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A blood biomarker of the pace of aging is associated with brain structure: replication across three cohorts

Ethan T. Whitman [a,*], Calen P. Ryan [b], Wickliffe C. Abraham [c], Angela Addae [a], David L. Corcoran [d], Maxwell L. Elliott [e], Sean Hogan [f], David Ireland [f], Ross Keenan [g,h], Annchen R. Knodt [a], Tracy R. Melzer [g,i], Richie Poulton [f], Sandhya Ramrakha [f], Karen Sugden [a], Benjamin S. Williams [a], Jiayi Zhou [b], Ahmad R. Hariri [a], Daniel W. Belsky [b,j], Terrie E. Moffitt [a,k,l,m,n], Avshalom Caspi [a,k,l,m,n], on behalf of the Alzheimer's Disease Neuroimaging Initiative [1]

[a] Department of Psychology and Neuroscience, Duke University, Durham, NC, USA
[b] Butler Columbia Aging Center, Columbia University Mailman School of Public Health, New York, USA
[c] Department of Psychology, University of Otago, Dunedin, New Zealand
[d] Department of Genetics, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA
[e] Department of Psychology, Center for Brain Science, Harvard University, Cambridge, MA, USA
[f] Dunedin Multidisciplinary Health and Development Research Unit, Department of Psychology, University of Otago, Dunedin, New Zealand
[g] Brain Research New Zealand-Rangahau Roro Aotearoa, Centre of Research Excellence, Universities of Auckland and Otago, New Zealand
[h] Christchurch Radiology Group, Christchurch, New Zealand
[i] Department of Medicine, University of Otago, Christchurch, New Zealand
[j] Department of Epidemiology, Columbia University Mailman School of Public Health, New York, USA
[k] Center for Genomic and Computational Biology, Duke University, Durham, NC, USA
[l] King's College London, Social, Genetic, and Developmental Psychiatry Centre, Institute of Psychiatry, Psychology, & Neuroscience, London, UK
[m] PROMENTA, Department of Psychology, University of Oslo, Norway
[n] Department of Psychiatry and Behavioral Sciences, Duke University, Durham, NC, USA

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ABSTRACT

Biological aging is the correlated decline of multi-organ system integrity central to the etiology of many age-related diseases. A novel epigenetic measure of biological aging, DunedinPACE, is associated with cognitive dysfunction, incident dementia, and mortality. Here, we tested for associations between DunedinPACE and structural MRI phenotypes in three datasets spanning midlife to advanced age: the Dunedin Study (age=45 years), the Framingham Heart Study Offspring Cohort (mean age=63 years), and the Alzheimer's Disease Neuroimaging Initiative (mean age=75 years). We also tested four additional epigenetic measures of aging: the Horvath clock, the Hannum clock, PhenoAge, and GrimAge. Across all datasets (total N observations=3380; total N individuals=2322), faster DunedinPACE was associated with lower total brain volume, lower hippocampal volume, greater burden of white matter microlesions, and thinner cortex. Across all measures, DunedinPACE and GrimAge had the strongest and most consistent associations with brain phenotypes. Our findings suggest that single timepoint measures of multi-organ decline such as DunedinPACE could be useful for gauging nervous system health.

* Corresponding author.
E-mail address: ethan.whitman@duke.edu (E.T. Whitman).

[1] Data used in preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). As such, the investigators within ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in analysis or writing of this report. A complete listing of ADNI investigators can be found at: http://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf

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1. Introduction

Aging is the primary risk factor for many prevalent diseases (Niccoli and Partridge, 2012). Indeed, geroscientists have begun to treat aging itself as a preventable cause of many aging-related diseases (Barzilai et al., 2018; Campisi et al., 2019; Matt et al., 2015). The *geroscience hypothesis* defines aging as the gradual, progressive, correlated biological decline of the entire body over decades (Kennedy et al., 2014; Gladyshev, 2016). Crucially, individuals of the same *chronological* age often vary in their rate of *biological* aging (Belsky et al., 2015). Despite its importance, there is still no agreed-upon measure of biological aging (Ferrucci et al., 2020). To address this, researchers have begun to use DNA methylation to quantify aging. DNA methylation is a highly age-sensitive epigenetic process wherein methyl groups are selectively added to DNA molecules to affect gene transcription. Attempts to develop measures of aging have often used blood DNA methylation because blood is the most widely profiled source of DNA and blood DNA methylation is a biological substrate that is sensitive to age-related changes across the body (Horvath and Raj, 2018; Levine et al., 2015).

In the past decade, several algorithms have been developed to estimate biological aging using DNA methylation (Rutledge et al., 2022). These algorithms are typically referred to as 'epigenetic clocks.' The first generation of epigenetic clocks was trained largely on chronological age (Hannum et al., 2013; Horvath, 2013). Subsequently, a second generation of clocks was trained on cross-sectional measures of current health that predict mortality such as C-reactive protein levels, white blood cell count, and smoking packs-per-year (Levine et al., 2018; Lu et al., 2019). This second generation includes clocks such as PhenoAge and GrimAge. Tests for associations between first- and second-generation epigenetic clocks and brain structure have yielded mixed results. Some studies reported large positive associations between accelerated epigenetic age and reduced total brain volume (Hillary et al., 2021), reduced hippocampal volume (Davis et al., 2017), increased white matter hyperintensities (Hillary et al., 2021), and thinner cortex (Proskovec et al., 2020). However, other studies found only small positive associations between accelerated epigenetic age and lower hippocampal volume (Milicic et al., 2022) and increased white matter hyperintensities (Raina et al., 2017). Still others reported null associations between accelerated epigenetic age and cortical thickness, cortical surface area, and cortical volume (Cheong et al., 2022), or even found that accelerated epigenetic age is associated with preserved white matter microstructure, specifically increased fractional anisotropy and reduced mean diffusivity (Chouliaras et al., 2018).

