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Women in aging neuroscience 2021

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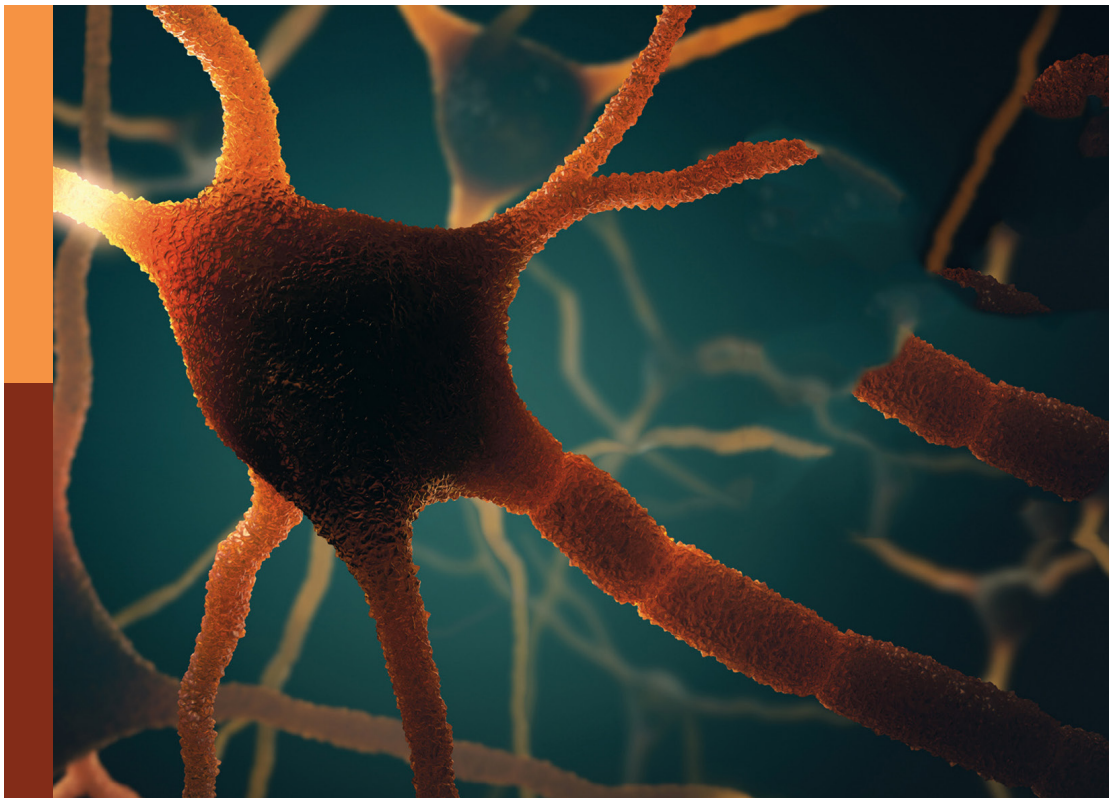

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Women in aging neuroscience 2021

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Table of contents

04 Editorial: Women in aging neuroscience 2021
Silvia Fossati

07 Parkinson's Disease Medication Adherence Scale: Conceptualization, Scale Development, and Clinimetric Testing Plan
Michelle H. S. Tosin, Christopher G. Goetz, Dharah P. C. F. Bispo, Henrique B. Ferraz, Marco Antonio A. Leite, Deborah A. Hall, Glenn T. Stebbins and Beatriz Guitton R. B. Oliveira

13 Selective Serotonin Reuptake Inhibitor-Treatment Does Not Show Beneficial Effects on Cognition or Amyloid Burden in Cognitively Impaired and Cognitively Normal Subjects
Yvonne Bouter and Caroline Bouter for the Alzheimer's Disease Neuroimaging Initiative

24 Psychobiological Evaluation of Day Clinic Treatment for People Living With Dementia – Feasibility and Pilot Analyses
Alexandra Wuttke-Linnemann, Svenja Palm, Katharina Geschke, Nadine Skoluda, Theresa Bischoff, Urs M. Nater, Kristina Endres and Andreas Fellgiebel

38 Sympathoexcitatory Responses to Isometric Handgrip Exercise Are Associated With White Matter Hyperintensities in Middle-Aged and Older Adults
Andrew G. Pearson, Kathleen B. Miller, Adam T. Corkery, Nicole A. Eisenmann, Anna J. Howery, Karly A. Cody, Nathaniel A. Chin, Sterling C. Johnson and Jill N. Barnes

52 Ovarian steroid hormones: A long overlooked but critical contributor to brain aging and Alzheimer's disease
Steven Jett, Eva Schelbaum, Grace Jang, Camila Boneu Yepez, Jonathan P. Dyke, Silky Pahlajani, Roberta Diaz Brinton and Lisa Mosconi

92 Feelings of loneliness and isolation: Social brain and social cognition in the elderly and Alzheimer's disease
Rosalba Morese and Sara Palermo

102 Neural basis of impaired narrative discourse comprehension in prodromal and mild dementia with lewy bodies
Anaïs Falque, Mélanie Jordanis, Lionel Landré, Paulo Loureiro de Sousa, Mary Mondino, Emmanuelle Furcieri and Frédéric Blanc

111 State-of-the-art review of the clinical research on menopause and hormone replacement therapy association with Parkinson's disease: What meta-analysis studies cannot tell us
Santiago R. Unda, Sabina Marciano, Teresa A. Milner and Roberta Marongiu

121 Association of cerebral spinal fluid copper imbalance in amyotrophic lateral sclerosis
Qiao Yi Chen, Peng Wu, Ting Wen, Xing Qin, Ronghua Zhang, Rui Jia, Jiaoting Jin, Fangfang Hu, Xiaoge Xie and Jingxia Dang

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Editorial: Women in aging neuroscience 2021

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KEYWORDS

female neuroscientists, sex differences, biomarkers, psychosocial evaluation, therapeutic strategies, neurodegenerative disorders

Editorial on the Research Topic
Women in aging neuroscience 2021

Introduction

While the number of women in neuroscience is gradually increasing, reaching over 50%, the proportion of women in aging neuroscience at higher career levels, such as Professors and Chairs, is still severely discrepant compared with their male counterpart. This is known as the "leaky pipeline," likely due to a combination of personal factors as well as a recognized gender inequality in the possibility to obtain grants and publish manuscripts in high impact journals (https://doi.org/10.1016/B978-0-12-819641-0.00007-4; https://www.frontiersin.org/articles/10.3389/fpsyg.2019.01297/full). While solving these well-recognized issues will require longer time frames, this Research Topic aims to highlight the wonderful and impactful work of women-neuroscientists.

Our contributors are female authors that work on aging neuroscience as well as neurodegenerative disorders. Even if still outnumbered by their male counterparts, these women are among the current and future leaders in the neuroscience and neurodegeneration fields. This important collection of papers clearly indicates that these impactful scientists, together with other established female colleagues, will represent influential role models to lead the path to a bright future for the next generation of women in aging neuroscience.

This diverse group of manuscripts highlights the high level of research lead by women neuroscientists. Interestingly, in some cases, this Research Topic also unveils differences in the incidence and causes of neurodegenerative disorders, such as Alzheimer's (AD) and Parkinson's disease (PD), in women compared to males, tackling the subject *of* "women in aging neuroscience" from two complementary sides.

Effect of hormones and menopause on AD and PD risk

One important aspect of sex differences in neurodegenerative diseases is due to the impact of steroid hormones and menopause on the risk to develop these age-associated disorders.

Two manuscripts on this subject were led respectively by Dr. Lisa Mosconi, and by Dr. Roberta Marongiu. Jett et al. elegantly summarize how 17β-estradiol, an ovarian hormone with multiple neuroactive properties, which has been called "the master regulator of the female brain," is involved in the neurobiology of aging, AD and cognitive impairment. The authors remind us that estradiol levels drop at menopause, in association with complaints of poor sleep, loss of thermoregulation, and impaired cognitive abilities. Importantly, as shown by

Dr. Mosconi and others, this was also linked to a disproportionate increase in AD-related imaging biomarkers in the brain of peri- and post-menopausal women compared to age-matched men. Especially, women at risk for AD, exhibited preclinical AD endophenotypes already during perimenopause. The fact that women constitute about 2/3 of AD patients (not only due to their longer life expectancy; https://www.alz.org/media/documents/alzheimers-facts-and-figures.pdf), highlights the importance of better understanding menopause-AD relationships. However, the debate remains open on whether menopausal hormone therapy has neuroprotective value.

In the second manuscript, Unda et al. tackle a similar subject in relation to PD. Interestingly, the authors acknowledge that women have a lower risk for PD compared to men, differently from AD, which raises the possibility that menopause and ovarian hormones may have very different mechanistic effects in brain areas more affected in AD or PD. The authors clarify that, due to the heterogeneity in study designs, the role for age at menopause, type of menopause and hormone replacement therapy on PD is still unclear. This study, while collecting the newest information on the subject, also highlights gaps in the literature and provides indications for the best ways to answer some of these open questions.

Development of Medication Adherence Scales for PD

Since suboptimal medication adherence in neurodegenerative diseases remains a problem, Tosin et al. outlined an objective design of the development and validation of the PD Medication Adherence Scale. This report demonstrates the feasibility of such an instrument for medication adherence process in people with PD, allowing the proper implementation of these techniques.

Stress, loneliness, and isolation factors in aging and dementia

Other manuscripts tackle the important subjects of psychosocial evaluations, stress, loneliness and isolation in elderly people with dementia. In the first of these papers, Wuttke-Linnemann et al. interviewed patients and caregivers in a day clinic context. This work is timely, as measure of success of AD/dementia therapies, especially for the psychosocial response, is often based on caregivers opinions. The authors highlight the possibility that physiological stress markers, such as hair cortisol, could help in complementing the evaluation of treatment effects. Not all participants were willing to collect physiological stress markers. However, hair samples were easier to obtain than saliva. Because of the inability or unwillingness for people with dementia to correctly describe their symptoms, collecting these biomarkers may be an important strategy to accurately measure stress values in research or clinical trials subjects.

In the opinion article, Morese and Palermo highlight how the impairment of cognitive function may have an impact on loneliness for older people, through changes in interaction with family and friends, or the perception of relationship satisfaction. Social isolation during life may also contribute, through induction of depression and other described biological mechanisms, such as inflammation, to an increased risk of AD and dementias. Notably, loneliness is a modifiable factor, which can be addressed during life to prevent the development of cognitive impairment and AD-related dementias.

In apparent contrast, the manuscript of Bouter and Bouter suggests that in cognitively impaired and cognitively normal participants from the Alzheimer's Disease Neuroimaging Initiative (ADNI) study, serotonin reuptake inhibitors (SSRI)-treatment, associated with depression improvement, did not show beneficial effects on amyloid load nor cognition. This may indicate that depression or social isolation must be treated earlier in life to prevent cognitive dysfunction, rather than as a therapy. Overall, controlled randomized prospective studies on the effect of SSRIs on AD-pathologies are necessary to overcome limitations of previous studies.

Relationship of sympathoexcitatory responses with white matter hyperintensities

In another valuable contribution of Pearson et al., the association between cardiovascular and cerebrovascular responses in hand grip exercises, post-exercise ischemia, and white matter hyperintensities (WMH) were assessed. This study suggests that individuals who show smaller increases in responses to sympathoexcitatory stress have greater WMH burden, strongly associating weaker peripheral cardiovascular responses to cerebrovascular dysfunction and WMH.

Changes in comprehension and CSF metals in neurodegeneration

This Research Topic spanned over multiple types of dementia. In Falque et al., comprehension impairment was found in mild dementia with Lewy bodies (DLB) subjects. There was also a correlation between striatal gray matter volumes and DLB patients' ability to organize information. This work points to the need of future research on the association of the striatum and striato-frontal processes with narrative comprehension in dementias.

Finally, the study, Chen et al. aimed to examine potential associations between cerebral spinal fluid (CSF) metals and amyotrophic lateral sclerosis (ALS) risk. The study found that Cu levels were lower in the ALS and spinal-onset groups, while Ni levels were higher in the spinal-onset group compared to the control and bulbar-onset groups, highlighting the differential association of CSF metals with neurodegeneration.

Author contributions

The author confirms being the sole contributor of this work and has approved it for publication.

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Conflict of interest

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Parkinson's Disease Medication Adherence Scale: Conceptualization, Scale Development, and Clinimetric Testing Plan

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Background: Medication adherence is a crucial component in the management of patients with chronic diseases needing a long-term pharmacotherapy. Parkinson's disease (PD) is a chronic, degenerative disease with complex drug treatment that poses challenging barriers to patient adherence. The adoption of best practices of scale development can contribute to generate solid concepts and, in the long run, a more stable knowledge base on the underlying constructs of medication adherence in PD measured by the items of the first scale to be created for this purpose.

Purpose: To present the development process and clinimetric testing plan of the Parkinson's Disease Medication Adherence Scale (PD-MAS).

Method: We adopted a hybrid approach plan based on the United States Food and Drug Administration and Benson and Clark Guide that will create a patient-reported outcome instrument. We presented an overview of consecutive and interrelated steps, containing a concise description of each one. International research centers from Brazil and United States were initially involved in the planning and implementation of the methodological steps of this study.

Results: We developed a four-phase multimethod approach for the conceptualization and the clinimetric testing plan of the PD-MAS. First, we describe the development process of the conceptual framework of the PD-MAS underpinning the scale construct; second, we formalized the development process of the first version of the PD-MAS from the generation of item pools to the content validation and pre-testing; third, we established the steps for the first pilot testing and revision; fourth, we describe the steps plan for the first pilot testing and revision, to finally describe its clinimetric testing plan and validation.

Conclusion: The overview presentation of the development phases and the clinimetric testing plan of the PD-MAS demonstrate the feasibility of creating an instrument to measure the multidimensional and multifactorial components of the medication adherence process in people with PD.

Keywords: scale development, measurement, psychometrics (MeSH), medication adherence (MeSH), Parkinson's disease

INTRODUCTION

Despite increased awareness, suboptimal medication adherence among people treating chronic diseases remains a global problem (Sabaté, 2003). Among people with Parkinson's disease (PD), suboptimal adherence to medications ranges from 10 to 67% (Davis et al., 2010), and is currently measured using two methods: 1. direct, defined as objective measurements of concentrations of the medication or its metabolites (e.g., measurement of drug and metabolite levels in the blood and/or urine); and 2. indirect, defined as subjective measures of proxies' observations (e.g., pill count, electronic monitoring devices, electronic health records, and rating scales) (Grosset et al., 2006; Kulkarni et al., 2008; Davis et al., 2010). So far, there is no standard procedure for measuring medication adherence, and both methods have advantages and disadvantages. In people with PD, the use of rating scales to accurately measure the behaviors influencing the medication adherence process may have additional disadvantages, because of the absence of an instrument developed specifically to measure this construct in this population (Lam and Fresco, 2015).

To date, the only rating scale created to assess medication adherence in PD, measures only one domain of this construct: the belief that people with PD have regarding their antiparkinsonian medications. Developed in 2016, the instrument "Parkinson's Disease Medication Beliefs Scale (PD-Rx)" was tested by scientists in a first pilot study. The scale aims to identify the beliefs that underlie drug phobia. However, in a pilot test of the clinimetric properties of the measure, the authors emphasize that the study had limitations related to both the sample (small and homogeneous), and the lack of more robust measures to test convergent validity (e.g., electronic drug monitoring devices) (Fleisher et al., 2016).

The challenge of developing and validating a rating scale to measure medication adherence specifically in people with PD, depends on the ability of this instrument to capture, in a reliable and valid way, the set of dimensions and factors involved in the medication adherence process, assembling the most appropriate items to constitute test questions. For example, the presence of non-motor symptoms of PD, such as cognitive impairment, depression, apathy, excessive daytime sleepiness, or concomitant psychosis, has been shown to influence adherence (Mendorf et al., 2020). Likewise, the use of different drug presentations (such as oral versus patch medications, fractionated versus single-dose medications) has been shown to be factors involved in the medication adherence process in this population (Mynors et al., 2007; Schnitzler et al., 2010). To capture these and other factors that could potentially influence adherence, it is recommended that a comprehensive measuring instrument follow an inclusive approach. This requires the scale developers to have specific theoretical, methodological, and statistical competencies in clinimetrics, as it involves the collection and analysis of primary and secondary data (Boateng et al., 2018). These competencies are not usually taught but are duplicated from procedures reported in scientific papers (Carpenter, 2018).

The proper construction of a scale has important implications for the inferences of the measure, as it affects both the quality and size of the effects obtained and the statistical significance of these effects, reflecting the precision and sensitivity of the instrument (Furr, 2018; Kyriazos and Stalikas, 2018). Precise measurements of the severity and impact of PD symptoms and response to drug treatment are necessary for the development of symptomatic and disease-modifying therapies (Espay et al., 2017; Pires et al., 2017; Paolini Paoletti et al., 2020) and the current literature is vast in evidence on clinical findings and their impacts in PD patients (Tan et al., 2014; Muzerengi et al., 2016; Kramer et al., 2018). However, studies that correlate these data to medication adherence, as measured using scales, are scarce, raising the need to create a rating scale that measures medication adherence specifically for the context of PD.

To produce more solid concepts in the long term with a more stable knowledge base on adherence to PD medication, in this study protocol, we followed the standard recommendations of the instrument developers (Benson and Clark, 1982; US Food and Drug Administration, 2009; Speight and Barendse, 2010), and outline a clear and objective design of the steps for the development and validation of the Parkinson's Disease Medication Adherence Scale (PD-MAS).

METHODS

Study Design

This is a clinimetric study protocol for rating scale development. We adopted the United States Food and Drug Administration (FDA) (Benson and Clark, 1982; US Food and Drug Administration, 2009) and Speight and Barendse (2010) guidance for developing a patient-reported outcome (PRO) instrument for use as a clinical trial. This guidance met both best practices for developing and validating rating scales for health, social, and behavioral research (Boateng et al., 2018), and the recommendations of the International Parkinson and Movement Disorders Society (MDS) Task Force on Rating Scales in Parkinson's Disease: clinical practice and research (Sampaio et al., 2012; **Figure 1**).

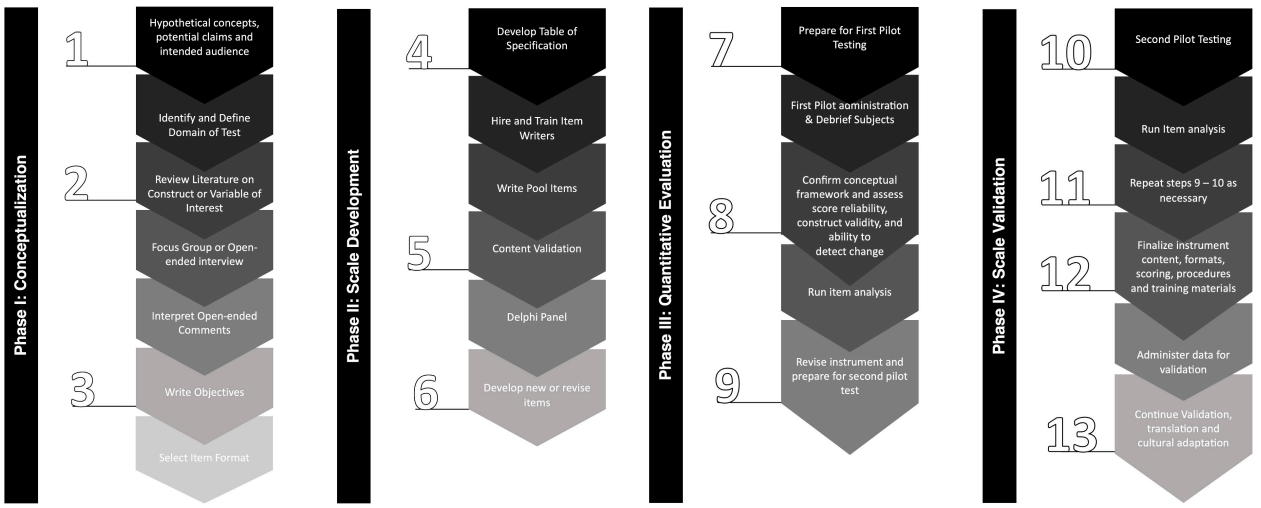

FIGURE 1 | Illustrative overview of a scale development and validation process.

Study Settings

International movement disorders outpatient clinics of the following university hospitals have been initially involved in the planning for future implementation of the methodological steps of this study: Antônio Pedro Hospital (HUAP), of the Fluminense Federal University (UFF), Niterói, Brazil; São Paulo Hospital, of the São Paulo Federal University (UNIFESP), São Paulo, Brazil; and Rush University Medical Center (RUMC), Chicago, IL, United States. These are tertiary centers specialized in the care of patients with movement disorders, including PD, with neurologists, nurses, ancillary health professionals, and a multidisciplinary team specialized in this area.

As these centers have different languages (Portuguese and English), the PD-MAS will be created in these two language versions, complying with parsimony criteria between the two versions.

RESULTS

The PD-MAS development process was designed to be implemented in four phases, including: (1) conceptualization; (2) scale development; (3) quantitative evaluation; and (4) validation. Each phase has interactive steps with a multimethod approach for the collection and analysis of primary and secondary data that will support the integral development of the PD-MAS. The aims and activities that will be carried out in each phase of the development of the PD-MAS are summarized in **Supplementary Table 1**.

Phase I: Conceptualization Overview

The conceptual framework basis of the PD-MAS will be determined following Delphi panel of experts based on the population of interest; objectives of the instrument; global domain; content areas; questions to be answered; purposes of the measurement items; and the indications for its use. Then, to adequately address the observable and relevant phenomena of the global domain and subdomains addressed by the PD-MAS, primary and secondary sources of information will be explored.

As a secondary source, a non-systematic review of the literature on the topic "medication adherence," "Parkinson's disease," and "medication adherence in people with PD" will provide a preliminary conceptual definition of the domain, and provide a preliminary assessment of the medication adherence scales used in studies with people with PD. Following this non-systematic review, a systematic review of the literature will identify the domains and subdomains assessed by the medication adherence rating scales used in studies with people with PD, and whether the essential components of medication adherence are adequately covered.

As a primary source, focus groups with people with PD and their caregivers will be conducted, and observable and relevant phenomena of medication adherence that may not have been identified in the secondary source will be captured. Given the heterogeneity of PD disabilities and impairments, such focus groups will need to cross different age groups, disease stages, family structures, cultural, financial, and health system differences.

Lastly, the data from both secondary and primary sources will be combined through data triangulation to allow quantitative and qualitative analysis that will establish the specific measurement objectives of the PD-MAS, that will form the basis of the preliminary scale development phase.

Phase II: Scale Development Overview

From the objectives of the PD-MAS obtained in the previous phase, we will elaborate the items that will constitute the PRO, considering the criteria determined for its format and scoring model (Benson and Clark, 1982). As recommended, we will develop an exhaustive number of items to ensure that enough for the final version of the scale (Natalio et al., 2014). The excessive creation of items will ensure that enough items are obtained

for the final version of the scale without the need to revisit the previous steps at a more advanced stage (Natalio et al., 2014).

Subsequently, a careful review of each item will be conducted according to pre-established criteria and will be checked if: they are clearly defined; they meet the selected format; the answer options are plausible and excluding; the terms used are adequate; the wording is clear; the phenomena identified in the three data sources are considered; they are consistent with their conceptual definitions; and they are representative and relevant to the global domain and the clinical interpretation (Benson and Clark, 1982; Natalio et al., 2014). The selected item will form the first prototype of the PD-MAS will be established. This phase will be concluded after a content validation from a Delphi panel of specialists and a cognitive pretesting with people with PD, their caregivers and movement disorders specialists. The cognitive pretesting will examine the extent to which the questions reflect the domain under study, and the extent to which the respondent feels comfortable with the response options and feels that the response options are appropriate and if answers to the questions produce valid measurements that are meaningful to the patient and/or caregiver.

We anticipate that the reporting source of information for the PD-MAS will need to be flexible to accommodate the patient and a primary caregiver. As such, we envision two validated versions, "PD-MAS: Patient" and "PD-MAS": caregiver. In cases where the management and administration of medication for PD is done in cooperation between the person with PD and their caregiver, both must respond to the scale independently, each using the most appropriate version of the PD-MAS.

Phase III: Quantitative Evaluation Overview

The testing plan of the PD-MAS is composed by sequential clinimetric phases of quantitative evaluation of the scale's measurement properties using Classical Test Theory (CTT) (DeVellis, 2006) and Item Response Theory Analysis (IRT) (Hays et al., 2000). Through large international cross-sectional study with people with PD, the measurement properties of the scale will be tested according to the follow criteria: reliability, structural validity, internal consistency, measurement error, criterion validity, hypotheses testing for construct validity and responsiveness of the scale. This phase of testing will entail healthcare professionals directly involved in the care of people with PD, and they will be trained to use the PD-MAS in their clinical and research practices. They will also be trained in entering data in the database that will be created specifically to store the data from these phases of the study. Demographic information about each patient who participates in these phases will identify profiles of people with PD who will predict the need to gather the questionnaire data through an interview based on evaluators. The added rater time will be calculated and incorporated into the clinimetric feasibility analysis. The version of the scale that meets the criteria of sufficient reliability, validity and responsiveness and accommodates maximum information without duplication will become PD-MAS in its final form.

Phase IV: Scale Validation Overview

It is possible that a second pilot testing will be done to fill in the gaps identified in the first pilot testing. Should it be required, the data will be analyzed again, and as necessary, some phases may be resumed as the version of the scale needs to meet the criteria of sufficient reliability, validity, and responsiveness. This process may be iterative and is designed to allow the scale to capture the maximum amount of unduplicated information in the final PD-MAS form.

From here, we intend not to restrict ourselves to the English and Portuguese versions only, but to translate the PD-MAS into other languages. Therefore, the translated versions will be submitted to a responsivity testing program for PD-MAS, as well as tested through qualitative cognitive assessments in a small number of patients for each language under consideration.

At the conclusion of each phase, we will assess the need for revisions of the PD-MAS. We recognize that the scale may in fact evolve over the phases of clinimetric testing and, therefore, we will not publish any draft before its final version. However, we anticipate that future projects offer an opportunity for individual researchers, societies, and industries to be part of the studies that will validate the first scale created to measure the multidimensional and multifactorial components involved in the medication adherence process in patients with PD.

DISCUSSION

Despite the availability of literature on the theory of development and validation of scales (Benson and Clark, 1982; McDowell, 2006; Stebbins, 2012; Boateng et al., 2018; Carpenter, 2018; Kyriazos and Stalikas, 2018), the conceptualization, design, clinimetric testing, and validation analysis of a new scale need to be done with a sensitivity to scientific rigor and practicality related to the population of interest, in this case, patients with PD.

According to COSMIN (COnsensus-based Standards for the selection of health Measurement INstruments), it is important that clinicians and researchers analyze at least six clinimetric criteria before selecting health measurement instruments, including: (1) a description of the conceptual framework that explains which concepts are being measured by scale; (2) what is the target population and their health condition; (3) how items should be weighted according to each of the scale's subdomains; (4) what is the mode of administration and data collection; (5) what are the response options and their appropriate scores; and finally, (6) the possibility of translation or cultural adaptation (Mokkink et al., 2018). To meet these criteria, this research devoted considerable resources of time to describe the development process and clinimetric testing plan of the PD-MAS, providing the international community with official definitions and technical procedures that will be used in all phases of development of this rating scale.

Because the development and validation phases will be conducted in multinational settings, including raters, patients and caregivers who are not native-English/Portuguese speaking,

this study protocol provides an assurance of homogeneity in the implementation of research procedures. Similar initiatives have been used in clinimetric studies in PD, and with this infrastructure work before the field tests, the scales that were finally tested on a large scale were successful, emphasizing the relevance that this methodological rigor has before the start of scale development (Goetz et al., 2014; Comella et al., 2015).

Finally, we expect that the information resulting from longitudinal research data on medication adherence in PD can be used to support disease-based outcomes and additional impacts on patients and their care partners, such as: quality of life, burden, functional level, levodopa equivalent daily dose (LEDD) and adverse effects. Furthermore, we expect that the data from the PD-MAS will support the results of studies that express other clinical indices, such as those related to motor and non-motor symptoms of PD.

CONCLUSION

As science advances and novel research questions are put forth, new scales become necessary. The use of multiple items to measure an underlying latent construct can account for, and isolate, item-specific measurement error, which leads to more accurate research findings. The development of a new scale is a challenging process given the involvement of several theoretical, methodological, and statistical competencies.

The overview presentation of the development phases and the clinimetric testing plan of the PD-MAS demonstrate the feasibility of creating an instrument to measure the multidimensional and multifactorial components of the medication adherence process in people with PD. With this report, researchers will be equipped for the proper implementation of the techniques of this long-term study.

AUTHOR CONTRIBUTIONS

All authors contributed to the conception and design of the study. MT, BO, and GS organized the database and the statistical analysis plan. MT wrote the first draft of the manuscript. All authors contributed to writing the manuscript, reviewing the statistical plan, and approving the submitted version.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnagi.2022.900029/full#supplementary-material

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Selective Serotonin Reuptake Inhibitor-Treatment Does Not Show Beneficial Effects on Cognition or Amyloid Burden in Cognitively Impaired and Cognitively Normal Subjects

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Preclinical studies indicate that selective serotonin reuptake inhibitors (SSRI) have beneficial effects on Alzheimer-related pathologies. Therefore, the aim of this study was to evaluate the influence of SSRI-treatment on amyloid burden in ^{18}F-Florbetapir-positron emission tomography (PET) and on cognition in cognitively normal and cognitively impaired subjects. We included n = 755 cognitively impaired and n = 394 cognitively normal participants from the Alzheimer's Disease Neuroimaging Initiative (ADNI) that underwent at least one ^{18}F-Florbetapir-PET. Standardized uptake ratios (SUVR) and the Alzheimer Disease Assessment Scale-cognitive subscale (ADAS) scores as well as follow-up results were compared between subgroups with a history of SSRI-treatment (SSRI+) and without SSRI-treatment (SSRI-) as well as in subgroups of SSRI+/Depression+ and SSRI+/Depression- and SSRI-/Depression+ and SSRI-/Depression-. ^{18}F-Florbetapir-PET did not show significant differences of SUVR between the SSRI+ and SSRI- groups in both, cognitively impaired and cognitively normal participants. There were no differences in subgroups of SSRI+/Depression+ and SSRI+/Depression- and SSRI-/Depression+ and SSRI-/Depression-. However, SUVR showed a dose-dependent inverse correlation to the duration of medication in cognitively normal and in cognitively impaired patients. SRRI-treatment did not show an effect on ADAS scores. Furthermore, there was no effect on follow-up SUVR or on follow-up ADAS scores. Overall, SSRI-treatment did not show beneficial effects on amyloid load nor on cognition.

Keywords: positron emission tomography, selective serotonin reuptake inhibitors, amyloid-PET imaging, florbetaben, Alzheimer's disease

INTRODUCTION

Alzheimer's disease (AD), the most common form of dementia, is a progressive neurogenerative disease characterized by memory loss and decline of cognitive function. Despite extensive treatment efforts, AD remains incurable and novel therapies to prevent, slow down or delay the onset of the disease are urgently needed. Without effective therapies, the number of patients with dementia worldwide is estimated to reach more than 130 million by 2050 (Cummings et al., 2016).

Given the need for disease-modifying therapies for AD, drug repurposing may be a promising approach. Preclinical studies indicate that antidepressants, particularly selective serotonin reuptake inhibitors (SSRI), have beneficial effects on AD-related biomarkers including amyloid plaques, one of the major pathological hallmarks in AD (Cirrito et al., 2011, 2020; Sheline et al., 2014). Neuritic plaques consist of aggregated Abeta peptides that are formed within neurons by sequentially cleavage of the amyloid precursor protein (APP). Dysregulation of Aβ production and Aβ clearance leads to accumulation of hydrophobic Aβ forms and the formation of extracellular plaques (Chen et al., 2017; Guo et al., 2020). While the pathway of APP processing is well-characterized, mechanisms of its regulation are not yet fully understood. APP processing can be modulated by several signaling pathways including NMDA, acetylcholine and serotonin signaling systems. Serotonin receptors (5-HTr) might affect APP processing and Aβ levels. *In vitro* studies could show that activation of 5-HTr2a, 5-HTr2c, and 5-HTr4 increases non-amylogenic APP processing (Nitsch et al., 1996; Robert et al., 2001; Pakaski et al., 2005; Shen et al., 2011). In addition, treatment with SSRI reduced Aβ levels and amyloid plaque burden in different mouse models of AD (Tucker et al., 2005; Nelson et al., 2007; Cirrito et al., 2011, 2020). Furthermore, beneficial effects of antidepressant medication on cognition could be shown in depressed patients, while there was no effect on non-depressed individuals (Prado et al., 2018).

However, whether chronic SSRI-treatment influences amyloid burden and cognition in humans remains unclear. A few studies suggest a possible positive benefit of SSRI treatment on the risk of developing AD, whereas the effect on amyloid burden remains controversial (Kessing et al., 2009; Cirrito et al., 2011; Bartels et al., 2018, 2020).

The aim of this study is the evaluation of the influence of SSRI-treatment on amyloid burden in ^{18}F-Florbetapir positron emission tomography (PET) in cognitively normal and cognitively impaired individuals.

MATERIALS AND METHODS

Data used in this study were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database.[1] The ADNI was launched in 2003 as a public-private partnership, led by Principal Investigator Michael W. Weiner, MD. The primary goal of ADNI has been to test whether serial magnetic resonance imaging (MRI), positron emission tomography (PET), other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of mild cognitive impairment (MCI) and early Alzheimer's disease (AD). ADNI is a multicentered project that aims to improve clinical research on AD unifying data on demographics, clinical and cognitive assessments as well as on genetic, biochemical and imaging biomarkers. Standardized protocols and unlimited data access allow analysis of an enormous amount of data from more than 1800 patients that have been included to the ADNI database to date. Groups of AD patients, patients with mild cognitive impairment (MCI), and cognitively normal elderly controls were included in four phases starting in 2004.

Study Sample

In this study, ADNI data from all four phases, ADNI-1, ADNI-GO, ADNI-2, and ADNI-3, were downloaded from the ADNI database (see text footnote 1) on May 7, 2021. Patients aged 57–93 with at least one available ^{18}F-Florbetapir-PET were downloaded (n = 1296; **Figure 1**). Patient characteristics, medical history, medication, Mini Mental State Examination (MMSE), Clinical Dementia Rating score (CDR), the Alzheimer's Disease Assessment Scale–Cognitive Subscale (ADAS), depression-history, education years, ApoE4 status and PET results were assessed. Patients with insufficient or inconsistent data on cognition or prior medication were excluded (n = 22). N = 20 patients were excluded as SSRI-treatment was paused before the baseline PET and n = 105 patients without significant objective amnestic dysfunction but with subjective memory concerns were excluded.

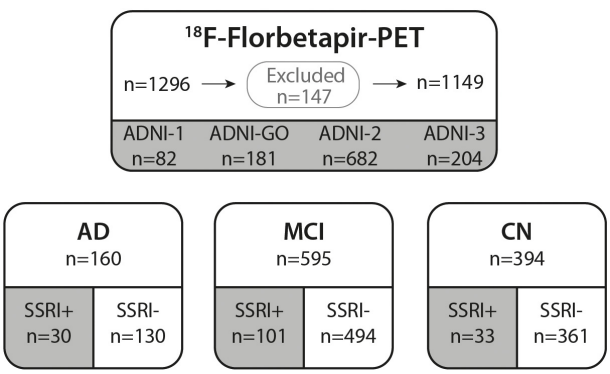

FIGURE 1 | ADNI cohort. ^{18}F-Florbetapir-PET was performed in n = 1296 ADNI participants. N = 147 subjects were excluded from our study due to missing data or stopped SSRI medication as well as patients with subjective memory deficits that did not meet criteria of AD or MCI. A total of n = 1149 subjects from ADNI phases 1, GO, 2 and 3 were included in this study. All subjects were categorized according to their diagnosis in AD, MCI, or CN. Groups were further subdivided in subjects with prior SSRI-treatment (SSRI+) and subjects with no history of SSRI-treatment (SSRI−). AD, Alzheimer's dementia; MCI, mild cognitive impairment; CN, cognitively normal controls.

[1] http://adni.loni.usc.edu

According to the ADNI protocol, participants were categorized as cognitively normal and cognitively impaired with subgroups of MCI and Alzheimer's dementia (AD). AD patients showed a significant subjective and objective memory loss (based on scores on the WMS-R Logical Memory II subscale) affecting activities of daily living with a MMSE below 26 and CDR of 0.5 or 1 plus. MCI patients were defined as patients showing significant memory issues with a MMSE from 24 to 30 and CDR of 0.5 plus an abnormal score on the WMS-R Logical Memory II subscale. Cognitively normal participants showed no signs of dementia with normal cognition (MMSE from 24 to 30 and a CDR of 0 plus a WMS-R Logical Memory II subscale score above education-adjusted cutoffs).

Detailed information about the ADNI inclusion and exclusion criteria can be found online.[2]

For our analysis, all groups and subgroups were divided into history of SSRI treatment (SSRI+) and without SSRI treatment (SSRI−).

A total of $n = 164$ patients were currently treated with an SSRI at the time of the PET (Citalopram: $n = 69$; Escitalopram $n = 16$; Fluoxetine $n = 24$; Paroxetine $n = 7$; Sertraline $n = 48$; **Table 1**).

Alzheimer's Disease Neuroimaging Initiative ^{18}F-Florbetapir-PET/CT

^{18}F-Florbetapir-PET/CT was performed according to a standardized imaging protocol with 4 × 5min frames acquired 50–70 min post-injection of 370 (± 10%) MBq ^{18}F-Florbetapir. All PET images were reviewed for quality control by the ADNI PET QC team and transmitted in DICOM format to the Laboratory of Neuroimaging (LONI) for storage. In order to uniformize data from different origins, PET image data were pre-processed with motion correction, time frame averaging, reorientation in a standardized 160 × 160 × 96 matrix with a voxel size of 1.5 mm and smoothing with a scanner-specific filter function determined from Hoffman phantom scans during the certification process (more details on adni.loni.usc.edu).

PET images were further processed with FreeSurfer v7.1.1 for an MRI-based definition of multiple cortical regions as well as reference regions for normalization.

Amyloid load was quantitatively analyzed using standardized uptake ratios (SUVR) of composite regions normalized by cerebellar uptake. In order to analyze possible region-specific differences, SUVR of frontal, parietal, temporal and cingulate cortex regions were also obtained. As described before, intensity normalization of SUVRs was performed using a FreeSurfer-defined whole cerebellum region for cross-sectional analysis and a FreeSurfer-defined composite reference region for longitudinal studies.

[2]https://adni.loni.usc.edu/study-design/

TABLE 1 | Selective serotonin reuptake inhibitor (SSRI) treatment.

	SSRI+		Duration of medication	Dose
	n	%	Months (SD)	mg (SD)
All	164	100	56.27 (64.92)	
Citalopram	69	42	43.75 (50.61)	19.64 (10.79)
Escitalopram	16	10	47.27 (47.41)	14.06 (6.88)
Fluoxetine	24	15	113.5 (94.96)	28.33 (14.94)
Paroxetine	7	4	39.0 (28.65)	25.71 (12.72)
Sertraline	48	29	54.23 (63.08)	72.86 (43.71)

Statistical Analysis

Statistical analysis was performed using SPSS Statistics version 27 (IBM, Armonk, NY, United States) and GraphPad Prism version 9 (GraphPad Software, San Diego, CA, United States).

Differences between groups were tested using non-parametric tests (as data did not pass normality tests, e.g., Shapiro–Wilk test $p = 0.0006$), therefore Mann–Whitney U test or Kruskal–Wallis test followed by *post hoc* multiple comparison were used as indicated. Univariate analysis of covariance (ANCOVA) was used for covariate adjustment as indicated. Chi-square test or Fisher's exact test were used for categorical variables. Relationships between two variables were assessed using Spearman correlation and simple linear regression.

For follow-up PET and ADAS data changes were calculated as absolute and relative change between baseline and follow-up (ΔSUVR/ΔADAS; Δ%SUVR/Δ%ADAS).

Significance levels are given as follows: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

RESULTS

Baseline Characteristics

A total of $n = 755$ cognitively impaired (AD and MCI patients) and $n = 394$ cognitively normal participants from ADNI phases ADNI-1 ($n = 82$), ADNI-GO ($n = 181$), ADNI-2 ($n = 682$), and ADNI-3 ($n = 204$) that underwent at least one ^{18}F-Florbetapir-PET were included in this study. Baseline characteristics for all patients categorized into diagnostic groups are shown in **Table 2**. Patients were categorized as AD ($n = 160$), MCI ($n = 595$) and cognitively normal ($n = 394$) according to clinical symptoms and results of neuropsychological assessments following the ADNI protocol (**Figure 1**).

The group of AD patients was slightly older compared to the MCI group (Kruskal–Wallis test with Dunn's multiple comparison test, $p = 0.0093$) while there were no differences between all other groups (Kruskal–Wallis test, $p > 0.07$). Groups of cognitively impaired patients showed a higher number of male patients compared to cognitively normal controls (Chi-square test, $p = 0.0002$).

MMSE, CDR and ADAS showed significant differences between AD and MCI as well as between AD or MCI compared to cognitively normal patients after adjusting for age and gender as covariates (ANCOVA, $p < 0.001$, **Table 2**).

There were no differences between age, gender, MMSE scores, ApoE4-allels or diagnosis between cognitively impaired SSRI+ and SSRI− patients ($p > 0.11$; **Table 2**). There were no differences between age, MMSE scores or ApoE4-allels between cognitively normal SSRI+ and SSRI− patients ($p > 0.19$; **Table 2**). Slight differences were observed in gender distribution with

TABLE 2 | Patient characteristics.

Characteristics	**Total**		**SSRI+**		**SSRI−**		
1. Cognitively impaired patients							
	Total *N* = 755		**SSRI+ *N* = 131**		**SSRI− *N* = 624**		
	Mean	***SD***	***Mean***	***SD***	***Mean***	***SD***	***p***
Age	73.59	7.9	73.04	7.6	73.7	8.0	0.4095
MMSE	25.96	3.9	25.52	4.01	26.1	3.8	0.2549
ADAS	19.66	11.2	22.43	12.1	19.0	10.8	0.0126
Education (years)	16	2.7	15.73	2.6	16.06	2.7	0.185
	N	***%***	***N***	***%***	***N***	***%***	
Diagnosis							>0.9999
AD	160	21	30	21	130	21	
MCI	595	79	101	79	494	79	
Gender							0.1146
Female	326	44	65	49	261	42	
Male	429	56	66	51	363	58	
ApoE4 alleles							0.2385
0	378	50	56	43	322	52	
1	287	38	57	43	230	37	
2	90	12	18	14	72	12	
SSRIs							
All			131	100			
Citalopram			52	40			
Escitalopram			9	7			
Fluoxetine			22	16			
Paroxetine			5	4			
Sertraline			39	32			
History of depression							<0.001
Depression	249	33	94	69	155		
No depression	506	67	37	31	469		
2. Objectively cognitively normal patients							
	Total *N* = 394		**SSRI+ *N* = 33**		**SSRI− *N* = 361**		
	Mean	***SD***	***Mean***	***SD***	***Mean***	***SD***	***p***
Age	74.44	7.13	72.08	5.8	74.2	6.9	0.1930
MMSE	28.99	1.21	28.68	1.7	29.0	1.2	0.3556
ADAS	9.3	5.0	11.5	7.8	9.1	4.5	0.0383
Education (years)	16.54	2.56	15.64	2.25	16.66	2.57	0.0138
	N	***%***	***N***	***%***	***N***	***%***	
Gender							0.0035
Female	215		26	79	189	52	
Male	179		7	21	172	48	
ApoE4 alleles							0.5615
0	282		24	73	258	72	
1	100		9	27	91	25	
2	12		–	0	12	3	
SSRI							
All			33	100			
Citalopram			17	52			
Escitalopram			7	21			
Fluoxetine			2	6			
Paroxetine			2	6			
Sertraline			5	15			
History of depression							<0.001
Depression	51	13	19	58	32	9	
No depression	343	87	14	42	329	91	

AD, Alzheimer's dementia; MCI, mild cognitive impairment. Gray shades highlight patients with SSRI medication.

proportionately more women in the SSRI+ group (Chi-square test; $p = 0.002$; **Table 2**) which was considered irrelevant for further analysis. Amyloid load did not differ between female and male patients (Mann–Whitney U test, $p = 0.1308$).

$N = 300$ patients had a history of depression. The amount of patients with a history of depression was significantly higher in the SSRI+ group compared to the SSRI− group (Chi-square test; $p < 0.001$; **Table 2**).

Positron Emission Tomography Results

A baseline PET was available in all patients ($n = 1149$). SUVRs were significantly higher in the AD subgroup compared to the MCI subgroup as well as in AD or MCI compared to cognitively normal controls after adjusting for age and gender as covariates (**Figure 2A**, ANCOVA, $p < 0.001$).

Selective Serotonin Reuptake Inhibitors Treatment

There were no significant differences of SUVR between the SSRI+ and SSRI− patients regardless of the diagnosis (**Figure 2A**, Mann–Whitney U test; AD: $p = 0.667$; MCI: $p = 0.169$; cognitively normal controls: $p = 0.188$).

SUVR showed a significant negative correlation with the duration of SSRI-treatment in cognitively normal SSRI+ as well as in cognitively impaired SSRI+ patients (**Figures 2B,C**, Spearman correlation, cognitive normal: $p = 0.0038$; $r = -0.4965$; cognitively impaired: $p = 0.025$; $r = -0.1958$).

SUVRs did not differ between groups treated with citalopram, escitalopram, fluoxetine, paroxetine or sertraline, neither in SSRI+ CI patients (Kruskal–Wallis test; $p = 0.3276$) nor in SSRI+ cognitive normal controls (Kruskal–Wallis test; $p = 0.8296$).

There was no dose-dependency of SUVR in any of the groups treated with citalopram, escitalopram, fluoxetine, paroxetine or sertraline, neither in SSRI+ cognitively impaired patients (Kruskal–Wallis test; $p > 0.14$) nor in SSRI+ cognitive normal patients (Kruskal–Wallis test; $p > 0.3$).

Regional Differences

Regional differences of amyloid load were analyzed in frontal, cingulate, parietal and temporal cortex. There were no significant differences between regional SUVR of SSRI+ and SSRI− regardless of the diagnosis (Kruskal–Wallis test, $p > 0.5$ in all groups).

History of Depression

^{18}F-Florbetapir uptake did not show significant differences between SSRI+ and SSRI− patients with and without history of depression regardless of the diagnosis (**Figure 3**; Kruskal–Wallis test; AD: $p = 0.8304$; MCI: $p = 0.5308$; cognitive normal controls: $p = 0.1937$).

