Research Partnership in Cognitive Aging

A report to the McKnight Brain Research Foundation

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REPORT SUMMARY

The Foundation for the National Institutes of Health (FNIH) is pleased to present the following Research Partnership in Cognitive Aging 2023 report to the McKnight Brain Research Foundation (MBRF). The report provides an update from the National Institute on Aging (NIA) on the Cognitive SuperAgers Networks, both supported through the Research Partnership in Cognitive Aging. The report also includes updates on the Mindfulness, EDucation, and EXercise for Age-Related Cognitive Decline (MEDEX) trial (now complete) continuation study, as well as two additional initiatives that stemmed from the Cognitive Aging Summit III.

The current centerpiece of the Research Partnership in Cognitive Aging between the NIA and MBRF, coordinated by the FNIH, is the research supported through the funding opportunity "Network for Identification, Evaluation, and Tracking of Older Persons with Superior Cognitive Performance for Their Chronological Age," RFA-21-015 (https://grants.nih.gov/grants/guide/rfa-files/RFA-AG-21-015.html). Updates to this research are provided below.

"Network for Identification, Evaluation, and Tracking of Older Persons with Superior Cognitive Performance for Their Chronological Age (U19 Clinical Trial Not Allowed)"

Resilience/Resistance to Alzheimer's Disease in Centenarians and Offspring (RADCO) U19AG073172

The RADCO cooperative agreement (U19AG073172), awarded to Drs. Thomas Perls (Boston University Medical Campus), Stacy Andersen (Boston University Medical Campus), and Susan Bookheimer (UCLA) is in the third year of award. The NIA is supporting a multi-year administrative supplement to enhance diversity and data capture, in the form of a fourth phenotyping and biospecimen core and neuroimaging core site at Georgia State University (GSU). The addition of the GSU site has enhanced the diversity of the RADCO cohort. The goal is to enroll 234 Black participants, thus increasing the Black participant proportion of the RADCO sample from 7.2% to 22.2%.

The abstract for U19AG073172:

DESCRIPTION (provided by applicant): Centenarians delay age-related diseases and disabilities into their mid-nineties. Some remain cognitively intact despite extreme exposure to the strongest risk factor for cognitive impairment and Alzheimer's disease (AD), aging. The overall hypothesis of this study, titled "Resilience/Resistance to AD in Centenarians and Offspring" (RADCO), is: centenarian cognitive SuperAgers and some of their offspring have protective factors that confer such resilience or, in some cases, even resistance against cognitive decline and dementia. RADCO assembles an unprecedentedly large sample

of prospectively studied centenarian cognitive SuperAgers (n=495, essentially, centenarians with cognitive function that falls within the norms of septuagenarians) along with offspring (n=600) and offspring spouses (n=120), who, via RADCO cores, undergo careful, comprehensive, and cutting-edge neuropsychological, biomarker, neuroimaging, and neuropathological phenotyping. These data are used by two projects with the overall scientific objective of gauging cognitive resilience in this sample, understanding the underlying protective biology and translating that into therapeutic targets. The Cognitive Resilience and Resistance Phenotypes Project (Project 1) gauges resilience by neuroimaging, plasma AD biomarkers risk and neuropathology, and therefore generates a range of resilience endophenotypes. The Protective Factors and Mechanisms Project (Project 2) is the translation arm of RADCO; it discovers genes, candidate biological pathways and sets of mi-RNA regulators associated with the resilience endophenotypes characterized in Project 1. In-vitro models of AD incorporate cortical neurons, microglial cells, and astrocytes created from centenarian cognitive superager induced pluripotent stem cell (iPSC) lines are used to test the candidate pathways for how they cause resilience against AD.

PUBLIC HEALTH RELEVANCE: Centenarian cognitive SuperAgers have exceptional cognitive function despite extreme exposure to the strongest risk factor for cognitive impairment and Alzheimer's disease, aging. The RADCO Study gauges cognitive resilience among centenarian cognitive SuperAgers and their offspring using cognitive testing, neuroimaging, blood biomarkers, and neuropathology. Translational studies will identify protective factors and underlying mechanisms that confer resilience or in some cases, even resistance against cognitive decline and dementia.

Study to Uncover Pathways to Exceptional Cognitive Resilience in Aging (SUPERAging) U19AG073153

The SUPERAging cooperative agreement (U19AG073153) awarded to Drs. Emily Rogalski, Marsel Mesulam, and Changiz Geula is in its third year of award. This year has seen a change in the locus of the primary award. Dr. Rogalski has transferred from Northwestern University to the University of Chicago. Dr. Mesulam has stepped down as one of the Multi-Principal Investigators (MPI) and remains active on the project as a co-investigator. Dr. Geula remains as an MPI. Both Drs. Geula and Mesulam are still on faculty at Northwestern. The team published findings in the Journal of the American Geriatrics Society in 2023 regarding the medication usage profiles of cognitive SuperAgers compared to age-peers. They reported that the medication profiles of cognitive SuperAgers showed no significant difference compared to cognitively average-for-age older controls in total medications, prescription medications, OTC medications, or in 10 medications/medication categories of interest. In another 2023 publication in Journal of the International Neuropsychological Society, they demonstrated that the episodic memory measure from the NIH Toolbox® is useful for differentiating cognitive SuperAgers from those with average-for-age cognition. These publications are attached to this report.

The abstract for U19AG073153:

DESCRIPTION (provided by applicant): The primary goal is to establish a multicenter SuperAging Consortium to identify behavioral, health, biologic, genetic, environmental, socioeconomic, psychosocial, anatomic and neuropathologic factors associated with SuperAging. These goals will be achieved through an organizational structure with 3 Cores (Administrative/Biostatistics, Clinical/Imaging, and Biospecimen/ Neuropathology) and 2 Research Projects. The Consortium will enroll 500 participants across 4 US Sites located in Illinois, Wisconsin, Michigan and Georgia, and the Canadian Site in Southwest, Ontario, with a focus on the enrollment of Black SuperAgers and Cognitively Average Elderly Controls with similar demographics (Controls). The Administrative/Biostatistics Core will provide governance and fiscal oversight, maintain scientific integrity, and create a centralized biostatistics and database infrastructure to harmonize the goals and activities of the Cores, Sites, and Projects, with each other, with the NIA, and with extramural collaborators. The Clinical/Imaging Core will standardize criteria for the uniform cross-site and multidisciplinary characterization of SuperAgers, streamline recruitment including that of Black participants, enter relevant information in the comprehensive database, support co-enrollment into Project 1, and encourage collaborative ventures aiming to understand the factors that promote SuperAging. The Biospecimen/Neuropathology Core will collect and bank brain tissue and blood products from SuperAging and Control cases, according to optimized procedures. It will render pathological diagnoses, quantitate selected markers of neurodegeneration and neuronal structure, coordinate the analyses of plasma biomarkers for Alzheimer's disease, and make specimens available for collaborative investigations. Project 1 will use state-of-the-art wearable technology to obtain real-time measurements in the course of everyday life to characterize quantitative parameters related to sleep, physical activity, autonomic responsivity, and social engagement to determine whether SuperAgers have relatively preserved and quantitatively determined physiologic and behavioral "complexity" compared to Controls. Project 2 will use transcriptomic, genetic, and protein profiling approaches to test the hypothesis that SuperAgers will demonstrate significant molecular differences in their central and peripheral immune and inflammatory system parameters compared to matched Control and Alzheimer's disease participants. By identifying neurobiologic features that contribute to superior memory performance in old age, outcomes from this Consortium will help isolate factors that promote successful cognitive aging and perhaps also prevent age-related brain diseases such as Alzheimer's disease.

PUBLIC HEALTH RELEVANCE: The proposed Consortium offers optimal organization for the accelerated recruitment of a racially diverse cohort of SuperAgers so that they can be more fully characterized neuropsychologically, neuropathologically, psychophysiologically, and molecularly. The planned activities of the Consortium will help isolate factors important for promoting successful cognitive aging and potentially also for avoiding age-related brain diseases such as Alzheimer's disease.

Follow-on Study of the MEDEX Clinical Trial

Participants in the MEDEX ("Remediating Age-related Cognitive Decline: Mindfulness-based Stress Reduction and Exercise") clinical trial are being followed through a new award (R01AG072694, "Resilience and Brain Health of Older Adults During the COVID-19 Pandemic") to Dr. Eric Lenze (PI of MEDEX; Washington University St. Louis), Dr. Breno Diniz (University of Connecticut School of Medicine), and Dr. Julie Wetherell (University of California San Diego).

The project goals are to elucidate whether exercise and mindfulness can mitigate the effects of stressors from the COVID-19 pandemic on cognitive function and emotional health in later life, including neurobiological measures of risk for Alzheimer's disease. By following the MEDEX participants, repeated sets of clinical, cognitive, molecular, and neuroimaging measures spanning 7.5 years and covering the pre-, during-, and post-pandemic period are being generated.

The abstract for R01AG072694:

DESCRIPTION (provided by applicant): Exercise and mindfulness are believed to be effective stress reduction interventions, but research to date has not been able to assess their benefits while individuals are coping with a major stressor in real time. The COVID-19 pandemic is an unwanted natural experiment in the deleterious effects of stress – especially social isolation (social disconnectedness and loneliness), a stressor particularly strongly associated with the pandemic – on older Americans' cognitive and emotional health and risk for Alzheimer's disease (AD). This project will elucidate whether exercise and mindfulness can mitigate the effects of pandemic stress on cognitive function and emotional health in later life, including neurobiological measures of risk for AD. We will leverage a unique resource: the NIH-funded trial, MEDEX. By leveraging MEDEX and following these participants, who continue to attend monthly booster sessions of their randomized condition remotely during the pandemic, we will have repeated sets of clinical, cognitive, molecular, and neuroimaging measures covering 7.5 years during the pre-, during-, and post-pandemic period. We can examine intervention effects, as well as individual factors such as resilience, on long-term outcomes. Among other innovative aspects of the project, we will analyze effects on two novel peripheral biomarkers: Senescence Associated Secretory Phenotype (SASP), which measures mechanisms of biological aging, and plasma amyloid Aβ42 and Aβ40, which measure AD risk. In the proposed project, (1) during the pandemic, we will use novel methods such as Ecological Momentary Assessment (EMA) to characterize social isolation both objectively (e.g., number of social contacts) and subjectively (e.g., loneliness), and its biological mechanisms on aging (such as elevations in SASP and plasma amyloid); (2) post-pandemic, we will assess downstream effects on cognitive function, emotional well-being, and brain health, including AD risk, using neuropsychological assessments, EMA, and neuroimaging. Outcomes include (Aim 1) changes in cognitive performance and emotional well-being and decline in emotional well-being measured by positive

and negative effect and sleep quality; increases in biological aging and decreasing AB42/40 ratio in the post-pandemic phase, indicating higher risk of AD; and atrophy in hippocampal and prefrontal volume (structural MRI) and reduced global functional connectivity (resting state fMRI). Modifiers of these effects (Aim 2) include exercise and mindfulness; psychological resilience; COVID-19 exposure; medical morbidities; and APOE genotype. Mechanisms of cognitive, emotional, and brain health changes (Aim 3) include amyloid (Aβ40 and Aβ42), SASP, DNA methylation, and cortisol during the pandemic. This project will advance our knowledge of the impact of social isolation and other stressors on older adults, including mechanisms by which these stressors produce deleterious cognitive, emotional, and brain health changes over time, and whether exercise and mindfulness have durable protective effects.

PUBLIC HEALTH RELEVANCE: As referenced above, the COVID-19 pandemic provides an undesired natural measure of the detrimental effects of stress and social isolation on older Americans' cognitive and emotional health and risk for Alzheimer's disease. This project will advance our knowledge of the impact of social isolation and loneliness on older adults, including mechanisms by which these stressors produce negative cognitive, emotional, and brain health changes and how we can prevent those negative effects.

Additional Initiatives Stemming from the Cognitive Aging Summit III

In addition to RFA-AG-21-015 providing support for the two network grants to identify, evaluate, and track cognitive SuperAgers, which was jointly sponsored by the MBRF and the NIA, the NIA launched two additional research initiatives based on knowledge gaps and research opportunities identified from the Cognitive Aging Summit III.