In contrast to these earlier epigenetic clocks, we recently developed a third-generation DNA methylation-based measure that is unique in estimating a person's rate of biological aging. The DunedinPACE (**P**ace of **A**ging **C**alculated from the **E**pigenome) algorithm was developed by first measuring people's rate of physiological change over time and then identifying the methylation patterns that optimally captured individual differences in their age-related decline (Belsky et al., 2022). Specifically, age-related decline was measured over ages 26, 32, 38, and 45 years in 19 biomarkers of the cardiovascular, metabolic, renal, immune, dental, and pulmonary organ systems among healthy midlife individuals of the same chronological age participating in the Dunedin Study (Elliott et al., 2021b). Methylation patterns at the end of the 20-year observation period were then identified that estimated how fast each participants' multi-organ decline occurred during the 20 years leading up to the point of measurement (Belsky et al., 2022). Thus, DunedinPACE was designed to capture methylation patterns reflecting individual differences in the rate of age-related multi-organ decline and it has been robustly associated with multimorbidity and mortality (Belsky et al., 2022; Bernabeu et al., 2023; Faul et al., 2023; Kuiper et al., 2023; Lachlan et al., 2022; McMurran et al., 2023). Importantly, DunedinPACE allows for readily measuring the pace of aging in individuals who lack data to implement longitudinal physiological profiling.

Of note, DunedinPACE was not trained on any measures of central nervous system decline. Thus, it is not clear whether DunedinPACE is associated with brain structure. Recent studies have used longitudinal multi-organ measurements to demonstrate that aging 'below the neck' is related to aging of the brain (Elliott et al., 2021a, 2021b; Tian et al., 2023). Additionally, prior work has found some correspondence between DNA methylation patterns in blood and brain tissue (Horvath et al., 2012), suggesting that DNA methylation in blood is a promising surrogate for relating the aging of body and brain (but see (Shireby et al., 2020). However, it is unknown whether a measurement from a blood sample at a single timepoint also captures the association between brain structure and body aging. DunedinPACE has been associated with cognitive and clinical measures that are thought to index health of the central nervous system. For instance, faster DunedinPACE has been associated with more rapid cognitive decline (Belsky et al., 2022; Reed et al., 2022), mild cognitive impairment, and dementia (Sugden et al., 2022). These findings suggest that DunedinPACE indexes typical decline of cognitive ability during typical aging as well as in neurodegenerative illness. Associations between DunedinPACE and these key cognitive and clinical phenotypes suggest that DunedinPACE may also be associated with brain structure, though this question has not been formally tested.

We examined associations between DunedinPACE and multiple measures of brain structure across three large datasets spanning mid- to late-life: the Dunedin Study (N = 770, mean age=45 years), the Framingham Heart Study Offspring Cohort (FHS-OC; N = 903, mean age=63.76 years), and the Alzheimer's Disease Neuroimaging Initiative (ADNI; N observations=1707, N individuals=649; mean age=75.41 years). Across all three datasets (N observations=3380; N individuals=2322), we tested for associations between DunedinPACE and measures of brain structure derived from high-resolution magnetic resonance imaging (MRI; Fig. 1) including: total brain volume (TBV), hippocampal volume (HC), white matter hypointensity volume (WMHypo), mean cortical thickness (CT), and total cortical surface area (SA). When available in the Dunedin Study and ADNI, we also tested for associations between DunedinPACE and white matter hyperintensity volume (WMHyper). In addition, we leveraged the longitudinal nature of ADNI to test for associations between DunedinPACE and age-related changes in brain structure. For comparison, we also tested associations between first- and second-generation epigenetic clocks and brain structure.

2. Methods

Data used in the current analyses were collected in the Dunedin Study (Poulton et al., 2023), the FHS-OC (Feinleib et al., 1975), and ADNI (Petersen et al., 2010). Further details on each of these studies is provided below. All analyses were checked for reproducibility by an independent data analyst who used the manuscript to derive code and reproduce statistics in an independent copy of the data.

2.1. Dunedin Study

The Dunedin Study is a longitudinal study of a population-representative birth cohort (N = 1037) born between April 1972 and March 1973 in Dunedin, New Zealand (Poulton et al., 2023). The cohort is primarily White (93 %, self-identified), matching South Island demographic characteristics. Assessments were carried out at birth and ages 3, 5, 7, 9, 11, 13, 15, 18, 21, 26, 32, 38, and most recently 45 years, when 94 % of living members took part. DNA methylation and MRI data reported here were collected at the age-45 assessment phase. The Dunedin Study was approved by the New Zealand Health and Disability Ethics Committee and the Duke University Institutional Review Board. All Study members provided written informed consent.

2.1.1. DNA methylation

DNA methylation was measured from whole blood using Illumina Infinium MethylationEPIC BeadChip Arrays and run at the Molecular

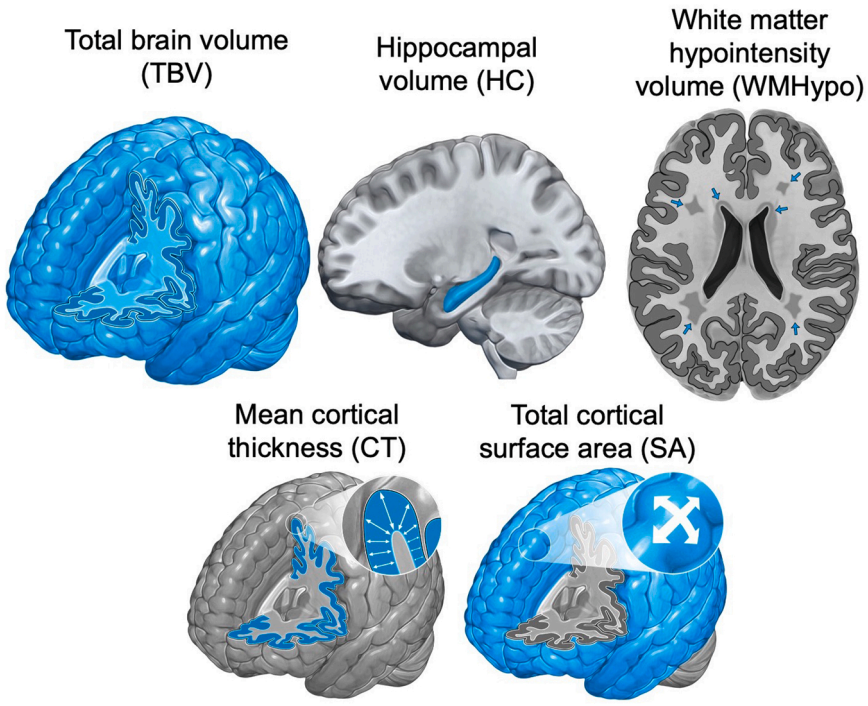

Fig. 1. Diagrams of MRI-derived brain structure measures. Phenotype of interest is represented in blue. Top row shows total brain volume (TBV), hippocampal volume (HC), and white matter hypointensity volume (WMHypo). Bottom row shows mean cortical thickness (CT) and total cortical surface area (SA).

