MCKNIGHT BRAIN RESEARCH FOUNDATION (MBRF)
Meeting of the Research Committee
of the Board of Trustees

Friday, January 20, 2023
4:00 pm ET – 5:00 pm ET

Members Attending: Dr. Madhav Thambisetty, Chair; Dr. Patricia Boyle, Trustee; Dr. Richard Isaacson, Trustee; and Dr. Sue Pekarske, Trustee

Absent: Dr. Mike Dockery, MBRF Chair

Also Attending: Dr. Lee Dockery, Chair Emeritus; Ms. Melanie Cianciotto, Corporate Trustee; Dr. Angelika Schlanger, Executive Director; and Ms. Valerie Patmintra, Senior Communications Advisor

AGENDA

4:00 pm ET	1.	Call to Order/Roll Call	Dr. Madhav Thambisetty
ACTION	2.	Approval of Minutes, October 17, 2022	Dr. Madhav Thambisetty
	3.	Updated Activity Timeline	Dr. Madhav Thambisetty
	4.	Current Grants/Programs a. MBRF Innovator Awards in Cognitive Aging and Memory Loss (AFAR) i. Status Update	Dr. Madhav Thambisetty
ACTION		ii. Proposed changes for 2023	
		b. MBRF Clinical Translational Research Scholarship in Cognitive Aging and Age-Related Memory Loss (ABF) i. Status Update ii. Proposed changes for 2023	
		c. FNIH/NIA 2022 Research Partnership In Cognitive Aging Report	
	5.	Pilot Grants – Leadership Council Update	Dr. Madhav Thambisetty
	6.	Cognitive Aging Summit Planning Update a. Planning meeting with FNIH and NIA schedule for Jan 31, 2023	Dr. Madhav Thambisetty
ACTION	7.	Adjourn	Dr. Madhav Thambisetty

MINUTES
MCKNIGHT BRAIN RESEARCH FOUNDATION (MBRF)
RESEARCH COMMITTEE
CONFERENCE CALL
October 17, 2022

The Research Committee of the MBRF was called to order at 4:00 pm EST on October 17, 2022, by Dr. Madhav Thambisetty.

The following members were present:

Dr. Madhav Thambisetty, Chair of the Research Committee, Trustee
Dr. Sue Pekarske, Trustee

The following members were absent:
Dr. Patricia Boyle, Trustee
Dr. Mike Dockery, MBRF Chair
Dr. Richard Isaacson, Trustee

Others attending:

Dr. Lee Dockery, Chair Emeritus
Ms. Melanie Cianciotto, Corporate Trustee
Dr. Angelika Schlanger, Executive Director

1. Call to Order

Dr. Thambisetty welcomed the members of the committee to the call.

2. Minutes of the October 21, 2021, Meeting

The minutes of the October 21, 2021, Research Committee Meeting (Attachment 1) were reviewed and approved as amended. The changes are:

Item 4 c - Add Dr. before Farah Lubin's name, add and after COVID-19

Action Item 3 add that after recommends, bold Action Item 3

Action Item 1: The minutes of the October 21, 2021, Research Committee Meeting were approved as amended (Attachment 1).

3. Updated Activity Timeline

The committee reviewed the updated Activity Timeline (Attachment 2) for information.

4. Current Grants/Programs

a. Status – MBRF Innovators Awards in Cognitive Aging and Memory Loss

The committee discussed the Review Committee Structure for the MBRF Innovator Awards in Cognitive Aging and Memory Loss.

The MBRF lnnovator Awards Committee List should show Dr. Patricia Boyle as a trustee of the McKnight Brain Research Foundation.

AFAR's timeline for announcement of the awards is very short and does not allow for review of the recommendations by the MBRF Research Committee prior to presenting to the full MBRF Board. Dr. Schlanger will reach out to AFAR to suggest they lengthen their timeline. If this is not feasible, a Research Committee meeting will be scheduled to allow time to review the recommendations and present them to the board.

The emphasis of these awards is to support both clinical translational research as well as research in understanding the basic biology of cognitive aging. The RFA clearly states that "One award will be made to support innovative studies focusing on clinical translational research and another will support innovative studies of basic biological mechanisms underlying cognitive aging and age-related memory loss." https://www.afar.org/grants/mcknight-award

The committee feels that the number of clinicians and/or clinician scientists on the review committee should be increased to ensure balance between assessment of proposals under each of these two categories. While we could request AFAR to nominate such experts from among their own grantees to serve as reviewers, we may also consider going outside of AFAR to find qualified candidates to serve on the review committee.

There were four applications divided into two pools for reviewing: Basic Science and Clinical Translational. An award will be made in each category. The committee discussed how to formalize the directive for clinical translational work.

b. Status – MBRF Clinical Translational Research Scholarship in Cognitive Aging and Age-Related Memory Loss

The committee discussed the MBRF Clinical Translational Research Scholarship (CTRS) in Cognitive Aging and Age-Related Memory Loss. There needs to be balance between the MBRF and non-MBRF reviewers to avoid the perception of a conflict especially when reviewing applicants from MBI candidates. The contract with the American Brain Foundation requires each application be reviewed by three reviewers. The committee discussed the lack of applications from the MBI's in this year's pool of applicants. The exact reasons for the lack of interest from the MBIs in putting forward candidates for these awards are unclear. The committee discussed whether this may be due to the

changes in the Leadership Council and whether specific discussions with council around ensuring greater participation in the Innovator and CTRS awards may be useful.

From the pool of nine applications this year at least three were focused exclusively on Alzheimer's disease and related dementias (ADRD). The RFA clearly states that: "the focus should NOT be solely on a neurodegenerative dementia (e.g. Alzheimer's disease); however, proposals that focus on a combined study of cognitive aging and neurodegenerative cognitive changes may be considered."

The committee discussed whether more prescriptive language should be added to the RFA next year to ensure compliance with this requirement. Dr. Thambisetty shared the Simon Foundation's program announcement which is very prescriptive about their guidelines and informs applicants their application will not be considered if it does not meet them. The committee feels this should be discussed in more detail at the October 27, 2022, Board of Trustees' Meeting and that the MBRF should be fair, open and transparent in what is funded.

5. Pilot Grants – Leadership Council Update

Dr. Mike Dockery has a follow-up meeting with Dr. Ron Lazar on October 20, 2022 and will provide an update on the Pilot Grants and Leadership Council at the October 27, 2022, Board of Trustees' meeting.

6. New Program Concepts

Dr. Thambisetty shared the Hevolution Foundation/AFAR Partnership (Attachment 4) with the committee. The committee discussed potential strategic partnerships with the purpose being to reach out to a more diverse talent pool of applicants and to leverage synergies with strategic partners. The committee feels the MBRF should wait until ongoing initiatives in education and public outreach are mature before reaching out to potential partners. The foundation must also be mindful about the current requirement for MBRF grantees to be located within accredited institutions within the United States.

7. Adjourn

Dr. Thambisetty asked if there was any further discussion. Hearing none, he called for adjournment of the meeting at 4:55 p.m. EST.

Summary of Action Items:

Respectfully Submitted,

Melanie A. Cianciotto
Corporate Trustee

Research Committee Activity Timeline

2022-2023

Updated January 10, 2023

Duty (from Committee Charter)	Activity/Action	Outcome	Date	Comments
"Encourage and assess research at the McKnight Brain Institutes (MBIs)"	Review of the Annual Reports of the MBIs	Information for scientific review includes: scientific achievements, publications, presentations, collaborations	DONE February 5, 2020 DONE June 15, 2020 DONE Feb. 26, 2021 Annual Reports were reviewed by the Trustees on Feb. 9, 2022	Reviewers presented at Feb. 2021 Trustees Meeting. Follow up letters were written and sent to each of the MBIs. All Requests of MBIs have been addressed by MBIs. MBRF/MBI Task Force was established April 2021 to streamline Annual Report Recommendations. Recommendations were reviewed Oct 28, 2021 by Trustees. New Template was used for 2021 Annual Reports
	Review of all New Funding Requests from MBIs. Most Funding Requests should be reviewed by the Interventional Core Committee of the MBIs first.	UM submitted a request for $200,000 for Neurocognitive Post-Doctoral Fellowship over the next two years Christian Agudelo, MD, was selected	October 23, 2019 Trustees voted to fund -- payable over two years. Position Start Date – July 2020	The notification letter mentioned that future funding should come from other sources (See "The Evelyn F. McKnight Neurocognitive Clinical Scholar in Brain Health and Aging" on page two)

		UA submitted a request for $244,400 for UM's participation in the Precision Aging Demonstration Pilot	The proposal was reviewed and approved by the Trustees on Feb 5, 2020. The budget was revised and approved June 2020	Dr. Mike Dockery notified UA of the Trustees' approval Trustees were notified of the revised budget and approved no-cost revisions
		A Funding Request "Centralized, telephone-based, computer-assisted…Spanish" for $129,000 was submitted in April 2021 by Dr. Ron Lazar	Reviewed by Cmte in July and not recommended	This request was reviewed by the Trustees in July 2021 and was denied. Suggestion was provided to Dr. Lazar to work though MBI Core Committee if he chooses to resubmit.
		UM submitted a request for $ 3 million to endow a Neurocognitive Training Fund in Brain Health and Aging. UM submitted a request for $250,000 to co-fund a fellowship over 5 years – The Evelyn F. McKnight Neurocognitive Clinical Scholar in Brain Health and Aging"	July 1, 2021 October 2021 Research Cmte reviewed on October 21, 2021; Recommended funding; Trustees reviewed and approved funding October 28, 2021 Grant Notification Memorandum was dated Nov. 10, 2021	This request was denied by Trustees on July 28, 2021, but Dr. Lee Dockery was asked to pursue conversations with UM about how they might proceed. Dr. Dockery had several conversations and exchanges with UM with ideas for strengthening the program infrastructure. A memorandum notifying UM of the approval for funding the Evelyn F. McKnight Neurocognitive Clinical Scholar in Brain Health and Aging for a total of $250,000 ($50,000 over 5 years) to be matched by UM was sent by Dr. Mike Dockery to UM and agreed to and signed by Drs. Sacco and Rundek.

"Encourage and assess research at the McKnight Brain Institutes (MBIs)" continued	Review of Travel Award Fund: Originally established to fund research scholars and faculty to visit other McKnight institutions.	Few applications for travel. The funds allocated for travel have been used to fund the activities of focus groups: Epigenetics, MRI standardization and cognitive test battery working group	Reviewed at each Trustees' Meeting ON HOLD DUE TO UNIVERSITY TRAVEL RESTRICTIONS	Approved in 2009 In the amount of $100,000 Approximately $30,000 remains in the fund
	Inter-institutional Block Grants	Cognitive Aging Core Working Groups	N/A	5 Areas: Brain and Cognitive Health Cognitive Aging & Memory Cognitive Testing Battery Epigenetics MRI standardization
	Inter-institutional Block Grants	Bio-Informatics Core (Epigenetics)	Funding period: 9/1/2013-8/31/2015	Tom Foster, UF still lead scientist.
	Inter-institutional Block Grants	Neuroimaging Core	Funding period: 1/1/2015 to 12/31/2017 $931,759.00	
	Inter-institutional Block Grants	Cognitive Assessment and Brain Registry Core	Funding period: 9/1/2015-8/31/2017 Request for another extension was approved at the Feb 5, 2020, Trustees' meeting.	No-cost Extension Request submitted for April 30, 2021. Trustees approved the extension.
	Review of Pilot Grants (Funding Requests and Progress Reports)	1)A Novel Invention Tool – Levin 2)Revitalizing Cognition in Older Adults – Bowers	1)Funding Period: 5/1/2018-4/30/2020 2)Funding period: 5/1/2018-4/30/2020	1)Funding for 2-years for total of $120,000 2)Funding for 2-years for total of $120,000

				No-cost Extension Request submitted and approved for April 30, 2021.
		3)Transcutaneous Vagal Nerve Stimulation and Cognition Training – Williamson/Alexander	3)Approved July 2019 Funding period: 10/1/2019-9/30/2021 Deadline was extended	3)Funding for 2-years for total of $120,000
			Request for no-cost Extension	Trustees approved at their August 29, 2022 meeting
	Applications for 2021 Pilot Grants	5 Letters of Intent were Submitted	Research Cmte Reviewed LOIs for 2020 Jan. 29, 2021.	Trustees approved 3 grants
		3 Grants were approved	Feb. 26, 2021	
		With Dr. Gomes-Osman's subsequent departure from UM, the Core Committee recommended the next application in line to replace Dr. Gomes-Osman's. This was submitted by Dr. Sonya Kaur "Sleep Intervention…"	The Research Cmte did not recommend funding the next-in-line proposal in its July 2021 meeting "Reuniting the Brain and Body to Understand Cognitive Aging: The Nexus of Geroscience and Neuroscience" pilot grant August 2022	The Trustees denied funding and setting this precedent in its July 2021 meeting. Dr. Rundek was notified. Interim Report submitted. Trustees reviewed and approved on August 29, 2022
	Checked RFA for 2022 before it was posted to be sure it stresses Junior Faculty. It does.	Drs. Lazar and Levin shared that only 1 LOI was received for 2022 funding cycle.	January 31, 2022 Leadership Council Meeting attended by Drs. Thambisetty and Mike Dockery and A. Porter	Several reasons for only 1 LOI were cited. The Leadership Council drafted a new RFA to address these reasons and broaden the scope of the research for Trustee review at their February meeting

			February 23, 2022 September 12, 2022	Dr. Mike Dockery, on behalf of the Trustees, responded to the LC and the members of the Core Committee that they did not wish to change the focus of the pilot grant program by changing the RFA Dr. Mike Dockery, on behalf of the Trustees, and Angelika Schlanger attended the Leadership Council meeting and asked the Council to follow up with the MBRF on the status of the Cognitive Aging and Memory Intervention Core Workgroup, in terms of its membership and plans to respond to the Memo from February 23, 2022.
"Identify opportunities…to foster greater interest in cognitive aging and age-related memory loss (in the scientific community)"	Research Partnership with the Foundation for NIH and the NIA. 1st cycle-2009, 2nd cycle-2014	Fund balance of $1 million from 2nd five-year partnership returned to MBRF Report received on all FNIH/MBRF activities RFA posted: "Network for Identification, Evaluation, and Tracking of Older Persons with Superior Cognitive Performance for Age" FNIH Report submitted	DONE August 2019 FNIH Report in October 2019 had an error. A corrected report resubmitted on Feb. 5, 2020.	History: Established 2009 $5 M over 5 years from MBRF; match from NIA and partners was $23 M for total of $28 M (17 five-year grants funded) 2014 Partnership renewal funded one 5-year project for $15 million with $5 M from MBRF and $10 M from NIA Valerie connected with Julie Wolf-Rodda and Molly Wagster on promoting STARRS study.

