Draft version August 4, 2025
Typeset using LaTeX **twocolumn** style in AASTeX631

Regional Brain Morphometry and Adaptive Foraging Reveal Age-Related Cognitive Flexibility and Resilience Trends in Egyptian Fruit Bats

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

ABSTRACT

Aging often leads to cognitive decline, yet some individuals maintain remarkable cognitive abilities despite advanced age—a phenomenon known as cognitive resilience. This study investigated the neural and behavioral correlates of cognitive resilience in 33 long-lived Egyptian fruit bats (DNAm age: 6.6-13.8 years), an excellent model for mammalian aging. We integrated refined behavioral phenotyping from a multi-phase spatial foraging task (quantifying spatial learning, perseveration, and adaptive shifting) with regional brain morphometry (volume and mean signal intensity) derived from b0 images of Diffusion Tensor Imaging (DTI) sequences across 24 atlas-defined regions. Statistical analyses employed multiple linear regressions to assess age effects and moderation models with False Discovery Rate (FDR) correction to identify brain-behavior interactions indicative of resilience. Results showed that older bats exhibited significantly fewer short-term perseverative errors, suggesting enhanced cognitive flexibility or strategy shifts with age. Concurrently, mean b0 signal intensity in ROI 14 significantly increased with DNAm age, potentially reflecting age-related microstructural changes. While no brain-behavior interactions achieved statistical significance after stringent FDR correction, an exploratory analysis revealed a compelling trend: higher b0 signal intensity in ROI 19 appeared to mitigate age-related declines in learning consolidation, a pattern consistent with cognitive resilience. These findings highlight the nuanced nature of cognitive aging in bats, revealing specific age-related behavioral adaptations and localized brain changes, and provide data-driven hypotheses for future research into neurobiological mechanisms supporting cognitive health in long-lived species.

Keywords: Astronomy data reduction, Computational astronomy, Normal distribution, Computational methods, Multivariate analysis

1. INTRODUCTION

Aging is a universal biological process characterized by progressive changes across multiple physiological systems, frequently culminating in a decline in cognitive functions such as memory, learning, and executive control. This age-related cognitive decline presents a significant challenge to individual well-being and public health globally. However, a remarkable phenomenon, termed cognitive resilience, highlights that not all individuals experience uniform decline; a subset maintains robust cognitive abilities well into advanced age, often despite the presence of age-related neuropathology. Understanding the neural and behavioral mechanisms underpinning this resilience is a critical, yet complex, endeavor in neuroscience and gerontology.

The inherent difficulty in unraveling cognitive resilience stems from several factors. Firstly, cognitive function is influenced by an intricate interplay of genetic predispositions, environmental factors, and lifestyle choices, making it challenging to isolate specific drivers of sustained health. Secondly, the precise quantification of nuanced cognitive abilities, particularly those related to adaptive flexibility and the capacity to overcome previously learned responses, requires sophisticated behavioral paradigms that reflect ecologically relevant challenges. Thirdly, and crucially, much of our understanding of mammalian cognitive aging has been derived from traditional rodent models or primates, which, while valuable, may not fully capture the ecological and neurobiological diversity relevant to understanding resilience across species. There is a pressing need for novel, long-lived mammalian models that exhibit complex, ecologically relevant cognition and for which comprehensive neuroimaging data can be acquired.

To address this gap, our study leverages the Egyptian fruit bat (*Rousettus aegyptiacus*) as an exceptional model for mammalian aging research. These bats pos-

sess a relatively long lifespan for their size (up to 25 years in captivity), exhibit sophisticated echolocation-guided spatial navigation, and engage in complex social and foraging behaviors. Their natural history necessitates continuous learning, memory updating, and adaptive decision-making in dynamic environments, providing a rich, ecologically valid context from which to derive refined measures of cognitive flexibility. By studying these long-lived bats, and utilizing their DNA methylation age (DNAm_age) as a precise biological age marker, we aim to uncover conserved neurobiological principles of cognitive resilience that may transcend specific species and provide insights relevant to human brain health.

This investigation integrates two complementary approaches: refined behavioral phenotyping of adaptive foraging and regional brain morphometry. For behavioral assessment, we utilized a multi-phase spatial foraging task designed to go beyond simple performance measures and capture nuanced aspects of cognitive flexibility. Specifically, we developed novel quantitative metrics to assess spatial learning efficiency (e.g., entries into incorrect boxes before the first correct entry in a new environment), short- and long-term perseveration (the tendency to revert to previously rewarded, but now incorrect, locations), and adaptive shifting speed to new reward contingencies. These metrics are crucial for discerning how individuals adapt their strategies in the face of changing environmental demands, a hallmark of cognitive flexibility that is often challenged in aging.

On the neurobiological front, given the specific characteristics of our diffusion tensor imaging (DTI) dataset (absence of b-vectors and b-values for full DTI analysis), we innovatively leveraged the anatomical information contained within the b=0 images. These non-diffusion-weighted images serve as high-quality T2-weighted anatomical proxies, allowing us to perform regional brain morphometric analysis. We quantified two key features: regional volumes and mean signal intensities across 24 atlas-defined brain regions. While regional volume reflects gross structural integrity and potential atrophy, the mean b0 signal intensity can provide insights into microstructural properties such as tissue water content, cellular density, or myelination, offering a unique window into age-related microstructural changes within specific brain areas.

Our core objective is to unravel how specific brain regions are preserved or altered with biological age (DNAm_age), and critically, how these structural properties relate to the maintenance of adaptive spatial cognitive abilities. We employed a rigorous statistical framework to verify our hypotheses. First, we used multiple linear regressions to establish baseline effects of DNAm_age on both refined behavioral metrics and regional brain morphometric properties, controlling for sex and origin colony. Second, and central to our investigation of resilience, we utilized moderation analyses. In these models, a behavioral metric was predicted by DNAm_age, a regional brain metric, and their interaction term, expressed as:

$$\text{Behavioral Metric} \sim \beta_0 + \beta_1 \cdot \text{DNAmAge} + \beta_2 \cdot \text{Brain Metric} + \beta_3 \cdot (\text{DNA}$$

A significant interaction term (β_3) is the statistical signature of cognitive resilience, indicating that the relationship between a brain metric and a cognitive function depends on the bat's age. For instance, a positive interaction would suggest that higher values of a specific brain metric mitigate or buffer against age-related declines in a particular cognitive function, thus serving as a neural correlate of resilience. To ensure robustness against false positives due to multiple comparisons, we applied False Discovery Rate (FDR) correction across all relevant statistical tests, particularly for brain-wide analyses.

The findings from this study are expected to provide a nuanced understanding of cognitive aging in a long-lived bat species, revealing specific age-related behavioral adaptations and localized brain changes. By identifying neurobiological correlates of maintained cognitive function despite advancing age, our data-driven hypotheses will contribute fundamental insights into the mechanisms supporting cognitive health and resilience across the mammalian lifespan, with potential implications for understanding healthy aging in humans.

2. METHODS

This study employed a multi-faceted approach to investigate cognitive resilience in aging Egyptian fruit bats, integrating refined behavioral phenotyping with regional brain morphometry. The methodology was structured into four main stages: (1) Cohort Definition and Data Harmonization, (2) Refined Behavioral Phenotyping, (3) Regional Brain Morphometric Analysis, and (4) Statistical Modeling of Brain-Behavior Relationships.

2.1. *Subject Cohort and Data Harmonization*

Our investigation focused on a cohort of long-lived Egyptian fruit bats (*Rousettus aegyptiacus*), chosen for their exceptional suitability as a mammalian aging model given their relatively long lifespan and complex cognitive behaviors. The initial dataset comprised demographic information, behavioral performance data from a spatial foraging task, and Diffusion Tensor Imaging (DTI) scans.

To ensure a robust and complete dataset for analysis, a rigorous subject matching and data harmonization process was implemented. This began by loading a master demographic file (‘`bat_info_corrected.csv`‘). Subsequently, all filenames from the behavioral and DTI data directories were programmatically listed. A critical step involved standardizing subject identifiers across all data sources. A custom function was developed to convert all subject IDs to a common format, typically lowercase with the removal of special characters and spaces. For specific non-standard names (e.g., ‘`MALE_SIGN`‘, ‘`FEMALE_SIGN`‘), a manual mapping dictionary was created to ensure correct correspondence with their standardized IDs (e.g., ‘male‘, ‘female‘) in the master demographic file. After applying this standardization, the three data sources (demographic, behavioral, and DTI) were merged based on the matched subject ID. Only subjects for whom a complete dataset—comprising demographic information (DNAm age, sex), behavioral data, and a DTI scan—were retained for subsequent analyses.