Genomics Shared Resource at the Duke Molecular Physiology Institute. Further details on DNA methylation methods in the Dunedin Study have been reported previously (Belsky et al., 2022; Sugden et al., 2020) and are presented in the supplemental materials.

2.1.2. MRI

T1-weighted and T2-weighted fluid attenuated inversion recovery (FLAIR) images were collected using a Magnetom Skyra 3 T scanner with a 64-channel head/neck coil (Siemens Healthcare GmbH). High-resolution structural images were obtained using a T1-weighted MP-RAGE sequence. TBV, HC, WMHypo, CT, and SA were generated from the T1-weighted images using The Human Connectome Project minimal preprocessing pipeline (Glasser et al., 2013) and WMHyper was derived from the T2-weighted images using the Unidentified Bright Object detector (Jiang et al., 2018) with visual confirmation of accuracy provided by a trained neuroradiologist. Further details on MRI methods in the Dunedin Study have been reported previously (Arbeloff et al., 2019), and are presented in the supplemental materials.

2.2. Framingham Heart Study – Offspring Cohort (FHS-OC)

The FHS tracks the development of cardiovascular disease in three generations of families from Framingham, Massachusetts, beginning in 1948 (Tsao and Vasan, 2015). We analyzed data collected from the second generation of participants, known as the Offspring Cohort (Feinleib et al., 1975). All DNA methylation data were collected at the Framingham Offspring 8th follow-up, and MRI data were collected during an independent schedule of assessments conducted approximate to the time of the 8th follow-up. We obtained FHS-OC data from the National Institutes of Health Database of Genotypes and Phenotypes (dbGaP) under accession phs000007.v33.p14. DNA methylation data are available as substudy phs000724.v10.p14. Brain imaging data are available as substudy phs002559.v1.p14. We analyzed DNA methylation data downloaded as phg000492.v5. FHS_DNAMethylation.raw-data-idat and brain imaging data downloaded as phs000007.v33.pht004364.v2.t_mrbrfs_2010_1_0900s. The FHS was approved by the Institutional Review Board for Human Research at Boston University Medical Center. All participants provided written informed consent.

2.2.1. DNA methylation

DNA methylation was measured from whole blood using Illumina Infinium HumanMethylation450 BeadChip Arrays and run at the University of Minnesota and The Johns Hopkins University (dbGaP phs000724.v9.p13). Further details of DNA methylation methods in the FHS-OC have been reported previously (Mendelson et al., 2017).

2.2.2. MRI

T1-weighted images were collected using a Magnetom 1.5 T scanner with T1-weighted coronal spoiled gradient-recalled echo acquisition sequence (Siemens Healthcare GmbH). TBV, HC, WMHypo, CT, and SA were generated using FreeSurfer version 5.3 (Fischl, 2012). T2-weighted FLAIR images were not available for the measurement of WMHyper. Further details on MRI methods in FHS-OC have been reported previously (McGrath et al., 2019; Van Lent et al., 2023).

2.3. Alzheimer's Disease Neuroimaging Initiative (ADNI)

The primary goal of ADNI is to test whether serial magnetic resonance imaging, positron emission tomography (PET), other biological markers, and clinical and neuropsychological assessments can be combined to measure the progression of neurodegeneration in individuals with mild cognitive impairment, Alzheimer's disease, and healthy older adults. DNA methylation data were downloaded from the ADNI data repository on June 3rd, 2020 and MRI data were downloaded on June 12th, 2022 (adni.loni.usc.edu). ADNI was approved by the Institutional Review Boards of all the participating institutions. All participants provided written informed consent.

2.3.1. DNA methylation

DNA methylation was measured from whole blood using the Illumina Infinium MethylationEPIC BeadChip Array and run at AbbVie. Further details on DNA methylation methods in ADNI have been reported previously (Sugden et al., 2022; Vasanthakumar et al., 2020).

2.3.2. MRI

T1-weighted and T2-weighted FLAIR images were collected using either 1.5 T or 3 T scanners. TBV, HC, WMHypo, CT, and SA were generated from the T1-weighted images using FreeSurfer version 4.3, 5.1, or 6.0 (Fischl, 2012). WMHyper were generated from the T2-weighted images using a previously described algorithm (Decarli et al., 1999). MRI acquisition parameters varied across ADNI sites and waves; however, the targets for acquisition were isotropic 1 mm^3 voxels. (Jack et al., 2008). All MRI data underwent centralized quality control by ADNI investigators prior to becoming available for download. ADNI WMHypo, CT, and SA data are distributed according to the FreeSurfer version used in processing; therefore, we used Longitudinal ComBat to harmonize across FreeSurver versions using all FreeSurfer observations with QC ratings of 'Pass' from individuals with DNA methylation data (Beer et al., 2020). Further details on MRI methods in ADNI can be found at adni.loni.usc.edu.

2.3.3. Cognitive status

ADNI participants were classified into cognitively normal (CN), mild cognitive impairment (MCI), or dementia groups by ADNI study physicians based on subjective memory complaints, multiple neurocognitive and behavioral assessment scores, and level of impairment in activities of daily living. Complete diagnostic criteria used by ADNI can be found at https://adni.loni.usc.edu/methods/documents/.