One of the recommendations from the 2017 Summit was to support a longitudinal study of rats that would closely track the animals throughout their lives. NIA's Intramural Research Program (IRP) implemented that recommendation via a longitudinal study in rodents, "Successful Trajectories of Aging: Reserve and Resilience in Rats" (STARRRS). The award was made to Dr. Peter Rapp in the IRP. The study is on track to generate state-of-the-art neuroimaging, along with phenotypic results, non-invasive biological samples, plus other indicators that NIA hopes will yield insight into the mechanisms of healthy neurocognitive aging. The overarching goal of STARRRS is to establish an open resource of longitudinal data from male and female rats, including detailed behavioral characterization and neuroimaging, tissues and other biospecimens, for research on mechanisms of reserve and resilience in aging, and to inform resilience to Alzheimer's disease and related dementias. As of the end of 2023, 440 animals have been enrolled into the project, including animals that now have completed or are nearing completion of the longitudinal study. Brain MRI scans have been collected on almost 100 animals at two time points, along with data from motor activity, memory, attention, olfaction, frailty, and anxiety assessments.

An additional recommendation from the 2017 Summit was to develop operational definitions of constructs such as cognitive reserve, resilience, compensation, etc., that could be used uniformly by researchers. The Summit brought together a multidisciplinary group of investigators with shared interest in research on age-related cognitive decline as well as cognitive reserve and resilience. There was unanimous agreement that a significant barrier to progress in the field was the lack of clear and universally accepted definitions of important concepts related to cognitive reserve and resilience, and that it was imperative to address this deficit. An RFA (RFA-AG-18-024) was released by NIA, and one award was made to Dr. Yaakov Stern and Columbia University Health Sciences for a network grant titled "Collaboratory on Research Definitions for Cognitive Reserve and Resilience" (R24 AG061421).

Through a no-cost extension this past year, Dr. Stern and his co-investigators (Drs. Marilyn Albert, Carol Barnes, Roberto Cabeza, Alvaro Pascual-Leone, and Peter Rapp) were able to continue work on this effort and to hold a fourth workshop. The website for the effort, https://reserveandresilience.com/, contains information for these four workshops, the latest being in early December 2023. The framework for operational definitions of reserve and resilience concepts was published in 2023 in Neurobiology of Aging, along with a Commentary by Dr. Wagster and Dr. King. Both publications are attached to this report. Besides conducting workshops and developing and publishing the framework, this grant allowed for the award of pilot grants to explore and expand the constructs of resilience and reserve in the service of the framework development as well as to establish resources for future exploration. A publication was generated in 2023, resulting in part from one of the pilot awards (see Appendices for full publication): Gray, D. T., et al., Extracellular matrix proteoglycans support aged hippocampus networks: a potential cellular-level mechanism of brain reserve, Neurobiology of Aging, 2023.

The abstract for R24AG061421:

DESCRIPTION (provided by applicant): Research indicates that specific life exposures and genetic factors contribute to some people being more resilient than others, with lower rates of cognitive decline with aging, and reduced risk of developing Alzheimer's disease and related dementias (ADRD). There are likely several complex and highly interactive mechanisms that lead to these individual differences in vulnerability to decline, probably reliant on both structural and functional brain mechanisms. Key concepts often used in research in this area are cognitive reserve, brain reserve and brain maintenance. However, the definitions of these concepts differ across researchers, and the translation from human to animal research is not well developed. Also, their relationship to other invoked concepts such as efficiency, capacity, and compensation are not well explicated. The goal of this project is to work towards achieving state-of-the-art definitions for these concepts to allow researchers to use common nomenclature. In addition, the goal is to validate approaches to help advance research on these approaches that will lead to better maintenance of brain and cognitive health and treatment and/or prevention of ADRD. To that end, we will hold three cross-discipline workshops that will bring together investigators to discuss and come to consensus on these concepts,

create focused workgroups that will examine each of these issues, fund pilot grants designed to further the understanding and research applicability of these concepts, and develop data sharing and information exchange platforms to help guide and promote research in this area.

PUBLIC HEALTH RELEVANCE: To achieve state-of-the-art definitions and research guidelines for key concepts associated with resilience against cognitive aging and Alzheimer's disease related dementias, this project will hold three multidisciplinary workshops, establish focused work groups, create a data sharing and information platform, and support pilot grants designed to further the understanding of these concepts.

APPENDIX

- Journal of the American Geriatrics Society: Medical characterization of cognitive SuperAgers: Investigating the medication profile of SuperAgers
- Journal of the International Neuropsychological Society: NIH Toolbox® Episodic Memory Measure Differentiates Older Adults with Exceptional Memory Capacity from those with Average-for-Age Cognition
- Neurobiology of Aging: A framework for concepts of reserve and resilience in aging
- Neurobiology of Aging: Lost – and Found – in Translation
- Neurobiology of Aging: Extracellular matrix proteoglycans support aged hippocampus networks: a potential cellular-level mechanism of brain reserve

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Medical characterization of cognitive SuperAgers: Investigating the medication profile of SuperAgers

INTRODUCTION

Aging is associated with decline in cognition, with episodic memory changes representing the most common complaint of older adults.[1] SuperAgers are 80+ years with episodic memory capacity at least equal to persons in their 50s to 60s.[2] Their youthful memory phenotype offers a unique model for identifying factors for optimizing healthspan. Initial investigations have identified biologic, genetic, and psychosocial features that distinguish SuperAgers from their average episodic memory peers.[2–4] However, medications have not been characterized.

Medications, both as therapies supporting cognition and as indicators of overall health may contribute to the youthful SuperAging phenotype. Polypharmacy (i.e., use of >5 medications), affects ~40% of US older adults and is associated with increased risk of adverse drug events, falls, and mortality. When considering medication type, opiates, benzodiazepines, and non-benzodiazepine hypnotics are on The American Geriatrics Society (AGS) Beers Criteria list of potentially inappropriate medications (PIMs) for older adults, in part due to their detrimental effects on cognition. Conversely, common medications from antihypertensives to statins to vitamin D have been investigated for possible memory benefits.[5,6]

This study examined whether medication profiles differed between SuperAgers and controls.

METHODS

Community-dwelling participants age 80+ were prospectively enrolled as SuperAgers or cognitively average older controls. Detailed inclusion criteria have been previously reported.[2] Briefly, SuperAgers must perform at or above average normative values for 50–65-year-olds in episodic memory and at least average-for-age normative values in other cognitive domains. Controls were required to perform average-for-age across cognitive domains. The study received institutional review board approval and informed consent was obtained.

Participants reported current medications and supplements, dosage, and duration for each medication/supplement. Staff verified responses. Two physicians independently categorized medications as prescription or OTC; discrepancies were adjudicated by consensus.

Secondary analysis further classified participants as users/non-users of 10 medications/medications classes. Aspirin, angiotensin-converting enzyme inhibitors/angiotensin receptor blockers, and statins were highlighted given their roles in cardiovascular health. Using the updated AGS Beers Criteria, diuretics, opiates, benzodiazepines, and non-benzodiazepines hypnotics were examined as PIMs. Vitamin D, metformin, and thyroid hormones were included for their potential role in supporting cognition.

Linear regression models were used to analyze differences in the number of medications (prescription, OTC, total medications) used, and logistic regression was used to model binary variables (use versus non-use) for 10 specific medications or medication classes. Race, gender, and age were included as covariates. Uncontrolled *t*-test and Fisher's exact tests were performed for continuous and binary variables respectively. Significance was set at $p = 0.05$.

RESULTS

Table 1 provides demographics and neuropsychological performance for 96 SuperAgers and 46 controls. No significant difference was detected in total, mean prescription, or OTC medication use between SuperAgers and controls in the uncontrolled *t*-test or the linear regression controlling for age, gender, and race (Figure 1). The specified medications/medication use categories also showed no significant difference between groups (Figure 1).

DISCUSSION

The medication profiles of SuperAgers, older adults with exceptional episodic memory, showed no significant difference compared to cognitively average-for-age older controls in total medications, prescription medications, OTC medications, or in 10 medications/medication categories of interest. On average, prescription medications were higher in the current study (SuperAgers: 3.48, controls 3.20) than in larger epidemiologic studies like the Bronx Aging Study (BAS: 2.3) and the Monongahela Valley Independent Elders Survey (MoVIES: 2.0).[7,8] Notably,

Janessa R. Engelmeyer and Alice Kerr contributed equally to the work.

TABLE 1 Demographics and neuropsychological performance.

Demographics	SuperAgers ($n = 96$) [range]	Cognitively normal controls ($n = 46$) [range]
Age (years)	82.3 ± 3.4 [80–101]	84.2 ± 4.7 [79–102]
Education (years)	16.3 ± 2.4 [12–20]	16.7 ± 2.9 [6–20]
Sex, men:women	26:70	17:29
Race, Caucasian:African American	85:11	42:4
Handedness, right:left:ambidextrous	90:4:2	44:2:0
Neuropsychological test performance		
RAVLT delay recall raw score	11.0 ± 1.9	5.7 ± 1.3
Trail making test B raw score	87.9 ± 3.4	106.6 ± 43.3
Category fluency: Animals raw score	21.4 ± 5.2	19.0 ± 5.0
Boston naming test (BNT) 30 item raw score	28.1 ± 2.3	26.9 ± 2.9
Weschler test of adult reading (WTAR) estimated FSIQ (80+)	115.5 ± 8.0	114.4 ± 9.1

Note: Data are shown as means ± standard deviations and [ranges] RAVLT delay: measure of episodic memory with possible scores ranging from 0 to 15. Trail making test B: timed measure of executive function, testing discontinued at 300 s. Category fluency: measure of semantic fluency in which participants list as many items as possible (animals) in 60 s. BNT: measure of object naming with possible scores ranging from 0 to 30. WTAR FSIQ: measure of premorbid intelligence.

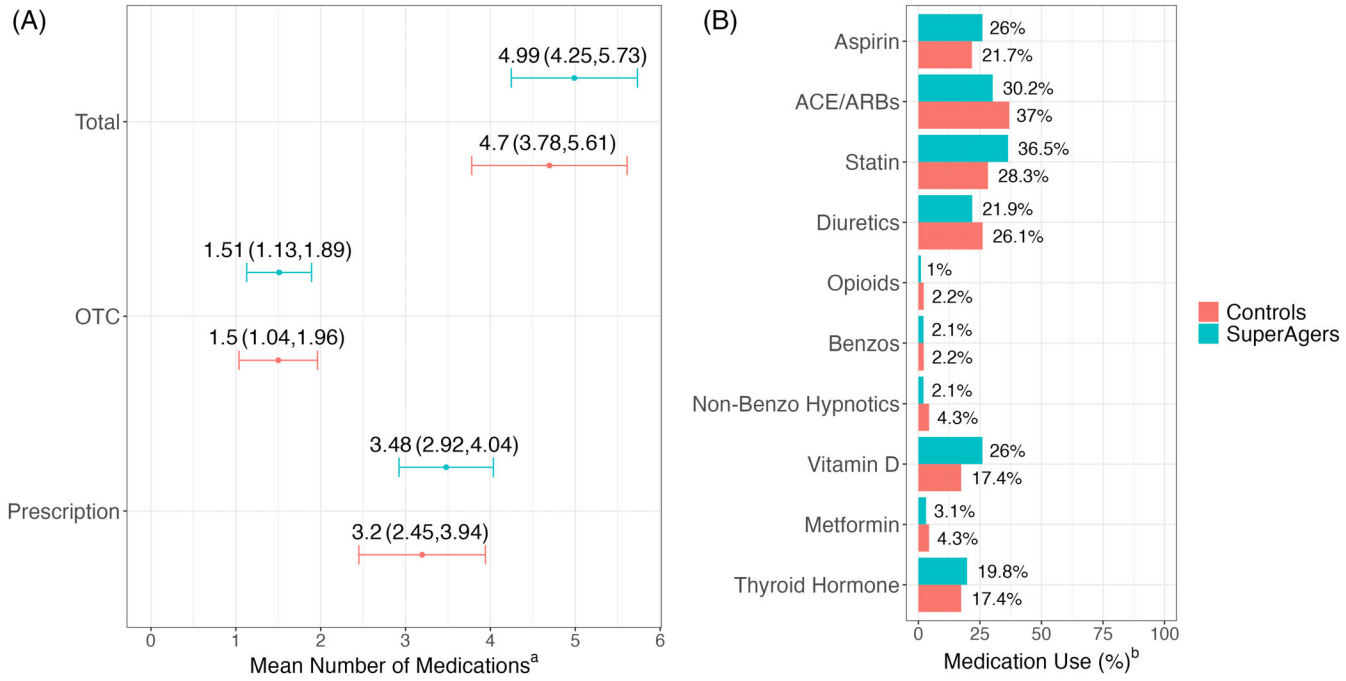

FIGURE 1 Medication use does not differ between SuperAgers and controls. (A) Mean number of medications did not differ between SuperAgers and controls (p values, total: 0.63, OTC: 0.97, prescription: 0.55; or controlled p-values, total: 0.37, OTC: 0.85, prescription: 0.37). (B) Percent of individuals taking a medication did not differ between SuperAgers and controls for p-values range: 0.29–0.82; controlled p-values range: 0.16–97). [a]p-value from a t-test for difference of means. Controlled p-value takes race, gender, and age into consideration using a linear regression analysis. [b]p-value from a Fisher's exact test. Controlled p-value takes race, gender, and age into consideration using a logistic regression analysis. ACE/ARBs, angiotensin-converting enzyme inhibitors/angiotensin receptor blockers; benzos, benzodiazepines; OTC, over the counter.