	3rd cycle approved 2019 to begin Spring of 2020	For information only	Posted Feb 2020; Deadline LOI Sept. 1; Application October 1, 2020 First payment was made to FNIH by March 31, 2021. Will continue until 2025 Dr. Molly Wagster will be attending the March 23-25 Inter-institutional Meeting at UA. The Trustees have invited her to present at their meeting on March 23, and to the idea of inviting the grantees for a video presentation. Dr. Julie Gerberding, Julie Wolf-Rodda, FNIH, and Dr. Molly Wagster, NIA, attended MBRF Trustees Meeting on October 27, 2022, in DC	NIA will provide $14M to be pooled with MBRF $5 M. A 2.8 Match. RFA was shared with Communications Working Group for posting and with Leadership Council. Two grants were provided from the Research Partnership ""Network for Identification, Evaluation and Tracking of Older Persons with Superior Cognitive Performance for their Chronological Age" to Dr. Thomas Perls, Boston University, and Dr. Emily Rogalski. Julie Wolf-Rodda, FNIH, Dr. Molly Wagster, NIA, and other members of the FNIH and NIA teams will be meeting by Zoom with Drs. Mike Dockery, Lee Dockery, Madhav Thamibisetty and Angelika Schlanger on January 31, 2023 to being planning the next Cognitive Aging Summit.
"Identify opportunities…to foster greater interest in cognitive aging and age-related memory loss (in	MBRF Innovators Awards in Cognitive Aging and Memory Loss	Program was Approved by the Trustees Potential administrative and/or funding partners were approached	October 14, 2020 December 2020	AFAR Review Committee: Chair: Dr. Anna Maria Cuervo Members: Dr. Rafa de Cabo

the scientific community)"	The McKnight Brain Research Foundation committed $4.5 million over the next five years to support outstanding mid-career scientists committed to researching the basic biological mechanisms underlying cognitive aging and memory loss.	American Federation of Aging Research (AFAR) was identified as an excellent partner organization. AFAR presented a proposal and draft contract for review Revised Agreement signed between AFAR and the MBRF	January 2021 February 2021 July 15, 2021 August 2021 Mid Oct. 2021 Dec. 15, 2021 March 2022 August 2, 2022 September 19, 2022 December 7, 2022	Dr. Thambisetty Dr. Boyle and Dr. Roz Anderson 2021 LOI Deadline – 9 LOIs Received LOI Review – 7 applicants asked to submit full application Application Deadline Award Announcement 2022 *LOI Submission and review was eliminated due to the small number of applicants in 2021* Application Deadline Application Review – 4 applied. Award Announcement
	Reserve & Resilience Workshop 2019 Reserve & Resilience Workshop Pilot Grants 2020 Final Reserve & Resilience Workshop 2021	Over 300 Attendees (8 MBI researchers) Organizers requested $30,000 to support (1 – 3) pilot grants	September 9 and 10th, 2019 Bethesda In-Person Meeting CHANGED TO VIRTUAL MTG September 14 and 15, 2020; Report Submitted Jan. 2021 Oct 31/Nov 1 Bethesda Meeting will be a hybrid – part virtual and part person. The program is posted on reserveandresilience.com.	This is an outcome from Cog. Aging Summit III held in 2017. Research Committee approved support in first and second years. Dr. Stern requested support for the Final R & R Workshop to take place Oct. 31/Nov. 1 in Bethesda. He did not request a specific amount but support MBRF provided last year was $30,000. Committee supports recommendation to fund at no more than $30,000.

			Of note, Jen Bizon and Tom Foster are panelists.	
"Encourage young investigators in this area of research"	McKnight Brain Research Foundation Clinical Translational Research Scholarship with American Academy of Neurology (AAN) and American Brain Foundation (ABF)	2021-2022 MBRF Reviewers are Dr. Boyle, Dr. Thambisetty, and Dr. Isaacson	Reviewers meet in Dec. Two Scholars are selected and alternates were identified. Awardees are notified in January. Funding starts July 1 of each cycle	<u>First Scholarships Awarded</u> January 2018 (McConnell, Albert) <u>Second Scholarships</u> Awarded January 2019 (Camargo, Sedaghat) <u>Third Scholarships</u> Awarded January 2020 (Baxter, Getz)
	McKnight Brain Research Foundation Clinical Translational Research Scholarship with American Academy of Neurology (AAN) and American Brain Foundation (ABF) (continued)		Edits to 2021 RFA were made and approved by Research Cmte. RFA was posted as of July 4, 2020, on AAN site. Advertising followed 2019 Plan for 2020 Award and begin in August, 2020. 8 applications for 2021 were received. October 14, 2020, Renewal for next five years was approved by the Trustees	<u>Fourth Scholarships</u> were Awarded in January 2021 to Dr. Wendy Yau Wai-Ying (Brigham and Women's) and ~~Dr. Matthew Burns (UF)~~ Dr. Reem Waziry (Publicly announced in April 2021 (Dr. Matthew Burns [UF] received a K-Award from NIA and had to decline the McKnight Scholarship.) <u>Fifth Scholarships</u> Advertising was conducted in August and September 5 Applications received Oct. 1. Review was in Dec. 2021

		Members of the 2022-23 Review Committee include Dr. Madhav Thambisetty and Dr. Patricia Boyle	2022-23 Deadlines September 1, 2022 Application Deadline November 3, 2022 Review Committee Meets January 9, 2023 Notification of Recipients Spring 2023 Announcement of Recipients	<u>Sixth Scholarships</u> New 2022-23 RFA Draft was reviewed and has been posted and advertised - 9 applications were reviewed
"Encourage young investigators…" Continued	Poster Reception at 2019 Society for Neuroscience annual meeting (Chicago) MBRF/MBI Poster Reception 2020 Society for Neuroscience (SfN) annual meeting in DC October 24 – 28, 2020 canceled due to DC pandemic closing guidelines Society for Neuroscience will meet in San Diego Nov 12 - 16		October 20, 2019 August 29, 2022 September 5, 2022 September 1, 2022	First Poster Reception held in 2008. (50 submissions received) Sponsored by MBRF. Hosted by Directors of MBIs. Submissions open to researchers at MBIs and invited guests only MBRF Trustees Decided not to host the MBRF/MBI Poster session at the 2022 meeting. Dr. Mike Dockery updated the Leadership Council on Sept. 12, 2022 by Zoom. Dr. Mike Dockery wrote to the Leadership Council to ensure it will take place in 2023. Ms. Porter wrote to Dr. Molly Wagster to alert her that the poster reception will not take place this year.

McKnight Brain Research Foundation Innovator Awards in Cognitive Aging and Memory Loss

- The Program
- Eligibility
- Selection Criteria
- Application Procedures
- Reporting Requirements
- Annual Meeting

The McKnight Brain Research Foundation (MBRF) and the American Federation for Aging Research (AFAR) will provide up to two 3-year awards of $750,000 (USD) each to advanced Assistant Professors and recently appointed Associate Professors (MDs and PhDs.) One award will be made to support studies focusing on clinical translational research and another award toward understanding basic biological mechanisms underlying cognitive aging and age-related memory loss.

The application deadline is ~~August 1, 2022~~July 31, 2023.

The Program

The major goal of the program is to identify emerging scientific leaders by building a cadre of outstanding research scientists across the United States to lead transformative research in the field of cognitive aging.

The program targets full-time independent investigators at the rank of Assistant Professor or Associate Professor (or equivalent) with established independent research programs who have already demonstrated a firm commitment to cognitive aging research. It will add substantial start-up support for a period of three years to help these investigators develop and/or expand an outstanding research program in cognitive aging and memory loss.

One award will be made to support innovative studies focusing on **clinical translational research** and another will support innovative studies of **basic biological mechanisms** underlying cognitive aging and age-related memory loss. It is expected that the proposed research will yield transformative discoveries and thus proposals are invited that are high risk/high gain in nature and that would be less suitable for conventional sources of funding. For example, this support could be deployed towards conducting a pilot clinical trial, developing proof-of concept interventions to ameliorate age associated cognitive impairment, gather preclinical data to accelerate testing of potential interventions, and further study the mechanistic basis of age-associated cognitive impairment in relevant experimental models with a view to identifying novel treatment targets. Scientists proposing to pursue basic research should clearly articulate the potential of their findings to be translated into clinically relevant strategies, and/or treatments. Research studies at the intersection of age-associated cognitive changes and disease-related cognitive impairment may be considered if a strong case can be made for their relevance to cognitive aging and age-related memory loss. However, research that is primarily focused on neurodegenerative diseases (e.g., Alzheimer's disease) will not be supported.

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Two 3-year awards of $750,000 (USD) each will be made in 2023~~2~~, of which a maximum of 10% may be used for indirect expenses or institutional overhead. To demonstrate a commitment to the investigator, the institution is asked to support the investigator's project through matching funds. The investigator needs to identify 50% in matching funds, which can only be from non-federal funds, and cannot be used by more than one project. This could be cash and/or in-kind matching, and can include faculty effort, and goods and services paid from departmental funds. For an in-kind match, the selection committee will determine whether this is equivalent to a monetary match.

Eligibility

To be eligible, the applicant must:

- Have completed research training prior to the beginning of this award (October 1, 2023~~2~~):
  o PhD candidates: no more than 7 years from the completion of formal post-doctoral research training post-PhD,
  o MD or combined degree candidates: no more than 12 years from the date when finished residency.
- Be an independent investigator at the rank of Assistant Professor or Associate Professor (appointed no earlier than October 1, 2020~~19~~), who has received R01 funding (or equivalent funding such as an NIH DP5, R35 or NSF Research awards.)
- Be tenure-track faculty or equivalent in an academic or non-profit institution with evidence of long-term institutional support as indicated by commitment of resources including independent laboratory space, start-up research funds and personnel. Candidates not in a tenure-track position are also eligible and should demonstrate similar evidence of long-term institutional support and not be in a time-limited appointment.
- Have a proven track record of research accomplishments in cognitive aging as indicated by their publications in high-impact journals, awards, and other metrics of peer recognition.
- Provide evidence of institutional matching funds as described in a **form completed by the Dean or Department Chair**.
- Be in full time employment at an academic or non-profit research institution in the United States.

The program **does not** provide support for:

- Senior faculty, i.e., at the rank of Associate Professor or higher who have held this position before October 1, 2020~~19~~.
- Assistant Professors who have not yet received R01 or equivalent extramural independent funding.
- Investigators who are conducting research at a federal government or for-profit institution.
- See comment above about disease specific research.

Questions about eligibility and suitability of research project can be addressed to **grants@afar.org**.

Selection Criteria

Five criteria are used to determine the merit of an application:

- Qualifications of the applicant;
- Quality and promise of the proposed research and its relevance to cognitive aging/age-related memory loss;
- Novelty/impact of the proposed research and potential to have transformative clinical impact;
- Excellence of the research environment;
- The commitment by the institution to provide matching funds.

Application Procedures and Timeline

Please refer to the **application instructions**. Incomplete applications cannot be considered. All applications must be submitted via email to **afarapplication@afar.org**.

The applications will be reviewed by a committee whose recommendations will be presented to MBRF and AFAR for final funding decisions.

Please review **this link** which includes suggestions for submitting an LOI or application to AFAR. Click **here** for our Frequently Asked Questions page. If you are using animals in your research, please review **Principles of Animal Use for Gerontological Research** or this recent webinar recording from the Nathan Shock Centers of Excellence: **https://nathanshockcenters.org...**

MBRF and AFAR will not provide reviewer critiques to any applicants at any review level.

Timeline:
Application deadline: ~~August 1, 2022~~July 31, 2023

Anticipated Award Announcement: September 15, 2023~~2~~

Award Start Date: October 1, 2023~~2~~

Reporting Requirements

Investigators will be required to submit a brief **narrative report** annually on the progress of their research. Final narrative and financial reports are required within three months following the end date of the award.

Annual Meeting

Recipients of this award are expected to attend the AFAR Grantee Conference. The purpose of the meeting is to promote scientific and personal exchanges among recent AFAR grantees and experts in aging research. Grantees are also expected to attend the annual inter-institutional meeting of the MBRF.

Funder

McKNIGHT BRAIN RESEARCH FOUNDATION

Preserving memory, enhancing life

Founded in 1999 by Evelyn McKnight, the Foundation's specific goal is to better understand and alleviate age-related cognitive decline and memory loss. Cognitive changes due to the normal aging process may affect up to 87 percent of people age 65 and older, impacting abilities like processing speed and decision-making and contributing to some types of memory loss. The **McKnight Brain Research Foundation** works to champion research to better understand age-related cognitive decline and memory loss and educate the public on the steps that can be taken to maintain cognitive and brain health and age successfully.

In its first 20 years, the Foundation established Evelyn F. McKnight Brain Institutes at the University of Alabama at Birmingham, the University of Arizona, and the University of Miami, and the Evelyn F. and William L. McKnight Brain Institute at the University of Florida.

By partnering with the Foundation for the National Institutes of Health, and with the support of three Cognitive Aging Summits and the National Academy of Medicine Cognitive Aging Report, we have made great progress to better understand the effects of age-related cognitive decline and memory loss over the last two decades.

The McKnight Brain Research Foundation and the McKnight Brain Institutes are leaders in cognitive aging research. By providing research funding to promising investigators as they continue to embark upon independent careers, the MBRF proposes to build a core group of outstanding research scientists across the United States to lead transformative research in the field of cognitive aging.

Research Partnership in Cognitive Aging

A report to the McKnight Brain Research Foundation

January 12, 2023

Foundation for the National Institutes of Health
National Institute on Aging

Report Summary

Two initiatives currently form the centerpiece of a Research Partnership in Cognitive Aging between the McKnight Brain Research Foundation (MBRF) and the National Institute on Aging (NIA), coordinated by the Foundation for the National Institutes of Health (FNIH) – "Plasticity and Mechanisms of Cognitive Remediation in Older Adults" and "Network for Identification, Evaluation, and Tracking of Older Persons with Superior Cognitive Performance for Their Chronological Age." One is drawing to a close now that the trial has ended and the results have been published. The other still is in the early stages and we look forward to continued progress over the next four years.

The FNIH is pleased to present this 2022 report to the MBRF. It provides updates from the NIA on the MEDEX trial and the Cognitive Superagers Networks, both supported through the Research Partnership in Cognitive Aging, as well updates on two additional initiatives that stemmed from the Cognitive Aging Summit III.

"Plasticity and Mechanisms of Cognitive Remediation in Older Adults (R01)"

Link to NIA Request for Applications: RFA-AG-14-016

Remediating Age-related Cognitive Decline: Mindfulness-based Stress Reduction and Exercise (MEDEX)

The MEDEX clinical trial (R01 AG049369), awarded to Eric Lenze, M.D. at Washington University in St. Louis, is complete and the results were published in *JAMA* in December 2022. Top line findings were that neither exercise nor mindfulness-based stress reduction, or the combination of the two, was better than placebo in impacting cognitive performance or brain structure in older adults. See Appendix A for the original abstract describing the plan for the MEDEX trial. The *JAMA* article describing the results is Appendix B. Washington University's press release about the results is at the following link: https://medicine.wustl.edu/news/exercise-mindfulness-dont-appear-to-boost-cognitive-function-in-older-adults/

Note:

The participants in the MEDEX trial will continue to be followed through a new award (R01AG072694, "Resilience and Brain Health of Older Adults During the COVID-19 Pandemic") to Dr. Lenze (Washington University St. Louis), Dr. Breno Diniz (University of Connecticut School of Medicine), and Dr. Julie Wetherell (University of California San Diego).

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 Alzheimer's Disease. The team will leverage the MEDEX trial. By doing so, and following the participants, who continue to attend monthly booster sessions of their randomized condition remotely during the pandemic, they will generate repeated sets of clinical, cognitive, molecular, and neuroimaging measures spanning 7.5 years and covering the pre-, during-, and post-pandemic period.

"Network for Identification, Evaluation, and Tracking of Older Persons with Superior Cognitive Performance for Their Chronological Age (U19 Clinical Trial Not Allowed)"
Link to NIA Request for Applications: RFA-AG-21-015

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 second year of award. The NIA is supporting a multi-year administrative supplement to enhance diversity and data capture. The supplement funds a fourth phenotyping and biospecimen core and neuroimaging core site at Georgia State University (GSU). Addition of the GSU site will enhance the diversity of the RADCO cohort by enrolling 234 African Americans, thus increasing the proportion of the RADCO sample that is African American 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 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 "Resilience/Resistance to AD in Centenarians and Offspring" (RADCO) Study gauges cognitive resilience among centenarian cognitive superagers and their offspring using cognitive testing, neuroimaging, blood biomarkers, and neuropathology and 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 (Northwestern University of Chicago) is in its second year of award. The team published

findings in *Journal of Neuroscience* (see Appendix C) in late 2022 regarding brain structural differences in cognitive SuperAgers vs cognitively normal older adults. They reported that the size of neurons in the entorhinal cortex (ERC) of cognitive SuperAgers is significantly larger than ERC neurons in adults with amnestic mild cognitive impairment, than those of age peers with normal cognition for their age, than those of adults in their 50s and 60s. The authors suggest that larger ERC neurons are a biological signature of the cognitive SuperAging trajectory.

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.

Additional Initiatives Stemming from the Cognitive Aging Summit III

Besides RFA-AG-21-015 that provided support for the two network grants to identify, evaluate and track cognitive superagers and 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. That recommendation is now an action. NIA's Intramural Research Program (IRP) has a longitudinal study underway - STARRRS— Successful Trajectories of Aging: Reserve and Resilience in RatS. 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. STARRS will create open- source data and a sample hub to be shared with the entire aging science community. The goal is to bring us closer to an understanding of the factors that contribute to successful versus unsuccessful neurocognitive aging. The first cohort of animals entered the study during the past year and data collection is underway.