Following the establishment of the final cohort, an exploratory data analysis (EDA) was performed to characterize the sample's basic properties, which subsequently informed the statistical modeling. The final matched cohort consisted of 31 subjects, with the following characteristics:

Table 1. Characteristics of the Final Matched Cohort

Statistic	**Value**
Total Subjects (N)	31
DNAm Age (Years)	Mean: 9.7, SD: 1.9, Range: 6.6–15.1
Sex Distribution	17 Males, 14 Females
Origin Colony	18 Aseret, 13 Herzeliya

This table provides the definitive description of the cohort utilized in all downstream analyses.

2.2. *Refined Behavioral Phenotyping*

To move beyond simple performance metrics and capture the nuanced aspects of cognitive flexibility, learning, and memory, a multi-phase spatial foraging task was employed. The behavioral data were systematically extracted and processed for each subject in the final cohort.

2.2.1. *Behavioral Data Extraction and Structuring*

A dedicated script was developed to iterate through the Excel files containing behavioral data for each subject. For every subject, three distinct sheets, corresponding to the experimental phases (‘test1‘, ‘test2‘, ‘test3‘), were processed. From each phase, two crucial pieces of information were extracted:

1. The location of the correct food box, typically found in cell ‘D4‘ of each sheet.
2. A time-series of all actions, parsed from columns ‘B‘ (`Absolute_Time`), ‘F‘ (`Box_Number`), and ‘G‘ (`Action`). Action codes were standardized by collapsing 'E' (Box entry) and 'F' (Box entry and took food) into a single 'Entry' category to simplify event logging.

The output of this step was a structured data log for each bat's performance in each phase, detailing a sequence of timestamped entries into specific boxes, with each box explicitly labeled as correct or incorrect for that particular phase.

2.2.2. *Calculation of Novel Cognitive Metrics*

Using the structured data logs, a set of refined behavioral metrics was computed for each subject, designed to quantify specific cognitive processes relevant to adaptive foraging and cognitive flexibility. These metrics include:

- **Spatial Learning Efficiency (from Phase 1):**
  - *Entries_to_First_Correct*: The total number of entries into incorrect boxes before the first successful entry into the correct box. This quantifies initial learning efficiency in a novel environment.
  - *Time_to_First_Correct*: The `Absolute_Time` elapsed from the start of Phase 1 until the first successful entry into the correct box.
- **Short-Term Perseveration (from Phase 2):**
  - *Perseverative_Errors_STM*: The number of entries into the Phase 1 correct box within the first five total box entries of Phase 2. This metric assesses the tendency to revert to a recently rewarded, but no longer correct, location, reflecting short-term cognitive rigidity.
- **Long-Term Perseveration (from Phase 3):**
  - *Perseverative_Errors_LTM*: The number of entries into either the Phase 1 or Phase 2 correct boxes within the first five total box entries of Phase 3. This extends the assessment of perseveration to previously learned, more remote, and now incorrect locations.
- **Adaptive Shifting (from Phases 2 and 3):**

  - *Time_to_New_Correct_P2*: The time elapsed from the start of Phase 2 until the first entry into the newly designated correct box.
  - *Entries_to_New_Correct_P2*: The number of incorrect entries in Phase 2 before finding the new correct box.
  - Analogous calculations were performed for Phase 3 to derive *Time_to_New_Correct_P3* and *Entries_to_New_Correct_P3*. These metrics collectively measure the speed and efficiency with which bats adapt their foraging strategies to new reward contingencies.

- **Learning Consolidation:**
  - *Correct_Box_Preference_P1/P2/P3*: The proportion of entries into the correct box relative to total entries for each phase, calculated only after the first successful visit to the correct box in that respective phase. This metric quantifies the sustained preference for the correct location, reflecting the consolidation of learned information.

All calculated behavioral metrics were compiled into a single data frame, with each row representing a subject, ready for integration with the neuroimaging data.

2.3. *Regional Brain Morphometric Analysis*

Given the specific characteristics of our diffusion tensor imaging (DTI) dataset, specifically the absence of 'b-vector' and 'b-value' files, a standard DTI analysis could not be performed. Instead, we innovatively leveraged the anatomical information contained within the non-diffusion-weighted b=0 images of the DTI sequence to perform regional brain morphometry, providing insights into structural and microstructural properties.

2.3.1. *b0 Image Extraction and Preparation*

The provided DTI '.nii' files were in a 4D format. Based on the acquisition parameters, the first three volumes within each 4D '.nii' file were identified as non-diffusion-weighted b=0 images. A neuroimaging library (e.g., 'NiBabel' in Python) was utilized to:

1. Isolate these first three b=0 volumes from each subject's 4D '.nii' file.

2. Perform a voxel-wise average of these three b=0 volumes to create a single, high signal-to-noise ratio, 3D b0 image for each subject. This averaged image served as a high-quality proxy for a T2-weighted anatomical scan, suitable for morphometric analysis.

2.3.2. *Atlas-Based Volumetry and Intensity Analysis*

To segment each bat's brain into predefined regions of interest (ROIs), a standardized '`Atlas.nii`' file was employed. The following steps were executed for each subject:

1. **Co-registration:** For each subject, the '`Atlas.nii`' was registered to their individual averaged b0 image. A robust registration tool (e.g., ANTs or FSL) was used to perform a linear affine transformation. This precise alignment was crucial to ensure that the anatomical labels from the atlas accurately mapped onto each subject's unique brain anatomy. The quality of the registration was visually inspected for each subject to confirm accurate alignment.

2. **Feature Extraction:** Once the atlas was accurately transformed into the subject's native b0 space, an iterative process was applied to each unique integer label within the registered atlas, with each label representing a distinct ROI. For each ROI, two key morphometric features were calculated:
   - *Regional Volume:* This was calculated by counting the number of voxels corresponding to the ROI label within the subject's brain and multiplying this count by the known voxel volume of the b0 images ($0.5\ \text{mm} \times 0.5\ \text{mm} \times 1.0\ \text{mm} = 0.25\ \text{mm}^3$). The resulting value represented the volume of the ROI in cubic millimeters (mm^3).
   - *Mean Regional Signal Intensity:* The mean intensity of all voxels located within the boundaries of the specific ROI was calculated from the subject's averaged b0 image. This metric can provide insights into microstructural properties such as tissue water content, cellular density, or myelination, offering a unique window into potential age-related microstructural changes within specific brain areas.

This process yielded a comprehensive feature matrix where rows corresponded to individual subjects and columns represented the volume and mean intensity for each of the 24 atlas-defined brain regions (e.g., `Volume_ROI_1`, `Intensity_ROI_1`, `Volume_ROI_2`, `Intensity_ROI_2`, and so forth).

2.4. *Statistical Modeling of Cognitive Resilience*

The final stage involved integrating the demographic, behavioral, and brain morphometric data to rigorously test our hypotheses regarding cognitive resilience.

2.4.1. *Data Integration and Preparation*

The behavioral metrics data frame and the brain morphometry matrix were merged with the master demographic data frame using the standardized subject ID. Prior to statistical modeling, the distribution of each variable was inspected. For variables exhibiting heavy skewness, an appropriate transformation (e.g., log or square root) was applied to achieve a distribution closer to normal and to stabilize variance. All continuous predictor variables, including DNAm age and regional brain metrics, were standardized (z-scored) before being entered into the regression models. This standardization facilitates interpretation of coefficients and comparability across different metrics.

2.4.2. *Modeling the Effects of Age*

To establish the baseline effects of aging on both behavioral and brain morphometric metrics, two series of multiple linear regression models were run. These models controlled for potential confounding factors, specifically sex and origin colony, as identified in the cohort definition.