2.4. DunedinPACE estimation

In all three datasets, we derived DunedinPACE from the DNA methylation data using a publicly available algorithm (https://github.com/danbelsky/DunedinPACE). Briefly, this algorithm was derived from elastic net regression to estimate the pace of biological aging from a set of 173 CpG sites (Belsky et al., 2022). We applied these regression weights to the methylation scores at these CpG sites to calculate the DunedinPACE score. Following prior work (Sugden et al., 2022), we residualized DunedinPACE for chronological age in the mixed-age FHS-OC and ADNI datasets. Because age is modestly correlated with DunedinPACE, we residualized DunedinPACE for age to yield the rate of aging while accounting for the expected rate of aging given a participant's chronological age. Because all Dunedin Study members were aged 45 at the time of their blood draw and MRI scans, age residualization was not necessary for analyses of Dunedin Study data. Within each study, DunedinPACE values were standardized to mean=0, standard deviation=1. Further details of estimating DunedinPACE in the Dunedin Study, FHS-OC, and ADNI have been reported previously (Belsky et al., 2022; Sugden et al., 2022).

To allow for comparison of DunedinPACE with other epigenetic clocks, we also report cross-sectional results for the Horvath, Hannum, GrimAge, and PhenoAge clocks (Hannum et al., 2013; Horvath, 2013; Levine et al., 2018; Lu et al., 2019). Additional details about the calculation of these epigenetic clocks can be found in the supplemental materials.

2.5. Primary analyses

We first conducted a regression analysis of linear associations between DunedinPACE and each MRI measurement in all three datasets. Diagrams of each MRI measurements are presented in Fig. 1. All regression models controlled for sex. Models using FHS-OC and ADNI data also included covariates for age, age^2, sex*age, and age^2*sex to account for the age variation in these cohorts. Controlling for age helps protect against residual confounding that arises from the multifaceted relationship between age, biological aging, and age-related outcomes. This step is recommended in standard analyses of epigenetic aging clocks (Krieger et al., 2023). We did not control for age in the Dunedin Study because all individuals were aged 45 at the time of blood draws and MRI. We also controlled for intracranial volume (ICV) for all analyses of TBV and HC. WMHypo and WMHyper volumes were log-transformed for normality prior to analyses. In analyses using ADNI data, we included multiple timepoints within individuals in our models and calculated robust standard errors to account for non-independence.

We leveraged the longitudinal nature of ADNI to test whether baseline DunedinPACE could predict the rate of subsequent change in brain structure. We restricted this analysis to data from a subset of ADNI participants who remained cognitively normal throughout enrollment (N = 153). This is because neurodegeneration in Alzheimer's disease may diverge from the normative aging patterns indexed by DunedinPACE. Next, we identified participants from this subset who had ≥ 3 timepoints for each MRI measure after their first DNA methylation timepoint (N = 107–147, Table 2). Using this subset of cognitively normal participants with sufficient longitudinal MRI data, we generated multilevel linear models for each MRI measure with random effects for both person and age. Using these models, we derived trajectories to track decline in each MRI measure for each person (i.e., a "decline" curve). To focus on relative changes in brain structure, we residualized TBV and HC for ICV prior to generating these curves. We then tested whether each person's initial DunedinPACE measure could predict their subsequent rate of brain structure change, after controlling for age, sex, and length of observation period.

2.6. Sensitivity analyses

We conducted three sensitivity analyses. First, white blood cell abundance is thought to affect whole-blood epigenetic clock estimates. Therefore, we repeated our analyses while controlling for white blood cell abundance (specifically plasmablasts, +CD8pCD28nCD45RA-T cells, naïve CD8 T cells, CD4 T cells, Natural Killer cells, monocytes, and granulocytes) estimated from DNA methylation (Horvath, 2013; Houseman et al., 2012). Second, carriership of the *APOE* ε4 risk allele has been associated with altered brain structure (Régy et al., 2022). Therefore, we repeated all analyses while including risk allele carriership as a covariate to test whether *APOE* ε4 could account for any observed associations between DunedinPACE and brain structure. We also computed results while stratifying by *APOE* ε4 carriership to discern how carriership of this allele affects the relationship between DunedinPACE and brain structure. Finally, we tested for cross-sectional associations between DunedinPACE and brain structure in ADNI while stratifying by diagnoses of MCI or dementia at the time of MRI scanning. The diagnosis-stratified results are presented in the supplemental materials.

3. Results

3.1. Demographic characteristics

In the Dunedin Study, 770 Study members (male=51.0 %) had both DNA methylation and MRI data available at age 45. The median number of days between blood sample collection and MRI was 1. More details on participants who had delays between blood sample collection and MRI can be found in the supplemental materials. Details showing that these Study members continue to represent the original birth cohort can also be found in the supplemental materials. Of note, 8 Dunedin Study members were missing *APOE* genotype data and were excluded from sensitivity analyses controlling for *APOE* ε4 carriership. In FHS-OC, 903 participants (male=42 %) had both DNA methylation and MRI data. The mean age at the time of blood sample collection was 63.76 years (SD=8.11 years, range=40.00–84.00 years) and the mean age at the time of MRI was 64.53 years (SD=8.14 years, range=40.98–84.68 years). The median number of days between blood sample collection and MRI scanning was 94. More details on participants who had delays between blood sample collection and MRI scanning can be found in the supplemental materials. Of note, only 820 participants in FHS-OC had *APOE* genotype data. Thus, sensitivity analyses controlling for *APOE* ε4

carriership in FHS-OC were performed on a smaller subsample than the main analyses. In ADNI, 649 participants (male=55.6 %) had DNA methylation data: 83 had only a baseline measurement, 121 had two measurements, 407 had three measurements, 29 had four measurements, and 9 had five measurements. This yielded 1707 overall DNA methylation samples. The mean age at blood sample collection was 75.41 years (SD=7.66 years). We paired DNA methylation timepoints with unique MRI observations that took place at a similar time (i.e., <6 months). The median number of days between blood sample collection and MRI scanning for each MRI phenotype was 0, although there were some MRI observations with greater delays from blood sample collection. Further information on these cases can be found in the supplemental materials. There was some variation in the total number of observations and individuals included for each MRI measure. Descriptions of the subsamples for each measure in ADNI are presented in Table 1. Age distributions for all three datasets are shown in Fig. 2.

