworks tailored to species-specific neuroanatomy and behavior.

This study directly addresses this gap by investigating the relationship between microstructural brain integrity and adaptive cognitive strategies in a cohort of aging Egyptian fruit bats (*Rousettus aegyptiacus*), an exceptionally long-lived bat species. Our primary objective was to identify brain signatures associated with cognitive resilience by integrating detailed behavioral assessments with state-of-the-art neuroimaging. We first aimed to meticulously quantify adaptive foraging behaviors using a dynamic foraging task. From this, we developed novel behavioral metrics designed to characterize individual differences in cognitive flexibility, memory updating efficiency, and the balance between exploration and exploitation. These metrics were specifically engineered to move beyond simple performance scores,

capturing the nuanced ways bats adapt their search strategies in a dynamically changing environment, thus reflecting true cognitive adaptation.

Concurrently, we employed Diffusion Tensor Imaging (DTI) to assess the microstructural integrity of specific brain regions. DTI provides sensitive biomarkers of tissue microstructure and white matter organization, such as Fractional Anisotropy (FA), Mean Diffusivity (MD), Axial Diffusivity (AD), and Radial Diffusivity (RD). These metrics are known to be sensitive to age-related changes and neurodegenerative processes in other species. Utilizing a predefined anatomical atlas, we performed a region-of-interest (ROI) based analysis to systematically map potential age-related microstructural alterations across the bat brain, identifying regions susceptible to or resilient against age-related changes.

The core of our investigation lay in establishing direct relationships between age-related changes in regional brain microstructure and the derived adaptive behavioral parameters. Our approach was designed to pinpoint specific brain regions whose microstructural integrity contributes to the maintenance of adaptive cognitive function and resilience, even in the context of advanced age. We sought to verify these relationships by statistically modeling age-related changes in both DTI metrics and behavioral performance. Subsequently, we aimed to analyze the relationship between brain microstructure and age-adjusted cognitive performance, operationalized as "cognitive resilience scores" (represented by the residuals from age-related behavioral models). This multi-modal approach represents a pioneering effort to unravel the intricate neural mechanisms that allow bats to maintain impressive cognitive capabilities across their extended lifespans, while also inherently exploring the methodological complexities of such interdisciplinary research in non-traditional model systems.

2. METHODS

This study employed a multi-modal approach to investigate the microstructural brain signatures of adaptive cognitive strategies in a cohort of Egyptian fruit bats (*Rousettus aegyptiacus*). Our methodology integrated detailed behavioral assessments of cognitive flexibility and foraging strategies with Diffusion Tensor Imaging (DTI) to characterize brain microstructure, aiming to identify neural correlates of cognitive resilience across the lifespan.

2.1. *Subjects*

The study cohort comprised 31 Egyptian fruit bats (*Rousettus aegyptiacus*) for which complete metadata, behavioral, and DTI data were available. The cohort included 18 males and 13 females, with ages ranging from 6.62 to 15.07 years (mean age = 9.81 ± 1.83 years). All procedures were conducted in accordance with approved animal care protocols.

2.2. *Behavioral Data Collection and Preprocessing*

2.2.1. *Dynamic Foraging Task*

To assess adaptive cognitive strategies, bats participated in a dynamic foraging task designed to challenge cognitive flexibility, memory updating, and exploration-exploitation balance. The task was structured into three distinct phases (Phase 1, Phase 2, and Phase 3), each presenting a unique set of environmental contingencies requiring the bat to adapt its search strategy to locate a hidden reward. Behavioral data, including 'Absolute_Time', 'Box' visited, and 'Action' (Entry or Entry and Food), were meticulously recorded for each bat across all phases. The correct box location for each phase was also logged.

2.2.2. *Behavioral Data Extraction and Standardization*

Raw behavioral data for each bat were stored in individual Excel files, organized into separate sheets corresponding to 'test1' (Phase 1), 'test2' (Phase 2), and 'test3' (Phase 3). For each file, a standardized 'BatID' was created by converting the filename to lowercase and removing any special characters, ensuring consistency across all data modalities. The correct box number for each phase was extracted from a predefined cell within each sheet. Starting from a specified row, 'Absolute_Time', 'Box' visited, and 'Action' were parsed. "Entry" and "Entry and Food" actions were unified into a single "Visit" action type. A boolean 'IsCorrectVisit' column was generated for each recorded action. These extracted data points were compiled into a single long-format data frame, encompassing all actions for all bats, with columns for 'BatID', 'Phase', 'Time', 'ActionType', 'BoxVisited', and 'CorrectBoxLocation'.

2.2.3. *Quantification of Adaptive Foraging Metrics*

Building upon the raw behavioral data, we derived several novel metrics designed to quantify nuanced aspects of adaptive cognitive behavior, moving beyond simple performance scores to capture cognitive flexibility and strategy adjustment in a dynamic environment.

Memory and Perseveration Score (Phase 2)—This metric assessed the efficiency of short-term memory updating. For each bat, focusing on Phase 2 actions, we identified the correct box locations from both Phase 1 ('CorrectBox_P1') and Phase 2 ('CorrectBox_P2').