Another 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).

Dr. Stern, and his co-investigators (Drs. Marilyn Albert, Carol Barnes, Roberto Cabeza, Alvaro Pascual-Leone, Peter Rapp), have completed the goals of the project. The website for the effort https://reserveandresilience.com/ contains information for three workshops that have been held to date, the latest being in late October 2021. The framework for operational definitions of reserve and resilience concepts are in press in *Neurobiology of Aging*, along with a Commentary by Dr. Wagster and Dr. King. Both should be published online shortly and will be made available to the Board within the next month.

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 to develop data sharing and information exchange platforms to help guide promote research in this area.

PUBLIC HEALTH RELEVANCE: In order to achieve state-of-the-art definitions and research guidelines for key concepts associated with resilience against cognitive aging and Alzheimer's disease related dementia, 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 A

MEDEX Trial Abstract (NIH Award R01AG049369):

DESCRIPTION (provided by applicant): The vast majority of older adults will suffer declines in cognitive functions such as memory and cognitive control (or executive function), interfering with their ability to participate and engage in meaningful activities. Importantly, the recent observation that the brain retains plasticity late into life suggests that timely and personalized interventions might remediate age- related cognitive decline. Two promising interventions are Mindfulness-Based Stress Reduction and Exercise, each of which appears to act in multi-modal ways to make plastic changes in CNS function to improve memory and cognitive control in older adults. Our research team has conducted several studies of these interventions, supporting their benefits and pathways to improved cognitive functioning. We propose a 2x2 factorial design RCT to definitively test MBSR and exercise for remediation of age-related cognitive decline. We will randomize 580 healthy community-living adults aged 65+ to one of four conditions: MBSR alone, exercise alone, MBSR + exercise, or health education (a control condition).
Participants will receive protocolized interventions for a six-month acute period, followed by a 12- month maintenance period. We will examine (1) cognitive improvements using a well-validated and sensitive neuropsychological battery focusing on memory and cognitive control; (2) mechanistic changes such as reduced cortisol and improved insulin sensitivity (3) neuroimaging markers of plasticity: structural and functional connectivity changes indicating plastic CNS changes underlying the cognitive improvements (4) individual variability that predicts response to the interventions. Our main goal is to carry out a high-quality clinical trial, such that data and biosamples will become a resource for the scientific community. Then, we can not only improve the lives of older adults in the near-term by matching individuals to readily available interventions that most benefit them, we can also understand the mechanisms of neuroplastic changes with interventions to rescue cognitive decline with aging, leading to a more active and vital senior community.

PUBLIC HEALTH RELEVANCE: The world is graying, and the vast majority of older adults will have declines in cognitive function, interfering with function, quality of life, and engagement in valued activities. We will test two promising interventions - Mindfulness Based Stress Reduction (MBSR) and Exercise - for their ability to remediate age-related cognitive decline. MBSR and exercise are both inexpensive, well-tolerated, safe, and highly scalable interventions; therefore, our project can demonstrate how effective they are, for whom, and by what mechanisms, in the near-term older adults could receive lifestyle strategies that would benefit their brain and cognitive functioning, staving off disability and dependence on others and maintaining engagement in life's most valued activities.

JAMA | Original Investigation

Effects of Mindfulness Training and Exercise on Cognitive Function in Older Adults
A Randomized Clinical Trial

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IMPORTANCE Episodic memory and executive function are essential aspects of cognitive functioning that decline with aging. This decline may be ameliorable with lifestyle interventions.

OBJECTIVE To determine whether mindfulness-based stress reduction (MBSR), exercise, or a combination of both improve cognitive function in older adults.

DESIGN, SETTING, AND PARTICIPANTS This 2 × 2 factorial randomized clinical trial was conducted at 2 US sites (Washington University in St Louis and University of California, San Diego). A total of 585 older adults (aged 65-84 y) with subjective cognitive concerns, but not dementia, were randomized (enrollment from November 19, 2015, to January 23, 2019; final follow-up on March 16, 2020).

INTERVENTIONS Participants were randomized to undergo the following interventions: MBSR with a target of 60 minutes daily of meditation (n = 150); exercise with aerobic, strength, and functional components with a target of at least 300 minutes weekly (n = 138); combined MBSR and exercise (n = 144); or a health education control group (n = 153). Interventions lasted 18 months and consisted of group-based classes and home practice.

MAIN OUTCOMES AND MEASURES The 2 primary outcomes were composites of episodic memory and executive function (standardized to a mean [SD] of 0 [1]; higher composite scores indicate better cognitive performance) from neuropsychological testing; the primary end point was 6 months and the secondary end point was 18 months. There were 5 reported secondary outcomes: hippocampal volume and dorsolateral prefrontal cortex thickness and surface area from structural magnetic resonance imaging and functional cognitive capacity and self-reported cognitive concerns.

RESULTS Among 585 randomized participants (mean age, 71.5 years; 424 [72.5%] women), 568 (97.1%) completed 6 months in the trial and 475 (81.2%) completed 18 months. At 6 months, there was no significant effect of mindfulness training or exercise on episodic memory (MBSR vs no MBSR: 0.44 vs 0.48; mean difference, –0.04 points [95% CI, –0.15 to 0.07]; $P = .50$; exercise vs no exercise: 0.49 vs 0.42; difference, 0.07 [95% CI, –0.04 to 0.17]; $P = .23$) or executive function (MBSR vs no MBSR: 0.39 vs 0.31; mean difference, 0.08 points [95% CI, –0.02 to 0.19]; $P = .12$; exercise vs no exercise: 0.39 vs 0.32; difference, 0.07 [95% CI, –0.03 to 0.18]; $P = .17$) and there were no intervention effects at the secondary end point of 18 months. There was no significant interaction between mindfulness training and exercise ($P = .93$ for memory and $P = .29$ for executive function) at 6 months. Of the 5 prespecified secondary outcomes, none showed a significant improvement with either intervention compared with those not receiving the intervention.

CONCLUSIONS AND RELEVANCE Among older adults with subjective cognitive concerns, mindfulness training, exercise, or both did not result in significant differences in improvement in episodic memory or executive function at 6 months. The findings do not support the use of these interventions for improving cognition in older adults with subjective cognitive concerns.

TRIAL REGISTRATION ClinicalTrials.gov Identifier: NCT02665481

JAMA. 2022;328(22):2218-2229. doi:10.1001/jama.2022.21680

Visual Abstract

Supplemental content

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Most older adults experience deteriorating cognitive function. Declines in episodic memory and executive function parallel volume losses in brain structures, such as the hippocampus and dorsolateral prefrontal cortex (DLPFC).[1,2] With the increasing age of the population, lifestyle interventions could provide a scalable means to target modifiable mechanisms of these cognitive and brain changes, thereby helping improve and maintain cognitive functioning.[3]

Two promising interventions are mindfulness training and exercise. Mindfulness-based stress reduction (MBSR) is a group-based intervention based on mindfulness meditation training.[4] From a mechanistic standpoint, practicing mindfulness may enhance cognitive processes such as working memory[5]; further, mindfulness techniques may reduce stress, thereby affecting physiological parameters such as cortisol levels and sleep.[6,7] Aerobic and strength training are both theorized to be associated with cognitive function[8]; some studies have found exercise-related cognitive changes together with structural brain changes.[9,10] Previous studies have suggested changes in insulin sensitivity, aerobic capacity, and body fat as some of the proposed mechanisms.[11] MBSR and exercise could have additive benefits because their putative mechanisms may be complementary. Accordingly, a randomized clinical trial was conducted to determine whether MBSR and exercise improve cognitive function and whether the combination of MBSR and exercise has greater benefits than either intervention alone.

Key Points

Question Does mindfulness training, exercise, or the combination of these interventions improve cognitive function in older adults with subjective cognitive concerns?

Findings In this randomized clinical trial that included 585 participants, mindfulness training, exercise, or both did not result in significant differences in improvement in episodic memory or executive function composite scores at 6 months.

Meaning The findings do not support the use of mindfulness training, exercise, or a combination of both for significantly improving cognitive function in older adults with subjective cognitive concerns.

Methods

Study Design

The MEDEX (Mindfulness, Education, and Exercise) study was a randomized clinical trial comparing MBSR and exercise, alone or in combination, with a robust control intervention (health education) designed to control for expectancy in older adults with subjective cognitive concerns and without dementia. Outcome assessments evaluated cognitive function and brain structure over 18 months of intervention. For full details of the trial design, protocol, and statistical analysis plan, see Wetherell et al[12] and Supplement 1. The study was conducted from 2015 to 2020 in St Louis, Missouri, and San Diego, California, with enrollment from November 19, 2015, through January 23, 2019, and final follow-up on March 16, 2020. Ethics approval was provided by the universities' institutional review boards. All participants provided written informed consent. Recruitment methods included use of press (eg, television, newspapers), online sources (eg, via social media, websites), printed flyers, presentations at community outreach events, and direct mailings.

Participants

From November 2015 to January 2019, the study enrolled community-dwelling older adults. Inclusion criteria were age 65 to 84 years; self-reported age-related changes in cognitive function, defined by a positive response to questions of whether they or others had noticed trouble with their memory or concentration; and being cognitively intact, defined as scoring less than 10 on the Short Blessed Test, for which scores greater than or equal to 10 suggest impairment consistent with dementia.[13] The study allowed mild cognitive impairment, and no clinical rating of dementia status was done. Exclusion criteria were neurodegenerative illness (eg, dementia, Parkinson disease, cerebrovascular disease); not sedentary (current moderate- to high-intensity exercise ≥1 h/wk or light activity ≥1 h/d; see eMethods 1 in Supplement 2 for details); current meditation practice or cognitive training; medical conditions that suggest shortened lifespan, or would prohibit safe participation, would prohibit safe participation in the interventions (eg, metastatic cancer, unstable cardiovascular disease), or would interfere with study assessments (eg, diabetes medication, systemic glucocorticoids, magnetic resonance imaging [MRI] contraindications, severe hearing/visual impairment); and nonfluent English-language speaker.

Randomization

After baseline assessment, participants were randomized to the following groups in a 1:1:1:1 ratio: MBSR alone, exercise alone, combined MBSR and exercise, and health education (control group). Using R software, the study statistician (M.D.Y.) generated the randomization sequence. The study primary investigator and coordinators were kept blinded to the randomization until the study coordinator was ready for the next group to be randomized. Participants learned their randomization assignment at the first intervention group meeting. Randomization was done in groups of approximately 15 individuals (range, 12-17) and was stratified by site.

Interventions

All interventions were conducted for 18 months, which consisted of a 6-month acute and 12-month maintenance phase.

The MBSR intervention matched the format of the consensus MBSR protocol[14]; after a brief introductory meeting, the intervention was conducted in 8 weekly 2.5-hour classes plus a half-day retreat. For the remainder of the 6-month acute phase and the subsequent 12 months of maintenance, MBSR classes met monthly. Content included instruction in mindfulness meditation practices and exercises to enhance mindfulness in everyday life. Participants also used *A Mindfulness-Based Stress Reduction Workbook*.[15] Participants received daily

at-home assignments with a goal of 60 minutes of daily at-home meditative practice. Additional details are provided in Supplement 2.

The exercise intervention was designed to improve aerobic fitness, strength, balance, mobility, and flexibility. It consisted of facility-based, instructor-supervised 1.5-hour classes twice weekly for 6 months. Sessions included aerobic exercise, resistance training, and functional exercises. Participants were prescribed home exercise with a goal of completing at least 300 minutes per week of combined class plus home exercise. Classes continued once per week during the 12-month maintenance phase with the same exercise goal of at least 300 minutes per week. Additional details are provided in Supplement 2.

Participants in the combined MBSR and exercise intervention underwent both MBSR and exercise, with the above-listed frequency of classes and goals for each intervention.

The health education intervention was an attention placebo to control for nonspecific factors (eg, time spent in groups) and expectancy.[16] It matched the MBSR intervention for group setting, class time, frequency of sessions, and attention with weekly assignments, but no goals, related to the amount of time engaged in them. It was based on the Stanford chronic disease self-management book *Living a Healthy Life with Chronic Conditions*,[17] omitting information on mindfulness and exercise.

To monitor fidelity, both sites utilized instructors trained in the respective interventions. Instructor fidelity was maintained by regular supervision calls, measuring session time and confirming adherence to the study protocol, and, in the case of MBSR, video recording sessions with review by MBSR experts according to published fidelity criteria for mindfulness-based interventions[18] (all sessions were rated as competent; Supplement 2).

To evaluate participant adherence, class attendance was monitored. Additionally, for MBSR and exercise interventions, home practice during the 6-month acute period was measured and reinforced using daily surveys sent to tablets or smartphones. During the maintenance phase, participants in the MBSR and exercise groups were asked if they had any breaks in their home practice.

Outcomes

All outcomes were measured by blinded assessors. The 2 primary outcomes were episodic memory and executive function (cognitive control) composites (standardized to a mean [SD] of 0 [1]; higher composite scores indicate better cognitive performance) at the 6-month end point. These composite scores were calculated from a neuropsychological test battery conducted at 0, 3, 6, and 18 months. The secondary end point was 18 months. These domains were selected based on previous research on the effects of mindfulness and exercise on cognitive function. Memory tests were immediate and delayed recall using a 16-item word list and 2 paragraphs developed for repeated administrations during longitudinal studies (ie, different lists and paragraphs at each time point)[19] and the Picture Sequence Memory Test from the National Institutes of Health (NIH) Toolbox.[20] Executive function tests were the Dimensional Change Card Sort test, Flanker Inhibitory Control and Attention Test, and List Sorting Working Memory Test from NIH Toolbox and the following 3 additional computer-based tests: the Consonant-Vowel Odd-Even Switching test,[21] the Sustained Attention to Response Test,[22] and the Stroop Test.[23] For each memory or executive function variable, a Z score was computed for each participant using the mean and SD of that variable computed on all randomized participants at baseline ([participant score − mean]/SD). Composite scores were then created by taking the mean of the Z scores of all available memory or cognitive control variables (additional details are provided in the statistical analysis plan [Supplement 1]). Composite scores, compared with individual test scores, improve both test-retest reliability and the ability to detect subtle changes in scores, as exemplified by the Preclinical Alzheimer Cognitive Composite (a clinical trial outcome that similarly combines multiple cognitive tests).[24] For interpretation purposes, if the intervention was effective in improving each individual measure that comprised the composite by 1 SD, the overall composite score would improve by 1 point (compared with the control). The correlations between the baseline (month 0) and 6-month composite scores were 0.81 for the memory composite and 0.80 for the executive function composite, suggesting high reliability.

Secondary outcomes (left and right hippocampal volume and left and right DLPFC surface area and cortical thickness) consisted of high-resolution T1-weighted MRI (MP-RAGE; 1 × 1 × 1 mm; TR = 2300 ms; TI = 900 ms; TE = 2.95 ms; flip angle = 9°), which were acquired at 0, 6, and 18 months. Longitudinal FreeSurfer[25] processing generated the measurements. The correlations between the baseline and 6-month MRI measures were 0.99 for hippocampal volume, 0.98 for DLPFC surface area, and 0.92 for DLPFC thickness. At the same time points, resting-state MRI data were collected; these data are presented in another report.[26]

Additional secondary cognitive outcomes included the Revised Observed Tasks of Daily Living[27] score, a performance-based measure of functional cognitive capacity (range, 0-28; higher values indicate greater ability to complete everyday activities) and the Quality of Life in Neurological Disorders Cognitive Function[28] score, a self-report measure of cognitive concerns (range, 18-90; higher values indicate better outcomes).