Follow-Up ^{18}F-Florbetapir-PET

At least one follow-up ^{18}F- Florbetapir-PET was available in $n = 650$ patients. Mean follow-up period was 26.49 months for one follow-up PET. A second or third follow-up was only available in the MCI and cognitively normal group with a mean period of 57.34 months for a second follow-up PET scan ($n = 361$) and 74.86 months for a third follow-up PET scan ($n = 184$).

In order to analyze changes of amyloid deposition, differences of SUVR between the baseline PET and available follow-up PETs were calculated (ΔSUVR). Longitudinal changes of SUVR did not show any significant differences between SSRI+ and SSRI− AD-patients after one follow-up (**Table 3** and **Figure 4A**; Mann–Whitney U test; $p = 0.4269$).

There were no significant differences between SSRI+ and SSRI− MCI-patients after the first, second or third follow-up (**Table 3** and **Figure 4A**; Mann–Whitney U test; first follow-up: $p = 0.9501$; second follow up: $p = 0.3405$; third follow-up: $p = 0.2387$). Furthermore, we did not detect significant differences between ΔSUVR in the cognitively normal group either (**Table 3** and **Figure 4A**; Mann–Whitney U test; first follow-up: $p = 0.6367$; second follow up: $p = 0.8637$; third follow-up: $p = 0.921$).

Cognition

In order to analyze differences in cognition as well as longitudinal changes of cognition between SSRI+ and SSRI− groups the Alzheimer Disease Assessment Scale-cognitive subscale (ADAS) scores were used. Baseline ADAS as well as follow-up scores were collected as close to the date of the corresponding baseline and follow-up PET scan as possible.

Baseline ADAS was available in all patients ($n = 1149$). ADAS scores correlated with amyloid burden in PET (**Figure 5A**, Spearman $r = 0.4396$; $p < 0.001$). ADAS was significantly higher in AD patients compared to MCI patients and in MCI and AD patients compared to cognitively normal controls after adjusting for age, gender and education as covariates (**Figure 5B**; ANCOVA; $p < 0.001$). There were no significant differences between SSRI+ and SSRI− subgroups after adjusting for age, gender and education as covariates (**Figure 5B**, ANCOVA, AD: $p = 0.238$; MCI: $p = 0.153$; NMC: $p = 0.063$).

At least one follow-up ADAS score was available in $n = 646$ patients with a mean follow-up of 25.5 months. A second and third follow-up ADAS score was available in the MCI and cognitively normal group with a mean period of 60.67 months for a second follow-up ($n = 356$) and 73.93 months for a third follow-up ($n = 179$). In order to analyze longitudinal changes of cognition, differences of ADAS between the baseline score and available follow-up scores were calculated (ΔADAS; **Table 3**).

There were no significant differences of the ADAS score between SSRI+ and SSRI− AD-patients after one follow-up (**Table 3** and **Figure 4B**; Mann–Whitney U test; $p = 0.2593$). Furthermore, we did not detect significant differences between ΔADAS in the MCI-group nor in the cognitively normal group after the first, second or third follow-up (**Table 3** and **Figure 4B**; Mann–Whitney U test; MCI: first follow-up: $p = 0.5651$; second follow up: $p = 0.5142$; third follow-up: $p = 0.2465$; cognitively normal group: first follow-up: $p = 0.2084$; second follow up: $p = 0.595$; third follow-up: $p = 0.359$).

ApoE4

ApoE4 carriers showed significantly higher SUVR compared to non-carriers in cognitively impaired patients as well as in cognitively normal subjects (Mann–Whitney U test; $p < 0.001$;

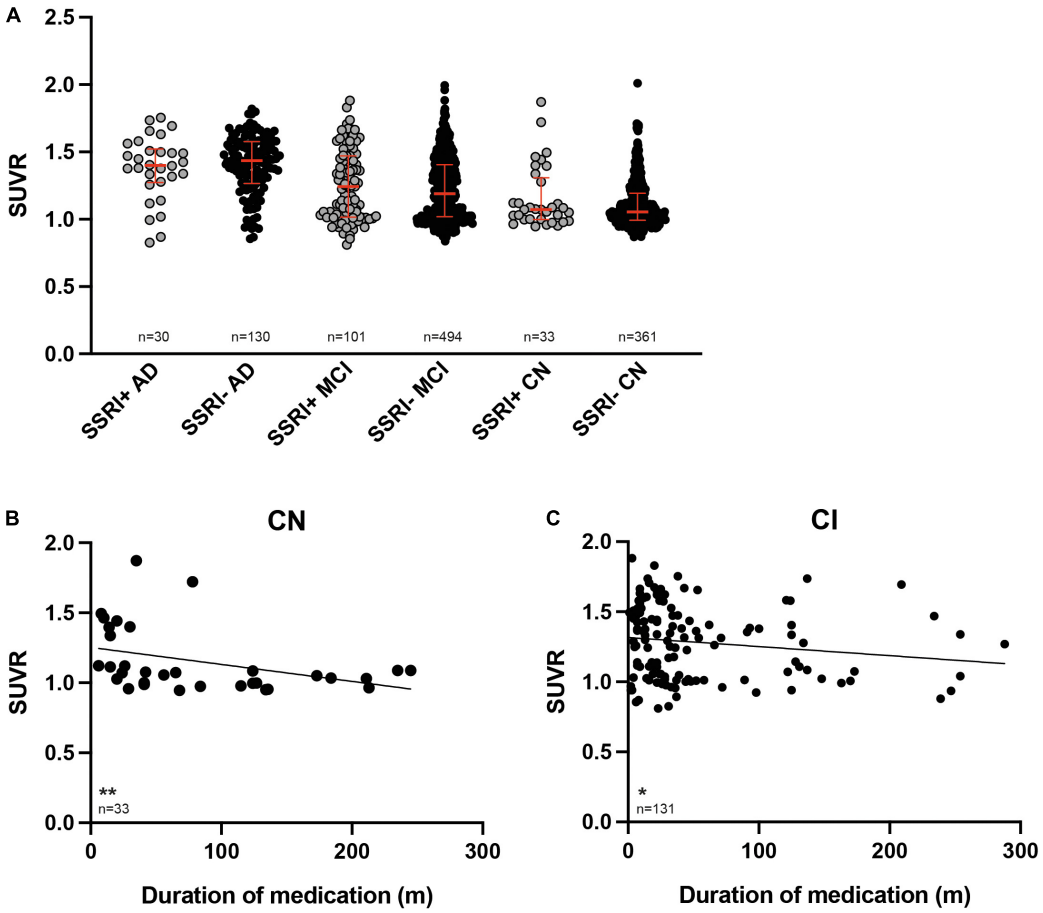

FIGURE 2 | ^{18}F-Florbetapir uptake in SSRI-treated and untreated subjects. **(A)** SUVR was significantly higher in AD-patients compared to MCI patients as well as between AD or MCI patients compared to CN (ANCOVA). SUVR did not show significant differences between SSRI-treated (SSRI+) and untreated (SSRI−) subjects regardless of the diagnosis (Mann–Whitney *U* test; red bars represent median ± interquartile range). **(B)** SUVR showed a time-dependent inverse correlation to the duration of medication in cognitively normal patients. **(C)** SUVR also showed a time-dependent negative relation to the duration of medication in cognitively impaired patients. Spearman correlation; $^{*}p < 0.05$; $^{**}p < 0.01$. SUVR, standard uptake value ratio; CN, cognitively normal controls; CI, cognitively impaired patients; AD, Alzheimer's disease; MCI, mild cognitive impairment.

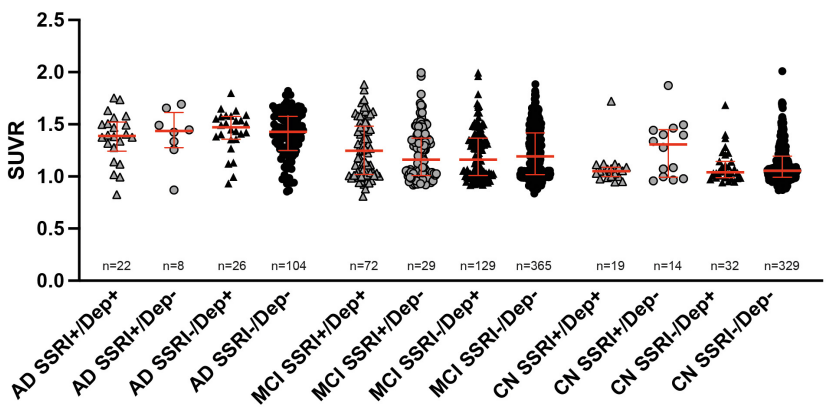

FIGURE 3 | ^{18}F-Florbetapir uptake in SSRI-treated and untreated subjects with and without history of depression. No significant differences between SSRI-treated and untreated subjects with or without depression were detected regardless of the diagnosis (Kruskal–Wallis test; red bars represent median ± interquartile range. AD, Alzheimer's disease; MCI, mild cognitive impairment; CN, cognitively normal controls.

TABLE 3 | Follow up SUVR and ADAS.

	Delta SUVR			Delta ADAS		
	FU1	FU2	FU3	FU1	FU2	FU3
SSRI+ AD	+0.039 (4%)	na	na	+9.5 (29%)	na	na
SSRI− AD	+0.018 (1.8%)	na	na	+4.4 (14%)	na	na
SSRI+ MCI	+0.014 (1.6%)	+0.029 (3.3%)	+0.045 (4.9%)	+2.6 (14%)	+4.3 (22%)	+6.9 (36%)
SSRI− MCI	+0.014 (1.6%)	+0.021 (2.5%)	+0.018 (2.1%)	+1.6 (10%)	+3.7 (23%)	+3.1 (19%)
SSRI+ CN	+0.007 (1%)	+0.023 (3%)	+0.03 (3.8%)	+1.4 (11%)	+1.7 (13%)	+6.7 (46%)
SSRI− CN	+0.013 (1.7%)	+0.019 (2.5%)	+0.02 (2.6%)	−0.1 (1.1%)	+0.9 (10%)	+1.5 (17%)

AD, Alzheimer's dementia; MCI, mild cognitive impairment; FU, follow up; CN, cognitively normal controls. Gray shades highlight patients with SSRI medication.

Figure 6). However, there were no differences in the SUVR between SSRI+ and SSRI− ApoE4 carriers regardless of the diagnosis (Mann–Whitney *U* test; AD: $p = 0.7909$; MCI: $p = 0.3644$; cognitively normal: $p = 0.9065$; **Figure 6**).

DISCUSSION

Amyloid plaques are a major pathological hallmark of AD and the modulation of plaques, APP processing, and related signaling pathways has been a research focus for novel AD-therapies for many years. The role of different neurotransmitter systems in the development of AD is still unclear. Recent evidence suggests that the serotonergic system, that has mainly been studied in mood disorders so far, might play a role in the pathogenesis of AD (Cirrito et al., 2011; Ramos-Rodriguez et al., 2013).

Mid-life or late-life depression is considered a risk factor for the development of AD. However, whether depression is a prodromal symptom or a true etiologic risk factor for AD remains controversial. Both, AD and depression share similar neuropathological changes as neuroinflammation, elevated oxidative stress markers and synaptic dysfunctions and an increased cortical amyloid burden is also seen in patients with a lifetime history of depression (Chung et al., 2015; Herbert and Lucassen, 2016; Mahgoub and Alexopoulos, 2016).

Therefore, the modulation of serotonergic systems with SSRIs might be a potential treatment option for AD patients with or without a history of depression.

Several preclinical studies demonstrated beneficial effects of SSRI-treatment on AD-pathologies in different mouse models of AD suggesting a modulation of APP processing by SSRIs. Cirrito et al. (2011) first showed a serotonin-dependent reduction of Aβ levels in the brain interstitial fluid (ISF) in APP/PS1 mice after short-term treatment with citalopram or fluoxetine. Furthermore, reduced cortical and hippocampal plaque burden as well as decreased Aβ levels in ISF and cerebrospinal fluid (CSF) were also detected after chronic citalopram administration (Cirrito et al., 2011). Beneficial effects on plaque load and Aβ-levels following citalopram treatment could also be confirmed by a couple of other studies (Sheline et al., 2014; Wei et al., 2017; Zhang et al., 2018). Sheline and colleagues demonstrated a reduced formation of plaques as well as decreased growth of preexisting plaques in APP/PS1 mice after 28 days of citalopram treatment (Sheline et al., 2014). Similar to the results obtained after citalopram treatment, several studies demonstrated that fluoxetine treatment decreases amyloid plaques and soluble Aβ levels in the brain in different AD mouse models (Cirrito et al., 2011; Wang et al., 2014, 2016; Qiao et al., 2016; Jin et al., 2017; Ma et al., 2017; Sun et al., 2017; Zhou et al., 2019). Furthermore, there is limited evidence that paroxetine has also beneficial effects on the AD pathology as Nelson et al. (2007) showed reduced accumulation of tau-protein and cortical Aβ1-40 levels after chronic paroxetine treatment in 3xTg mice and Olesen et al. (2017) detected a reduction of plaque load in the hippocampus of APP/PS1-mice (Nelson et al., 2007; Olesen et al., 2016, 2017). In addition, escpitalopram was able to reduce tau-hyperphosphorylation in cultures of Aβ1-42 treated hippocampal neurons of fetal brains obtained from rats (Wang et al., 2016) and in a recent study, Cirrito and colleagues showed a significantly reduced plaque load in APP/PS1 mice after 28 days of escpitalopram treatment (Cirrito et al., 2020).

While these preclinical results hint to a beneficial effect of SSRIs on AD-pathologies, studies on their impact in humans are very limited. Bartels et al. (2018) showed that long-term use of SSRIs delayed the conversion from MCI to AD in patients with a previous depression for 3 years (Bartels et al., 2018). Similarly, Burke et al. (2018) showed that the risk of cognitively normal patients with a prior history of depression developing AD was neutralized by SSRI treatment compared to untreated patients (Burke et al., 2018). A retrospective study of Down syndrome patients with a previous history of depression showed that SSRI use for more than 90 days significantly delayed the onset of dementia (Tsiouris et al., 2014).

Sheline et al. (2014) demonstrated a significant reduction of Aβ production and CSF Aβ concentration after a single high dose application of citalopram in healthy volunteers (Sheline et al., 2014). However, it should be noted that the study was performed on young participants, presumably before plaque formation while our cohort was much older. However, in a recent study, the same group showed that short-term longitudinal treatment of escitalopram decreases CSF Aβ42 levels in cognitively normal older adults (Sheline et al., 2020).

In the current study, we could not detect any differences of amyloid burden or cognition between patients with SSRI treatment compared to untreated patients regardless of a previous depression.

So far, only two studies have evaluated the effects of SSRI-treatment on amyloid burden using amyloid-PET showing

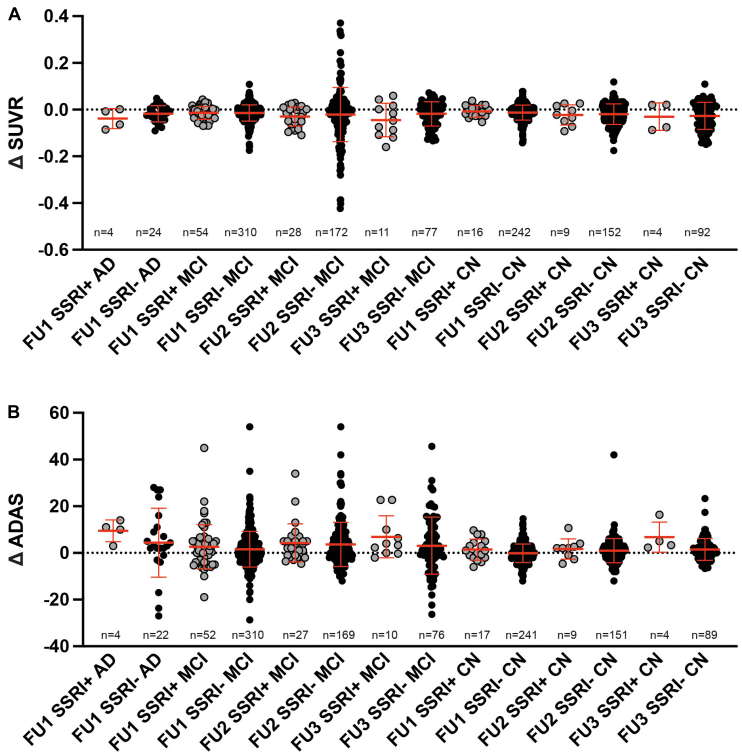

FIGURE 4 | Follow up. **(A)** Differences of SUVR between follow-up and baseline PET did not show significant differences between SSRI-treated and untreated subjects (Mann–Whitney *U* test). **(B)** Differences of ADAS between follow-up and baseline examination did not show significant differences between SSRI-treated and untreated subjects (Mann–Whitney *U* test). Red bars represent median ± interquartile range.

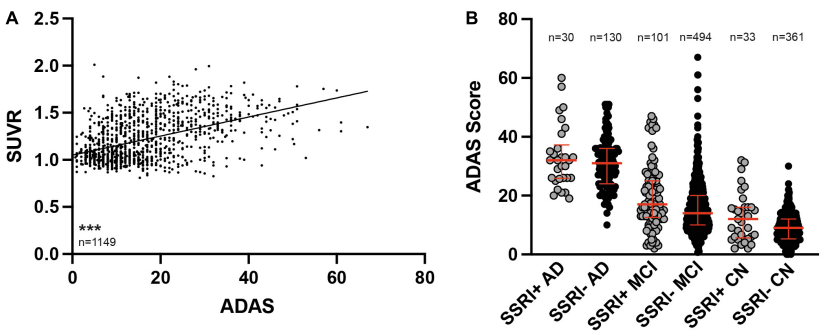

FIGURE 5 | ADAS cognition test results. **(A)** ADAS scores correlated to SUVR results (Spearman correlation; $^{***}p < 0.001$). **(B)** SSRI-treatment did not influence cognition. ADAS was significantly higher in AD patients compared to MCI and both, AD patients and MCI patients showed higher ADAS scores compared to CN (ANCOVA). Red bars represent median with interquartile range. AD, Alzheimer's disease; MCI, mild cognitive impairment; CN, cognitively normal controls.

controversial findings. Brendel et al. (2018) showed no significant reduction of amyloid deposition in ^{18}F-Florbetapir-PET between SSRI-treated and untreated MCI and AD patients with a prior history of depression (Brendel et al., 2018). Results are in line with our findings, as we did not detect significant differences in amyloid burden between SSRI-treated and untreated patients in a comparable patient cohort. In addition, we did not find any significant differences in MCI and AD groups without prior depression nor in cognitively normal patient groups with or without depression. Consistent with our findings, in a previous study by Bartels et al. (2018) CSF Aβ levels were unaffected by SSRI treatment in MCI patients with a history of depression.

In contrast to those findings and to our findings, Cirrito et al. (2011) showed a significant lower cortical binding of the ^{11}C-labeled amyloid tracer Pittsburgh Compound-B (^{11}C-PiB) in cognitively normal patients that have received fluoxetine, citalopram or escitalopram treatment (mean duration 35 months) compared to patients with no history of SSRI use. The Thioflavin-S derivate ^{11}C-PiB was the first amyloid tracer applied in human PET studies with high sensitivity and specificity for

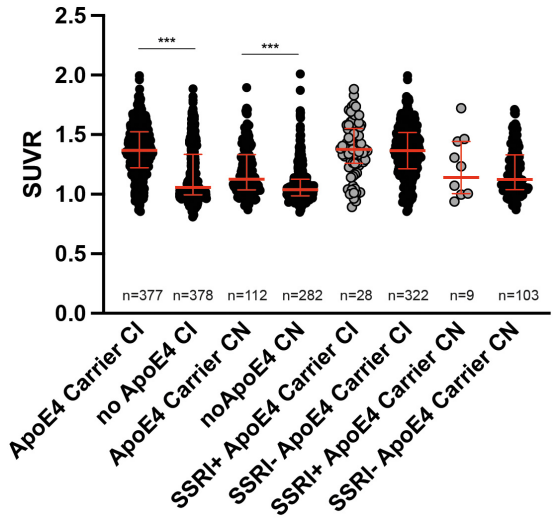

FIGURE 6 | ApoE4 carriers. ApoE4 carriers showed significantly higher SUVR compared to non-carriers in cognitively impaired patients as well as in cognitively normal subjects (Mann–Whitney *U* test; ***$p < 0.001$). There were no differences between ApoE4 carriers in SSRI+ and SSRI− groups (Mann–Whitney *U* test). Red bars represent median ± interquartile range.

the detection of amyloid plaques. Limitations of the ^{11}C-labeld tracer due to its availability and short half-life were overcome with the development of ^{18}F-labeled amyloid tracers, including ^{18}F-Florbetapir. ^{18}F-labeled tracers show comparable binding properties and clinical performance to ^{11}C-PiB and therefore, differences cannot be mainly explained by the use of different amyloid tracers (Johnson et al., 2013; Barthel and Sabri, 2017; Villemagne et al., 2017). In the study by Cirrito et al. (2011), all participants that took SSRIs had a history of depression while our cohort also included patients that used SSRI due to other reasons than depression which might be the main reason of discrepant results. However, after separating participants with and without history of depression, there were no differences between SSRI+ and SSRI− subjects in our cohort.

We could show that the duration of SSRI treatment correlated inversely with amyloid burden in both, cognitively normal and cognitively impaired patients, consistent with the findings of Cirrito et al. (2011). The time of onset and duration of exposure to SSRIs seems to be a crucial factor as SSRI-treatment might have preventive properties reducing Aβ accumulation. Hypothesizing that SSRIs influence Aβ metabolism promoting the non-amylogenic APP processing pathway leading to a lower amount of soluble neurotoxic Aβ forms and lower amyloid plaque burden, only long-term treatment might contain AD pathologies in a protective way. Furthermore, the development of AD pathologies takes many years and only short-term treatment might not be sufficient in order to reduce Aβ accumulation. This theory is supported by the results of a large Danish population-based study in which patients who received only one prescription of SSRI antidepressants had an increased rate of dementia compared to subjects unexposed to antidepressants, while continued long-term antidepressant treatment (six to nine prescriptions) was associated with a reduced rate of dementia (Kessing et al., 2009). Additional factors that might be modulated by long term SSRI-treatment include depressive symptoms, stress and neuroinflammation with a possible beneficial effect on cognition.

Preclinical studies indicate that the decrease of Abeta levels after SSRI treatment is dose-dependent (Cirrito et al., 2011; Sheline et al., 2014). A dose-dependent decrease of Abeta levels was first demonstrated by Cirrito et al. (2011) as they showed a reduction of ISF Aβ-levels by 12–16% using 5 mg/kg of citalopram while 10 mg/kg reduced Aβ-levels by 24%. However, in our cohort, we could not detect a dose-effect on the cerebral amyloid load in ^{18}F-Florbetapir-PET. One possible explanation of theses divergences might be an inter-individual variation in the efficacy of SSRI-treatment. Thus, the dose of SSRI treatment may vary greatly between individual patients and bias a grouped analysis.

It has previously been shown that the ApoE ε4 allele influences the response to different antidepressants in geriatric depression. Murphy et al. (2003) showed that patients carrying the ε4 allele showed a rapid onset of the noradrenergic and specific serotonergic antidepressant mirtazapine action, whereas paroxetine-treated patients with the ε4 allele were slow to respond (Murphy et al., 2003). However, ApoE genotype had no effect on the treatment outcome with SSRIs in the current study. Although, ApoE4 carriers showed significantly higher SUVR compared to non-carriers in cognitively impaired patients as well as in cognitively normal subjects.

Limitations of this study include the retrospective setting. Especially inconsistencies in SSRI-treatment protocols (doses, type of SSRI, duration of treatment) have to be considered as an important limitation. Furthermore, more detailed information on the number and extent of depressive episodes were not available in the dataset. In addition, inconsistencies between the groups regarding the history of depression might have led to a possible bias. Another limitation is the limited number of patients in some of the studied sub-groups.

Overall, the effect of SSRI-treatment on AD-pathologies in humans remains controversial. While preclinical studies and first clinical data showed promising results, a beneficial effect neither on amyloid load nor cognition could be confirmed so far.

CONCLUSION

The effect of SSRI-treatment on AD-pathologies remains unclear. However, preclinical data and the negative correlation between the time of SSRI treatment and amyloid load continue to suggest a possible beneficial effect of SRRI-treatment on the pathogenesis of AD. A controlled randomized prospective study on the effect of SSRIs on AD-pathologies is necessary in order to overcome limitations of previous studies evaluating treatment effects in a dose- and time-controlled manner. Furthermore, more information of the mechanism of action of SSRI-treatment are needed, including secondary and tertiary preventive properties, e.g., possible modulation of depressive symptoms and stress of SSRIs in the development of AD.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

All ADNI participants provided informed written consent, which was approved by each site's Institutional Review Board. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

CB and YB designed the project, analyzed the data, and wrote the manuscript. Both authors contributed to revising the manuscript and approved the final version.

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Psychobiological Evaluation of Day Clinic Treatment for People Living With Dementia – Feasibility and Pilot Analyses

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Background: Hospitalization is often stressful and burdensome for people living with dementia (PwD) and their informal caregivers (ICs). Day clinic treatment may provide a suitable alternative, but is often precluded by a diagnosis of dementia. Furthermore, it is often caregiver-based ratings that measure treatment success as the validity of self-reports in PwD is critically discussed. We therefore set out to examine the feasibility of psychobiological stress measures in PwD and ICs and to evaluate treatment trajectories considering both the day clinic context and the daily life of the dyads.

Method: A total of 40 dyads of PwD (mean age: 78.15 ± 6.80) and their ICs (mean age: 63.85 ± 13.09) completed paper-and-pencil questionnaires (covering stress, depressive symptoms, and caregiver burden among others) in addition to the measurement of hair cortisol concentrations (HCC) at admission, discharge, and follow-up 6 months after day clinic treatment. As part of an ambulatory assessment, for 2 days at the beginning and 2 days at the end of the day clinic treatment, PwD and ICs collected six saliva samples per day for the analysis of salivary cortisol (sCort) and alpha-amylase (sAA).

Results: Paper-and-pencil questionnaires and HCC assessments were more feasible than the ambulatory assessment. We found discrepancies between subjective and physiological markers of stress in PwD. Whereas HCC decreased over time, self-reported stress increased. Child–parent dyads reported decreases in neuropsychiatric symptoms, associated burden, and self-reported stress from admission to follow-up. In daily life, both PwD and ICs showed characteristic diurnal profiles of sAA and sCort, however, we found no differences in summary indicators of salivary stress markers over time.

Discussion: The psychobiological evaluation was feasible and added informative value, underlining the potential of physiological stress markers to complement self-reports

on stress in PwD and to objectively evaluate treatment trajectories. In this sample, HCC was more feasible and acceptable as biological marker of stress compared to saliva samples. Concerning treatment trajectories, differential effects on the dyads were found, with child–parent dyads benefiting more from day clinic treatment compared to spousal dyads.

Keywords: aging, autonomic nervous system, HPA axis, neurodegeneration, psychophysiology, psychiatric day unit, geriatric psychiatry, memory clinic

INTRODUCTION

Stress in People Living With Dementia and Their Informal Caregivers

Stress, and particularly chronic stress, has harmful effects on people living with dementia (PwD) and their informal caregivers (ICs). Adverse health effects are reported in both, with chronic stress leading to faster disease progression in PwD (Csernansky et al., 2006) and diminished health in their ICs (Bauer et al., 2000). From a dyadic perspective, however, stress not only affects the individual, but rather affects both members of the dyad (Wuttke-Linnemann et al., 2019). For PwD and their ICs, this dyadic interplay can result in toxic exacerbations and escalations: A common source of stress for ICs is the presentation of behavioral and psychological symptoms of dementia (BPSD) in PwD (e.g., hallucinations, delusions, sleep disturbances, and aggression), which challenge ICs' resources (Feast et al., 2016). Often, ICs do not have adequate means to meet the needs underlying the BPSD, resulting in further manifestations of and deteriorations in BPSD, and in turn leading to additional stress in ICs. Thus, PwD and ICs are trapped in a vicious cycle that frequently leads to poor outcomes for both parties: Caregivers often present with fatigue and exhaustion, reduced quality of life, increased depression, poorer health, and lower income (Kales et al., 2015; Feast et al., 2016); PwD experience higher rates of emergency hospital admissions and disruptions in care, early admission to nursing homes, faster disease progression, and increased morbidity and mortality (Kales et al., 2015).

(Emergency) Hospital Admissions and Disruptions in Care

PwD are more often admitted to hospital than age-matched patients without dementia (Joyce et al., 2007; Frytak et al., 2008), and BPSD, associated with high caregiving burden, place PwD at a particularly increased risk of hospitalization (Toot et al., 2013). However, these hospital admissions have a series of adverse health outcomes. Compared to older adults without dementia, hospitalization of PwD is accompanied by increased rates of complications, including a higher prevalence of delirium, dehydration, and pain, worsened cognitive and physical status, increased behavioral and psychological symptoms, as well as increased morbidity (e.g., urinary tract infections, decubitus, pneumonia, metabolic imbalance, sepsis, heart failure, myocardial infarction, anemia, complications after surgery, and thrombotic events) and mortality (Morrison and Siu, 2000; Robinson et al., 2009; Sampson et al., 2009; Ehlenbach et al., 2010; Wilson et al., 2012; Bail et al., 2013, 2015; Rao et al., 2016; Hessler et al., 2018). Moreover, PwD have longer hospital stays (Zekry et al., 2009; Mukadam and Sampson, 2011; Motzek et al., 2017), more readmissions, and more transfers to nursing home care (Draper et al., 2011). Nevertheless, about a quarter of hospital admissions of cognitively impaired older adults are caused by ambulatory care-sensitive conditions (Wolf et al., 2019), which are primarily treatable on an ambulatory basis. This implies that improved ambulatory care might reduce the frequency of hospitalizations, which is of particular importance in cognitively impaired older persons.

Day Clinic Treatment as an Alternative

Hospital treatment should be adapted to the needs of PwD in order to reduce the negative consequences of a hospital stay. Day clinic treatment might therefore present a possible alternative to maintain as much daily routine for the PwD as possible while allowing for as much recovery as possible for the ICs. Although day clinic treatment for psycho-geriatric patients in Germany began in 1976 (Wächtler et al., 1994), it still does not play a significant role in the health care system for these patients in Germany. So far, the number of empirical studies on psychiatric day clinics for PwD remains limited.

There is empirical evidence that interprofessional specific programs for PwD in a day clinic lead to a clear improvement in behavioral symptoms and positively influence the distress of caring relatives (Johansson and Gustafson, 1996; Hoe et al., 2005; Weber et al., 2009; Wunner et al., 2020). Furthermore, day clinic treatment of PwD was found to reduce the 1-year hospital readmission risk, as compared to an increased risk among inpatients (Steinkamp and Werner, 1998; van de Vorst et al., 2018). In a previous study by our work group, we compared sociodemographic and clinical characteristics of voluntarily treated PwD and their ICs between an inpatient setting and a day clinic setting (Linnemann et al., 2018). PwD did not substantially differ in these characteristics between the two settings and the treatment effects were similar. However, concerning ICs, there were significant differences between the two settings. ICs of day clinic patients were significantly older, showed a higher burden due to practical caring responsibilities, lower physical health, and a higher rate of depressive syndromes at follow-up compared to caregivers of inpatients.

While the aforementioned study provided evidence that day clinic treatment is feasible in PwD, it did not address specific research questions. First, all assessment instruments were based on self-report for ICs and on informant-based ratings for PwD. Thus, the perspective of the PwD was not directly included.

Second, although stress often endangers the stability of care, leading to hospital admissions, the study did not assess stress *per se*.

Psychobiological Stress Markers in People Living With Dementia – Need to Complement Subjective Reports With Physiological Markers

The assessment of physiological stress markers allows complementing the subjective perspective of PwD (Wuttke-Linnemann et al., 2019, 2022). Particularly given the questionable validity of self-reports in PwD that may arise due to cognitive impairments resulting in anosognosia (Wilson et al., 2016), physiological stress markers might enable the psychobiological stress experience to be captured more comprehensively. In this regard, we recently found discrepancies between subjective and physiological markers of stress in PwD when evaluating the treatment success of a dyadic home-based psychosocial intervention. Whereas subjective stress did not decrease over time, PwD reported lower secretion of cortisol after each home visit (Wuttke-Linnemann et al., 2022).

While elevated levels of the stress hormone cortisol have been identified as risk factor for the development of dementia (Ouanes and Popp, 2019) and for a faster disease progression in PwD (Csernansky et al., 2006), physiological stress markers are seldom used to evaluate treatment effects in PwD. However, this would be particularly relevant in PwD as the hippocampus is sensitive to chronic stress and glucocorticoids (Conrad, 2008) facilitating further neurodegeneration. Chronic stress has deleterious and neurotoxic effects on the brain with dysregulations in glucocorticoids increasing allostatic load (McEwen, 2001). These adverse health effects of stress on health are mediated by changes in the stress-sensitive systems of the body (McEwen, 1998). Chronic stress increases allostatic load mediated by dysfunctions and dysregulations in the hypothalamic-pituitary-adrenal axis and the autonomic nervous system. This has particular relevance as these stress-sensitive systems interact with the immune system and thus shape the organism's response to adversity in daily life. However, although the assessment of physiological stress markers holds the potential to inform diagnosis and treatment of PwD, they are seldomly used in PwD and their ICs.

In a feasibility study of salivary cortisol as objective measure for physiological stress in nursing home residents with dementia, Pu et al. (2020) report multiple challenges and high number of missing values most often due to cognitive impairments of the PwD. Hair cortisol might be a promising marker in this regard, as it can be assessed unintrusively and retrospectively captures long-term cortisol secretion. Particularly in older age, the potential of hair cortisol to assess cortisol as a risk factor and as a biomarker to evaluate the effectiveness of stress reduction interventions has been discussed (Wright et al., 2015). However, so far, physiological stress markers such as hair cortisol have most often been assessed in caregivers of PwD rather than directly in PwD (Stalder et al., 2014; Rippon et al., 2021). Empirical evidence on intervention effects as captured by physiological stress markers in PwD is in its beginning. The empirical evidence so far covers a wide and heterogenous range of interventions such as dyadic psychosocial intervention (Wuttke-Linnemann et al., 2022), hand massage (Schaub et al., 2018), acupressure (Kwan et al., 2017), touch (Woods et al., 2009), robot companions (Liang et al., 2017), art interventions (D'Cunha et al., 2019), music therapy (Chu et al., 2014; de la Rubia Ortí et al., 2018), dance therapy (Ho et al., 2020), or exercise (Venturelli et al., 2016). Most often salivary cortisol is assessed as outcome measure with only few studies that simultaneously assessed salivary cortisol and salivary alpha-amylase (Schaub et al., 2018; Wuttke-Linnemann et al., 2022). Methodological aspects vary among these studies considering the study population (severity of dementia), the setting of the study (hospital, nursing home, and home), the number of saliva samples obtained, sampling pattern and collection method, thus limiting comparisons across studies. Nevertheless, in the majority studies point to beneficial effects on salivary cortisol, although often challenges with collecting sufficient valid saliva samples are reported (Kwan et al., 2016). Beneficial effects present themselves by pre to post differences showing decreases in salivary cortisol after participation in a dyadic psychosocial intervention (Wuttke-Linnemann et al., 2022), acupressure (Schaub et al., 2018), hand massage (Kwan et al., 2017), and exercise- and cognitive-based treatments (Venturelli et al., 2016). Whereas one study finds decreases in salivary cortisol after music therapy (de la Rubia Ortí et al., 2018), another one did not (Chu et al., 2014). Also beneficial effects on diurnal rhythms are reported with increases in characteristic markers such as morning-to-evening ratio (D'Cunha et al., 2019) and slope (Ho et al., 2020) and decreases in total daily output as measures by area-under-the-curve (Wuttke-Linnemann et al., 2022).

Effects Beyond the Clinic Setting – Ambulatory Assessment to Capture Daily Life Experiences

A study by Fonareva et al. (2012) showed that stress ratings differed between research center and home environments, rendering it necessary to assess the effects of an intervention in daily life as well. However, whereas the research center environment has the advantage of monitoring the assessments more closely, control mechanisms cannot be implemented so easily in home environments. In particular, the collection of saliva samples might be less feasible at home than in a research center. Hodgson and Granger (2013) presented recommendations on how to assess saliva samples in older frail patients using the caregivers' assistance and encouraged to assess these markers in this vulnerable population.

Overall, the empirical evidence points to the dilemma that PwD are often admitted to hospitals even though hospitalization has adverse health effects on them. Alternatives such as day clinic treatment are available, but the evaluation of these alternatives is most often based on informant-based ratings rather than self-report. We therefore set out, to evaluate the feasibility of psychobiological stress markers in both PwD and ICs in the day clinic context as well the home environment by means of an ambulatory assessment approach. Furthermore, we then

evaluated the effectiveness of day clinic treatment and treatment trajectories concerning both PwD and ICs over time (admission, discharge, six months follow-up).

MATERIALS AND METHODS

Procedure

The study took place in a day clinic for PwD located in Munich, Germany. Prior to elective admission to the day clinic, all PwD and ICs were informed about the possibility to participate in a scientific evaluation of the day clinic treatment. Inclusion criteria for ICs were age ≥18 years, fluency in the German language, role as the primary informal caregiver, regular contact with the PwD (at least twice a week), and no cognitive impairment (MMSE ≥ 24). Inclusion criteria for PwD were fluency in the German language and a firm or suspected diagnosis of dementia. Concerning the assessment of salivary stress markers, further exclusion criteria were defined for both PwD and ICs: intake of any medication with an effect on the neuroendocrine system, chronic disease affecting the neuroendocrine system, psychiatric condition (substance dependence, psychosis), smoking, body mass index (BMI) ≥ 30, hair shorter than 1 cm. However, to encourage as many dyads as possible to participate in the study, the measurement of physiological stress markers (i.e., saliva and hair samples) was optional.

PwD and their ICs who expressed an interest in participating were informed about the study in a personal meeting, which was scheduled within the first 5 days after admission. It was stressed that participation was voluntary and would not affect treatment in the day clinic. Written informed consent was obtained from both PwD and ICs before participation. PwD with legal guardians were only included if they had basic cognitive capacity and if implicit intentional behavior to participate in the study was shown.

After inclusion in the study, baseline sociodemographic variables were collected by the study personnel, who also performed the Mini-Mental State Examination (MMSE; Folstein et al., 1983) with the PwD. Next, a hair sample was taken from both dyad members by trained study personnel. PwD and ICs were then asked to complete questionnaires in the subsequent 5 days. If PwD did not have the mental capacity to complete the questionnaires, study personnel were available to provide support. We specifically asked caregivers to refrain from assistance in order to prevent biases. As part of the ambulatory assessment, on the 2 days after study inclusion, subjective stress ratings were gathered and saliva sampling took place, consisting of six daily assessments (awakening, 30 min after awakening, 10 am, 2 pm, 6 pm, and 9 pm) for the analysis of salivary cortisol (sCort) and alpha-amylase (sAA). Directly before discharge, PwD and ICs were asked to complete questionnaires again and complete ambulatory assessment on the 2 days before discharge from the day clinic. Hair samples were retaken at discharge. After 6 months, paper-and-pencil questionnaires were sent out by mail, and both PwD and ICs were invited to an outpatient session at the clinic, where a third hair sample was taken from both dyad members. The study procedure is illustrated in **Figure 1**. The ethics committees of the Landesärztekammer Bayern (as the day clinic was situated in Munich) and the Landesärztekammer Rheinland-Pfalz (as the evaluation was coordinated in Mainz) approved the study protocol.

Therapeutic Rationale of the Day Clinic

The day clinic offers 20 outpatient places for patients with memory disorders and dementia. Only voluntarily treated patients can be electively admitted, as there are no closed wards. Treatment and travel costs (transportation by a special driving service) are usually covered by health insurance companies. At night and on weekends, patients are at home. Support is offered in organizing assistance or planning a care concept for the time at night and weekends. Based on guideline-oriented diagnostics and treatment, a holistic approach to treatment is taken, focusing on the needs of the individual patient. In most cases, treatment is scheduled to last 4–6 weeks. During this time, the patients take part in various therapeutic offers from Monday to Friday, according to an individual therapy plan. Treatment is scheduled workdays from 8.30 am to 5.00 pm (Fridays 8.30 am to 4.00 pm). A variety of non-pharmacological treatments are offered, such as music therapy, occupational therapy, art therapy, movement therapy and psychotherapy among others. Additionally, the patients also receive an individually tailored drug treatment plan according to guidelines. Further, one focus of the day clinic is the inclusion of the ICs and the social environment in order to strengthen the sustainability of the therapy.

Assessment Instruments

Psychometric Test Battery

PwD and ICs completed the following paper-and-pencil scales at each assessment point (admission, discharge, follow-up):

To assess depressive symptoms, we used the short form of the Geriatric Depression Scale (GDS-15) (Sheikh and Yesavage, 1986). The GDS-15 is validated for older populations with an MMSE score of 10 or more (Conradsson et al., 2013) and is also regularly used for the assessment of depressive symptoms in caregivers of PwD (Covinsky et al., 2003). The scale comprises 15 items rated using a dichotomous response format (yes/no). After recoding five reverse-coded items, a sum score is calculated, with sum scores ≥5 indicating clinically relevant depressive symptoms.

Subjectively perceived stress levels were assessed using the Perceived Stress Scale (PSS-10, Cohen, 1988). Chwalisz and Kisler (1995) found the PSS to be particularly suitable in the context of caregiving compared to traditional measures on caregiver burden. Deeken et al. (2018) demonstrated good psychometric properties of the PSS in both PwD and ICs. Participants are asked to rate ten items on a scale ranging from 0 (never) to 4 (often) referring to the past 4 weeks. After recoding four reverse-coded items, a sum score is calculated, with higher scores representing higher stress.

We used the Screening Scale for Chronic Stress (SSCS) from the Trier Inventory for the Assessment of Chronic Stress (TICS) (Schulz et al., 2004). The SSCS is a summary scale based on the 12 items that loaded highest on the first factor in the validation of the TICS. It comprises items from five different types of

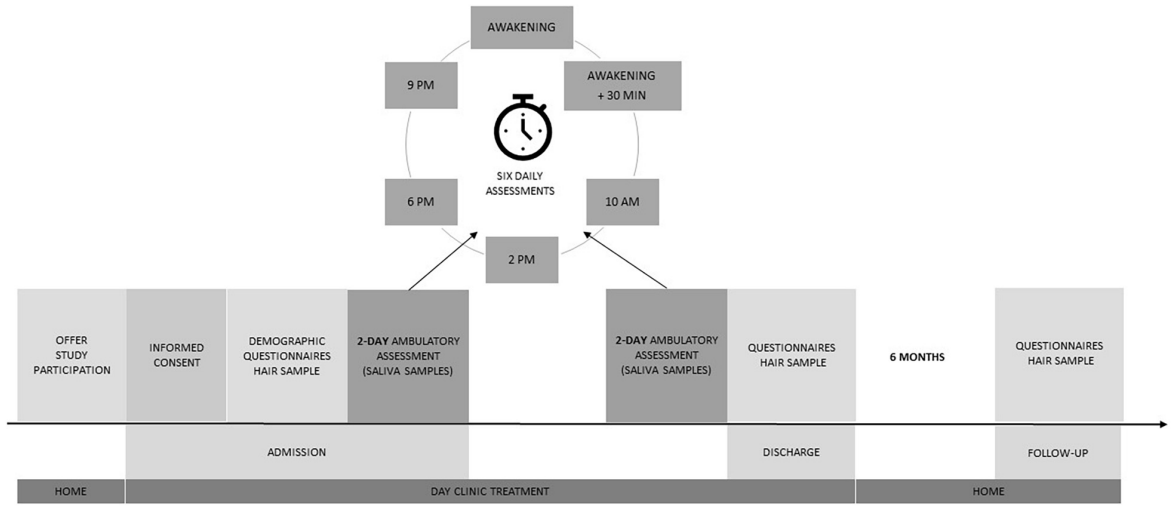

FIGURE 1 | Study procedure.

stress: sorrow, work overload, social stress, work discontent, and lack of social recognition. Higher sum scores indicate higher chronic stress.

The German version of the Brief Resilience Scale (BRS) was used to measure trait resilience (Chmitorz et al., 2018). The scale comprises six items rated on a scale ranging from 1 (strongly disagree) to 5 (strongly agree). After recoding reverse-coded items, a mean score is calculated.

Additionally, ICs were asked to rate BPSD and functional independence of the PwD at all three assessment points:

To assess behavioral symptoms of dementia, we used the 12-item version of the Neuropsychiatric Inventory (NPI) (Cummings et al., 1994). In this interview, caregivers are asked to rate the occurrence of 12 domains of behavioral symptoms (e.g., delusions, hallucinations, depression, anxiety, and sleep disturbances). If any of the domains occur, caregivers are asked to rate the frequency and severity of each symptom on a scale ranging from 1 to 4 for frequency (1 = occasionally, less than once per week to 4 = very frequently) and 1 to 3 for severity (1 = mild, 2 = moderate, 3 = severe). A total score is calculated as the sum of the product frequency*severity, which can vary between 0 and 144, with higher scores indicating higher frequency and severity of BPSD. We further included the Caregiver Distress Scale (Kaufer et al., 1998), on which caregivers are asked to rate their distress on a scale ranging from 0 (=no distress) to 5 (=very severe or extreme stress) for each confirmed domain. Summing this distress rating for each domain, one can calculate the NPI caregiver distress score, which can vary between 0 and 60.

The Bayer Activities of Daily Living Scale (B-ADL) is an informant-rated scale on impairments in activities of daily living in older people with cognitive impairments (Hindmarch et al., 1998). With a total of 25 items, informants are asked to rate the frequency of problems in everyday life functioning on a 10-point scale ranging from 1 (never) to 10 (always), with higher scores thus indicating higher impairments in activities of daily living.

The Barthel index is an informant-rated scale on independence in activities of daily living (Mahoney and Barthel, 1965), thus indicating the degree of care needs. A total of 10 items are rated, with higher scores indicating higher autonomy.