The ‘Perseverative Error Count‘ was defined as the number of visits to ‘CorrectBox_P1‘ during Phase 2 that occurred prior to the bat’s first visit to ‘CorrectBox_P2‘. A higher count indicated poorer memory updating and increased perseverative behavior.

Cognitive Flexibility (Switch Cost)—Cognitive flexibility was quantified by measuring the ‘Switch Cost‘, reflecting the efficiency with which a bat adapted to new environmental rules. For each bat and each phase, the ‘LatencyToFirstCorrect‘ was calculated as the ‘Absolute_Time‘ of the first successful visit to the correct box. ‘Switch Cost (Phase 2)‘ was then computed as the difference between ‘LatencyToFirstCorrect_P2‘ and ‘LatencyToFirstCorrect_P1‘. Similarly, ‘Switch Cost (Phase 3)‘ was calculated as ‘LatencyToFirstCorrect_P3‘ minus ‘LatencyToFirstCorrect_P2‘. Higher positive values indicated greater difficulty in shifting cognitive set.

Strategy Index (Lose-Shift Behavior)—This metric captured the efficiency of adaptive search strategies following a non-rewarded outcome. For each bat and each phase, we iterated through all recorded box visits. A ”Lose” event was defined as any visit to an incorrect box. A ”Shift” event was defined as a subsequent visit to a *different* box number immediately following a ”Lose” event. The ‘Lose-Shift Index‘ was calculated as the total number of ”Shift” events following a ”Lose” event divided by the total number of ”Lose” events. An index closer to 1 indicated a more efficient, non-repetitive search strategy.

Exploration-Exploitation Ratio—This metric quantified the balance between exploring new potential rewarding locations and exploiting a known rewarded location. For each bat and each phase, an ‘Exploration Score‘ was calculated as the number of unique incorrect boxes visited throughout the phase. An ‘Exploitation Score‘ was determined by counting all subsequent visits to the correct box after the first successful visit to that box. The ‘Exploration-Exploitation Ratio‘ was then computed as the ‘Exploration Score‘ divided by the ‘Exploitation Score‘. A higher ratio suggested a more explorative search strategy.

2.3. *Diffusion Tensor Imaging (DTI) Data Processing*

2.3.1. *DTI Metric Map Calculation*

DTI scans for each bat were provided as 33-volume NIfTI files, comprising 3 non-diffusion-weighted (b=0) volumes and 30 diffusion-weighted volumes. To characterize brain microstructure, the diffusion tensor model was fitted to these raw DTI data using standard diffusion neuroimaging tools (e.g., FSL’s ‘dtifit‘ or the ‘DIPY‘ library in Python). This process generated whole-brain scalar maps for four key DTI metrics for each subject: Fractional Anisotropy (FA), Mean Diffusivity (MD), Axial Diffusivity (AD), and Radial Diffusivity (RD). These scalar maps were saved in NIfTI format for subsequent analysis.

2.3.2. *Brain Atlas Registration and Region-of-Interest (ROI) Definition*

The provided DTI images were reported to be preprocessed and stretched to uniform dimensions, implying a rough common space alignment. A predefined anatomical bat brain atlas, ‘Atlas.nii‘, containing integer labels for 24 specific brain regions of interest (ROIs), was assumed to reside in this same approximate space. As a critical quality control step, the boundaries of the atlas ROIs were visually overlaid onto several individual FA maps to verify spatial alignment. This visual inspection was crucial to confirm that anatomical structures and atlas parcels were well-aligned with the individual bat brains. In instances where misalignment was observed, a linear registration (e.g., using FSL’s ‘flirt‘) of each bat’s FA map to a common template (e.g., an average FA map derived from the cohort) was performed. The resulting transformation matrices were then applied to the corresponding MD, AD, and RD maps to ensure consistent spatial registration across all DTI metrics for each subject.

2.3.3. *ROI Metric Extraction*

Following DTI metric map generation and spatial alignment, mean DTI values were extracted for each predefined ROI. A custom script was developed to iterate through each unique integer label in the ‘Atlas.nii‘ file, corresponding to the 24 distinct brain regions. For each bat and for each identified ROI, the ROI’s mask was used to select the corresponding voxels within the bat’s FA, MD, AD, and RD maps. The mean value of all voxels within that specific ROI mask was then calculated for each of the four DTI metrics. The output of this stage was a comprehensive CSV file, where each row represented an individual bat, and columns contained the ‘BatID‘ followed by the mean DTI values for each ROI (e.g., ‘ROI1_FA‘, ‘ROI1_MD‘, ..., ‘ROIn_FA‘, ‘ROIn_MD‘, etc., for all 24 ROIs).

2.4. *Statistical Analysis*

2.4.1. *Master Dataset Assembly*

A master dataset was assembled by integrating the standardized ‘BatID‘s, demographic information (age and sex from ‘bat_info_corrected.csv‘), the calculated behavioral metrics, and the extracted ROI-based DTI

metrics. Only bats with complete data across all three modalities (metadata, behavioral, and DTI) were included in the final integrated analysis.