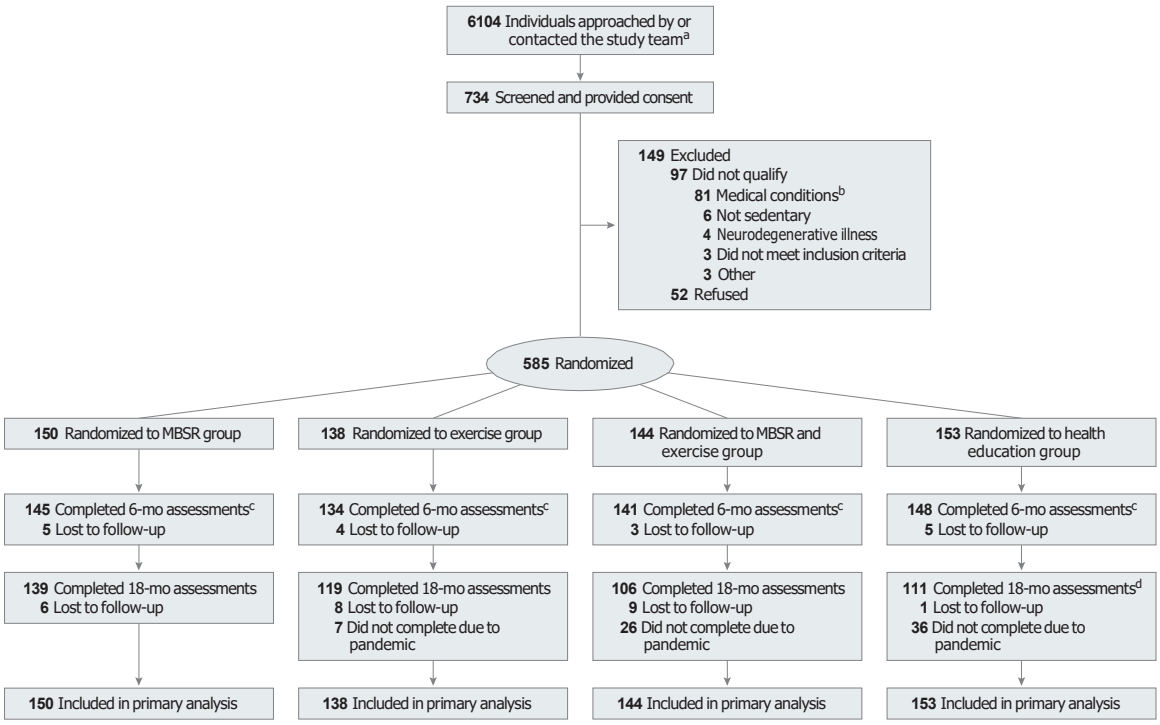
To assess mechanisms of exercise- and mindfulness-induced cognitive benefits, several physiological and performance measures at 0, 6, and 18 months were tested (details of measurement are provided in Supplement 2): aerobic fitness, insulin sensitivity and resistance, body fat and fat-free masses, physical performance, plasma cortisol levels, physical activity, time to fall asleep and total sleep time, mindfulness state, and upper- and lower-body strength.

Race and ethnicity were self-reported by participants based on fixed categories to understand the diversity of enrolled participants and for potential future subgroup analyses examining differences in results based on these characteristics.

Sample Size Calculation

A target sample size of 580 participants was determined based on 80% power to detect either main effects or an interaction of

Figure 1. Participant Flow in a Study of the Effect of Mindfulness-Based Stress Reduction (MBSR) and Exercise on Cognitive Function



[a] Unless individuals were screened, they were not fully assessed for eligibility; as such, the study team does not have the results (eg, why they were excluded or declined) for all of these individuals.

[b] Conditions that would suggest shortened lifespan or would prohibit safe participation in the interventions (eg, metastatic cancer, unstable cardiovascular disease) or would interfere with study assessments (eg, diabetes medication, systemic glucocorticoids, magnetic resonance imaging contraindications, severe hearing/visual impairment).

[c] Unless they officially withdrew, participants who missed the 6-month assessment were not out of the study; they could rejoin for the 18-month assessment.

[d] A higher number of participants (n = 36) in the health education intervention group were unable to complete the 18-month assessments due to the COVID-19 pandemic because of the randomization schedule (eg, these intervention groups were the last groups to be randomized in the trial). For example, 3 of the last 4 groups randomized in the trial were health education.

at least a small effect size, of 0.2 (Cohen *d*). The study was not designed to detect a specific minimal clinically important difference. All power analyses were conducted with G*Power, version 3.1, and assumed 15% attrition for power calculations.

Statistical Analyses

See Supplement 1 for the complete statistical analysis plan. A marginal model was fit for the repeated measures analyses. The model included the between-participant main effects of MBSR and exercise, their interaction, and the 2- and 3-way interactions between time and the between-participant effects. Time (0, 3 [cognitive measures only], 6, and 18 months) was a within-participants effect with an unspecified covariance matrix due to uneven time intervals between visits. Site, age, and sex were included as covariates in the models. Clustering by site was accounted for because site was a factor in all primary and secondary outcome models.

The primary test of effectiveness of each intervention was the change in the composite scores from baseline to 6 months in the participants randomized to undergo the intervention compared with those not receiving the intervention, as computed with the appropriate contrast (eg, MBSR vs no MBSR). The 2 × 2 factorial design was analyzed with the 2 main effects of exercise (underwent exercise intervention vs did not undergo exercise) and MBSR (underwent MBSR intervention vs did not undergo MBSR).

All randomized individuals were included in the primary analysis (Figure 1). Participants were analyzed according to their randomization group. A Bonferroni-adjusted 2-tailed significance level of .025 was used for each of the 2 primary outcomes. Effect sizes with 95% CIs for 6- and 18-month effects for all primary and secondary outcomes were computed. Because of the potential for type I error due to multiple comparisons, findings for analyses of secondary time points and secondary outcomes should be interpreted as exploratory. The mixed-model analytic approach used is robust in accounting for missing data. Participants were included in the analytic model if they had data for at least 1 time point.

Given neutral findings for the primary outcomes, the importance of post hoc analyses became clear. Subsequent per-protocol analyses were conducted, as were subgroup tests examining changes in cognitive outcomes among those who

showed the most vs the least change in the physiological and performance markers described above. The 2 per-protocol groups were defined post hoc based on examination of attendance data and home practice data: participants reporting home practice of their randomized intervention (MBSR or exercise) on at least 70% of days and participants attending at least 70% of classes. Both groups also excluded individuals who participated in interventions to which they were not randomized (Supplement 2). Additionally, given that the primary outcomes showed no intervention effect, the original plan to examine MRI structural changes as part of a mediator analysis was modified: rather than examining MRI structural changes as mediators, they were analyzed as secondary outcomes. All analyses were conducted in SAS, version 9.4 (SAS Institute).

Results

Enrollment and Participant Characteristics

A total of 6104 individuals were approached by or directly contacted the study team and 734 completed baseline screening and provided written informed consent; of these individuals, 149 did not qualify or wish to participate further. Thus, 585 individuals met all study criteria and were randomized and included in the analysis. A total of 97.1% of participants completed 6-month assessments and 81.2% completed 18-month assessments (Figure 1).

The full sample had a mean (SD) age of 71.5 (4.8) years and education level of 16.2 (2.2) years and 424 (72.5%) were women, 2 (0.3%) were American Indian, 27 (4.6%) were Asian, 69 (11.8%) were Black, 477 (81.5%) were White (the remaining individuals were unknown or >1 race), and 39 (6.7%) were Hispanic/Latino. Demographic information and other baseline characteristics were well-balanced across intervention groups (Table).

Primary Outcomes

Figure 2 shows changes over 18 months in the 2 primary outcome measures: composite variables of memory and executive function. At 6 months, there were no significant differences in these measures when comparing participants with and without MBSR (memory composite score, 0.44 vs 0.48; mean difference, –0.04 points [95% CI, –0.15 to 0.07]; $P = .50$; executive function score, 0.39 vs 0.31; mean composite difference, 0.08 [95% CI, –0.02 to 0.19]; $P = .12$) and with vs without exercise (memory composite, 0.49 vs 0.42; mean difference, 0.07 points [95% CI, –0.04 to 0.17]; $P = .23$; executive function composite, 0.39 vs 0.32; mean difference, 0.07 points [95% CI, –0.03 to 0.18]; $P = .17$).

Secondary Outcomes

There were also no significant differences at 18 months (secondary end point) for the composite variables of memory (MBSR vs no MBSR: 0.61 vs 0.53; mean difference, 0.08 [95% CI, –0.04 to 0.19]; $P = .18$; exercise vs no exercise: 0.55 vs 0.59; mean difference, –0.04 [95% CI, –0.15 to 0.07]; $P = .47$) and executive function (MBSR vs no MBSR: 0.27 vs 0.31; mean difference, –0.04 [95% CI, –0.15 to 0.07]; $P = .44$; exercise vs no exercise: 0.28 vs 0.29; mean difference, –0.01 [95% CI, –0.12 to 0.11]; $P = .93$)

Secondary outcomes included structural MRI measures (Figure 3) and additional cognitive outcomes (Supplement 2). At 6 months, there were no significant intervention effects on hippocampal volume (MBSR vs no MBSR: difference, –3.46 mm^3 [95% CI, –14.27 to 7.34]; $P = .53$; exercise vs no exercise: difference, 3.04 mm^3 [95% CI, –7.76 to 13.85]; $P = .58$), DLPFC surface area (MBSR vs no MBSR: difference, 22.71 mm^2 [95% CI, –22.95 to 68.36]; $P = .33$; exercise vs no exercise: difference, –17.18 mm^2 [95% CI, –62.83 to 28.48]; $P = .46$), or cortical thickness (MBSR vs no MBSR: difference, –0.01 mm [95% CI, –0.02 to 0.01]; $P = .37$; exercise vs no exercise: difference, 0.01 mm [95% CI, 0.00-0.02]; $P = .21$). At the secondary time point of 18 months, there was also no significant intervention effects on DLPFC surface area (MBSR vs no MBSR: difference, 25.35 mm^2 [95% CI, –23.18 to 73.88]; $P = .31$; exercise vs no exercise: difference, 21.11 mm^2 [95% CI, –27.41 to 69.64]; $P = .39$) or cortical thickness (MBSR vs no MBSR: difference = –0.01 mm, [95% CI, –0.02 to 0.00], $P = .10$; exercise vs no exercise: difference, –0.01 mm [95% CI, –0.02 to 0.00]; $P = .09$). One exception was that hippocampal volume showed a significantly greater reduction over 18 months with MBSR compared with no MBSR (difference, –20.16 mm^3 [95% CI, –33.88 to –6.44]; $P = .004$), contrary to the hypothesized direction of change; however, there was no significant intervention effect with exercise compared with no exercise (difference, –6.26 mm^3 [95% CI, –19.98 to 7.46]; $P = .37$). There was also a main effect of time for hippocampal volume ($P < .001$) and DLPFC cortical thickness ($P < .001$) (but not DLPFC surface area [$P = .68$]), which declined in all groups over 18 months. There were no significant intervention effects on the secondary cognitive outcomes (Observed Tasks of Daily Living or Neurological Disorders Cognitive Function score; eFigure 2 in Supplement 2).

Tests of Combination MBSR and Exercise and Intervention Interactions

Interactions between the 2 factors in the 2 × 2 design (MBSR vs no MBSR and exercise vs no exercise) were tested. Because none of the interaction test results were significant at 6 months (memory composite, $P = .93$; executive function composite, $P = .29$; hippocampal volume, $P = .76$; DLPFC surface area, $P = .19$; and DLPFC cortical thickness, $P = .52$), the primary analyses described above were conducted by pooling the factorial groups. eTable 1 in Supplement 2 presents a 4-group analysis (MBSR alone, exercise alone, combined MBSR and exercise, and health education), along with full data on the 3-way interactions tested for the primary outcomes and secondary MRI outcomes. This comparison shows that combined MBSR and exercise showed no significant improvement compared with MBSR alone, exercise alone, or health education (eFigure 1 in Supplement 2).

Adherence to the Interventions and Per-Protocol Analysis

Participants had a median (IQR) attendance of 90% (80.0%-100.0%) at MBSR classes and 83.3% (71.7%-91.7%) at exercise classes in the first 6 months. eFigure 3 in Supplement 2

Table. Baseline Characteristics by Intervention Group

Characteristic	MBSR (n = 150)	Exercise (n = 138)	MBSR and exercise (n = 144)	Health education (n = 153)
Age, mean (SD), y	71.2 (4.2)	71.1 (4.9)	72.4 (5.3)	71.1 (4.6)
Sex, No. (%)				
Women	108 (72.0)	108 (78.3)	102 (70.8)	106 (69.3)
Men	42 (28.0)	30 (21.7)	42 (29.2)	47 (30.7)
Race, No. (%)				
American Indian or Alaska Native	0	1 (0.7)	1 (0.7)	0
Asian	3 (2.0)	9 (6.5)	10 (6.9)	5 (3.3)
Black or African American	18 (12.0)	14 (10.1)	18 (12.5)	19 (12.4)
Native Hawaiian or Other Pacific Islander	0	0	0	0
White	127 (84.7)	109 (79.0)	112 (77.8)	129 (84.3)
More than 1 race	1 (0.7)	0	3 (2.1)	0
Unknown/not reported	1 (0.7)	5 (3.6)	0	0
Hispanic, No. (%)	7 (4.7)	9 (6.5)	14 (9.7)	9 (5.9)
Current smoker, No. (%)	4 (2.7)	1 (0.7)	0	5 (3.3)
Education level, mean (SD), y	16.0 (2.2)	16.6 (2.2)	16.0 (2.3)	16.1 (2.1)
APOE*_E4_–positive, No. (%)	49/149 (32.9)	44 (31.9)	37/143 (25.9)	44 (28.8)
CIRS-G Score, mean (SD)[a]	6.7 (2.9)	6.7 (2.8)	6.9 (2.8)	6.7 (3.0)
Comorbidities, No. (%)				
Arthritis	69 (46.0)	73 (52.9)	67 (46.5)	63 (41.2)
Hypertension	53 (35.3)	58 (42.0)	60 (41.7)	73 (47.7)
High blood cholesterol	46 (30.7)	56 (40.6)	62 (43.1)	71 (46.4)
Credibility and expectations for improvement, mean (SD)[b]				
Credibility	30.1 (6.8) [n = 143]	33.5 (5.8) [n = 123]	32.9 (6.4) [n = 127]	26.6 (7.4) [n = 143]
Improvement	59.4 (24.2) [n = 143]	61.9 (23.5) [n = 123]	68.4 (23.0) [n = 127]	56.1 (22.5) [n = 139]
BMI classification, No. (%)[c]				
Normal (16-24.9)	57 (38.0)	34 (24.6)	35 (24.3)	47 (30.7)
Overweight (25-29.9)	51 (34.0)	51 (37.0)	63 (43.8)	54 (35.3)
Obese (≥30.0)	42 (28.0)	53 (38.4)	46 (31.9)	52 (34.0)
WTAR standard score, mean (SD)[d]	113.6 (10.6)	114.1 (10.2)	113.4 (10.4)	112.2 (10.3)
SPPB modified score, mean (SD)[e]	8.8 (1.8)	8.7 (1.9) [n = 137]	9.0 (2.0)	8.8 (2.0)
Paragraph recall score, mean (SD)				
Immediate[f]	43.0 (10.7) [n = 149]	43.4 (10.2) [n = 137]	41.9 (10.8)	42.6 (9.8)
Delayed[f]	36.4 (12.0)	37.5 (10.8)	35.8 (11.3)	36.7 (10.1)
Word List score, mean (SD)				
Learning[g]	32.4 (7.4)	31.3 (7.3)	31.2 (7.6)	31.1 (7.4)
Recall[h]	6.9 (3.1)	7.1 (3.2)	6.6 (3.0)	6.5 (3.2)

(continued)

Table. Baseline Characteristics by Intervention Group (continued)

Characteristic	MBSR (n = 150)	Exercise (n = 138)	MBSR and exercise (n = 144)	Health education (n = 153)
Neuro-QoL Cognitive Function score, mean (SD)[i]	62.4 (12.6) [n = 149]	63.2 (12.1)	65.5 (11.4)	63.3 (11.4)
OTDL score, mean (SD)[j]	20.7 (3.2)	20.3 (3.5)	20.3 (3.5)	20.2 (3.7)
CAMS-R score, mean (SD)[k]	38.1 (6.2)	37.0 (5.6)	37.8 (5.9)	36.7 (5.6)
NIH Toolbox Fluid Composite score, mean (SD)[l]	92.0 (8.7)	92.0 (10.0)	91.4 (8.4)	91.5 (9.1)
Cortisol area under curve, mean (SD)[m]	5580 (2471) [n = 134]	5703 (2606) [n = 116]	6565 (2950) [n = 123]	5749 (2500) [n = 133]
Insulin sensitivity, mean (SD)				
HOMA-IR[n]	2.5 (1.7) [n = 149]	3.0 (2.2) [n = 135]	3.0 (2.1) [n = 143]	3.1 (2.3)
OGIS, mL/min^{-1}/m^{-2}[o]	352 (61) [n = 139]	344 (63) [n = 128]	348 (67) [n = 136]	346 (67) [n = 145]

Abbreviations: MBSR, mindfulness-based stress reduction; NIH, National Institutes of Health; OTDL, Observed Tasks of Daily Living.