these studies were completed over 20 years prior with younger participants (average age: 79.2, 73.1 years, respectively). Higher use in the current study likely reflects temporal changes rather than intrinsic medical differences, given that prescription use increases over the life span and in recent decades.[9]

Use of potentially inappropriate medications tended to be lower in this study than National Social Life, Health, and Aging Project (NSHAP) cohort, a representative sample of adults aged 57–85 at enrollment.[10] Statins were the most commonly used medications for both SuperAgers (36.5%) and controls (28.3%), while the NSHAP was 46.2%. Similarly, the NSHAP participants reported higher aspirin use (40.2%) compared to SuperAgers (26.0%) and controls (21.7%). Definitive conclusions cannot be drawn without statistical comparison; however, higher use of these medications in larger, representative samples of older adults relative to this study raises the possibility our controls may not represent typical older adult medication use.

In summary, while SuperAgers differ in memory performance from controls, their medication use—total, prescription, and pre-specified subclasses of medication use—did not differ. Thus, distinctive medication profiles cannot fully account for memory performance differences between SuperAgers and cognitively average older adults. However, our previous findings point to slower brain atrophy and psychosocial factors, as potential contributors to youthful memory performance.[2–4]

AUTHOR CONTRIBUTIONS

Janessa R. Engelmeyer contributed to data acquisition, analysis, and drafted the manuscript. Alice Kerr contributed to data analysis, interpretation and drafted the manuscript. Beth A. Makowski-Woidan contributed to data acquisition and critical revision of the manuscript. Nathan P. Gill and Hui Zhang contributed to data analysis, interpretation, and critical revision of the manuscript. Lee Lindquist contributed to analysis, interpretation, and critical revision of the manuscript. M.-Marsel Mesulam, Sandra Weintraub, and Emily J. Rogalski contributed to the study conception and design, data acquisition, and critical revision. All authors gave final approval and agreed to be accountable for all aspects of the work.

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CONFLICT OF INTEREST STATEMENT

Emily J. Rogalski, M.-Marsel Mesulam, Hui Zhang, Nathan P. Gill, Lee Lindquist, and Sandra Weintraub report NIH funding. Emily J. Rogalski, M.-Marsel Mesulam, and Sandra Weintraub report receiving honoraria.

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The sponsor was not involved in the design, methods, subject recruitment, data collection, analysis, or preparation of the manuscript.

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BRIEF COMMUNICATION

NIH Toolbox® Episodic Memory Measure Differentiates Older Adults with Exceptional Memory Capacity from those with Average-for-Age Cognition

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Abstract

Objective: Older adults with exceptional memory function, designated "SuperAgers," include individuals over age 80, with episodic memory at least as good as individuals ages 50s–60s. The Northwestern University SuperAging cohort is defined by performance on an established test of verbal memory. The purpose of this study was to determine if superior verbal memory extends to nonverbal memory in SuperAgers by examining differences in the National Institutes of Health Toolbox® (NIHTB) between older adults with exceptional memory and those with average-for-age cognition. **Method:** SuperAgers ($n = 46$) and cognitively average-for-age older adults ($n = 31$) completed a comprehensive neuropsychological battery and the NIHTB Cognition module. Multiple linear regressions were used to examine differences on subtests between groups. **Results:** There was a significant effect of group on the Picture Sequence Memory score, ($p = .007$), such that SuperAgers had higher scores than cognitively average-for-age older adults. There were no other group effects across other non-episodic memory NIHTB Cognition measures. **Conclusions:** Findings from this study demonstrated stronger performance on the memory measure of the NIHTB in SuperAgers compared to cognitively average-for-age older adults demonstrating superior memory in not only verbal but also nonverbal episodic memory in this group. Additionally, this study adds to the literature validating the NIHTB in older adults, particularly in a novel population of adults over age 80 with exceptional memory.

Keywords: "SuperAgers", normal aging, NIH Toolbox, memory, cognition, dementia

INTRODUCTION

Decline in memory functions is often accepted as part of "normal" aging, with mild changes beginning in mid-life and more accelerated changes occurring over the age of 60 (Nyberg et al., 2012). However, at the Northwestern Mesulam Center for Cognitive Neurology and Alzheimer's disease, we have identified a group of individuals that we designated "SuperAgers," who are over age 80 and able to maintain superior memory performance compared to their same age peers and at a level that is at least "average" for 50- and 60-year-olds (Rogalski et al., 2013). Longitudinal follow-up of these individuals suggests that superior memory performance can be maintained over time, providing additional support for their resistance to the typical age-related decline (Gefen et al, 2014; Rogalski et al., 2019). With respect to psychological factors, SuperAgers report greater levels of social relationships compared to cognitively average-for-age peers (Cook Maher et al., 2017). Neuroimaging studies have demonstrated greater cortical integrity and slowed rates of atrophy compared to cognitively average age-matched peers and thicker anterior cingulate cortex compared to

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50-–65-year-olds (Harrison et al., 2012; Cook et al., 2017). Further, post-mortem studies suggest a lower frequency of Alzheimer neuropathology and higher density of von Economo neurons in the anterior cingulate compared to cognitively average older adults and individuals with amnestic mild cognitive impairment (Gefen et al., 2015).

The operationalization of memory capacity in SuperAgers was defined on the basis of scores on the Rey Auditory Verbal Learning Test (RAVLT), a difficult 15-item list-learning test of verbal episodic memory, which is widely used and has good psychometric properties. However, performance on tests of other episodic memory measures, including memory tests that place less emphasis on verbal abilities, has not been systematically investigated in this population. A recent research tool that was designed to measure cognitive functions in adults is the National Institutes of Health Toolbox® for Assessment of Neurological and Behavioral Function (NIHTB; Gershon et al., 2013; Weintraub et al., 2014). Traditionally, the evaluation of cognitive abilities in older adults has included either brief cognitive screening measures or lengthy neuropsychological batteries that often require clinical expertise and these batteries frequently differ across studies, making direct comparisons difficult to conduct. The NIHTB is a computerized suite of tests that measure cognitive, emotional, motor and sensory domains in individuals aged 3–85, and was designed to be used across a variety of settings, particularly in longitudinal research studies so that findings across studies could be conveniently compared. The Cognition Module includes a test of episodic memory, the Picture Sequence Memory test, that relies less heavily on verbal abilities, requiring participants to recall sequences of pictured actors and actions in the order they were originally learned over several trials.

The present study examined differences across all subtests of the Cognition module of the NIHTB between older adults with exceptional memory and those who are cognitively average-for-age. Our particular focus was to determine if the NIHTB episodic memory test specifically, which is less reliant on verbal abilities in comparison to our gold-standard of memory capacity, would be sensitive to differences between SuperAgers and "normal" agers. This is important as it would extend the contexts in which SuperAgers display superior episodic memory performance and opens the possibility of using the NIHTB as an efficient tool for future identification of SuperAgers. This study is also the first characterization of NIHTB Cognition module in an established cohort of older adults with exceptional memory. Given that SuperAgers display superior memory capacity, we hypothesized that the SuperAger group would demonstrate greater performance on the episodic memory test of the NIHTB.

METHODS

Participants

Participants 80 years or older were recruited through the Mesulam Center and Northwestern's Alzheimer's Disease Research Center Clinical Core, community lectures, and/or word of mouth. SuperAgers were referred on the basis of high memory scores and the absence of impairment in any other cognitive domain but were not necessarily superior in non-memory domains. Inclusion criteria for SuperAgers included: (1) score at or above the average level for 50–65-year-olds (equivalent to the Superior range for their own age) on the delayed recall condition of the (RAVLT; Schmidt, 1996), a 15-word list-learning memory test; and (2) performance within one standard deviation of the average range for their age on nonmemory measures including the Trail Making Test Part-B, Category Fluency Test, and 30-item Boston Naming Test according to published normative data (Heaton, 2004, Randolph, 1998, Mack et al., 1992). Inclusion criteria for Cognitively Average-for-Age Older Adults included: performance within the average-for-age normative range on the RAVLT and on all non-memory tests administered in the study. Full scale IQ was measured using the Wechsler Adult Intelligence Scale, Third Edition (WAIS-III). Additional inclusion criteria for both groups were that all participants maintained their cognitive status (as measured by neuropsychological battery described above) from their visit to the time the NIH Toolbox® was administered to maintain the integrity of our SuperAger sample. The administration of the NIHTB and collection of the neuropsychological battery occurred no more than three months apart. Additionally, all participants were required to have preserved activities of daily living. Participants with significant neurologic or psychiatric illnesses were excluded. All participants provided written informed consent. The Institutional Review Board at Northwestern University approved all study procedures. Research was completed in accordance with the Helsinki Declaration.

Study Measures

As described in previous studies (Gefen et al., 2014), all participants underwent a neuropsychological battery, including measures of attention, executive functions, language, and episodic memory. Participants completed the Cognition module of the NIH Toolbox® as part of the biyearly standardized battery. The Cognition Battery consists of tests assessing Executive Function and Attention (Dimensional Change Card Sort Test and Flanker Inhibitory Control and Attention Test), Episodic Memory (Picture Sequence Memory Test), Language (Oral Reading Recognition Test and Picture Vocabulary Test), Processing Speed (Pattern Comparison Processing Speed Test), and Working Memory (List Sorting Working Memory Test) (Weintraub et al., 2014). In addition to individual test scores, Cognitive Function, Fluid Cognition, and Crystallized Cognition composite scores are computed. In the Picture Sequence Memory Test, participants are shown a series of pictures depicting a sequence of events, for example, playing in the park. Then, the pictures are assembled in the center of the screen and participants are asked to reproduce the spatial placement of the previously demonstrated sequence of pictures. For additional details on these modules, refer to the original publications (Weintraub et al., 2013; Gershon et al., 2010; Gershon et al., 2013) and the NIHTB website (nihtoolbox.org).

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Table 1. *Study sample characteristics and NIHTB subtest scores*

	SuperAgers ($n = 46$)	Cognitively average-for-age 80+ year-olds ($n = 31$)	Test statistic (t or χ^2)
Demographics and estimated IQ			
Age, mean (SD)	84.2 (3.3)	84.0 (3.9)	.3
Sex, M:F	10:21	16:30	.1
Race, CA:AA	43:3	24:7	.3
Years of education, mean (SD)	17.2 (2.1)	16.5 (2.0)	1.3
WAIS-FSIQ, mean (SD)	133.0 (12.2)	118.7 (15.2)	4.5***
Performance on Standardized Neuropsychological Measures used for Study Inclusion			
RAVLT Delay Raw, mean (SD)	11.0 (1.8)	5.2 (1.4)	1.3***
BNT-30 Raw, mean (SD)[a]	28.2 (1.6)	26.3 (2.8)	3.5**
Animal Fluency Raw, mean (SD)[b]	22.7 (4.4)	19.8 (5.3)	2.6**
Trails B Raw (s), mean (SD)	78.9 (28.3)	104.0 (42.9)	2.9**
NIH toolbox performance			
Oral reading recognition (Language), mean (SD)	7.4 (1.3)	6.2 (2.1)	.03
Picture vocabulary (Language), Mean (SD)	7.8 (1.7)	6.9 (2.0)	.1
Flanker inhibitory control (Executive Attention), mean (SD)	7.4 (.6)	7.2 (.9)	.5
Dimensional Card Sort, (Executive Switching), mean (SD)	7.5 (.7)	7.4 (.8)	.5
Pattern comparison (Processing Speed), mean (SD)	35.5 (9.4)	33.5 (8.0)	.0
List sorting (Working Memory), mean (SD)	15.5 (2.4)	14.6 (3.1)	.1
Picture sequence memory (Episodic Memory), mean (SD)	441.2 (65.8)	388.0 (58.9)	7.7**

*$p < .05$, **$p < .01$, ***$p < .001$
SD = standard deviation; M = male; F = female; CA = Caucasian; AA = African American.
*Note two participants (1 control, 1 SuperAger) had low Boston Naming Test (BNT) scores at the time of NITB but met qualifying criteria at their initial visit. Four participants (3 Controls, 1 SuperAger) had consistently low BNT scores, for one English was a second language and may have contributed to lower scores. These participants were retained in all analysis as there were no other objective or subjective reports of difficulty with language.