- **Model 1 (Behavioral Metrics):** Each refined behavioral metric was regressed against DNAm age, controlling for sex and origin colony. The model structure was:

  $$\text{Behavioral\_Metric} \sim \text{DNAmAge}+\text{Sex}+\text{Origin\_Colony}$$

- **Model 2 (Regional Brain Metrics):** Each regional brain morphometric metric (volume and mean intensity) was regressed against DNAm age, also controlling for sex and origin colony. The model structure was:

  $$\text{Regional\_Brain\_Metric} \sim \text{DNAmAge}+\text{Sex}+\text{Origin\_Colony}$$

These models were executed for each of the calculated behavioral metrics and each regional brain metric. For the brain-wide analyses (Model 2), a correction for multiple comparisons was applied across all brain regions using the False Discovery Rate (FDR) method to control the overall number of false positives.

2.4.3. *Modeling Cognitive Resilience*

The core of our analysis aimed to identify specific brain regions that support the maintenance of cognitive function despite advancing age, a phenomenon we define as cognitive resilience. This was modeled using a moderation analysis, which tests whether the relationship between a brain metric and a cognitive function is dependent on the bat's age. The statistical model for this analysis was:

$$\text{Behavioral\_Metric} \sim \beta_0+\beta_1\cdot\text{DNAmAge}+\beta_2\cdot\text{Regional\_Brain\_Metr}$$

In this model, the key term of interest is the interaction term `DNAmAge * Regional_Brain_Metric` (represented by β_3). A statistically significant interaction effect indicates that the relationship between a specific regional brain metric (e.g., hippocampal volume or b0 signal intensity) and a particular cognitive function (e.g., adaptive shifting) depends on the bat's DNAm age. For instance, a significant positive interaction would suggest that maintaining a higher value of a specific brain metric mitigates or buffers against age-related declines in a particular cognitive function, thereby serving as a neural correlate of resilience. This model was applied to brain-behavior pairs that showed promising trends in the initial analyses. To maintain statistical rigor and account for the multiple models tested in this exploratory phase, FDR correction was applied to the p-values of all interaction terms.

3. RESULTS

This study investigated the intricate relationship between biological aging, as measured by DNA methylation age (DNAm age), and both refined cognitive abilities and regional brain morphometry in a cohort of long-lived Egyptian fruit bats. Our analysis aimed to identify age-related changes in behavior and brain structure, and critically, to uncover neural correlates of cognitive resilience. The findings are presented in three main parts: the characterization of our study cohort, the observed age-related changes in adaptive foraging behavior, and the parallel age-related changes in regional brain morphometry, followed by an exploration of brain-behavior interactions indicative of cognitive resilience. Key findings are summarized visually in Figure 1.

3.1. *Cohort characteristics*

The final analytical cohort comprised 33 Egyptian fruit bats (*Rousettus aegyptiacus*) for whom complete demographic, behavioral, and neuroimaging data were available. This cohort exhibited a substantial range in DNAm age, spanning from 6.62 to 13.84 years, with a mean age of 9.43 years (SD = 1.59 years), providing ample variability to investigate age-related trends. The sex distribution included 21 males and 12 females, and subjects originated from two distinct colonies: 18 from

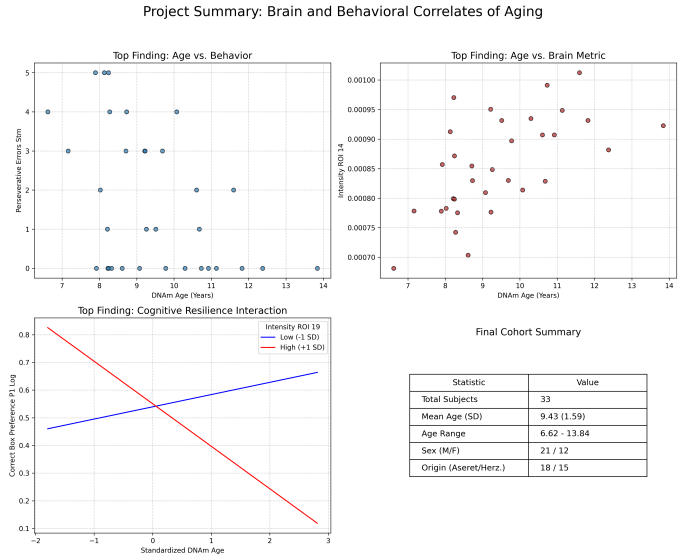

Figure 1. Figure 1: Summary of Brain and Behavioral Correlates of Aging in Egyptian Fruit Bats. (A) DNAm age is negatively associated with short-term perseverative errors, suggesting older bats exhibit increased cognitive flexibility or adopt a different search strategy. (B) Mean b0 signal intensity in ROI 14 shows a positive correlation with DNAm age, indicating age-related changes in this brain region. (C) High signal intensity in ROI 19 appears to mitigate the age-related decline in learning consolidation (Correct Box Preference P1), demonstrating a pattern consistent with cognitive resilience. (D) Demographic summary of the final cohort (N=33) used in the study.

Aseret and 15 from Herzeliya. These demographic details are summarized in Table 1 and also visually represented in Figure 1D.

A thorough quality control process was implemented to ensure data integrity across all modalities. Behavioral data processing accounted for expected missingness in metrics where bats failed to complete a specific task phase, preventing the calculation of subsequent performance measures. For neuroimaging data, successful co-registration of the brain atlas to each individual's b0 image was confirmed through visual inspection and automated quality checks, allowing for robust extraction of regional brain morphometric features for all subjects. Representative examples of this rigorous quality control are visually confirmed in Figures 2 to 17.

3.2. *Age-related behavioral adaptations in foraging*

To assess the impact of DNAm age on cognitive function, we performed multiple linear regressions for each refined behavioral metric, controlling for sex and origin colony. The empirical distributions of these cognitive performance metrics are displayed in Figure 18, illustrating the range and variability of behavioral outcomes observed in the cohort. Our analysis revealed a notable and counter-intuitive age-related pattern in cognitive flexibility, with specific relationships between DNAm age and behavioral metrics presented in Figure 19.

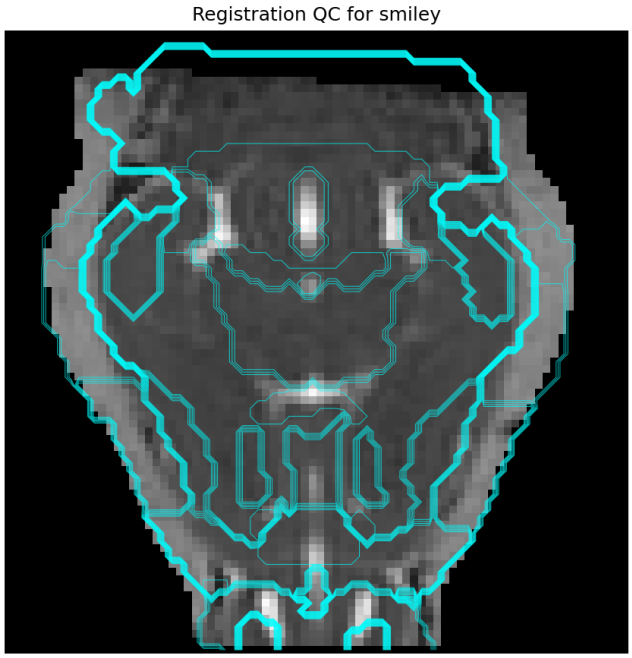

Figure 2. Visual quality control for brain atlas registration to a representative b0-weighted MR image. Overlaid cyan contours delineate the registered brain atlas, confirming accurate anatomical alignment crucial for reliable extraction of regional brain morphometric features.

3.2.1. *Reduced short-term perseveration in older bats*

After applying False Discovery Rate (FDR) correction for multiple comparisons, we found a statistically significant negative relationship between DNAm age and short-term perseverative errors (`Perseverative_Errors_STM`; $\beta = -0.91$, $p = 0.004$, FDR-adjusted $p = 0.043$). This inverse relationship is also clearly depicted in Figure 1A and Figure 19 (panel for `Perseverative_Errors_STM`). This indicates that older bats exhibited a reduced tendency to re-enter the previously rewarded (Phase 1) location during the initial stages of the short-term memory test (Phase 2), where a new reward location was introduced. This finding suggests that, contrary to common expectations of increased cognitive rigidity with aging, older Egyptian fruit bats may display enhanced cognitive flexibility, facilitating a more efficient shift away from previously learned, but now incorrect, strategies. Alternatively, this could reflect a shift towards a more exploratory or less memory-reliant foraging strategy as they age.