[a] The Cumulative Illness Rating Scale-Geriatric (CIRS-G) is a 14-item instrument that measures the number and severity of physical health problems (13 organ systems; 0-4 score for each system; overall range, 0-56). Higher scores are indicative of more comorbidities and severe medical conditions. The mean score was between 6.7 and 6.9 (dependent on the intervention group), which suggests the sample was generally healthy, with approximately 3 moderate-severity medical conditions per participant.

[b] The Credibility and Expectations Questionnaire[35] was administered after the first intervention class to evaluate participants' perception of the credibility of the intervention to which they were assigned (4 questions; score range, 4-40; higher scores indicate greater credibility), and their expectations for improvement (1 question; range, 0%-100%; higher percentages indicate expectations for greater improvement). Participants generally rated the credibility of the interventions as high, with mean ranges above 30 for all intervention groups except for health education (mean [SD] of 26.6 [7.4]). Expectations for improvement were above 50% for all groups.

[c] The percentage of people with body mass index (BMI) >30.0 in this study is slightly lower than the value reported for older adults (>60 y) of 41.5% in the National Health and Nutrition Examination Survey 2017-2020.[36]

[d] Wechsler Test of Adult Reading (WTAR) measures intelligence and has 50 items. The standard score ranges from 52 to 128, with higher scores indicating higher estimated IQ; a standard score is equivalent to an IQ score. The normative score is 100. Based on the WTAR, this sample was above-normal in terms of IQ; this aligns with the advanced educational levels.

[e] The Short Physical Performance Battery (SPPB) assesses walking speed, lower extremity strength, and balance. Modified scoring was used (range, 1-12; higher scores indicate better physical functioning). The sample had mean scores between 8.7 and 9.0, suggestive of relatively high physical functioning.

[f] The Paragraph Recall task is used to quantify memory performance. This measure involved the participant listening to 2 stories and being asked to recall and report as many of the paragraph elements as possible, with each story having 44 elements (range, 0-88 for immediate and delayed recall tests; higher scores are better). The immediate positive total mean score was slightly higher across all intervention groups (mean, 41.9-43.4) than the delayed positive total mean score (mean, 35.8-37.5).

[g] This task involved the participant recalling as many words as possible from a list of 16 words (4 learning trials were presented) (range, 0-64; higher scores are better). Across all intervention groups, the mean score was between 31.1 and 32.4, suggesting that the sample was able to recall slightly less than half of the words over the course of 4 trials.

[h] This task occurs 20 minutes from the learning task. The participant was asked to recall as many words as possible from the 16-word list (range, 0-16; higher scores are better). The mean score was between 6.5 and 7.1 across all intervention groups, suggesting that the sample was able to recall less than half of the words after the delayed period.

[i] The Quality of Life in Neurological Disorders Cognitive Function (Neuro-QoL) is an 18-item self-report measure that assess health-related quality of life (range, 18-90; higher scores are better). The mean score across all interventions was between 62.4 and 65.5, suggesting that in general participants had only mild decrements in self-reported everyday cognitive function.

[j] The Revised Observed Tasks of Daily Living measures functional capacity and has a range of 0 to 28. Higher scores suggest better functional capacity. Across all intervention groups, the mean score was between 20.2 and 20.7. This suggests high functional capacity at baseline.

[k] The self-report Cognitive and Affective Mindfulness Scale-Revised (CAMS-R) measures state mindfulness (range, 12-48; higher scores indicate greater state of mindfulness). The mean score across intervention groups ranged from 36.7 to 38.1, indicating this sample reported a high level of state mindfulness at baseline.

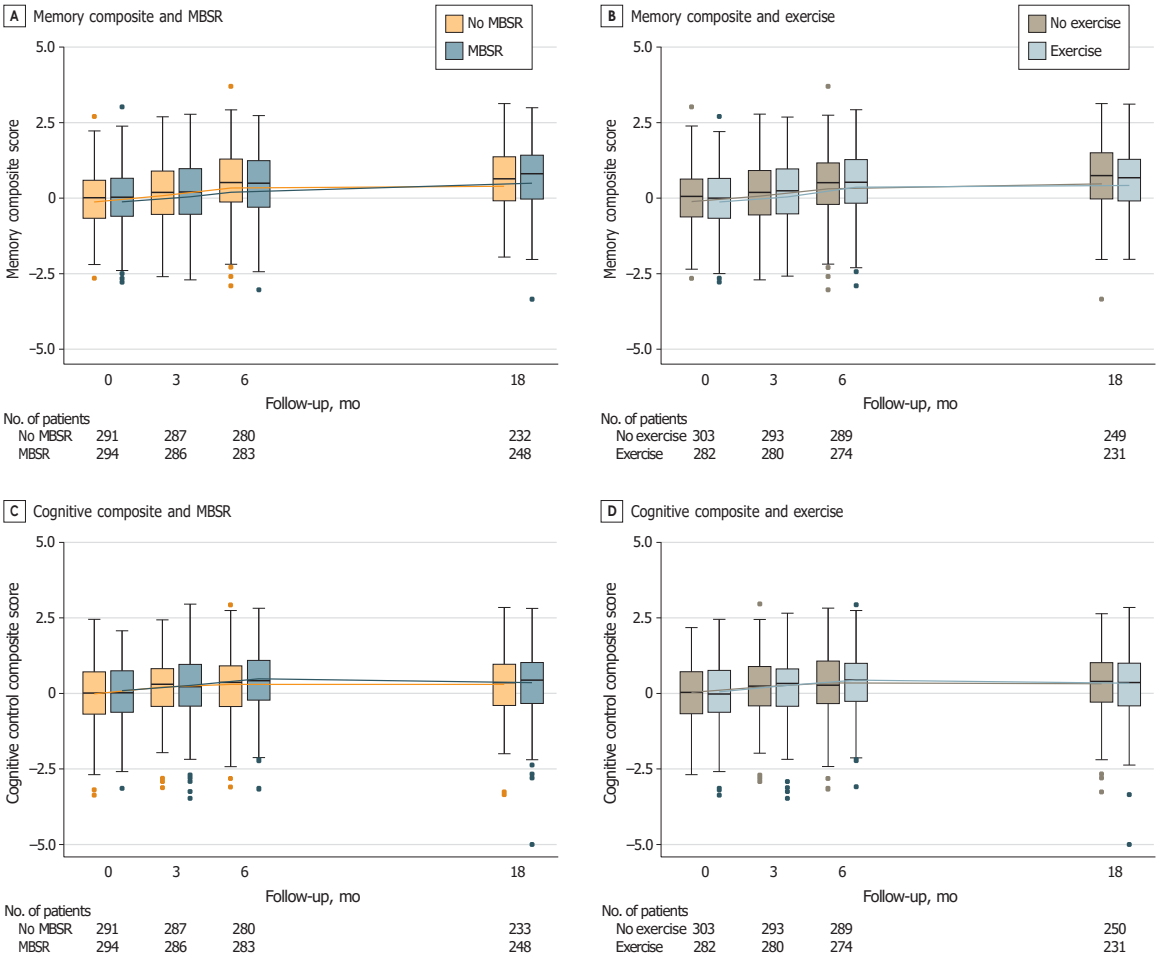
[l] This score was derived from the mean standard scores of the Flanker, Dimensional Change Card Sort, Picture Sequence Memory, List Sorting, and Pattern Comparison tasks and then deriving standard scores based on this new distribution. An uncorrected standard score at or near 100 indicates ability that is average compared with others nationally. A standard score of approximately 85 suggests significantly below-average fluid cognitive ability. The mean score of this sample was 91.4 and 92.0.

[m] Cortisol area under the curve is based on salivary measurements collected at waking, 30 minutes after waking, and bedtime on 3 consecutive days. There is no normative range for cortisol AUC for this specific assay.

[n] Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) calculated using fasting glucose and insulin. A HOMA-IR less than 1.0 indicates insulin sensitivity. The mean HOMA-IR was above 1.0 across all intervention groups.

[o] Oral Glucose Insulin Sensitivity (OGIS) calculated using a 75 g 2-hour oral glucose tolerance test obtained at the 0, 90, and 120 time points. The higher the OGIS index, the more insulin sensitive an individual is. An OGIS score of 302 mL/min^{-1}/m^{-2} (+/− 17) suggests impaired glucose tolerance. The mean OGIS score was greater than 302 mL/min^{-1}/m^{-2} across all intervention groups.

Figure 2. Memory and Executive Function Composite Changes Over 18 Months



The composite scores were the standardized mean of several neuropsychological test scores for the domain of interest. A Z score was computed for each participant ([participant score − mean]/SD), using the mean and SD of that variable computed on all randomized participants at baseline. For example, the memory composite variable was created by the mean Z scores of all available memory variables. For composite interpretation purposes, if the intervention was effective in improving each individual measure that comprised the composite by 1 SD, the overall composite score would improve by 1 point (compared with the control). The ranges for memory and executive function are −3.3 to 3.7 and −5.0 to 3.0, respectively. See eTable 2 in Supplement 2 for numerical/model data of intervention effects. The boxplot inner horizontal lines represent the median values, the boxes represent the IQR (25% and 75%), the vertical whiskers extend to the upper and lower adjacent values (the furthest points within 1.5 IQRs of the 25th and 75th percentiles), and the dots indicate outlier values.

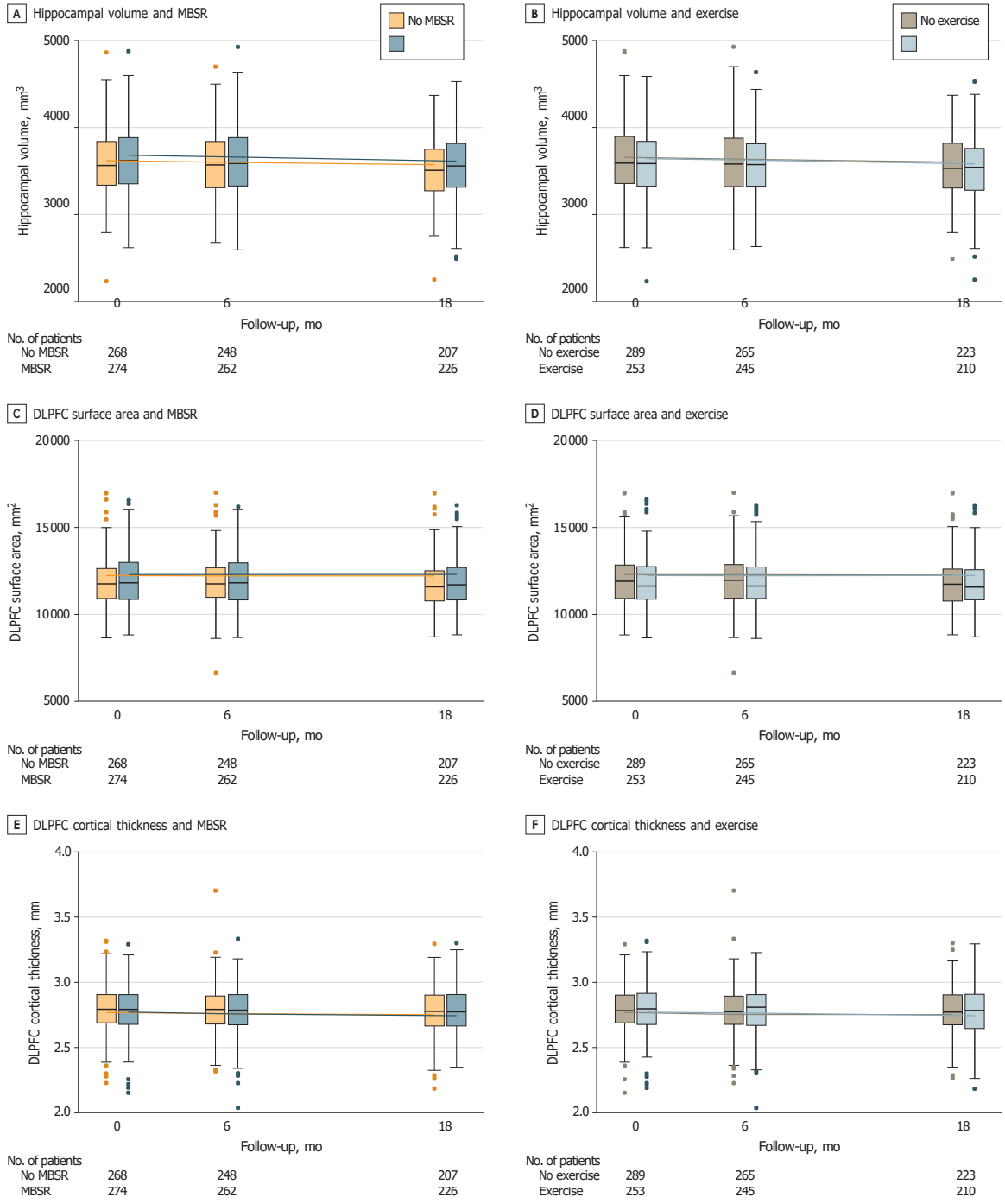
shows adherence to the interventions based on home practice and class attendance. eTable 2 in Supplement 2 compares intervention effects in the entire sample and the per-protocol subgroups; results are unchanged for all primary and secondary outcomes.

Post Hoc Analysis of Subgroups That Showed Putatively Beneficial Effects of Interventions

eTable 3 in Supplement 2 shows the effects of the interventions on multiple performance and physiological measures. Physical performance, aerobic fitness, and strength increased and sleep quality significantly improved (sleep latency was reduced and total sleep time was increased) with exercise (eTable 3A in Supplement 2). No variables were influenced by MBSR, including self-reported mindfulness (eTable 3B in Supplement 2).

Subgroups of participants who had the most change (top tertile) vs those who had the least change (bottom tertile) in these performance and physiological variables were then evaluated in terms of changes in their cognitive performance. eTable 4 in Supplement 2 quantifies these tertiles and eFigure 4 in Supplement 2 compares their episodic memory and executive function changes over 18 months. As shown in eFigure 4 in Supplement 2, there were, at most marginal, and, in the majority of cases, no differences in subgroups, which suggests limited to no evidence that MBSR or exercise

Figure 3. Structural Brain Changes Over 18 Months



Shown are the mean of the right- and left-sided brain structures. The volumes of the brain regions in this article are somewhat dependent on the measurement technique; existing literature has found that both the volumes and their rate of change are consistent with studies in healthy aging. For example, Fraser et al[37] found a rate of hippocampal atrophy of approximately 1% per year and Frangou et al[38] reported a frontal cortical thickness change of 0.005 mm per year. These are within the range of changes reported in the current sample. The ranges for hippocampal volume, DLPFC surface area, and DLPFC cortical thickness are 2232 to 4926; 6642 to 16 992; and 2.0 to 3.7, respectively. See eTable 2 in Supplement 2 for numerical/model data of intervention effects. The boxplot inner horizontal lines represent the median values, the boxes represent the IQR (25% and 75%), the vertical whiskers extend to the upper and lower adjacent values (the furthest points within 1.5 IQRs of the 25th and 75th percentiles), and the dots indicate outlier values.

differentially affected cognitive performance of participants in the top vs bottom tertiles; therefore, no inferential statistics were calculated.