Statistical Analyses

Differences in participant demographics were assessed using two-sample *t*-tests. NIH Toolbox® scores were summarized using frequencies and percentages for categorical variables or mean and standard deviation for continuous variables. Histograms and scattered plots of each NIH Toolbox® measure were examined to explore the shape of distributions and identify potential outliers. For the Cognition Module, computed scores were calculated for Flanker, Dimensional Change Card Sort, Pattern Comparison, and Picture Sequence Memory subtests, theta scores were calculated for Reading and Vocabulary, and raw scores were used for List Sorting. For details regarding computation of theta and computed scores, refer to NIHTB Scoring and Interpretation Guide (http://www.healthmeasures.net/images/nihtoolbox/Training-Admin-Scoring_Manuals/NIH_Toolbox_Scoring_and_Interpretation_Manual_9-27-12.pdf). Composite scores were calculated by averaging the normalized scores of each measure, and then deriving scale scores based on this new distribution. For each NIH Toolbox® measure, multiple linear regressions were used to examine differences between groups. Covariate adjustments included Wechsler Adult Intelligence Scale -Full Scale Intelligence Quotient (WAIS-FSIQ), sex, age, and education. Linear regression model fit was assessed using measures of collinearity and non-linearity, including residuals *versus* fits plots, histograms, Q-Q plots of residuals, and Dfbeta statistics. Adjusted R2 values were used to summarize variability explained in the linear models. All analyses were conducted in R 3.5.3 software.

RESULTS

Groups did not differ with respect to age, race, years of education, or sex (*ps* > .05) (Table 1). SuperAgers had a higher WAIS-FSIQ than cognitively average-for-age older adults ($p < .001$). Across all linear models, r-squared values ranged from .03–.34. Collinearity was not a concern, with pairwise correlations ranging from 0 to .56 for model covariates. Multicollinearity was not a concern, with variance inflation factor ranging from 1.03 to 1.79. Dfbeta statistics indicated there are some observations of influences. In order to improve linearity for measures that were positively skewed, scores were log-transformed, which included scores on the Picture Sequence Memory test.

Within the Cognition Module, there was an effect of group on Picture Sequence Memory scores (F (1,63) = 7.7, $p = .007$, $\beta = .10$), such that the SuperAger group had higher scores than the Cognitively Average-for-Age Older Adults (Figure 1). The effects of sex, age, education level, and FSIQ were not significant in this model. There were no other

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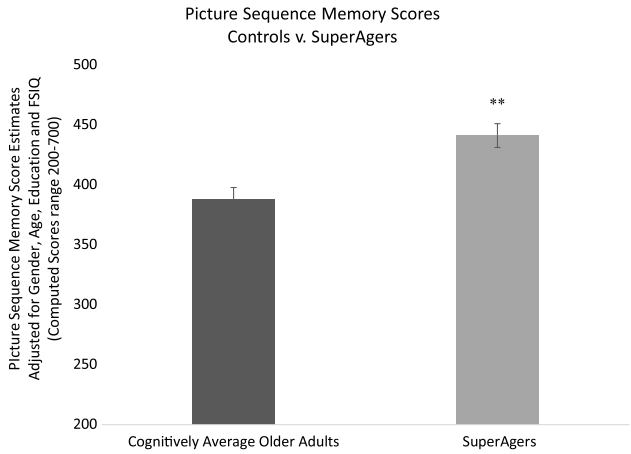

Fig.1. SuperAgers perform significantly better than cognitively average-for-age 80+ year-olds on the NIH Toolbox® Picture Sequence Memory. $^{**}p < .01$

effects of group across all other NIHTB Cognition tests of non-memory domains or on Composite Scores (all *p*s > .05).

DISCUSSION

This study sought to extend the superiority of episodic memory compared with other cognitive domains in the Northwestern University SuperAging cohort by comparing SuperAgers with cognitively average-for-age older adults on the tests of the NIHTB Cognition Battery. Findings of this study demonstrated greater performance on a test reliant on nonverbal episodic memory, the Picture Sequence Memory Test, in the SuperAging group compared to cognitively average-for-age older adults. Performance across all other measures of cognition on the NIHTB were comparable between groups. These findings confirm the exceptional episodic memory in SuperAgers.

The criteria for inclusion in the Northwestern University SuperAging research program involve completion of neuropsychological measures examining multiple aspects of cognition, with particular emphasis on memory abilities. SuperAging status requires performance at or above the average level of 50–65-year-olds on the RAVLT, a well-established measure of verbal episodic memory shown to be sensitive to early changes in memory and structural brain changes in Alzheimer's disease (e.g., Estévez-González et al., 2003). Longitudinal studies of SuperAgers have demonstrated that superior memory performance tends to be stable, suggesting that exceptional memory capacity is not necessarily a function of superior premorbid cognitive abilities, but rather a resistance to age-related cognitive changes (Gefen et al., 2014; Rogalski et al., 2019). The finding that performance on the NIHTB Picture Sequence Memory, a measure of episodic memory that places less emphasis on verbal memory abilities, also differentiates the SuperAging group and cognitively average-for-age older adults is further confirmation of the memory superiority in SuperAgers. This suggests that superior memory capacity in SuperAgers may not be specific to the list learning of the RAVLT, but is more general to episodic memory. Additionally, scores on all other NIHTB measures were similar between groups, which mirrors our criteria that SuperAgers may score in at least the average range on all other measures of cognition, including object naming, semantic fluency, and executive attention. Although it is unclear why there were no differences at the group level across other NIHTB subtests, it is possible there may be nuanced profiles at the individual level; this is similar to what was observed in recent work from the SuperAging Research Program, which demonstrated significant intragroup variability on multiple cognitive domains (Maher et al., 2021). Additional explanations include differences in the specific domains assessed by our neuropsychological measures *versus* the NIHTB (i.e. verbal fluency), as well as differences in tests used in this study compared to the neuropsychological tests used to validate the NIHTB (Weintraub et al., 2013).

The NIHTB has been validated in older adults without cognitive impairment and has also been investigated in older adults with varying degrees of cognitive impairment. In particular, one study examined the psychometric properties of the NIHTB cognition module in cognitively intact older adults and found acceptable test-retest reliability over a one-year period, as well as a relationship between NIHTB Fluid Composite and cerebral volumes, and a strong correlation between Fluid and Crystallized Composites with their respective gold standard composites (Scott et al., 2019). In a study of older adults with subjective decline, mild cognitive impairment, or mild dementia, performance on the NIHTB Cognition module was consistent with performance on traditional neuropsychological tests and had greater discriminative ability when supplemented with RAVLT delayed recall performance (Hackett et al., 2018). Further, neuroimaging studies have demonstrated relationships between NIHTB performance and hippocampal volume and tau deposition in older adults (O'Shea et al. 2016; Snitz et al., 2020). One important limitation is that the sample was a predominantly white, well-educated group, and therefore replication with a more diverse sample is needed. The present study is one of the first, to our knowledge, to examine performance in the NIHTB in an established cohort of adults over age 80 with exceptional memory and adds to the utility of using the NIHTB to measure cognitive functioning in the oldest of old age groups.

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CONFLICTS OF INTEREST

The authors have no conflicts of interest to disclose.

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Commentary

A framework for concepts of reserve and resilience in aging

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ABSTRACT

The study of factors, across species, that allow some individuals to age more successfully than others has important implications for individual wellbeing as well as health education, policy and intervention. Design of studies and communication across investigators in this area has been hampered by a diversity of terminology. The Collaboratory on Research Definitions for Reserve and Resilience in Cognitive Aging and Dementia was funded by the National Institute on Aging and established in 2019 as a 3-year process of developing consensus definitions and research guidelines. The proposed Framework is based on an iterative process including 3 annual Workshops, focused workgroups, and input from numerous international investigators. It suggests the overarching term: resilience, and presents operational definitions for 3 concepts: cognitive reserve, brain maintenance, and brain reserve. Twelve pilot studies that integrate these definitions are presented. The use of a common vocabulary and operational definitions will facilitate even greater progress in understanding the factors that are associated with successful aging.

1. Introduction

The study of factors that allow some individuals to age more successfully than others, including for example genetics and life exposures, has important implications for individual wellbeing as well as health education, policy and intervention. Moreover, identifying factors that are relevant across species (i.e., humans and nonhumans) is fundamentally necessary to facilitate studies of the neurobiological underpinnings of such factors.

In this context, overarching concepts like reserve and resilience are often invoked for capturing differential susceptibility to brain aging and disease. However, design of studies and communication across investigators in this area has been hampered by a diversity of terminology. Several groups have published proposed nomenclature and operational definitions for concepts including resilience, cognitive reserve (CR), brain reserve (BR), brain maintenance (BM), compensation, scaffolding, resistance, and resilience. Across these papers there are often disparate definitions for the same term. In addition, most of these papers focus on human studies, so the definitions and nomenclature are not optimally suitable for nonhuman studies.

The Collaboratory on Research Definitions for Reserve and Resilience in Cognitive Aging and Dementia was funded by the National Institute on Aging of the National Institutes of Health in the USA and established in 2019 as a 3-year process of developing consensus definitions and research guidelines for CR and related concepts. The present document is the result of an iterative process including 3 large annual Workshops, input from focused workgroups, and the extensive participation and consultation of over 40 selected, international expert investigators who utilize multiple research approaches and study both humans and nonhumans. Here we present a framework that includes definitions for 3 concepts, CR, BM, and BR, along with suggested operational definitions to help guide the design of research investigating these concepts. We also include resilience as an overarching term that subsumes all of the concepts presented.

Our aim is to present a well-defined set of operational definitions in order to encourage, advance, and develop research on

Abbreviations: CR, Cognitive reserve; BM, Brain maintenance; BR, Brain reserve.

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these concepts. At the same time, we want to encourage investigators who have different views or use a given concept differently to note how their definitions relate or differ with one of those described here. Similarly, this framework provides a basis for describing how the operational definition of another concept differs from those suggested here.

Our intention is not to limit the creativity or ingenuity of investigators, or to claim that the framework presents the only way to investigate these important concepts. We hope to encourage research that provides either evidence-based support for these concepts or that presents data that cannot be accommodated by the proposed operational definitions of these concepts. We also hope that referring to this framework will facilitate collaboration and comparison of findings across studies and species.

The Collaboratory also sponsored 12 studies that were intended to implement the suggested research guidelines and thus provide experimental examples of their operational utility. This disparate set of studies incorporates humans and nonhumans, as well as multiple approaches including epidemiologic, neuroimaging, and interventions. We include in the supplementary material descriptions of the pilot projects as wells as a table that summarizes the projects and how they incorporate the framework presented here. These provide useful real-world examples that illustrate how study designs can incorporate the suggested framework.

Our hope for this framework is that the use of a common vocabulary and operational definitions will facilitate even greater progress in understanding the factors that are associated with successful aging.

2. Resilience

The term resilience has been used in many contexts. Here we consider it a general term that subsumes any concept that relates to the capacity of the brain to maintain cognition and function with aging and disease. There can be substantial variability in the mechanisms underlying resilience. Here, we present 3, CR, BM, and BR.

3. CR

3.1. Definition

CR is a property of the brain that allows for cognitive performance that is better than expected given the degree of life-course related brain changes and brain injury or disease:

- Property of the brain refers to multiple potential mechanisms including molecular, cellular and network levels. The working hypothesis is that these mechanisms help cope with or compensate for brain changes and the consequences of brain injury or disease.
- These mechanisms can be characterized via biological or cognitive-experimental approaches.
- Better than expected cognitive performance refers to differences ideally measured longitudinally.