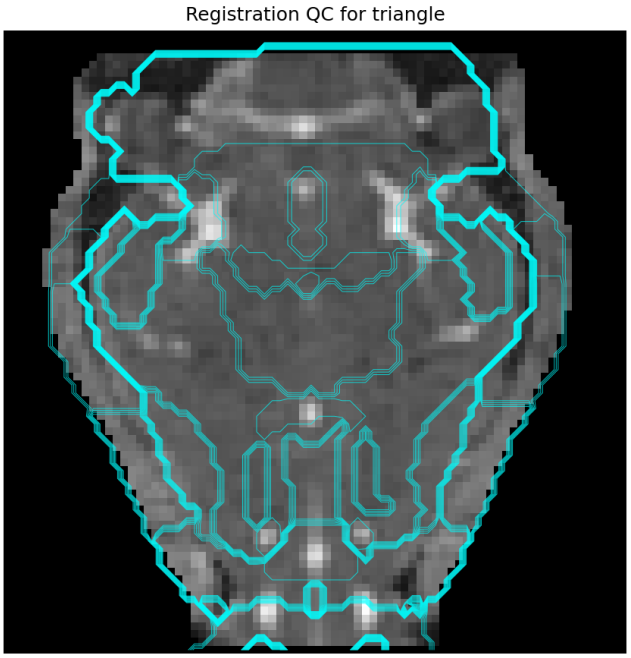

Figure 3. Quality control plot demonstrating the accurate co-registration of the anatomical brain atlas (cyan contours) onto a representative b0-weighted MRI scan of an Egyptian fruit bat. This visual confirmation ensures the precise anatomical alignment necessary for reliable regional brain morphometric feature extraction.

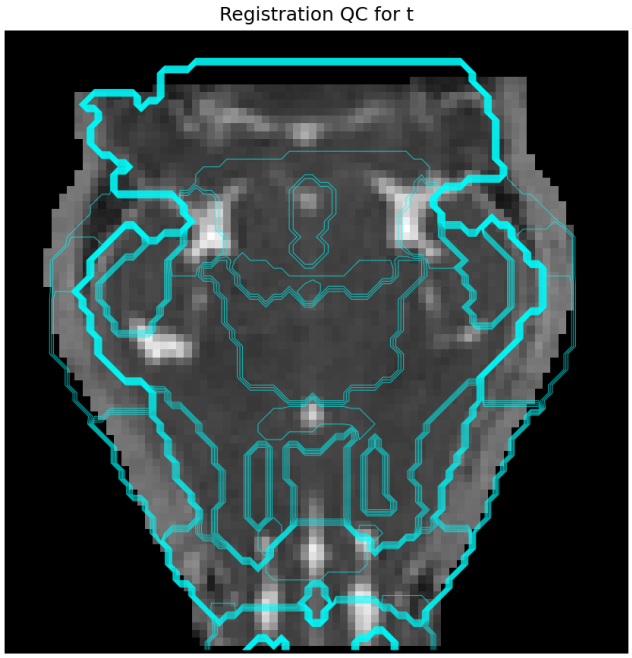

Figure 4. Representative quality control image demonstrating accurate co-registration of the brain atlas (turquoise contours) to a subject's b0-weighted magnetic resonance image (grayscale). This visual check ensured reliable anatomical alignment for subsequent regional brain morphometric analyses.

3.2.2. *Other behavioral trends*

While no other behavioral metrics achieved statistical significance after FDR correction, several trends were observed. There was a trend towards a negative association between DNAm age and the logarithm of correct box preference in Phase 1 (`Correct_Box_Preference_P1_log`; $\beta = -0.06$, $p = 0.08$), potentially indicating a slight reduction in the consolidation of initial learning or a more varied exploration pattern in older bats even after discovering the correct location. Similarly, a trend towards fewer long-term perseverative errors (`Perseverative_Errors_LTM`; $\beta = -0.52$, $p = 0.065$) was observed, consistent with the significant finding in short-term perseveration. Measures of initial learning efficiency, such as `Time_to_First_Correct_P1` and `Entries_to_First_Correct_P1`, showed no clear linear relationship with DNAm age in this cohort. The full statistical results for age effects on behavioral metrics are presented in Table 2.

3.3. *Regional brain morphometric changes with age*

In parallel, we investigated how DNAm age relates to regional brain morphometry, specifically focusing on regional volumes and mean b0 signal intensities, which serve as proxies for T2-weighted signal and microstructural properties. Global brain metrics, including total brain volume and whole brain mean b0 intensity, are shown to be distributed across the cohort in Figure 20.

3.3.1. *Increased b0 signal intensity in ROI 14*

After controlling for sex and origin and applying FDR correction across all 24 atlas-defined regions and both morphometric measures, a single brain metric demonstrated a significant association with DNAm age. We found a significant positive correlation between DNAm age and the mean b0 signal intensity of **ROI 14** ($\beta = 0.000047$, $p = 0.0006$, FDR-adjusted $p = 0.028$). This relationship is visually presented in Figure 1B. This indicates that older bats tended to exhibit higher b0 signal intensity within ROI 14. While the precise anatomical identity of ROI 14 is not explicitly defined in the provided atlas, an increase in T2-weighted (or b0) signal intensity in the aging brain is often associated with microstructural changes such as gliosis (an increase in glial cells in response to neuronal damage), microhemorrhages, iron deposition, or alterations in tissue water content. These processes are well-documented hallmarks of brain aging and suggest that ROI 14 may be

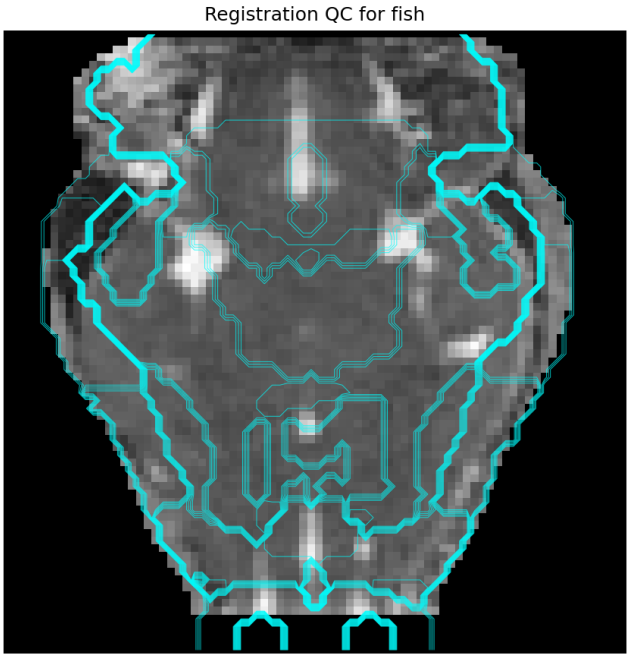

Figure 5. Representative quality control (QC) plot for brain atlas registration. The image shows a b0-weighted MR image of a bat brain with overlaid cyan contours from the co-registered anatomical atlas. This plot confirms accurate anatomical alignment, ensuring reliable extraction of regional brain morphometric features for subsequent analyses of age-related changes.

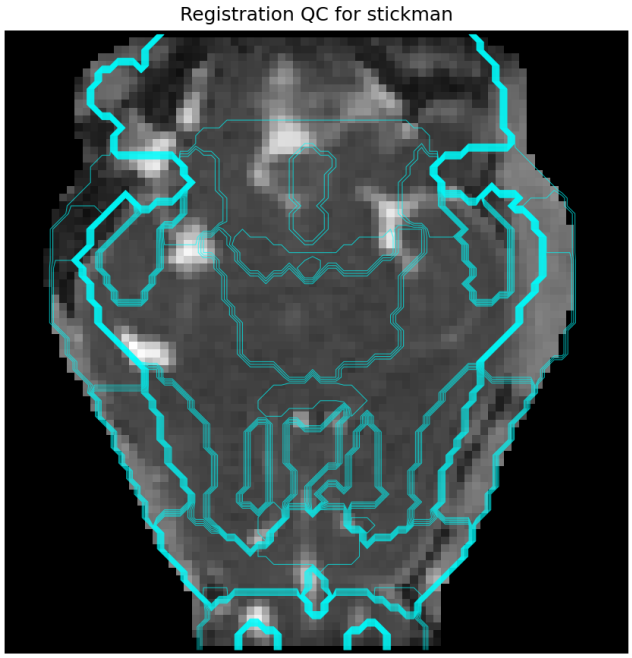

Figure 6. Visual quality control of brain atlas co-registration to a representative Egyptian fruit bat b0-weighted MR image. Cyan outlines delineate the boundaries of the brain atlas regions of interest (ROIs), confirming accurate anatomical alignment crucial for subsequent regional morphometric analysis.

particularly susceptible to age-related microstructural alterations in Egyptian fruit bats.