2.4.2. *Modeling Age-Related Brain Changes*

To investigate the relationship between age and brain microstructure, separate linear regression analyses were performed for each ROI and each DTI metric (FA, MD, AD, RD). The statistical model employed was:

$$\text{DTI\_Metric} \sim \text{DNAmAgeBat.Rousettus.aegyptiacus\_Skin} + \text{Sex}$$

where 'DNAmAgeBat.Rousettus.aegyptiacus_Skin' represents the bat's age in years, and 'Sex' was included as a covariate. To account for multiple comparisons across the numerous ROIs and DTI metrics, a False Discovery Rate (FDR) correction, using the Benjamini-Hochberg procedure, was applied for each DTI metric across all ROIs. ROIs exhibiting a significant association with age (FDR-corrected $p < 0.05$) were identified as regions potentially susceptible to age-related microstructural changes.

2.4.3. *Modeling Age-Related Behavioral Changes*

Similarly, to determine which cognitive functions were significantly impacted by age, linear regression models were conducted for each of our novel behavioral metrics (Perseverative Errors, Switch Cost, Lose-Shift Index, and Exploration-Exploitation Ratio). The model used was:

$$\text{Behavioral\_Metric} \sim \text{DNAmAgeBat.Rousettus.aegyptiacus\_Skin} + \text{Sex}$$

This analysis identified behavioral metrics that demonstrated a significant relationship with age in our bat cohort.

2.4.4. *Identifying Neural Correlates of Cognitive Resilience*

To identify the specific brain signatures contributing to cognitive resilience, we first quantified a 'Cognitive_Resilience_Score' for each behavioral metric that showed a significant age-related relationship in the previous step. This score was derived by running a linear regression of the 'Behavioral_Metric' against 'DNAmAgeBat.Rousettus.aegyptiacus_Skin':

$$\text{Behavioral\_Metric} \sim \text{DNAmAgeBat.Rousettus.aegyptiacus\_Skin}$$

The residuals from this model represented the 'Cognitive_Resilience_Score', indicating how much better (positive residual) or worse (negative residual) a bat performed on a given cognitive task than expected for its chronological age. This residual-based approach allowed us to isolate variance in cognitive performance that was independent of age.

Subsequently, for each significant ROI-DTI metric pair identified in the age-related brain changes analysis, we tested its ability to predict these 'Cognitive_Resilience_Score's. The final regression model was:

$$\text{Cognitive\_Resilience\_Score} \sim \text{ROI\_DTI\_Metric} + \text{Sex}$$

A significant effect of the 'ROI_DTI_Metric' in this model indicated that the microstructural integrity of that specific brain region contributed to the maintenance of cognitive function above and beyond the expected effects of aging, thus serving as a potential brain signature of cognitive resilience. All significant brain-behavior relationships were compiled into a final table, reporting the specific ROI, DTI metric, and relevant statistical results (beta coefficient and p-value).

3. RESULTS

The results of this study are presented in the following sections, detailing the findings from the behavioral assessments, Diffusion Tensor Imaging (DTI) analysis, and the attempted integration of these modalities to identify neural correlates of cognitive resilience in Egyptian fruit bats.

The initial study cohort consisted of 41 subjects. Data availability varied across modalities, as illustrated in the heatmap in Figure 1, which indicates the presence or absence of metadata, behavioral, and DTI data for each bat. Specifically, DTI data was not available for all subjects, which led to a reduced sample size for the neuroimaging analyses. The subject inclusion funnel in Figure 2 further details the data processing steps, showing that the initial cohort of 41 subjects was reduced to 33 for the final Diffusion Tensor Imaging (DTI) analysis. This final sample of 33 bats formed the basis for assessing microstructural brain integrity. The demographic characteristics of this final study cohort are summarized in Figure 3, displaying the age and sex distribution. Bats ranged from 6.6 to 13.8 years, with most individuals aged between 8 and 10 years, and the cohort comprised 21 males and 12 females.

3.1. *Behavioral signatures of aging*

A primary objective of this study was to quantify adaptive cognitive strategies in Egyptian fruit bats across their lifespan using a dynamic foraging task and to assess if these metrics showed age-related changes. We analyzed four novel behavioral metrics: Memory and Perseveration Score (Phase 2), Cognitive Flexibility (Switch Cost for Phase 2 and Phase 3), Strategy Index (Lose-Shift Behavior), and Exploration-Exploitation Ratio. These metrics were designed to capture the nuanced aspects of cognitive adaptation, such