CR can be influenced by multiple genetic and environmental factors, operating at various points or continuously across the lifespan.

3.2. Operational definition: general considerations

Research aimed at further elucidating CR requires the inclusion of 3 components:

1. Measures of life course-related brain changes, insults, disease, or risk factors that theoretically impact cognitive outcomes,
2. Measures of associated change in cognition, and
3. A variable that influences the relationship between 1 and 2.

Ideally, the aim is to demonstrate that any proposed CR measure (e.g., a sociocultural or functional brain measure) moderates the relationship between 1 and 2. For example, in an analysis where change in brain atrophy/pathology measures (component 1) predict change in cognition (component 2), and includes education as a hypothesized CR proxy (component 3), there is a statistical interaction between brain measures and education, such that level of education significantly moderates the association of brain measures with cognitive change.

Even without evidence for moderation, it can also be sufficient to demonstrate that a hypothesized CR proxy or measure is associated with cognitive performance over and above (e.g., after adjusting for) the effects of brain change, pathology, or insult. For example, in a multiple regression analysis of change in cognition that includes brain atrophy/pathology measures and a hypothesized CR proxy, the proxy should account for variance in cognitive performance. In this analysis, the CR proxy simply adds predictive information (a protective factor), a weaker form of CR evidence than moderation.

All 3 components are needed when investigating CR. For example:

Demonstrating that expression of higher connectivity within a specific resting BOLD network is associated with slower cognitive decline is not sufficient to conclude that expression of this network reflects CR. To make a claim about CR it must also include measures of age-related brain change, insult or disease that theoretically impact cognitive outcomes.

Similarly, a relationship between a particular genotype and rate of cognitive decline would not be sufficient to conclude that this genotype is associated with CR. It would be important to demonstrate that the genotype's relationship to reduced rates of cognitive decline is expressed through moderation of age-related brain change or reduction of the expected impact on cognitive performance of a given brain insult or disease.

3.3. Specification of the 3 components needed to elucidate CR

3.3.1. Measures of life course-related brain developmental changes, injury, or disease that theoretically impact cognitive outcomes

This could consist, for example, of measures of anatomic changes such as loss of brain volume or white matter tract integrity, or onset and progression of disease pathology such as biomarkers of neurodegenerative disease.

These changes could be more extensively specified. Measures/mechanisms underlying aging that impact cognitive outcomes could include change in structure or function of synapses, oxidative damage/stress, impaired stress response signaling, Ca2+ dyshomeostasis and/or dysregulation, mitochondrial function, impaired waste disposal, inflammation, epigenetics, stem cell depletion, and altered neuronal activity/connectivity.

It is likely that unmeasured or unknown brain or pathologic changes contribute to inter-individual variance in the cognitive outcomes. Their eventual inclusion would increase the precision of elucidating CR.

3.3.2. Measures of cognition

This term encompasses measures of cognition and day-to-day function that change with aging and disease. When possible, it would be useful to adopt cognitive tests that show changes with age or brain disease, and that can be used across species. In this case, it is important to be mindful that formal operational similarity between human and nonhuman tasks is not sufficient, or even necessary; the tasks need to tap similar underlying neural systems.

3.3.3. CR proxy/mechanism:*a hypothesized variable that influences the relationship between 1 and 2*

As the definition of CR states, these mechanisms can be characterized via biological or cognitive experimental approaches.

Proxies for CR in human studies have included features associated with both endowment and experience, including early age IQ, cognitively stimulating exposures across the age span, education, occupational exposures, leisure activity, social networks, or other exposures, hypothesized or to be discovered, that might impart CR. Similar proxies such as behavioral training, physical exercise, environmental enrichment, social housing, or diet are applicable to nonhuman studies.

In addition, the nature of the CR proxy or mechanism that influences the relationship between component 1 and 2 can be explored. For example, investigators might explore whether differential expression of a specific functional network is associated the degree of sustained cognitive function in the face of age-related brain changes that impact cognition. More generally, mechanisms underlying CR could be specified at the molecular, cellular or network levels.

3.4. Example of studies of CR

In studies of CR, longitudinal designs optimally address the 3 features underlying the concept of CR. However, rich information can be gained from cross-sectional studies including discovering variables that appear to be critical for CR, establishing preliminary observations, providing insight into neurobiological mechanisms and developing research or conceptual approaches.

3.4.1. Longitudinal study incorporating measures of brain and cognitive change

In a longitudinal study, one could explore whether some life exposure conceptually linked to CR moderates the relationship between change in brain status (e.g., volume, white matter tract integrity, white matter hyperintensity burden) and change in cognition. For example, one could establish a relationship between age-related changes in cortical thickness/surface area, brain volume, and white matter tract integrity with changes in cognition. The potential moderation by education of this relationship could then be explored. Such moderation would provide support for the idea that higher education is associated with CR.

Some longitudinal studies may have no direct measures of brain change. Analyses that assume parity across all followed individuals or incorporate risk factors for brain changes could suggest hypotheses and guide subsequent studies.

3.4.2. Neural implementation of CR

Although variables such as IQ, education, occupational attainment etc. can be associated with CR as described in 4.1, that is, moderating between measures that theoretically impact cognitive outcomes (component 1) and measures of associated change in cognition (component 2), more insight into the mechanisms underlying CR might be obtained from studies that directly examine neural mechanisms. In both human and nonhuman studies, imaging techniques including functional MRI (fMRI), spectroscopy, and EEG are uniquely suited for longitudinal measurements, providing in-depth assessments of brain structure, neural activity, and the chemistry in the aging brain. CSF, plasma/serum, and extracellular vesicle biology in blood are advancing rapidly and may provide a translatable fluid biopsy for relevant brain changes in this context.

Thus, one goal might be to identify functional networks or circuits, whose differential expression moderates the relationship between age-related brain changes that impact cognitive outcomes and the associated change in cognition. For example, longitudinal studies of aging or neurodegenerative disease can investigate how the relationship between changes in structure/function and cognition/clinical status can be moderated by proposed reserve-related networks. It would be of interest to determine whether differential expression of this network is related to life exposures such as education or occupational experience. This would create a relationship between a proxy for CR and a potential brain mechanism underlying that proxy.

3.4.3. Intervention studies and natural experiments

Intervention studies can most directly test whether some exposure or mechanism underlies CR by examining whether the intervention moderates the effect of age-related brain changes on cognitive outcomes. These studies can help explore mechanisms underlying CR.

Similarly, controlled perturbations such as transcranial magnetic or direct current stimulation could model brain insult, stressor or disease. Alternately, they could be used to modulate activity in networks/circuits associated with CR, and by suppressing it or facilitating it, gain causal and mechanistic insights, and even potentially explore therapeutic interventions.

Sometimes, environmental changes can be used as natural experiments. A natural experiment is a situation when some change occurs in the environment that is not under experimental control and approximates random assignment. An example of such a natural experiment is changes to compulsory schooling laws. Conversely, animal models that feature increased individual differences in cognitive aging, under conditions of tightly controlled life-course exposures, can test for inherent genetic and biological moderators or mediators of CR. Quasi-experimental twin design is closest to this experimental design in humans. This design can utilize cotwins with different levels of exposures such as educational or occupational attainment.

4. BM

4.1. Definition

BM refers to the relative absence of changes in neural resources or neuropathologic change over time as a determinant of preserved cognition in older age.

BM can be influenced by multiple genetic and environmental factors, operating at various points across the lifespan.

4.2. Operational definition

BM is influenced by factors (genes, sex, early life influence or differential experiences) that slow or prevent brain changes associated with aging and disease. The emphasis centers on change over time. Thus, BM may be operationalized as minimal changes in brain markers of aging or disease associated with preservation of cognitive function.

Research aimed at further elucidating BM requires the inclusion of 3 components.

1. Measures of age-related brain changes, injury or disease that theoretically impact cognitive outcomes,
2. Measures of change in cognition.

Demonstrating a link between less change in 1 and less change in 2 would be evidence of BM.

To investigate potential mechanisms of individual differences in BM one could examine:

1. A hypothetical variable that influences 1.

This variable can encompass many of the same exposures potentially associated with CR. However, their impact on BM in this context would be specific to maintaining the structural and functional integrity of the brain.

4.3. Example studies of BM

BM is optimally ascertained in longitudinal designs. A single time point measurement cannot definitively differentiate people who have maintained their brain from those who did not but started at a higher baseline level. In both human and nonhuman studies this issue can be addressed to some degree by determining what level of brain status is expected for a particular age, or considering a given brain measure relative to the distribution seen in younger subjects. However, longitudinal designs are preferable to examine factors underlying interindividual differences in the change in neural resources that are in turn associated with differences in cognitive outcomes.

4.3.1. Longitudinal study of BM

A general approach to studying BM would be to examine longitudinally whether individual differences in the rate of age- or disease-related brain anomalies accumulated over time are related to individual differences in the rate of cognitive change.

4.3.2. Exposures related to BM

An extension of study 3.1 would be to assess potential proxies or mechanisms (e.g., genetic, lifestyle, neural) that are associated with these different trajectories of BM/change.

In summary, BM and CR are complementary concepts. BM accounts for individual differences in cognitive trajectories that are associated with differences in rate of brain change. In contrast, CR addresses individual differences in cognitive trajectories controlling for changes in neural resources or neuropathology.

5. BR

5.1. Definition

BR has been used to reflect the neurobiological status of the brain (numbers of neurons, synapses, etc.) at any point in time. BR does not involve active adaptation of functional cognitive processes in the presence of injury or disease as does CR.

5.2. Operational definition and example studies

Research aimed at further elucidating BR requires the inclusion of 2 components:

1. Measures of brain features that theoretically are associated with cognition.
2. Associated measures of cognition.

5.3. Example studies of BR

Longitudinally, differences in BR at a point in time could account for the observation that individuals starting at a different level of cognition may show the same rate of age- or disease-related cognitive decline. This could reflect different initial levels (intercepts) due to variation in BR, but similar rates of change (slopes) due to similar depletion of BR. This is distinguished from BM, where slopes would differ as a function of the degree of BM.

BR has also been associated with individual differences in level of cognition given a specific amount of brain change, injury or disease, such as amyloid plaques and neurofibrillary tangles. This association could rely on a threshold model, where a specific amount of depletion of neurobiological capital results disease-related changes. Those who initially have a higher BR can tolerate more depletion before they show symptoms.

6. Conclusion

Here we present a framework that includes well defined operational definitions for 3 concepts: CR, BM and BR. We also propose the term resilience to subsume all of the concepts presented. The operational definitions were carefully designed to be applicable to both human and nonhuman studies.

We believe that the use of a common vocabulary and operational definitions will facilitate research design and communication. The framework also provides a basis for describing how the operational definition of another concept differs from those suggested here.

Our hope for this framework is that a common vocabulary and operational definitions will facilitate even greater progress in understanding the factors that are associated with successful aging and lifelong brain health.

Disclosure statement

None of the authors have actual or potential conflicts of interest to disclose.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.neurobiolaging.2022.10.015. This includes: 1. Aknowledgment list 2. descriptions of funded pilot projects and 3,a table that summarizes the projects and how they incorporate the framework presented here.

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Commentary

Lost – and Found – in Translation

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In 2017, the National Institute on Aging (NIA) with the support of the Foundation for the National Institutes of Health on behalf of the McKnight Brain Research Foundation, held the Cognitive Aging Summit III. This third Summit was focused on cognitive reserve and resilience, highlighting the increasing importance of these concepts towards understanding age- and pathology-related cognitive change. At the same time, the Summit also highlighted important gaps in our shared understanding of how to characterize terms such as *reserve, resilience*, and *maintenance*; moreover, these gaps appeared to result from a lack of clear and consistent definitions of these and related terms. Disagreements over nomenclature and terminology are hardly new in psychology and neuroscience; indeed, the recognition of Jingle/Jangle fallacies in the measurement literature (Kelley, 1927) has long exposed the obstacles to knowledge advancement that these create. Especially clear at the Summit was that differences in usage of key terms had become serious enough to be more than just a nuisance, impeding (among other things) the ability of researchers using human vs. animal models, cross-sectional vs. longitudinal designs, or neuroimaging vs. neuropsychological approaches to appreciate each other's scientific contributions, much less potentially collaborate. In order to know whether and how a lifestyle, a gene or genes, a personality trait, other factors, or all of the aforementioned confer cognitive reserve, brain maintenance, or resilience to neurodegenerative disease, we need common definitions and a universal language.