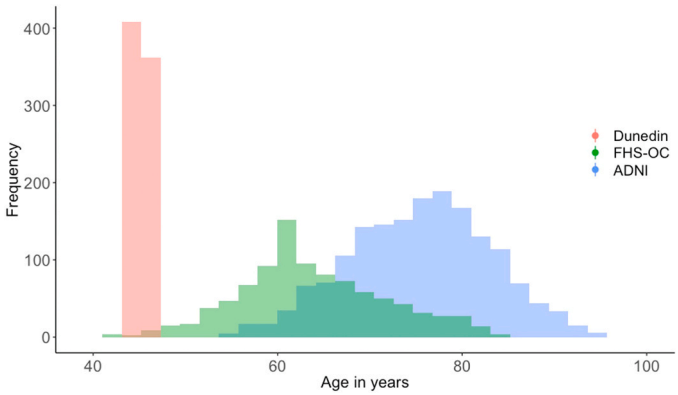

Fig. 2. Age distributions in the Dunedin Study, FHS-OC, and ADNI. Histograms showing the age distributions in years at the time of blood draw for DNA methylation analysis in each dataset. Abbreviations: ADNI = Alzheimer's Disease Neuroimaging Initiative; FHS-OC: Framingham Heart Study – Offspring Cohort.

3.2. Total brain volume

Across all three datasets, people with accelerated biological aging, as indexed by faster DunedinPACE, had lower TBV (Dunedin Study: $\beta = -.06$, $p < .001$, 95 % CI: [−.09, −.03]; FHS-OC: $\beta = -.04$, $p = .03$, 95 % CI: [−.07, −.004]; ADNI: $\beta = -.06$, $p = .005$, 95 % CI: [−.10, −.02]; Fig. 3). These associations were robust to white blood cell abundance and *APOE* ε4 carriership (see supplemental materials).

3.3. Hippocampal volume

Across all three datasets, people with faster DunedinPACE had lower HC (Dunedin Study: $\beta = -.06$, $p = .02$, 95 % CI: [−.12, −.01]; FHS-OC: $\beta = -.07$, $p = .02$, 95 % CI: [−.13, −.01]; ADNI: $\beta = -.10$, $p = .004$, 95 % CI: [−.17, −.03]; Fig. 3). These associations were robust to white blood cell abundance and *APOE* ε4 carriership (see supplemental materials).

3.4. White matter microlesions

Across all three datasets, people with faster DunedinPACE had greater WMHypo volume (Dunedin Study: $\beta = .08$, $p = .02$, 95 % CI: [.01,.15]; FHS-OC: $\beta = .09$, $p = .01$, 95 % CI: [.02,.16]; ADNI: $\beta = .11$, $p = .004$, 95 % CI: [.03,.18]; Fig. 3). These associations were robust to white blood cell abundance and *APOE* ε4 carriership (see supplemental materials). Faster DunedinPACE was also associated with greater WMHyper volume in the Dunedin Study, though this effect was attenuated in ADNI (see supplemental materials for details).

3.5. Cortical thickness

Across all three datasets, people with faster DunedinPACE had thinner cerebral cortex (Dunedin Study: $\beta = -.18$, $p < .001$, 95 % CI: [−.24, −.11]; FHS-OC: $\beta = -.09$, $p = .01$, 95 % CI: [−.16, −.03]; ADNI: $\beta = -.10$, $p = .02$, 95 % CI: [−.19, −.01]; Fig. 3). These associations were robust to white blood cell abundance and *APOE* ε4 carriership (see supplemental materials).

3.6. Cortical surface area

In the Dunedin Study, people who were aging faster, as measured by DunedinPACE, tended to have less total cortical surface area ($\beta = -.08$, $p = .01$, 95 % CI: [−.14, −.02], Fig. 3). However, we did not observe this association in FHS-OC or ADNI (FHS-OC: $\beta = -.01$, $p = .75$, 95 % CI: [−.07,.05]; ADNI: $\beta = .01$, $p = .81$, 95 % CI: [−.07,.08]; Fig. 3). These results were not affected by white blood cell abundance or *APOE* ε4 allele carriership (see supplemental materials).

Table 1
Demographic information for the ADNI DNA methylation sample. Leftmost columns show the sample size for paired observations from ADNI. We have included both the total number of unique observations as well as the number of individuals. The third from left column presents the mean and standard deviations of the age in years at which the blood draw occurred. The fourth from the left column shows the time in days between the blood draw and MRI. Rightmost three columns show the proportion of most recent diagnostic status (cognitively normal, mild cognitive impairment, or dementia) at the time of the blood draw.

	N						
	Observations	Individuals	Mean age at blood draw (SD, Min-Max)	Blood draw/MRI mean interval (days)	CN (%)	MCI (%)	Dementia (%)
DNA methylation	1707	649 (55.6 % male)	75.4 (7.66, 55.0-95.6)	-	31.90 %	48.10 %	19.90 %
Overlap with:							
TBV	1426	604 (55.0 % male)	74.6 (7.50, 55.0-95.6)	2.0	31.90 %	50.90 %	17.20 %
HC	1358	581 (54.9 % male)	74.4 (7.43, 55.0-95.6)	3.6	31.90 %	51.20 %	16.90 %
WMHypo	1078	446 (51.8 % male)	74.4 (7.37, 55.0-93.1)	10.1	31.60 %	50.60 %	17.70 %
CT	1078	446 (51.8 % male)	74.4 (7.37, 55.0-93.1)	10.1	31.60 %	50.60 %	17.70 %
SA	1078	446 (51.8 % male)	74.4 (7.37, 55.0-93.1)	10.1	31.60 %	50.60 %	17.70 %

Abbreviations: ADNI = Alzheimer's Disease Neuroimaging Initiative; SD = standard deviation; CN = cognitively normal; MCI = mild cognitive impairment; TBV = total brain volume; HC = hippocampal volume; WMHypo = white matter hypointensities; CT = mean cortical thickness; SA = total cortical surface area.