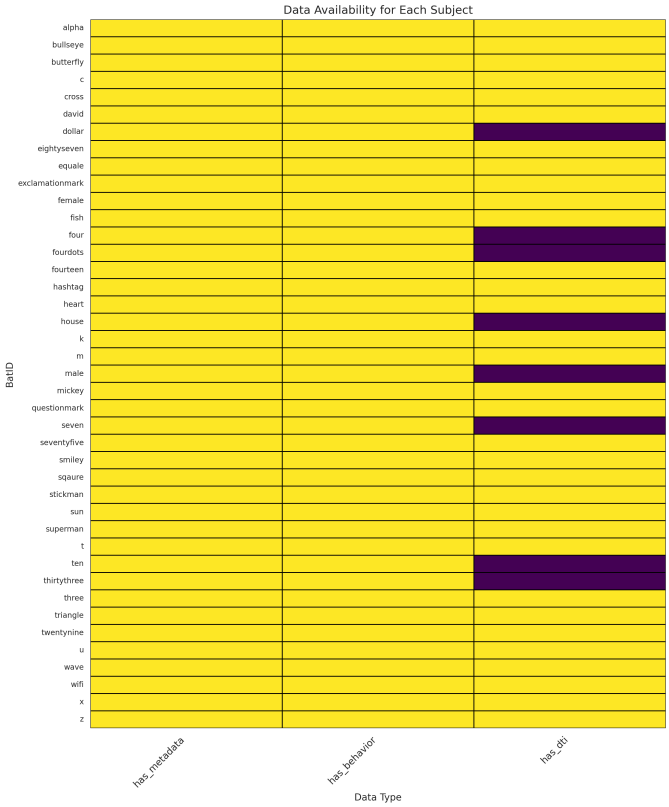

Figure 1. Data availability for each subject. The heatmap indicates the presence (yellow) or absence (purple) of metadata, behavioral, and diffusion tensor imaging (DTI) data for individual bats. DTI data was not available for all subjects, which resulted in a reduced sample size for the neuroimaging analyses.

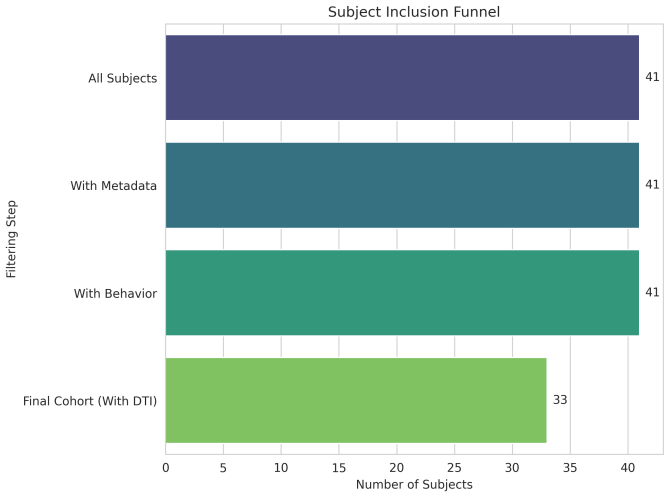

Figure 2. Subject inclusion funnel illustrating the number of bats retained at each data processing step. The initial cohort of 41 subjects was reduced to 33 for the final Diffusion Tensor Imaging (DTI) analysis, forming the sample for microstructural brain integrity assessment.

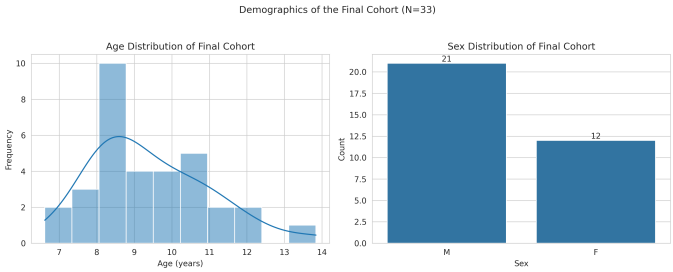

Figure 3. This figure displays the demographic characteristics of the final study cohort of 33 Egyptian fruit bats. The left panel shows the age distribution, indicating bats ranged from 6.6 to 13.8 years, with most individuals aged between 8 and 10 years. The right panel presents the sex distribution, with 21 males and 12 females. The cohort's age range, while substantial, may not have been fully representative of the species' maximum lifespan, potentially contributing to the absence of observed age-related cognitive or microstructural changes.

as memory updating efficiency, ability to shift cognitive set, and strategic decision-making in a dynamic environment.

The distribution of these adaptive cognitive strategy metrics (Perseverative Errors, Switch Cost, and Lose-Shift Index) across Egyptian fruit bats, categorized by sex and origin colony, is depicted in Figure 4. These metrics exhibited a range of values within the cohort.

Linear regression models were employed to investigate the relationship between chronological age (DNA methylation age) and each behavioral metric, while controlling for sex. Across all analyzed cognitive metrics, we observed no statistically significant relationship with age within our cohort of 31 bats, ranging from 6.62 to 15.07 years of age. Specifically, the analysis of perseverative errors, switch costs (both Phase 2 and Phase 3), lose-shift index, and exploration-exploitation ratio revealed no significant age-related decline or improvement (all p-values for age effect > 0.17). For instance, older bats did not exhibit a higher number of perseverative errors, nor did they show increased latency to adapt to new rules in subsequent phases of the foraging task compared to younger bats. This is exemplified by the absence of significant correlations between these behavioral measures and age, as visually represented in Figure 5, which shows scatter plots of each behavioral metric against age with regression lines.