Discussion

In this multicenter trial involving older adults with subjective cognitive concerns, mindfulness training, exercise, or both did not result in significant differences in improvement in episodic memory or executive function composite scores at 6 months. In secondary analyses, there were no significant improvements due to the interventions at 18 months in secondary outcomes, including structural brain measures of hippocampus and DLPFC. The findings do not support the hypothesis that these interventions improve cognitive performance in older adults.

These null findings differ from positive findings in some randomized clinical trials of exercise[29] and epidemiological data that have suggested that exercise was associated with improved cognitive and brain health in older adults,[30] as well as a smaller body of literature supporting the beneficial role of mindfulness.[31] There are several potential causes for these null findings. First, all groups showed increases in cognitive performance over time, so it could be posited that all interventions (including health education) benefited participants equally and these increases reflect those benefits, and thus the study failed due to lack of a proper negative control. Arguing against this idea is that the health education intervention was designed for this study so that it would not specifically target cognition (eg, it did not include a mindfulness or exercise regimen). Further, if cognitive performance increases represented true benefits, one would expect to see a reflection of those benefits in brain structures (ie, increase or attenuated decrease in the size of hippocampus and DLPFC, structures involved in episodic memory, and executive function), yet both structures showed longitudinal declines with all conditions, consistent with age-related atrophy not attenuated by the interventions. In addition, the combination of MBSR and exercise showed no greater change than each intervention alone. Thus, the increases in cognitive performance likely reflect expectancy or practice effects from repeated exposure to the assessments.

Another potential cause of the null findings was failure in target engagement (ie, failure in having the desired effect from the interventions), which could result from poor participant adherence, low intervention fidelity by instructors, low intensity of interventions, or low reliability of outcome measures. However, none of these problems were apparent: participants demonstrated high adherence and retention in the study, instructors were trained and supervised for fidelity, the intensity of interventions was similar to that in prior trials, and outcome reliability was good. Furthermore, per-protocol analyses of participants that were more highly adherent to the interventions showed no significant differences from the overall sample. In the exercise intervention, physiological and performance changes suggest participants benefited from exercise. Thus, the findings are similar to the Lifestyle Interventions and Independence for Elders Study, which showed a beneficial effect of 24 months of exercise on disability prevention, but not cognitive performance.[32] In contrast, MBSR was not associated with significant change in any physiological or performance measure, which raises the question of whether the implementation of MBSR was sufficient; however, given adequate instructor fidelity, participant class attendance, and home practice, the lack of a measurable effect of mindfulness training may reflect a lack of clearly-measurable targets in mindfulness-based intervention.

Another possibility accounting for lack of detectable effect of interventions is that participants were generally healthy and potentially insufficiently sedentary at baseline, thereby limiting potential for benefiting from lifestyle interventions. To test this, subgroup analyses of those who showed the greatest changes in physiological or performance variables posited to underlie cognitive health (eg, improved insulin sensitivity) were conducted. These analyses found that, even when the interventions produced beneficial changes in these putative mechanisms, they still did not lead to significant cognitive benefits. Thus, the health of the participants does not appear to explain the null results. As a whole, these results suggest that the underlying hypothesis is unsupported.

Limitations

This study has several limitations. First, the participants were largely White and the majority were college-educated; this limited diversity reduces generalizability of findings. Second, the study focused on structural characteristics of hippocampus and DLPFC as proxy measures of the brain's health; other regions or assessment techniques might be more sensitive to intervention effects.[33] Third, the study tested interventions over 18 months; a longer period of intervention may be needed to show beneficial effects. Fourth, the study focused on healthy older adults who were objectively cognitive intact; some studies have found beneficial effects of exercise on cognitive function in more physically or cognitively ill and frail older adults,[34] as well as benefits of MBSR in older adults with depression and anxiety.[7] Fifth, individuals with subjective cognitive concerns are a heterogeneous group that could include those with incipient dementia as well as individuals experiencing the influence of medications, medical conditions, or nutrition status. These and other potentially remediable mechanisms beyond cortisol, insulin sensitivity, and aerobic fitness were not examined in this study and should be considered in future research.

Conclusions

Among older adults with subjective cognitive concerns, mindfulness training, exercise, or both did not result in significant differences in improvement in episodic memory or executive function composite scores at 6 months. The findings do not support the use of these interventions for improving cognition in older adults with subjective cognitive concerns.

ARTICLE INFORMATION

Accepted for Publication: November 5, 2022.

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Obtained funding: Lenze, Barch, Depp, Head, Wetherell.
Administrative, technical, or material support: Barch, Depp, Diniz, Eyler, Foster, Gettinger, Klein, Nicol, Nishino, Rodebaugh, Schweiger, Shimony, Sinacore, Snyder, Tate, Wu, Wetherell.
Supervision: Lenze, Ances, Balota, Foster, Schweiger, Shimony, Sinacore, Tate, Wu, Wetherell.
Other - Served as Research Coordinator for study: Voegtle.
Other - Design, implementation, and evaluation of exercise intervention: Nichols.
Other - Data analysis concerning insulin kinetics and sensitivity: Patterson.

Conflict of Interest Disclosures: Dr Lenze receiving personal fees from Boehringer Ingelheim, IngenioRx, Merck, Pritikin ICR, and Prodeo and grants from COVID Early Treatment Fund, Fast Grants, and Janssen Biotech, outside the submitted work. Dr Barch reported receiving grants from the National Institute of Mental Health and the National Institute on Drug Abuse during the conduct of the study. Dr Foster reported receiving grants from the National Institutes of Health during the conduct of the study and grants from the American Occupational Therapy Foundation and Washington University School of Medicine outside the submitted work. Dr Gettinger reported receiving grants from the National Institutes of Health during the conduct of the study and personal fees from Pritikin ICR for consulting outside the submitted work. Dr Head reported receiving grants from the National Institutes of Health, McKnight Brain Research Foundation, and Taylor Family Institute for Innovative Psychiatric Disorders at Washington University during the conduct of the study. Dr Nichols reported receiving grants from UC San Diego during the conduct of the study. Dr Nicol reported receiving grants from Alkermes, personal fees from Alkermes and Novartis, and nonfinancial support from Usona outside the submitted work. Dr Nishino reported receiving grants from the National Institute on Aging during the conduct of the study. Dr Patterson reported receiving grants from Washington University in St Louis during the conduct of the study. Dr Rodebaugh reported receiving grants from the National Institutes of Health during the conduct of the study. Dr Schweiger reported grants from the National Institutes of Health during the conduct of the study. Dr Snyder reported receiving grants from Washington University for National Institutes of Health funding for this research during the conduct of the study. Dr Tate reported receiving grants from the National Institutes of Health during the conduct of the study. Dr Wing reported receiving grants from the National Institute of Mental Health during the conduct of the study. Dr Wetherell reported receiving grants from the National Institutes of Health during the conduct of the study and receiving a salary from the Department of Veterans Affairs outside the submitted work. No other disclosures were reported.

Funding/Support: This project was funded by NIH R01 AG049689 and the McKnight Brain Research Foundation. Additional funding was from the Taylor Family Institute for Innovative Psychiatric Disorders at Washington University, and NIH grants P50MH122351, P50 HD103525, P30 DK056341 (Washington University Nutrition and Obesity Research Center), and UL1 TR000448 (Washington University Institute of Clinical and Translational Sciences). Dr Eyler was additionally supported by the Desert-Pacific VA Mental Illness Research Education and Clinical Center and Dr Twamley was additionally supported by a VA Rehabilitation Research and Development Research Career Scientist Award.

Role of the Funder/Sponsor: The funders had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Data Sharing Statement: See Supplement 3.

Additional Contributions: We express appreciation to the MEDEX trial participants, members of the data and safety monitoring board (Paul Newhouse, MD [Vanderbilt University School of Medicine; chair]; Eric Rodriguez, MD [University of Pittsburgh Medical Center; safety officer]; Elizabeth Hoge, MD [Georgetown University Medical Center]; Helena Kraemer, PhD [Stanford University School of Medicine]; and Jeffrey Burns, MD [University of Kansas Medical Center]), and the entire MEDEX research team. MEDEX trial participants and the research team received compensation for their role in the study. The data and safety monitoring board members did not receive compensation.

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The Journal of Neuroscience, November 9, 2022 · 42(45):8587–8594 · 8587

Neurobiology of Disease

Integrity of Neuronal Size in the Entorhinal Cortex Is a Biological Substrate of Exceptional Cognitive Aging

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Average aging is associated with a gradual decline of memory capacity. SuperAgers are humans ≥80 years of age who show exceptional episodic memory at least as good as individuals 20–30 years their junior. This study investigated whether neuronal integrity in the entorhinal cortex (ERC), an area critical for memory and selectively vulnerable to neurofibrillary degeneration, differentiated SuperAgers from cognitively healthy younger individuals, cognitively average peers ("Normal Elderly"), and individuals with amnestic mild cognitive impairment. Postmortem sections of the ERC were stained with cresyl violet to visualize neurons and immunostained with mouse monoclonal antibody PHF-1 to visualize neurofibrillary tangles. The cross-sectional area (i.e., size) of layer II and layer III/V ERC neurons were quantified. Two-thirds of total participants were female. Unbiased stereology was used to quantitate tangles in a subgroup of SuperAgers and Normal Elderly. Linear mixed-effect models were used to determine differences across groups. Quantitative measurements found that the soma size of layer II ERC neurons in postmortem brain specimens were significantly larger in SuperAgers compared with all groups ($p < 0.05$)—including younger individuals 20–30 years their junior ($p < 0.005$). SuperAgers had significantly fewer stereologically quantified Alzheimer's disease-related neurofibrillary tangles in layer II ERC than Normal Elderly ($p < 0.05$). This difference in tangle burden in layer II between SuperAgers and Normal Elderly suggests that tangle-bearing neurons may be prone to shrinkage during aging. The finding that SuperAgers show ERC layer II neurons that are substantially larger even compared with individuals 20–30 years younger is remarkable, suggesting that layer II ERC integrity is a biological substrate of exceptional memory in old age.

Key words: Alzheimer's disease; entorhinal cortex; neurofibrillary tangles; neuronal integrity; SuperAging

Significance Statement

Average aging is associated with a gradual decline of memory. Previous research shows that an area critical for memory, the entorhinal cortex (ERC), is susceptible to the early formation of Alzheimer's disease neuropathology, even during average (or typical) trajectories of aging. The Northwestern University SuperAging Research Program studies unique individuals known as SuperAgers, individuals ≥80 years old who show exceptional memory that is at least as good as individuals 20–30 years their junior. In this study, we show that SuperAgers harbor larger, healthier neurons in the ERC compared with their cognitively average same-aged peers, those with amnestic mild cognitive impairment, and – remarkably – even compared with individuals 20–30 years younger. We conclude that larger ERC neurons are a biological signature of the SuperAging trajectory.

Received Apr. 5, 2022; revised Sep. 7, 2022; accepted Sep. 13, 2022.

Author contributions: M.-M.M., E.R., C.G., and T.G. designed research; C.N., A.K., I.A., G.M., R.A.S., and A.Z. performed research; C.N., A.K., I.A., G.M., N.P.G., R.A.S., A.Z., R.K., H.Z., Q.M., E.R., C.G., and T.G. analyzed data; C.N., A.K., I.A., G.M., N.P.G., R.A.S., A.Z., R.K., H.Z., Q.M., M.E.F., E.H.B., M.-M.M., E.R., C.G., and T.G. wrote the paper.

This study was supported by the National Institute on Aging of the National Institutes of Health (Grants P30-AG-013854, R01-AG-062566, R01-AG-067781, R01-AG-045571, R56-AG-045571, U19-AG-073153). National Institute on Aging (P30 AG072977, F31AG07631), the National Institute of Neurological Disorders and Stroke (T32NS047987), and a National Science Foundation Graduate Research Fellowship (DGE-1842165). We thank the research participants of the Northwestern University SuperAging Research Program and the Northwestern University Alzheimer's Disease Research Center for invaluable contributions to this study and the brain donation program.

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The authors declare no competing financial interests.

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https://doi.org/10.1523/JNEUROSCI.0679-22.2022

Introduction

Memory capacity declines during the course of average aging. The entorhinal cortex (ERC) and hippocampus, two areas critical for episodic memory, are selectively susceptible to neurofibrillary tangle (NFT) formation. This phenomenon starts as an age-related process and intensifies in a prodromal stage of Alzheimer's disease (AD), known as amnestic mild cognitive impairment (aMCI), reaching its peak in the dementia of AD. Even age-related memory decline could therefore reflect the emergence of neurofibrillary degeneration in the hippocampus and entorhinal cortex (Balasubramanian et al., 2012; Koen and Yonelinas, 2016; Gefen et al., 2018). While this age-related decline is common, it is not necessarily inevitable.

The Northwestern University SuperAging Research Program investigates a unique trajectory that reflects the resistance and resilience to the involutional process characteristic of the MCI–AD continuum. SuperAgers (SAs) are defined as individuals ≥80 years old who demonstrate episodic memory performance at least as good as what would be considered normal for individuals 20–30 years younger (Harrison et al., 2012; Rogalski et al., 2013; Gefen et al., 2014). Of the first 10 cases that came to autopsy, the hippocampus and ERC contained a low to intermediate density of NFTs (Braak stages II–III), whereas healthy cognitively average age-matched control subjects ("Normal Elderly") had an NFT density range that extended into Braak stage IV (Merrill et al., 2000; Rogalski et al., 2019). Even the NFT-containing limbic areas in SuperAgers contained many healthy-appearing neurons and the neocortex was generally free of neurofibrillary degeneration (Rogalski et al., 2019). The status of ERC neurons is of particular interest in light of the exceptional memory performance in SuperAgers despite old age. A central question is whether SuperAgers are resistant to neurofibrillary degeneration or are resilient to the effects of NFT on neuronal number and size.

Prior research has shown that the number of cortical neurons does not display age-related changes in cognitively intact elderly free of dementia (Stark et al., 2007; Freeman et al., 2008). Recent work on postmortem cases with non-AD dementia demonstrated a tight concordance between shrinkage of neuronal soma and the manifestation of clinical symptoms (Kim et al., 2018). In an effort to understand the factors that contribute to the preservation of memory and to the SuperAging phenotype, the current study investigated the cross-sectional area (i.e., size) of neurons in the ERC in a rare series of autopsies. Stellate cells in layer II and pyramidal cells in layer III/V of the ERC were targeted for measurement given their pivotal role in the reciprocal transfer of information between association cortex and the hippocampal formation (Van Hoesen and Hyman, 1990; Van Hoesen and Solodkin, 1993; Canto et al., 2008), their position along the perforant pathway (Hyman et al., 1984, 1986; Witter, 2007), and the relative paucity of ERC NFTs in SuperAgers compared to their cognitively average peers (Gefen et al., 2021). Stereological quantitation was also performed in a subset of specimens to determine the relationship between NFT formation and neuronal size in layer II of the ERC. The result of this investigation includes the unexpected finding that ERC neuronal size is significantly larger in SuperAgers compared with younger neurologically healthy individuals, in addition to their same-aged elderly peers. This outcome raises fundamental questions regarding the nature of the age-related involutional phenomena in SuperAgers and their relationship to superior memory capacity.

Materials and Methods

Participant characteristics

All participants were required to demonstrate preserved activities of daily living. All participants were also required to lack clinical evidence or history of neurologic or psychiatric disease. The autopsied brains of six participants characterized as "Cognitive SuperAgers" from the Northwestern University SuperAging Research Program were identified from the Northwestern University Alzheimer's Disease Research Center Brain Bank. As comparison, the autopsied brains of seven "cognitively average elderly" ["Normal Elderly"(NE)] participants from the Northwestern University SuperAging Research Program, six healthy younger adults ["Younger Controls" (YCs)], and five participants with antemortem aMCI were additionally identified. Written informed consent and agreement to enter the brain donation program were obtained from all participants in the study, and the study was approved by the Northwestern University Institutional Review Board and in accordance with the Helsinki Declaration (https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/). Samples from representative brain regions of each participant were surveyed qualitatively and found to be free of significant neurodegenerative pathology other than amyloid plaques and neurofibrillary tangles. Braak staging (Braak and Braak, 1991b; Braak et al., 1993) was surveyed in each participant to identify the degree of tangle involvement. Apolipoprotein E (ApoE) genotype was assessed using DNA extracted from blood samples provided by each participant according to enrollment procedures into the Northwestern University SuperAging Research Program. See Table 1 for participant characteristics.