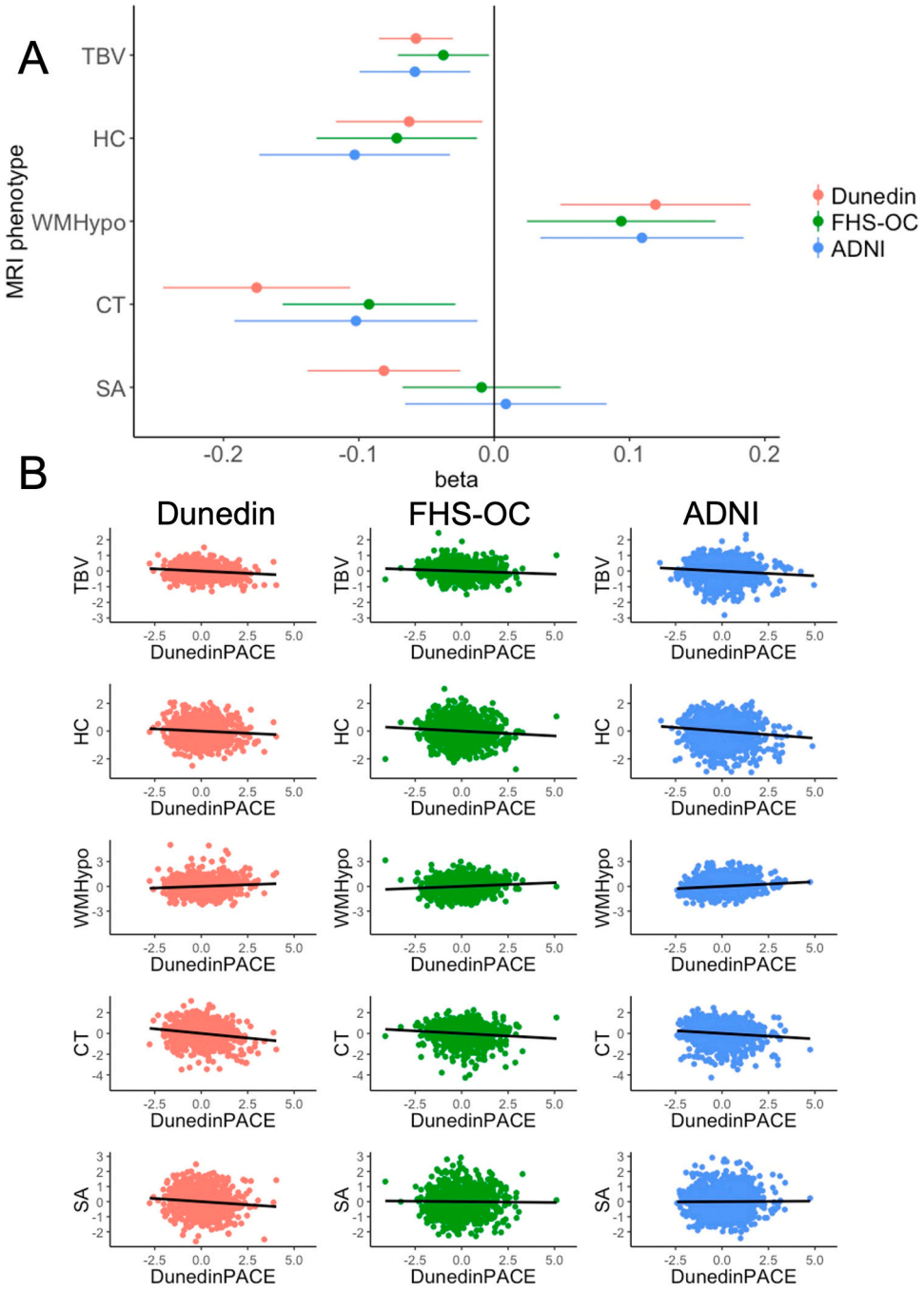

Fig. 3. Associations between DunedinPACE and brain structure. DunedinPACE was negatively associated with TBV, CT, and HC across all three datasets, and positively associated with WMHypo volume across all three datasets. A) Forest plot of all associations reported as standardized betas (error bars represent 95 % confidence intervals). B) Scatterplots of all associations. X-axes represent standardized scores of MRI measures after residualizing for age and sex. Y-axes represent standardized values for DunedinPACE. Abbreviations: ADNI = Alzheimer's Disease Neuroimaging Initiative; FHS-OC: Framingham Heart Study – Offspring Cohort; TBV = total brain volume; HC = hippocampal volume; WMHypo = white matter hypointensities, CT = mean cortical thickness, SA = total cortical surface area.

3.7. Change in brain structure

In ADNI, 153 participants with DNA methylation data remained cognitively normal throughout enrollment. Of these, varying numbers had MRI measures at a minimum of three timepoints allowing for calculation of change trajectories (Table 2). Analyses of data from these participants revealed expected age-related changes in all MRI measures (see supplemental materials). We did not observe significant associations between baseline DunedinPACE and subsequent change in MRI phenotypes (TBV: $\beta = -.01$, $p = .92$, 95 % CI: [−.18,.16]; HC: $\beta = -.01$, $p = .95$, 95 % CI: [−.18,.17]; WMHypo: $\beta = .08$, $p = .38$, 95 % CI: [−.10,.28]; CT: $\beta = -.06$, $p = .57$, 95 % CI: [−.27,.15]; SA: $\beta = -19$, $p = .06$, 95 % CI: [−.38,.004]).

3.8. Comparison with other epigenetic clocks

For comparison purposes, we repeated our cross-sectional analyses replacing DunedinPACE with each of four well-studied epigenetic clocks: first-generation Horvath and Hannnum clocks, and second-generation PhenoAge and GrimAge clocks. Associations of first-generation clocks with MRI measures were mostly null. PhenoAge generally had non-significant associations that were smaller than

Table 2
Demographic description for the ADNI longitudinal MRI sample. Sample size, sex, average age, and average observation length for ADNI participants who remained cognitively normal during ADNI enrollment and with ≥ 3 MRI time-points after DNA methylation observation.

MRI phenotype	N	Mean age at baseline (SD, Min-Max)	Mean observation length (years)
TBV	128 (48 % male)	74.94 (6.67, 62.3-93.5)	5.54
HC	122 (48 % male)	74.52 (6.31, 62.3-93.5)	5.61
WMHypo	92 (46 % male)	74.96 (6.33, 63.4-91.1)	4.73
CT	92 (46 % male)	74.96 (6.33, 63.4-91.1)	4.73
SA	92 (46 % male)	74.96 (6.33, 63.4-91.1)	4.73

Abbreviations: ADNI = Alzheimer's Disease Neuroimaging Initiative; TBV = total brain volume; HC = hippocampal volume; WMHypo = white matter hypointensities; CT = mean cortical thickness; SA = total cortical surface area

DunedinPACE (Fig. 4). GrimAge had significant associations of similar magnitude to DunedinPACE in the Dunedin Study and FHS-OC; however, GrimAge only had a significant association with CT in ADNI (Fig. 4). Complete details of these analyses are presented in supplemental materials.