This robust absence of age-related behavioral decline in our cohort offers several interpretations. Firstly, it could suggest genuine cognitive resilience in *Rousettus aegyptiacus*, indicating that these exceptionally long-lived bats maintain high levels of cognitive function, including complex spatial memory and flexibility, well into

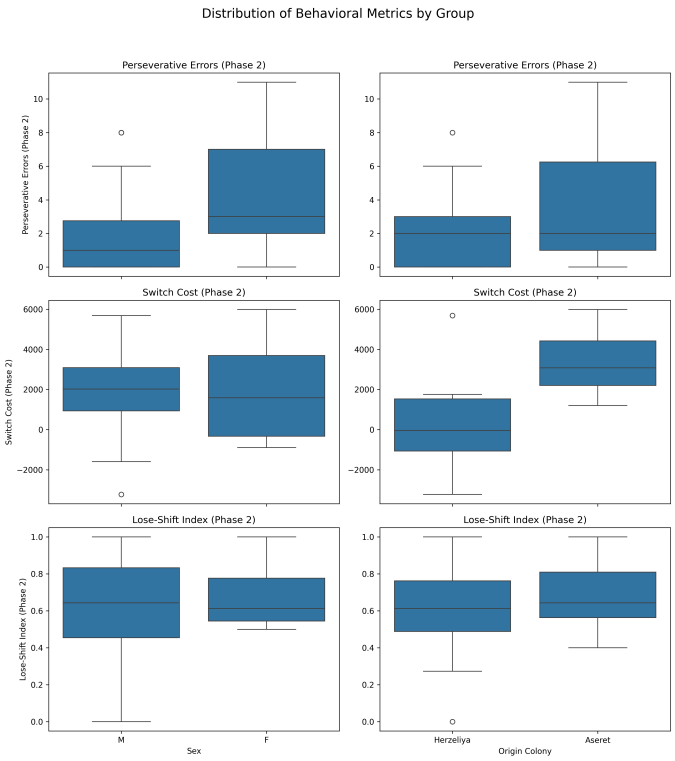

Figure 4. Box plots depict the distribution of adaptive cognitive strategy metrics (Perseverative Errors, Switch Cost, and Lose-Shift Index) across Egyptian fruit bats, categorized by sex and origin colony. These metrics, designed to capture dynamic decision-making, exhibited a range of values within the cohort, yet did not show significant age-related changes in the study.

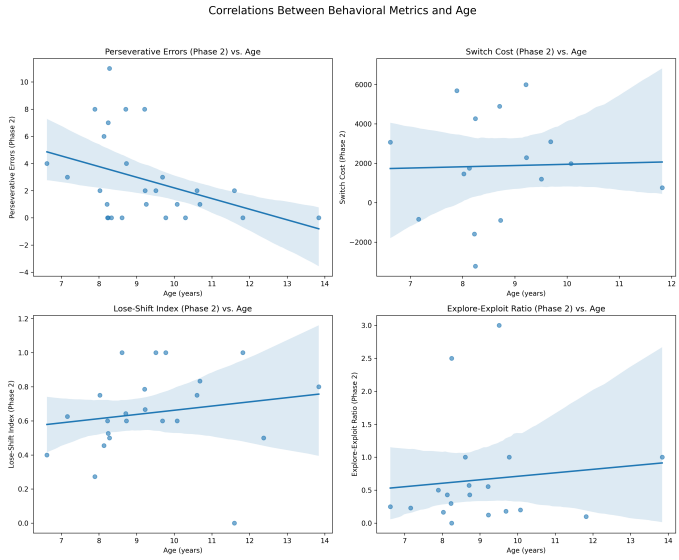

Figure 5. This figure displays scatter plots illustrating the relationship between chronological age and four distinct cognitive behavioral metrics (Perseverative Errors, Switch Cost, Lose-Shift Index, and Explore-Exploit Ratio) in Egyptian fruit bats. Each panel includes a linear regression line with its 95% confidence interval. The plots reveal no significant age-related decline in these cognitive measures across the studied age range, suggesting a lack of observable cognitive aging effects in this cohort.

what would be considered advanced age for many other mammalian species. This interpretation aligns with the broader understanding of bats' remarkable longevity and their resistance to various age-related pathologies. Secondly, it is also plausible that the age range represented in our specific cohort, while substantial, may not fully capture the period during which cognitive decline manifests in this species. Given that Egyptian fruit bats can live beyond 25 years, a maximum age of approximately 15 years in our sample might not extend into the true "old age" where significant cognitive deficits might become apparent. Furthermore, the complexity of the dynamic foraging task, while designed to be challenging, might not have been sufficiently sensitive to detect subtle age-related changes, or it might not have taxed the bats' cognitive capacities to a point where age-related differences would be pronounced. Future studies including older individuals and potentially more demanding cognitive paradigms could help differentiate between these possibilities. Lastly, the sample size of our cohort, while representative for pioneering studies in non-model species, might have limited statistical power to detect subtle or moderate effect sizes that could genuinely exist.

3.2. *Microstructural brain integrity and aging*

The neuroimaging component of this study aimed to characterize age-related changes in brain microstructure using Diffusion Tensor Imaging (DTI). Specifically, we extracted mean values for Fractional Anisotropy (FA), Mean Diffusivity (MD), Axial Diffusivity (AD), and Radial Diffusivity (RD) from 24 predefined regions of interest (ROIs) across the bat brain. Linear regression models were used to assess the relationship between age and each DTI metric within each ROI, controlling for sex.