Inclusion criteria

Cognitive SuperAgers. Detailed inclusion criteria have been reported previously (Rogalski et al., 2013). Briefly, all participants were community-dwelling, English-speaking adults ≥80 years of age who were free of significant neurologic or psychiatric illness. Inclusion criteria also included neuropsychological test performance criteria, which were chosen for their relevance for cognitive aging and their sensitivity to detect clinical symptoms associated with dementia of the Alzheimer's type (Weintraub et al., 2009). The delayed recall score of the Rey Auditory Verbal Learning Test (RAVLT; Schmidt, 2004) was used as a measure of episodic memory, and SuperAgers were required to perform at or above average normative values for individuals in their 50s and 60s (midpoint age, 61 years; RAVLT delayed recall raw score, ≥9; RAVLT delayed recall scaled score, ≥10; for more information, see Gefen et al., 2015).

Cognitively average Normal Elderly individuals. Cognitively average elderly individuals were community-dwelling, English-speaking adults ≥80 years of age who were free of significant neurologic or psychiatric illness and were enrolled into the Northwestern University SuperAging Research Program as cognitively average control subjects based on neuropsychological performance. Specifically, these individuals were required to fall within 1 SD of the average range for their age and education before death (Ivnik et al., 1996; Heaton et al., 2004; Shirk et al., 2011). Criteria are in accordance with the National Institute on Aging and Alzheimer's Association (NIA-AA) for elderly individuals considered "not demented" (Albert et al., 2011).

Younger cognitively average individuals (Younger Controls). Ages of the younger cognitively average participants ranged from 26 to 61 years. Clinical records were available for each participant and were assessed carefully for evidence of cognitive deficits. If the clinical history did not definitively validate normal cognitive function, this information was obtained from the next of kin.

Individuals with aMCI diagnosis. Participants received a diagnosis of aMCI during life based on the criteria proposed by the NIA-AA (Albert et al., 2011). Individuals with an antemortem diagnosis of aMCI were required to show clear impairment on neuropsychological tests of memory and no impairment in other cognitive domains.

Tissue processing and histopathology

Postmortem intervals (PMIs) ranged from 3 to 58 h. After autopsy, each specimen was cut into 3–4 cm coronal blocks and fixed in 4%

Table 1. Participant characteristics

Participant	Age at death (years)	Sex	Education (years)	PMI (h)	Brain weight (g)	Braak staging	ApoE	Non-AD pathology
SA 1	99	F	16	58	1020	III	3, 3	Multiple cortical microinfarcts (nonsignificant); 1 remote lacunar infarct, left putamen; ARTAG, AGD
SA 2	90	F	18	4	990	III	3, 3	ARTAG, AGD, 1 remote lacunar infarct, left globus pallidus
SA 3	90	F	14	4.5	1100	II-III	2, 3	PART (definite), Lewy body in dorsal motor nucleus of vagus (incidental), ARTAG
SA 4	95	F	18	5	1241	0	NA	NA
SA 5	92	M	16	11	1247	I	3, 3	Medial temporal TDP-43 pathology; moderate cerebrovascular disease, non-occlusive
SA 6	82	F	14	24	1241	I	3, 3	Lewy bodies in substantia nigra and locus coeruleus, incidental; mild cerebrovascular disease, nonocclusive
NE 1	96	F	14	5	NA	IV	NA	NA
NE 2	88	M	12	12	1250	III-IV	NA	NA
NE 3	82	M	NA	24	NA	III-IV	NA	NA
NE 4	95	F	12	3.25	1096	III	2, 3	NA
NE 5	89	F	16	9	1180	II	3 ,3	NA
NE 6	88	M	20	9	1490	I	3, 3	Glioblastoma, WHO grade IV, 9.0 cm in greatest dimension, left parieto-occipital region; amygdala-only Lewy body disease
NE 7	87	F	16	16	1183	I	3, 3	Moderate vascular disease
aMCI 1	89	F	18	4.5	1280	II	NA	None
aMCI 2	99	F	13	5	1060	III-IV	3, 3	None
aMCI 3	92	F	12	3.5	1084	III-IV	3, 3	None
aMCI 4	90	M	14	3	1380	III	3, 3	None
aMCI 5	92	M	16	4.5	1100	V	3, 4	Superficial contusion in occipital lobe
YC 1	45	M	NA	13	1650	0	NA	NA
YC 2	61	F	NA	22	1080	I	NA	NA
YC 3	50	F	NA	48	1150	0	NA	NA
YC 4	57	F	NA	6	1100	0	NA	NA
YC 5	59	F	NA	20	1300	NA	NA	NA
YC 6	26	M	NA	8	1560	0	NA	NA

ARTAG, aging-related tau astrogliopathy; AGD, argyrophilic grain disease; PART, primary age-related tauopathy; CHD, coronary heart disease; NA, not available; F, female; M, male; WHO, World Health Organization; None, TDP-43 staining was not available. Braak staging followed published guidelines (Braak and Braak, 1985, 1991a,b; Braak et al., 1993).

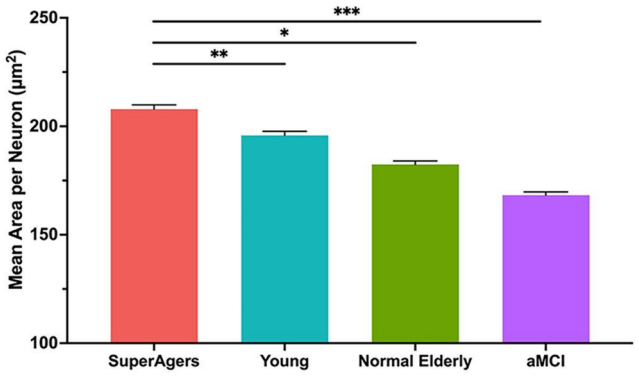

Figure 1. Mean cross-sectional area per neuron in layer II of the entorhinal cortex. Heights of bars represent the difference in mean cross-sectional area per squared micrometer of layer II neurons in the entorhinal cortex between SuperAgers ($N = 6$), Younger Controls ($N = 6$), Normal Elderly ($N = 7$), and individuals with aMCI ($N = 5$). An overall average of ; 1044 neurons (SD, 229) were measured per group. SuperAgers showed a significantly larger mean area of layer II ERC neurons compared with Normal Elderly, aMCI individuals, and Younger Controls. There were no significant differences in the mean area of layer II neurons between Normal Elderly, aMCI individuals, and Younger Controls. Statistical significance was assessed using a linear mixed-effect model. Error bars represent the SEM. *p , 0.05; **p , 0.01; *** p , 0.001.

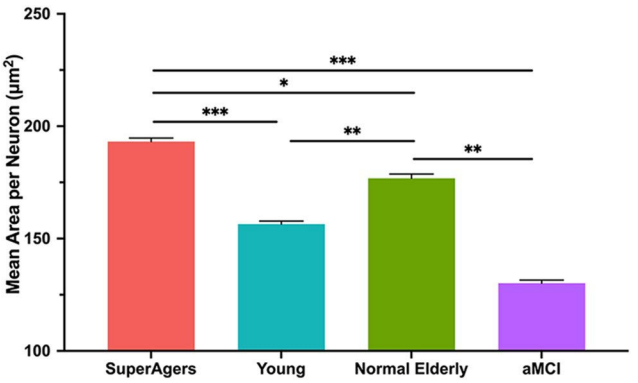

Figure 2. Mean cross-sectional area per neuron in layer III/IV of the entorhinal cortex. Heights of bars represent the mean cross-sectional area per square micrometer of layer III/V neurons in the entorhinal cortex among SuperAgers ($N = 6$), Younger Controls ($N = 6$), Normal Elderly ($N = 7$), and individuals with aMCI ($N = 5$). An overall average of ; 1052 neurons (SD, 53) were measured per group. Mean area of layer III/V ERC neurons was significantly larger in SuperAgers compared with Normal Elderly, aMCI individuals, and Younger Controls. Normal Elderly also showed larger neuronal cross-sectional area in layer III/V of the ERC compared with aMCI individuals and Younger Controls. Statistical significance was assessed using a linear mixed-effect model. Error bars represent the SEM. *p , 0.05; **p , 0.01; ***p , 0.001.

paraformaldehyde for 30–36 h at 4°C, and then taken through sucrose gradients (10–40% in 0.1 M sodium phosphate buffer, pH 7.4) for cryoprotection and stored at 4°C. Blocks were sectioned at a thickness of 40 μm on a freezing microtome and stored in 0.1 M phosphate buffer containing 0.02% sodium azide at 4°C until use. Regions were equivalent across tissue blocks taken from the anterior entorhinal cortex. Up to six sections (intersection interval, 24 or 54) of the entorhinal cortex were collected, and a 1.0% cresyl violet Nissl stain was used to visualize neurons. All specimens were evaluated grossly for cortical, caudate, cerebellar, and brainstem atrophy, as well as vascular pathology. Braak staging (Braak et al., 1993) was surveyed in each case to identify NFT involvement in transentorhinal/entorhinal cortex, other limbic cortical areas, and neocortical regions. SuperAger, Normal Elderly, and aMCI specimens were also evaluated microscopically for Lewy and non-Lewy α-synucleinopathies, vascular pathologies, frontotemporal lobar degeneration-tau, and pathologic TDP-43 (Table 1).

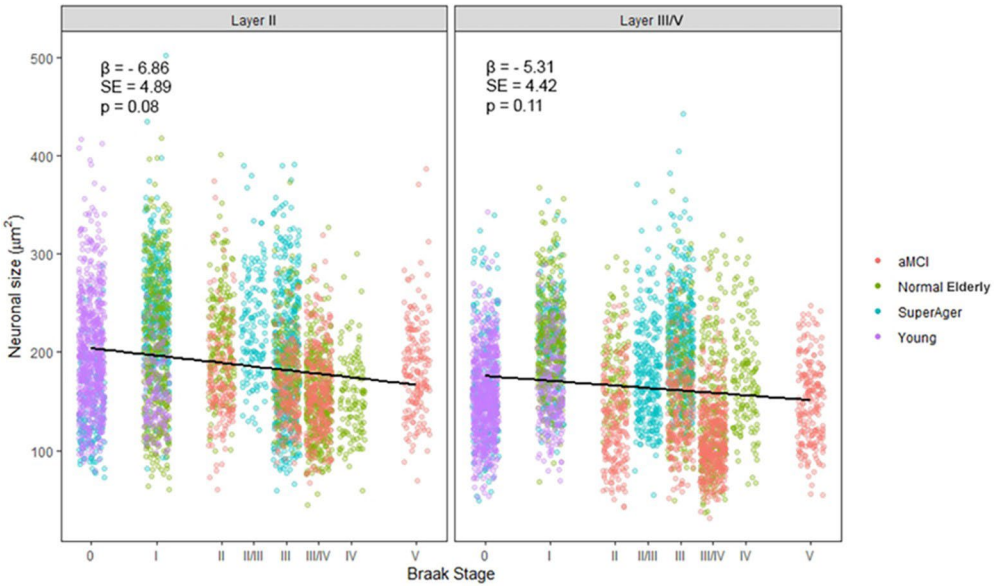

Figure 3. Relationship between the cross-sectional area of layer II ERC neurons and Braak stage. Moderate evidence for a negative association of increasing Braak stage on neuronal cross-sectional area (in μm^2) was found, yet it did not quite reach statistical significance in ERC layer II ($p = 0.08$; $b = 6.86$) and in ERC layer III/V ($p = 0.11$; $b = 5.31$). Statistical significance was assessed using a linear mixed-effect model. b, Regression coefficient; SE, SE of the regression coefficient.

Measurement of cross-sectional area of neurons in the ERC

The cross-sectional area of neurons in layer II and layer III/V of each ERC region per participant was measured. Layer II neurons were identified by their stellate appearance and their arrangements in islands of neurons. Layer III/V neurons were identified by their pyramidal shape and the orientation of their apical dendrite toward the cortical surface. To measure the cross-sectional area of neurons, 6–10 photomicrographs were obtained randomly from sections spanning layer II and layer III/V per each ERC layer and analyzed at 20× magnification. Analysis of the cross-sectional area of neurons in the ERC was conducted by an individual blinded to group affiliation; a second rater performed analyses of the area of ERC neurons on three cases to ensure consistency in measurement. Analysis was conducted using the image analysis software ImageJ (version 1.53). Within ImageJ (RRID:SCR_003070), the tracing function was used to measure the area of the neuron at 5.5 pixels/μm (image size, 1600 × 1200 pixels). The area was obtained for at least 100 neurons per layer (II and III/V independently) of the ERC region per participant. Mean total area of neurons was calculated in layer II and layer III/V of the ERC, per case, and evaluated for differences across groups.

Modified stereological analysis of NFT pathology in layer II of ERC

In a subset of cases (five SuperAgers and five Normal Elderly) with tissue available, modified stereological methods were used to estimate the density of PHF-1-stained tangles in layer II. Thioflavin-S-positive NFT counts in the ERC without specific quantitation of laminar patterns are reported in the study by Gefen et al. (2021). Whole-hemisphere sections were selected that contained ERC with clear layer II cell islands and were immunostained with the mouse monoclonal antibody PHF-1 (P. Davies, Albert Einstein College of Medicine, New York, NY; catalog #PHF1; RRID:AB_2315150). PHF-1 recognizes tau phosphorylated at Ser396/404 and allows for visualization of tangles and pretangles in the ERC. Briefly, layer II of the ERC was traced at 2.5× magnification and analyzed at 40× magnification by an individual blinded to group. Analysis was performed using the fractionator method and StereoInvestigator software (MBF Bioscience; RRID:SCR_004314). The sections used in analysis were treated as adjacent sections, allowing for calculation of the density in the total volume within the sections. The top and bottom 10 μm of each section were set as the guard height. The dimensions of the counting frame chosen were 225 × 225 μm, based on trials. The coefficient of error was calculated, and sampling parameters were adjusted so that the coefficient of error was ≤0.1. Data were expressed as counts per cubic millimeter based on planimetric calculation of volume by the fractionator software. Mean NFT densities were compared between the two groups.

Experimental design and statistical analysis

The study used a cross-sectional experimental design based on autopsied specimens. A Kolmogorov–Smirnov test for equality of distributions was used to confirm consistency between two raters of neuronal size. The test failed to reject the null hypothesis of equal distributions, indicating agreement (i.e., consistency) between raters. Differences among age at death, education, and PMI were determined using a one-way ANOVA. A linear mixed-effect model with a random intercept for subject was used to compare the mean neuronal cross-sectional area among the four groups for layer II and layer III/V. The same model was used to determine differences in area between layer II and layer III/V neurons across all cases. Postmortem interval, age at death, and Braak stage, were included as covariates. The p-value for rejecting the null hypothesis was set at 0.05. Linear mixed-effect modeling was also used to test whether there was an association among age at death, cross-sectional area of neurons, and Braak staging (layer II and layer III/V, independently). Braak staging was treated as a continuous variable. Statistical analyses were performed using RStudio Software (version 4.0.3; RRID:SCR_000432). A Welch's t test was used to compare the densities of NFTs in layer II between SuperAgers and Normal Elderly.