4. Discussion

By aggregating three large datasets, we assembled the largest sample to date for examining associations between epigenetic clocks and brain anatomy. Using this sample, we showed that DunedinPACE, an epigenetic index of an individual's pace of biological aging derived by tracking multi-organ decline from age 26 to 45 in a same-age birth cohort of healthy people, is associated with several MRI measures of brain structure in three independent datasets encompassing mid- to late-life. In all three datasets, people with accelerated biological aging, as indexed by higher DunedinPACE scores, had smaller total brain volume, smaller hippocampal volume, greater burden of white matter micro-lesions, and thinner cerebral cortex. These results are consistent with the geroscience hypothesis of correlated decline across the entire body (Kennedy et al., 2014). The geroscience hypothesis argues that aging is a stochastic process leading to gradual loss of system integrity across all organ systems. We showed that faster decline of non-nervous organ systems (i.e., cardiovascular, metabolic, renal, immune, dental, and pulmonary) is associated with individual differences in brain structure during midlife and older age, supporting the idea that aging simultaneously affects organ systems across the entire body. Our findings also align with recent work showing that aging of body organ systems is associated with aging of the brain (Elliott et al., 2021a; Tian et al., 2023), and further demonstrate that this association can be captured using a blood sample from a single timepoint.

The associations between DunedinPACE and MRI measures of brain structure were consistent across our three datasets despite differences in cohort demographics. The Dunedin Study, FHS-OC, and ADNI differ substantially in participant age (Dunedin Study: age=45; FHS-OC mean age=64; ADNI mean age=75; Fig. 2). ADNI is also highly enriched for Alzheimer's disease and related dementias, whereas the Dunedin Study and FHS-OC are not. Approximately 18 % of the ADNI participants in our cross-sectional analyses had been diagnosed with dementia at the time of assessment. In contrast, no participants in either the Dunedin Study or FHS-OC datasets were diagnosed with dementia. Notably, associations between DunedinPACE and brain structure across all three datasets are similar in magnitude to those between direct, longitudinal measures of organ-system decline and MRI measures in the Dunedin Study (Elliott et al., 2021b). Thus, a single timepoint calculation of DunedinPACE closely replicates associations between brain and body aging estimated from longitudinal measurements of multiple organ systems. The fact that the observed effect sizes are generally consistent between the Dunedin Study, FHS-OC, and ADNI further suggests good generalizability of DunedinPACE across age and cognitive status.

Our findings indicate that DunedinPACE had strong and consistent associations with brain structure relative to first-generation epigenetic clocks. Across all three datasets used here, associations between first-generation epigenetic clocks and brain structure were null or very

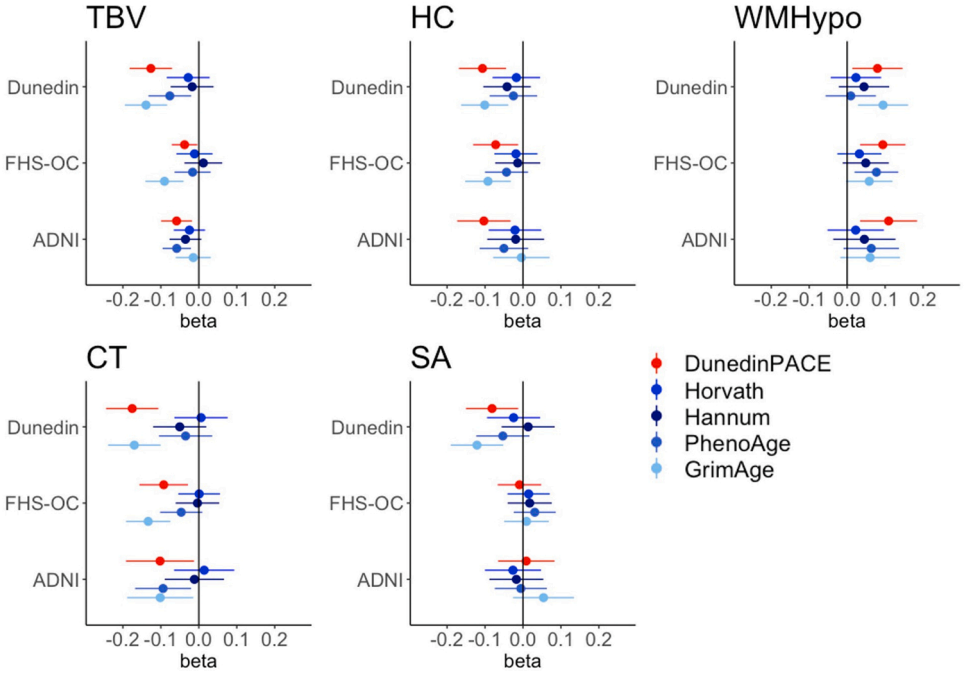

Fig. 4. Comparison between epigenetic clocks. Forest plots of associations between each epigenetic clock and TBV, HC, WMHypo, CT, and SA in the Dunedin Study, FHS-OC, and ADNI. Abbreviations: ADNI = Alzheimer's Disease Neuroimaging Initiative; FHS-OC = Framingham Heart Study – Offspring Cohort; TBV = total brain volume; HC = hippocampal volume; WMHypo = white matter hypointensities; CT = mean cortical thickness; SA = total cortical surface area.

close to zero. Previous neuroimaging research using first-generation epigenetic clocks has yielded inconsistent associations with brain structure (Cheong et al., 2022; Chouliaras et al., 2018; Davis et al., 2017; Proskovec et al., 2020; Raina et al., 2017). This is likely due to underpowered study designs (Liu et al., 2023). One study also using the ADNI dataset reported an association between hippocampal volume and the Hannum clock (Milicic et al., 2022); however, this association was restricted to a sample of 34 amyloid-β+, cognitively unimpaired individuals with > 3 timepoints of MRI data. Of note, that study did not find a significant association between the Hannum clock and brain structure, including hippocampal volume, in the larger ADNI sample (Milicic et al., 2022). While we find that first-generation clocks tend to show null associations with MRI measures, a second-generation clock, GrimAge, was associated with brain structure in both the Dunedin Study and FHS-OC, although, GrimAge associations with TBV and HC were null in ADNI. Overall, our findings suggest that newer epigenetic clocks such as DunedinPACE and GrimAge may have greater promise for gauging brain structure relative to first-generation epigenetic clocks. This is in line with the growing consensus that aging biomarkers trained only on chronological age have limited ability to detect age-related health outcomes whereas aging biomarkers trained on the *rate* of biological aging are more associated with to age-related health outcomes (Moqri et al., 2023; Zhang et al., 2019).