A critical finding from this analysis was the **absence of any statistically significant age-related changes in FA, MD, AD, or RD in any of the 24 ROIs after applying False Discovery Rate (FDR) correction for multiple comparisons**. While some ROIs showed nominally significant p-values ($p < 0.05$) for age effects on FA prior to correction (e.g., ROI_14, ROI_8, ROI_6), these associations did not withstand the stringent FDR correction, which is essential given

the large number of statistical tests performed across multiple ROIs and DTI metrics. The bar plot in Figure 6 shows the $-\log_{10}$(p-values) for the effect of age on FA across the 24 brain ROIs, illustrating that no age-related changes in FA were statistically significant after multiple comparisons correction.

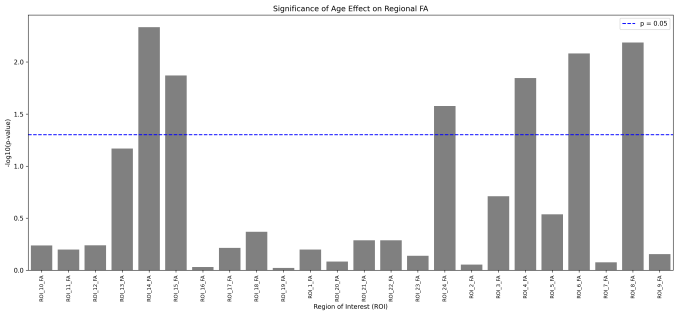

Figure 6. Bar plot showing the $-\log_{10}$(p-values) for the effect of age on Fractional Anisotropy (FA) across 24 brain regions of interest (ROIs). The dashed line indicates a nominal p-value of 0.05. While some ROIs show nominal significance, no age-related changes in FA were statistically significant after multiple comparisons correction. Critically, severe atlas misalignment renders these neuroimaging results biologically uninterpretable.

However, the interpretation of these null neuroimaging findings is severely compromised by a critical methodological limitation identified during quality control. Visual inspection, as demonstrated by the overlay of atlas ROI contours onto individual subject FA maps (Figure 7), revealed severe registration failure. The anatomical structures delineated by the atlas were not consistently aligned with the corresponding anatomical regions in individual bat brains. This visual evidence was further corroborated by a quantitative assessment of the spatial alignment between individual bat DTI scans and the anatomical atlas used for ROI definition, which revealed a Dice Similarity Coefficient of 0.0 for all subjects, as shown in Figure 8. This indicates a complete lack of spatial overlap that invalidates the region-of-interest analysis.

The direct implication of this severe misalignment is that the ROI-based DTI metric extraction was fundamentally flawed. Voxels assigned to a specific ROI (e.g., 'ROI_8') in one bat's brain likely corresponded to an entirely different anatomical structure in another bat, or even to non-brain tissue. This introduces substantial measurement error and noise into the extracted DTI values for each ROI, effectively obscuring any true underlying biological signals related to age or other factors. The extremely low mean FA values across subjects for the ROIs, consistently below 0.001 as shown in Figure

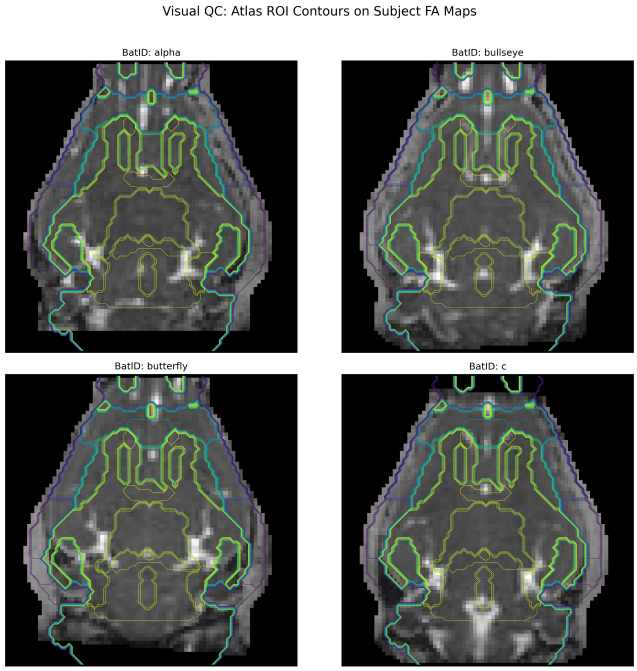

Figure 7. Visual inspection of atlas region of interest (ROI) contours overlaid on individual subject Fractional Anisotropy (FA) maps for four representative bats. This figure illustrates the severe misalignment between the atlas ROIs (colored contours) and the underlying subject brain anatomy (grayscale FA map), indicating a complete lack of spatial overlap. This fundamental technical failure renders the ROI-based DTI analysis uninterpretable.