Physiological Stress Markers

Hair cortisol concentrations (HCC): One to three hair strands were cut as close as possible to the scalp from the posterior vertex region of the head by trained study personnel. For determination of HCC, the first proximal 2 cm segment was used which is thought to reflect the cumulative cortisol secretion of the past 2 months (Wennig, 2000). Hair wash and cortisol extraction procedures based on laboratory protocol by Stalder et al. (2012), with minor modifications. In brief, hair samples were washed twice in a glass vial for 3 min using 3 mL isopropanol. For cortisol extraction, 7.5 $\pm$ 0.5 mg whole, finely cut hair were incubated in 1.8 mL methanol in a glass vial. After incubation for 18 h at room temperature, 1.6 mL were transferred in another glass vial. Then, 1.6 mL of the supernatant was evaporated at 50°C until samples were completely dried. Finally, the samples were resuspended with 225 μL ultra-pure water and immediately vortexed for 20 s. For cortisol determination, a commercially available cortisol luminescence immunoassay was used (LIA; IBL International, a Tecan Group Company, Hamburg, Germany). Inter- and intra-assay coefficients of variation were 3.4 and 6.0%, respectively.

The ambulatory assessment combined momentary subjective stress ratings with the measurement of salivary cortisol (sCort) secretion and salivary alpha-amylase (sAA) activity at each time point. Momentary subjective stress was assessed using a one-item approach to keep participant burden to a minimum. Using printed questionnaires, participants were asked to rate the item 'At this moment, I feel stressed' on a visual analog scale (VAS) ranging from 0 ('not at all') to 100 ('completely'). Furthermore, at each of the time points, PwD and ICs were asked to transfer accumulated saliva into pre-labeled vials using SaliCaps (IBL International, a Tecan Group Company, Hamburg, Germany).

Participants were asked to store the saliva samples as cool as possible at home in their freezer and to bring them to the day clinic at their earliest convenience, whereupon the samples were frozen at −80°C. Additionally, participants were asked to refrain from eating, drinking (except for water or tea without sugar), or intensive physical activity for 1 h prior to sampling. As a compliance check, participants had to document whether they had eaten, drunk or engaged in intensive physical activity prior to saliva sampling. Two of the daily saliva samples of the PwD fell within the time of the day clinic stay (10 am and 2 pm). These two samples were thus collected by nursing staff of the day clinic and directly stored at −80°C. For the remaining saliva samples, ICs were asked to assist the PwD in collection, as recommended by Hodgson and Granger (2013). sCort levels were measured using a commercially available enzyme-linked immunoassay (IBL International, a Tecan Group Company, Hamburg, Germany). sAA activity was measured using a kinetic colorimetric test and reagents obtained from DiaSys Diagnostic Systems (Holzheim, Germany). Inter-assay variance was 13.3% for sAA and 12.6% for sCort, and intra-assay variance was 14.2% for sAA and 1.67% for sCort. Summary indices (area-under-the-curve with respect to ground, AUCg) were calculated according to the formula provided by Pruessner et al. (2003) including the six daily assessments at admission and discharge each. Cortisol awakening response (CAR) was calculated as percentage rise in sCort secretion from awakening to 30 min after awakening.

Statistical Analysis

Analyses were performed using SPSS (IBM SPSS Statistics for Windows, Version 23.0, IBM Corporation, Armonk, NY, United States). Concerning feasibility, we calculated the amount of missing values per parameter and report reasons for this if applicable. In addition, we tested whether the missing values in our data set were associated with specific characteristics of the sample. We created dummy variables with a 0 (value missing)/1 (value present) coding for HCC and saliva sample measures. We then calculated the correlation with potential influencing variables (Time, Person, Gender, Relationship, MMSE, NPI, and GDS) with these dummy variables. Additionally, we tested further for dependencies among these variables by means of a series of 2 × 2 chi-square tests (person × GDS/PSS/SCSS/BRS; sAA/sCort/CAR/HCC × GDS/PSS/SCSS/BRS; sAA × HCC) separately for each time point (baseline, discharge, and follow-up). Since very small cell abundances were expected in some comparisons, we report the results of the Fisher exact test. Concerning the evaluation of treatment trajectories over time, we estimated multilevel models (MLM) using the MIXED function. Construction of the models and subsequent interpretation was done in accordance with Bolger and Laurenceau (2013). In a stepwise procedure, we first created a base model that solely included the outcome variable (scores from paper-and-pencil questionnaires, HCC, ambulatory assessment data). As the intraclass correlation (ICC) (=the amount of variance between second-level units in relation to the total variance) was >0.20 for all base models, we estimated follow-up MLMs. In these MLMs, we first tested the model for a random effect of the variable Time. If the inclusion of the random effect (Time) did not improve model fit, all further models were calculated with fixed effects only. Concerning the outcome variables Bayer-ADL, NPI Burden and HCC, including Time as random effects variable improved model fit. These random effects models can be found in **Supplementary Material A** as we report fixed effects models focusing on between-subject differences here in the manuscript. We went on including further predictors into the model in a stepwise manner: 1st: Time, 2nd: Person (IC vs. PwD), 3rd: control variables (Gender; in the case of psychobiological outcome measures we further included age and BMI), 4th: Relationship (spousal dyad vs. child–parent dyad) as fixed effects variables due to known associations with the outcome measures. Comparison between the models and the base model was made by comparing model information criteria. Specifically, we interpreted the Bayesian Information Criterion (BIC), with lower figures representing better model fit. We stopped the inclusion of additional predictors when the model fit was worsened by the inclusion of additional predictors.

RESULTS

Participants

A total of 40 dyads of PwD and their IC participated in the study. PwD (17 female) were 78.15 ± 6.80 years old (range: 57–94) and ICs (31 female) were 63.85 ± 13.09 years old (range: 36–84). A total of 39 PwD had already a secure diagnosis of dementia at admission, whereas one patient was diagnosed with a suspected diagnosis of dementia that was confirmed during the day clinic stay. The majority of PwD was diagnosed with Alzheimer's disase (F00.1, $n = 22$; F00.2, $n = 8$; F00.0, $n = 1$) followed by unspecified dementia (F03, $n = 4$), dementia in other diseases classified elsewhere (F02.3, $n = 3$; F02.0, $n = 1$), and vascular dementia (F01.9, $n = 1$). Dyads were either married couples (59.2%) or child–parent constellations (40.8%). The mean MMSE sum score of PwD was 16.10 ± 6.57. PwD reported that they had suffered from cognitive symptoms for 44.62 ± 34.31 months, ranging from 1 to 132 months. At baseline, a total of 28 PwD had been assessed as requiring care (mean care level: 2.32 ± 1.06, range 0–5). ICs reported a mean of 54.93 ± 61.50 h per week spent on caregiving (range 2.50–168 h). A total of 15 out of 40 ICs (37.5%) reported using support services. Concerning the work situation of the ICs, 50.7% were retired, 39.1% were in employment, 7.2% stayed at home, and 2.9% were unemployed.

Feasibility Aspects – Missing Values and Completeness

Concerning the paper-and-pencil psychometric test battery, missing values of each test varied between 0 and 12 at baseline resulting in completion rates of 85.00–100.00%. At discharge missing values varied between 6 and 19 per test (76.25–92.50% completion rate), and at follow-up between 6 and 23 (71.25–92.50% completion rate). Completeness of assessment varied between PwD and ICs, with the completeness of assessments of PwD varying between 22 and 40 PwD per test (55.00–100.00%) and completeness of ICs varying between 34 and 39 ICs per test (85.00–97.50%).

TABLE 1 | Results from psychometric test battery over time.

	PwD			IC		
	Admission	**Discharge**	**Follow-up**	**Admission**	**Discharge**	**Follow-up**
	***X* ± *SD* (*n*)**	***X* ± *SD* (*n*)**	***X* ± *SD* (*n*)**	***X* ± *SD* (*n*)**	***X* ± *SD* (*n*)**	***X* ± *SD* (*n*)**
GDS-15	4.80 ± 3.42 (40)	3.68 ± 3.56 (34)	5.92 ± 3.87 (25)	3.85 ± 2.83 (34)	4.29 ± 3.07 (35)	4.35 ± 3.33 (37)
PSS-10	15.27 ± 6.96 (37)	14.12 ± 6.26 (33)	19.41 ± 7.11 (22)	19.10 ± 6.93 (39)	17.91 ± 6.88 (35)	17.26 ± 7.88 (35)
SSCS	10.82 ± 9.57 (33)	8.48 ± 7.11 (27)	16.40 ± 10.18 (20)	22.63 ± 10.24 (38)	20.21 ± 9.46 (34)	20.65 ± 10.04 (37)
BRS	3.63 ± 0.67 (33)	3.51 ± 0.68 (32)	2.93 ± 0.72 (20)	3.23 ± 0.81 (39)	3.31 ± 0.80 (36)	3.28 ± 0.75 (37)
Bayer ADL	8.11 ± 1.76 (39)	7.59 ± 2.21 (36)	8.30 ± 2.00 (37)	–	–	–
Barthel Index	74.36 ± 22.04 (39)	75.14 ± 21.33 (35)	67.92 ± 23.92 (36)	–	–	–
NPI sum	29.05 ± 18.14 (40)	23.60 ± 15.77 (37)	26.60 ± 18.48 (37)	–	–	–
NPI burden	–	–	–	16.21 ± 9.96 (34)	14.00 ± 10.00 (32)	16.90 ± 12.84 (30)

PwD, people living with dementia; IC, informal caregiver; GDS-15, Geriatric Depression Scale; PSS-10, Perceived Stress Scale; SSCS, Screening Scale for Chronic Stress taken from the Trier Inventory for the Assessment of Chronic Stress; BRS, Brief Resilience Scale; ADL, activities of daily living; NPI, neuropsychiatric inventory; X, mean; SD, standard deviation; n, sample size.

With regard to HCC, a total of nine participants (six PwD, three ICs) did not provide hair samples at any of the three time points (11.25%). Another individual 19 hair samples could not be collected. The reason for this was most frequently insufficient amount of hair or lack of possibility to be present at the on-site appointment. No participant reported reservations or objections. Thus, a total of 194 hair samples was sent to the laboratory for analysis. There, a further 17 samples were excluded [too short hair (<2 cm, $n = 12$), insufficient amount of collected hair ($n = 4$), one outlier (>3 SD) in HCC (216.07 pg/mg)]. Thus, a total of 177 (out of 240 possible) hair samples were available for analysis, equaling 73.75% of overall completion. Sixty-four samples were entered into the analysis at baseline, 58 at discharge, and 55 at follow-up. Considering the sub-sample that provided hair samples, completion rates varied between 77.46 and 90.14% at each assessment point. However, only 16 (out of 40) dyads provided hair samples at all three assessment points. Missing values in HCC were correlated with GDS-15 sum score in that higher depression scores were related to missing values ($r = -0.195$, $p = 0.003$).

In terms of the ambulatory assessment, five dyads, three individual ICs, and three individual PwD did not collect saliva samples at all, resulting in the exclusion of 16 out of 80 participants (20.00%) in the respective analyses. Furthermore, three dyads and one individual IC only provided saliva samples at the beginning of day clinic treatment (but not at the end). Thus from a potential of 1,920 saliva samples, only 1,452 saliva samples were possible. Considering this sub-sample that provided saliva samples, a total of 1,139 cortisol values (78.44% completeness) and 1,086 alpha-amylase values (74.79% completeness) were entered into the analysis. Reasons for missing values were either that they could not be collected in daily life (interference with current activity) or that they were excluded in the lab due to insufficient amount of saliva. A total of 9 PwD was not able to collect saliva samples at home and thus only provided those saliva samples that were collected by the day clinic personal during the day clinic stay. Subjective stress ratings were available in 948 cases (65.29% completeness). The area-under-the curve (AUC), as a summary indicator, could only be calculated in the case of six complete assessments per day. This occurred for 114 days concerning VAS, 103 days concerning sCort, and 91 days concerning sAA (out of 256 possible days). Correlation analyses show that missing values for CAR, sCort and sAA occurred more often in PwD ($p < 0.01$), more often at discharge ($p < 0.01$), and were more often in child–parent dyads than spousal dyads ($p < 0.01$). Further, the amount of missing values increase with decreasing MMSE ($p < 0.01$) and increasing GDS-15 sum sore ($p < 0.05$). Results of the correlation analyses can be found in **Supplementary Material B**. The results of the chi-square tests indicate that there are statistical dependencies between the missing values of the questionnaires and the person (more frequent in PwD) especially at later time points and between missing values of the questionnaires and missing values in the biomarkers also at later time points (**Supplementary Material C**).

Feasibility Aspects – Reliability of Paper-and-Pencil Questionnaires

Indicators of reliability concerning the paper-and-pencil questionnaires were at least good in both PwD and ICs. The reliability of GDS-15 was even higher in PwD ($\alpha = 0.84$, $\omega = 0.84$) than in ICs ($\alpha = 0.79$, $\omega = 0.79$). Concerning the PSS, reliability was high in both PwD ($\alpha = 0.79$ and $\omega = 0.80$) and ICs ($\alpha = 0.87$ and $\omega = 0.87$), as compared to $\alpha = 0.84$ in the German validation study (Klein et al., 2016). It is of note, that in the present sample, the mean sum score of PSS was 18.13 ± 7.2 in ICs and 15.85 ± 6.99 in PwD compared to normative data from a German validation study in which the mean score on the PSS in the subgroup of participants aged ≥60 years lay at 11.94 ± 6.14 (compared to $\bar{x} = 12.57$ in the total sample). Concerning chronic stress levels, reliability was high in ICs ($\alpha = 0.93$ and $\omega = 0.94$) and in PwD ($\alpha = 0.91$ and $\omega = 0.91$), as compared to $\alpha = 0.91$ in the TICS validation study. At baseline, PwD had a mean score of 10.82 ± 9.57 and IC had a mean score of 22.63 ± 10.24, as compared to 14.37 ± 8.22 in the TICS validation study. Concerning BRS, reliability was higher in ICs ($\alpha = 0.82$; $\omega = 0.83$) than in PwD ($\alpha = 0.64$, $\omega = 0.65$), as compared to $\alpha = 0.85$ in the validation study. In the present study, the mean BRS value at baseline was 3.23 ± 0.81 in ICs and 3.63 ± 0.67 in PwD. In a population-based validation study, mean values for the

TABLE 2 | Results of linear mixed models predicting changes in psychometric test battery depending on time (admission, discharge, and follow-up), person (PwD and IC), and relationship (spousal dyad and child–parent dyad).

	GDS-15	PSS-10	SSCS	BRS	Bayer ADL	Barthel-Index	NPI sum	NPI burden
	Estimate (*SE*), *t*	Estimate (*SE*), *t*	Estimate (*SE*), *t*	Estimate (*SE*), *t*	Estimate (*SE*), *t*	Estimate (*SE*), *t*	Estimate (*SE*), *t*	Estimate (*SE*), *t*
Base model								
BIC	1029.786	1320.499	1337.736	416.235	826.643	1877.921	1896.713	1401.411
Intercept	4.51 (0.34), 13.464***	16.99 (0.69), 24.667***	16.85 (1.17), 14.456***	3.35 (0.07), 45.013***	8.01 (0.20), 39.823***	71.16 (2.37), 29.986***	26.74 (1.67), 16.046***	15.52 (1.09), 14.300***
Full model								
BIC	971.693	1234.869	1234.055	400.832	809.334	1782.340	1803.133	1336.187
Intercept	4.29 (1.13), 3.797***	17.18 (2.37), 7.250***	20.41 (3.52), 5.802***	2.90 (0.25), 11.555***	8.05 (0.36), 22.198***	−74.52 (4.76), 15.657***	22.02 (3.25), 6.770***	15.17 (1.93), 7.876***
Time	0.48 (0.37), 1.287	0.46 (0.95),0.490	−0.28 (1.19), −0.238	0.13 (0.10), 1.251	0.06 (0.13),0.443	−3.02 (1.70), −1.776	1.86 (1.40), 1.324	0.98 (0.91), 1.077
Gender	0.45 (1.27),0.353	2.15 (2.67),0.805	1.42 (3.97),0.359	0.28 (0.28),0.982	0.00 (0.51),0.001	1.79 (6.65),0.269	−0.24 (4.52), −0.053	−0.36 (2.71), −0.133
Person	−0.07 (1.25), −0.057	−3.49 (2.64), −1.323	−8.48 (3.97), −2.133*	0.49 (0.28), 1.749	–	–	–	–
Relationship	−2.35 (1.55), −1.519	0.91 (3.31),0.276	2.09 (4.99),0.418	0.80 (0.35), 2.242*	−1.34 (0.71), −1.887	9.68 (8.99), 1.077	4.96 (6.07),0.818	−4.84 (3.64), −1.329
Time*Gender	−0.28 (0.39), −0.721	−0.38 (1.01), −0.374	0.85 (1.26),0.678	−0.09 (0.11), −0.791	−0.00 (0.17), −0.008	−1.83 (2.15), −0.854	0.25 (1.78),0.139	0.77 (1.17),0.657
Gender*Relationship	1.28 (1.53),0.836	−2.25 (3.26), −0.690	−1.58 (4.98), −0.318	−0.46 (0.35), −1.300	1.16 (0.87), 1.335	−12.62 (10.77), −1.172	10.27 (7.19), 1.428	5.90 (4.48), 1.318
Time*Relationship	−0.46 (0.36), −1.280	−2.29 (0.91), −2.503*	−2.97 (1.12), −2.642**	−0.14 (0.10), −1.440	0.03 (0.17),0.154	−0.45 (2.11), −0.215	−7.87 (1.77), −4.460***	−3.19 (1.22), −2.609*
Time*Person	0.54 (0.35), 1.535	2.55 (0.93), 2.726**	2.98 (1.19), 2.504*	−0.32 (0.10), −3.126**	–	–	–	–
Gender*Person	−3.80 (1.50), −2.536*	−5.51 (3.17), −1.738	−7.39 (4.88), −1.512	0.54 (0.34), 1.570	–	–	–	–
Relationship*Person	5.24 (1.46), 3.600***	6.41 (3.09), 2.074*	2.53 (4.76),0.531	−0.72 (0.34), −2.133*	–	–	–	–

*BIC, Bayesian Information Criterion; PwD, people living with dementia; IC, informal caregiver; GDS-15, Geriatric Depression Scale; PSS-10, Perceived Stress Scale; BRS, Brief Resilience Scale; ADL, activities of daily living; NPI, neuropsychiatric inventory; Gender: 0 male, 1 female; Person: 0 IC, 1 PwD; Relationship: 0 spousal dyad, 1 child–parent dyad; SE, standard error; t, t-value; ***p < 0.001, **p < 0.01, *p < 0.05, fixed effects are reported here.*

BRS in two general population samples with a mean age of 42.56 ± 26.52 and 51.05 ± 17.90 were 3.58 and 3.37 (Chmitorz et al., 2018). In a study examining effects of a home-based dyadic psychosocial interventions of PwD and ICs, we found mean BRS values at baseline of 3.13 ± 0.78 for ICs and 3.00 ± 0.38 for PwD (Wuttke-Linnemann et al., 2020).

Clinical Characteristics Over Time – Subjective Ratings

The mean duration of stay in the day clinic was 33.13 ± 10.53 days (including weekends). The mean values of the psychometric test battery and physiological stress markers over time can be found in **Table 1**.

Using MLM, we tested whether the scores on each of the psychometric test battery changed over time depending on the person (IC vs. PwD) and relationship (spousal vs. child–parent dyad) while controlling for gender. However, we found no significant main effect of Time in any of the models. Only in the models concerning the SSCS and BRS did a significant main effect of the Person (SSCS) and Relationship (BRS) emerge. These main effects can be interpreted such that PwD reported lower SSCS sum scores than did ICs, and that child–parent dyads reported higher resilience than did spousal dyads. All results can be found in **Table 2**.

Furthermore, a series of interaction terms were significant. The Time*Relationship interaction was significant regarding the NPI sum score, NPI burden, the PSS-10, and the SSCS. Graphical illustration of these interactions show an effect of Time for ICs of child–parent dyads, with the sum scores on all of these scales decreasing from admission to follow-up, representing decreases in BPSD and associated burden as well as reduced subjective stress levels. Furthermore, significant Time*Person interactions were found concerning the PSS-10, SSCS, and BRS in that that perceived stress levels and chronic stress increased in PwD over time while resilience decreased. These figures remained stable from admission to discharge, and changed particularly from discharge to follow-up. Furthermore, the interaction term Relationship*Person was significant concerning the GDS-15, PSS-10, and BRS, showing that PwD in child–parent dyads had on average higher depression scores, higher perceived stress, and lower resilience compared to PwD in spousal dyads.

In sum, when evaluating the treatment trajectories based on subjective data there were no significant changes over time in

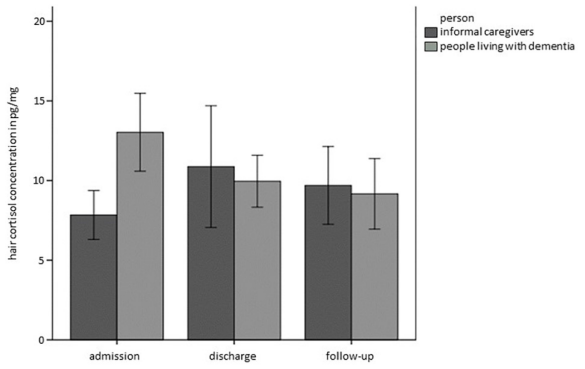

FIGURE 2 | Hair cortisol concentrations (HCC) over time separately for PwD and ICs. Error bars represent standard error of the mean.

scores concerning the psychometric test battery in the total sample. However, there were differential effects as PwD reported lower chronic stress than ICs while perceived stress levels and chronic stress increased in PwD and resilience decreased over time. Further, there were significant differences between child–parent dyads and couples: child–parent dyads reported higher resilience than couple dyads. Furthermore, for child–parent dyads decreases in behavioral symptoms of dementia and associated burden and reduced subjective stress levels were found from admission to follow-up. At the same time, PwD in child–parent dyads had overall higher depression scores, higher perceived stress, and lower resilience.

A graphical illustration of all significant interaction terms can be found in **Supplementary Material D**.

Physiological Stress Markers Over Time – Hair Cortisol Concentrations and Ambulatory Assessment Data

Concerning HCC, the full model showed a better fit than the base model (BIC 1406,537$_{basemodel}$ vs. BIC$_{fullmodel}$ 1167,034). Whereas there was no main effect of Time, Relationship or Person, there was a significant interaction of Time*Person (*Estimate* = −6.34, $SE = 2.83$, $t = -2.241$, $p < 0.05$), as graphically depicted in **Figure 2**. This interaction term can be interpreted such that PwD showed decreases in HCC over time, while there were no significant differences in HCC over time in ICs.

Data from the ambulatory assessment are descriptively presented in **Table 3**. A visual inspection of the data shows normal diurnal rhythms of IC and PwD regarding cortisol secretion as both show typical cortisol awakening response (CAR) at the beginning and end of day clinic treatment. ICs further showed a typical awakening response in alpha-amylase at both time points. In contrast, PwD only showed the typical decline in sAA after awakening only at the beginning of day clinic treatment, but not at discharge from the day clinic.

The models of the MLMs predicting differences in summary indicators concerning alpha-amylase did not deliver results as the models had too many missing values. Models regarding summary indicators of cortisol secretion (CAR and AUCg) and momentary subjective stress (VAS) did not yield any significant differences. The complete statistics concerning these models can be found in the **Supplementary Material E**.

In sum, complementing the findings on subjective ratings with physiological stress markers, discrepancies between subjective and physiological markers of stress emerged in PwD: we found significant differences in HCC, with PwD showing decreases in HCC over time while reporting subjectively increased chronic stress over time. From a descriptive perspective, moreover, HCC at baseline was higher in PwD than in ICs, although PwD subjectively reported lower chronic stress than did ICs. Data from the ambulatory assessment revealed characteristic diurnal profiles for both PwD and ICs, however, there were no significant differences in summary indicators over time.

DISCUSSION

Summary

We set out to evaluate the feasibility of a psychobiological evaluation of day clinic treatment in PwD and their ICs to evaluate treatment trajectories considering both the day clinic context and the daily life of the dyads. The feasibility of psychobiological measures varied between the different outcome variables. Whereas all participants were willing to provide subjective reports, not all participants were willing or had the possibility to collect physiological stress markers. Concerning HCC, 11.25% of the sample did not provide hair samples and concerning saliva samples as part of the ambulatory assessment, 20.00% of the sample did not provide saliva samples at all. However, when considering the sub-samples that provided physiological stress markers, completion rates were comparable between paper-and-pencil questionnaires (71–100%) and HCC (77–90%), but lower in saliva samples (75–78%) making HCC a particular promising physiological stress marker in PwD and IC. Validity of self-reports in PwD was high in for the GDS-15, PSS-10, and SSCS and only moderate in BRS.

Concerning treatment trajectories we found discrepancies between subjective and physiological markers of stress in PwD with decreases in HCC over time while reporting subjectively increased chronic stress over time. Although feasibility was high in subjective reports, PwD might use different time perspectives than described in the instruction of the respective scales. Despite the challenges in collecting physiological stress markers in this particular population, the discrepancy between subjective and physiological markers emphasizes the added value of assessing physiological markers of stress to complement the evaluation of PwDs' and ICs' stress experience. Thus, physiological stress markers hold the potential to inform diagnosis and treatment trajectories in both PwD and ICs.

Feasibility and Added Value of Psychobiological Stress Markers

The assessment of psychobiological stress markers in PwD was feasible. Although Arsenault-Lapierre et al. (2012) reported that anosognosia in PwD was correlated with anosognosia for perceived stress, the validity of self-reports was high for

TABLE 3 | Ambulatory assessment data.

	Awakening	+30 min	10 am	2 pm	6 pm	9 pm
	X ± *SD* (*n*)	*X* ± *SD* (*n*)	*X* ± *SD* (*n*)	*X* ± *SD* (*n*)	*X* ± *SD* (*n*)	*X* ± *SD* (*n*)
IC						
Admission						
VAS	21.74 ± 22.21 (54)	27.89 ± 26.64 (53)	25.56 ± 21.88 (46)	27.80 ± 27.16 (50)	28.86 ± 27.13 (54)	19.33 ± 24.21 (52)
sCort	0.29 ± 0.35 (62)	0.47 ± 0.30 (61)	0.16 ± 0.14 (58)	0.13 ± 0.12 (55)	0.07 ± 0.07 (59)	0.06 ± 0.06 (57)
sAA	217.30 ± 268-03 (54)	132.62 ± 151.66 (54)	224.62 ± 216.40 (59)	220.63 ± 202.08 (55)	249.85 ± 216.96 (58)	277.53 ± 254.74 (59)
Discharge						
VAS	18.83 ± 21.51 (41)	22.07 ± 21.85 (42)	30.11 ± 26.61 (39)	23.15 ± 23.34 (38)	27.70 ± 26.87 (38)	21.53 ± 22.27 (36)
sCort	0.26 ± 0.23 (48)	0.50 ± 0.32 (51)	0.16 ± 0.12 (51)	0.14 ± 0.18 (50)	0.07 ± 0.05 (48)	0.05 ± 0.04 (46)
sAA	299.03 ± 355.95 (43)	145.91 ± 206.05 (44)	213.28 ± 240.74 (47)	319.09 ± 273.46 (46)	274.33 ± 286.68 (47)	242.46 ± 208.73 (47)
PwD						
Admission						
VAS	16.68 ± 26.56 (28)	15.67 ± 15.73 (27)	14.69 ± 19.06 (48)	13.58 ± 19.93 (50)	18.04 ± 19.29 (28)	19.52 ± 22.10 (27)
sCort	0.47 ± 0.38 (34)	0.56 ± 0.36 (37)	0.22 ± 0.15 (55)	0.20 ± 0.20 (56)	0.18 ± 0.33 (36)	0.11 ± 0.12 (35)
sAA	172.63 ± 253.42 (31)	153.41 ± 218.70 (33)	208.39 ± 211.53 (58)	303.27 ± 291.94 (57)	260.45 ± 259.00 (36)	266.08 ± 285.92 (35)
Discharge						
VAS	14.65 ± 21.86 (26)	23.54 ± 25.24 (27)	18.37 ± 26.47 (47)	17.67 ± 25.83 (44)	21.18 ± 23.37 (28)	20.19 ± 25.40 (25)
sCort	0.33 ± 0.18 (31)	0.41 ± 0.24 (34)	0.21 ± 0.14 (55)	0.18 ± 0.17 (54)	0.09 ± 0.08 (33)	0.10 ± 0.08 (33)
sAA	134.63 ± 212.70 (27)	175.50 ± 261.22 (29)	252.56 ± 268.82 (54)	316.90 ± 345.33 (52)	255.97 ± 251.77 (30)	200.15 ± 199.09 (31)

PwD, people living with dementia; IC, informal caregiver; VAS, visual analog scale on subjective, momentary stress ranging from 0 (not at all) to 100 (completely); sCort, salivary cortisol secretion in μg/dl; sAA, salivary alpha-amylase activity in U/ml; X, mean; SD, standard deviation; n, sample size.

subjective stress measures in PwD. Nevertheless, it has to be critically discussed, that we used statistical measures to evaluate the validity of self-reports in PwD, whereas Arsenault-Lapierre et al. (2012) considered the discrepancy between self-report and caregiver-based ratings as a measure for anosognosia. This raises the question of whether it is possible to rate perceived stress levels externally. Regarding the validity of informant-based and self-reported ratings, Kaiser et al. (2022) found a high discrepancy between observer- and patient-reported outcomes when evaluating the success of depression treatment, thus emphasizing the need to directly include the perspective of the patient. As the potential to include the patient's perspective is particularly limited in the case of a dementia diagnosis, our data support the notion of adding physiological markers to complement the patient's perspective. Likewise, Arsenault-Lapierre et al. (2012) found no association between anosognosia and cortisol levels, leading to the assumption that physiological stress markers are particularly robust in PwD. In the present study, we also found a higher completion rate for HCC compared to the ambulatory assessment data, possibly because HCC was assessed at three time points by study personnel, in comparison to participants being required to collect saliva samples independently, or with the assistance of ICs, over 24 different points in time in daily life as part of the ambulatory assessment. Both the assistance of study personnel and the day clinic context might have reduced burden for the participant in comparison to the ambulatory assessment at home. This makes HCC a particular promising physiological stress marker in PwD, as it can complement subjective reports on chronic stress levels while keeping participant burden to a minimum. This converges with Wright et al. (2015) who describe the potential of HCC as retrospective biological marker of stress in older adults.

Although perceived momentary stress levels should be complemented by ambulatory assessment data, the high number of missing values in our study limits the informative value in this regard. Interestingly, completeness was higher in spousal dyads than child–parent dyads. From this, it might be recommended that PwD need assistance and support in collecting saliva samples, which is probably more likely to be the case with spouses than with children due to physical proximity. Another way to increase feasibility might be the use of electronic devices that emit prompts to remind participants to complete the assessments. However, we decided against the use of technical equipment in order not to overburden the participants. Interestingly, whereas almost 30% of the sample decided not to provide saliva samples, the remaining participants completed around 80% of saliva samples. This might be interpreted as suggesting that higher completeness and feasibility are associated with more selective study samples. Thus, one might need to weigh the specificity of a sample against its generalizability. Further, larger study samples are necessary to detect effects in daily life by means of ambulatory assessment data.

Comparing the feasibility of our psychobiological assessment to the existing literature, different settings and populations need to be kept in mind. Studies with high numbers of missing values were most often set in nursing homes addressing agitated residents with dementia: Pu et al. (2020) analyzed saliva samples from 8 out of 43 PwD, Kwan et al. (2016) analyzed 161 out of 360 saliva samples. Although these studies were set in a controlled setting where study personnel was available to assist in the collection of saliva samples, high amounts of missing values were found due to refusal of PwD to collect saliva samples, cognitive impairments, or inadequate saliva volume. In contrast, missing values in PwD from residential age care without agitation and home dwelling PwD were lower compared to agitated PwD in nursing homes, as D'Cunha (2019) was able to analyze saliva samples from 22 out of 25 PwD. These comparisons stress the fact that the feasibility of saliva samples is closely linked to the

severity of dementia. Likewise, in our study missing values in saliva samples increased with dementia severity. On the other hand, missing values in HCC were not related to disease severity but depressive symptoms. This emphasized the potential of HCC in patients with severe dementia to complement informant-based ratings with physiological data on stress markers.

In terms of content, we found further evidence for the added value of psychobiological markers in PwD. In a recent study by our work group (Wuttke-Linnemann et al., 2022), we also found discrepancies between subjective and physiological markers of stress concerning the evaluation of a home-based dyadic psychosocial intervention for patients with mild to moderate dementia and their ICs. The discrepancy between HCC and subjective stress measures found in this study supports the notion that physiological stress markers complement subjective measures in all stages of the disease. This is in line with Stalder et al. (2017) who reports no consistent correlations among HCC and subjective stress measures, specifically in chronically stressed populations. Various reasons can be assumed for this, and in particular in the context of dementia care, this discrepancy might have important implications. Based on our data, we cannot answer whether this means that the subjective data are less valid than assumed, but we can use disease-specific knowledge to generate hypotheses for future studies. In particular, HCC was higher in PwD than in ICs at admission to the clinic, even though PwD reported lower subjective chronic stress. This could be interpreted as suggesting that PwD tend to downplay their symptoms in order to avoid attracting attention, particularly in the context of a clinic admission, which brings about unwanted disruptions to one's daily routine, thus implying motivational reasons for this discrepancy. Another explanation might concern the cognitive deficits. As dementia involves memory impairments, it might be the case that PwD rate their momentary stress level as they cannot accurately remember how their stress level has been within the last 4 weeks. This would explain why validity was statistically high although PwD might have used a different time scale when answering the items on subjective chronic stress. Accordingly, this explanation would allude to cognitive deficits and reduced informative value of self-reports in PwD. Furthermore, HCC decreased over time in PwD despite the fact that subjective stress reports increased. Again, this might suggest that conceptually, self-reports do not match physiological stress markers in PwD very well, as they assess different stress constructs and different time scales.

Day Clinic Treatment Trajectories in People Living With Dementia and Informal Caregivers

Overall, the assessed variables remained stable over time, with the exception of subjective stress levels, which increased in PwD over time while resilience decreased. However, this does not imply that day clinic treatment does not work for PwD. Our previous study also found differences over time in dementia-specific assessment instruments (Linnemann et al., 2018) as well as no differences in treatment trajectories between day clinic and inpatient settings, rendering day clinic treatment a valid treatment alternative. In the context of a neurodegenerative disorder like dementia, stabilizing effects are considered to be worthwhile as well (Linnemann et al., 2018). Indeed, the finding that autonomy and BPSD did not worsen over a time period of 6 months can be seen as a success.

Nevertheless, in the present study, treatment trajectories differed according to dyad type, with child–parent dyads benefiting more from day clinic treatment than spousal dyads. In fact, child–parent dyads even showed a reduction in BPSD and associated burden and stress from admission to follow-up. Furthermore, PwD from child–parent dyads were more affected by depression and increased stress. This is reminiscent of the results of the aforementioned previous study (Linnemann et al., 2018), which reported that caregivers of PwD from a day clinic were older and thus more often represented spousal than child–parent dyads. The spousal caregivers were also more physically and psychologically impaired, both at baseline and at follow-up. Our results thus add the insight that day clinic treatment might be more feasible and effective in child–parent dyads than in spousal dyads. We can only speculate on the underlying reasons for this finding. One explanation might be that spousal dyads most often live together and share the majority of daily life. In line with this, Kürten et al. (2021) found that the time ICs need to supervise the PwD predicted depressiveness in ICs. This might explain the differences in treatment trajectories that we found between child–parent and spousal dyads, as spousal dyads may be required to spend more time on caregiver duties, if residing in the same household as the care recipient. Accordingly, spousal caregivers might thus experience day clinic treatment as less relieving compared to inpatient treatment, as the morning and evening/night-time hours still have to be covered by the ICs.

Limitations

Although the study protocol combined subjective and physiological markers of stress repeatedly over time in a difficult-to-reach vulnerable population, certain methodological issues warrant critical attention: First of all, the assessments at admission and discharge were scheduled in the days *after* admission and the days *prior* to discharge. Thus, we cannot draw any conclusions about how psychobiological measures might have varied *before* admission and *after* discharge. It is possible that the mere admission to the day clinic already relieved certain symptoms and that the assessment of our variables in the week before admission might have led to a different profile. Furthermore, as we collected data at discharge, it is possible that the imminent discharge brought about an exaggeration of possible effects of treatment. Moreover, we do not know how the transition back to the home environment affected the dyads. These are important research questions that should be addressed in future studies. In addition, the higher ecological validity of the ambulatory assessment data is accompanied by lower internal validity. Specifically, as the PwD needed assistance from their ICs when collecting the saliva samples, it remains unclear whether this affected the dyad. In particular, we do not know whether the ICs perceived assisting in collecting saliva samples from the PwD, while also collecting their own saliva samples, as stressful. Future investigations need to evaluate methods to perform

ambulatory assessment in PwD and their ICs in a non-intrusive manner. Furthermore, our study sample was selective, with an overrepresentation of female ICs, thus limiting conclusions on male ICs. However, this gender distribution is often found in studies, as ICs of PwD are predominantly female. Finally, the study was exploratory in nature, with no control group, thus preventing conclusions on causality. Future studies are necessary to compare characteristics, treatment trajectories, and treatment effects between day clinic and inpatient settings in a randomized controlled design.

Conclusion

The feasibility of the psychobiological evaluation of the day clinic treatment varied according to the different stress measures. The highest feasibility was found for subjective stress measures and hair cortisol concentrations. Despite cognitive deficits, subjective stress reports showed high validity although due to the memory deficits in PwD they may represent different time perspective than stated in the instruction. Ambulatory assessment data showed many missing values, with 20% of the sample unwilling or unable to collect saliva samples. This high number of missing values limits the informative value regarding effects in daily life. However, when dyads did decide to collect saliva samples, they showed high rates of completion. Thus, in selective samples the assessment of salivary stress measures was feasible, whereas in more heterogenous samples the collection of hair cortisol concentrations might be preferred as less active engagement of the participant is necessary. There was a discrepancy between subjective and physiological stress markers in PwD, emphasizing the fact that physiological markers complement subjective reports in a meaningful way. Treatment trajectories revealed stabilizing effects for PwD over time, but differed between spousal and child–parent dyads, with child–parent dyads generally appearing to benefit more from day clinic treatment compared to spousal dyads. Overall, the psychobiological evaluation of day clinic treatment was feasible for PwD and ICs, and future studies need to corroborate these findings in larger samples. Physiological stress markers, particularly hair cortisol, hold the potential to become an objective marker of stress that is relevant in diagnosis and treatment of dementia both from a preventive as well as disease modifying perspective.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committees of the Landesärztekammer Bayern (as the day clinic was situated in Munich) and the Landesärztekammer Rheinland-Pfalz (as the evaluation was coordinated in Mainz). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

AW-L and AF conceived the study and were in charge of overall direction, coordination, and planning. TB assisted in collecting the data and entered the data into a database. SP prepared the data for data analysis. SP and AW-L performed the data analysis. KE supervised the analysis of salivary markers. UN and NS supervised the analysis of hair cortisol samples. AW-L wrote the first draft of the manuscript with support from KG and SP. All authors provided critical feedback and helped to shape the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnagi.2022.866437/full#supplementary-material

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Sympathoexcitatory Responses to Isometric Handgrip Exercise Are Associated With White Matter Hyperintensities in Middle-Aged and Older Adults

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Vascular dysfunction may occur prior to declines in cognitive function and accumulation of neuropathology. White matter hyperintensities (WMH) develop due to cerebral ischemia and elevated blood pressure in midlife. The purpose of this study was to evaluate associations between cardiovascular and cerebrovascular responses to sympathoexcitatory stimuli and WMH burden in cognitively unimpaired middle-aged and older adults. Sixty-eight adults (age = 63 ± 4y, men = 20, women = 48) participated in this study. Participants completed isometric handgrip exercise (IHG) exercise at 40% of maximal voluntary contraction until fatigue followed by a 90s period of post-exercise ischemia. Heart rate (HR), mean arterial pressure (MAP), middle cerebral artery blood velocity (MCAv), and end-tidal CO_2 were continuously measured throughout the protocol. Cerebrovascular resistance index (CVRi) was calculated as MAP/MCAv. WMH lesion volume and intracranial volume (ICV) were measured using a FLAIR and T1 scan on a 3T MRI scanner, respectively. WMH fraction was calculated as (WMH lesion volume/ICV)*100 and cubic root transformed. Multiple linear regressions were used to determine the association between cardiovascular and cerebrovascular responses to IHG exercise and post-exercise ischemia and WMH fraction. Multiple linear regression models were adjusted for age, sex, apolipoprotein ε4 status, and total work performed during IHG exercise. During IHG exercise, there were significant increases from baseline in HR (25 ± 12%), MAP (27 ± 11%), MCAv (5 ± 10%), and CVRi (22 ± 17%; $P < 0.001$ for all). During post-exercise ischemia, HR (8 ± 7%), MAP (22 ± 9%), and CVRi (23 ± 16%) remained elevated ($P < 0.001$) while MCAv (0 ± 10%) was not different compared to baseline. There was an inverse association between the percent change in HR (r = −0.42, $P = 0.002$), MAP (r = −0.41, $P = 0.002$), and CVRi (r = −0.31,

P = 0.045), but not MCAv (r = 0.19, P = 0.971) in response to IHG exercise and WMH fraction. There were no associations between responses to post-exercise ischemia and WMH fraction. Lower sympathoexcitatory responses to IHG exercise are associated with greater WMH burden in middle-aged to older adults. These findings suggest that individuals who demonstrate smaller increases in HR, MAP, and CVRi in response to sympathoexcitatory stress have greater WMH burden.

Keywords: blood pressure, cerebrovascular disease, white matter, cerebral artery blood velocity, cerebrovascular resistance

INTRODUCTION

Identification of novel vascular risk factors is necessary to understand vascular contributions to the progression of cognitive decline and dementia (Gorelick et al., 2011). Specifically, investigating mechanisms underlying the reduction in cerebral blood flow and changes in blood pressure (BP) during the presymptomatic phase of dementia could help to elucidate the link between vascular risk and dementia (Snyder et al., 2015). Impaired peripheral and cerebral vascular regulation as a consequence of chronic hypertension could contribute to the reduction in cerebral blood flow and progression of cognitive decline (Farkas and Luiten, 2001). Indeed, vascular dysregulation and impaired hemodynamic responses to stress may occur early in the progression of cognitive decline, prior to clinically-relevant declines in cognitive function (Iadecola, 2004; Iturria-Medina et al., 2016). In fact, vascular risk scores have been used to predict future risk of dementia 20 years later in middle-aged adults (Kivipelto et al., 2006). Further, in individuals with Alzheimer's disease (AD), vascular factors including systolic hypertension and angina at the time of AD diagnosis are associated with a more rapid decline in cognitive function (Mielke et al., 2007).

White matter hyperintensities (WMH) are areas of increased brightness and signal density observed on MRI scans indicative of small vessel cerebrovascular disease which are associated with cognitive decline and increased severity of dementia in individuals with AD (Heyman et al., 1998). Longitudinal studies suggest an association between WMH burden and cardiovascular risk factors (Breteler et al., 1994; Jeerakathil et al., 2004), lower total cerebral perfusion (Vernooij et al., 2008), and lower cerebral blood flow (Promjunyakul et al., 2018). Indeed, cerebral blood flow is lower in areas with WMH compared to areas without WMH (Brickman et al., 2009; Stewart et al., 2021). Cerebral blood flow is also lower in adults with WMH compared to those without (Fazekas et al., 1988; O'Sullivan et al., 2002). In addition, lower middle cerebral artery blood velocity (MCAv) and higher cerebral pulsatility index (PI) has been reported in adults with greater severity of WMH burden (Puglisi et al., 2018). Importantly, during the presymptomatic phase of dementia, WMH volumes are associated with increased risk of progression to MCI (Soldan et al., 2020).

Longitudinal studies have demonstrated that elevated BP and systolic blood pressure (SBP) variability in midlife increases the risk of developing WMH (Dufouil et al., 2001; Havlik et al., 2002; Wartolowska and Webb, 2021). In addition to vascular risk factors, the physiological response to repeated acute or chronic physiological and psychological stressors likely contribute to future risk of disease (McEwen and Stellar, 1993). The response to acute physiological stressors may therefore reveal dysfunction in the systemic and cerebral circulation that could affect white matter health. For example, a greater increase in BP in response to mental stress is associated with WMH burden (Waldstein et al., 2004) and impaired hypercapnic cerebrovascular reactivity is associated with white matter damage (Sam et al., 2016). Isometric handgrip (IHG) exercise represents an acute sympathoexcitatory stressor indicative of activities of daily living as heart rate (HR), BP, and sympathetic nerve activity increase in response to sustained isometric contractions (Lind and McNicol, 1967; Mark et al., 1985). Post-exercise ischemia occludes blood flow to the previously contracted muscle leading to sustained elevations in BP and sympathetic activity, in the absence of muscle contraction (Rowell and O'Leary, 1990). Together, IHG exercise and post-exercise ischemia provide insight into the cardiovascular responses to muscle mechanoreflex and metaboreflex activation. Isometric and rhythmic handgrip exercise responses have been used to evaluate cardiovascular risk in a variety of populations (Ide et al., 1998; Giller et al., 2000; Ranadive et al., 2017; Miller et al., 2019; Tarumi et al., 2021). We have previously reported that women with a history of preeclampsia (who have an elevated risk of cerebrovascular disease and cognitive decline) demonstrated a greater cerebral blood flow velocity response to IHG exercise (Miller et al., 2019), despite similar increases in BP (Ranadive et al., 2017), compared with women without a history of preeclampsia. However, the cardiovascular and cerebrovascular response to IHG exercise and post-exercise ischemia have not been linked to white matter health. The purpose of this study was to evaluate associations between cardiovascular and cerebrovascular responses to sympathoexcitatory stimuli and WMH burden in a cohort of cognitively unimpaired middle-aged and older adults. We hypothesized that both a greater cardiovascular and cerebrovascular response to IHG exercise and post-exercise ischemia would be associated with greater WMH burden.

MATERIALS AND METHODS

Ethics Statement

All study procedures were approved by the University of Wisconsin-Madison Institutional Review Board and performed

according to the Declaration of Helsinki by obtaining written informed consent from each participant.

Participants

Participants were recruited from cohorts within the Wisconsin Alzheimer's Disease Research Center (ADRC). These cohorts included the Investigating Memory in Preclinical Alzheimer's Disease – Causes and Treatments (IMPACT) cohort and the Healthy Older Controls cohort. Within the IMPACT cohort, participants had either 1) a parent clinically diagnosed with AD clinical syndrome according to the self-report of the adult child, 2) a biological mother or father that lived to at least 75 years old or 70 years old, respectively, without symptoms of dementia, or 3) indeterminate parental history of dementia (i.e., parent did not live to age limits described above, parent has or had MCI, or participant is adopted and biological family history is unknown, etc.). Within the Healthy Older Control cohort, participants were at least 65 years old and did not have a family history of dementia. Family history of dementia was determined using a self-report dementia questionnaire for each parent or an autopsy report when available (Johnson et al., 2017). Participants were considered to be cognitively unimpaired based on a consensus diagnosis as previously described (Sager et al., 2005; Rivera-Rivera et al., 2016). Briefly, at least one physician and at least one neuropsychologist met to determine the stage of dementia severity using the Clinical Dementia Rating scale (Morris, 1997). Additionally, participants underwent a blood draw and MRI (Rivera-Rivera et al., 2016).