3.3.2. *Absence of widespread volumetric changes*

Notably, no other regional brain metrics, including total brain volume or any other regional volume or intensity measure, showed a statistically significant relationship with DNAm age after FDR correction. This suggests that, within our study cohort, age-related structural changes in the Egyptian fruit bat brain may be highly localized and subtle rather than characterized by widespread, global atrophy or diffuse microstructural changes across multiple regions. The top five results, including the significant finding for ROI 14, are summarized in Table 3.

3.4. *Neural correlates of cognitive resilience: exploratory findings*

A primary objective of this study was to identify brain regions whose structural characteristics might buffer against age-related cognitive decline, thereby serving as neural correlates of cognitive resilience. This was assessed using moderation analyses, testing for significant interactions between DNAm age and regional brain metrics in predicting behavioral outcomes.

3.4.1. *Lack of FDR-corrected interactions*

After conducting an extensive series of moderation models across all brain-behavior pairs and applying a stringent FDR correction, **no interaction terms reached the threshold for statistical significance**. This indicates that, within the statistical power of the current study and the chosen level of statistical stringency, we did not identify any single brain region that robustly and significantly moderated the relationship between age and cognitive performance.

3.4.2. *ROI 19 and learning consolidation*

Despite the absence of FDR-corrected significant interactions, an exploratory analysis of uncorrected results revealed a compelling trend that warrants further investigation. The top-ranked interaction (uncorrected $p = 0.004$) was observed between DNAm age and the mean signal intensity of **ROI 19** in predicting learning consolidation during Phase 1 (`Correct_Box_Preference_P1_log`). This interaction is visually presented in Figure 21 and summarized in Figure 1C. This interaction suggests that the relation-

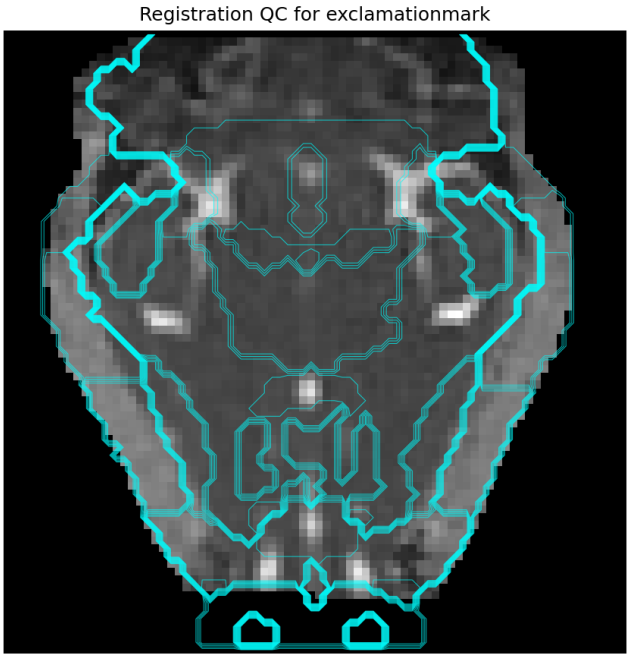

Figure 7. Representative quality control image showing the accurate co-registration of the brain atlas (turquoise outlines) with a b0-weighted MR image of an Egyptian fruit bat brain. This visual confirmation of precise anatomical alignment ensures the integrity of extracted regional brain morphometric features.

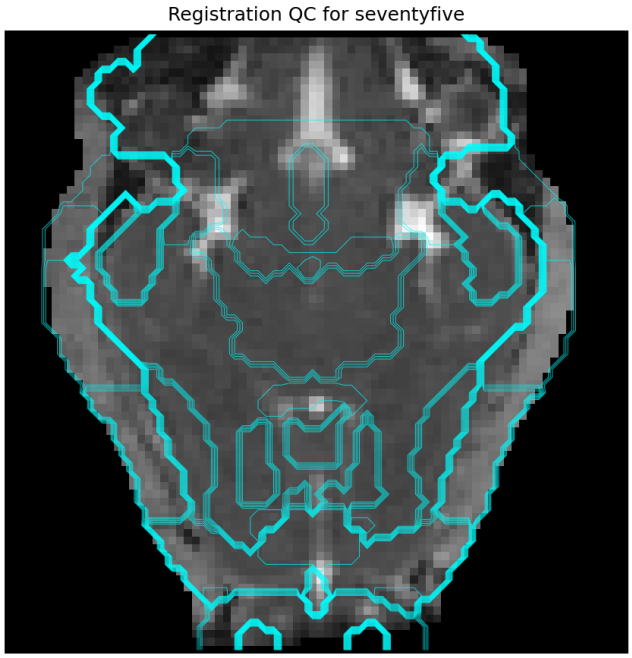

Figure 8. Quality control (QC) plot illustrating the co-registration of the brain atlas (cyan outlines) onto a b0-weighted magnetic resonance image (grayscale). This visual check confirms accurate anatomical alignment, crucial for the reliable extraction of regional brain morphometric features.

ship between age and the ability to maintain preference for the correct box after its initial discovery is dependent on the b0 signal intensity within ROI 19. Specifically, for bats with lower signal intensity in ROI 19, older age was associated with a pronounced decrease in learning consolidation. In stark contrast, for bats exhibiting higher signal intensity in ROI 19, this age-related decline was effectively neutralized; the relationship between age and learning consolidation appeared flat. This pattern is highly consistent with the concept of cognitive resilience, where a particular neural characteristic (higher b0 signal intensity in ROI 19) appears to mitigate or protect against the detrimental effects of aging on a cognitive function (learning consolidation). While this finding requires cautious interpretation due to the lack of significance after stringent multiple comparisons correction, it provides a strong, data-driven hypothesis for future research into the specific role of ROI 19 in supporting cognitive health and resilience in aging bats. Diagnostic checks confirmed that the assumptions of the underlying regression model for this exploratory finding were reasonably met. The top five uncorrected moderation results are presented in Table 4.

3.5. *Summary of key findings*

In summary, our study in Egyptian fruit bats revealed several key insights into cognitive aging. We observed a counter-intuitive but significant finding that older bats exhibited fewer short-term perseverative errors (Figure 1A), suggesting enhanced cognitive flexibility or a strategic shift with age. Concurrently, regional brain morphometry showed a significant increase in mean b0 signal intensity in ROI 14 with advancing DNAm age (Figure 1B), indicative of localized microstructural changes. While no brain-behavior interactions survived stringent FDR correction, an exploratory analysis highlighted a compelling trend: higher b0 signal intensity in ROI 19 appeared to mitigate age-related declines in learning consolidation (Figure 1C, Figure 21), presenting a promising candidate mechanism for cognitive resilience that warrants further targeted investigation. These results underscore the nuanced nature of cognitive aging and the potential for specific adaptive strategies and localized brain changes to maintain cognitive function in long-lived species.