When we use the same term to refer to different things (the Jingle Fallacy) or different terms to refer to the same thing (the Jangle Fallacy), fundamental knowledge accumulation and progress predictably grind to a halt. Given the gravity of the inconsistencies in the field, the NIA published a funding opportunity announcement (RFA-AG-18-024, "Collaboratory on Research Definitions for Cognitive Reserve and Resilience to Alzheimer's Disease") to drive the development of operational definitions for the constructs of resilience and reserve, as well as related and often used terms such as compensation, brain maintenance, and even resistance.

In 2018, an award was made to support a unique structure to develop uniform, research-based definitions. Designated as the Collaboratory, the award allowed the investigators to use multiple routes to build definitions and consensus by forming working groups and holding workshops, and to enable the cross-validation of proposed definitions and concepts by supporting small pilot projects.

Besides fostering a common language and thus understanding among researchers for these constructs, the framework developed by the team and presented in this issue of *Neurobiology of Aging* will have far-reaching impact. Understanding, treating, and preventing Alzheimer's disease and Alzheimer's disease related dementias is a national priority for the U.S. If we can harness the knowledge of what confers reserve and resilience, treatments could be targeted to mimic these factors and hopefully prevent or slow disease progression.

The paper in this issue describes the thought processes in which the blue-ribbon Executive Committee for the Collaboratory, aided by many experts around the globe, engaged to reach consensus and provide distilled guidance for the scores of researchers hoping to crack the code on how to gain or maintain successful cognitive performance and brain function with age. As the authors note, these definitions may be subject to revision as we gain more knowledge, but it is the generation of operational definitions like

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these in this framework that will help us more clearly communicate.

Uniformity has its benefits - a common language, operational definitions, and a platform from which to propose alternatives. If we aren't speaking the same language, we will be lost in the translation. This effort insures we can find a path forward.

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Regular article

Extracellular matrix proteoglycans support aged hippocampus networks: a potential cellular-level mechanism of brain reserve

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ABSTRACT

One hallmark of normative brain aging is vast heterogeneity in whether older people succumb to or resist cognitive decline. Resilience describes a brain's capacity to maintain cognition in the face of aging and disease. One factor influencing resilience is brain reserve—the status of neurobiological resources available to support neuronal circuits as dysfunction accumulates. This study uses a cohort of behaviorally characterized adult, middle-aged, and aged rats to test whether neurobiological factors that protect inhibitory neurotransmission and synapse function represent key components of brain reserve. Histochemical analysis of extracellular matrix proteoglycans, which play critical roles in stabilizing synapses and modulating inhibitory neuron excitability, was conducted alongside analyses of lipofuscin-associated autofluorescence. The findings indicate that aging results in lower proteoglycan density and more lipofuscin in CA3. Aged rats with higher proteoglycan density exhibited better performance on the Morris watermaze, whereas lipofuscin abundance was not related to spatial memory. These data suggest that the local environment around neurons may protect against synapse dysfunction or hyperexcitability and could contribute to brain reserve mechanisms.

1. Introduction

One often held misconception of brain aging is that neurodegenerative disease is inevitable; however, only 10%–14% of individuals over 65–70 years of age in the United States are demented (Manly et al., 2022; Plassman et al., 2007). This is not to imply that there are no cognitive changes that occur in normative aging. Rather, the extent of the decline and the domains impacted vary widely across healthy older individuals (e.g., Nyberg et al., 2012; Ryan et al., 2019). These individual differences may be a result of resilience or the capacity of the brain to maintain cognition and function across the lifespan (Stern et al., 2022). While there is still considerable debate regarding the exact nature of resilience, it can be achieved both through active adaptation and by tapping into existing neuronal resources (e.g., Cabeza et al., 2018; Stern et al., 2019, 2022). The factors that facilitate the engagement of brain reserve mechanisms can be either intrinsic to the brain or result from life exposure and can arise through a variety of mechanisms that range from molecular to cellular to network levels.

It has proven relatively challenging to identify robust intrinsic neurobiological features associated with resilience, particularly at cellular and molecular levels of analysis. One major reason for this challenge is that it is difficult to study mechanisms of resilience in animal models. This is because resilience is optimally assessed either through longitudinal study designs that can assess whether life exposure moderates the relationship between brain status and cognition or through cross-sectional studies that require very large sample sizes to appropriately sample the variability in cognitive outcomes necessary to study resilience (Stern et al., 2019, 2022). Despite these challenges, the much wider array of tools available to study the brain at multiple levels of analysis in nonhuman animals compared to humans highlights the importance of developing research aimed at understanding variability in cognitive function in these models of human aging. In rodents and nonhuman primates, one of the neurobiological features that most closely predicts cognitive performance is the number or function of synapses (e.g., Burke and Barnes, 2006; Dumitriu et al., 2010; Hara et al., 2012; Morrison

Abbreviations: CA3, cornu Ammonis 3; CIPL, Corrected Integrated Path Length; NDS, Normal Donkey Serum; OCT, Optimal Cutting Temperature; PBS, Phosphate Buffered Saline; WFA, *wisteria floribunda agglutinin*

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and Baxter, 2012; Smith et al., 2000). Thus, understanding the details of the variables that impact synapse health is critical to elucidating the biological mechanisms of cognitive resilience in aging. To this end, the present study combines histochemical and image-based analyses of tissue from a relatively large cohort of young adult, middle-aged, and aged rats to investigate potential neurobiological correlates of brain reserve, which is one mechanism by which resilience is thought to be accomplished.

Brain reserve refers to a neurobiological status that allows certain older individuals to evade robust declines in cognition. For a neurobiological factor to be considered brain reserve, it must be associated with some aspect of cognitive function independently of age (Stern et al., 2019, 2022). For example, it has been demonstrated that, regardless of age, rodents with more durable long-term potentiation (LTP) also have more durable memory (Bach et al., 1999; Barnes, 1979; Dieguez and Barea-Rodriguez, 2004). Furthermore, work from rodents, monkeys, and humans has shown that neuronal hyperexcitability of the CA3-dentate gyrus region of the hippocampus emerges with advanced age, most prominently in individuals that exhibit the greatest degree of memory impairment (Spiegel et al., 2013; Thomé et al., 2016; Wilson et al., 2005; Yassa et al., 2011). Immunohistochemical labeling and slice physiology of CA3 neurons indicate that age-associated deficits in inhibitory neurotransmission likely play central roles in this hyperexcitability (Spiegel et al., 2013; Thomé et al., 2016; Tran et al., 2018). These observations predict that neurobiological factors that work to maintain neuronal plasticity and excitatory-inhibitory balances in the hippocampus may represent a form of brain reserve for hippocampus-dependent aspects of cognition.

In this study, we provide evidence that the status of the brain extracellular matrix may represent a form of brain reserve, and we suggest that this is likely accomplished by mediating the relationship between altered inhibitory signaling, CA3/dentate gyrus hyperexcitability (e.g., Small et al., 2002; Thomé et al., 2016; Wilson et al., 2005; Yassa et al., 2011), and memory abilities later in life. The extracellular matrix is a network of proteins and sugars that are secreted from multiple cell types and play critical roles in regulating tissue hydration, ionic balances, and neuronal plasticity (Dityatev et al., 2010). Specialized insoluble aggregations of extracellular matrix proteoglycans called perineuronal nets have garnered significant attention due to their roles in regulating inhibitory neuron function (Sorg et al., 2016). Age-associated reductions in perineuronal nets around inhibitory neurons have been observed in the retrosplenial cortex (Gray et al., 2022; Ueno et al., 2019), which is a region that is critically involved in mnemonic processing and exhibits increased cFos expression (Haberman et al., 2017) and reduced functional connectivity (Ash et al., 2016) in memory-impaired aged rats. While perineuronal net structures in the cortex preferentially aggregate around inhibitory neurons (Brückner et al., 1993; Härtig et al., 1992), the proteoglycans in the hippocampus preferentially accumulate around the somas of CaMKII-expressing excitatory neurons in the pyramidal cell layers of CA2 and CA3 (Lensjø et al., 2017). Whether age impacts hippocampal perineuronal net structures distinctly from those in the cortex has not been assessed. Here, we examine the impact of age on extracellular matrix proteoglycans, specifically in CA3, of behaviorally assessed rats at different points of the lifespan.

2. Materials and methods

2.1. Subjects

A total of 29 adult (6–8 months), 33 middle-aged (15–17 months), and 34 aged (23–25 months) male F344 rats (Harlan Sprague-Dawley, Indianapolis, IN, USA) participated in the study. Rats were housed individually in Plexiglas guinea pig tubs on a reverse 12-hour light-dark cycle and were given free access to food and water. Rats were handled between 5 and 10 minutes per day following their arrival prior to behavioral testing. All protocols described adhered to NIH guidelines and guidelines set by the Animal Care and Use Committee at the University of Arizona (Tucson, AZ).

2.2. Behavioral testing

Rats were tested on the spatial and visually cued versions of the Morris watermaze (Morris, 1984). Rats that were not able to perform the visually cued version of the Morris watermaze were excluded from participation in the study due to the visual demands of this paradigm. The watermaze apparatus consisted of a circular pool with an approximate diameter of 120 cm and a depth of 36 cm. An escape platform was hidden beneath the surface of the water for the spatial version and just above the surface of the water for the cued version (Fig. 1A). Water was made opaque using nontoxic white Crayola paint and maintained at a temperature range between 26°C and 28°C. The apparatus sat in the center of a 2.3 × 2.73 × 2.5 m room that contained several visual stimuli on the walls as distal cues. A chair and a metal board were placed adjacent to the pool as proximal cues.

The full protocol for the Morris watermaze procedure used in this study has been published previously (Barnes et al., 1996). Briefly, the spatial version of the watermaze consisted of 4 sequential days of testing with each rat performing 6 trials per day. For each trial, the rats were given 1 minute to locate the escape platform. If the rat failed to locate the platform after 1 minute, it was guided and placed on the platform for 30 seconds. The procedure for the cued version of the watermaze was identical to the spatial version with the exception that testing was conducted over 2 consecutive days instead of 4. A corrected integrated path length (CIPL) measure was calculated as described in detail elsewhere (Gallagher et al., 1993; Shen and Barnes, 1996; Vorhees and Williams, 2014). Briefly, this measure is derived by calculating an average swimming speed for each animal on each trial as well as the amount of time required to swim to the escape platform in a straight line at that speed. This speed-corrected optimal pathlength is then removed from the record to get the CIPL score. This procedure corrects for the locations of the different start location entry points (some being closer to the platform than others). To evaluate improvement in task performance, a within-subject measure was derived by subtracting the average session 4 CIPL from the average session 1 CIPL (Day 1 – Day 4).

2.3. Histological preparations

Following behavioral testing, all rats were anesthetized with 5% isoflurane and decapitated using a rodent guillotine. The brains were rapidly extracted, flash-frozen in isopentane, cooled over an ethanol bath containing dry ice, and stored at −80°C until cryosectioning. For sectioning, brains were blocked in optical cutting temperature (OCT) compound such that an adult, middle-aged, and aged brain were included on the same slide to reduce technical variability. Sections of 20 µm in thickness were then cut and mounted using a cryostat. Slides were stored at −80°C.