We did not find evidence that baseline DunedinPACE predicted subsequent risk-related change in brain structure in ADNI participants who remained cognitively normal throughout enrollment. However, the analytic sample with the requisite three MRI scans to model trajectories of reliable change in brain structure was small. This reduced statistical power and limited our ability to detect associations. Notably, in all three datasets, DunedinPACE was associated with TBV controlling for ICV, which is thought to represent the 'maximal' size a person's brain reached during their life as the cranial cavity does not shrink during aging (Royle et al., 2013). "TBV controlling for ICV" is sometimes interpreted as a proxy measure for longitudinal atrophic decline in TBV since childhood (Royle et al., 2013). While speculative, this suggests the hypothesis that faster DunedinPACE could potentially be associated with accelerated neurodegeneration. Even so, without longitudinal data we cannot rule out that this association simply reflects natural variability in brain-to-head size ratio. Research using larger longitudinal samples is needed to test this hypothesis.

Our findings are consistent with a recent report using data from the UKBiobank (Tian et al., 2023) to reveal that change in multiple individual organ systems (i.e., musculoskeletal, cardiac, metabolic, and pulmonary) each mapped onto change in the brain. Here we demonstrate a similar brain-body connection using DunedinPACE, a single timepoint DNA methylation measure trained on longitudinal decline in multiple organ systems. These two reports using complementary designs show that MRI measures of brain structure are correlated with progressive, multi-organ decline in the rest of the body. Future studies should longitudinally measure multiple organ systems to address which is more relevant for brain aging: decline unique to individual organs, or the shared decline across organs predicted by the geroscience hypothesis and operationalized through DunedinPACE.

Our study has limitations. First, most of the data analyzed were derived from White participants reflecting the paucity of racial and ethnic diversity in datasets that have both DNA methylation and brain MRI. Notably, there is evidence that DunedinPACE can index health outcomes amongst both Black and Asian individuals (Graf et al., 2022; Schmitz et al., 2022; Schmitz and Duque, 2022; Shen et al., 2023). Second, DunedinPACE does not necessarily represent methylation of specific genes thought to contribute to neurodegeneration or aging progression, and is best thought of as a non-causal statistical indicator of multi-organ decline. Third, although we observed a robust three-dataset association between DunedinPACE and WMHypo volume, the association between DunedinPACE and WMHyper was only observed in one out of the two studies where this measure was available. While these two measures are sometimes thought to be roughly equivalent (Wei et al., 2019), there is some evidence to suggest that T1-hypointense white matter lesions could represent the more severe and clinically relevant components of white matter damage (Melazzini et al., 2021). Finally, we were only able to assess change in brain structure in one dataset, ADNI, which had the smallest number of individuals without dementia and who were advanced in age (i.e., age>80 years). We recommend larger, more diverse, and more population-representative studies to assess how DunedinPACE is related to change in brain structure.

In summary, we present evidence that accelerated biological aging, as indexed by faster DunedinPACE, is consistently associated with brain structure across three large datasets spanning midlife to older age. This suggests that the geroscience hypothesis of correlated, progressive decline of organs includes the central nervous system. We build on prior work by demonstrating that associations between brain and body aging can be detected using a single timepoint whole blood measure of DNA methylation. Collectively, these findings reinforce that aging is a whole-body process and suggest that aging neuroscience research stands to benefit from further attention to organs 'below the neck'.

CRediT authorship contribution statement

Belsky Daniel W.: Writing – review & editing, Formal analysis, Conceptualization. **Hogan Sean:** Writing – review & editing, Data curation. **Moffitt Terrie E.:** Writing – review & editing, Project administration, Funding acquisition, Data curation, Conceptualization. **Ireland David:** Writing – review & editing, Data curation. **Zhou Jiayi:** Writing – review & editing, Formal analysis. **Corcoran David L.:** Writing – review & editing, Formal analysis. **Hariri Ahmad R.:** Writing – review & editing, Project administration, Funding acquisition, Data curation, Conceptualization. **Elliott Maxwell L.:** Writing – review & editing, Formal analysis. **Sugden Karen:** Writing – review & editing, Formal analysis. **Abraham Wickliffe C.:** Writing – review & editing, Data curation. **Williams Benjamin S.:** Writing – review & editing, Formal analysis. **Addae Angela:** Writing – review & editing, Formal analysis. **Poulton Richie:** Writing – review & editing, Project administration, Funding acquisition, Data curation. **Ramrakha Sandhya:** Writing – review & editing, Data curation. **Ryan Calen P.:** Writing – review & editing, Visualization, Formal analysis. **Melzer Tracy R.:** Writing – review & editing, Data curation. **Whitman Ethan T.:** Writing – review & editing, Writing – original draft, Visualization, Formal analysis, Conceptualization. **Caspi Avshalom:** Writing – review & editing, Project administration, Funding acquisition, Data curation, Conceptualization. **Keenan Ross:** Writing – review & editing, Data curation. **Knodt Annchen R.:** Writing – review & editing, Formal analysis, Data curation.

Conflict of interest

K. Sugden, A. Caspi, D. W. Belsky, D. L. Corcoran, R. Poulton, and T. E. Moffit are listed as inventors of DunedinPACE, a Duke University and University of Otago invention licensed to TruDiagnostic Inc. All other authors report no conflict of interest.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.neurobiolaging.2024.01.008.

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