Ninety-five cognitively unimpaired adults (32 men, 63 women) between 55 and 69 years of age participated in this study. The distribution of men and women in this study is similar to a previously published study using the Wisconsin ADRC IMPACT cohort (Hoscheidt et al., 2016). All participants had a body mass index (BMI) less than or equal to 34.9 kg/m^2. Women were postmenopausal for >1 year and were not currently taking oral menopausal hormone therapy. Exclusion criteria consisted of a confirmed diagnosis of MCI or dementia of any kind, uncontrolled hypertension, significant surgical history, history of clinically significant stroke, cerebrovascular disease, or other major neurological disorders. Participants with controlled hypertension were included in the study.

Experimental Procedures

Participants visited the Bruno Balke Biodynamics Laboratory at the University of Wisconsin-Madison on two separate occasions for a screen day and experimental study day. On the experimental study day, participants arrived at the laboratory after a 4-h fast and having refrained from performing strenuous exercise in the previous 24 h. Participants were instructed to avoid consumption of caffeine or chocolate in the previous 12 h, alcohol in the previous 24 h, and avoid the use of aspirin or non-steroidal anti-inflammatory drugs for 48 h prior to the study visit. Participants were asked to withhold over-the-counter medications on the experimental study day. All experimental study day procedures were performed while participants lied supine in a dimly lit, temperature-controlled room kept between 22 and 24°C. On a separate day, participants visited the Wisconsin Institutes for Medical Research in Madison, WI for an MRI scan.

Screen Day Measurements

Upon arrival to the laboratory, height and weight were obtained using a standard scale and stadiometer. The screen day visit consisted of a brief familiarization with study procedures, a supine arterial BP measurement using a brachial cuff (Datex Ohmeda, GE Healthcare, Fairfield, CT, United States) after resting quietly in a dimly lit room for 10 min, and middle cerebral artery (MCA) screening using transcranial Doppler ultrasound (TCD). MCA imaging was conducted to ensure proper angle of the probe, and the anatomical location, depth of the signal, and mean velocity were recorded. Maximal voluntary contraction (MVC) was determined from the average of two brief maximal handgrip contractions of the left hand separated by 1 min of rest.

Experimental Study Day Measurements

Following 10 min of supine rest, arterial BP was measured in triplicate using a brachial BP cuff and the average value was recorded. HR was measured using a 3-lead electrocardiogram (CardioCap, Datex Ohmeda, GE Healthcare, Fairfield, CT, United States). Mean arterial blood pressure (MAP), SBP, and diastolic blood pressure (DBP) were continuously measured using a non-invasive finger cuff (NOVA, Finapres Medical Systems, Amsterdam, The Netherlands). End-tidal CO_2 ($ETCO_2$) was measured using a nasal cannula. MCAv of the left MCA was measured using a 2-MHz TCD (Spencer Technologies, Seattle, WA, United States). The ipsilateral MCA was used to measured MCAv to represent global changes in cerebral blood flow. Quality MCAv signals were obtained using established guidelines (DeWitt and Wechsler, 1988) and signal quality was confirmed prior to beginning the IHG exercise protocol.

Experimental Study Day Protocol

Participants were fitted with a BP cuff on the upper left arm (D. E. Hokanson, Inc., Bellevue, WA) and a handgrip dynamometer (AD Instruments, Colorado Springs, United States) was placed in their left hand. Due to the experimental setup, all participants performed the IHG exercise protocol using their left hand apart from two participants due to medical history. Prior to exercise, participants rested quietly in the supine position for 3 min while baseline measurements were collected. Then, participants completed an IHG exercise protocol at 40% of their previously determined MVC until fatigue as previously described (Ranadive et al., 2017; Miller et al., 2019). Fatigue was defined as the time at which participants were no longer able to maintain 40 $\pm$ 5% of their MVC despite verbal encouragement by the researchers. Upon reaching fatigue, a BP cuff was rapidly inflated to a pressure 100 mmHg above resting SBP to elicit a period of post-exercise ischemia. Following 90 s of cuff inflation, participants rested quietly for 2 min. All cardiovascular and cerebrovascular variables were closely monitored throughout the experimental protocol. The percentage of MVC was continuously monitored throughout the protocol and participants were verbally instructed and encouraged to maintain the target intensity. Grip force (kg) was

recorded throughout the protocol. An experimental study day timeline is shown in **Figure 1**.

MRI Measurements

On a separate visit, MRI brain scans were done on a 3T clinical MRI scanner (MR750, GE Healthcare, Waukesha, WI, United States) at the Wisconsin Institutes for Medical Research. In the supine position, participants were fitted and imaged with a 32-channel or 48-channel head coil (Nova Medical Head Coil, Nova Medical, Wilmington, MA, United States) with a gradient strength of 50 mT/m and a gradient slew rate of 200 mT/m/ms. Intracranial volume (ICV) was measured using a T1-weighted structural brain volume (BRAVO) scan with the following parameters: fast spoiled gradient echo sequence, inversion time = 450 ms, repetition time = 8.1 ms, echo time = 3.2 ms, flip angle = 12°, acquisition matrix = 256 × 256, field of view = 256 mm, slice thickness = 1.0 mm, and scan time ∼8 min. Total WMH lesion volume was measured using a FLAIR scan.

Apolipoprotein E Genotyping

Apolipoprotein E (*APOE*) is a genetic risk factor for Alzheimer's disease and presence of one or more copies of the ε4 allele is associated with increased risk of cognitive decline (Liu et al., 2013). *APOE* status was determined using competitive allele-specific polymerase chain reaction-based genotyping assays (LGC Genomics, Beverly, MA) as previously described (Johnson et al., 2017). Participants were considered *APOE* ε4 positive if they had one or more copies of the ε4 allele.

Data Analysis

Cardiovascular and cerebrovascular variables were recorded using LabChart 8 at 250 Hz (AD Instruments, Dunedin, New Zealand) and stored offline for analysis. Cardiovascular variables included: HR, cardiac output (CO), MAP, SBP, DBP, and pulse pressure (PP). Cerebrovascular variables included: MCAv, cerebral PI, and cerebrovascular resistance index (CVRi). CO was calculated as HR x stroke volume. Stroke volume was derived from finger BP using Modelflow analysis (NOVA, Finapres Medical Systems). Cerebral PI was calculated as (systolic MCAv – diastolic MCAv)/mean MCAv. CVRi was calculated as MAP/MCAv. $ETCO_2$ was measured throughout the protocol. Baseline measurements were recorded during the middle 60 s of the 3 min baseline period prior to exercise. Variables were continuously recorded during the IHG exercise and post-exercise ischemia protocols. Time to fatigue was recorded for each participant in seconds. Due to individual differences in handgrip MVC and time to fatigue, work performed was derived by calculating the integral of grip force throughout the IHG exercise protocol. To account for individual differences in the time to fatigue during IHG exercise and consistent with our previous work, data was divided into tertiles for analysis (Ranadive et al., 2017; Miller et al., 2019). Briefly, the time to fatigue for each participant was recorded during the IHG exercise protocol using an electronic handgrip device interfaced with LabChart. Then, the response to IHG exercise was divided into tertiles for analysis. Cardiovascular and cerebrovascular variables were averaged during each tertile of IHG exercise. The response to post-exercise ischemia during the final 60 s was used for analysis. The percent change from baseline during the IHG exercise and post-exercise ischemia protocol was calculated for all cardiovascular and cerebrovascular variables and the average of each variable of interest was used for analysis.

WMH were assessed using a lesion prediction algorithm from the Lesion Segmentation Tool (LST) in SPM (Schmidt, 2017). The LST lesion prediction algorithm uses a FLAIR scan (with the optional co-registration and resampling to the resolution of a T1-weighted reference image) to estimate the lesion probability at each voxel, outputting a lesion probability map. In the quantification step, the lesion probability was thresholded to 0.5 and constrained to voxels at least 4mm from the estimated edge of brain tissue. The resulting lesion probability map for each scan underwent visual quality assessment by trained reviewers, where the probability map was overlayed onto the FLAIR scan and compared side by side for accuracy to the unsegmented FLAIR scan. As needed, segmentations were secondarily reviewed by an experienced neuroradiologist and excluded when warranted. WMH fraction was calculated by dividing WMH lesion volume (mm^3) by ICV (mm^3) and multiplying by 100. As WMH lesion volume and fraction are highly skewed measures, data were cubic root transformed for analysis to produce a normal distribution and reduce skewness (Habes et al., 2016).

Statistical Analysis

Normality of all variables was assessed using Shapiro-Wilk tests and visually inspected using histograms and QQ plots. Equal variance across time points (baseline, each tertile of IHG exercise,

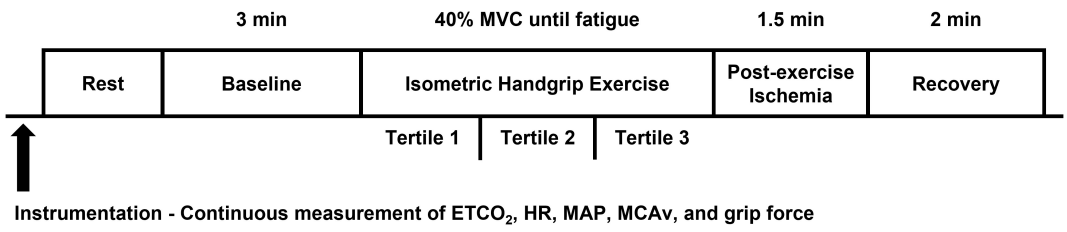

FIGURE 1 | Experimental study day timeline. $ETCO_2$, end-tidal CO_2; HR, heart rate; MAP, mean arterial pressure; MCAv, middle cerebral artery blood velocity.

and post-exercise ischemia) for each variable was assessed using Brown-Forsythe tests. One-way repeated measures ANOVA were performed to evaluate if raw cardiovascular and cerebrovascular variables differed from baseline during the IHG exercise and post-exercise ischemia protocol. One-way repeated measures ANOVA were performed to evaluate if the percent change from baseline in cardiovascular and cerebrovascular variables differed between time points (each tertile of IHG exercise and post-exercise ischemia). Pairwise comparisons for each variable were assessed using Bonferroni *post hoc* testing and effect sizes were calculated as eta squared. All statistical analyses were completed using R software. Means ± standard deviation for all variables are presented. Statistical significance was set *a priori* at $P < 0.05$.

To determine the association between the cardiovascular and cerebrovascular response to IHG exercise and post-exercise ischemia and WMH fraction, multiple linear regression was performed. The final tertile of IHG exercise was used for statistical analysis as the final tertile represents the greatest sympathoexcitatory stress experienced by participants prior to onset of fatigue as indicated by a progressive increase in HR and MAP in all participants (**Figures 2**, **3** and Ranadive et al., 2017). In each multiple linear regression analysis, independent variables of age at MRI, sex, *APOE* ε4 status, and work performed during the IHG exercise protocol were added to the model as independent variables. Age at MRI was included as an independent variable in the model due to increases in WMH burden with age (de Leeuw et al., 2001). For sex, women were assigned a value of 1 and men assigned a value of 0 as WMH burden is greater in women compared to men (Fatemi et al., 2018). WMH burden is associated with *APOE* status such that homozygous *APOE* ε4 individuals have greater rates of WMH accumulation compared to individuals without a copy of the *APOE* ε4 allele (Sudre et al., 2017). Accordingly, participants with one or more copies of the *APOE* ε4 allele were considered *APOE* ε4 positive and assigned a value of 1 while participants without a copy were considered *APOE* ε4 negative and assigned a value of 0 (Kaufman et al., 2021). Finally, the pressor response to handgrip exercise may be dependent upon absolute contraction loads during exercise (Lee et al., 2021). Therefore, work performed during the IHG exercise protocol was added as an independent variable to account for inter-individual differences in MVC and absolute contraction load during exercise.

Participants with controlled hypertension were included in the study. As such, we also evaluated the effect of controlled hypertension on our results. To determine the influence of controlled hypertension on our results, participants with controlled hypertension ($n = 13$) were assigned a value of 1 while normotensive participants ($n = 55$) were assigned a value of 0 (Dufouil et al., 2001) when added to the multiple linear regression models.

Due to the uneven distribution of men and women, we did not evaluate sex differences in the cardiovascular and cerebrovascular response to IHG exercise and post-exercise ischemia or associations with WMH fraction *a priori*.

RESULTS

Participants

Of the 95 participants recruited for this study, 27 participants were not included in the final analysis due to not completing the IHG exercise protocol ($n = 2$), inadequate data quality (i.e., MAP or MCAv signal loss; $n = 8$), incomplete *APOE* genotype data ($n = 7$), or incomplete WMH data ($n = 9$). In addition, one participant was excluded from analysis due to having a WMH lesion volume > 7 standard deviations above the mean (mean WMH lesion volume = 776.0 mm^3). Participant characteristics and cardiovascular variables at rest in the 68 participants (20 men, 48 women) with complete data are located in **Table 1**. The average age of participants during their most recent MRI was 62 ± 4 years. The average difference between the ages of participants at their most recent MRI to the experimental study day visit in the laboratory was 0.2 years. The average WMH lesion volume was 776.0 ± 1118.4 mm^3, average ICV was $1.4 \times 10^6 \pm 1.4 \times 10^5$ mm^3, and average WMH fraction was 0.05 ± 0.07. Sixty-two participants (91%) were right hand dominant.

Cardiovascular and Cerebrovascular Responses to Sympathoexcitatory Stimuli

The average handgrip MVC for participants was 25.3 ± 10.0 kg. The average time to fatigue during IHG exercise was 153.6 ± 49.1 s and average work performed during IHG exercise was 1,371.4 ± 539.8 kg/s. During IHG exercise, HR, CO, MAP, PP, MCAv, and CVRi increased compared with baseline ($P < 0.001$; **Figure 2** and **Table 2**), as expected. Cerebral PI decreased during IHG exercise compared with baseline ($P < 0.001$; **Table 2**). During post-exercise ischemia, HR, CO,

TABLE 1 | Participant characteristics and selected cardiovascular variables at rest.

Variable	Value
Sex (M/W)	20/48
Age (y)	63 ± 4
Education (y)	17 ± 3
Weight (kg)	76 ± 15
Height (cm)	168 ± 8
Body mass index (kg/m^2)	27 ± 4
Heart rate (bpm)	60 ± 7
Mean arterial blood pressure (mmHg)	93 ± 10
Systolic blood pressure (mmHg)	126 ± 15
Diastolic blood pressure (mmHg)	76 ± 8
MoCA	28 ± 2
Family history positive (n,%)	48, 71
APOE ε4 positive (n,%)	27, 40
Hypertension (n,%)	13, 19
Diabetes (n,%)	2, 3

Values expressed as mean ± SD. MoCA, Montreal Cognitive Assessment; APOE, apolipoprotein E.

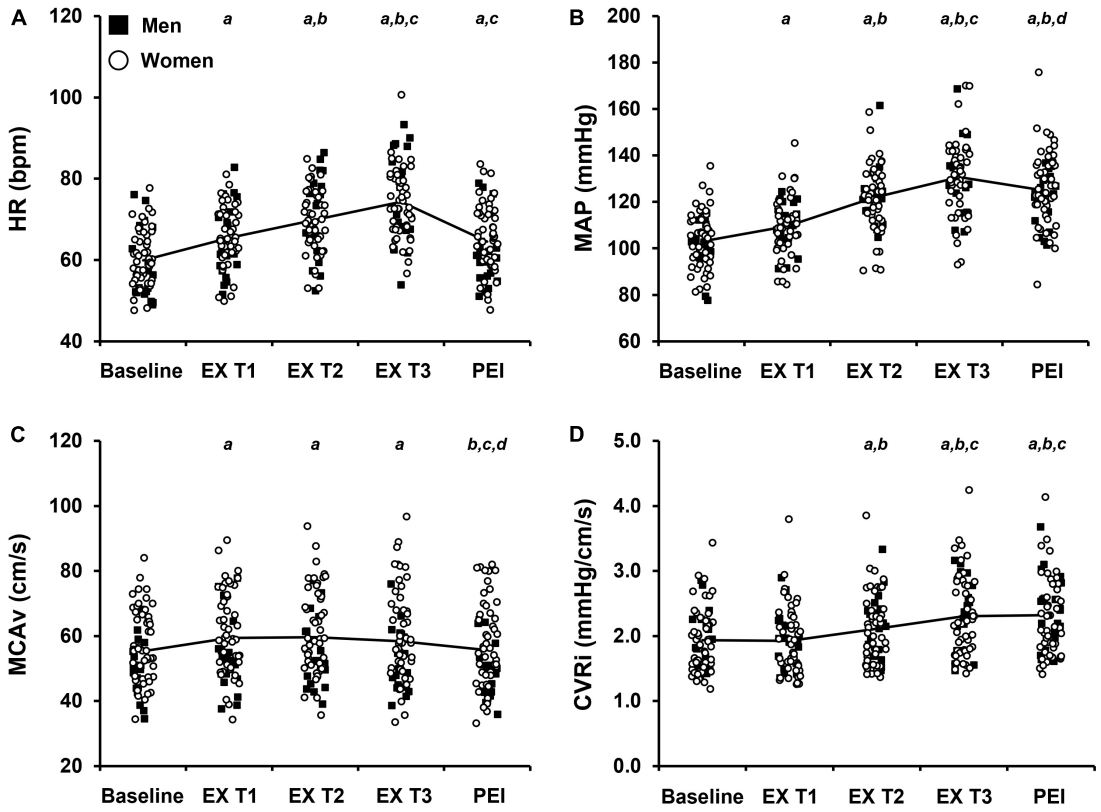

FIGURE 2 | Raw values of cardiovascular and cerebrovascular variables at baseline and during each tertile of isometric handgrip exercise (EX T1, EX T2, EX T3, respectively) followed by a period of post-exercise ischemia (PEI). Means and individual data presented. The solid black line represents mean values. **(A)** Heart rate, HR; **(B)** Mean arterial pressure, MAP; **(C)** Middle cerebral artery blood velocity, MCAv; **(D)** Cerebrovascular resistance index, CVRi. Significance is indicated by the following: $^{a}P < 0.05$ vs. baseline, $^{b}P < 0.05$ vs. exercise T1, $^{c}P < 0.05$ vs. exercise T2, $^{d}P < 0.05$ vs. exercise T3 (n = 68; One-way repeated measures ANOVA with Bonferroni *post hoc* testing).

MAP, PP, and CVRi remained elevated compared with baseline ($P < 0.001$) whereas MCAv did not differ compared with baseline values ($P > 0.05$; **Figure 2** and **Table 2**). Cerebral PI increased during post-exercise ischemia compared with baseline ($P < 0.001$, **Table 2**). $ETCO_2$ was similar between baseline and IHG exercise until the final tertile of IHG exercise when $ETCO_2$ decreased and remained lower during post-exercise ischemia ($P < 0.001$, **Table 2**). The percent change from baseline in HR, MAP, MCAv, and CVRi during IHG exercise and post-exercise ischemia are presented in **Figure 3**. The percent change from baseline in all cardiovascular and cerebrovascular variables during IHG exercise and post-exercise ischemia are located in **Table 3**.

Associations Between Cardiovascular and Cerebrovascular Responses to Sympathoexcitatory Stimuli and White Matter Hyperintensities

All multiple linear regressions between cardiovascular and cerebrovascular variables at rest and WMH fraction were adjusted for age at MRI, sex, and *APOE* ε4 status. HR ($P = 0.006$), MAP ($P < 0.001$), SBP ($P = 0.002$), DBP ($P < 0.001$), and PP ($P < 0.001$) at rest were positively associated with WMH fraction. There were no significant associations between the cerebrovascular variables at rest (MCAv, cerebral PI, or CVRi) and WMH fraction ($P > 0.05$ for all). All multiple linear regressions between cardiovascular and cerebrovascular responses to sympathoexcitatory stimuli and WMH fraction were adjusted for age at MRI, sex, *APOE* ε4 status, and work performed during the IHG exercise protocol. Results of multiple linear regression analyses during the final tertile of IHG exercise and WMH fraction are presented in **Table 4**. There was a negative association between the percent change in HR ($P = 0.002$), MAP ($P = 0.002$), SBP ($P = 0.005$), DBP ($P = 0.002$), and CVRi ($P = 0.045$) during the final tertile of IHG exercise and WMH fraction (**Table 4**). Unadjusted individual data between the percent change in HR, MAP, MCAv, and CVRi during the final tertile of IHG exercise and WMH fraction are presented in **Figure 4**. There were no significant associations between cardiovascular and cerebrovascular variables during post-exercise ischemia and WMH fraction (**Table 5**).

Additionally, we evaluated the effect of controlled hypertension on our findings. After correction for age at MRI, sex, *APOE* ε4 status, and controlled hypertension, HR ($P = 0.023$), MAP ($P = 0.006$), SBP ($P = 0.012$), and DBP

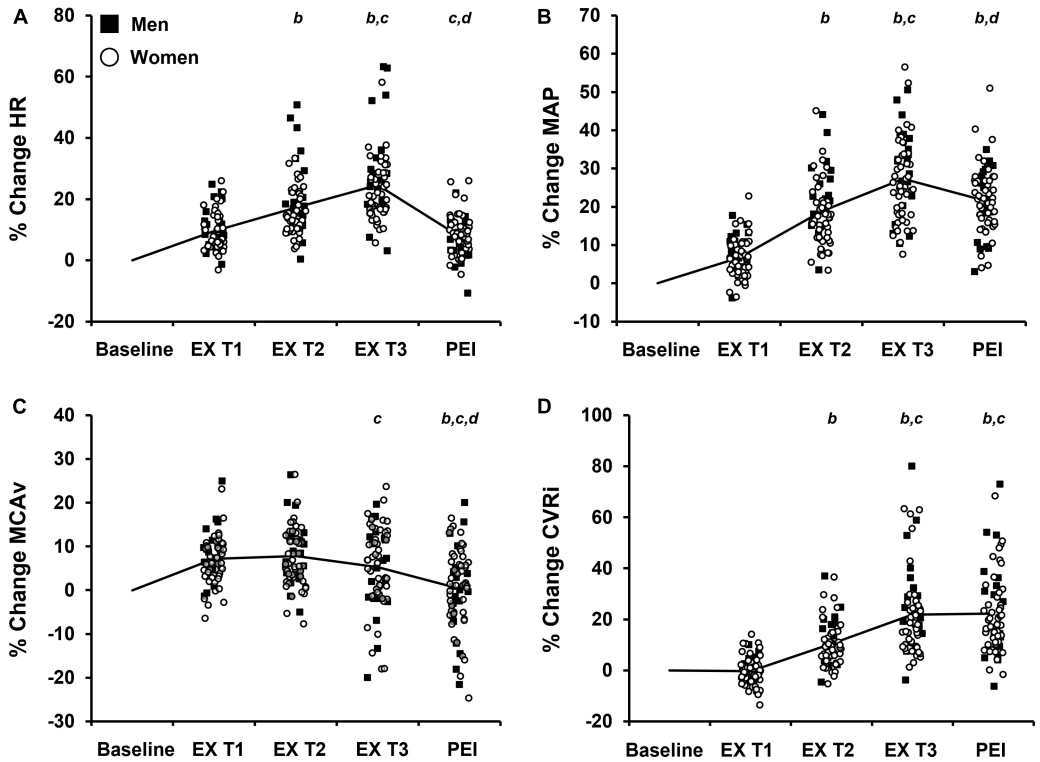

FIGURE 3 | Percent change (% change) from baseline values in cardiovascular and cerebrovascular variables during each tertile of isometric handgrip exercise (EX T1, EX T2, EX T3, respectively) followed by a period of post-exercise ischemia (PEI). Means and individual data presented. The solid black line represents mean values. **(A)** % change in heart rate, HR; **(B)** % change in mean arterial pressure, MAP; **(C)** % change in middle cerebral artery blood velocity, MCAv; **(D)** % change in cerebrovascular resistance index, CVRi. Significance is indicated by the following: $^{b}P < 0.05$ vs. exercise T1, $^{c}P < 0.05$ vs. exercise T2, $^{d}P < 0.05$ vs. exercise T3 (n = 68; One-way repeated measures ANOVA with Bonferroni *post hoc* testing).

TABLE 2 | Cardiovascular and cerebrovascular variables during isometric handgrip exercise and post-exercise ischemia.

Variable	Baseline	Exercise T1	Exercise T2	Exercise T3	PEI	*P*-value (effect size)
HR (bpm)	60 ± 7	65 ± 8^{a}	70 ± 8^{a,b}	74 ± 9^{a,b,c}	65 ± 8^{a,c,d}	**<0.001** (0.266)
CO (L/min)	5.3 ± 1.2	5.3 ± 1.2	5.6 ± 1.3^{a,b}	6.0 ± 1.4^{a,b,c}	5.5 ± 1.2^{a,b,d}	**<0.001** (0.045)
MAP (mmHg)	103 ± 12	110 ± 12^{a}	122 ± 14^{a,b}	131 ± 16^{a,b,c}	125 ± 15^{a,b,d}	**<0.001** (0.359)
SBP (mmHg)	143 ± 18	150 ± 19^{a}	166 ± 21^{a,b}	178 ± 23^{a,b,c}	175 ± 23^{a,b,c}	**<0.001** (0.310)
DBP (mmHg)	76 ± 9	81 ± 9^{a}	89 ± 11^{a,b}	95 ± 13^{a,b,c}	89 ± 10^{a,b,d}	**<0.001** (0.312)
PP (mmHg)	67 ± 13	69 ± 14^{a}	77 ± 15^{a,b}	82 ± 15^{a,b,c}	86 ± 16^{a,b,c,d}	**<0.001** (0.199)
MCAv (cm/s)	55 ± 11	59 ± 13^{a}	60 ± 13^{a}	58 ± 14^{a}	56 ± 13^{b,c,d}	**<0.001** (0.021)
Cerebral PI (A.U.)	0.80 ± 0.09^{a}	0.76 ± 0.09^{a}	0.75 ± 0.09^{a}	0.75 ± 0.09^{a}	0.83 ± 0.10^{a,b,c,d}	**<0.001** (0.096)
CVRi (mmHg/cm/s)	1.9 ± 0.48	1.9 ± 0.49	2.1 ± 0.51^{a,b}	2.4 ± 0.60^{a,b,c}	2.4 ± 0.60^{a,b,c}	**<0.001** (0.112)
$ETCO_2$ (mmHg)	39 ± 3	39 ± 4	38 ± 4	37 ± 4^{b,c}	38 ± 3^{a,b,c}	**<0.001** (0.021)

Values are expressed as means ± SD. Data for isometric handgrip exercise are divided into tertiles (Exercise T1, T2, and T3). Data for post-exercise ischemia (PEI) presented during the final 60s of PEI. Cerebral PI, cerebral pulsatility index; CO, cardiac output; CVRi, cerebrovascular resistance index; DBP, diastolic blood pressure; ETCO2, end-tidal CO2; HR, heart rate; MAP, mean arterial blood pressure; MCAv, middle cerebral artery blood velocity; PP, pulse pressure; SBP, systolic blood pressure. Bolded P-values indicate a significant effect of condition. [a] P < 0.05 vs. baseline, [b] P < 0.05 vs. exercise T1, [c] P < 0.05 vs. exercise T2, [d] P < 0.05 vs. exercise T3; (n = 68; repeated measures ANOVA with Bonferroni post hoc testing. Effect sizes calculated as eta squared).

($P = 0.008$) at rest remained positively associated with WMH fraction. There were no significant associations between PP, MCAv, cerebral PI, or CVRi at rest and WMH fraction after correction for confounding variables ($P > 0.05$ for all). The results of multiple linear regression analyses corrected for age at MRI, sex, *APOE* ε4 status, work performed during the IHG exercise protocol, and controlled hypertension during IHG exercise and post-exercise ischemia are located in **Supplementary Tables 1**, **2**. Following correction for controlled hypertension, the inverse associations between the percent change in HR ($P = 0.014$), MAP ($P = 0.013$), SBP ($P = 0.032$), and DBP ($P = 0.008$) during the final tertile of IHG exercise and

TABLE 3 | Percent change from baseline in cardiovascular and cerebrovascular variables during isometric handgrip exercise and post-exercise ischemia.

Variable	Exercise T1	Exercise T2	Exercise T3	PEI	*P*-value (effect size)
HR (%)	10 ± 6	17 ± 9[b]	25 ± 12[b,c]	8 ± 7[c,d]	**<0.001** (0.348)
MAP (%)	7 ± 5	19 ± 9[b]	27 ± 11[b,c]	22 ± 9[b,d]	**<0.001** (0.432)
SBP (%)	5 ± 5	17 ± 8[b]	25 ± 10[b,c]	23 ± 9[b,c]	**<0.001** (0.465)
DBP (%)	7 ± 6	19 ± 10[b]	26 ± 12[b,c]	18 ± 9[b,d]	**<0.001** (0.356)
PP (%)	3 ± 6	12 ± 8[b]	7 ± 5[b,c]	29 ± 14[b,c,d]	**<0.001** (0.555)
MCAv (%)	7 ± 6	8 ± 7	5 ± 10[c]	0 ± 10[b,c,d]	**<0.001** (0.115)
Cerebral PI (%)	−5 ± 4	−6 ± 6	−5 ± 7	4 ± 7[b,c,d]	**<0.001** (0.312)
CVRi (%)	0 ± 6	11 ± 9[b]	22 ± 17[b,c]	23 ± 16[b,c]	**<0.001** (0.354)

Values are expressed as means ± SD. Data for isometric handgrip exercise are divided into tertiles (Exercise T1, T2, and T3). Data for post-exercise ischemia (PEI) presented during the final 60s of PEI. Cerebral PI, cerebral pulsatility index; CVRi, cerebrovascular resistance index; DBP, diastolic blood pressure; HR, heart rate; MAP, mean arterial blood pressure; MCAv, middle cerebral artery blood velocity; PP, pulse pressure; SBP, systolic blood pressure. Bolded P-values indicate a significant effect of condition. [b]P < 0.05 vs. exercise T1, [c]P < 0.05 vs. exercise T2, [d]P < 0.05 vs. exercise T3; (n = 68; repeated measures ANOVA with Bonferroni post hoc testing. Effect sizes calculated as eta squared).

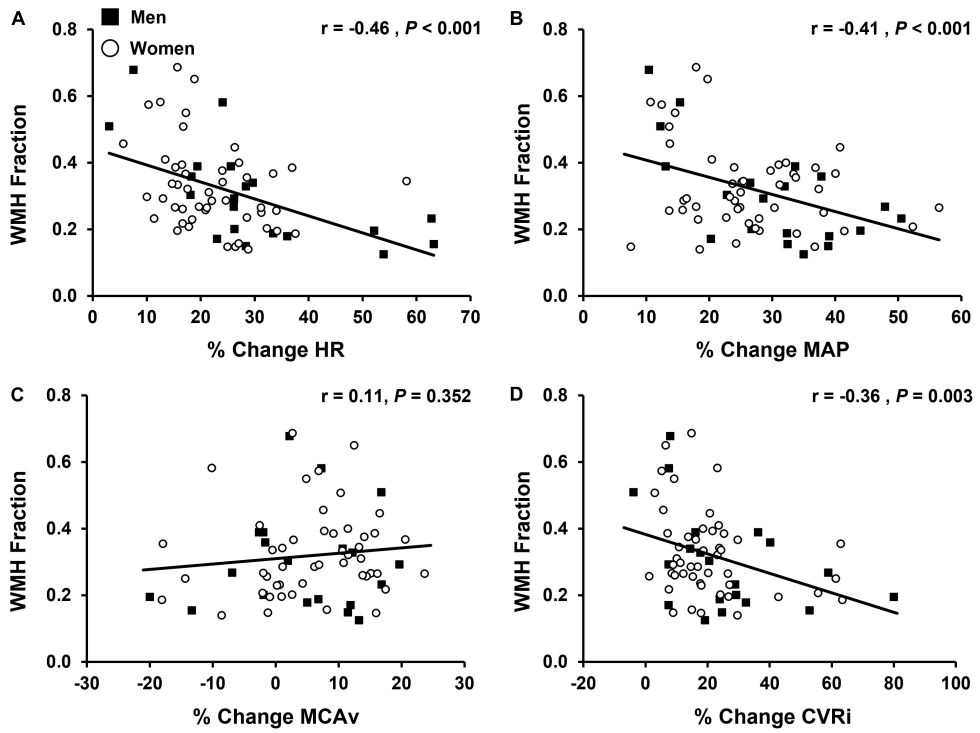

FIGURE 4 | Associations between the percent change (% change) from baseline values in cardiovascular and cerebrovascular variables during the final tertile of isometric handgrip exercise (EX T3) and white matter hyperintensity (WMH) fraction (n = 68). WMH fraction was calculated by dividing WMH lesion volume by intracranial volume, converting to a percentage, and applying a cubic root transformation to reduce skewness. Men are shown in black squares and women are shown in open circles. **(A)** % change in heart rate, HR vs. WMH; **(B)** % change in mean arterial pressure, MAP vs. WMH; **(C)** % change in middle cerebral artery blood velocity, MCAv vs. WMH; **(D)** % change incerebrovascular resistance index, CVRi vs. WMH. Note: data and statistics shown in figure have not been adjusted for age at MRI, sex, APOE 4 status, or work performed during isometric handgrip exercise. After adjustment for confounding variables, there was a negative association between the % change in HR ($P = 0.002$), MAP ($P = 0.002$), and CVRi ($P = 0.045$) during the final tertile of isometric handgrip exercise and WMH fraction. Corresponding results from multiple linear regression analyses are presented in **Tables 4**, **5**.

WMH fraction remained significant (**Supplementary Table 1**). However, there was no longer a negative association between the percent change in CVRi during the final tertile of IHG exercise and WMH fraction ($P = 0.136$, **Supplementary Table 1**). There were no significant associations between cardiovascular and cerebrovascular variables during post-exercise ischemia and WMH fraction (**Supplementary Table 2**).

DISCUSSION

The primary aim of this study was to evaluate associations between cardiovascular and cerebrovascular responses to sympathoexcitatory stimuli and WMH burden. The novel finding of this study was that a lower percent change in HR, BP, and CVRi in response to IHG exercise was associated with

TABLE 4 | Results of multiple linear regression analysis between cardiovascular and cerebrovascular variables during isometric handgrip exercise and white matter hyperintensity fraction.

Variable	Standardized β	*P*-value
HR		
HR (bpm)	0.067	0.586
% Change HR	−0.432	**0.002**
MAP		
MAP (mmHg)	−0.018	0.886
% Change MAP	−0.380	**0.002**
SBP		
SBP (mmHg)	0.031	0.806
% Change SBP	−0.361	**0.005**
DBP		
DBP (mmHg)	−0.094	0.479
% Change DBP	−0.383	**0.002**
PP		
PP (mmHg)	0.119	0.348
% Change PP	−0.110	0.406
MCAv		
MCAv (cm/s)	−0.022	0.868
% Change MCAv	−0.005	0.971
Cerebral PI		
PI (A.U.)	0.127	0.350
% Change PI	0.181	0.145
CVRi		
CVRi (mmHg/cm/s)	0.005	0.973
% Change CVRi	−0.271	**0.045**

Data presented as standardized β estimates for raw values and the percent change (% change) from baseline during the final tertile of isometric handgrip exercise. Cerebral PI, cerebral pulsatility index; CVRi, cerebrovascular resistance index; DBP, diastolic blood pressure; HR, heart rate; MAP, mean arterial blood pressure; MCAv, middle cerebral artery blood velocity; PP, pulse pressure; SBP, systolic blood pressure. White matter hyperintensity (WMH) fraction was calculated by dividing WMH lesion volume by intracranial volume, converting to a percentage, and applying a cubic root transformation to reduce skewness. Linear regression estimates adjusted for age at MRI (y), sex (Women = 1; Men = 0), APOE ε4 status (APOE ε4 positive = 1; APOE ε4 negative = 0), and work performed during the isometric handgrip exercise protocol (kg/s). (n = 68. Bolded p-values indicate a significant association between the cardiovascular or cerebrovascular variable and WMH fraction).

TABLE 5 | Results of multiple linear regression analysis between cardiovascular and cerebrovascular variables during post-exercise ischemia and white matter hyperintensity fraction.

Variable	Standardized β	*P*-value
HR		
HR (bpm)	0.232	0.059
% Change HR	−0.135	0.265
MAP		
MAP (mmHg)	0.180	0.153
% Change MAP	−0.143	0.256
SBP		
SBP (mmHg)	0.181	0.149
% Change SBP	−0.146	0.251
DBP		
DBP (mmHg)	0.141	0.270
% Change DBP	−0.149	0.231
PP		
PP (mmHg)	0.171	0.170
% Change PP	−0.105	0.408
MCAv		
MCAv (cm/s)	0.034	0.799
% Change MCAv	0.116	0.356
Cerebral PI		
PI (A.U.)	−0.037	0.775
% Change PI	−0.037	0.759
CVRi		
CVRi (mmHg/cm/s)	0.061	0.647
% Change CVRi	−0.183	0.162

Data presented as standardized β estimates for raw values and the percent change (% change) from baseline during the finals 60s of post-exercise ischemia. Cerebral PI, cerebral pulsatility index; CVRi, cerebrovascular resistance index; DBP, diastolic blood pressure; HR, heart rate; MAP, mean arterial blood pressure; MCAv, middle cerebral artery blood velocity; PP, pulse pressure; SBP, systolic blood pressure. White matter hyperintensity (WMH) fraction was calculated by dividing WMH lesion volume by intracranial volume, converting to a percentage, and applying a cubic root transformation to reduce skewness. Linear regression estimates adjusted for age at MRI (y), sex (Women = 1; Men = 0), APOE ε4 status (APOE ε4 positive = 1; APOE ε4 negative = 0), and work performed during the isometric handgrip exercise protocol (kg/s). (n = 68).

greater WMH burden in middle-aged and older adults, which was opposite of our original hypothesis. These results persisted when accounting for potentially confounding variables including age at MRI, sex, *APOE*ε4 status, and work performed during the IHG exercise protocol. Our results indicate that individuals with greater WMH burden may have impaired cardiovascular and cerebrovascular responses to sympathoexcitatory stress. Together, our findings suggest that blunted HR, BP and CVRi in response to acute sympathoexcitatory stress are associated with greater WMH burden in cognitively unimpaired middle-aged and older adults.

We have previously reported a 10-14% increase in HR, 7-20% increase in MAP, and 9-13% increase in MCAv in response to IHG exercise at 30% of MVC until fatigue in postmenopausal women with or without a history of hypertensive pregnancy (Ranadive et al., 2017; Miller et al., 2019). Consistent with our previous work and others, we observed similar sustained increases in cardiovascular (Lewis et al., 1983; Sander et al., 2010; Ranadive et al., 2017; Lee et al., 2021) and cerebrovascular variables (Miller et al., 2019) during IHG exercise and post-exercise ischemia. A unique aspect of this study is the inclusion of cerebral hemodynamics (MCAv and CVRi) to assist in interpretation of hemodynamic changes in the cerebral vessels in response to two sympathoexcitatory stressors. In response to IHG exercise at 40% MVC, we observed a 25% increase in HR, a 27% increase in MAP, a 5% increase in MCAv, and a 22% increase in CVRi during the final tertile of IHG exercise. Compared to our previous work, the larger increases in HR, MAP, SBP, and DBP observed in this study are likely due to the inclusion of both men and women as well as a greater relative workload (40 vs. 30% MVC). Our findings build upon our previous work in this area by including a larger sample size of both sexes and evaluating associations between cardiovascular and cerebrovascular responses to acute

sympathoexcitatory stimuli and WMH. To our knowledge, this is the first study to examine associations between cardiovascular and cerebrovascular responses to sympathoexcitatory stimuli and WMH burden. All participants in this study were enrolled in Wisconsin ADRC cohorts. Participants enrolled in these cohorts are part of a larger longitudinal study of over 1,200 individuals focused on early detection of AD, identification of both protective and risk factors, and developing strategies to delay onset and progression of AD (Johnson et al., 2017). The participants recruited for this study were relatively young and were healthy, free of cardiovascular disease (other than controlled hypertension n = 13), dementia of any kind, and history of clinically significant stroke, cerebrovascular disease, or other major neurological disorders. Therefore, collecting physiological data in this cohort provides insight beyond aging alone. As a result, the stressors used in this study in cognitively unimpaired middle-aged and older adults have the potential to serve as useful tools for early detection and identification of risk factors associated with cognitive decline and dementia.

The relationship between elevated BP at rest and WMH burden has been previously reported (van Dijk et al., 2004) and elevated BP and BP variability during the middle-aged years are associated with increased WMH burden (Dufouil et al., 2001; Havlik et al., 2002; Wartolowska and Webb, 2021). Elevated BP at midlife is also associated with increased risk of cognitive decline (Swan et al., 1998; DeCarli et al., 2001), dementia (McGrath et al., 2017; Walker et al., 2019), and AD (Lennon et al., 2019; Ou et al., 2020). However, epidemiological and longitudinal studies only provide insight into associations between BP values at rest and WMH burden or retrospective analysis based on historical BP measurements. Cardiovascular and cerebrovascular responses to acute or chronic physiological stimuli in healthy adults may reveal dysfunction in the systemic and cerebral circulation prior to significant damage to or changes in brain volumes. Indeed, greater BP responses to mental stress are associated with poor performance on cognitive challenges (Waldstein and Katzel, 2005) and WMH burden (Waldstein et al., 2004). Acceleration of WMH burden occurs prior to presentation of MCI (Silbert et al., 2012) and midlife represents a unique period during the presymptomatic phase of dementia in which intervention may be beneficial to delay the onset of cognitive decline. As such, identifying potential mechanisms contributing to the increase in WMH burden during midlife may be important for understanding the progression from normal cognition to presentation of symptoms of cognitive decline.

Isometric handgrip exercise followed by a period of post-exercise ischemia represent acute physiological stimuli that elicit a marked increase in HR, BP, and sympathetic nervous system activity. In agreement with our original hypothesis, the expected response to a sympathoexcitatory stimulus is a substantial increase in HR, BP, and CVRi. Indeed, we observed a 25% increase in HR, 27% increase in MAP, 25% increase in SBP, 26% increase in DBP, and a 22% increase in CVRi during the final tertile of IHG exercise. Moreover, each of these variables remained elevated during post-exercise ischemia. Contrary to our hypothesis, however, a lower percent change in HR, MAP, SBP, DBP, and CVRi in response to IHG exercise was associated with greater WMH burden in middle-aged and older adults. One explanation for these findings is that failure to increase resistance in the cerebral circulation (i.e., CVRi) may lead to propagation of highly pulsatile blood flow into the delicate microcirculation suggesting that individuals with greater WMH burden may have impaired regulation of cerebral blood flow. Along these lines, following an acute hypertensive stimulus (a single bout of resistance training), older adults demonstrated a greater increase in cerebral PI despite no change in mean MCAv compared with young adults (Rosenberg et al., 2020). These results suggest that cerebral blood flow regulation may be impaired following an acute hypertensive stimulus in older adults, which may lead to greater transmission of pulsatile flow into the microcirculation and increased risk of end-organ damage.

Consistent with this idea, carotid artery PP, carotid PI, cerebral PI, and aortic stiffness are associated with increased risk for silent subcortical infarcts (Mitchell et al., 2011). Additionally, aortic stiffness is associated with higher WMH volume and carotid PI is associated with lower gray and white matter volumes (Mitchell et al., 2011). In the cerebral circulation, cerebral PI is positively associated with greater WMH volume in middle-aged and older adults (Tarumi et al., 2014). These findings suggest that aortic stiffness and transmission of pulsatile flow into the microcirculation may lead to quantifiable changes in brain volume and increased WMH burden. A recent study conducted by Tarumi et al. in young adults investigated cardiovascular variables and cerebral blood flow in response to repeated bouts of rhythmic handgrip exercise using phase-contrast MRI (Tarumi et al., 2021). Using a similar relative intensity of handgrip exercise (30-40% MVC), HR, BP, CVRi, cerebral blood flow, and respiratory rate increased during rhythmic handgrip exercise despite no change in vessel cross-sectional area (Tarumi et al., 2021). Thus, higher resistance in the cerebral vessels may attenuate an increase in cerebral blood flow and prevent cerebral hyperperfusion during rhythmic handgrip exercise, which may represent a compensatory myogenic response to a hypertensive stimulus in order to dampen pulsatile flow. Failure to increase resistance in the cerebral vessels (indicated by a smaller increase in CVRi) during an acute sympathoexcitatory stimulus suggests a potential lack of active vasoconstriction occurring in the cerebral circulation that may allow for propagation of pulsatile flow into the microcirculation. Broadly, acute instances of physiological stressors that affect BP and cerebral blood flow, experienced across the lifespan, may affect white matter health. We report that individuals who demonstrated greater increases in HR, BP, and CVRi in response to IHG exercise had lower WMH burden, which was opposite of our hypothesis. It is possible that increases in CVRi during a sympathoexcitatory stress, indicating active vasoconstriction, may protect the cerebral circulation from increases in pulsatile flow. For example, in spontaneously hypertensive rats, smooth muscle hypertrophy in the cerebral arteries occurs, leading to increased cerebrovascular resistance (Tayebati et al., 2012). Changes in structure and function in the cerebral circulation may protect the brain from high perfusion pressures during periods of elevated BP and augmented cerebral blood flow (Faraci and Heistad, 1990; Tayebati et al., 2012). Alternatively, individuals with greater WMH burden or

reduced white matter integrity (which was not measured in the present study) may have impaired cardiovascular responses to sympathoexcitatory stress. However, this is unlikely as WMH volumes in the present study are low, likely due to the average age of participants in this study being only ∼63 years.

In agreement with the findings from Tarumi et al., we report significant increases in HR, BP, MCAv, and CVRi during IHG exercise in middle-aged and older adults. Yet, the cardiovascular and cerebrovascular responses were highly variable. While IHG exercise was performed at the same relative intensity for all participants, we observed a wide range of responses in HR (3-63% increase), MAP (8-57% increase), MCAv (−20 to + 23% change), and CVRi (−4 to + 80% change) during the final tertile of IHG exercise. In agreement with our findings, the percent change in MAP and MCAv in response to rhythmic handgrip exercise appears to be heterogeneous (Giller et al., 2000) whereby MCAv may decrease in some individuals. Our findings indicate that middle-aged and older adults demonstrate varying responses to an acute sympathoexcitatory stimulus performed at the same relative intensity. IHG exercise represents an acute sympathoexcitatory stimulus that may be comparable to carrying an object for an extended period of time. As these types of activities are performed frequently throughout activities of daily living, evaluating the cardiovascular and cerebrovascular response to repeated bouts of acute sympathoexcitatory stress may be important for understanding changes in the brain prior to presentation of symptoms of cognitive decline. Altered expected responses to acute physiological stressors such as IHG exercise, which induce brief, large increases in BP, may be associated with measurable changes in brain volume and WMH.