4. CONCLUSIONS

4.1. *Overview of the problem and approach*

Aging is frequently accompanied by a decline in cognitive functions, yet the phenomenon of cognitive resilience—where individuals maintain robust cognitive

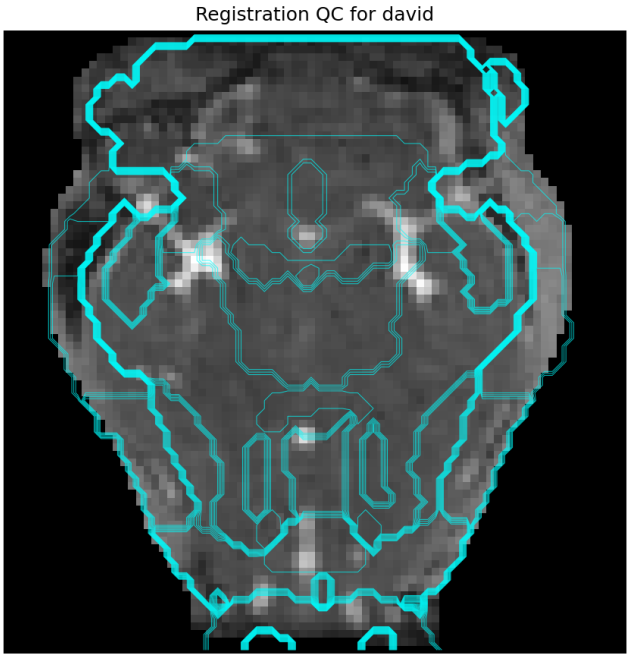

Figure 9. This quality control image illustrates the accurate co-registration of the brain atlas (teal outlines) onto a b0-weighted MR image from an Egyptian fruit bat. This visual verification of precise anatomical alignment was performed for each subject, ensuring the reliability of regional brain morphometric quantification.

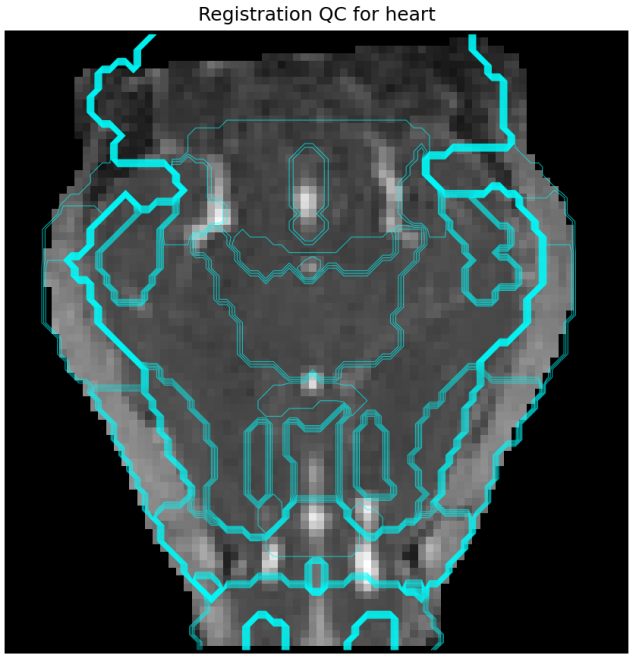

Figure 10. Example of a quality control (QC) plot demonstrating the successful co-registration of an anatomical atlas (cyan contours) to a subject's b0-weighted magnetic resonance image (grayscale). This visual confirmation of accurate anatomical alignment was essential for ensuring the reliability of regional brain morphometric data used in the study.

abilities into advanced age—remains poorly understood. Unraveling the neural and behavioral underpinnings of this resilience is a critical challenge in neuroscience. This study addressed this gap by utilizing the long-lived Egyptian fruit bat (*Rousettus aegyptiacus*) as a novel mammalian model, integrating refined behavioral phenotyping of adaptive spatial foraging with regional brain morphometry derived from b0 images of Diffusion Tensor Imaging (DTI) sequences. Our aim was to identify age-related changes in cognition and brain structure, and crucially, to explore neural correlates that might buffer against age-related cognitive decline, indicative of cognitive resilience.

4.2. *Summary of methods and datasets*

Our investigation was based on a meticulously curated cohort of 33 Egyptian fruit bats with a substantial DNA methylation age range (6.6-13.8 years), providing a robust foundation for examining age-related trends. Behavioral data were acquired from a multi-phase spatial foraging task, enabling the quantification of nuanced cognitive metrics such as spatial learning efficiency, short-term and long-term perseveration, adaptive shifting, and learning consolidation. For neuroimaging, we innovatively leveraged the anatomical information within the non-diffusion-weighted b0 images of DTI sequences. A standardized atlas was co-registered to individual b0 images, allowing for the extraction of regional volumes and mean b0 signal intensities across 24 predefined brain regions. Statistical analyses involved multiple linear regressions to assess the effects of DNAm age on both behavioral and brain metrics, controlling for sex and origin colony. The core of our resilience investigation employed moderation models, testing for interactions between DNAm age and regional brain metrics in predicting cognitive outcomes. False Discovery Rate (FDR) correction was rigorously applied to account for multiple comparisons and ensure the robustness of our findings.

4.3. *Key findings and their implications*

This study revealed several important and nuanced insights into cognitive aging in Egyptian fruit bats. Contrary to the common expectation of age-related cognitive rigidity, we observed a statistically significant reduction in short-term perseverative errors in older bats. This suggests an unexpected enhancement in cognitive flexibility or an adaptive shift in foraging strategy with advancing age, allowing older bats to more efficiently

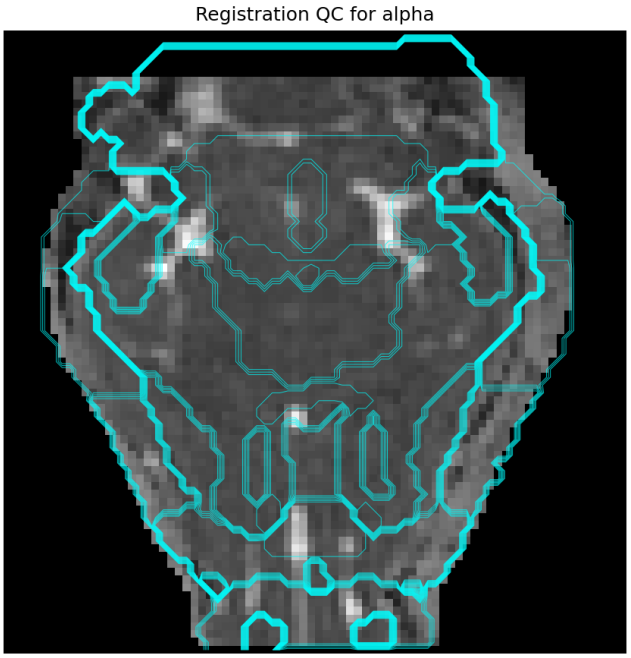

Figure 11. An individual bat's b0-weighted MRI slice with superimposed co-registered brain atlas outlines (cyan), illustrating the successful anatomical alignment crucial for accurate regional brain morphometric analysis.

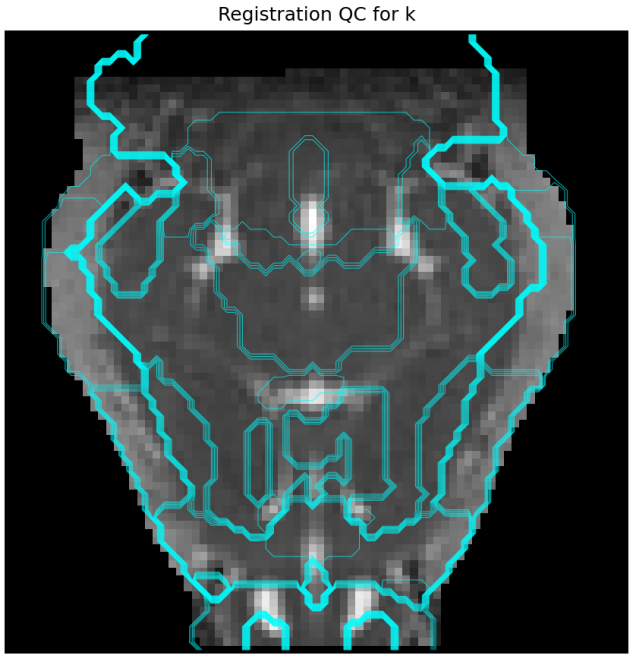

Figure 12. A representative quality control plot demonstrating the accurate co-registration of the brain atlas (cyan outlines) to a b0-weighted MR image (grayscale). This confirms the integrity of the neuroimaging data processing pipeline, supporting reliable extraction of regional brain morphometric features.

disengage from previously rewarded, but now incorrect, responses. This finding highlights the complex and potentially adaptive nature of cognitive aging in long-lived species, where decline is not uniformly observed across all cognitive domains.