The day before staining, the slides were moved into a −20°C freezer. On the first day of the histological protocol, slides were first removed from the freezer and allowed to thaw for approximately 20 minutes. The tissue was then fixed by submerging slides in 4% paraformaldehyde in phosphate buffer saline (PBS; Sigma-Aldrich, P-5368, 0.01 M, pH 7.4) for 30 minutes. Slides were rinsed in PBS buffer containing 0.01% Triton-X (PBS-TX) and then incubated for 1 hour in a blocking solution consisting of 1% normal donkey serum (NDS; Sigma-Aldrich, D9663) in PBS-TX. A hydrophobic pen was used to

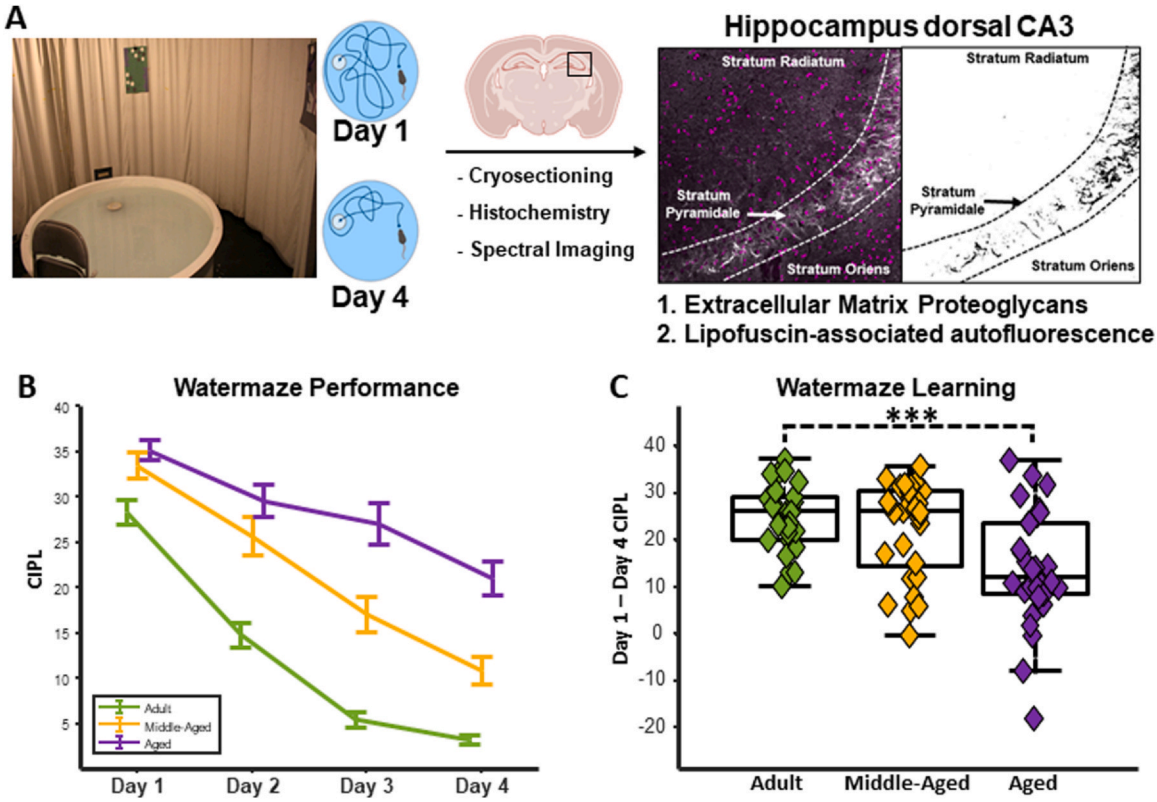

Fig. 1. Experimental design and age comparisons of spatial memory on the Morris watermaze. (A) Image of the Morris watermaze testing environment. The watermaze apparatus consisted of a circular pool with an approximate diameter of 120 cm and a depth of 36 cm and an escape platform hidden beneath the surface of the water for the spatial version and just above the surface of the water for the cued version. Rats underwent 4 sequential days of testing with each rat performing 4 trials per day, and a corrected integrated path length (CIPL) to the escape platform was calculated (see "Methods"). Brains from all animals underwent histological and image-based analyses of extracellular matrix proteoglycan abundance (*wisteria floribunda agglutinin*) and lipofuscin-associated autofluorescence. (B) Average CIPL scores across the 4 days of testing for the adult, middle-aged, and aged rats used in the histochemical analyses. Green represents adult rats, yellow represents middle-aged rats, and purple represents aged rats. Both aged and middle-aged rats exhibited higher CIPL scores than did young across all sessions, and aged rats exhibited higher CIPL scores on the third and fourth days of testing compared to middle-aged rats. (C) The difference measured between day 1 and day 4 of testing. Boxplots denote the middle 50% of the data, and horizontal lines indicate the median of each distribution. Aged rats exhibited a smaller difference, indicating poorer spatial learning. **, $p < 0.01$.

draw a perimeter around all the tissue on the slide, and the blocking solution was pipetted onto the slide. Following incubation in the block solution, the sections were incubated overnight in a solution containing 1% NDS, 0.01% PBS-TX, and biotinylated wisteria floribunda agglutinin (WFA; 1:50; Vector Laboratories, B-1355), which selectively labels N-acetylgalactosamines beta 1 (GalNAc beta 1–3 Gal) residues of glycoproteins within the extracellular matrix (Hilbig et al., 2001). The slides were then washed in PBS-TX (3 × 5 minutes) and incubated in a solution consisting of 1% NDS, PBS-TX, and streptavidin-conjugated Cy5 (peak emission: 670 nm, 1:50; Vector Laboratories, Burlingame, CA, United States; SA-1500). Slides were washed again in PBS (3 × 5 minutes) and coverslipped using 80% glycerol (Vector Laboratories, Burlingame, CA, United States, H-1200-10).

2.4. Confocal imaging and image processing

Brain sections were first imaged with a 40x/1.3 oil objective using a Zeiss LSM 880 inverted confocal microscope to obtain images of native tissue autofluorescence. Image acquisition was performed using a 405-nm laser (Diode 405-30), a 488-nm laser (Argon), and a 633-nm laser (Helium-Neon). All 3 laser lines were used simultaneously in Lambda collection mode using ZEN Black 2.1 imaging software. Lambda collection mode imaging enables the collection of pixel intensity data along the full emission spectrum (410–695 nm) in 32 distinct bins (8.9 nm/bin). For each tissue section, a Z-stack was collected centered on the pyramidal cell layer of the CA3 region of the dorsal hippocampus (Fig. 1A), and these images were fed into a linear unmixing analysis to classify and separate the native tissue autofluorescence from fluorophore fluorescence. The linear unmixing analysis was conducted using ZEN Blue software. The analysis uses a least-squares fit-based algorithm to classify individual pixels into different channels based on their relative contribution to each channel's reference spectrum (Mansfield et al., 2005; Zimmermann, 2005). Unlabeled brain sections were imaged in Lambda mode using the 405-, 488-, and 633-nm lasers to obtain autofluorescence reference spectra, and the reference spectra for each fluorophore were obtained by imaging brain sections with just a single fluorophore. A full description of the linear unmixing procedure was previously published (Pyon et al., 2019). All brain sections were imaged a second time using a 20x objective on a Zeiss Apotome microscope to obtain images of WFA. Again, Z-stacks centered on the CA3 region of the dorsal hippocampus were obtained.

2.5. Image analysis

Image analysis was performed using custom-written macros in Fiji (ImageJ; Schneider et al., 2012) image analysis software. Images of the native tissue autofluorescence and WFA were imported into Fiji, and their contrast was enhanced using the "Enhance Contrast" command. The images were then thresholded using the

"setAutoThreshold" command with the "RenyiEntropy dark" setting with a lower pixel intensity cutoff of 145. The thresholded images were binarized, and the proportion of pixels above the threshold was extracted as an estimate of native autofluorescence abundance and WFA deposition.

2.6. Statistical analyses

Analysis of Morris watermaze performance between age groups was performed using a 2-way analysis of variance (ANOVA) with age as a factor and an α-level of 0.05. *P* values underwent a Bonferroni-Holm correction for post-hoc comparisons. An analysis of WFA deposition and the abundance of autofluorescence were also assessed using a 2-way ANOVA with age as a factor. Individual relationships between anatomical variables and Morris watermaze performance were assessed using a robust regression analysis, also with an α-level of 0.05. All analyses were performed using R Studio statistical analysis software (Boston, MA) or MATLAB (Natick, MA).

3. Results

3.1. Spatial memory deficits increase as a function of age

All rats underwent testing on the spatial version of the Morris watermaze across 4 consecutive sessions. There was a significant effect of the session on the average CIPL scores across sessions, indicating that the animals learned the location of the escape platform (repeated measures AVOVA; $F(3255) = 92.03$; $p < 0.0001$; Fig. 1B). A significant age-by-session interaction was also observed, indicating that older rats exhibit poorer spatial learning across sessions (repeated measures AVOVA; $F(3255) = 11.73$; $p < 0.0001$; Fig. 1B). Post-hoc analyses indicate that middle-aged rats exhibited higher CIPL scores than did young adults across all sessions (*t*-test, Bonferroni-Holm correction; Day 1: $t(53) = -2.65$, $p = 0.01$; Day 2: $t(53) = -4.15$, $p < 0.0001$; Day 3: $t(53) = -5.34$, $p < 0.0001$; Day 4: $t(53) = -3.11$, $p < 0.0001$), as did the aged rats (*t*-test, Bonferroni-Holm correction; Day 1: $t(56) = -4.13$, $p < 0.0001$; Day 2: $t(56) = -6.19$, $p < 0.0001$; Day 3: $t(56) = -8.00$, $p < 0.0001$; Day 4: $t(56) = -8.28$, $p < 0.0001$). Post-hoc comparison also indicates that aged rats exhibited a higher CIPL than did the middle-aged rats on the third and fourth sessions (*t*-test, Bonferroni-Holm correction; Day 1: $t(59) = -0.98$, $p = 0.33$; Day 2: $t(59) = -1.39$, $p = 0.16$; Day 3: $t(59) = -3.29$, $p < 0.01$; Day 4: $t(59) = -4.10$, $p < 0.001$). The difference between day 1 and day 4 CIPL scores was calculated as an estimate of spatial learning, with the older animals showing lower scores (ANOVA; $F(2, 95) = 6.34$, $p = 0.003$; Fig. 1C). Post-hoc tests confirm that aged rats exhibited a smaller difference in performance between day 1 and day 4 compared to young adults (*t*-test; $t(61) = 4.01$, $p = 0.00037$). Although not statistically significant, the same trend was observed when directly comparing middle-aged and aged rats (*t*-test; $t(65) = 2.04$; $p = 0.09$). On the cued version of the watermaze, there was an effect of day of training in all age groups (ANOVA, $F(1, 95) = 72.4$, $p < 0.0001$), indicating that the animals improved performance over days. There was no overall statistically significant difference between age groups (ANOVA, $F(2, 95) = 3.07$, $p = 0.0512$), nor was there an age-by-day interaction (ANOVA, $F(2, 95) = 0.84$, $p = 0.43$).

3.2. Reduced proteoglycan density is associated with spatial memory only in aged rats

An age-associated decrease in WFA deposition was observed (2-way ANOVA; age: $F(1, 84) = 10.187$, $p = 0.002$; Fig. 2B). Post-hoc tests confirm that WFA deposition was lower in aged rats compared to young adults (*t*-test; $t(56) = 3.77$; $p = 0.00079$) but was not different between middle-aged and aged rats (*t*-test; $t(60) = 1.25$; $p = 0.42$), nor between adult and middle-aged rats (*t*-test; $t(54) = 1.73$; $p = 0.17$), although middle-aged rats appeared to show WFA coverage that was intermediate between the young adult and aged rats. When WFA deposition was analyzed with respect to watermaze learning scores, no relationship was observed in the young-adult or middle-aged groups (robust regression; adult: $t(24) = -1.11$, $p = 0.27$; middle-aged: $t(28) = -0.59$, $p = 0.56$; Fig. 2C). Among the aged rats, however, the animals with more WFA in CA3 exhibited better watermaze learning (robust regression; *t*