Hypertension is associated with increased risk of developing WMH (Dufouil et al., 2001). Although we may be underpowered to adequately control for controlled hypertension (n = 13, 19% of participants) in our population of middle-aged and older adults, associations with WMH fraction were evaluated with controlled hypertension included in the linear model (in addition to age at MRI, sex, *APOE* ε4 status, and work performed during the IHG exercise protocol). After adjusting for controlled hypertension, negative associations between the percent change in HR, MAP, SBP, and DBP during IHG exercise and WMH fraction remained significant. Yet, there was no longer an association between the percent change in CVRi during IHG exercise and WMH fraction. Although the individuals with controlled hypertension were evenly distributed throughout the entire group for the variables of interest, previous work has suggested that older hypertensive adults demonstrate greater increases in raw MAP values and sympathetic nerve activity in response to IHG exercise performed at 30 and 40% MVC compared with normotensive older adults (Delaney et al., 2010). Additionally, older hypertensive adults exhibit a more rapid increase in MAP and sympathetic nerve activity (within the first 10s of muscular contraction) compared with normotensive older adults (Greaney et al., 2015). In the aforementioned studies, older adults in the hypertensive group currently taking antihypertensive medication (80% of participants) were instructed to refrain from taking medication for two days prior to the experimental study (Delaney et al., 2010; Greaney et al., 2015). In the present study, there were no differences in the change in raw MAP and CVRi values between adults with controlled hypertension and those who were normotensive ($P > 0.05$ for both), although the aims of the present study did not include evaluating the effects of controlled hypertension. One possible explanation for the discrepancy between previous studies (Delaney et al., 2010; Greaney et al., 2015) and our findings is that individuals with uncontrolled hypertension were excluded from our study. Additionally, participants in the present study were instructed to take prescription medication on the day of the study, which could have influenced the response to IHG and post-exercise ischemia. Nevertheless, when controlled hypertension was added to the linear model, the inverse association between the percent change in HR, MAP, SBP, and DBP during IHG exercise and WMH fraction remained significant ($P < 0.05$ for all; **Supplementary Table 1**). These results suggest that regardless of controlled hypertension status, an attenuated increase in HR and BP in response to IHG exercise is associated with greater WMH burden in middle-aged and older adults.

LIMITATIONS

In order to determine CVRi, we measured the cerebral hemodynamic response to IHG exercise in the MCA using TCD. A major assumption of TCD is that the MCA diameter remains constant at rest and during a stimulus. Previous work evaluating MCAv and MCA diameter in response to rhythmic handgrip exercise has demonstrated that MCA diameter may decrease by ∼ 2% in adults aged 20-59 years old suggesting active vasoconstriction in the cerebral circulation during rhythmic handgrip exercise (Verbree et al., 2017). However, the cross sectional area of the internal carotid and vertebral arteries do not change in response to rhythmic handgrip exercise performed at 30-40% MVC in young adults (Tarumi et al., 2021). It is possible that IHG exercise causes vasoconstriction of the MCA which may cause an increase in MCAv despite little or no change in cerebral blood flow (Giller et al., 2000), though the effect of isometric versus rhythmic handgrip exercise on MCA diameter is currently unknown. While the present study evaluated MCAv in response to IHG exercise in a robust sample size of middle-aged and older adults, the cerebrovascular response to IHG exercise and potential associations with WMH burden should be interpreted with caution. Additionally, all participants performed the IHG exercise protocol using their left hand, regardless of handedness. Due to the experimental setup, we evaluated MCAv in the left (ipsilateral) MCA. When MCAv was evaluated in both the right and left MCA during rhythmic handgrip exercise in adults aged 21-43 years old, previous studies have shown that MCAv increased in the contralateral side only (Jørgensen et al., 1993; Linkis et al., 1995). In the present study, we report a significant, albeit variable, increase in MCAv in the ipsilateral MCA during IHG exercise in adults aged 55-69 years old, which we interpret as a global response. It is possible that we could have observed a greater increase in MCAv in the contralateral (right) MCA during IHG exercise, and follow-up studies should perform bilateral MCA assessments. Lastly, arterial CO_2 is a powerful regulator of cerebral vascular tone (Brian, 1998) and

we utilized $ETCO_2$ as a surrogate measure of arterial CO_2. A reduction in $ETCO_2$ may cause cerebral vasoconstriction and potentially a decrease in cerebral blood flow. We observed a small (1-2 mmHg), but significant, reduction in $ETCO_2$ during the final tertile of IHG exercise which persisted during post-exercise ischemia. Similarly, reductions in $ETCO_2$ have also been observed during isometric (Muza et al., 1983; Fontana et al., 1993), and rhythmic handgrip exercise (Jørgensen et al., 1993; Tarumi et al., 2021). It is possible that a 1-2 mmHg decrease in $ETCO_2$ could have resulted in a decrease in MCAv in this study.

CONCLUSION

In summary, IHG exercise performed at 40% MVC until fatigue elicited significant increases in HR, BP, MCAv, and CVRi in cognitively unimpaired middle-aged and older adults. Individuals with greater WMH burden demonstrated a lower percent change in HR, BP, and CVRi in response to IHG exercise. Together, these results indicate an inverse association between WMH burden and cardiovascular and cerebrovascular responses to a sympathoexcitatory stimulus.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by University of Wisconsin-Madison Institutional Review Board. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

JB conceived and designed the research. AP, KM, AC, NE, AH, and JB performed the experiments. AP analyzed the data. AP, KC, and JB interpreted the results of experiments. AP prepared the figures. AP and JB drafted the manuscript. All authors edited and revised the manuscript, and approved the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnagi.2022.888470/full#supplementary-material

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Ovarian steroid hormones: A long overlooked but critical contributor to brain aging and Alzheimer's disease

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Ovarian hormones, particularly 17β-estradiol, are involved in numerous neurophysiological and neurochemical processes, including those subserving cognitive function. Estradiol plays a key role in the neurobiology of aging, in part due to extensive interconnectivity of the neural and endocrine system. This aspect of aging is fundamental for women's brains as all women experience a drop in circulating estradiol levels in midlife, after menopause. Given the importance of estradiol for brain function, it is not surprising that up to 80% of peri-menopausal and post-menopausal women report neurological symptoms including changes in thermoregulation (vasomotor symptoms), mood, sleep, and cognitive performance. Preclinical evidence for neuroprotective effects of 17β-estradiol also indicate associations between menopause, cognitive aging, and Alzheimer's disease (AD), the most common cause of dementia affecting nearly twice more women than men. Brain imaging studies demonstrated that middle-aged women exhibit increased indicators of AD endophenotype as compared to men of the same age, with onset in perimenopause. Herein, we take a translational approach to illustrate the contribution of ovarian hormones in maintaining cognition in women, with evidence implicating menopause-related declines in 17β-estradiol in cognitive aging and AD risk. We will review research focused on the role of endogenous and exogenous estrogen exposure as a key underlying mechanism to neuropathological aging in women, with a focus on whether brain structure, function and neurochemistry respond to hormone treatment. While still in development, this research area offers a new sex-based perspective on brain aging and risk of AD, while also highlighting an urgent need for better integration between neurology, psychiatry, and women's health practices.

KEYWORDS

hormones, menopause, estrogen, neuroimaging, Alzheimer's disease, hormone therapy, menstrual cycle, pregnancy

Introduction

Sex is a genetic modifier of brain aging and risk of neurodegenerative disease

Sex differences in disease prevalence, manifestation, and response to treatment are rooted in genetic and hormonal differences between men and women. The effects of sex on neural aging phenotypes are often as large as, if not larger than the effects of other important variables (Cahill, 2006). In fact, female sex is the second greatest risk factor for late-onset Alzheimer's disease (AD), second only to the aging process itself (Farrer et al., 1997). Moreover, susceptibility to aging-related neurodegenerative diseases and mental health conditions is greater in women than men, whereas men exhibit higher rates neuropsychiatric and learning disorders with developmental origins (Jazin and Cahill, 2010; McCarthy, 2016; Mauvais-Jarvis et al., 2020).

For decades, the general mindset was that sex differences in brain structure and function were controlled by a unitary program: genetic sex as the determinant of gonadal sex, and gonadal hormones as the determinants of brain sexual differentiation and subsequent neurophysiological and behavioral outcomes (McCarthy, 2016). Evidence has accumulated that numerous sex-specific factors, including hormonal, but also genetic and environment-driven epigenetic mechanisms, act in concert to provoke or eliminate sex differences in brain (McCarthy et al., 2009; Giatti et al., 2019). The combination of all these genetic and hormonal variables generates two different neurobiological systems in men and women. Starting at puberty, cells with androgen or estrogen receptors will be affected differently in men and women (McEwen, 2002), eliciting differences in disease predisposition, manifestation, and response to treatment. Overall, genetic sex is an important modifier of neurophysiology and neuropathology via genetic, epigenetic, and hormonal regulations (Cahill, 2006).

The value of understanding sex differences in brain aging and neurodegenerative disease is as self-evident as it is underappreciated. Historically, for multiple reasons, including the purported safety of women and their offspring, women of childbearing age were excluded from clinical trials (Clayton, 2016). As a result, for several decades, evidence-based medicine was defined by male physiology. In 1993, the US National Institutes of Health (NIH) mandated the inclusion of women in NIH-funded clinical trials, but many investigators did not follow this mandate (Schiebinger et al., 2016). This was followed by a 2014 mandate to consider sex as a biological variable in basic research (Mazure and Jones, 2015). However, based on arguments that ovarian hormone fluctuations made female animals too volatile to assess, preclinical research and drug development studies have also predominantly used male animal models (McCarthy et al., 2017). Today, even though women are included in biomedical research, the data from both clinical trials and research studies is rarely broken down by sex.

The field of cognitive aging and AD is no exception as sex and gender are more likely to be used as confounders than predictors. As of 2022, of all clinical trials of AD, none has set out to examine sex differences in efficacy or outcomes (Ferretti et al., 2018). Recent research, however, has elucidated the important neuroprotective role of ovarian steroid hormones and their receptors for cognitive aging and AD (Morrison et al., 2006; Brinton et al., 2015).

Focus of this review

This review explores the role of ovarian steroid hormones, especially 17β-estradiol, as contributors of brain aging and risk of AD or dementia. Endogenous exposures to ovarian hormones include chiefly pubertal timing, the menstrual cycle, pregnancy, and menopause. Exogenous hormonal exposures include chiefly use of hormonal therapy such as oral contraceptives and menopause hormone therapy (MHT). Throughout the review, the emphasis is on studies that used brain biomarkers of AD, primarily brain imaging, conducted in cisgender women with sound methodology.

Search strategy and selection criteria

We conducted a systematic review of neuroimaging studies of the menstrual cycle, oral contraceptives, menopausal status, and randomized clinical trials of MHT, as well as of imaging studies of pubertal timing and pregnancy as related to cognitive aging and AD risk. We also provide a narrative review of psychometric studies of all exposures. We searched PubMed and the Web of Science for papers published in English between 1998 and 2022, using "estrogen," "sex steroids," "ovarian hormones," or "sex hormones," all exposures and outcomes as search terms. Although we tried to cite seminal studies as necessary, because of space limitation, representative reviews were also selected. We also provide general information on the actions of ovarian steroid hormones in brain to provide context for the research findings linking these hormones to brain aging and AD risk.

Action of ovarian steroid hormones in brain

The brain is a target for ovarian steroid hormones

The primary hormones secreted by the ovaries are 17β-estradiol (estradiol, E2), the most prevalent form of estrogen

produced before menopause, and progesterone, a type of progestagen. Both hormones pass through the blood–brain barrier and have receptors throughout the brain (McEwen, 2002). As reviewed below, estradiol receptors (chiefly ERα and ERβ) are present throughout areas of the brain involved in both reproductive and cognitive functions (McEwen et al., 2001). However, there is controversy regarding estrogen receptor expression across species, especially ERβ, due to limited ERβ antibody specificity (Maioli et al., 2021). Validated techniques have confirmed ERβ in rodent but not human brain (Maioli et al., 2021). Development of ERβ antibodies with higher binding specificity is needed to resolve this inconsistency, as discussed elsewhere (Andersson et al., 2017). Moreover, despite animal research demonstrating the presence of progesterone receptors (PRA and PRB) in brain regions involved in cognition, little is known about their location or function in the human brain (Brinton et al., 2008). As such, here we focus primarily on the action of estradiol on brain structure and function.

Estradiol is a steroid hormone synthesized in a series of enzymatic steps beginning with the conversion of cholesterol into pregnenolone in the mitochondria (McEwen, 2002). The final enzymatic step, the conversion of testosterone into estradiol, is catalyzed by the enzyme aromatase, or estrogen synthase (McEwen, 2002). In neurons and astrocytes, depending on tissue and time period, estradiol can also be synthesized from androstenedione and estrone (E1) (Cui et al., 2013). Starting at puberty, and for the duration of a woman's reproductive life, estradiol is mainly produced in the ovary. Its levels in plasma change during development, fluctuate cyclically during the menstrual cycle, increase dramatically during pregnancy, drop during lactation, and eventually decline after menopause (McEwen, 2002).

Estradiol is also locally synthesized in different tissues, including brain. Recent research demonstrates that the brain is a steroidogenic organ (Arevalo et al., 2015) expressing the molecules and enzymes necessary for the conversion of endogenous cholesterol into local estradiol. As a result, the brain is a target for the action of both peripheral estradiol and neuroestradiol, e.g., estradiol synthesized in neural cells (Arevalo et al., 2015). There is emerging evidence that both types of estradiol, from ovaries and brain, control various neurobiological processes, including sexual behavior, but also neurological functions such as regulation of body temperature and blood pressure, response to stress, some aspects of mood and of cognition (Lupien et al., 2009). Importantly, brain steroidogenesis is regulated independently of peripheral steroidogenesis, and brain steroid levels do not correlate with plasma steroid levels in animals (Caruso et al., 2013).

Moreover, there is some evidence that the brain upregulates synthesis of neurosteroids in response to the drop in estrogen following oophorectomy as a compensatory adaptive reaction (Caruso et al., 2010). This suggests that similar mechanisms might be in place in response to naturally occurring declines in ovarian hormones following spontaneous menopause, though this remains to be confirmed.

Estradiol: The "master regulator" of the female brain

Estradiol has been called the "master regulator of the female brain" (Rettberg et al., 2014) due to its wide range effects on neuronal structure and function. Its neuroprotective role is of particular relevance for cognitive aging and AD. In mouse models of AD, decreasing estradiol levels in plasma following oophorectomy exacerbate brain damage under neurodegenerative conditions (Azcoitia et al., 1999), trigger decrease cerebral glucose metabolism (CRMglc) (Ding et al., 2013), and increase amyloid-β fibrillization (Yue et al., 2005).

Estrogen therapy reduces such damage (Azcoitia et al., 1999), normalizing CMRglc and reducing Aβ oligomers in oophorectomized mice (Yue et al., 2005). Estradiol's neuroprotective action may be related to its role in maintaining metabolic homeostasis in body and brain (Frank et al., 2014; Rettberg et al., 2014). In brain, estradiol regulates glucose metabolism, glycolysis, oxidative phosphorylation and subsequent ATP generation in neurons (Rettberg et al., 2014). Substantial evidence indicates that metabolic alterations play a role in neurodegenerative diseases including AD (Lin and Beal, 2006).

Additionally, genetic studies have identified variants of the gene encoding for the aromatase enzyme that are associated with an increased risk for AD (Iivonen et al., 2004; Huang and Poduslo, 2006; Medway et al., 2014) These genetic variants may result in decreased estradiol synthesis in brain, which, together with decreased serum estradiol levels in post-menopausal women may increase the risk for AD (Huang and Poduslo, 2006). Aromatase expression is indeed increased in prefrontal cortex of patients with severe AD, a phenomenon that has been interpreted to be part of a "rescue program" (Luchetti et al., 2011).

Estrogen receptors (ERs) also coordinate multiple neuroprotective signaling cascades, either via direct activation or by the interaction of ERs with the receptors for other neuroprotective factors. Estradiol action in brain can be both delayed in onset and prolonged in duration ("genomic") or rapid in onset and short in duration ("non-genomic") (McEwen and Milner, 2017). Both ERα and ERβ are expressed in regions including hippocampus, amygdala, and hypothalamus, their distribution density differs. ERα shows greater distribution in hypothalamic nuclei associated with sexual behavior, whereas ERβ is expressed more in areas associated with cognition such as basal forebrain, prefrontal cortex, temporal and parietal regions, and posterior cingulate (Foster, 2012). Additionally, while both ERα and ERβ participate in the overall

neuroprotective action of the estradiol, ERα is more closely involved in neuroprotection, as demonstrated by animal models of focal ischemia (Dubal et al., 2001), whereas ERβ has been shown to be involved in cognition, thought to promote learning and memory, neural plasticity, and regulating neurotrophic factors (Zhao et al., 2015). The G-protein coupled estrogen receptor (GPER1) shows widespread brain distribution, with heavy concentration in key brain regions including hippocampus and amygdala (Hadjimarkou and Vasudevan, 2018) and play a key role in mediating the rapid action of estradiol.

ERα and ERβ are also implicated in modulating the immune system. Both receptors are expressed on microglia and astrocytes, both involved in neuroinflammation and implicated in Alzheimer's disease (Mishra and Brinton, 2018). Activation of ERα and ERβ via estradiol treatment has been reported to decrease inflammatory responses such as phagocytosis and cytokine secretion, ultimately having an anti-inflammatory and neuroprotective effect (Mishra and Brinton, 2018). Activation of ERα has also been reported to shorten the inflammatory response to infection in preclinical studies (Villa et al., 2015). There is increasing evidence that chronic inflammatory processes are activated during midlife chronological and endocrine aging, which ultimately limit the clearance capacity of microglia and lead to immune senescence (Mishra and Brinton, 2018). The inflammatory immune response is a possible unifying factor that bridges across the three major risk factors for AD in women: aging, menopause, and ApoE epsilon 4 (ApoE4) genotype (Mishra and Brinton, 2018).

Influence of sex hormones across a woman's life

Ovarian hormones affect the nervous system in ways that extend beyond their essential actions of regulating gonadotropin secretion and modulating sexual behavior. As reviewed below, at a neurological level, estrogens are involved in regulation of thermoregulation, mood, sleep, and cognitive abilities, among other factors (McEwen et al., 1997). From a cognitive aging perspective, both estradiol and progesterone influence verbal memory, fluency, performance on spatial tasks, and fine motor skills (Maki and Henderson, 2016). Declines in these hormones with menopause have been associated with an increased risk of cognitive impairment, affective disorders, and AD pathology (Rahman et al., 2019; Jett et al., 2022).

In what follows, we review research elucidating the role of ovarian steroid hormones in cognitive aging and AD risk across the female lifespan, including studies of puberty, menstrual cycle, hormonal contraceptive use, the menopause transition, and hormone therapy for menopausal symptoms.

Pubertal timing and menstrual cycle

Puberty is characterized by surges in the production of sex hormones, which in turn prompt dramatic organizational changes in the brain, followed by transformative changes in cognition and behavior (Sisk and Foster, 2004). For girls, the maturation of the ovaries with the subsequent production of estradiol and progesterone typically occurs around age 11–12 years, ranging from 10 to 18 years (Anderson et al., 2003). This results in the development of secondary sexual characteristics and of menarche, or the first menstrual bleeding.

There is ample evidence that ovarian sex hormones influence brain development and cognition during adolescence. While reviewing these findings is beyond the scope of this review, we recommend prior reviews on the topic (Giedd et al., 1999; Sisk and Foster, 2004; Blakemore, 2008). Herein, we focus on links between early hormonal exposures and cognitive aging in midlife and older age. Of all the factors involved in puberty and adolescence, two have been consistently examined as possible predictors of future cognitive impairment and AD or dementia: pubertal timing and the menstrual cycle.

Pubertal timing and age at menarche

A recent hypothesis posits that the brain has declining sensitivity to sex hormones throughout adolescence, such that females who mature early have greater effective ovarian hormone exposure than those who mature late (Schulz et al., 2009). The age at which a woman enters menarche has gained attention for a possible relationship with cognition in later life due to longer estrogen exposure when menarche occurs at a younger age (Bernstein et al., 1991). While research on this topic is scant, some studies indicate associations between an early age at menarche and greater white matter integrity in frontal cortex in adolescence (Chahal et al., 2018). Thus, pubertal timing may facilitate brain maturation due to longer exposure to ovarian sex hormones, which may in turn confer greater brain reserve later in life.

Nonetheless, the majority of studies so far indicate null associations between age at menarche and cognitive impairment or AD risk (Geerlings et al., 2001; Henderson et al., 2003; Colucci et al., 2006; Fox et al., 2013; Prince et al., 2018; Najar et al., 2020; Song X. et al., 2020). On the other hand, in some studies, a younger age at menarche correlated with better visual memory performance on Benton's visual retention test and psychomotor speed on a trail making task (task A) (Ryan et al., 2009), and with a reduced risk of dementia or AD in later life (Rasgon et al., 2005a; Gilsanz et al., 2019). Additionally, the Gothenburg H70 Birth Cohort study reported associations between a younger age at menarche and lower CSF $A\beta_{42/40}$ ratio and higher hyperphosphorylated tau levels among older post-menopausal

women free of dementia (Najar et al., 2021). More studies of pubertal timing, ideally spanning puberty and young adulthood to midlife and beyond, and including the use of AD biomarkers, are needed to clarify the strength and reproducibility of these associations.

Menstrual cycle

The typical menstrual cycle is 28 days long, with normal variation ranging from 22 to 35 days (Reed and Carr, 2000; Grieger and Norman, 2020). Menstruation is generally considered the beginning of the cycle, which is divided into two phases, follicular and luteal. The follicular phase begins after the first day of menstruation and is characterized by initial low levels of both estradiol and progesterone followed by rising estradiol. Estradiol levels peak before ovulation (∼day 14), triggering the release of luteinizing hormone (LH). The luteal phase begins after ovulation and is characterized by a decrease in estradiol that settles at moderate levels, while progesterone begins to rise. If the egg is not fertilized, estradiol and progesterone decline during the second half of the phase (i.e., premenstrual phase), triggering menstruation and a new cycle. As these phases are relatively easy to pinpoint, studies of the menstrual cycle offer a unique opportunity to clarify the influence of ovarian hormones on neuronal circuits implicated in the regulation of cognitive and emotional processing.

Seminal animal studies from the early 1990's demonstrated that estradiol levels regulate synaptogenesis and synapse density on excitatory spines in hippocampal CA1 pyramidal neurons in female rats (Woolley and McEwen, 1992), which have been since replicated by many investigators (for example, Hara et al., 2015; McEwen and Milner, 2017; Sheppard et al., 2019). Fluctuations in synaptogenesis occur throughout the estrous cycle, with increases in synapses on dendritic spines after estrogen treatment, along with decreases in spine synapse density that occurs between the days of proestrus and estrus (Woolley and McEwen, 1992). Consistent with these observations, neuroimaging and cognitive studies provide evidence for changes in brain structure, function, and cognitive performance across the menstrual cycle or as a function of ovarian hormones.

The long-held view is that verbal memory and implicit memory are enhanced in the late follicular and midluteal phase, when estradiol is high (Hampson, 1990; Maki et al., 2002; Pletzer et al., 2011), whereas spatial and numerical abilities are enhanced in the early follicular phase, when estradiol is low (Hausmann et al., 2000; Courvoisier et al., 2013). Nonetheless, results are generally inconsistent (Sacher et al., 2013; Sundström Poromaa and Gingnell, 2014). Specifically for brain aging and AD, only one study to date has investigated possible associations of menstrual cycles and AD risk (Fox et al., 2013). In a cohort of 89 elderly British women, Fox et al. (2013) reported a marginally significant association between the number of menstrual cycles, defined as the number of months between menarche and menopause, free from oral contraceptive use, pregnancy, breastfeeding, and post-partum anovulation, and a lower risk of AD. Each additional month of having a menstrual cycle corresponded to a 0.3% reduction in risk of AD.

Neuroimaging studies of the menstrual cycle are summarized in **Table 1**. Several structural MRI studies report changes in the volume of several cortical and subcortical regions across the menstrual cycle (**Figure 1**). Most studies indicate increased hippocampal or amygdala volumes during the late follicular phase, when estradiol levels are rising and progesterone is low (Protopopescu et al., 2008; De Bondt et al., 2013a; Lisofsky et al., 2015; Pletzer et al., 2018), with some exceptions (Ossewaarde et al., 2013). Two studies also demonstrate a direct association between higher estradiol levels and larger hippocampal volume (Barth et al., 2016; Pletzer et al., 2018), while another study found a positive association between estradiol levels and the volume of another limbic structure, the parahippocampal gyrus (Lisofsky et al., 2015). Insular volume has also been reported, being positively associated with estradiol levels and higher during the follicular phase (De Bondt et al., 2016). Prefrontal cortex volume and thickness also appear to be positively associated with estradiol levels (Dubol et al., 2021).

On the contrary, the volume of the basal ganglia and anterior cingulate cortex (ACC) is reduced during the late follicular phase, opposite the pattern observed for the hippocampus (Protopopescu et al., 2008; De Bondt et al., 2013a). In the mid-luteal phase, when estradiol levels are moderate and progesterone levels are high, ACC volume increased. The increase in ACC volume was inversely correlated with estradiol levels, and positively correlated with progesterone levels (De Bondt et al., 2013a; Pletzer et al., 2018). Other regions, including fusiform gyrus, insula, and some parts of the temporal and frontal cortices, also change in size across the menstrual cycle (Pletzer et al., 2018).

Functional MRI studies also provide evidence of differential activation patterns during the menstrual cycle. A recent systematic review of neuroimaging studies indicates increased prefrontal cortical activity during cognitive tasks during the mid-luteal phase (Dubol et al., 2021). There is mixed evidence for preferential ACC activation exhibits greater activation and functional connectivity during the early follicular (menstrual) phase and late follicular phase compared with the midluteal phase (Thimm et al., 2014), or in the midluteal compared to the late follicular phase (Diekhof and Ratnayake, 2016). Activity in hippocampus (Pletzer et al., 2019b) and insular cortex (Dubol et al., 2021) during cognitive activities tend to be greater during the follicular phase.

Additionally, resting state fMRI studies indicate that some regions within the Default Mode Network (DMN) are more connected in the early follicular phase, when estradiol and progesterone levels are low (Petersen et al., 2014;

TABLE 1 Summary of studies investigating the effects of the menstrual cycle and of use of hormonal contraceptives on neuroimaging outcomes.

Study	Participants	Age, years	Exposure	Neuro-imaging technique	Cognitive measures	Study design	Main findings
Marečková et al. (2012)	10 naturally cycling women, 10 OC users	18–29	Menstrual cycle, OC	fMRI	Emotional processing	Repeated measures analysis	OC users exhibited higher BOLD signals in right FFA to ambiguous and angry faces vs. naturally cycling women Higher BOLD signal in FFA when observing angry faces during menstruation Longer OC duration was associated with higher BOLD signal in left FFA during ambiguous and angry face conditions
De Bondt et al. (2013b)	15 naturally cycling women, 15 OC users	18–28	Menstrual cycle, OC use	DTI	–	Repeated measures analysis	Higher mean diffusivity in fornix in OC users vs. naturally cycling women during the luteal phase Mean diffusivity in fornix was negatively associated with luteinizing hormone and estradiol
Bayer et al. (2014)	22 naturally cycling women	19–33	Menstrual cycle	fMRI	Emotional processing	Repeated measures analysis	No effect of menstrual cycle on recognition accuracy Higher recollection performance for negative items during early follicular phase vs. luteal phase Greater activity in HIP and ACC during both positive and negative emotional stimuli during early follicular phase vs. luteal phase Greater activity in bilateral ACC during positive emotional stimuli during early follicular phase vs. luteal phase Greater activity in left AMY during negative emotional stimuli during luteal phase vs. early follicular phase
Gingnell et al. (2014)	16 naturally cycling women, 17 women with PMDD	34 (9)	Menstrual cycle	fMRI	Emotional processing	Repeated measures analysis	No cycle phase difference on functional connectivity PMDD women rated social stimuli as more negative than controls during luteal phase PMDD women exhibited higher activity in AMY and insula, and lower activity in ACC toward social stimuli than controls during luteal phase. No group differences during follicular phase
Hjelmervik et al. (2014)	16 naturally cycling women	23 (5)	Menstrual cycle	Resting state fMRI	–	Repeated measures analysis	No effects of menstrual cycle on resting state connectivity
Petersen et al. (2014)	46 women using OCs (22 during placebo pill phase; 24 during active pill phase) vs. 45 naturally cycling women (20 in early follicular phase 25 in midluteal phase)	18–40	Menstrual cycle and OC use	Resting state fMRI	–	Group comparisons of menstrual cycle phases and OC pill phases	Greater connectivity of DMN regions in early follicular vs. midluteal phase and vs. OC users Greater connectivity of ECN regions in early follicular vs. midluteal phase and active OC users Greater connectivity of ECN regions in placebo vs. active OC users
Pletzer et al. (2014)	14 women using combined OCs vs. 16 naturally cycling women	25 (5)	OC use	fMRI	Number tasks	Group comparisons	Lower FPN activation in OC users vs. natural cyclers in follicular phase Greater PFC and inferior parietal lobe activation in OC users vs. natural cyclers in midluteal phase
Thimm et al. (2014)	21 naturally cycling women	18–34	Menstrual cycle	fMRI and rsfMRI	Cognitive control/attention	Repeated measures analysis	Greater ACC activity during menstrual and late follicular vs. midluteal phase Greater connectivity between FPN regions during menstrual vs. luteal phase
Albert et al. (2015)	28 naturally cycling women	18–45	Menstrual cycle	fMRI	Montreal Imaging Stress Task	Between-group comparison	Greater left HIP activity during psychosocial stress during ovulation vs. early follicular phase Bilateral HIP activity during stress was positively associated with estradiol levels

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10.3389/fnagi.2022.948219

TABLE 1 (Continued)

Study	Participants	Age, years	Exposure	Neuro-imaging technique	Cognitive measures	Study design	Main findings
Biegon et al. (2015)	10 PRE, 10 POST	23–67	Menstrual cycle, menopause status	^{11}C-vorozole PET	–	Group comparison	Aromatase activity did not differ between cycle phases POST had lower Aromatase enzyme activity vs. PRE
De Bondt et al. (2015a)	10 naturally cycling women, 21 OC users	18–30	Menstrual cycle, OC use	^{1}H MRS	–	Repeated measures analysis	Higher GABA+/Cr ratios in PFC during ovulation vs. follicular phase, luteal phase, active and inactive OC phase No difference in GABA+/Cr ratios in PFC between active or inactive OC phase vs. follicular or luteal phases No difference in GABA+/Cr ratios in PFC between active vs. inactive OC phase
De Bondt et al. (2015b)	19 women using monophasic OCs vs. 18 naturally cycling women	24 (3)	Menstrual cycle and OC use	Resting state fMRI	–	Repeated measures analysis and group comparisons	No effects of menstrual cycle or OC use on resting state connectivity
Diekhof and Ratnayake (2016)	15 naturally cycling women	25 (2)	Menstrual cycle	fMRI	Reinforcement learning	Repeated measures analysis	Greater ACC activity to negative feedback during midluteal vs. late follicular phase ACC activity correlated with avoidance learning during midluteal phase Greater avoidance learning during midluteal vs. late follicular phase
Franke et al. (2015)	7 naturally cycling women	21–31	Menstrual cycle	Structural MRI, BrainAGE	–	Repeated measures analysis	No differences in GMV, WMV or CSF volume over the menstrual cycle Lower BrainAGE scores during ovulation vs. menses Higher estradiol levels associated with lower BrainAGE scores
Frokjaer et al. (2015)	60 naturally cycling women	24 (5)	Menstrual cycle	[^{11}C]DASB PET	–	Double-blind, randomized placebo-controlled study	No changes in serotonin activity Increases in depressive symptoms correlated positively with increase in serotonin binding within the GnRHa treated group
Henningsson et al. (2015)	56 naturally cycling women	24 (5)	Menstrual cycle	fMRI	Emotional processing	Double-blind, randomized placebo-controlled study	No effects of GnRHa vs. placebo
Jacobs et al. (2015)	13 naturally cycling women, 11 women with remitted MDD	43–50	Menstrual cycle	fMRI	Emotional processing	Repeated measures analysis	Reduced brain activity in left HIP, right AMY and hypothalamus during late follicular phase vs. early follicular phase in healthy controls after stress challenge No differences in brain activity for MDD women between early or late follicular phase
Lisofsky et al. (2015)	21 naturally cycling women (11 controls; 10 PMDD patients),	22–31	Menstrual cycle	Structural MRI	–	Repeated measures analysis	Larger HIP GMV in late vs. early follicular phase Estradiol levels positively correlated with PHG GMV
Petersen et al. (2015)	21 women in follicular phase, 25 women in luteal phase, 22 OC users in active phase, 22 OC users in inactive phase	18–40	Menstrual cycle, OC use	Structural MRI	–	Group comparison	Larger global GMV in naturally cycling women vs. OC users PCC and orbitofrontal cortex thickness greater in naturally cycling women vs. OC users Greater cortical thickness in follicular phase, luteal phase, and OC inactive phase vs. OC active phase
Pletzer et al. (2015)	22 women using antiandrogenic OCs vs. 18 women using androgenic OCs vs. 20 naturally cycling women in menstrual or early follicular phase	25 (6)	OC use	Structural MRI	–	Group comparisons	Larger FFA and PHG GMV in users of antiandrogenic OCs vs. naturally cycling women Smaller frontal GMV in users of androgenic OCs vs. naturally cycling women No group differences in HIP, PHG, and ACC

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10.3389/fnagi.2022.948219

TABLE 1 (Continued)

Study	Participants	Age, years	Exposure	Neuro-imaging technique	Cognitive measures	Study design	Main findings
Zhu et al. (2015)	10 naturally cycling women	18–38	Menstrual cycle	fMRI	Mental rotation task	Repeated measures analysis	Greater left superior parietal cortex activity during late follicular phase associated with decreased errors in mental rotation task vs. early follicular phase Greater right superior parietal and superior frontal cortex activity associated with longer reaction time during late follicular phase vs. early follicular phase
De Bondt et al. (2016)	24 naturally cycling women, 23 androgenic OC users, 10 anti-androgenic OC users	18–34	Menstrual cycle, OC use	Structural MRI	–	Repeated measures analysis	Larger insula GMV during ovulation vs. luteal phase No differences between androgenic OC users vs. anti-androgenic OC users No difference between follicular phase vs. OC use Somatic premenstrual symptoms were associated with frontal cortex GMV in androgenic OC users
Lisofsky et al. (2016)	28 naturally cycling women, 28 OC users	16–33	Menstrual cycle, OC use	Structural MRI, rsfMRI	Emotional processing, episodic verbal memory, working memory, spatial memory	Repeated measures analysis	Lower positive affect in OC users vs. naturally cycling women No changes in cognitive performance in either group Lower left AMY and PHG volume in OC users vs. naturally cycling women Negative functional connectivity between AMY, PHG and DLPFC in OC users vs. naturally cycling women
Pletzer et al. (2016)	16 women using androgenic OCs vs. 16 using antiandrogenic OCs vs. 18 naturally cycling women	25 (6)	Menstrual cycle and OC	rsfMRI	–	Repeated measures and group comparisons	Greater temporal-to-DMN connectivity during late follicular vs. menstrual/early follicular phase Greater connectivity of DMN during midluteal phase vs. menstrual/early follicular phase Increased PFC-to-DMN connectivity in androgenic OC users vs. menstrual/early follicular phase Increased basal ganglia-to-DMN connectivity in antiandrogenic OC users vs. menstrual/early follicular phase
Arnoni-Bauer et al. (2017)	18 naturally cycling women, 11 OC users	18–35	Menstrual cycle, OC	fMRI	–	Repeated measures analysis	Greater activity in AMY, ACC, insula, and hypothalamus during luteal phase and OC users vs. follicular phase No difference for OC users
Syan et al. (2017)	25 naturally cycling women	16–45	Menstrual cycle	rsfMRI	–	Repeated measures analysis	No differences in connectivity between menstrual cycle phases Progesterone positively correlated with connectivity of FPN and DMN regions in late luteal phase
Donishi et al. (2018)	93 naturally cycling women	18–24	Menstrual cycle	rsfMRI	–	Group comparison	Higher percentage of global hubs in frontal medial cortex during the follicular phase vs. luteal phase Global hubs in sensorimotor cortex were greater during luteal phase vs. follicular phase
Engman et al. (2018)	18 naturally cycling women, 17 OC users, all who had previously experienced OC-related negative affect	25 (4)	Menstrual cycle, Oral contraceptives	fMRI, rsfMRI	–	Double-blind, randomized placebo-controlled trial	Naturally cycling women exhibited higher RSFC in AMY to middle and superior frontal gyri, paracentral lobule, and cerebellum, and higher RSFC in dorsal ACC to middle frontal, superior and transverse temporal, postcentral gyri, during the luteal phase vs. follicular phase OC users exhibited higher dorsal ACC RSFC in superior frontal gyrus and precuneus and lower RSFC in AMY to postcentral gyrus during treatment vs. follicular phase Naturally cycling placebo users exhibited higher AMY RSFC in postcentral gyrus and cuneus vs. OC users during treatment

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10.3389/fnagi.2022.948219

TABLE 1 (Continued)

Study	Participants	Age, years	Exposure	Neuro-imaging technique	Cognitive measures	Study design	Main findings
Hjelmervik et al. (2018)	15 naturally cycling women	23 (5)	Menstrual cycle	^{1}H MRS	–	Repeated measures analysis	Higher creatine levels in left PFC vs. right PFC during follicular and menstrual phases, no hemisphere differences during luteal phase
Petersen et al. (2018)	18 naturally cycling women, 18 women with PMDD	18–41	Menstrual cycle	fMRI	Emotion regulation task	Repeated measures analysis	Women with PMDD exhibited lower negative emotion regulation during the luteal phase vs. follicular phase or naturally cycling luteal phase Lower activation in right DLPFC during emotion regulation task in women with PMDD during luteal phase vs. follicular phase and naturally cycling women in luteal phase No group or cycle phase differences in AMY activation
Pletzer et al. (2018)	55 naturally cycling women	18–35	Menstrual cycle	Structural MRI	–	Repeated measures analysis	Larger HIP GMV in late follicular phase vs. menstrual/early follicular and midluteal phases, which positively correlated with estradiol levels Greater basal ganglia GMV in menstrual/early follicular vs. late follicular phase, which positively correlated with progesterone levels
Dan et al. (2019)	20 naturally cycling women	21–29	Menstrual cycle	fMRI	Emotional face matching task	Repeated measures analysis	No significant difference between brain activation to negative emotional faces between mid-follicular vs. late-luteal phases
Petersen et al. (2019)	18 naturally cycling women, 17 women with PMDD	18–44	Menstrual cycle	fMRI	Emotion-regulation task	Repeated measures analysis	No effect of menstrual phase on resting-state functional connectivity Greater connectivity between middle temporal cortex and left ECN in PMDD women vs. controls Greater connectivity between left AMY and PCC, mid-cingulate cortex, and right angular gyrus, and between right AMY and middle temporal cortex during follicular phase vs. luteal phase
Pletzer et al. (2019a)	131 naturally cycling women (79 past OC users, 52 non-users)	18–35	Previous OC use	Structural MRI	–	Group comparison	No GMV difference between OC past users and non-users Positive association between past OC duration and bilateral HIP and basal ganglia GMV Negative association between time since OC discontinuation and bilateral HIP and basal ganglia GMV Associations between OC duration and HIP GMV non-significant after controlling for time since OC discontinuation No difference between androgenic vs. anti-androgenic OC
Pletzer et al. (2019b)	36 naturally cycling women	25 (4)	Menstrual cycle	fMRI	Spatial navigation and verbal fluency	Repeated measures analysis	Increased HIP/PHG activity in preovulatory phase during navigation and fluency, which positively correlated with estradiol levels Increased caudate and DLPFC activity in midluteal phase during navigation and fluency, which positively correlated with progesterone levels
Sundström Poromaa et al. (2019)	90 naturally cycling women	18–49	Serum Allopregnanolone	^{11}C DASB PET	–	Group comparison	Negative association between serum allopregnanolone levels and serotonin binding in PFC
Şafak (2019)	32 naturally cycling women	20–40	Menstrual cycle	ADC	–	Group comparison	No differences between follicular phase vs. luteal phase
Weis et al. (2019)	19 naturally cycling women	18–34	Menstrual cycle	rsfMRI	–	Repeated measures analysis	Greater frontal-to-DMN connectivity during menstrual/early follicular vs. late follicular phase

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TABLE 1 (Continued)

Study	Participants	Age, years	Exposure	Neuro-imaging technique	Cognitive measures	Study design	Main findings
Herrera et al. (2020)	20 OC users	18–28	OC use	fMRI	n-back working memory task	Repeated measures analysis	Greater task load-related deactivation in frontal pole, PCC, and middle temporal gyrus during hormone-present phase vs. hormone-absent phase
Hidalgo-Lopez et al. (2020)	60 naturally cycling women	18–35	Menstrual cycle	rsfMRI	–	Repeated measures analysis	Decreased intrinsic connectivity in the right angular gyrus with medial prefrontal and posterior cingulate/precuneus areas during the luteal phase vs. pre-ovulatory phase Increased HIP EC during luteal phase vs. pre-ovulatory phase Higher ALFF in caudate during luteal phase vs. pre-ovulatory phase and menses Increased connectivity between right caudate and right middle frontal gyrus during pre-ovulatory phase vs. menses Increased connectivity between left putamen and contralateral dorsomedial thalamus during luteal phase vs. menses
Larsen et al. (2020)	16 OC users, 37 non-users (8 with IUD)	26 (5)	OC use	^{11}C SB207145-PET	–	Cross-sectional	9–12% reduced global serotonin binding in OC users vs. non-users, with largest difference in HIP
Meeker et al. (2020)	14 naturally cycling women	18–45	Menstrual cycle	fMRI, rsfMRI, and structural MRI	–	Repeated measures analysis	Greater GMV in parietal cortex during menstrual phase vs. follicular, ovulatory, and luteal phases Greater parietal WMV during ovulatory and luteal phases vs. follicular and menstrual phases Greater primary somatosensory cortex GMV during menstrual phase vs. follicular phase Greater WMV in right hemisphere during follicular phase vs. luteal phase Greater functional connectivity between left IPL and right visual cortex during ovulatory phase vs. luteal phase Greater functional connectivity between right and left IPL during ovulatory phase vs. follicular phase Greater functional connectivity between right IPL and left medial PFC during luteal phase vs. menstrual phase
Nasseri et al. (2020)	24 OC users (monophasic HC)	18–35	OC use	rsfMRI	–	Repeated measures analysis	Greater functional connectivity between left AMY and right VMPFC during hormone-present phase vs. hormone-absent phase after a stress test Greater functional connectivity between left PHG and right superior lateral occipital cortex during hormone-absent phase vs. hormone-present phase No differences in HIP functional connectivity between hormone-present phase vs. hormone-absent phase
Sharma et al. (2020)	48 naturally cycling women, 27 OC users	18–26	OC use	fMRI and structural MRI, DTI	Emotional n-back test	Group comparison	Lower GMV in right putamen in OC users vs. naturally cycling women Higher WMV in left PHG, HIP, right AMY, putamen, and rectus in OC users vs. naturally cycling women Higher FA in left HIP in OC users vs. naturally cycling women Higher brain activity in left lingual gyrus, paracentral lobule, right insula, frontal cortex, supplementary motor area in OC users vs. naturally cycling women during negative stimuli memory task No group difference in errors made during memory task

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TABLE 1 (Continued)

Study	Participants	Age, years	Exposure	Neuro-imaging technique	Cognitive measures	Study design	Main findings
Zhuang et al. (2020)	16 naturally cycling women	20–24	Menstrual cycle	fMRI and rsfMRI	Intertemporal binary choice task	Repeated measures analysis	Greater activation in bilateral lingual gyrus, calcarine gyrus, left middle and inferior occipital gyri during the mid-luteal phase vs. late follicular phase More activity in left putamen, HIP, insula, bilateral caudate and visual areas during delay discounting during late follicular phase vs. mid-luteal phase Greater activity in bilateral putamen when choosing short-term reward during late follicular phase vs. mid-luteal phase During the late follicular phase, greater dorsal striatum activity was associated with short-term reward choices. During the mid-luteal phase, greater DLPFC activity was associated with delayed reward choices Greater functional connectivity between left putamen and DLPFC during the mid-luteal phase vs. late follicular phase
Zhuang et al. (2020)	49 naturally cycling women	19–28	Menstrual cycle	rsfMRI	–	Group comparison	Greater activity in right DLPFC during mid-luteal phase vs. late follicular phase During the late follicular phase, relative progesterone levels were positively associated with ALFF in right HIP, thalamus, precuneus, and left angular gyrus. No associations between estradiol and brain activation During the mid-luteal phase, estradiol was positively associated with bilateral DLPFC and superior medial PFC ALFF. Relative progesterone levels positively correlated with temporal cortex ALFF
Menting-Henry et al. (2022)	18 naturally cycling women, 16 androgenic OC users, 16 anti-androgenic OC users	25 (6)	Menstrual cycle, OC use	fMRI, structural MRI	Emotion recognition	Group comparison	No group differences in emotion recognition performance No group differences in AMY GMV Lower ALFF in left PCC was associated with higher recognition of disgust in anti-androgenic OC users Right superior parietal lobe ALFF during sadness recognition was positively associated in naturally cycling women and negatively associated in anti-androgenic OC users Left AMY and ACC connectivity was negatively associated for naturally cycling women during fear recognition Right AMY and left middle frontal gyrus connectivity during fear recognition was negatively associated in naturally cycling women and positively associated in anti-androgenic OC users
Noyan et al. (2022)	13 control subjects and 13 subjects with Schizophrenia	18–45	Menstrual cycle	rsfMRI	–	Repeated measures analysis	No differences in functional connectivity between groups or cycle phases Estradiol levels positively correlated with connectivity of auditory network in the left AMY during the early follicular phase in schizophrenia patients Progesterone levels positively correlated with connectivity between left FPN and precuneus during the early follicular phase Progesterone levels negatively correlated with connectivity between the ECN in right superior frontal gyrus. No associations between estradiol and functional connectivity

Only studies since 2012 are included in the table.ACC, anterior cingulate cortex; ADC, apparent diffusion coefficient; ALFF, amplitude of low-frequency fluctuations; AMY, amygdala; BOLD, blood-oxygen-level-dependent; BrainAGE, Brain Age Gap Estimation; Cr, creatine; CSF, cerebrospinal fluid; DLPFC, dorsolateral prefrontal cortex; DMN, default mode network; EC, eigenvector centrality; ECN, executive control network; FA, fractional anisotropy; FFA, fusiform face area; fMRI, functional magnetic resonance imaging; FPN, frontoparietal network; GABA, gamma aminobutyric acid; GMV, gray matter volume; GnRHa, gonadotropin-releasing hormone agonist; HC, hormonal contraceptives; HIP, hippocampus; MDD, major depressive disorder; IPL, inferior parietal lobule; IUD, intrauterine device; MRI, magnetic resonance imaging; OC, oral contraceptives; PCC, posterior cingulate cortex; PET, positron emission tomography; PFC, prefrontal cortex; PHG, parahippocampal gyrus; PMDD, premenstrual dysphoric disorder; POST, post-menopausal; PRE, pre-menopausal; RSFC, resting-state functional connectivity; rsfMRI, resting-state functional magnetic resonance imaging; VMPFC, ventromedial prefrontal cortex; WMV, white matter volume.