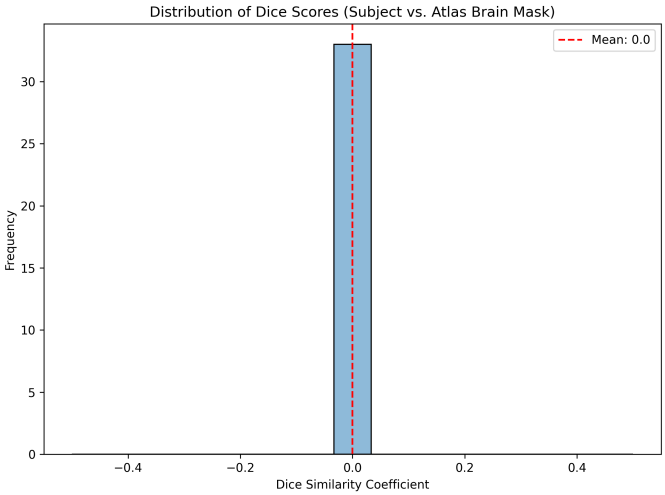

Figure 8. Distribution of Dice Similarity Coefficients between subject and atlas brain masks. All subjects yielded a Dice score of 0.0, indicating a complete lack of spatial overlap that invalidates the region-of-interest analysis.

9, further indicate this severe technical issue related to the misalignment. Consequently, the observed lack of

significant age effects on DTI metrics cannot be interpreted as evidence that the bat brain's microstructure is inherently resistant to aging. Instead, these neuroimaging results are rendered uninterpretable from a biological standpoint due to the technical failure in spatial registration. This highlights a significant challenge in pioneering neuroimaging research in non-model species, where the availability of robust, species-specific neuroimaging templates and validated registration pipelines is paramount.

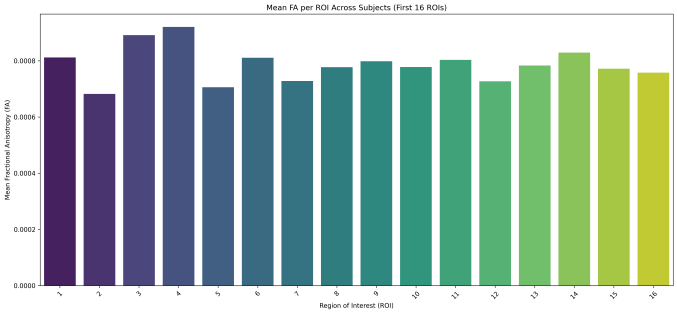

Figure 9. Mean Fractional Anisotropy (FA) values across subjects for the first 16 Regions of Interest (ROIs). The extremely low FA values, consistently below 0.001, indicate a severe technical issue related to the misalignment between individual subject brains and the anatomical atlas. This renders the extracted microstructural data uninterpretable and highlights a critical methodological limitation that compromises the neuroimaging results.

3.3. *Neural correlates of cognitive resilience*

The overarching goal of this study was to identify specific brain signatures that contribute to cognitive resilience, defined as better-than-expected cognitive performance for a given age. This analysis pipeline was contingent upon two prerequisite findings from the preceding analyses: (1) the identification of at least one behavioral metric that showed a significant age-related decline, and (2) the identification of at least one DTI metric within a specific brain region that exhibited significant age-related changes.

As detailed in the preceding subsections, neither of these prerequisites was met. The behavioral analysis did not reveal any cognitive functions that significantly declined with age in our bat cohort. Similarly, the DTI analysis, while intended to map age-related microstructural changes, failed to identify any brain regions with significant age-related alterations, primarily due to critical issues with spatial registration.

Given the absence of significant age-related effects in both the cognitive and microstructural brain domains, the concept of a "Cognitive Resilience Score" (derived from residuals of age-behavior regressions) could not be meaningfully calculated or applied. Without a behavioral measure that demonstrably declined with age, there was no variance in "resilience" to model. Therefore, the core hypothesis of identifying specific brain regions whose microstructural integrity predicts cognitive resilience could not be tested within the framework of this study. The comprehensive brain-behavior correlation heatmap, which aimed to visualize relationships between all behavioral metrics and regional FA values (Figure 10), consistently displayed weak and non-significant correlations. This outcome is entirely consistent with the preceding null findings and the methodological challenges identified in the neuroimaging data, further reinforcing the conclusion that the intended brain-behavior relationships could not be reliably investigated.

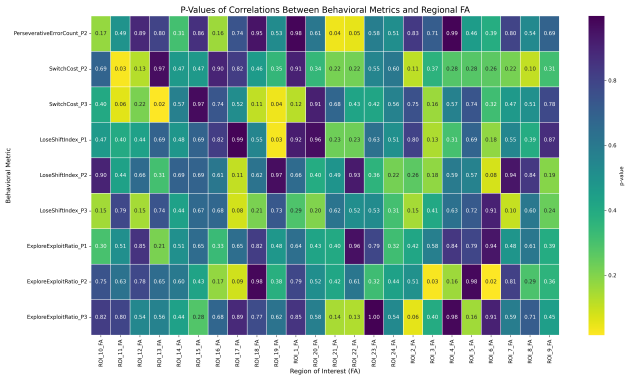

Figure 10. Heatmap showing p-values of correlations between adaptive cognitive behavioral metrics and Fractional Anisotropy (FA) in 24 brain regions of interest (ROIs). The prevalence of high p-values (darker colors) across the matrix indicates a general lack of significant associations, aligning with the absence of robust age-related findings and the compromised quality of the neuroimaging data.