Results

In accordance with criteria, the mean age of Younger Controls (mean age, 49.67 years; SD, 13.05) was significantly lower than those of SuperAgers (mean age, 91.33 years; SD, 5.72), Normal Elderly (mean age, 89.29 years; SD, 4.82), and aMCI (mean age, 92.40 years; SD, 3.91; p < 0.05); there were no significant differences in age among other groups. No group differences were found among years of education, PMI, or brain weight. Only one aMCI case (aMCI 5) carried an APOE-4 allele, a known risk factor for Alzheimer's disease (Saunders et al., 1993; Roses, 1996). The Braak staging of NFTs in aMCI subjects ranged from II to V, in Normal Elderly from I to IV, and in SuperAgers from 0 to III. All Younger Controls showed a

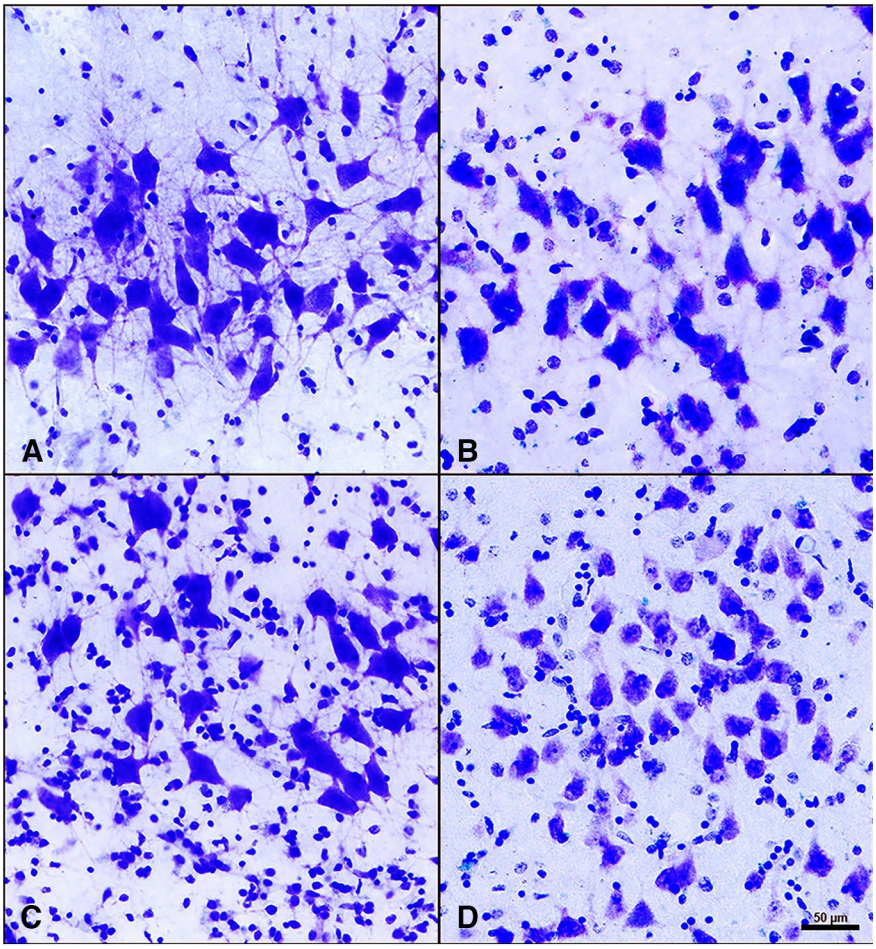

Figure 4. Layer II neurons of the entorhinal cortex in SuperAgers, Younger Controls, Normal Elderly, and aMCI individuals. *A–D*, Layer II neurons in SuperAgers, Younger Controls, Normal Elderly, and individuals with aMCI visualized with cresyl violet staining. *A*, SA 2, a 90-year-old female SuperAger. *B*, YC 4, a 57-year-old female young control subject. *C*, NE 6, an 88-year-old male elderly control subject. *D*, aMCI 1, an 89-year-old female with aMCI. Scale bar: (in *D*) *A–D*, 50 μm. *A–D*, SuperAger (*A*) shows a significantly larger mean area of layer II ERC neurons compared with Younger Controls (*B*), Normal Elderly (*C*), and individual with aMCI (*D*).

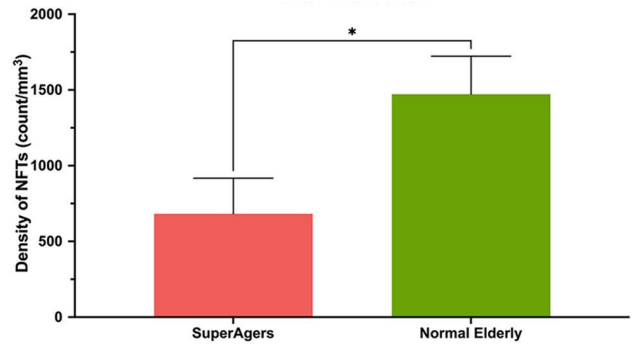

Figure 5. Density of NFTs in layer II of the entorhinal cortex heights of bars represent the mean density per cubic millimeter of NFTs in layer II neurons in the entorhinal cortex between SuperAgers ($N = 5$) and Normal Elderly ($N = 5$). Density of NFTs in layer II ERC neurons was significantly smaller in SuperAgers (mean, 682; SEM, 235) compared with Normal Elderly (mean, 1472; SEM, 251; p , 0.05). This relationship held when controlled for Braak staging. Statistical significance was assessed using a Welch's *t* test. Error bars represent the SEM.

Braak stage of 0 with the exception of participant "YC 2" (stage I; Table 1).

For each group, the mean cross-sectional area of neuronal soma was significantly greater in layer II compared with layer III/V (SuperAgers, Younger Controls, aMCI: p , 0.001; Normal Elderly: $p = 0.005$). Mean soma area of layer II ERC neurons was significantly larger in SuperAgers compared with Normal Elderly (p , 0.05), aMCI individuals ($p = 0.001$), and, remarkably, Younger Controls (p , 0.005). There were no significant differences in the mean area of neurons among Normal Elderly, Younger Controls, and aMCI individuals in layer II of the ERC [Fig. 1 (see also Fig. 4)]. At the individual-case level, there was some overlap in layer II soma sizes, highlighting variability; for example, two SuperAgers (SA 1 and SA 4) showed soma sizes that fell below the average size of their same-age peers, and the inverse was true for two Normal Elderly (NE 5 and NE 6). The mean area of layer III/V ERC neurons followed the same trend, where SuperAgers showed a larger soma area than Normal Elderly (p , 0.05), Younger Controls (p , 0.001), and aMCI individuals (p , 0.001). However, Normal Elderly showed a significantly larger soma area of layer III/V neurons compared with aMCI individuals ($p = 0.003$) and, unexpectedly, Younger Controls ($p = 0.01$). This is also likely because of the presence of variability in soma size among individual cases given the small groups of postmortem brain tissue, particularly in the Normal Elderly (Fig. 2).

Analyses were performed to determine whether there was a relationship between age at death and the cross-sectional area of neurons within layer II and layer III/V of the ERC, regardless of group affiliation. There was no evidence of an association between age at death and area of layer II and III/V neurons of the ERC. The same relational analyses were performed to determine the relationship between cross-sectional area of neurons and Braak staging (Figs. 3, 4). There was moderate evidence for a negative association of increasing Braak stage on neuronal cross-sectional area that did not quite reach statistical significance in layer II ($p = 0.08$; $b = 6.86$) or layer III/V ($p = 0.11$; $b = 5.31$) of the ERC.

Finally, modified unbiased stereology was performed in ERC sections stained immunohistochemically with PHF-1 to visualize layer II pre-NFTs and mature NFTs in a subset of five SuperAgers (SA 1–5) and five Normal Elderly (NE 1, 2, 3, 6, and 7). The estimated PHF-1-positive NFT densities in layer II were significantly higher in Normal Elderly (; 1500/mm³) compared with SuperAgers (; 700/mm³; p , 0.05), by a difference of about twofold (Figs. 5, 6).

Discussion

Cognitive SuperAgers are individuals 2: 80 years of age who appear to be resistant to the deleterious effects of aging on memory function. Thus far, prior research reported that compared with same-aged peers, SuperAgers show less white matter neuroinflammatory markers (Gefen et al., 2019) and acetylcholinesterase activity (Janeczek et al., 2018), higher cortical volumes (Harrison et al.,

2012; Rogalski et al., 2013; Sun et al., 2016), lower rates of atrophy (Cook et al., 2017), and higher density of Von Economo neurons in the anterior cingulate cortex (Gefen et al., 2018) when compared with Normal Elderly. Additional neuropathologic studies of successful agers found associations between preservation of cognition and decreased levels of neurofibrillary tangles (Kawas et al., 2015), amyloid plaques (Kawas et al., 2021), and TDP-43 inclusions (Nelson et al., 2022) in postmortem samples. In a recent study, we reported that SuperAgers harbor fewer NFTs in the entirety of the ERC compared with Normal Elderly and individuals with aMCI (Gefen et al., 2021). The current study extended these findings through an examination of neuronal size as a proxy for cellular integrity of the ERC in SuperAgers. There were four novel findings. First, SuperAgers displayed significantly larger neuronal cross-sectional area in layer II of the ERC when compared with Normal Elderly, individuals with aMCI, and, most remarkably, even relative to the mean ERC cell size of Younger Controls, some of whom were nearly 60 years their junior. Second, a small to moderate negative effect was found between age at death and neuronal size in layer II among all cases, a trend that was recapitulated by the relationship between Braak stage and size as well. Third, we found that SuperAgers harbored significantly fewer NFTs in layer II alone than Normal Elderly. Finally, and in accordance with prior literature (Krimer et al., 1997; Merrill et al., 2000), within the ERC and across all groups, layer II perikarya were larger than those that dwell in layer III/V. Taken together, we can conclude that the integrity of neuronal size in the entorhinal cortex is a biological substrate of exceptional cognitive aging. The inverse is also true: that is, neuronal atrophy appears to be a characteristic marker of normal and pathologic aging. We suspect that this process is a function of neurofibrillary formation in the affected cells (Fig. 7), leading to compromised memory abilities in older age.

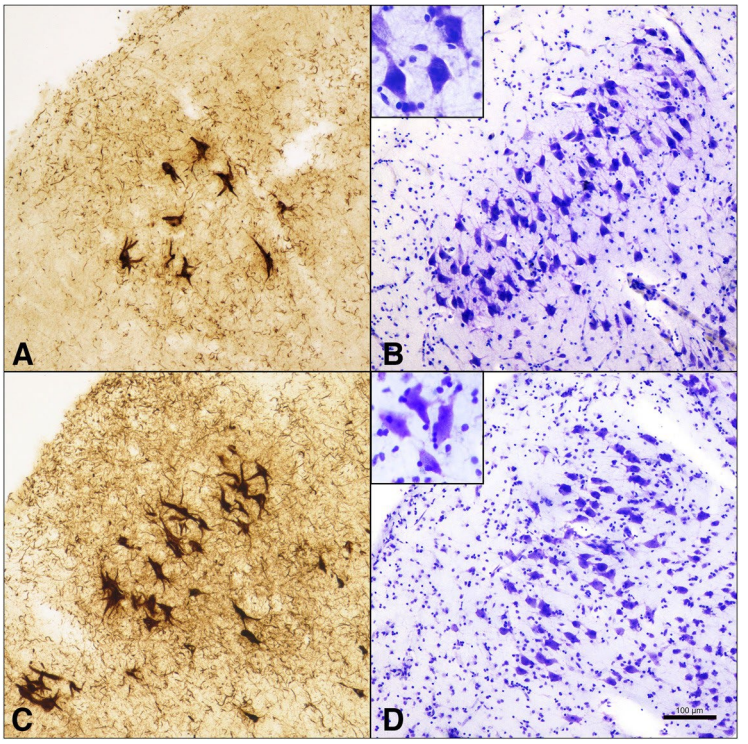

Figure 6. NFTs in layer II ERC neurons compared with neuronal size in SuperAgers and Normal Elderly. *A*, *B*, SA 2, a 90-year-old female SuperAger. *C*, *D*, NE 6, an 88-year-old male elderly control. SuperAger shows significantly fewer layer II NFTs (*A*) and larger layer II soma size (*B*) compared with Normal Elderly (*C*, *D*). Scale bar, 100 µm.

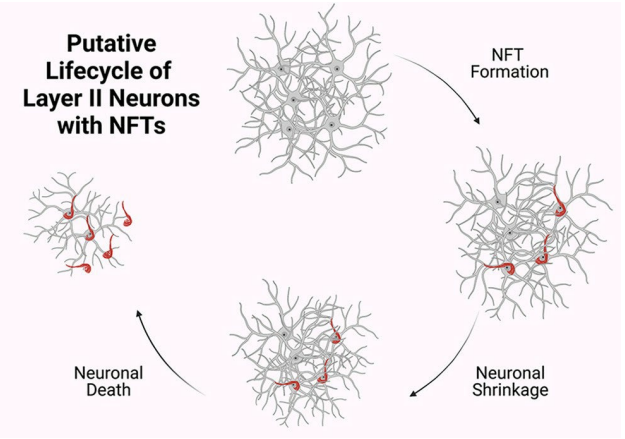

Figure 7. Putative life cycle of layer II neurons with NFTs. Results suggest that in stellate neurons in layer II of ERC, NFT formation leads to neuronal shrinkage. As previously understood, NFTs undergo biochemical changes and remain as "ghost tangles" after their associated neurons dies. Neuronal shrinkage may be an initial mechanism along the course toward age-related cognitive impairment. Created with BioRender.com.

For reasons that remain unknown, cell populations in the ERC are selectively vulnerable to NFT formation during normal aging and in early stages of AD (Braak and Braak, 1985, 1991a). In contrast, SuperAgers either resist the neuropathologic changes of aging and AD or are resilient to cognitive impairment despite demonstrating pathologic brain changes (Rogalski et al., 2013, 2019; Gefen et al., 2021). In a previous report, despite an overlap in Braak staging, NFT burden in SuperAgers was unusually low in the ERC given their age with approximately three times fewer NFTs compared with Normal Elderly across the entire ERC (Gefen et al., 2021). In the current study, Braak staging ranged from 0 to III in SuperAgers and from I to IV in Normal Elderly, again with overlap. When viewed in relation to layer II cell size, there was evidence to suggest that increased NFT pathology is a biological driving force leading to neuronal shrinkage. This was most apparent in the aMCI group, where AD is pathologically present and cell size is significantly lower compared with other groups. To our knowledge, this is one of the first reports to suggest that neuronal shrinkage is a biological substrate of NFT degeneration and poor memory functioning. Current findings showing that SuperAgers resist layer II NFTs in the ERC strongly suggest that a neuron spared from tangle formation can maintain its structural integrity. The remarkable observation that SuperAgers showed larger layer II neurons than their younger peers may imply that large ERC stellate cells were present *de novo* and are maintained structurally throughout life.

Future in-depth studies are needed to examine possible mechanisms of neuronal, axonal, synaptic, and dendritic integrity in larger samples of SuperAgers across corticolimbic regions. The nucleus basalis of Meynert, for example, contains a population of

cholinergic neurons (Ch4) that are distinctly magnocellular, and project to the olfactory bulb, the amygdala, and the entire cortical mantle (Mesulam et al., 1983). Early pretangles form first in Ch4 neurons in parallel with layer II neurons of the ERC over the course of aging and AD, then spread to other limbic/paralimbic areas, then to neocortex. The cause of vulnerability is not presumed to be the cholinergic nature of Ch4 but rather its location within a continuous band of limbic structures (Mesulam, 2013). The investigation of dendritic and axonal integrity, synaptic abnormalities, and genetic and metabolomic factors in any of these anatomically vulnerable limbic regions are all viable avenues of exploration. In the hippocampus proper, synaptic loss in particular is highly correlated with cognitive decline in AD (Honer et al., 1992; Colom-Cadena et al., 2020). Such decline is thought to be because of the loss of afferents from layer II ERC neurons that span to the outer molecular layer of the dentate gyrus (Scheff et al., 2006). In animal models of successful aging, the preservation of postsynaptic densities in the molecular layer correlated with better spatial learning ability in cognitively intact rats (Smith et al., 2000; Morrison and Baxter, 2012). Less is known, however, about the status of synaptic integrity in limbic systems in human specimens procured from successful agers. A fruitful future study involves the measurement of synaptic proteins in layer II pyramidal neurons and throughout hippocampal subfields to establish a putative link between strong synaptic currents and neuronal integrity. Thus far, the study of SuperAgers has led to the conclusion that these unique individuals carry with them a biological signature that now comprises a finding of larger, and healthier, ERC neurons relatively void of tau pathology. With time, it is likely that other factors that promote resistance and resilience to aging-related involutional phenomena will be discovered.

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