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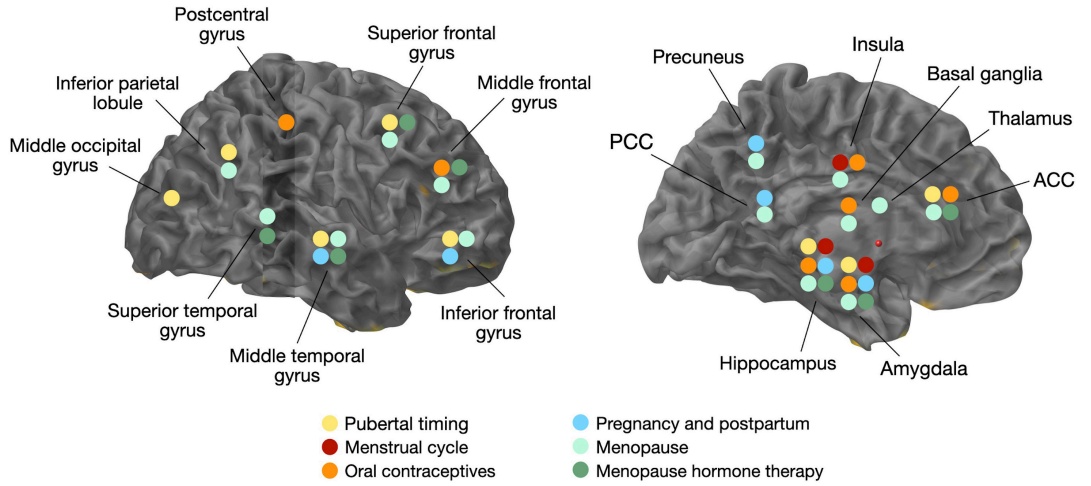

FIGURE 1
Overview of volumetric gray matter changes related to changes in estradiol levels and/or hormonal transitions. ACC, anterior cingulate cortex; PCC, posterior cingulate cortex.

Weis et al., 2019). Instead, another study reported no impact of menstrual cycle phase on DMN connectivity but increased connectivity between basal ganglia and frontoparietal attention network in midluteal phase, when both progesterone and estradiol are high (Pletzer et al., 2016). Some studies showed higher functional connectivity between amygdala and cingulate cortex, and amygdala with middle frontal gyrus (Petersen et al., 2019), and between ACC and the executive control network during the follicular phase as compared to the luteal phase, whereas dorsolateral prefrontal cortex and sensorimotor cortex are more connected with hippocampus (Arélin et al., 2015), resulting in greater activity in response to stimuli (Dubol et al., 2021), during the luteal phase compared to the follicular phase. Another study has reported that the hippocampus has greater whole brain functional connectivity at rest during the mid-luteal phase (Hidalgo-Lopez et al., 2020). Finally, a study comparing all three phases of the menstrual cycle showed higher hippocampal activation during the pre-ovulatory phase (e.g., higher estradiol) and higher fronto-striatal activation during the luteal cycle phase (e.g., higher progesterone) (Pletzer et al., 2019b). However, other studies comparing the three phases of the menstrual cycle did not confirm these associations (Hjelmervik et al., 2014; De Bondt et al., 2015b). Additionally, a study comparing the early follicular and mid-luteal phases found increased connectivity between angular gyrus and DMN, and between ACC with executive control network (ECN), during the follicular phase as compared to the mid-luteal phase (Petersen et al., 2014). A smaller study comparing mid-follicular and late luteal phases found no functional connectivity differences between menstrual phases (Syan et al., 2017). However, progesterone levels were positively correlated with connectivity of frontoparietal network (FPN) and DMN regions during the late luteal phase.

Although PET studies of the menstrual cycle are scarce and limited by small sample sizes, they did provide evidence for bioenergetic changes over the menstrual cycle, and limited to no effects on neurotransmitter activity. On [^{18}F]fluorodeoxyglucose (FDG) PET, cerebral glucose metabolism (CMRglc) was higher in thalamic, prefrontal, temporoparietal, and inferior temporal regions in the mid-follicular as compared to the luteal phase, whereas CMRglc in superior temporal, occipital, cerebellar, cingulate and anterior insular regions was higher in the luteal as compared to the follicular phase (Reiman et al., 1996). There were also no differences in overall brain glucose metabolic activity between the follicular and luteal phases as measured via FDG-PET (Rapkin et al., 2011). There is no evidence for changes in D2 dopamine receptor density during different phases of the menstrual cycle on [^{11}C]raclopride PET (Nordström et al., 1998), or for differences in serotonin binding between men and women in the follicular phase on [^{11}C]WAY-100635 PET (Stein et al., 2008). One study of [^{11}C]vorozole PET found no differences in aromatase activity between midcycle and late luteal phases (Biegon et al., 2015). A double-blind, randomized, placebo-controlled study investigating the effects of a gonadotropin-releasing hormone agonist (GnRHa) used [^{11}C]DASB PET to image serotonin transporter (SERT) binding during the follicular phase in naturally cycling women (Frokjaer et al., 2015). The researchers found that increased SERT binding in neocortex and lower estradiol levels in the GnRHa group was associated with depressive symptoms as compared to placebo (Frokjaer et al., 2015). Another study using [^{11}C]DASB PET reported that lower serum levels of allopregnanolone, which typically occurs during the follicular phase, was associated with greater SERT binding in prefrontal cortex (PFC) (Sundström Poromaa et al., 2019). However, several studies did not perform

follow-up scans during different menstrual cycle phases (Frokjaer et al., 2015; Sundström Poromaa et al., 2019), thus additional work is needed to elucidate the relationship between menstrual cycle effects on PET brain imaging.

Altogether, neuroimaging results indicate that hormonal changes during the menstrual cycle may impact widespread networks involved in memory, learning, attention, and emotion. It is possible that, as effects of ovarian hormones on synaptic activity are generally subtle, neuroimaging might be more sensitive to detecting these changes than cognitive tests. Since most fMRI studies show no links to cognitive performance despite detecting activational changes during the menstrual cycle, it's been hypothesized that not all effects of ovarian hormones might immediately translate to changes in cognition (Pletzer et al., 2019b). It is also possible that the brain compensates for cycling variations in ovarian hormone levels, leaving cognitive performance broadly unchanged throughout the menstrual cycle. Further, recent reviews suggest that menstrual cycle-related changes in cognition may be smaller than those in affective function and mood (Sacher et al., 2013). It is well established that the risk of depression becomes higher in women than in men starting at puberty (McGuire et al., 2019), and midlife depression is a risk factor for AD in turn (Livingston et al., 2020). Whether links between menstruation and mood are predictors of cognitive vulnerability later in life is under investigation.

Oral contraceptives

Hormonal contraceptives consist either of a synthetic progesterone (i.e., progestin), or a progestin and a synthetic estrogen (e.g., combined formulation). These exogenous hormones control ovulation by inhibition of follicular development, and suppression of the production of endogenous estradiol and progesterone (Taylor et al., 2021). Hormonal contraceptives have various routes of administration, including oral, transdermal, intrauterine, and transvaginal. The most common form of birth control is by means of oral contraceptives (OC), which are used by over 85% of women in the United States (Taylor et al., 2021). Most OC formulations contain 21 active pills followed by seven placebo pills, which do not halt menstruation. Placebo pills are placeholders meant to help you stay on track by taking one pill every day until the next month starts. Some formulations have longer or shorter pill phases. Other formulations contain 28 active (monophasic) pills, which halt menstruation. Most OC contain ethinylestradiol, a potent form of estradiol, and synthetic progestins with different hormone derivatives. As a result, pills can either be androgenic or anti-androgenic (Pletzer et al., 2019a; Taylor et al., 2021).

Given the effects of ovarian hormones on brain structure and function, examination of the effects of OCs on cognitive aging and AD risk provides important information for preventative efforts. Nonetheless, few studies have investigated whether OC use influences cognition. Most of these studies were conducted on young adult women, while a handful examined associations between OC use in young adulthood and midlife, and future risk of cognitive decline in older age. While some studies report no differences in cognitive performance between young adult women with natural cycles and OC users (Lisofsky et al., 2016), others suggest that OC therapy supports verbal memory (Warren et al., 2014; Beltz et al., 2015) but not verbal fluency (Griksiene et al., 2018). Users of pills with androgenic progestins may also show increased spatial ability (Griksiene et al., 2018). For the long-term, some studies report higher performance on cognitive testing (Egan and Gleason, 2012; Karim et al., 2016) or a reduced risk of cognitive impairment (Li et al., 2016; Song X. et al., 2020) in midlife women taking OC. One study reported an almost 50% lower risk of cognitive impairment in women aged 60 or older who had used birth control as compared to never-users (Li et al., 2016). The remaining studies report no associations between OC use and cognitive performance (Ryan et al., 2009; Tierney et al., 2013), cognitive decline (McLay et al., 2003), or dementia incidence (Najar et al., 2020). Inconsistent findings may be a result of discrepancies in several factors including the age of initiation, OC formulations, dosage and duration of use (Taylor et al., 2021).

Neuroimaging studies of OC use are summarized in **Table 1**. Generally, structural MRI studies of young adult women indicate that OC users have larger regional gray matter (GM) volumes than natural cycling women, chiefly in frontal, temporal and anterior cingulate cortices, as well as hippocampus, parahippocampal gyrus, and cerebellum (Pletzer et al., 2010, 2015; De Bondt et al., 2013a; **Figure 1**). Limited data from longitudinal studies suggest that frontal and ACC volumes may be larger during the active phase compared with the placebo phase, during which no hormones are given (Pletzer et al., 2010; De Bondt et al., 2013a). Another study observed larger hippocampal volume with longer duration of OC treatment in young adult women, although the associations were mild (Pletzer et al., 2019a). In a recent MRI study of midlife women at risk for AD, OC users exhibited greater GM volume in medial temporal lobe, precuneus, fusiform gyrus, parietal and frontal cortex as compared to never-users (Schelbaum et al., 2021), which is in line with findings in younger women (Pletzer and Kerschbaum, 2014). However, other studies reported reduced GM volume of amygdala, parahippocampal gyrus, hypothalamus, pituitary gland, posterior cingulate cortex and orbitofrontal cortex of OC users compared to non-users (Petersen et al., 2015; Lisofsky et al., 2016; Chen et al., 2021). When comparing the follicular phase of naturally cycling women with the inactive phase of androgenic progestins or antiandrogenic pills, OC users had lower GM volume in cingulate gyrus and bilateral culmen, although these effects did not survive correction for multiple

comparisons (De Bondt et al., 2016). The OC formulation also seems to matter, as women taking pills with androgenic progestins demonstrated smaller frontal volume and lower face recognition performance as compared to non-users, whereas those taking antiandrogenic pills had larger parahippocampal and fusiform volumes and better cognitive scores (Pletzer et al., 2015).

Most fMRI studies report an overall lack of performance differences between OC users and naturally cycling women during processing tasks (Brønnick et al., 2020; Taylor et al., 2021), although some studies indicate reduced frontoparietal activation in OC users compared with non-users in the follicular phase, and greater medial PFC and inferior parietal activation in OC users compared with non-users in the midluteal phase (Pletzer et al., 2014). Resting state fMRI studies have also produced mixed results, as some studies report no differences between women using OC and naturally cycling women (De Bondt et al., 2015b), whereas others report mixed effects (Brønnick et al., 2020; Taylor et al., 2021). On Diffusion Tensor Imaging (DTI), young OC users exhibited higher mean diffusivity (MD) when compared to naturally cycling women in the luteal phase (De Bondt et al., 2013b). Another study of 45–80 year old women reported reductions in fractional anisotropy (FA) with duration and age at onset of OC use (Nabulsi et al., 2020), while a separate study reported higher FA in younger OC users compared to naturally cycling women (Sharma et al., 2020).

Overall, research concerning OC effects on cognitive aging is just emerging. Although samples are small and differences between OC formulations were not reported in most studies, there is some indication that exogenous hormones influence brain volumes among young adult OC users, and may play a role in verbal functions, consistent with research on the menstrual cycle. Future systematic work is needed to better elucidate androgenic vs. anti-androgenic OC effects on cognitive health, and to probe between OC use pre-menopause and cognition post-menopause. Given the widespread use of OC, this work carries significant implications.

Pregnancy

Pregnancy induces significant changes in endogenous estrogen levels, with reported effects on brain structure and function (de Lange et al., 2020). High levels of estradiol observed during pregnancy may lend neuroprotective support due to cumulative estrogen exposure (Deems and Leuner, 2020). However, the neurological impact of pregnancy is multifaceted and the biological mechanisms impacting cognitive aging remain to be elucidated. On one hand, compared to women who have never been pregnant, the levels of circulating estrogen are lower in women who have experienced pregnancy, a difference which extends into menopause (Bernstein et al., 1985). On the other hand, brain sensitivity to estrogen is increased in pre-clinical models of pregnancy, as evidenced by increased numbers of ERα positive cells in parous rats compared to nulliparous rats (Byrnes et al., 2009). Reports also suggest these effects may be evident in the human brain, as parity has been associated with increased responsiveness to estrogen in older aged women (de Lange et al., 2019).

Nonetheless, the vast majority of studies have focused on the short-term effects of pregnancy and postpartum on brain structure, function, and cognition, with the longest follow-ups conducted at 2–6 years postpartum (Brunton and Russell, 2008; Barth and de Lange, 2020). Studies investigating the long-term effects of pregnancy and childbearing on cognitive aging and AD risk are scant, as summarized below.

There is some evidence for a positive effect of pregnancy on cognitive aging. Several studies have reported that midlife women who had experienced pregnancy exhibited better cognitive performance in verbal and visual memory performance (Henderson et al., 2003; Ning et al., 2020), and another reported lower AD risk in later life (Fox et al., 2018). Studies examining gravidity (total number of pregnancies including stillbirth, miscarriage, and/or abortion) have reported a reduced risk of AD in elderly women who had spent more cumulative months pregnant and breastfeeding throughout their life (Fox et al., 2013, 2018). Another study supported these findings in reporting protection against AD dementia with longer breastfeeding duration (Heys et al., 2011). During lactation, estrogen levels are lower, and thus there are likely other factors contributing to these associations.

However, other studies report detrimental effects of pregnancy on cognitive aging. Compared to nulliparous women, parous women had greater cognitive decline on Mini-Mental State Examination (MMSE) scores (McLay et al., 2003), increased AD risk (Colucci et al., 2006) and AD onset at a younger age (Ptok et al., 2002), which may be limited to non-carriers of the ApoE4 gene (Corbo et al., 2007). A post-mortem study reported no clear associations between cognition and parity, though parity was associated with higher levels of AD-related neuropathology (Beeri et al., 2009).

Other studies have reported null associations between parity and cognitive performance or dementia risk (Ptok et al., 2002; Corbo et al., 2007; Ryan et al., 2009; Bae et al., 2020). In the Rancho Bernardo Study, 1,025 women between the ages of 44–99 who were followed over time showed no long-term effect on cognitive performance in relation to their prior pregnancies (Ilango et al., 2019).

Discrepancies may be in part due to how studies define parity. Studies defining parity as the number of childbirths or time spent pregnant more commonly report associations with cognition as compared to studies defining parity as parous vs. nulliparous. The number of children may play an important role, studies report having 1–4 children provides neuroprotection in women (Heys et al., 2011; Ning et al., 2020;

Song X. et al., 2020), having 5 or more children, or grand multiparity, has been linked to negative effects as measured by cognitive performance or dementia risk (Rasgon et al., 2005a; Bae et al., 2020; Song X. et al., 2020).

While neuroimaging results are also mixed, MRI studies generally report positive effects of pregnancy and parity on structural brain aging (**Figure 1**). Two large studies reported that in comparison to nulliparous women, parous women, especially with a higher number of childbirths, exhibited less apparent brain aging as predicted via MRI-based machine learning models (de Lange et al., 2020; Ning et al., 2020). A recent volumetric MRI study of cognitively normal midlife individuals at risk for AD reported positive associations between number of children (between 2 and 5) and larger GM volume in frontal and temporal regions in women, whereas no associations were observed among men (Schelbaum et al., 2021). While there was no direct association between cognitive performance and number of children, there was a positive association between temporal cortex GMV with memory and global cognition performance, which suggests a mediation effect of pregnancy on cognition (Schelbaum et al., 2021).

Overall, studies investigating the associations between pregnancy and later life cognition are limited by small samples, heterogeneity of cognitive assessments and diagnostic criteria, possible inclusion of non-biological children, and different exposure variables. Pregnancy-related factors, including age at first birth, breastfeeding, or complications such as gestational diabetes or pre-eclampsia, have rarely been considered yet may have significant contributions. Later life cognitive testing or dementia diagnosis may also contribute to contrasting results, as the effects of pregnancy are likely more apparent closer to the time of childbirth than many years later after cumulative experiences have affected the brain.

The menopause transition

Menopause represents the permanent cessation of ovulation and menstrual cycles, which is defined retrospectively, after 12 months of amenorrhea without obvious pathologic cause (Harlow et al., 2012). Hormonally, menopause is characterized by drastic reductions in estradiol and progesterone levels and elevated levels of gonadotropins follicle-stimulating hormone (FSH) and luteinizing hormone (LH) (Santoro, 2005). Menopause occurs either as the result of a natural midlife aging process (spontaneous menopause) or iatrogenically, via surgical or pharmacological intervention (induced menopause). In most cases, induced menopause results from bilateral oophorectomy or salpingo-oophorectomy, which lead to an abrupt cessation of ovarian estrogen production. Hysterectomy without oophorectomy can reduce ovarian estrogen production by disturbing blood flow to the ovaries, thus indirectly influencing the onset of menopause (Jett et al., 2022). Endocrine therapy for cancer and radiation therapies can also damage the ovaries and precipitate menopause (Jett et al., 2022). The reduction in ovarian hormones, particularly estradiol, is thought to elicit vasomotor (e.g., hot flashes) and urogenital (e.g., vaginal dryness) symptoms, while also increasing risk for cardiovascular disease and osteoporosis (Harlow et al., 2012), as well as neurological and psychiatric disorders including depression, anxiety, and dementia (Monteleone et al., 2018).

The average age at spontaneous menopause in industrialized countries is 49–51 years (Monteleone et al., 2018). Therefore, women live at least a third of their lives in a hypogonadal state, and that number increases to up to half for women with induced menopause (Monteleone et al., 2018). Recent evidence that AD starts in midlife (Sperling et al., 2013), thus proximate to the menopause transition, has highlighted a previously overlooked connection between menopause and AD risk. Currently, menopause is the most widely investigated female-specific risk factor for AD (Rahman et al., 2020). Estrogen withdrawal during menopause has been linked to accelerated brain cellular aging, possibly increasing risk of neurodegenerative events and AD later in life (Wang et al., 2020b; Mosconi et al., 2021).

Spontaneous menopause is a normal physiological event without long-term adverse effects for the majority of women (Monteleone et al., 2018). However, as high as 80% of women are vulnerable to the neurological shifts that can occur during this transition (Brinton et al., 2015), experiencing not only vasomotor symptoms such as hot flashes, but also "brain fog" and cognitive complaints. While the term "brain fog" is not a medically accepted entity, it reflects the common self-reported awareness of a decline in memory, attention and concentration during the menopause transition (Gold et al., 2000). While statistics on this vary, over 60% of women report changes in their ability to think clearly, concentrate, remember, or make use of new information during the menopause transition (Greendale et al., 2020). Most women experience a 15–20% increase in forgetfulness during perimenopause relative to pre-menopausal levels (Gold et al., 2000).

Nonetheless, whether menopause-related cognitive complaints can be confirmed objectively is a topic of debate (Mitchell and Woods, 2011; Weber et al., 2012). The first evidence for associations between menopause and memory decline stemmed from studies of oophorectomy, which reported an almost doubled long-term risk of dementia in oophorectomized women (Rocca et al., 2007, 2014; Phung et al., 2010; Bove et al., 2014). Dementia risk is generally highest following bilateral oophorectomy, intermediate with unilateral oophorectomy, and lowest but significant following hysterectomy without oophorectomy (Yaffe et al., 1998; Hogervorst et al., 2000; LeBlanc et al., 2001; Rocca et al., 2007; Phung et al., 2010; Bove et al., 2014; Gilsanz et al., 2019). For example, The Mayo Clinic Cohort Study of Oophorectomy (MCSO) observed an 84% higher risk of dementia for women

who underwent unilateral oophorectomy with or without hysterectomy before age 42 years, and a 70% to double higher risk in women who underwent bilateral oophorectomy before the onset of natural menopause (Rocca et al., 2012). Phung et al. (2010) reported a 38% higher risk of dementia before the age of 50 for hysterectomy alone [RR = 1.38, 95% confidence interval (CI) = 1.07–1.78], and over double the risk with unilateral oophorectomy (RR = 2.10, 95% CI = 1.28–3.45) and bilateral oophorectomy (RR = 2.33, 95% CI = 1.44–3.77) (Phung et al., 2010). Dementia risk increases with younger age at the time of surgery (Rocca et al., 2008; Phung et al., 2010), which has also been associated with an increased burden of AD neuropathology at post-mortem (Bove et al., 2014; Agca et al., 2020). Surgical menopause may also have more severe consequences on cognitive function, including lower performance in verbal learning, visual memory (Rocca et al., 2007), and delayed word recall tasks (Zhou et al., 2011). Decline in short-term verbal memory was more severe in women who had greater than 50% decline in serum estradiol levels following surgery (Nappi et al., 1999; Farrag et al., 2002).

Overall, studies including surgical and spontaneous menopause cases indicate measurable, yet modest declines in verbal episodic memory on delayed recall tests, or lack of improvement in verbal memory and processing speed with repeated testing (Fuh et al., 2006; Greendale et al., 2009, 2011; Bromberger et al., 2010; Berent-Spillson et al., 2012; Epperson et al., 2013; Weber et al., 2013). In some studies, peri-menopausal women exhibited declines in working memory and complex attention rather than verbal episodic learning or memory (Weber et al., 2012), suggesting that operations demanding higher cognitive effort contribute to women's perception of cognitive difficulties.

Some studies indicate that cognitive changes are possibly transient, as evidenced by longitudinal reports suggesting that they are mostly present at the peri-menopausal and early post-menopausal stages, with a rebound to almost pre-menopausal levels after menopause (Greendale et al., 2009; Weber et al., 2013). In the Study of Women Across the Nation (SWAN), over 2,300 midlife women followed for 4 years showed a decrease in verbal memory and processing speed in perimenopause compared to their pre-menopausal scores (Greendale et al., 2009). These declines resolved post-menopause, when cognitive performance returned to pre-menopausal levels, or closer to baseline (Greendale et al., 2009). In the Rochester Investigation of Cognition Across Menopause, peri-menopausal and early post-menopausal women had lower verbal memory, attention, and working memory scores, which improved in late postmenopause (Weber et al., 2013). However, other studies report conflicting results of reduced memory still in postmenopause (Epperson et al., 2013). While cognitive effects are for the most part independent of non-cognitive menopausal symptoms such as anxiety and disturbed sleep (Greendale et al., 2010), frequent hot flashes and a negative mood have been linked with more severe cognitive disturbances (Maki et al., 2008; Drogos et al., 2013).

Importantly, memory declines during perimenopause and early postmenopause ranged from subtle to moderate, and remained within normal limits for age and education in most studies (Maki and Henderson, 2016). Moreover, women maintain an advantage in verbal memory as compared to age-controlled men regardless of menopausal status (Rentz et al., 2017), which strongly argue for development of gender-specific tests that also take account women's reproductive stage. Generally, cognitive complaints during menopause are unlikely to result in objectively measured impairments, thus often falling under the diagnostic category of subjective cognitive decline (SCD). Current evidence suggests that people ages 65 and older experiencing SCD may be at higher risk for MCI and dementia (Jessen et al., 2014), especially women (Pérès et al., 2011).

Although neuroimaging research of menopause is scant, and the majority of studies has been carried out in women who had already transitioned through the menopause, recent translational neuroimaging studies corroborate animal findings by showing associations between menopause and biomarker indicators of AD risk in midlife women (Rahman et al., 2019; Jett et al., 2022). Neuroimaging studies of menopause status are summarized in **Table 2**.

Recent multi-modality neuroimaging investigations targeting women at different menopausal stages (pre-menopausal, peri-menopausal, and post-menopausal), all carrying risk factors for AD, such as ApoE4 genotype and a family history of late-onset AD, demonstrate emergence of AD endophenotypes in women of peri-menopausal age (Mosconi et al., 2017, 2018a,b, 2021; Rahman et al., 2020). AD endophenotypes included higher Aβ load, lower CMRglc, and lower GM and WM volume in brain regions vulnerable to AD, chiefly posterior cingulate, precuneus, medial temporal, parieto-temporal, and frontal cortices as compared to pre-menopausal women and to age-controlled men, independent of age and midlife health indicators (Mosconi et al., 2017, 2018a,b, 2021; Rahman et al., 2020; **Figure 1**). Biomarker abnormalities increased post-menopause (Mosconi et al., 2017, 2018a,b, 2021; Rahman et al., 2020). Additionally, peri-menopausal and post-menopausal women positive for ApoE4 genotype exhibited the highest Aβ burden (Mosconi et al., 2017, 2021), supporting the notion that ApoE4 genotype exacerbates AD-related brain changes in women with onset in the perimenopause (Riedel et al., 2016). While menopause effects on Aβ deposition were overall mild, the earlier onset and longer exposure to Aβ pathology could help account for the higher prevalence of AD in women.

Longitudinal evaluations showed progressive AD biomarker abnormalities in the menopause transition, including chiefly declines in hippocampal and temporal lobe GM volumes, CMRglc declines in temporal regions and PCC, and increased Aβ deposition in frontal cortex (Mosconi et al., 2018b, 2021).

TABLE 2 Observational studies of menopause status and menopausal hormone therapy (MHT) use on neuroimaging outcomes.

Study	Exposure	Participants	Age, years	Imaging modality	Cognitive measures	Study design	Main findings
Eberling et al. (2000)	MHT use	8 MHT users, 5 non-users, 13 AD patients	74 (8)	FDG-PET	–	Group comparison	Higher CMRglc in MHT users vs. AD patients No CMRglc difference between non-users and AD patients
Maki and Resnick (2000)	MHT use	12 MHT users, 16 non-users	55+	^{15}O-water PET	Verbal Memory, Visual Memory, Psychomotor Speed	2 year longitudinal study	Greater increases in relative CBF in MTL, insula, cerebellum, frontal, and temporal cortex in MHT users vs. non-users Greater CBF increase in ACC in non-users vs. MHT users Greater CBF increases in insula, HIP, and temporal cortex during verbal memory task in MHT users vs. non-users Better performance on neuropsychological memory tasks in MHT users vs. non-users
Słopień et al. (2003)	Menopause status, MHT use	10 PRE, 20 POST	PRE: 33 (13), POST: 49 (5)	SPECT	–	Group comparison; follow-up SPECT on 10 women with low CBF who were put on MHT	Lower CBF in POST vs. PRE Ventricular CBF improved after 1 year of MHT use
Erickson et al. (2005)	MHT use	16 current MHT users, 14 past MHT users, 13 non-users (all POST)	57–79	Structural MRI	–	Group comparison	Larger GMV in frontal, temporal, and parietal cortex in all MHT users vs. non-users MHT duration positively associated with GMV in PFC, parietal and temporal cortex Larger WMV in medial temporal lobe in all MHT users vs. non-users
Rasgon et al. (2005b)	MHT use	11 MHT users, 9 non-users	50–84	FDG-PET	MMSE, Buschke-Fuld total recall, Delayed paragraph recall, Benton visual errors	2 year longitudinal study	PCC CMRglc decline in non-users vs no decline in MHT users No differences in cognitive performance
Boccardi et al. (2006)	MHT use	16 current tE2 users, 7 past MHT users, 17 non-users (all POST)	50+	Structural MRI	–	Group comparison	Larger global GMV in tE2 users vs. non-users, with peaks in cerebellum, middle temporal and inferior frontal gyri Larger GMV in cerebellum, middle temporal and inferior frontal gyri of past users vs. non-users
Gleason et al. (2006)	MHT use	4 opposed E2 users, 10 opposed CEE users, 9 non-users	59 (5)	fMRI	Auditory Verbal Learning Test; fMRI: line drawing task	Group comparison	Greater HIP activation in MHT users vs. non-users Estradiol users exhibited the best verbal memory performance, non-users intermediate, and CEE users the worst performance
Greenberg et al. (2006)	MHT use	41 current MHT users, 51 non-users (all POST)	60+	Structural MRI	MMSE, extensive neuropsychological evaluation including verbal fluency, verbal memory, visual memory	Group comparison	Smaller GMV and larger non-ventricular CSF volume in MHT users vs. non-users No differences in cognitive performance

(Continued)

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TABLE 2 (Continued)

Study	Exposure	Participants	Age, years	Imaging modality	Cognitive measures	Study design	Main findings
Low et al. (2006)	MHT use	64 current MHT users, 69 past MHT users, 80 non-users (all POST)	60–64	Structural MRI	Verbal intelligence	Group comparison	No differences in GMV Past MHT users exhibited the highest verbal intelligence, non-users intermediate, and current MHT users the lowest
Lord et al. (2008)	MHT use	16 current unopposed estrogen users, 10 past MHT users, 15 non-users (all POST)	50–74	Structural MRI	–	Group comparison	Larger right HIP GMV in current ET users vs. past users and non-users Negative association between HIP GMV and ET duration in current users but not past users No group differences in AMY GMV
Berent-Spillson et al. (2010)	MHT use	13 current MHT users vs. 24 past MHT users vs. 18 non-users (all POST)	60+	fMRI	Visual Delayed Matching to Sample task	Group comparison	No group difference on visual memory performance Greater activation in HIP, insula, PCC, ACC, parietal and frontal cortex for MHT users vs. non-users Greater activation in HIP, insula, frontal and parietal cortex in EPT users vs. non-users Greater activation in left parietal cortex and PHG for EPT users vs. ET users Greater activation in right parietal and frontal cortex in ET vs. EPT users Greater activation in right PFC for past MHT users vs. current users
Maki et al. (2011)	MHT use	13 MHT users vs. 12 non-users (all POST)	56–67	fMRI	Verbal memory: CVLT-II, EBMT, Unrelated Word List, Wechsler Memory Scale-III Faces subtest	Group comparison	POST women who had initiated MHT during PERI exhibited greater activation in left HIP but lower activity in bilateral PHG during recognition and match conditions of verbal memory tasks vs. non-users Better verbal recognition task performance in MHT users vs. non-users
Silverman et al. (2011)	MHT use	53 POST	49–69	FDG-PET	Auditory Consonant Trigrams, Benton Visual Retention Test, Boston Naming Test, Trail Making Test Rey-Osterrieth Complex Figure Test, Logical Memory	Baseline results from 2 year prospective randomized study	Higher CMRglc in left parietotemporal cortex and right temporal gyrus in 17β-estradiol users vs. CEE users 17β-estradiol users scored significantly higher on verbal memory performance vs. CEE users Positive association between verbal memory performance and CMRglc in Wernicke's and auditory association areas in E2 users Positive association between verbal memory performance and CMRglc in right superior frontal gyrus in CEE users Higher CMRglc in bilateral temporal cortex and frontal cortex in unopposed MHT users vs. opposed MHT users

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10.3389/fnagi.2022.948219

TABLE 2 (Continued)

Study	Exposure	Participants	Age, years	Imaging modality	Cognitive measures	Study design	Main findings
Shafir et al. (2012)	MHT use	15 CEE users, 20 CEE + MPA, 17 non-users (all POST)	60–81	fMRI	Emotional processing	Group comparison	Lower activation in left medial frontal gyrus and anterior cingulate during positive stimuli processing in ET users vs. non-users Lower activation in right posterior insula during positive stimuli processing in EPT users vs. non-users Greater activation in right entorhinal cortex during negative stimuli processing in ET users vs. non-users No brain activation differences between ET and EPT users Greater activation in right HIP during positive stimuli processing in all current MHT users vs. all past users
(Ryan et al., 2014)	MHT use	62 current users, 60 past users, 173 non-users (all POST)	68–75	Structural MRI	–	Group comparison	Smaller total GMV in current users vs. past and non-users No differences in HIP, corpus callosum, or white matter lesion volume
Stein et al. (2014)	Menopause status	16 PRE, 28 POST	PRE: 20–35; POST: 50–65	^{11}C WAY-100635 PET	–	Group comparison	Negative associations between progesterone levels and 5-HT1A serotonin binding in ACC in POST but not PRE Negative associations between DHEAS levels and 5-HT1A binding in AMY in POST but not PRE No association between estradiol levels and 5-HT1A binding in PRE or POST
Jovanovic et al. (2015)	MHT use	10 POST (all surgical)	40–65	^{11}C-MADAM PET	Trail Making Tasks A + B, "Reading the mind of the eyes" (social cog. Test), controlled oral word association test (FAS and categories)	6 month longitudinal study	Significant decrease in 5-HTT serotonin binding in frontal, parietal, occipital, temporal cortex, MTL and basal ganglia during MHT use vs. baseline Women with tE2 + testosterone treatment performed better on verbal fluency tasks vs. baseline
Thurston et al. (2015)	Hot flashes number and severity	3 PERI, 17 POST	40–60	rsfMRI	–	Association study	Positive association between physiologically-monitored hot flashes and DMN connectivity
Jacobs et al. (2016)	Menopause status	32 PRE, 29 PERI, 31 POST	45–55	fMRI and rsfMRI	Digit span, Controlled Oral Word Association Test, American National Adult Reading Test, 12-item Face Name Associative Memory Exam, 6-trial Selective Reminding Test	Group comparison	Decreased HIP activation but greater HIP connectivity during verbal processing for POST vs. PRE and PERI HIP activity positively correlated and HIP connectivity, and negatively correlated with declining estradiol
Thurston et al. (2016)	Hot flashes number and severity	3 PERI, 16 POST	40–60	Structural MRI (WMHV)	–	Association study	Positive association between physiologically-monitored night sweats and WMHV
Vega et al. (2016)	Menopause	31 POST	50–60	rsfMRI	Cognitive complaints	Association study	Positive association between cognitive complaints and ECN nodes, but not DMN nodes
Berent-Spillson et al. (2017)	Menopause status	15 PRE, 11 PERI, 28 POST	42–61	fMRI	Cognitive control of emotion processing	Group comparison	No group differences. On *post hoc* analysis, PERI group activated right TPO junction, while POST group activated PFC, PCC and TPO junction during emotion processing

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TABLE 2 (Continued)

Study	Exposure	Participants	Age, years	Imaging modality	Cognitive measures	Study design	Main findings
Braden et al. (2017)	MHT use	32 current MHT users, 41 past users, and 21 non-users (all POST)	73–91	Structural MRI	CVLT-II or Rey Auditory Verbal Learning Test	Group comparison	No differences in HIP volume in MHT users vs. non-users HIP volume correlated with verbal memory for non-users but not for MHT users
Jacobs et al. (2017)	Menopause status	26 PRE, 25 PERI, 20 POST	46–53	fMRI and rsfMRI	Verbal working memory	Group comparison	During verbal working memory task, increased DLPFC activation, but attenuated HIP deactivation across menopausal transition, which correlated with declining estradiol Greater DLPFC-HIP connectivity for POST vs. PRE, which correlated with verbal working memory for POST women only
Mosconi et al. (2017)	Menopause status	15 PRE, 13 PERI, 14 POST	40–60	Structural MRI, PiB-PET, FDG-PET	Digit symbol substitution, paired associates delayed recall, paragraph delayed recall, designs, object naming, WAIS vocabulary	Group comparison	Lower GMV and WMV in frontal cortex of PERI and POST vs. PRE Lower CMRglc in PCC, temporal and parietal cortex of PERI and POST vs. PRE Higher amyloid burden in PERI and POST vs. PRE ApoE4 + POST exhibited greatest amyloid burden in frontal cortex of all groups
Berent-Spillson et al. (2018)	MHT use	38 long-term MHT users vs, 19 non-users (all POST)	60+	fMRI	Verbal processing	Group comparison	Greater frontal activation during verbal processing in MHT users vs. non-users Longer response times during verbal discrimination and recall tasks in MHT users vs. non-users
Kim et al. (2018)	Menopause status	20 PRE, 20 POST	PRE: 40 (9) vs. POST: 56 (2)	Structural MRI	–	Group comparison	Reduced GMV in SMA, frontal and temporal regions of POST vs. PRE GMV differences correlated with estradiol levels
Mosconi et al. (2018a)	Menopause status	15 PRE, 14 PERI, 14 POST	40–60	FDG-PET and plasma COX	Digit symbol substitution, paired associates delayed recall, paragraph delayed recall, designs, object naming, WAIS vocabulary	Group comparison	Lower CMRglc in PCC, frontal, parietal and temporal cortex of POST vs. PRE Lower CMRGlc in PCC, temporal and frontal cortex in POST vs. PERI Lower CMRglc in PCC, temporal and parietal cortex of PERI vs. PRE Reduced COX activity in PERI and POST vs. PRE Lower verbal memory scores in POST vs. PRE

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10.3389/fnagi.2022.948219

TABLE 2 (Continued)

Study	Exposure	Participants	Age, years	Imaging modality	Cognitive measures	Study design	Main findings
Mosconi et al. (2018b)	Menopause status	15 PRE, 14 PERI, 12 POST	40–60	Structural MRI, PiB- and FDG-PET	Digit symbol substitution, paired associates delayed recall, paragraph delayed recall, designs, object naming, WAIS vocabulary	Group comparison over 3 years	Greater rates of amyloid accumulation in frontal cortex and PCC in POST vs. PRE Greater rates of amyloid accumulation in frontal cortex in PERI vs. PRE Greater rates of CMRglc and HIP GMV decline in frontal cortex in POST vs. PRE and PERI Higher rates of decline in higher-order processing in POST vs. PRE and PERI
Zhang et al. (2018)	Menopause status	44 PRE, 43 POST	45–50	rsfMRI	Attention Network Task, Stroop Test, One-back working memory task	Group comparison	Higher DC in AMY, and lower DC in middle occipital gyrus in POST vs. PRE In POST group, AMY-PFC connectivity was positively associated with executive function accuracy In POST group, decreased connectivity between middle occipital gyrus and inferior parietal gyrus associated with lower working memory scores Longer reaction times and lower accuracy on cognitive tests for POST vs. PRE
Seitz et al. (2019)	Menopause status	33 PRE, 29 PERI, 32 POST	46–53	Structural MRI	Digit span, Controlled Oral Association Test for verbal fluency of letters and categories, American National Adult Reading Test, Buschke Selective Reminding Task, Face Name Associative Memory Task	Group comparison	Positive associations between GMV in ACC with HIP, inferior parietal cortex, and DLPFC in POST vs. no associations in PERI In POST group, women exhibiting higher associations between ACC and HIP performed better on Buschke memory task vs. those exhibiting lower associations
Nabulsi et al. (2020)	MHT use	3,106 MHT users vs. 5,195 non-users (PRE and POST)	45–80	DTI	–	Group comparison	Slower decline in WM fiber coherence loss with age in MHT users vs. non-users WM preservation in ET users vs. EPT users
Rahman et al. (2020)	Menopause status, MHT use	16 PRE, 27 PERI, 42 POST	40–65	Structural MRI, PiB-PET, FDG-PET	Digit symbol substitution, paired associates delayed recall, paragraph delayed recall, designs, object naming, WAIS vocabulary	Group comparison	Higher CMRglc in frontal and parietal cortex, and lower amyloid burden in orbitofrontal gyrus in MHT users vs. non-users
Boyle et al. (2021)	MHT use	562 POST	71–94	Structural MRI	Modified MMSE, Benton Visual Retention Test, Digit Symbol Substitution Test	Group comparison	Larger total GMV in CEE MHT users vs. non-users
He et al. (2021)	Menopause status	32 PRE, 25 PERI	45–55	rsfMRI	MMSE	Group comparison	Increased ReHo in lingual gyrus and lower ReHo in superior frontal gyrus of PERI vs. PRE In PERI group, ReHo in frontal areas positively correlated with MMSE score

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TABLE 2 (Continued)

Study	Exposure	Participants	Age, years	Imaging modality	Cognitive measures	Study design	Main findings
Liu et al. (2021)	Menopause status	25 PRE, 25 PERI	45–55	rsfMRI	Stroop test	Group comparison	Increased ALFF in gyrus rectus and decreased ALFF in inferior frontal gyrus, insula and superior temporal gyrus of PERI vs. PRE Lower GMV in gyrus rectus and superior temporal gyrus of PERI vs. PRE Slower reaction rates in PERI vs. PRE
Mosconi et al. (2021)	Menopause status	30 PRE, 57 PERI, 74 POST	40–65	Structural MRI, ^{31}P-MRS, PiB-PET, FDG-PET	Memory (immediate and delayed recall of a paragraph and paired associates), higher-order processing (block design tests), and language (object naming)	Group comparison, including 2-year longitudinal component	POST group exhibited lower GMV and higher ATP/PCr in temporal vs. PRE; lower WMV and CMRGlc in parietal and temporal vs. PRE and PERI; higher CBF in frontal, temporal, and parietal cortex vs. PERI PERI group exhibited lower GMV in precuneus and fusiform vs. POST; lower CMRglc in temporal cortex vs. PRE ApoE4 + POST and PERI exhibited greater amyloid burden vs. other groups POST group exhibited GMV increase in precuneus and stable WM and CMRglc measures at 2-year follow-up
Schelbaum et al. (2021)	Menopause status, MHT use	15 PRE, 35 PERI, 49 POST	40–65	Structural MRI	Rey Auditory Verbal Learning Test and Wechsler Memory Scale logical memory delayed recall tests, executive function (Trail Making Test B and F-A-S), and language (object naming) tests	Group comparison and associations	Lower GMV in frontal and temporal cortex of POST and PERI vs. PRE Larger GMV in fusiform, frontal, and temporal cortex of MHT users vs. non-users
Wisch et al. (2021)	MHT use	70 MHT non-users, 16 MHT users	Non-users: 68 (7), users: 70 (8)	Structural MRI, Tau-PET, PiB-PET	Free and Cued Selective Reminding Test, Logical Memory IIa subtest (Wechsler Memory Scale), Digit Symbol Substitution Test, MMSE	Group comparison	Better cognitive performance in MHT users vs. non-users Lower tau burden in MHT users vs. non-users
Zhang S. et al. (2021)	Menopause status	54 PRE, 45 early POST	45–51	Structural MRI	Stroop Test, Two-back working memory task	Group comparison	Lower AMY GMV in POST vs. PRE Longer reaction rates and lower Two-back working memory scores in POST vs. PRE

ACC, anterior cingulate cortex; AD, Alzheimer's disease; ALFF, increased amplitude of low-frequency fluctuation; AMY, amygdala; ApoE, apolipoprotein E; ATP, adenosine triphosphate; CBF, cerebral blood flow; CEE, conjugated equine estrogen; CMRglc, cerebral metabolic rates of glucose; COX, cytochrome oxidase; CSF, cerebrospinal fluid; CVLT, California Verbal Learning Test; DLPFC, dorsolateral prefrontal cortex; DC, degree centrality; DMN, default mode network; ECN, executive control network; ET, estrogen therapy; EPT, estrogen + progesterone therapy; E2, estradiol; FA, fractional anisotropy; fMRI, functional MRI; GMV, gray matter volume; HIP, hippocampus; 5-HTT, serotonin transporter protein; MHT, menopausal hormone therapy; MMSE, Mini-Mental State Examination; MPA, medroxyprogesterone acetate; MRI, magnetic resonance imaging; MTL, medial temporal lobe; PCC, posterior cingulate cortex; PCr, phosphocreatine; PERI, peri-menopausal; PET, positron emission tomography; PFC, prefrontal cortex; PHG, parahippocampal gyrus; POST, post-menopausal; PRE, pre-menopausal; ReHo, Regional homogeneity; ROI, region of interest; rsfMRI, resting state fMRI; SMA, supplementary motor area; SPECT, single photon emission computed tomography; tE2, transdermal estradiol; WAIS, Wechsler Adult Intelligence Scale; WM, white matter; WMHV, white matter hyperintensity volume; WHV, white matter volume.