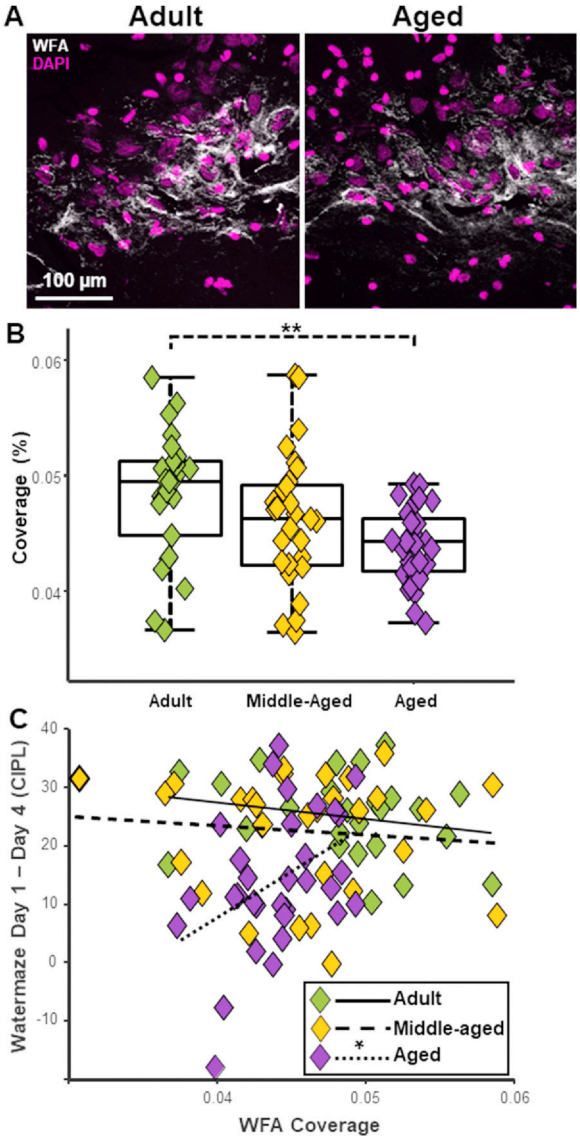

Fig. 2. Aged rats with more extracellular matrix in CA3 exhibited better spatial memory. (A) Representative photomicrograph of *wisteria floribunda agglutinin* (WFA) labeling in the CA3 region of the dorsal hippocampus from a young-adult and aged rat. (B) WFA coverage of dorsal CA3 separated by age. Aged rats exhibited lower WFA coverage than young-adult rats. Boxplots denote the middle 50% of the data, and horizontal lines indicate the median of each distribution. (C) Scatter plot of WFA coverage and Morris watermaze learning scores (Day 1 – Day 4 CIPL difference). There was no significant relationship observed between WFA coverage and CIPL difference scores for the adult and middle-aged rats. Amongst the aged rats, however, the animals with the most WFA coverage showed the best learning on the Morris watermaze. ***, $p < 0.001$.

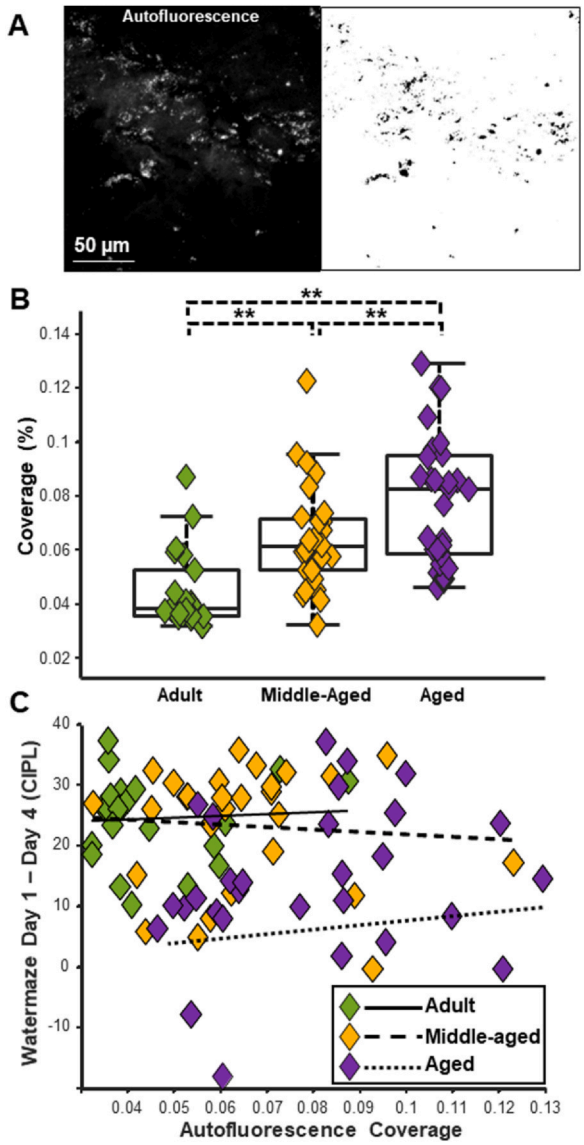

Fig. 3. Lipofuscin increases with age but is not associated with spatial memory. (A) (Left) Representative photomicrograph of lipofuscin-associated autofluorescence in the CA3 region of the dorsal hippocampus. (Right) A threshold image of the same micrograph to show the lipofuscin-associated autofluorescence. (B) Lipofuscin-associated autofluorescent coverage of dorsal CA3 separated by age. Boxplots denote the middle 50% of the data, and horizontal lines indicate the median of each distribution. The older animals exhibited more lipofuscin than did middle-aged or young-adult animals. Middle-aged animals showed more lipofuscin than did young adults. (C) Scatter plot of lipofuscin coverage and Morris watermaze learning scores (Day 1 – Day 4 CIPL difference). There was no significant relationship observed between lipofuscin coverage and CIPL difference scores for any age group. *, $p < 0.05$; ***, $p < 0.001$.

(30) = 2.06; $p = 0.04$; Fig. 2C). These data suggest that extracellular matrix proteoglycans in CA3 represent a biological variable that both changes with advanced age and has an impact on cognitive outcomes.

3.3. Age-associated increases in lipofuscin-associated autofluorescence are not associated with memory

The proportion of a field of view that was covered by lipofuscin-associated autofluorescence (Fig. 3A) was greater in the aged rats compared to young-adult and middle-aged rats (2-way ANOVA; age: $F(1, 75)$: 36.51, $p < 0.0001$; Fig. 3B). Post-hoc tests indicate that tissue from the middle-aged rats contained more lipofuscin compared to young adults (*t*-test; $F(48) = -4.04$, $p = 0.00038$; Fig. 3B), and aged tissue had more lipofuscin than both adult tissue (*t*-test; $F(48) = -5.95$, $p < 0.0001$) and middle-aged tissue (*t*-test; $F(55) = -2.44$, $p = 0.036$). No significant relationships were observed for any age group when lipofuscin coverage was analyzed with respect to watermaze learning scores (robust regression; adult: $t(20) = 0.29$, $p = 0.77$; middle-aged: $t(26) = -0.30$, $p = 0.76$; aged: $t(27) = 0.99$, $p = 0.33$; Fig. 3C). These data indicate that lipofuscin-associated autofluorescence represents a biological variable that changes with advanced age but has no impact on cognitive outcomes.

4. Discussion

The primary finding in this experiment is that aged rats with greater extracellular matrix proteoglycan deposition across the CA3 region of the hippocampus exhibited better spatial memory abilities compared to aged rats with less extracellular matrix protein. Conversely, age-related increases in lipofuscin-associated autofluorescence showed no relationship with spatial memory abilities in any of the age groups. Importantly, the relationship between greater proteoglycan deposition and spatial memory was only observed in aged rats and not in adult or middle-aged rats. This constellation of results fulfills the requirements of the operational definition of brain reserve that was proposed by Stern et al. (2022), which requires measures of aging brain features to be significantly related to cognitive phenotypes expressed in aged animals. In addition, the case for the extracellular matrix being an important component of brain reserve is made stronger by demonstrating the selectivity of this relationship, as age-associated increases in lipofuscin were not related to behavior.

4.1. The extracellular matrix regulates neuronal excitability

Age-associated hyperexcitability in the CA3-dentate gyrus region is thought to arise from a combination of factors, including decreases in inhibitory neuron drive onto excitatory neurons (Spiegel et al., 2013). Proteoglycans in the extracellular matrix play important roles in regulating processes that impact neural network excitability through a variety of different mechanisms. For example, enzymatic degradation of the extracellular matrix results in a significant reduction in inhibitory neuron firing (Balmer, 2016). Proteoglycans likely modulate firing properties due to their strong negative charge, which impacts the local electric field around neurons (Morawski et al., 2015). With age, as proteoglycan deposition decreases, it is possible that the voltage dependence of ion channels is impacted. One hypothesis that emerges from the present data is that, as deficits in inhibitory signaling begin to accumulate in aged brains, the proteoglycans of the extracellular matrix act as a buffer to maintain the network in a healthy physiological state. By extension, aged animals that maintain higher proteoglycan levels may avoid spatial memory impairments. This would be consistent with the idea that the preservation of the extracellular environment may represent a critical aspect of brain reserve.

Enzymatic degradation of extracellular matrix proteoglycans also results in the diffusion of AMPA receptors away from postsynaptic sites, which could alter postsynaptic sensitivity to glutamate release (Frischknecht et al., 2009). At glutamatergic synapses onto inhibitory neurons, the extracellular matrix stabilizes interactions between AMPA receptors and secreted scaffolding proteins such as neuronal pentraxins (Chang et al., 2010). Thus, another hypothesis that emerges from these data is that a breakdown of the hippocampus extracellular matrix and neuronal pentraxins decreases the stability of the glutamatergic drive onto inhibitory neurons. In humans, neuronal pentraxin 2 has been shown to decline in healthy aging and

to be substantially reduced in individuals with Alzheimer's disease. Importantly, in those cases where cognition is within a normal range but the brains show histopathological markers of Alzheimer's disease, neuronal pentraxin 2 is at age-matched control levels (Soldan et al., 2019; Xiao et al., 2017). These authors suggest that this protein protects cognitive function by maintaining a normal excitatory-inhibitory balance in these neural circuits.

4.2. The extracellular matrix regulates synapse function

With age, hippocampal neurons become susceptible to alterations in plasticity by becoming less able to maintain LTP (Bach et al., 1999; Barnes, 1979), more prone to long-term depression (Norris et al., 1996), and LTP reversal (Burke and Barnes, 2006; Norris et al., 1996). In addition to its role in regulating network excitability patterns that impact thresholds for the induction of different forms of plasticity, the extracellular matrix is also positioned to regulate mechanisms of structural synapse plasticity (Dityatev et al., 2010; Nguyen et al., 2020; Sorg et al., 2016). In the adult brain, extracellular matrix structures are thought to inhibit structural plasticity because they emerge toward the end of developmental critical periods and because genetic depletion of critical proteoglycans reinstates these critical periods (Lensjø et al., 2017; Pizzorusso et al., 2002, 2006). Furthermore, it has been shown that the release of matrix metalloproteinases and other degradative enzymes that target the extracellular matrix is necessary for experience-dependent plasticity (Nguyen et al., 2020; Wlodarczyk et al., 2011). Thus, in the adult brain, one function of the extracellular matrix might be to maintain a plasticity threshold that allows neuronal circuits to separate relevant from interfering stimuli. This would fit with the idea that one feature of brain reserve is the ability to preserve homeostatic balance within cells, which serves to optimize circuit function, thereby facilitating higher levels of cognition.

4.3. Extracellular matrix integrity may represent a form of brain reserve

The present findings indicate that it is the relative lack of change in the aged hippocampus extracellular matrix that provides some of the support necessary to maintain spatial memory in aged rats, consistent with a brain reserve mechanism. It is not possible to determine from the present data, however, whether contributions from cognitive reserve or brain maintenance as operationally defined by Stern et al. (2022) also contribute to our findings. A longitudinal design would be necessary to determine whether these other mechanisms of resilience were at play. For example, if the preserved extracellular matrix structure was a result of active adaptation to life-course exposures resulting in better-than-expected cognitive performance given the degree of brain insults sustained, then this would be consistent with a cognitive reserve mechanism. On the other hand, an absence of age-associated changes in the extracellular matrix over time would be consistent with a brain maintenance mechanism. Future longitudinal study designs in nonhuman models of aging will be necessary for determining which mechanisms beyond brain reserve may be playing a role. This would require extremely large initial cohorts to be examined at different time points across the lifespan. Such a study design should be able to capture the true impact that the extracellular matrix, or any other relevant neurobiological variable, has on cognitive outcomes across time.

Authors contributions

DTG conceptualized the experiment, oversaw imaging data collection, performed statistical analyses, and wrote the manuscript. MZ collected and analyzed behavioral data and revised the manuscript. NC collected and analyzed behavioral data. SK collected imaging and behavioral data. IS performed histochemistry, collected imaging data, and revised the manuscript. LMDB provided imaging resources and revised the manuscript. CAB conceptualized the experiment, oversaw behavioral data collection, and wrote the manuscript.

Verification

This manuscript is original, has not been published, and is not under consideration for publication elsewhere. We have no conflicts of interest to disclose.

Disclosure statement

The authors do not have any conflicts of interest.

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