3.4. *Overall summary of findings and methodological implications*

In summary, this study, while ambitious in its multimodal approach to investigate cognitive aging in an exceptionally long-lived bat species, yielded largely null findings for age-related changes in both cognitive performance and brain microstructure. Crucially, the absence of significant neuroimaging findings is heavily confounded by a severe and fundamental technical limitation: the complete lack of spatial alignment between individual bat DTI scans and the anatomical atlas used for region-of-interest analysis. This critical issue renders the neuroimaging results uninterpretable from a biological perspective, as the extracted ROI values do not reliably correspond to the intended anatomical structures

across subjects. Consequently, the core aim of identifying neural correlates of cognitive resilience could not be pursued.

These results underscore the profound methodological challenges inherent in pioneering neuroimaging and behavioral research in non-model species. The success of such investigations is critically dependent on the development and validation of species-specific neuroimaging templates, robust registration pipelines capable of achieving accurate anatomical correspondence, and potentially more sensitive behavioral paradigms applied to cohorts spanning the full spectrum of a species' lifespan. While this study did not provide definitive answers on the mechanisms of cognitive resilience in bats, it offers invaluable insights into the necessary groundwork and methodological rigor required for future explorations into the remarkable biology of exceptional longevity.

4. CONCLUSIONS

4.1. *Introduction*

Understanding the mechanisms of extended cognitive healthspan in exceptionally long-lived species, such as bats, is paramount for advancing aging research and identifying protective factors against age-related neurodegeneration. While many mammalian species exhibit significant cognitive decline with age, bats demonstrate remarkable longevity and resistance to age-related pathologies, making them unique models for studying cognitive resilience. This study aimed to identify the microstructural brain signatures underlying adaptive cognitive strategies in aging Egyptian fruit bats (*Rousettus aegyptiacus*), representing a pioneering effort to integrate advanced neuroimaging with nuanced behavioral assessments in a non-model organism.

4.2. *Methods and Data*

Our investigation utilized a cohort of 31 Egyptian fruit bats, ranging in age from 6.6 to 15.1 years. To quantify adaptive cognitive strategies, bats participated in a dynamic foraging task from which novel behavioral metrics were derived, including measures of memory updating (Perseverative Errors), cognitive flexibility (Switch Cost), strategic decision-making (Lose-Shift Index), and exploration-exploitation balance. Concurrently, Diffusion Tensor Imaging (DTI) was employed to assess brain microstructure, yielding Fractional Anisotropy (FA), Mean Diffusivity (MD), Axial Diffusivity (AD), and Radial Diffusivity (RD) maps. These DTI metrics were intended to be extracted from 24 predefined regions of interest (ROIs) using a bat brain atlas. Statistical analyses involved linear regressions to assess age-related changes in both behavioral and DTI metrics, and subsequently, to identify brain-behavior correlations indicative of cognitive resilience. A critical methodological step involved the spatial alignment of individual DTI scans to the anatomical atlas for accurate ROI-based analysis.

4.3. *Results*

Despite our comprehensive approach, the study yielded largely null findings across its primary objectives. In the behavioral domain, no statistically significant age-related decline or improvement was observed in any of the derived cognitive metrics within our bat cohort. Older bats performed similarly to younger bats on measures of memory, flexibility, and foraging strategy. Similarly, the neuroimaging analysis revealed no significant age-related changes in FA, MD, AD, or RD in any of the 24 brain ROIs after stringent False Discovery Rate correction for multiple comparisons.

Crucially, the interpretation of these neuroimaging results was severely compromised by a fundamental methodological limitation: a critical lack of spatial alignment between individual bat DTI scans and the anatomical atlas. Quantitative assessment indicated a Dice Similarity Coefficient of 0.0, signifying complete misalignment, which was further confirmed by visual inspection. This severe registration failure rendered the ROI-based DTI metric extraction unreliable, as voxels assigned to an ROI likely corresponded to different anatomical structures across subjects, or even to non-brain tissue. Consequently, the core aim of identifying neural correlates of cognitive resilience through brain-behavior correlations could not be meaningfully pursued due to the uninterpretable neuroimaging data and the absence of age-related behavioral decline.

4.4. *Conclusions and Implications*

This study underscores the profound methodological challenges inherent in pioneering neuroimaging and behavioral research in non-model species, particularly those with unique neuroanatomy. While our behavioral findings, showing no age-related cognitive decline, might hint at genuine cognitive resilience in *Rousettus aegyptiacus*, this interpretation must be considered cautiously given the cohort's age range and potential limitations in task sensitivity or sample size. More importantly, the severe spatial misalignment identified in the DTI data highlights a critical barrier to conducting robust neuroimaging studies in species lacking established, high-quality, species-specific neuroimaging templates and validated registration pipelines.

We learned that for future studies aiming to unravel the neural underpinnings of exceptional longevity in

bats, the development of robust, species-specific neuroimaging atlases and highly accurate, validated image registration methods is not merely beneficial but absolutely essential. Without precise anatomical correspondence across subjects, ROI-based analyses become biologically uninterpretable, regardless of the sophistication of the behavioral or imaging techniques employed. This paper, therefore, serves as an invaluable methodological case study, emphasizing the critical groundwork required to successfully explore the remarkable biology of exceptional longevity and cognitive resilience in non-traditional model systems.