10.3389/fnagi.2022.948219

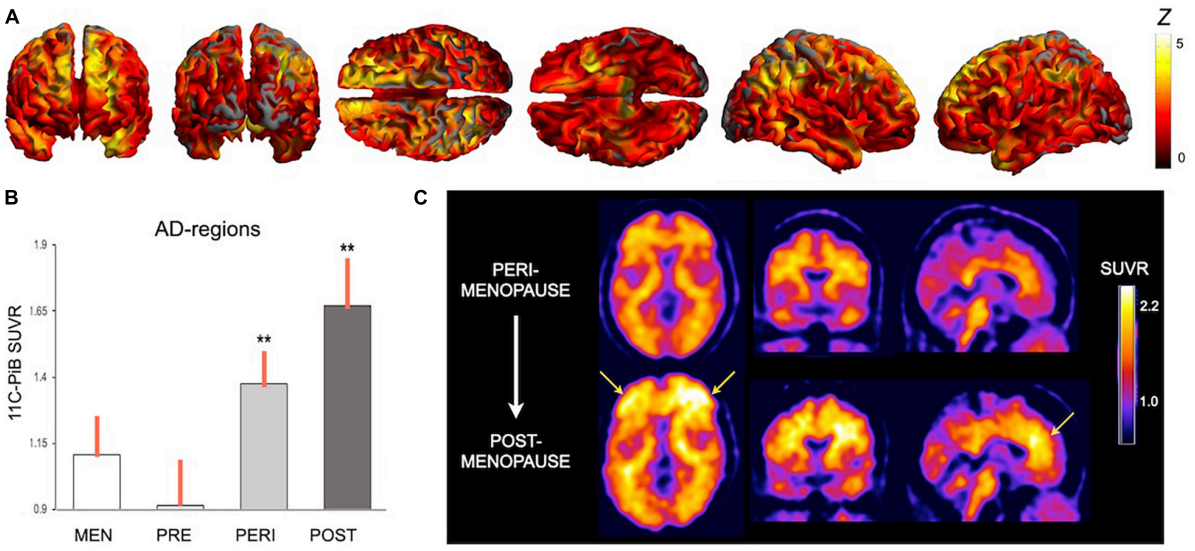

FIGURE 2
Effects of menopause on brain amyloid-beta deposition. Summary of Pittsburgh compound B PET (PiB-PET) studies showing menopause status effects on Aβ deposition: **(A)** Statistical parametric maps showing higher PiB uptake, a marker of Aβ load, in key brain regions for AD in a group of post-menopausal and peri-menopausal women vs. age-controlled men (Z scores > 2 correspond to $p < 0.001$). **(B)** In these regions, Aβ load was associated with menopausal status, e.g., was highest post-menopause, intermediate in peri-menopause, and lowest pre-menopause (**different from men at $p < 0.001$). **(C)** Aβ deposition is progressive during the menopause transition, as evidenced in a representative case who underwent PiB-PET at baseline, when she was peri-menopausal, and 3 years later, when she was post-menopausal. Images are adapted from data presented in **(A)** Mosconi et al. (2021), **(B)** Mosconi et al. (2017), and **(C)** Mosconi et al. (2018b). PiB, Pittsburgh compound B; SUVR, standardized uptake value ratio.

Figure 2 provides an overview of menopause effects on Aβ deposition among midlife women.

Nonetheless, a follow-up study provided preliminary evidence for biomarker stabilization or recovery in late post-menopause (Mosconi et al., 2021). For example, GM volume declined during peri-menopausal and early post-menopausal stages (Mosconi et al., 2018b), but plateaued in temporal cortex, and showed a rebound in precuneus in late post-menopause (Mosconi et al., 2021). WM declines in major WM tracts and CMRglc in parieto-temporal areas also appeared to plateau in late post-menopause (Mosconi et al., 2021). Additionally, cerebral blood flow (CBF) measured by means of Arterial Spin Labeling (ASL) was higher in the post-menopausal group as compared to pre-menopausal controls and to age-controlled men, and so was the ratio of adenosine triphosphate (ATP) to phosphocreatine (PCr) levels measured by means of ^{31}Phosphorus Magnetic Resonance Spectroscopy (31P-MRS), reflecting higher ATP synthesis (Mosconi et al., 2021). Importantly, cognitive performance was intact post-menopause, which correlated with GM volume and ATP levels (Mosconi et al., 2021). Biomarker "recovery" was, however, attenuated in peri-menopausal and post-menopausal ApoE4 carriers (Mosconi et al., 2021). Overall, while these findings need to be replicated in larger samples, in keeping with preclinical work (Wang et al., 2020c), they suggest presence of compensatory mechanisms that allow brain adaptation to the hypo-estrogenic post-menopausal state, at least in some women. Brain adaptation may also account for cognitive preservation and for the easing of menopausal symptoms observed in the late post-menopausal stage (Monteleone et al., 2018).

Other natural history studies indicate lower GM volume in post-menopausal as compared with pre-menopausal women in frontal and temporal regions, which positively corelated with estradiol levels (Kim et al., 2018). The type of menopause also seems to have an impact, as induced menopausal cases exhibited smaller medial temporal lobe volume as compared to spontaneous post-menopausal cases (Zeydan et al., 2019). Moreover, physiologically-monitored night sweats correlated with estrogen levels and white matter hyperintensities (Thurston et al., 2016). Albeit limited by the small samples and by the fact that cognition was not studied (Thurston et al., 2016), this study suggests a link between vasomotor symptoms and cerebral small vessel disease, a risk factor for later stroke and dementia (Debette and Markus, 2010).

fMRI studies also provide emerging evidence for menopause-related changes in brain activation during verbal tasks and emotion processing. In some studies, post-menopausal women showed the least hippocampal activation, in spite of increased hippocampal connectivity, during verbal processing (Jacobs et al., 2016, 2017). Post-menopausal women also exhibited increased dorsolateral prefrontal cortex activation during verbal working memory (Jacobs et al., 2017).

There is also evidence that post-menopausal women exhibited increased activation of regions involved in cognitive control during emotion decision making, such as the PFC, posterior cingulate, and temporoparietal junction, but not in limbic system (Berent-Spillson et al., 2017). Finally, presence of subjective cognitive complaints was associated with increased connectivity of the prefrontal cortex (Vega et al., 2016) while physiologically-monitored hot flashes were linked to increased DMN connectivity (Thurston et al., 2015).

Overall, a growing literature indicates that ovarian steroid hormones, particularly declines in estradiol, reshape the landscape of the female brain during the menopause transition. Some aspects of memory, such as verbal memory, are negatively impacted by menopause, along with more variable declines in processing speed, attention, and verbal fluency. These effects are, however, mild and tend to resolve in the late post-menopausal stage, e.g., approximately 6 years after the last menstrual period. Novel neuroimaging data also suggest that negative effects of menopause on neurophysiology may be transient, with the exception of women at risk for AD, who exhibit preclinical AD endophenotypes already during perimenopause. However, given that all women experience menopause but only a fraction will develop dementia, more work is warranted to elucidate which protective mechanisms may offset the effects of menopause on AD risk. Population-based studies indicate that over 30% of all AD cases could potentially be prevented by addressing modifiable medical and lifestyle factors such as smoking, depression, obesity, diabetes, and lack of physical activity (Livingston et al., 2020). Many of these factors also impact the age of onset and severity of menopause (Monteleone et al., 2018). More studies are needed to examine the effects of lifestyle and medical comorbidities on the brain changes occurring during menopause in association with future AD risk.

It also remains unclear whether altered brain biomarkers and memory fluctuations during perimenopause are predictive of dementia in later life. There are also preliminary findings of links between white matter hyperintensities and vasomotor symptoms, and of menopause-related changes in cognitive processing during emotion identification and in resting state networks, which need further clarification. While available findings need to be replicated in larger samples with longitudinal follow-ups and with the use of AD biomarkers, the evidence so far indicates that window of opportunity for support of estrogen-based neuroplasticity is early in the endocrine aging process.

Menopausal hormone therapy

Menopause hormonal therapy (MHT) includes oral and transdermal preparations thought to have systemic effects, and localized administrations (e.g., vaginal creams) that do not have systemic effects. Herein, we focus on systemic MHT. The treatment of choice for women with a uterus consists of combined (opposed) estrogens and progestins. The treatment of choice for women without a uterus is unopposed, or estrogen-only therapy. Estrogens can be estradiol or conjugated equine estrogens (CEE). Progestins vary in their hormone derivatives, as in hormonal contraceptives. The most commonly used are medroxyprogesterone acetate (MPA) and micronized progesterone.

There is a relatively large literature on MHT effects on brain and cognitive aging. In spite of this, results of whether MHT is viable for support of cognitive function and AD risk reduction are mixed. The hypothesis that MHT might protect against AD arose in large part from early observational studies and small clinical trials demonstrating a protective effect of MHT on cognitive function and AD risk among MHT users compared with never-users (Kawas et al., 1997; Zandi et al., 2002; Paganini-Hill et al., 2006; Sherwin, 2006; Whitmer et al., 2011), especially among younger, 50–59 year-old women (LeBlanc et al., 2001). Positive effects were particularly consistent with estrogen-only, or unopposed MHT for hysterectomized women (Sherwin and Phillips, 1990; Henderson et al., 2005; Rocca et al., 2007, 2010; Whitmer et al., 2011; Shao et al., 2012). Recent observational studies continue to provide conflicting results. For example, an analysis of health insurance claims from nearly 400,000 women reported a protective effect against AD and other neurodegenerative diseases with use of MHT (Kim et al., 2021). Compared with non-users, MHT users exhibited a 57% reduced risk of AD (Kim et al., 2021), with the greatest risk reduction for long-term MHT users (Kim et al., 2021). On the contrary, a population study in Finland of nearly 170,000 women reported that MHT use was associated with a 9–17% increased risk of AD, with higher risk for opposed MHT (Savolainen-Peltonen et al., 2019). In women younger than 60 at hormone therapy initiation, the increase in AD risk was mostly associated with MHT exposure over 10 years (Savolainen-Peltonen et al., 2019). However, ApoE4 status was not evaluated in this study. As Finland has a higher rate of ApoE4 carriers than most countries, with nearly 20% frequency, this is an important confounder as the effectiveness of MHT may be impacted by ApoE4 status (Depypere et al., 2016). Additionally, there is some evidence that oophorectomy before the natural age of menopause, but not after, is associated with an increased risk of AD (Rocca et al., 2007), which is mitigated by post-operative MHT (Sherwin and Phillips, 1990; Henderson et al., 2005; Rocca et al., 2007, 2010; Whitmer et al., 2011; Shao et al., 2012).

Randomized, placebo-controlled clinical trials of MHT for AD prevention have also provided conflicting results. The first large trial to test MHT for dementia prevention was the Women's Health Initiative (WHI). The WHI included two studies, the WHI Estrogen-plus-Progestin Study, in which women with a uterus were randomly assigned to receive either combined MHT (Prempro) or a placebo; and the WHI Estrogen-Alone Study, in which women without a uterus were

randomly assigned to receive either estrogen-alone therapy (Premarin) or a placebo. Cumulatively, the WHI showed some benefits related to use of MHT, including one-third fewer hip and vertebral fractures, and one-third lower risk of colorectal cancer relative to placebo (Rossouw et al., 2002; LaCroix et al., 2011). However, the trials were stopped prematurely as both MHT types were associated with an increased risk of coronary artery disease, stroke and blood clots (Rossouw et al., 2002; Anderson et al., 2004). Additionally, the Estrogen-plus-Progestin arm of the study initially showed an increased risk of cancer (Rossouw et al., 2002; Anderson et al., 2004), although subsequent analysis found no increase in risk (Anderson et al., 2012; Lobo, 2017).

The WHI included an additional arm, the WHI Memory Study (WHIMS), which examined the impact of MHT for dementia prevention among women ages 65 and older, thus in late post-menopause. These studies focused on oral CEEs in women with prior hysterectomy, and CEE/MPA in naturally post-menopausal women (Shumaker et al., 2003). Although AD was the *a priori* primary outcome of interest, all-cause dementia became the default primary outcome because of the lack of a sufficient number of AD cases at follow-up. In a sample of 2,947 post-menopausal women with prior hysterectomy, there was no evidence that CEE lowered the risk of all-cause dementia (Espeland et al., 2004; Shumaker et al., 2004). However, in a sample of 4,532 spontaneous post-menopausal women, CEE/MPA doubled the risk for all-cause dementia (Shumaker et al., 2003). Thus, the WHIMS study demonstrated no protective effects of unopposed MHT, and a substantial increase in dementia risk with opposed MHT among late post-menopausal women.

In terms of MHT effects on cognition, the WHIMS trial found that both opposed and unopposed therapy were associated with slightly worse mean scores in global cognitive function compared to placebo (Rapp et al., 2003; Espeland et al., 2004). These effects were observed within the first 3–4 years of the trial follow-up and remained fairly constant several years thereafter. The subsequent Women's Health Initiative Study of Cognitive Aging (WHISCA) study examined whether MHT influenced domain-specific cognitive function at initial assessment, an average of 3 years after randomization to MHT or placebo, and after an additional ∼3 year of on-trial follow-up. Among the 2,304 participants, only small mean differences in cognitive test scores changes were noted (Resnick et al., 2006, 2009). Together, these findings suggest that if MHT use produces an initial decrement in at least some aspects of cognitive function, this decrement does not markedly widen or diminish thereafter. Notably, all the above studies involved post-menopausal women above age 65, thus possibly already harboring pre-existing cardiovascular or neurodegenerative conditions. As such, it may have been too late for MHT to prevent those conditions. These considerations, together with evidence from observational studies, has led to the understanding that the efficacy of MHT depends on the timing of initiation and the use of progestogens (LeBlanc et al., 2001; Manson et al., 2006; Maki, 2013; Bove et al., 2014).

However, the newer Early versus Late Intervention Trial with Estradiol (ELITE)-cog and Kronos Early Estrogen Prevention Study (KEEPS) trials have reported no beneficial or adverse effects of MHT on cognition among recently post-menopausal women within 6 years of the menopause diagnosis (Gleason et al., 2015; Henderson et al., 2016; Miller et al., 2019). Nonetheless, MHT reduced the progression of subclinical atherosclerosis when therapy was initiated soon after menopause (Hodis et al., 2016), which has been linked to a 30% reduced number of heart attacks and cardiac deaths (Salpeter et al., 2009).

To date, eight meta-analysis have examined the neuroprotective effects of MHT on AD risk (Yaffe et al., 1998; Hogervorst et al., 2000; LeBlanc et al., 2001; Lethaby et al., 2008; O'Brien et al., 2014; Song Y. J. et al., 2020; Zhang G. Q. et al., 2021). Early meta-analyses were based almost entirely on observational studies, and indicated a 29–35% reduced risk of AD in MHT users (Yaffe et al., 1998; LeBlanc et al., 2001). However, the large majority of women in those studies had started MHT before they experienced natural or surgical menopause, generally used estrogen-only therapy (typically CEEs), and stopped using MHT after age 60. As such, the hypothesis that MHT protects against AD was developed based on studies of estrogen-only therapy beginning in early post-menopause (or prior) and stopping a few years post-menopause. In fact, MHT initiated more than 10 years after menopause did not protect against AD (Zandi et al., 2002). Rather, women who initiated MHT between ages 61 and 68 had about double the risk of developing AD as compared to those who had begun MHT at younger ages (Zandi et al., 2002). Today, although results are still mixed, MHT use remains more consistently associated with reduced risk of AD or all-cause dementia as compared to placebo and/or lack of use, especially for estrogen-alone therapy, although all reports indicated substantial heterogeneity and large variability (Hogervorst et al., 2000; Lethaby et al., 2008; O'Brien et al., 2014; Song Y. J. et al., 2020; Zhang G. Q. et al., 2021). As possible biases and lack of control for potential confounders limit interpretation of these studies, more work is warranted to better clarify the role of MHT for AD prevention and preservation of cognitive function.

There is some evidence that MHT may facilitate maintenance of some aspects of cognition when initiated in early post-menopause or prior. Verbal memory is consistently seen to be maintained or sometimes enhanced with estrogen-alone treatment. A review of randomized, placebo-controlled trials of MHT and verbal memory indicate a beneficial effect of estrogen alone therapy in women younger than age 65, especially surgically post-menopausal cases (Maki and Sundermann, 2009). Additionally, different forms of progestogen may have different effects, with negative effects of CEE/MPA on verbal

memory in younger women (Maki et al., 2007). There is also indication of positive, yet mild effects of MHT on learning and processing speed (Maki and Sundermann, 2009). Effects vary, however, with MHT type and timing, and there are individual differences, in particular related to time since menopause, type of menopause, and overall neurocognitive health prior to menopause.

Clinical trials using brain scans as endpoints lend support to the hypothesis that both age at treatment initiation and type of MHT are important factors to consider. As summarized in **Table 3**, the first generation of neuroimaging studies of MHT indicated a generally stimulating or preserving effects of MHT on CBF and CRMglc (Eberling et al., 2000; Maki and Resnick, 2000; Słopień et al., 2003; Rasgon et al., 2005b, 2014; Silverman et al., 2011). Among for women at risk for AD, PET studies provided evidence of differential changes in CMRglc as related to MHT use (Rasgon et al., 2005b, 2014; Silverman et al., 2011). A 2-year longitudinal study showed that non-users exhibited significant CMRglc declines in PCC, whereas MHT users did not exhibit significant CMRglc changes (Rasgon et al., 2005b). Two subsequent prospective, randomized clinical trials investigated post-menopausal women who were taking estrogen-alone MHT for at least 1 year prior to enrollment in the study, and were then randomized to continue or discontinue therapy. Over a 2-year period, women randomized to continue MHT exhibited a relative preservation of frontal and parietal CMRglc as compared with those randomized to discontinue MHT (Silverman et al., 2011; Rasgon et al., 2014). In addition, those continuing unopposed estradiol-based MHT showed additional preservation of CMRglc in PCC and precuneus (Rasgon et al., 2014). Additionally, unopposed MHT use was associated with higher CMRglc in frontal and temporal cortices, as well as better cognitive performance, as compared to opposed MHT, suggesting regionally specific neuroprotective effects (Eberling et al., 2000; Maki and Resnick, 2000; Silverman et al., 2011).

Structural MRI studies reported less consistent evidence of protective effects of MHT. Some report greater GM volumes in MHT users versus non-users (Erickson et al., 2005; Boccardi et al., 2006; Lord et al., 2008) or versus placebo (Eberling et al., 2003; Albert et al., 2017), mostly localized in frontal and temporal cortices, and hippocampus. In some studies, hippocampal volume was positively linked to verbal memory in treated post-menopausal women (Zhang et al., 2016; Braden et al., 2017). However, there are just as many contradictory reports showing decreased frontal GM volume in MHT users versus non-users (Coker et al., 2014; Ryan et al., 2014; Zhang et al., 2016), and decreased hippocampal volume in MHT users versus non-users (Greenberg et al., 2006; Low et al., 2006; Resnick et al., 2009) and in MHT users versus placebo, although there was no further decline from 1–3 to 6–7 years post-treatment (Coker et al., 2014). Notably, reports of positive effects of MHT focused on post-menopausal women in their 60s, whereas negative reports included mostly women of advanced age (71–89 years), sometimes with scanning conducted years after MHT ended. Additionally, two MRI studies showed no differences comparing current or past MHT users to non-users (Ryan et al., 2014; Braden et al., 2017). However, these studies were based on longitudinal WHIMS data collected several years after MHT cessation, and grouped users of estrogen-only and combined therapies, therefore not taking into account possible effects of MHT formulation (Ryan et al., 2014; Braden et al., 2017).

There are also reports of increased white matter hyperintensities with MHT use (Kantarci et al., 2016b) although results on this are mixed (Coker et al., 2014; Zhang et al., 2016) suggesting that effects of MHT on WMH are either small or moderated by confounders, such as age and overall cardiovascular health before treatment.

In addition, randomized controlled trials that incorporated fMRI indicated a higher activation of fronto-cingulate regions and hippocampus during verbal, non-verbal and spatial working memory tasks, although results are not always consistent (Shaywitz et al., 1999; Joffe et al., 2006; Smith et al., 2006; Dumas et al., 2010; Davison et al., 2013; Thomas et al., 2014; Berent-Spillson et al., 2015; Girard et al., 2017). Since these studies reported MHT-related effects in absence of differences in cognitive performance, it remains unclear whether higher activation during task performance reflects a beneficial response or a less efficient use of neuronal resources (Shaywitz et al., 1999; Thomas et al., 2014; Girard et al., 2017).

Overall, brain imaging studies of MHT suggest a putative positive role of estrogen against regional cerebral atrophy and metabolic decline, with an advantage of unopposed over combined MHT (Silverman et al., 2011; Rasgon et al., 2014), and of transdermal estradiol over oral CEE (Resnick et al., 2009; Zhang et al., 2016; Kantarci et al., 2018). However, brain imaging data suffers from several limitations (Comasco et al., 2014). Most studies are statistically under-powered due to relatively small samples and high heterogeneity, including differences in study design (controlled randomization vs. cross-sectional trials, parallel vs. cross-over design, baseline vs. placebo control state), different duration of MHT use, different routes of administration and posology/dose, and different type of therapy (unopposed vs. combined MHT), differences in the timing of initiation with respect to age and/or the menopausal transition, as well as use of different neuroimaging techniques, different neuropsychological paradigms in activation studies, and different processing and analysis pipelines.

In conclusion, active debate remains on whether MHT has value for neuroprotection. Natural history studies and some clinical trials suggest that MHT may support cognition and brain function in peri-menopausal and recently post-menopausal women. However, most studies demonstrating

TABLE 3 Clinical trials of menopausal hormone therapy (MHT) effects on neuroimaging outcomes.

Study	Participants	Age at baseline, years	MHT type	Imaging modality	Cognitive measures	Study design	Main findings
Shaywitz et al. (1999)	46 POST (last menstrual period > 5 months before enrollment)	33–61	1.25 mg CEE vs. placebo	fMRI	Verbal memory	∼2-month randomized, double-blind, placebo-controlled crossover study	Greater activation in inferior parietal lobule and superior frontal gyrus during verbal and non-verbal retrieval task in treated vs. placebo phase
Joffe et al. (2006)	50 PERI and POST (26 estrogen-treated and 24 placebo-treated women)	40–60	26 tE2 0.05 mg users vs. placebo	fMRI	CVLT, WMS-R, Rey-Osterreith Complex Figure Test	3-month randomized double-blind, placebo-controlled study	Fewer perseverative errors during verbal recall task in MHT users vs. placebo Greater activity in inferior frontal and parietal cortex during verbal memory task in MHT users vs. placebo Greater activity in frontal cortex, posterior cingulate and parietal cortex during spatial memory tasks in MHT users vs. placebo Greater activation in left posterior parietal and left inferior frontal cortices during verbal recall and visual memory task, respectively, in placebo vs. MHT users
Smith et al. (2006)	10 POST [7 (3) years since menopause]	50–60	5 μg ethinyl estradiol and 1 mg norethindrone acetate vs. placebo	fMRI	Visual Delayed Matching to Sample Task	3-month randomized, double-blind, placebo-controlled crossover study	MHT users exhibited higher bilateral prefrontal cortex activation vs. placebo No difference in task performance between active and placebo phase
Coker et al. (2009)	1,403 POST	65–79	257 CEE users, 436 CEE + MPA users, 710 placebo	Structural MRI	Modified Mini-Mental State Exam	Randomized, double-blind, placebo-controlled study (analysis of 1–6 years post-treatment)	No group differences in ischemic lesion volume
Persad et al. (2009)	10 POST	56–60	5 μg ethinyl estradiol + 1 mg norethindrone acetate vs. placebo	fMRI	Verbal memory	3-month randomized, double-blind, placebo-controlled crossover study	Greater activation in left and medial PFC, dorsal anterior cingulate, posterior cingulate, and left parietal cortex in MHT vs. placebo No group differences for verbal memory performance
Resnick et al. (2009)	1,403 POST	65–79	436 0.625 mg CEE with 2.5 mg MPA users vs. 257 0.625 mg CEE alone users vs. 710 placebo	Structural MRI	Modified Mini-Mental State Exam	Randomized, double-blind, placebo-controlled study (analysis of 1–6 years post-treatment)	Reduced hippocampus, frontal cortex, and global GMV in MHT users with large ischemic lesion volume Reductions in hippocampus GMV greatest in MHT users with low baseline cognitive scores
Dumas et al. (2010)	20 POST	59 (6)	10 users 1 mg oral 17β-estradiol vs. placebo	fMRI	Visual verbal n-back task (working memory)	3 month randomized, double-blind, placebo-controlled study	Greater BOLD signal in frontal cortex and precuneus during high word-load condition in MHT users vs. placebo No group differences in performance

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10.3389/fnagi.2022.948219

TABLE 3 (Continued)

Study	Participants	Age at baseline, years	MHT type	Imaging modality	Cognitive measures	Study design	Main findings
Love et al. (2010)	10 POST	56–60	5 μg ethinyl estradiol + 1 mg norethindrone acetate vs. placebo	fMRI	Emotional processing task	3 month randomized, double-blind placebo-controlled crossover study	Greater activation to negative stimuli in left occipital cortex, right precentral gyrus, PCC, and bilateral orbitofrontal cortex in MHT vs. placebo Reduced activation to negative stimuli in DLPFC, postcentral gyrus, and dorsal anterior cingulate in MHT vs. placebo Reduced activation to positive stimuli in left medial frontal cortex in MHT vs. placebo
Davison et al. (2013)	13 POST (no more than 5 years of amenorrhea at randomization)	49–55	6 E_2D users (continuous-combined estradiol 1 mg/drospirenone 2 mg) vs. placebo	fMRI	Visual attention/vigilance, psychomotor function/speed of processing, paired associates, list learn and recall, Groton Maze learning task and recall	6 month randomized, triple-blind placebo-controlled study	No significant group difference in BOLD signal during verbal fluency or mental rotation tasks No group difference for verbal fluency or mental rotation task performance Higher detection speed in placebo vs. MHT group
Coker et al. (2014)	729 POST	65+	127 CEE, 229 CEE + MPA, 373 placebo	Structural MRI	–	Randomized, double-blind placebo-controlled study (analysis of 1–3 to 6–7 years after treatment)	No group differences in brain or ventricular volume change Smaller frontal GMV in both treated groups vs. placebo at baseline CEE treated patients with a history of cardiovascular disease had greater increases in WMHV and total brain lesion volume No effects of MHT formulation
Kranz et al. (2014)	30 POST	47–64	10 oral estradiol users, 10 oral estradiol + micronized progesterone, 10 placebo	5-HT_1A PET	–	56–98 day randomized, double-blind, placebo-controlled study	No group differences for 5-HT_1A serotonin receptor binding

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TABLE 3 (Continued)

Study	Participants	Age at baseline, years	MHT type	Imaging modality	Cognitive measures	Study design	Main findings
Rasgon et al. (2014)	45 POST (28 continued MHT, 17 discontinued MHT following an average of 10 years of use)	50–65	16 17β-estradiol users (12 with concurrent progestin), 12 CEE users	FDG-PET	–	2 year Randomized, double-blind placebo-controlled study	Greater rates of CMRglc decline in medial PFC, left frontoparietal area, and right inferior parietal cortex in women who discontinued MHT vs. women continuing MHT In ApoE4 non-carriers, greater rates of CMRglc decline in medial PFC and left temporo-occipital area in women who discontinued MHT vs. women continuing MHT Women who discontinued 17β-estradiol, CMRglc decline was greatest in precuneus and PCC, while women who continued 17β-estradiol exhibited no CMRglc decline in precuneus or PCC bilaterally Women who continued CEE exhibited CMRglc decline in bilateral precuneus and PCC Greater rates of CMRglc decline in precuneus and PCC with continuation of 17β-estradiol or CEE with concurrent progestin
Thomas et al. (2014)	13 PERI	48–55	Micronized oral 17β-estradiol subsequently combined with progesterone and placebo	fMRI	Reward processing	4 month randomized, double-blind crossover study	Greater putamen and PFC activity during reward processing in treated group vs. placebo, which correlated with estradiol levels
Berent-Spillson et al. (2015)	29 PERI and POST (6–38 months since last menstrual period)	45–55	1 mg oral estrogen or 200 mg progesterone and placebo	fMRI	Verbal processing and visual working memory	3 month randomized, double-blind crossover study	PFC activity during verbal processing increased by estradiol treatment and decreased by progesterone treatment Decreased HIP activation with estradiol treatment vs. placebo Increased PFC and HIP activation during visual working memory with progesterone treatment
Kantarci et al. (2016a)	68 PERI and POST	52–65	17 oCEE + micronized progesterone, 21 tE2 + micronized progesterone, 30 placebo	PiB PET	CVLT, New York University Paragraphs	4 year randomized, double-blind, placebo-controlled trial	oCEE-treated group had lower CVLT total score compared to placebo Among ApoE4 carriers, tE2 group had lower amyloid burden compared to both placebo and CEE group
Kantarci et al. (2016b)	95 POST (within 5–36 months past their last period)	42–56	29 oCEE + micronized progesterone, 30 tE2 + micronized progesterone, 36 placebo	Structural MRI	Global cognitive function	4 year randomized, double-blind, placebo-controlled trial	Larger ventricular volume in oCEE users vs. placebo Women initiating oCEE later into menopause had larger ventricular volume increases Greater WMHV increase in CEE group at 48 months and in tE2 group at 18 months vs. placebo No group differences in cognition

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TABLE 3 (Continued)

Study	Participants	Age at baseline, years	MHT type	Imaging modality	Cognitive measures	Study design	Main findings
Zhang et al. (2016)	1,365 POST	65+	254 CEE, 420 CEE + MPA, 691 placebo	Structural MRI	–	Randomized, double-blind, placebo-controlled trial (analysis of 1–3 years post-treatment data)	Reduced frontal GMV in treated groups vs. placebo, especially estrogen-only users No group differences in white matter volume
Albert et al. (2017)	75 POST	51–74	33 high dose oral 17β-estradiol vs. 21 low dose MHT vs. 21 placebo	Structural MRI	–	3 month repeated measures of dose-dependent estradiol treatment vs. placebo	Increased hippocampal GMV with high dose estradiol vs. low dose and vs. placebo
Girard et al. (2017)	12 early POST (6–24 months of amenorrhea after the last menstrual period)	48–55	17β estradiol vs. 17β estradiol + progesterone vs. placebo	fMRI	Cognitive control	4 month randomized, double-blind crossover study	Greater PFC and ACC activation during task switching than in the control condition in active vs placebo phase No differences in task performance
Kantarci et al. (2018)	75 POST	42–56	20 oCEE + micronized progesterone, 22 tE2 + micronized progesterone, 33 placebo	Structural MRI	Global cognitive function	3-year follow-up of Kantarci et al. (2016)	Greater WMHV in oCEE group vs. placebo Slower rates of DLPFC volume decline in tE2 group vs. placebo No group differences in ventricular volumes or cognition
Jayachandran et al. (2020)	95 PERI and POST (within 6 months to 3 years past the last menstrual period)	42–59	29 oCEE + micronized progesterone, 30 tE2 + micronized progesterone, 36 placebo	Structural MRI (WMHV)	–	4 year randomized, double-blind, placebo-controlled trial	No difference between groups for WMHV changes over time
Kling et al. (2020)	78 PERI and POST	42–58	23 oCEE + micronized progesterone, 24 tE2 + micronized progesterone, 31 placebo	Structural MRI	–	4 year randomized, double-blind, placebo-controlled trial	In both treated groups, a greater increase in estrone (E1) associated with smaller increase WMH volume vs. placebo In tE2 group, greater decreases in FSH associated with smaller WMHV increases

ACC, anterior cingulate cortex; ApoE, apolipoprotein E; BOLD, blood-oxygen-level-dependent; CMRglc, cerebral metabolic rates of glucose; CVLT, California Verbal Learning Test; DLPFC, dorsolateral prefrontal cortex; fMRI, functional MRI; GMV, gray matter volume; HIP, hippocampus; MHT, menopausal hormone therapy; MPA, medroxyprogesterone acetate; MRI, magnetic resonance imaging; oCEE, oral conjugated equine estrogen; PCC, posterior cingulate cortex; PERI, peri-menopausal; PFC, prefrontal cortex; POST, post-menopausal; rsfMRI, resting state fMRI; tE2, transdermal 17β-estradiol; WM, white matter; WMHV, white matter hyperintensity volume; WMS-R, Wechsler Memory Scale-Revised.

10.3389/fnagi.2022.948219

benefits are based on observational studies, or studies of younger women which may have better captured the critical window for MHT action vs. larger clinical trials of older post-menopausal women. Observational studies are subject to bias as women who choose to use MHT have in general higher education, and tend to have healthier lifestyles and better overall health before and after taking MHT than women who do not (Matthews et al., 1996). Taking MHT may therefore be associated with a healthier lifestyle which in turn might be driving cognitive function. In addition, despite estrogen's biologically plausible mechanisms for supporting brain aging, most reviews have concluded that many observational studies and clinical trials are limited by methodological problems such as small size and short duration, and display substantial heterogeneity.

Conclusion

Understanding sex-driven effects of ovarian hormones on dementia risk is a crucial step toward development of precision medicine strategies for AD prevention. In recent years, significant progress has been made in discovering how ovarian steroid hormones influence cognitive aging, prompted in part by advancements in the research on sex differences in AD. Across the female lifespan, there is compelling evidence that estradiol levels influence brain structure, function, and biochemistry in many regions affected by AD. There are also increasing indications of complex interactions of estradiol with other sex hormones, chiefly progesterone and androgens.

In this review we examined the effects of puberty, the menstrual cycle, hormonal contraceptives, menopause, and MHT on cognitive aging and neuroimaging biomarkers of AD. While this field is still in its infancy, there is increasing evidence for associations between indicators of estrogen exposure, such as pubertal timing, menstrual cycle frequency, number of pregnancies, and OC use, and cognitive function over the course of a woman's life (Egan and Gleason, 2012; Li et al., 2016).

More work has been done to investigate changes in cognition and AD biomarkers during the transition to menopause, and more so as due to MHT use. Clinical studies indicate a dip in cognitive performance, mostly verbal memory, during peri-menopause, possibly followed by a rebound post-menopause. Significant heterogeneity has been noted as related to age, menopause status, use of MHT, and genetic risk factors. Hardly any clinical studies analyzed data in relation to women's existing genetic predisposition to AD or other neurological conditions. On the other hand, neuroimaging studies of midlife women at genetic risk for AD have provided robust evidence for emergence of AD endophenotypes with onset in peri-menopause among natural cyclers (Mosconi et al., 2017, 2018a,b, 2021; Rahman et al., 2020). Surgically induced menopause is also associated with a higher risk of AD, especially in presence of an earlier age at oophorectomy (Bove et al., 2014). Across studies, the risk of AD is over 30% higher following hysterectomy alone, and over two times higher in presence of oophorectomy relative to spontaneous menopause. For contrast, women's risk of AD is increased 4- and 12–15-fold with one or two ApoE4 alleles, respectively (Riedel et al., 2016). More work is needed to examine the combined effects of ApoE4 and hysterectomy/oophorectomy status prior to menopause on AD biomarkers, and whether the associations are modified by MHT use.

Menopause hormone therapy use has been heavily scrutinized due to the disparity between basic science, observational studies, and large randomized clinical trials. Overall, MHT action on brain is dependent on multiple factors, including chronological age, stage of reproductive aging, duration of hypogonadism, and presence of symptoms, as well as the formulation of MHT, route of administration, and the health status of the brain. Currently, MHT is not indicated to alleviate cognitive complaints or for AD prevention. However, some argue that MHT given to healthy peri-menopausal and early post-menopausal women under age 60 for about 5 years may be recommended for support of cognitive function with careful consideration of other risks (Stuenkel et al., 2015; Baber et al., 2016). There is mounting evidence that MHT use during early menopause, and in presence of symptoms, may help sustain neurological health and reduce the risk of AD (Brinton, 2008), whereas MHT initiated >5 years after menopause may be less beneficial if not detrimental as in the case of combined therapy (Shumaker et al., 2003). Personalized physician advice which takes into consideration key factors including age, menopausal stage, symptoms, and comorbidities, may offer a greater look at how MHT impacts AD risk as compared to the one-size-fits-all approach of randomized clinical trials, and argues for a precision medicine approach to MHT use (Kim and Brinton, 2021; Kim et al., 2021). More research is warranted to further understand this critical window of estrogen sensitivity.

Previous work has shown that, *in vitro* and *in vivo*, ApoE expression can be differentially regulated either by 17-beta-estradiol or specific agonists, depending on activation of ER subtypes (Wang et al., 2006). These data suggest that use of ER-selective ligands might provide therapeutic benefit to reduce AD risk by decreasing ApoE expression in ApoE4 allele carriers. Moreover, because ERβ promotes estrogen-mediated neuronal plasticity and memory function, a formula that selectively targets ERβ may be a novel and plausible solution for menopause-related vasomotor symptoms and cognitive impairment. In 2022, we obtained NIH funds to carry out a Phase IIb randomized, placebo-controlled clinical trial testing the efficacy of PhytoSERM, a selective estrogen receptor beta (ERβ) modulator comprised of three phytoestrogens: genistein, daidzein, and S-equol (Zhao et al., 2009), for AD prevention in midlife women. The PhytoSERM formulation has been shown to promote estrogenic action in brain while remaining

largely inactive or inhibitory in reproductive tissue (Zhao et al., 2009). The initial phase Ib/IIa clinical trial (ClinicalTrial.gov ID: NCT01723917) demonstrated safety and established the pharmacokinetics profile of PhytoSERM (Wang et al., 2020a). Results of the ongoing Phase IIb trial will become available by 2026.

In conclusion, ovarian steroid hormones are long overlooked but critical contributors to brain aging and AD risk. While the neurobiological consequences of hormonal activity have only begun to be understood, converging evidence supports a role for cumulative estrogen exposure in reducing risk of developing AD later in life. This strongly argues for continued examination of sex hormones and reproductive history factors in AD prevention strategies for women. There is an urgent need for prospective epidemiological, clinical and biomarkers studies with data taken at several time-points starting at midlife that examine the associations between lifetime estrogen exposure and neurological function in later life. Understanding the dynamic interplay between sex, chronological aging, endocrine aging, and additional AD risk factors is crucial to inform and justify primary precision-medicine strategies for AD prevention.

Author contributions

LM and SJ discussed the concepts and wrote the manuscript. ES, GJ, CB, JD, SP, and RD reviewed the literature and provided critical revision of the manuscript for important intellectual content. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Feelings of loneliness and isolation: Social brain and social cognition in the elderly and Alzheimer's disease

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From attachment styles to interpersonal neurobiology

Interpersonal neurobiology presents an integrated view of the development of the human mind, investigating how this occurs from the mutual influence between human relationships and brain structure and function: the focus of this approach is to understand how the brain gives rise to mental processes and how it is directly shaped by interpersonal experiences (Siegel, 1999). Through this approach we can understand what processes are useful in facilitating cognitive-behavioral development, emotional and psychological wellbeing, and resilience certainly during early childhood but probably throughout life. Underlying the mentioned processes is indeed a fundamental mechanism of integration that can be examined at different levels, from interpersonal to neurological (Siegel, 2001). Interpersonal neurobiology proposes an interpretation of mental processes whose main characteristics are (1) being both embodied, in the body, and relational; (2) the smooth flow of metabolites and information; and (3) the flow of information in the percipient and between people (Siegel, 2001). Knowing how to control and knowing how to modify this flow of energy and information, the basis of healthy regulation, are skills that are acquired in families with secure attachment (Bowlby, 1969; Siegel, 2001; Bowlby and King, 2004).

The child comes into the world genetically programmed to establish attachment bonds with its caregivers who will become, thus, the child's attachment figures (Bowlby, 1969; Cassidy and Shaver, 1999; Bowlby and King, 2004). The attachment system is considered a motivational system: an innate, adaptive, biologically determined system that drives a child to create certain selective attachments in his or her life. Although the attachment system is programmed at the brain level, the experiences a child has throughout his or her childhood go a long way toward shaping that system (Bowlby, 1969; Cassidy and Shaver, 1999; Bowlby and King, 2004). The succession of relational

experiences prompts the activation of brain neurons that respond to sensory events from the outside world or internally generated images from the brain itself (Gazzaniga, 1995; Kandel et al., 2012). The process is used to create a mental image, sensory image or linguistic representation of a concept or object (Siegel, 2001; Kandel et al., 2012). According to Siegel (2001), the neural substrate also allows the formation of an emergent self, a proto-self, determined largely by genetic and constitutional characteristics. This sense of self is embedded in the brain as well as in its interactions with the environment: *internal working model* (Siegel, 2001). On the other hand, the child's mind seems to develop a fundamental process in which the other's mental states (the so-called Theory of Mind, ToM) are also represented within the neuronal functioning of the brain (Stone et al., 1998). The sense of acting, coherence, affectivity and even the continuity of the self (memory) are therefore influenced by interaction with others. In this way, experience shapes the function of neural activity and can potentially shape the evolving structure of the brain throughout the lifespan. Developmental stages and aging change the concept of security from childhood's pursuit of physical proximity to sophisticated forms of relating and representing others. Through the process of creating new meanings from memories, internal working models reorganize themselves according to new relational experiences, thereby assisting in constructing a consistent self-model through adulthood (Bowlby, 1969; Cassidy and Shaver, 1999; Bowlby and King, 2004).

The attachment theory of John Bowlby is applicable to every age group, but researchers have been slow to study attachment in older adults (Bradley and Cafferty, 2001). Study findings suggest, however, that attachment issues could be relevant for the elderly, given that aging can be associated with separation, loss, and vulnerability (Bradley and Cafferty, 2001). As a matter of fact, the personal attachment style is associated with a range of outcomes in later life (such as reactions to the loss of a loved one, general wellbeing and adjustment to chronic illness and caregiver burden). The implications of attachment styles and the questions raised by interpersonal neurobiology regarding social isolation and loneliness on the directions of aging and the acceleration of any neurodegenerative process are discussed here.

Theoretical framework: Social brain and social cognition

Networks of relationships are important to Siegel (2001) since neural networks appear strongly influenced by relations with others, beyond genetic influences. In his words: "human connections shape neural connections, and each contributes to mind. Relationships and neural linkages together shape the mind. It is more than the sum of its parts; this is the essence of emergence" (Siegel, 2001, p. 3). The horizon of interpersonal neurobiology thus allows for a broad perspective that is reflected in neuroimaging investigations. Thus, social neuroscience hypothesizes brain evolution on the level of intersubjective actions. Due to the social environment in which primates live, specific selective pressures have led to the evolution of neurocognitive mechanisms capable of handling the challenges of social interaction (Adenzato and Enrici, 2005). Humans' social cognition consists of psychological processes that allow us to make inferences about what others are thinking and feeling (Adenzato and Enrici, 2005; Adolphs, 2009). The way social information is processed is divided into automatic and stimuli-driven processes, and those that are deliberate and controlled, but sensitive to context and strategy (Adolphs, 2009). In their proposal, the "social brain hypothesis," Byrne and Whiten (1988) were among the first to argue that complex social environments serve as a dominant selective pressure for human brain size. By appealing to particular pressures that a species adapted to social interactions would have faced, the social brain hypothesis attempts to explain the extraordinary size and complexity of the human brain (Barrett and Henzi, 2005; Dunbar and Shultz, 2007a,b).

In everyday life, social interaction is one of the most complex mental activities in which humans engage. The high cognitive load is necessary to predict the behavior of people involved in social interaction. In particular, the functions involved in the social brain relate to social cognition, which is important for sociability. The term social cognition refers to the set of abilities that enables an individual to construct mental representations of his or her relationships with others and to use these representations to adapt behaviors to the context (Adolphs, 2001). Social groups are complex in nature, and it is their complexity that has led to the advancement of prefrontal brain functioning and specialization (Adenzato and Enrici, 2005). Not only the prefrontal areas but also other cortical and subcortical structures are involved in the processing of social stimuli. Social information activates complex neural circuits that connect cortical and subcortical regions, including those usually thought to be involved in the emotional processing of stimuli, such as the amygdala, as well as those usually thought to be involved in the cognitive processing of stimuli, such as the temporo-occipital junction and the medial prefrontal cortex (Van Overwalle, 2009). This widespread neural involvement reverberates the fact that social cognition is a high-order function. Indeed, social cognition is broad and varied; it refers to all mental processes useful in social interaction, among which the ToM and mentalizing play a significant role. Premack and Woodruff (1978) define ToM as the ability t to attribute to other individuals' mental states that are different from one's own. Mentalisation is an inherently imaginative activity involving the attribution of intentional mental states based on clues. In mentalisation, it is recognized that a person's actions are autonomous and can be explained by his or her internal state (McLaren and Sharp, 2020).

According to Dodich et al. (2015), we deal with a multidimensional process in which different components are integrated. Among these, attribution of emotions and intentions is a very important component during the representation of mental states. Other authors use this term to refer to thinking or feeling about others' mental states (Saxe et al., 2006a,b; Van Overwalle, 2009). Some neural structures, such as the anterior cingulate cortex (ACC) (Palermo, 2017, 2020b), the medial prefrontal cortex (MPFC), the temporoparietal junction (TPJ), the posterior cingulate cortex (PCC), and the superior temporal sulcus (STS) are known to play a role (Adolphs, 2001; Saxe and Kanwisher, 2003). On the other hand, Sebastian et al. (2012) used functional magnetic resonance imaging (fMRI) on healthy subjects which showed that some brain areas involved with mentalization and perspective-taking, like the temporoparietal junction and the ventromedial prefrontal cortex, are recruited when affective stimuli are present.

In fact, it is only one behavioral domain, that of social cognition (Laird et al., 2011), that is strongly and exclusively associated with a neural network that closely resembles the default mode network (DMN), demonstrating bilateral activation of the inferior parietal/TPJ, posterior precuneus/cingulate, and medial frontal (Smith et al., 2009; Mars et al., 2012). Similar conclusions had previously been reached by Schilbach et al. (2008). When they examined the DMNs' responses to different types of cognitive stimulation some activations were quite similar to those observed in various aspects of social cognition: the left angular gyrus/TPJ in differentiating between self and others (Vogeley and Fink, 2003); the anterior cingulate in monitoring action in self and others (Amodio and Frith, 2006); the precuneus in social interactions (Schilbach et al., 2006). According to the authors, the biological "baseline" of the human brain corresponds to a psychological "baseline," our predisposition to engage in social cognition by default (Schilbach et al., 2008; Mars et al., 2012). Cognitive processes geared toward self-reflection, such as introspection and autobiographical memory, have been also linked to the DMN, and its integrity is now considered crucial to mental health (Grieder et al., 2018).

Loneliness and its impact on psychological wellbeing and social cognition

Loneliness is a negative emotional state experienced when there is a discrepancy between the relationships one would like to have and that one perceives to have (Alberti, 2019). This condition does not so much concern the amount of time spent with other people as the quality of the relationships themselves.

In industrialized countries about one-third of people are affected by this condition, with one in severely affected, with these proportions constantly increasing (Cacioppo and Cacioppo, 2018). Loneliness is to such an extent a painful companion for many people that an editorial in the New York Times on the issue was entitled, "Is Loneliness a Health Epidemic?" (Klinenberg, 2018). Those who are most likely to report a significant feeling of loneliness tend to belong to the most vulnerable social groups, such as the young, the elderly, the poor, the chronically ill, and the mentally ill (Hawkins-Elder et al., 2018).

Importantly, loneliness has a profound impact on physical and psychological health, often leading to negative outcomes; loneliness and social isolation would appear to be associated with a reduction in lifespan like that caused by smoking 15 cigarettes a day, with a 27% increased risk of premature mortality (Holt-Lunstad et al., 2010). On the other hand, establishing strong relationships would lead to a reduced risk of mortality (Holt-Lunstad et al., 2017).