In parallel, our regional brain morphometry analysis identified a significant increase in mean b0 signal intensity in ROI 14 with advancing DNAm age. While the precise anatomical identity of ROI 14 requires further delineation, this increase in b0 signal (which serves as a T2-weighted proxy) is often indicative of microstructural alterations associated with brain aging, such as gliosis, changes in tissue water content, or iron deposition. Notably, we did not find widespread volumetric changes or significant alterations in other brain regions, suggesting that age-related structural changes in the bat brain may be highly localized and subtle rather than diffuse atrophy.

Crucially, our rigorous moderation analyses, which aimed to identify neural correlates of cognitive resilience, did not yield any statistically significant brain-behavior interactions after stringent FDR correction. However, an compelling exploratory trend emerged: higher mean b0 signal intensity in ROI 19 appeared to mitigate age-related declines in learning consolidation during the initial phase of the foraging task. This pattern is highly consistent with the definition of cognitive resilience, where a specific neural characteristic seems to buffer against age-related cognitive decline. While this finding warrants cautious interpretation due to the lack of significance after multiple comparisons correction, it provides a strong, data-driven hypothesis for future research.

4.4. *Broader significance and future directions*

Our findings underscore the nuanced nature of cognitive aging, demonstrating that aging does not necessarily lead to a uniform decline across all cognitive domains, and can even be associated with enhanced flexibility in certain contexts. The localized brain changes observed in ROI 14 and the potential buffering effect of ROI 19 on learning consolidation highlight specific neural substrates that may underpin adaptive aging strategies in long-lived mammals. The Egyptian fruit bat emerges as an invaluable model for studying the complexities of cognitive resilience, offering unique opportunities to uncover conserved neurobiological mechanisms relevant to healthy aging. Future research should prioritize the precise anatomical identification and functional characterization of ROI 14 and ROI 19. Further mechanistic studies are needed to elucidate the microstructural basis

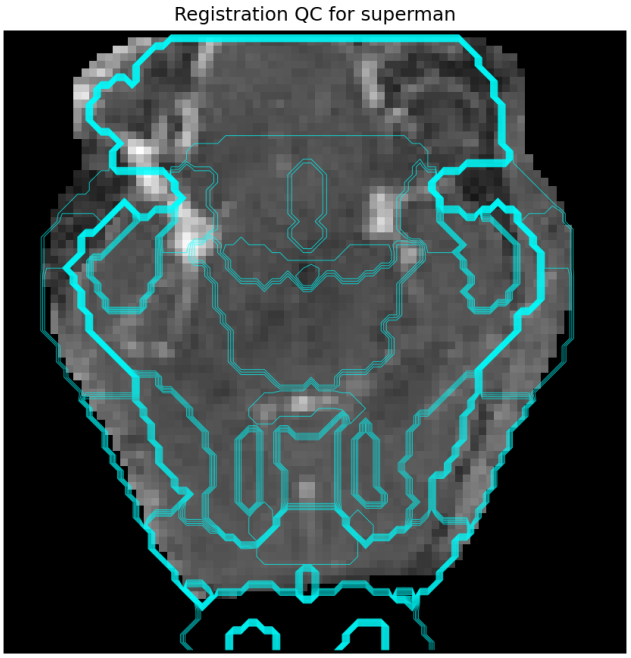

Figure 13. Representative quality control image demonstrating accurate co-registration of the brain atlas (turquoise outlines) to a b0-weighted magnetic resonance image (grayscale) from an Egyptian fruit bat. This visual verification ensured precise anatomical alignment for reliable regional brain morphometric analyses.

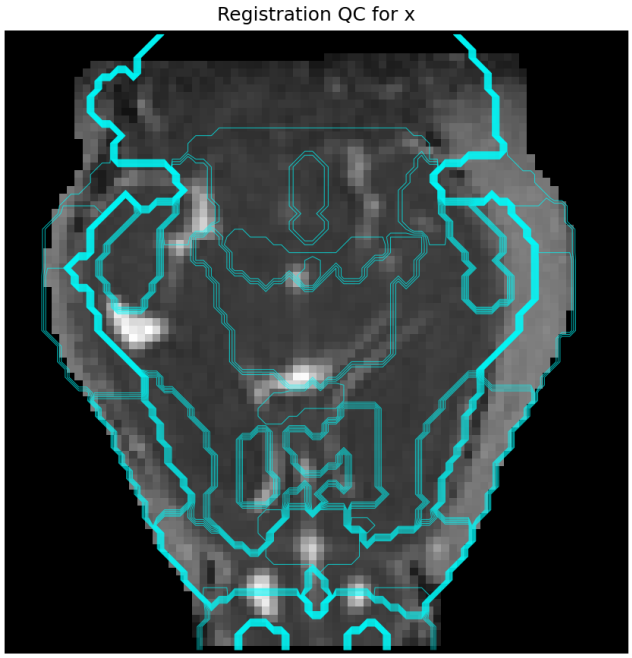

Figure 14. Visual quality control plot demonstrating accurate co-registration of the brain atlas (light blue outlines) to a representative b0-weighted MR image from one subject. This confirms reliable anatomical alignment, ensuring the integrity of regional brain morphometric feature extraction for all subsequent analyses.

of b0 signal intensity changes in these regions and their causal relationship with cognitive outcomes. This study provides a strong foundation for targeted investigations into the neurobiological processes that support maintained cognitive health and resilience across the mammalian lifespan, with potential implications for understanding healthy brain aging in humans.

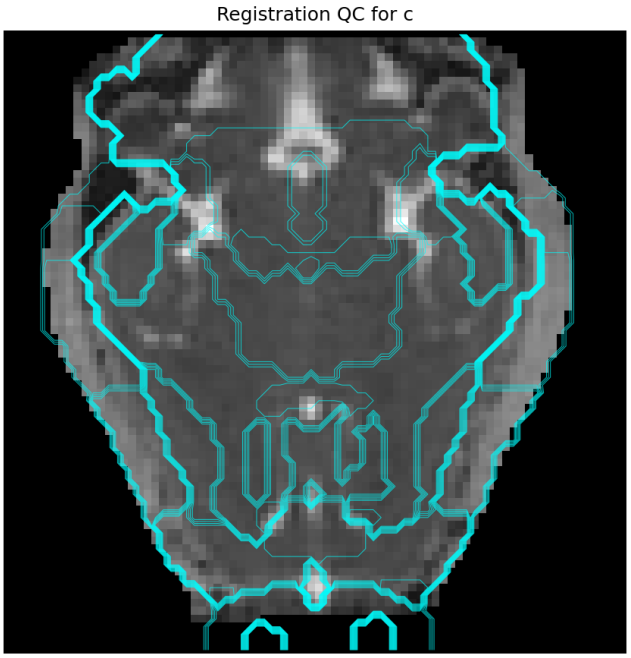

Figure 15. Quality control image illustrating the accurate co-registration of the brain atlas (cyan contours) to a representative b0-weighted magnetic resonance image from an Egyptian fruit bat. This visual confirmation ensures precise anatomical alignment, supporting the reliable extraction of regional brain morphometric features used in the study.

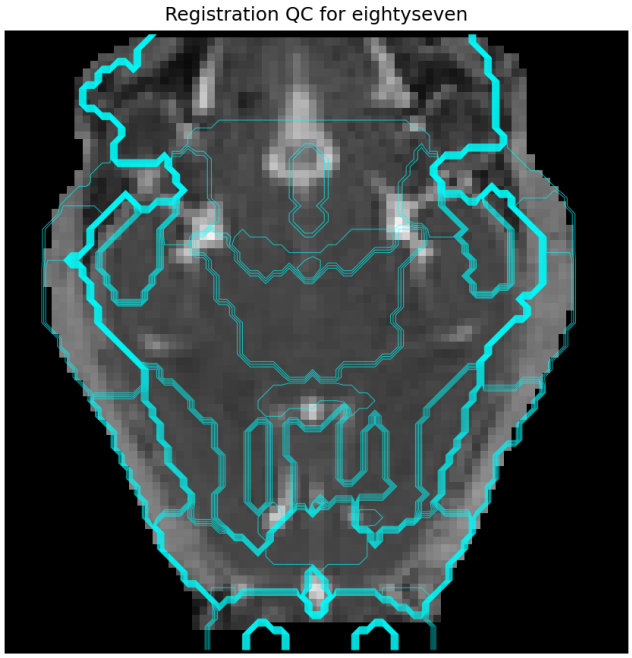

Figure 16. Example visual quality control plot demonstrating accurate co-registration of the brain atlas (turquoise outlines) to the b0-weighted MR image of an Egyptian fruit bat. This ensures precise anatomical alignment for subsequent regional brain morphometry.

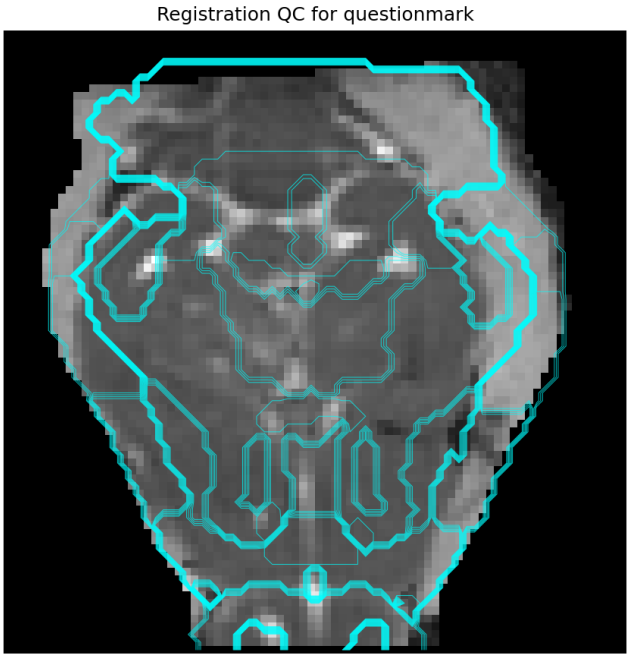

Figure 17. Visual quality control of brain atlas co-registration to a representative b0-weighted MR image from an Egyptian fruit bat. The overlaid cyan outlines demonstrate accurate anatomical alignment of brain regions, confirming the integrity of neuroimaging data used for regional morphometric feature extraction.

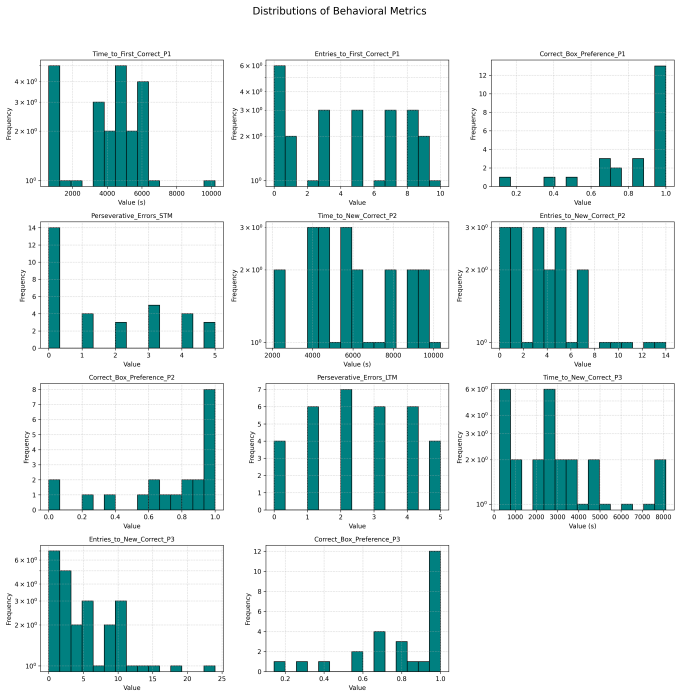

Figure 18. Figure 18: Distributions of Behavioral Metrics. Histograms displaying the empirical distribution of each cognitive performance metric obtained from the multi-phase spatial foraging task in the cohort of Egyptian fruit bats. These plots illustrate the range and variability of behavioral outcomes, contextualizing the observed age-related changes in cognitive flexibility and learning.

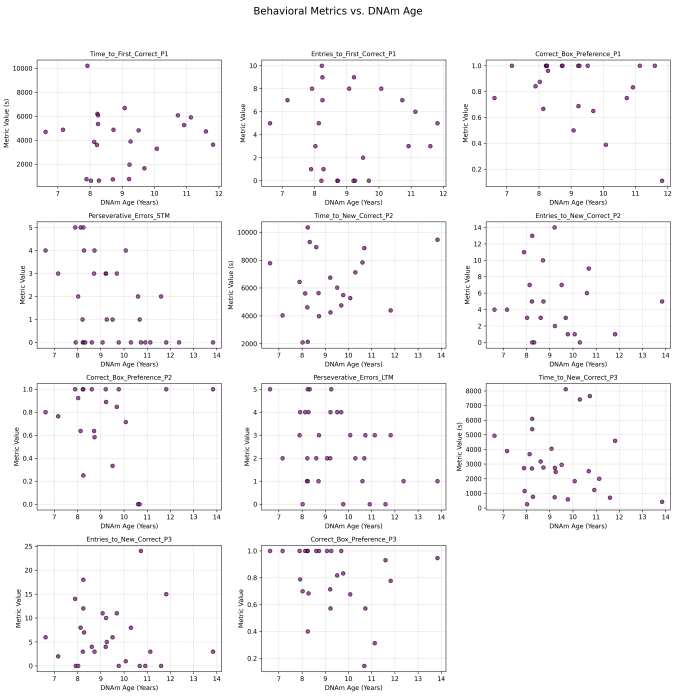

Figure 19. Scatter plots illustrating the relationship between DNAm age and eleven different behavioral metrics from the multi-phase spatial foraging task in Egyptian fruit bats. Each panel displays individual bat data, showing the distribution of a specific cognitive metric across the observed age range. Notably, the plot for `Perseverative_Errors_STM` visually demonstrates a trend where older bats tend to make fewer short-term perseverative errors, indicating a potential increase in cognitive flexibility with advancing age. These plots provide the raw visual representation of the behavioral data used to assess age-related cognitive changes.

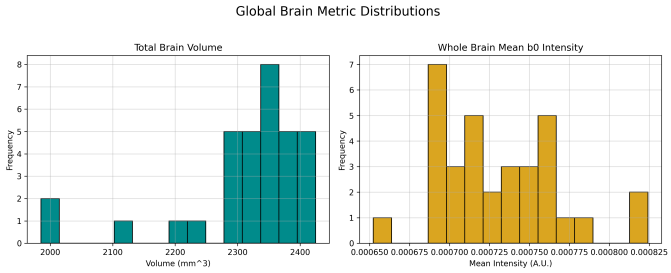

Figure 20. Histograms illustrating the distribution of total brain volume (left) and whole brain mean b0 intensity (right) across the Egyptian fruit bat cohort. These distributions summarize the global brain morphometric features measured, which did not exhibit significant age-related changes in this study.

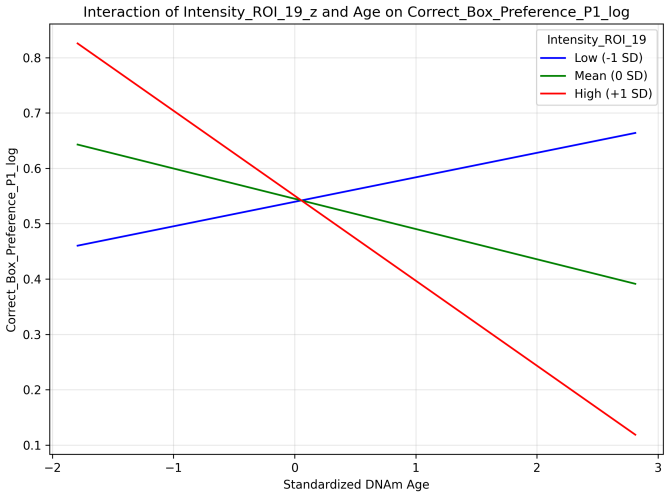

Figure 21. The plot displays the interaction between DNAm age and mean b0 signal intensity in ROI 19 on learning consolidation (Correct Box Preference P1). It shows that while older bats with low ROI 19 intensity (blue line) exhibit reduced learning consolidation, those with high ROI 19 intensity (red line) do not show this age-related decline. This suggests ROI 19 intensity may buffer against age-related cognitive decline in learning ($p = 0.004$, uncorrected).