Several studies on the effects of loneliness on the health of the general population have been conducted over time. Loneliness is known to affect mental influence mental health, by leading to depression (Alpass and Neville, 2003; Cacioppo et al., 2006a; Hawkley and Cacioppo, 2010). Indeed, loneliness precedes mood disorder in time, proving to be a key factor in the onset of the disorder (Cacioppo et al., 2010): loneliness seems to mediate the anxiety-depression relationship, with loneliness potentially resulting from anxiety and subsequently being able to sequentially activate depressive symptoms (Ebesutani et al., 2015). The process behind this phenomenon is quite complex. It is believed that oxytocin and arginine vasopressin act as key mediators of social behavior in non-human mammals and human (Heinrichs and Domes, 2008). Oxytocin reduces behavioral and neuroendocrine responses to social stress and, as a result, may allow animals to overcome their natural aversion to close proximity and inhibit defensive behavior, thus facilitating approaches (Heinrichs and Domes, 2008). Seven primary emotional processes have been described by affective neuroscience: SEEKING, RAGE, FEAR, sexual LUST, maternal CARE, separation-distress PANIC/GRIEF and joyful PLAY (Panksepp, 1998; Zellner et al., 2011). Social loss, perhaps the biggest epidemiological determinant of depression, may promote deflection of mood through overactivity of separation-distress PANIC/GRIEF and hypoactivity of SEEKING networks (Panksepp, 1998, 2003; Zellner et al., 2011). Endogenous opioids, which may mediate attachment and separation distress *via* oxytocin pathways, contribute to initiating depressive cascades through decreased SEEKING (Gunnar and Quevedo, 2007; Heinrichs and Domes, 2008; Nolte et al., 2011). Thus, altered affective networks occurring in depression may explain psychological pain and dysphoria. Human health, including the need for social relationships, is largely driven by the endogenous opioid hormonal system (Johnson et al., 2014). Illness may result from disrupting this system.

Indeed, from a biomedical point of view, loneliness has been associated also with poor self-rated health (Stickley et al., 2013).

Consistent with this, persistent loneliness has been associated with physical health problems (Newall et al., 2014) and sleep disorders (Cacioppo and Cacioppo, 2014). Moreover, it has also been linked with negative health habits, such as alcohol consumption (Stickley et al., 2013; Arpin et al., 2015) and smoking (Stickley et al., 2013).

In the neuropsychiatric field, loneliness has also been linked to obsessive-compulsive disorder (Timpano et al., 2014), social anxiety (Lim et al., 2016), and paranoia (Jaya et al., 2017). Loneliness has been associated with psychological distress (Stickley et al., 2013) but the most dramatic outcome of loneliness is suicide especially in populations at risk such as adolescents and old adults (Stravynski and Boyer, 2001; Morese et al., 2019a; Morese and Longobardi, 2020; Morese et al., 2020), an act almost constantly associated with the idea of being left alone and no longer able to receive help from anyone (De Leo and Diekstra, 1990). Indeed, strong associations among suicide ideation, parasuicide, and different ways of being lonely and alone were verified (Stravynski and Boyer, 2001). Importantly, the prevalence of suicide ideation and parasuicide increased with the degree of loneliness with differences between men and women (Stravynski and Boyer, 2001).

From a neuropsychological perspective, perceived social isolation (i.e., loneliness) is a risk factor for - and may contribute to - poorer overall cognitive performance, faster cognitive decline, poorer executive functioning, greater sensitivity to social threats, which is a confirmation bias in social cognition (Cacioppo and Hawkley, 2009). Therefore, social worlds tend to be perceived as threatening and punishing by lonely people (Cacioppo and Hawkley, 2009). Researchers have found that manipulating feelings of loneliness causes people to feel more anxious, fear negative evaluation, and act more coldly toward others (Cacioppo et al., 2006b), while also making them feel colder (Zhong and Leonardelli, 2008). Also, lonely people are more likely to form negative social impressions of others, and their expectations, attributional reasoning, and behavior toward others are less charitable than those of non-lonely individuals (Cacioppo and Hawkley, 2005). As a result of negative social expectations being validated by others, these expectations are reinforced and an individual is more likely to behave in ways that distance them from the very people they want to be close to better meet their social needs (Cacioppo and Hawkley, 2009). Hence, lonely individuals may perceive themselves as passive victims in their social world, yet they are active agents through their self-destructive interactions with others (Cacioppo and Hawkley, 2005).

Pandemic conditions have exacerbated these effects today as social distancing, fiduciary isolation, and quarantine have been imposed. The consequences on the elderly population are of particular interest (Morese et al., 2019b; Palermo, 2020a, 2021a; Amanzio et al., 2021).

Loneliness and social cognition in old age

Since the second half of the 20th century, advances in healthcare and nutrition have led to an increase in the number of older adults in Western societies. Research in developmental and health care is challenged by this rapid increase. Aging involves new definitions of both self and relational issues in many developmental models (Blatt, 2008), since it concurs to psycho-physical, cognitive, and social impairment (Maylor et al., 2002). Moreover, as older adults age, they become increasingly confronted with the loss of loved ones.

Loneliness and social isolation are growing public health concerns in our aging society (Akoya et al., 2020). The prevalence of loneliness among the population is high. Eighty per cent of those under 18 years of age and 40% of those over 65 years of age report feeling lonely at least occasionally (Giné-Garriga et al., 2021). A growing number of older people in the EU are living alone: they form a particularly vulnerable group in society, with an increased risk of social exclusion or poverty (Eurostat, 2020). Seniors who live alone are often facing complex dynamics that rarely have an easy explanation: loneliness can occur due to the natural events of life, age-related challenges, and changes in social life (Savikko et al., 2005). The most common causes include family crisis, physical and motor limitations, death of many peers, widowhood, limiting housing conditions, increased use of communication through electronic devices rather than face-to-face.

Women suffer from this condition more often than men. Indeed, women tend to live longer than men and are more likely to experience any of the above-listed situations. In 2018, the share of older women (aged 65 years or more) in the EU-27 living alone was 40 % (Eurostat, 2020). Older women living alone reported severe difficulties and are hence more likely to be frail. Loneliness, together with age, chronic pathologies, and non-self-sufficiency, must be considered a risk factor for the frailty process. A longitudinal study on a sample of 1,600 respondents, found that 43% of the elderly lived in a condition of loneliness and, 6 years after the first interview, the researchers discovered that those who were lonely had a 45% higher risk of mortality, with a worsening of the quality of life and personal autonomy (Perissinotto et al., 2012).

According to large-scale surveys, loneliness increases the risk of mortality because it increases the presence of diseases as well as the use of pharmaceuticals and health services, thereby increasing the costs of public health (Holwerda et al., 2012; Banks et al., 2016).

Just for an example, the 4-years long-term effects of loneliness on health include increased blood pressure, depression, weight gain, smoking alcohol/drug use, alone time and decreased physical activity, cognition,

heart health, sleep, stroke, and coronary heart disease (Berg-Weger and Morley, 2020).

Loneliness also appears to be associated with cognitive decline and the onset of major neurocognitive disorder (Donovan et al., 2016; Rafnsson et al., 2017; Luchetti et al., 2020). It is precisely in neurocognitive diseases that the importance of social relations has been studied and how they can modulate and enhance the neural correlates of the circuits that create wellbeing in feeling that one belongs to a specific social group (Morese et al., 2018; Morese and Palermo, 2020) (Figure 1). Several studies have shown that the social brain network, associated with a positive feeling of wellbeing and pleasure ("warm glow"), is the one that is activated when people feel part of their communities and experience social support (Morese et al., 2016, 2019a; Lo Gerfo et al., 2019; Auriemma et al., 2020; Longobardi et al., 2020; Morese and Longobardi, 2022). This might suggest that older adults' ToM is driven by the retrieval of information relevant to isolation (Beadle et al., 2012).

Given the above, loneliness is a factor that should not be taken lightly in the daily lives of older people, since this existential condition may have affected their health by initiating an iatrogenic cascade process on all the body's physiological systems (Morese et al., 2019b; Palermo, 2021b). Elderly people living alone are undoubtedly made more frailty by a possible deprivation of a social support network (family or friends) that they can rely on in times of need. In many western countries, this issue is becoming increasingly important: this is undoubtedly due to demographic concerns linked to an aging population and increased life expectancy (Palermo, 2020a).

An example of social brain disruption in aging: The case of Alzheimer's disease

As cognitive aging progresses, it is normal that there will be a progressive decline of social cognition (Moran et al., 2012). Performance during social-cognitive tasks was impaired and selectively accompanied by age-related decreases in the functional response of the dorsomedial prefrontal cortex. Based on these findings, age-related deficits in mentalizing are localized to circumscribed subregions of the default mode network, independent of tasks (Moran et al., 2012). The overlap between the social brain and the DMN have potential implications on neurodegenerative process. Indeed, several modifications of this network have been reported in in healthy older adults as well as in populations at risk for Alzheimer's disease (AD) (Mevel et al., 2011). In particular, decreased signal complexity in DMN nodes might contribute to cognitive decline in AD, which leads to even more dysfunctional DMN connectivity (Grieder et al., 2018) and potential effects on social cognition (Mars et al., 2012).

Concerning the perception of affiliation and isolation (i.e., loneliness), age-related similarities in the recruitment of brain regions involved in the ToM and self-referentiality (e.g., temporal pole, medial prefrontal cortex) are known (Beadle et al., 2012). Specifically, in response to isolation vs. affiliation imagery, older adults show greater recruitment than younger adults of the temporal pole, a region that is important for retrieving personally relevant memories used to understand the mental states of others (Beadle et al., 2012). A notable example of the effects of damage in that brain region is AD, which affects social functioning primarily through atrophy of the medial temporal lobe. Few studies have investigated ToM in AD patients using questionnaires, PET, and MRI/rs-fMRI. Yet no study has examined the neural correlates recruited during a social situation that involved the Theory of Mind. To measure the two components of the ToM, the attribution of emotions and intentions, Dodich et al. (2016) administered the Story-based Empathy task to patients with different neurodegenerative diseases. Both performances on the attribution of emotions and intentions were low in AD patients. Their poor performance may be due to a general cognitive decline, according to the authors (Dodich et al., 2016). The impairment of social skills in AD has a strong impact on interpersonal relationships, but to date, no neurophysiology studies deeply investigated the cognitive and emotional processes. Patients who were poorly involved in social activities and interactions tended to show more psychological and behavioral symptoms at baseline than socially involved patients (Arai et al., 2021). In addition, poor communication with family at baseline was associated with increased severity of psychiatric and behavioral disorders after 1 year (Arai et al., 2021). It, therefore, seems crucial to maximizing patients' involvement, as well as their opportunities for socialization and interaction, to prevent the exacerbation of symptoms over time (Arai et al., 2021).

An impairment of cognitive function may have an impact on loneliness for older people as it can hinder social interaction with family and friends, or interfere with judgements regarding relationship satisfaction (Burholt et al., 2017). The iatrogenic process tends to be self-sustaining. While alterations in social cognition occur with physiological aging, at the same time social isolation is itself inherently depressogenic and results in cascading effects on neurophysiological systems (Heinrichs and Domes, 2008; Nolte et al., 2011). Depressogenic mechanisms that emerge from loss of sociality include alterations in the stress axis, disinhibition of pro-inflammatory signals (Slavich and Irwin, 2014), particularly of the innate branch of the immune system (Cañas-González et al., 2020; Palermo, 2020a). The process contributes to the dysregulation of the stress axis, the decline in neurotrophins (also associated with increased inflammation), in opioids and oxytocin, which imay recursively contribute to a new reduction in neurotrophins (Heinrichs and Domes, 2008; Nolte et al., 2011).

The above fits into the context of the *social safety theory* (Slavich, 2020), which hypothesizes that the development and maintenance of friendly social ties is a fundamental

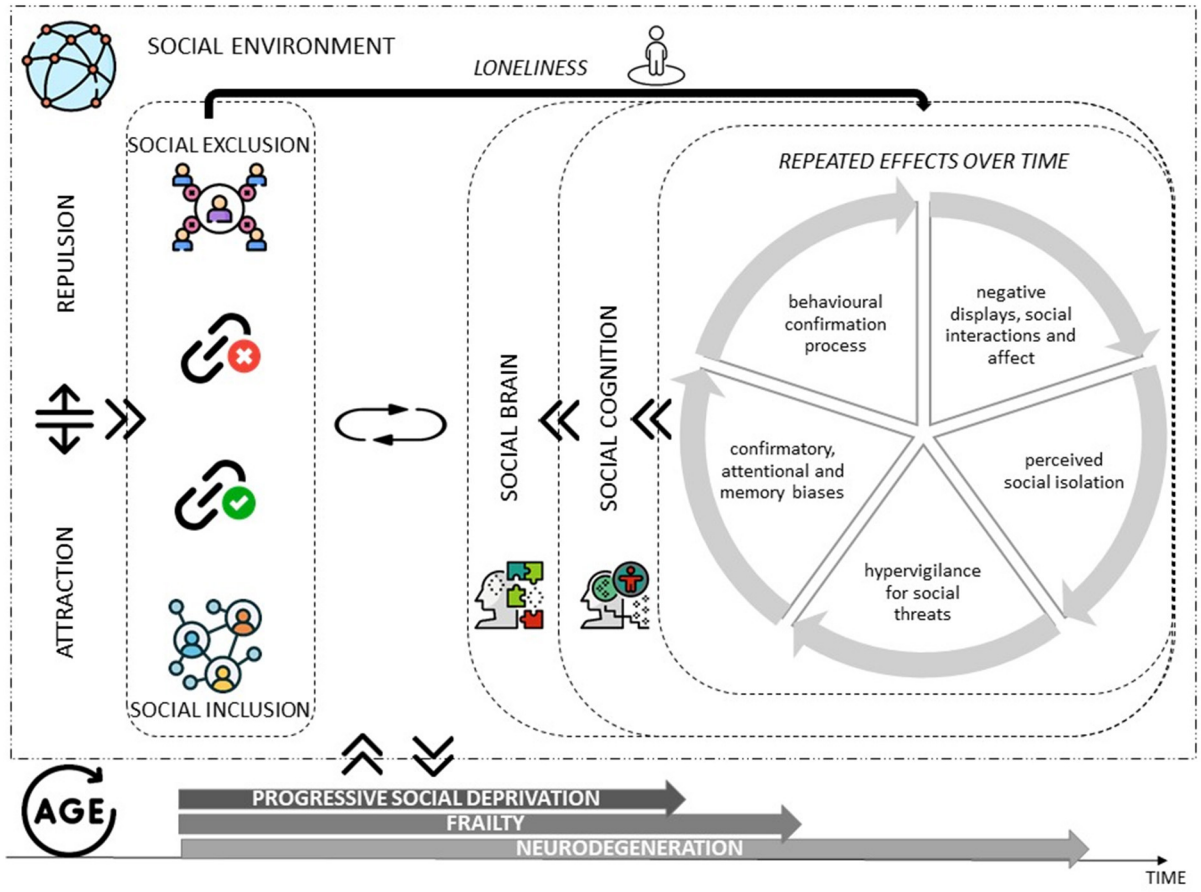

FIGURE 1
Theoretical model summarizing the relationships that exist between the dimensions of interest, the interactions between the individual and the social environment, and the dynamics of mutual interchange that affect the social cognition and social brain along the passage of time and the increasing age of the individual.

organizing principle of human behavior and that threats to social security are a critical feature of psychological stressors that increase the risk of illness. It is likely that anticipatory neural-immune reactivity to social threat is highly conserved due to situations of social conflict, isolation, devaluation, rejection, and exclusion historically increasing risk of physical injury and infection (Slavich, 2020). Survival ultimately depends on humans' ability to elaborate symbolically and respond to a potential danger situation, which is a neurocognitive and immunological function (Slavich, 2020). Positive and negative social experiences can be explained by the social safety theory on a biological and evolutionary basis, allowing us to explain why certain stressors are particularly harmful. The framework also provides a multilevel approach to explore the biopsychosocial determinants of health and aging disparities, physical and cognitive frailty, and interpersonal behavior impairment (Slavich, 2022).

Social isolation – through the biological routes described by the *social safety theory* - may contribute, through the primary induction of depression, to the increased risk of AD (Kuo et al., 2020; Drinkwater et al., 2021). Indeed, socially isolated people had lower volume in the brain's gray matter in brain regions involved with learning and thinking. Social isolation counts for a 26 percent increase in the likelihood of dementia onset (Shen et al., 2022). Due to their complex relationship, the association between dementia and late-life depression is still unclear (Kuo et al., 2020). Indeed, it has been proposed that to understand the pathogenic mechanisms of AD, it is necessary to consider its multifactorial nature considering all together risk factors such as hypertension, social engagement, obesity, education level, or physical inactivity (Kuo et al., 2020). There could therefore be "softer" secondary pathways to neurodegeneration even for those who do not become formally depressed, but simply for those who were isolated

and for dysphoric without being formally depressed. Just as an example, there is a well-known association between social isolation, loneliness, and cardiovascular disease (Golaszewski et al., 2022). Epidemiological studies report an independent association between dementia and cardiovascular disease, suggesting that stroke and dementia may share overlapping molecular mechanisms (Stakos et al., 2020; Leszek et al., 2021). Indeed, the pathogenesis of cardiovascular disease and AD is influenced by low-grade inflammation (Stakos et al., 2020). In particular, one of the major risk factors found to affect the cardiovascular system as well as the nervous system is ApoEε4 (Leszek et al., 2021).

Conclusions

Attachment theory highlights the existence in humans of an innate need to seek protective closeness and intimacy with significant figures during times of crisis, suffering, need or distress. However, this innate need is supplemented from an early age with experiences resulting from the environment in which the individual is immersed. Accordingly, the human tendency to desire and seek the closeness of attachment figures corresponds to an innate schema whose full operationalization is dependent on concrete experiences in relationships. In this sense, children's first relationships with caregivers influence the development of internal working models, expectations of themselves and others, and provide the basis for learning and social interactions. Being able to experience meaningful affective relationships and the quality of these relationships is essential both for maintaining self-confidence and emotional stability in the elderly and for coping with the traumas associated with neuropsychological and physical frailty. How the elderly person becomes available for the type of care they will receive depends on the relationship they have with their reference figures from the past. People who have a history of insecure attachments may be less likely to trust caregivers and medical personnel.

Often the lack of meaningful interpersonal relationships in old age results in loneliness, which some studies show is related to particular attachment styles (Cicirelli, 2010). It is not simply "being alone" that is an indicative or predictive factor of loneliness. Instead, the perception of loneliness is influenced by expectations about oneself and others. These expectations are the results of internal working models, which function in accordance with the person attachment style.

Social cognition, in turn, is associated with social inclusion; impairment of social cognition associated with social brain dysfunctioning is associated with loneliness (Figure 1). One might ask whether experiences of loneliness have increased and whether loneliness is a characteristic of modern societies, or it has always existed to a certain extent. It is not possible to answer this question because there is still no international standard for the definition of loneliness (and therefore the available data often do not distinguish between social isolation, living alone, and loneliness), and because past epidemiological investigations did not pay attention to this issue. However, the general feeling is that the phenomenon is increasing and has important consequences for the psychophysical health of individuals, especially the elderly. A modifiable factor, loneliness, can be ameliorated before the development of severe impairment or neurodegenerative disorders likely-due-to-AD. Indeed, inflammation-related diseases and viral infections are among the most prevalent forms of morbidity and mortality associated with this multilevel biological threat response due to social stress and according to the *social safety theory* (Slavich, 2022). Therefore, this theoretical paper's primary aim is to underline the importance of the social neuroscience perspective to study the social brain and social cognition in the older population, to draw useful indications for preventing the iatrogenic effects of isolation on psychophysical wellbeing and acceleration of neurodegeneration-related processes.

Author contributions

RM conceived the content of the article, participated in writing and supervised the manuscript for the parts falling within her competence. SP conceived the content of the article, participated in writing the first draft of the manuscript, wrote the last version, produced the infographics and supervised the entire manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Neural basis of impaired narrative discourse comprehension in prodromal and mild dementia with lewy bodies

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Narrative discourse (ND) comprehension is a complex task that implies not only linguistic abilities but also other cognitive abilities, including efficient executive functioning. An executive dysfunction has been described in dementia with Lewy bodies (DLB) from the early stage. Here, we question the link between executive dysfunction in DLB and narrative comprehension. The aim of our study was to evaluate ND comprehension and to investigate the neuroanatomical basis for its impairment in the early stage of DLB. DLB patients (N = 26) and controls (N =19) underwent the ND comprehension test of the Montreal Protocol for Evaluation of Communication (MEC). An additional, qualitative analysis was conducted on their verbal productions. Cognitive tests assessing verbal episodic memory, executive functions, naming and oral syntactic comprehension were also performed. Brain gray matter correlates of the ND comprehension test were examined using voxel-based morphometry (VBM). An ND comprehension impairment was found for prodromal and mild DLB patients as compared to controls. These difficulties were correlated with the Frontal Assessment Battery (FAB) score. ND comprehension impairment in DLB was further characterized by a deficit in the organization and the logic of the discourse. Moreover, VBM analysis revealed a correlation between striatal gray matter volumes and DLB patients' ability to extract and organize relevant information ($p < 0.05$, FDR correction, cluster level). The ND comprehension impairment in DLB patients could be related to their executive dysfunction through a deficit of information selection and organization that correlates with the volumetric reduction of striatal gray matter.

KEYWORDS

prodromal dementia with lewy bodies, narrative discourse, executive functions, voxel-based morphometry, striatum

Introduction

Verbal comprehension is a complex interpretative activity that leads to the conception of coherent mental representations. Comprehension implies not only linguistic abilities but also attention, memory, pragmatics and executive skills (Kim et al., 2019). Coherence is guaranteed by theme maintaining, adequacy between information and the congruence between mental representation and personal knowledge. The coherent representation of a discourse is ensured by the generation of inferences (Silagi et al., 2014). Coherence is established on two levels; local coherence corresponds to the microstructure of the discourse, and global coherence corresponds to its macrostructure (Kintsch, 1988). Most importantly, the continuous integration of both local and global coherence in a situational model integrating one's general knowledge about the world and context is required for a complete comprehension of the discourse.

At the brain level, several regions have been suggested as supporting discourse comprehension beyond the left dorsomedial prefrontal cortex (Ferstl et al., 2007). The processing of narrative coherence would be specifically associated with cingulate cortex, medial frontal (Ferstl et al., 2007) and parietal regions, while posterior parietal regions would be involved in the construction of the situational model, and frontal-temporal networks would support its maintenance over time (Yarkoni et al., 2008). More specifically, inference elaboration would rely on temporal regions (Virtue et al., 2006; Kim et al., 2019).

Discourse comprehension implies numerous cognitive abilities that may be impaired during aging, which is why a growing number of studies have investigated the evolution of comprehension in aging. Indeed, an aging effect is observed on inference generation in healthy adults (Williams et al., 2012). In neurodegenerative diseases, comprehension is further reduced (Murray and Stout, 1999; Welland et al., 2002; Monetta et al., 2009; Gaudreau et al., 2013). Detail and implicit information processing, like inferences, is impaired in early stage Alzheimer's disease (AD; Welland et al., 2002; Gaudreau et al., 2013), Parkinson's disease (PD; Murray and Stout, 1999; Monetta et al., 2008, 2009) and Huntington's disease (HD; Murray and Stout, 1999; Saldert et al., 2010). However, to date, little is known about narrative discourse (ND) comprehension in dementia with Lewy bodies (DLB).

Dementia with Lewy bodies is a cognitive neurodegenerative disease considered as the second leading cause of dementia, representing 15–20% of all dementia. DLB diagnosis is characterized by cognitive impairment plus the presence of two of the core clinical features, namely attentional fluctuations, visual hallucinations, REM sleep behavior disorder and elements of parkinsonism (McKeith et al., 2017). At the cognitive level, DLB impacts several functions: patients display cognitive and attentional fluctuations (Ballard et al., 2001, 2002), an early visuoconstructive impairment (Kemp et al., 2017), an executive dysfunction characterized by a lack of flexibility and thought mobility (Petrova et al., 2015; Kemp et al., 2017) and a moderate alteration of semantic memory as compared to AD (Calderon et al., 2001). At the brain level, Lewy body spectrum disorders (LBSD) such as PD, Parkinson's disease with dementia (PDD) and DLB are characterized by diffuse brain lesions related to the progression of the abnormal aggregation of alpha-synuclein, associated with pervasive cellular death in dopaminergic regions of the brain. However, contrary to other synucleinopathies, like PD, the pattern of alteration seems to spread to more cortical regions, such as the insula (Roquet et al., 2017), and to be associated with both phosphorylated tau and beta-amyloid deposits in the affected regions (Patterson et al., 2019).

Studies investigating language in DLB are scarce and tend to include other LBSD, such as PD and PDD. It has, however, been demonstrated that DLB patients have a reduced speech rate as compared to PD, PDD and control subjects, especially when the syntactic complexity increases, independently of language skills and possibly due to an executive dysfunction (Ash et al., 2012b). Moreover, it has been demonstrated that although the lexicon seems to be preserved in LBSD (Ash et al., 2012a; Boschi et al., 2017), action fluency would be particularly impaired in DLB (Delbeuck et al., 2013), suggesting an alteration of syntactic processes. Accordingly, in LBSD, and more particularly in DLB, studies found a syntactic impairment in both production (Ash et al., 2012a; Boschi et al., 2017) and comprehension, characterized by a deficit in syntactic ambiguity processing (Grossman et al., 2012). More generally, the organization of ND would be impaired in LBSD: the appreciation of the internal structure of scripts, theme maintaining, and coherence are disturbed. These deficits would be related to the executive dysfunction (Ash et al., 2012b; Gross et al., 2013; Grossman et al., 2017). Indeed, Ash et al. (2012a) found frontal and temporal cortical atrophy to be linked to the judgment of script organization. This led them to propose the involvement of the frontal ventrolateral cortex in ND coherence given that this region is involved in both executive functioning and ND. Moreover, Gross et al. (2013) highlighted the role of basal ganglia and frontal-striatal networks in temporal sequencing and selection of competing actions processes, which led them to suggest the potential contribution of a subcortical dysfunction in the script processing impairment in LBSD.

Accordingly, the goal of this study was to investigate the link between early executive dysfunction in DLB and discourse comprehension impairment. We predicted that early stage DLB patients would be more impaired in a narrative comprehension task than control subjects. The DLB narrative deficit would be related to DLB patients' executive dysfunction through the disorganization of ND. Moreover, the ND comprehension

Abbreviations: ND, narrative discourse; GM, gray matter; MEC, Montreal protocol for the evaluation of communication; FAB, frontal assessment battery.

deficit would be supported by a gray matter loss in frontal regions, which support executive functioning.

Participants and methods

Participants

Forty-five older adults participated in the study, comprising 26 patients with probable DLB and 19 controls. The experimentations were conducted in Strasbourg at geriatric day hospital of the Hôpital Saint François (France) from September 2018 to March 2019. All participants were fluent French speakers, free from any non-compensated hearing loss or language impairment that would impede comprehension of instructions, and free from noticeable anomia (see below for details of neuropsychological testing). DLB participants were included as follows: diagnosis of probable DLB according to the criteria of McKeith et al. (2017) for mild DLB and McKeith et al. (2020) for prodromal DLB, Mini-Mental State Examination (MMSE) score superior or equal to 19 in the absence of a comorbid neurodegenerative disorder. Controls were excluded if they presented an MMSE score below 26, a subjective cognitive complaint or a history of brain disease.

Procedure

All participants underwent a neuropsychological examination on the day of testing. Global cognitive functioning was assessed by MMSE, oral syntactic comprehension by GREMOTs (Bézy et al., 2016), image denominations by DO80 (Deloche and Hannequin, 1997), episodic memory by the French version of the free and cued selective reminding test (FC-SRT) called RL-RI-16 (Van der Linden et al., 2004), and executive functioning by Frontal Assessment Battery (FAB; Dubois et al., 2000).

Narrative discourse comprehension was assessed using the ND test taken from the Montreal Protocol for the Evaluation of Communication (MEC; Joanette et al., 2004), which is aimed at investigating inferential processes involved in access to the meaning of the story. In this test, a 214-word-long story consisting of 5 paragraphs (corresponding to the narrative structure; initial situation, complications, transforming action, denouement, final situation) is read to the participant twice. The first reading is fragmented by paragraph and participants are asked after each paragraph to immediately recall as many elements as they can. Thirty elements are expected to be recalled for the whole story (total information score/30), out of which 17 are considered to be principal elements (principal ideas score/17). The second reading is performed in its entirety without pause, after which the participant is instructed to recount the story, out of which 13 key elements are expected (entire recall score/13). Finally, participants are asked 12 comprehension questions to estimate their global understanding of the story (comprehension questions score/12).

A qualitative grid, inspired by several narrative analysis grids (Boutard Corinne, 2010; Bézy et al., 2016), was created to evaluate recall coherence. It assesses the reconstruction of the narrative by the participant throughout the story recall. Each point evaluated in this grid is representative of narrative coherence and involves executive functioning. The grid is comprised of 7 sub-items, distributed in 4 groups:

- Narrative theme.
- Discourse organization: respect of narrative structure, actions chronology and main ideas/details proportion.
- Logic of discourse: sequence of actions and relation markers.
- Discourse coherence: references.

Each sub-item was evaluated using ordinal scale from 0 (highly impaired) to 3 (normal performance). A narrative coherence score (out of 21) was finally obtained. Each examination was recorded and transcribed in order to evaluate the productions.

Neuroimaging

Whole-brain isotropic high-resolution T1-weighted MPRAGE images (TR = 1.9 s; Flip angle = 9°; TE = 2.53 ms; TI = 900 ms; FOV = 192 × 192 × 176; voxel size = 1 mm^3) were acquired for 23 of the 26 DLB patients during the same day as the behavioral testing using a 3T Siemens Magnetom VERIO MRI (Siemens, Erlangten, Germany) equipped with a 32-channel coil.

Data analysis was carried out using the Statistical Parametric Mapping package (SPM12; Wellcome Trust Centre for Neuroimaging). Briefly, after visual inspection and quality control, the images were segmented into 6 different maps of tissue class probabilities, including gray and white matter. These maps were subsequently spatially normalized using non-linear deformations (DARTEL algorithm) to obtain a study-specific template, which was in turn coregistered to the MNI152 template. The DARTEL flow fields were subsequently used to create a normalized gray matter (GM) map for each participant, in which the voxel-wise value of GM probability was modulated depending on the amount of warping applied during normalization in order to preserve global GM quantities, and smoothed using an 8 mm^3 full width at half-maximum (FWHM) Gaussian filter. Statistical analysis was then carried out on DLB patients' brain MRIs using a general linear model GLM model including 4 regressors of interest (principal ideas, total information, entire recall and comprehension questions) and a regressor of non-interest (total GM volume), with an uncorrected voxel-wise

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threshold of $p < 0.005$, cluster-wise corrected at $p < 0.05$ false discovery rate FDR.

Statistical analysis

The group differences between the control participants and the DLB participants, concerning ND and narrative coherence, were evaluated using Student and Wilcoxon-Mann-Whitney statistical tests (significance level set at 0.05). Statistical correlations were evaluated in the DLB group between narrative discourse score, narrative coherence score, MMSE, age and neuropsychological tests using Pearson's and Spearman's correlation tests (for interval and ordinal scale variables, respectively).

Results

Participants' characteristics

The two groups, DLB and controls, were not statistically different regarding age, sex, oral syntactic comprehension score and memory scores (Table 1). However, as expected, there was a significant difference between the two groups for the MMSE, DO-80 and FAB scores, with DLB participants performing less well than controls.

Intergroup comparisons

Globally, DLB patients performed worse than control participants in the ND tasks. DLB participants recalled less information than controls [principal ideas score T (43) = 3.37; $p = 0.002$ and total information score T (43) = 3.17; $p = 0.003$].

TABLE 1 Comparison of participants' characteristics between the DLB and control groups.

Characteristics	DLB group	Control group	Statistics	*P*-value
Group (*n*)	26	19		
Age *(sd)*	71.15 *(10.43)*	66.37 *(8.58)*	T (43) = −1.63	0.109
Sex ratio (F/M)	15–11	13–6	Chi^2 (1) = 0.54	0.463
MMSE *(sd)*	26.08 *(3.02)*	28.80 *(1.13)*	T (43) = −3.72	**0.00057***
Oral syntactic comprehension *(sd)*	5.85 *(0.61)*	6 *(0)*	T (43) = −1.09	0.281
DO80 *(sd)*	76.64 *(4.25)*	79.42 *(0.84)*	T (42) = −2.80	**0.0076***
FAB *(sd)*	15 *(2.91)*	17.47 *(0.96)*	T (43) = −3.55	**0.0009***
Free total recall–RL-RI/16 *(sd)*	24.23 *(8.79)*	28.05 *(6.60)*	T (39) = −1.56	0.128
Delayed total recall–RL-RI/16 *(sd)*	14.65 *(2.48)*	15.5 *(1.20)*	T (36) = −1.32	0.196

F/M, female/male; MMSE, mini–mental state examination; FAB, frontal assessment battery.
*Indicates significance at $p < 0.05$.

The entire recall and comprehension mean Z-scores of DLB patients were lower than those of controls [entire recall score T (43) = 2.91; $p = 0.006$ and comprehension questions T (43) = 3.76; $p = 0.0005$], with a marked variability of the DLB performances; Thirty-one percent of DLB patients were within the pathological range concerning entire recall, and 38.5% of them exceeded the pathological cut-off concerning comprehension questions.

Narrative coherence scores were significantly lower in the DLB group than in the control group ($U = 83$; $p = 0.0003$).

In terms of "actions chronology," there was no statistical difference between the DLB and control groups ($U = 167$; $p = 0.114$). DLB patients obtained significantly lower scores than controls on "narrative themes" ($U = 152$; $p = 0.0101$), "respect of narrative structure" ($U = 125.5$; $p = 0.006$), "relations markers" ($U = 122$; $p = 0.002$) and "references" ($U = 142$; $p = 0.0099$). DLB group scores were much lower than those of the control group for "main ideas/details proportion" ($U = 93$; $p = 0.0008$) and "sequence of actions" ($U = 111$; $p = 0.0009$) (Figure 1).

In the control group, narrative coherence scores were homogeneous and tended to reach the maximum, with most points lost in the "main ideas/details proportion" section (Figure 1). In the DLB group, narrative coherence impairments were particularly present in 3 sub-items: "main ideas/details proportion," "respect of narrative structure," and "sequence of actions" (Figure 1).

Clinical correlations

In the control group, no statistical correlation was observed between the scores of the ND test and the neuropsychological examination. In the DLB group, the entire recall score correlated significantly with the MMSE score [r(24) = 0.61; $p = 0.00098$], age [r(24) = −0.5; $p = 0.01$] and with the free total recall of RL-RI/16 [r(20) = 0.61; $p = 0.0026$]. The comprehension questions score correlated with the MMSE score [r(24) = 0.5; $p = 0.009$] and with the entire recall of the ND test of the MEC protocol [r(24) = 0.696; $p = 0.000079$]. There was no significant correlation between the comprehension questions score and age [r(24) = −0.38; $p = 0.055$], the FAB score [r(24) = 0.32; $p = 0.11$] or the total delayed recall of RL-RI/16 [r(18) = 0.09; $p = 0.70$]. The qualitative narrative coherence score correlated with the FAB score [r(21) = 0.5; $p = 0.014$]. The Principal ideas score of the MEC protocol correlated with the FAB score [r(24) = 0.52; $p = 0.007$] but did not significantly correlate with the total free recall of RL-RI/16 [r(20) = 0.29; $p = 0.19$].

Those DLB patients who did not produce the final inference performed significantly lower at comprehension questions than the DLB patients who produced the final inference ($U = 12$; $p = 0.0005$), suggesting a link between a high score on comprehension questions and the generation of the final inference. There was no statistical difference between DLB

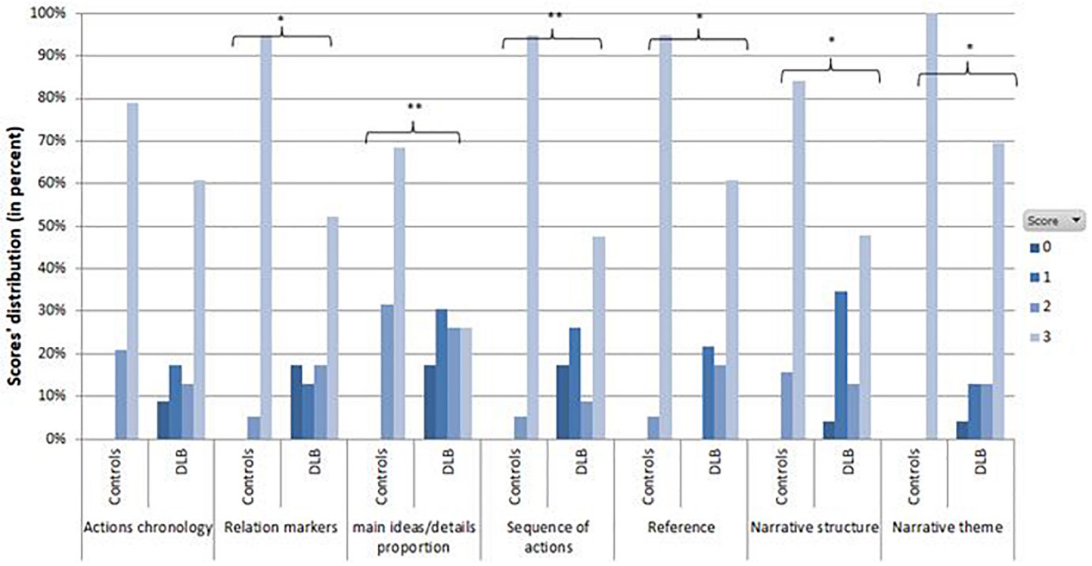

FIGURE 1
Distribution of each sub-item of narrative coherence in DLB patients and controls (Significance: *$p < 0.05$; **$p < 0.001$).

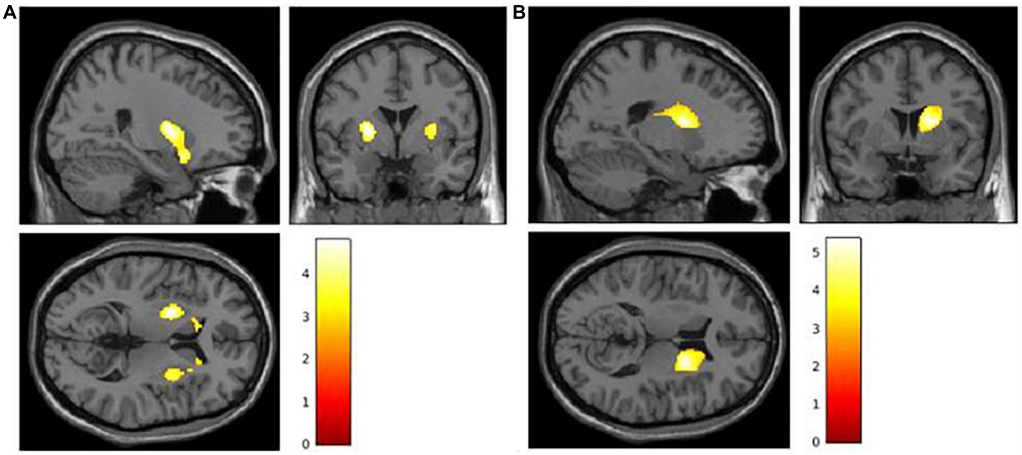

FIGURE 2
(A) Correlation between gray matter volume and principal ideas score. **(B)** Correlation between gray matter volume and entire recall Z-score. Colored voxels show regions for which statistical analysis is significant at $p < 0.005$ at the voxel level and FDR-corrected at $p < 0.05$ at the cluster level.

patients producing the final inference and those not producing it in terms of the FAB score ($U = 60$; $p = 0.382$).

Voxel-based morphometry correlations

Gray matter density in bilateral putamen and bilateral caudate nuclei (i.e., bilateral striatum) was found to be positively correlated in DLB participants with their principal ideas score ($p < 0.05$, cluster-wise FDR-corrected, extend threshold 1889 voxels; Figure 2A). Additionally, a positive relationship was found between the gray matter volume in the right caudate nucleus and the entire recall Z-score in DLB participants ($p < 0.05$, cluster-wise FDR-corrected; Figure 2B). The correlation between the entire recall and the caudate nuclei becomes bilateral when the cluster-wise threshold is lowered (extend threshold 500 voxels), which seems to indicate that the left caudate nucleus is also involved. Other variables (total information score and comprehension Z-score) did not display a significant correlation with the gray matter volume of either of these cerebral areas.

Discussion

The aim of the present study was to investigate to what extent the executive deficit associated with the early stage of DLB would influence the comprehension of ND in DLB patients. Our results confirmed an impairment of narrative comprehension in DLB patients and a strong correlation between their level of executive functioning and both their narrative coherence and principal ideas scores, which indicates that their capacity to re-elaborate the narrative meaning could be related to the efficiency of their executive functioning. The impairment of narrative comprehension further appeared related to a lower volume of gray matter in the striatum of patients.

Several studies have also shown deficits in narrative organization between DLB patients. Gross et al. (2013) found that DLB and PDD patients were less sensitive to the events' order of a script than PD patients and controls, which highlights the deficits of DLB and PDD patients in processing the structure of a script. Likewise, Ash et al. (2012b) demonstrated a reduced sensitivity to the hierarchical organization of a narrative in DLB and PDD patients, as compared to both controls and PD patients. Interestingly, in their study, theme maintaining and connections between items, which rely the most on executive functioning, were also the most affected domains. In addition, the narrative organization was more impaired in DLB subjects than in PDD subjects. Ash et al. (2012a) concluded that these difficulties in the comprehension and production of the organization of the narrative result from an executive control deficit.

Accordingly, our qualitative findings indicate that patients in the DLB group were particularly impaired on the sub-items "main ideas/details proportion" and "sequence of actions," indicating that their profile is characterized by a deficit in prioritizing and organizing information. Thus, DLB patients' dysexecutive syndrome could be involved in their deficit of selection and organization of relevant information affecting the link between microstructure and macrostructure, and ultimately coherence. Accordingly, DLB patients show difficulties with both local and global coherence processing as compared to control subjects (Boschi et al., 2017).

We expected that the narrative comprehension deficit would be related to frontal gray matter loss. Indeed, the narrative comprehension literature highlights the implication of the dorsomedial prefrontal cortex as an essential neural substrate of coherence processes (Ferstl et al., 2007; Yarkoni et al., 2008; Ash et al., 2012b; Noh et al., 2014). Difficulties in narrative discourse in neurodegenerative diseases such as corticobasal syndrome are reported to be related to atrophy patterns in frontal and parietal regions compatible with executive functioning (Gross et al., 2010). In the latter study, the deficit in narrative discourse was underlain by an alteration of the integration of the information into a meaningful whole, which is central to the entire recall and relies heavily on multiple round trips between the micro and macro level. These elements are congruent with our own behavioral results since the principal idea and entire recall scores involve the selection of relevant information through the same processes.

Our volumetric analyses did not find any significant link with cortical regions' gray matter density, but rather revealed a correlation between the gray matter density in the striatum of DLB participants and their principal ideas score as well as their entire recall score, more specifically in the bilateral putamen and the right caudate nucleus, respectively. Although the role of the striatum in motor control is now well accepted, several studies have highlighted the implication of the putamen and the caudate nucleus in language processes such as speech (Viñas-Guasch and Wu, 2017), syntax (Teichmann et al., 2006; Long et al., 2019) and high-order language processes (Moretti et al., 2017; Viñas-Guasch and Wu, 2017). Most importantly, Moretti et al. (2017) reported that basal ganglia enhance cortical efficiency in language processing, especially through their role in selecting relevant signals. Indeed, functional models of basal ganglia describe the extensive functional interconnection between the striatum and the prefrontal cortex (PFC; Monchi et al., 2001, 2006; Moretti et al., 2017).

Simard et al. (2011) distinguished two different loops participating to the executive processing of verbal material: one involved in planning processes, implicating the caudate nucleus and the ventrolateral PFC, and the other, involved in execution processes, implicating the putamen and the posterior PFC. Furthermore, according to Provost et al. (2010), although all monitoring processes (both self-ordered and externally triggered) involve the dorsolateral PFC, only self-ordered monitoring specifically requires the caudate nucleus. Finally, Gross et al. (2013) assumed the implication of striato-frontal networks and the basal ganglia in temporal sequencing and competing actions selection. Overall, our results therefore suggest a link between the alteration of striatal regions involved in specific executive components of language processing as a source for narrative comprehension impairment in early DLB patients.

Accordingly, we found correlations between some qualitative indicators of narrative comprehension and behavioral measures of executive functioning. However, we assessed the latter as a whole using a screening test (FAB), which did not allow for an exploration of specific executive components involved in the effect. Future studies should therefore include various executive tests such as the Trail Making Test (TMT) or Stroop test in order to investigate this matter more thoroughly.

Moreover, our samples were relatively small, and there was no other degenerative disease group that would have allowed us to assert the specificity of this link. We thus cannot rule out the possibility that the overall cognitive impairment resulting from

dementia is not responsible for the alteration of ND. Grossman et al. (2017) argued that LBD patients have more difficulties in assessing ND than non-demented LBD patients, indicating a progression of ND alteration throughout the development of the disease. Nonetheless, these difficulties in narrative organization were not correlated with the level of dementia (MMSE score), neither could they be entirely explained by a linguistic deficit.

Additionally, given that the deficit in ND was found on all of the subscores of the MEC scale, one could argue that the poor performance in ND was related to memory deficits. However, our participants did not display an impairment of episodic memory according to their scores in RL/RI 16, which tends to indicate that these narrative comprehension difficulties were not particularly due to verbal episodic memory deficits.

Another limit of our study is that we did not exclude patients with a psychiatric onset of prodromal DLB, even though all of our prodromal DLB patients had MCI. It is of relevance, as the affection of nigrostriatal pathways in either conditions (cognitive or psychiatric onset) can be different (Hansen et al., 2021), possibly in relation with a malfunction of the locus coeruleus (Hansen, 2021) in psychiatric onset patients. Since the locus coeruleus modulates executive functions through the noradrenergic system, and psychiatric symptoms are frequent at the prodromal stage of DLB (Blanc et al., 2022), future studies should explore potential implications of the type of onset of DLB (that is, cognitive, psychiatric or mixed) in this matter. Overall, our results are therefore consistent with the hypothesis that executive dysfunctioning in DLB patients could lead to difficulties in planning and reasoning about the narrative structure. We have demonstrated an impairment of narrative understanding in prodromal and mild DLB patients. Moreover, and contrary to our expectations, we have shown a strong correlation between these difficulties in narrative comprehension and the gray matter density of the striatum. This would appear to be the first demonstration of such a correlation. According to Yang et al.'s (2019), comprising a meta-analysis of 78 functional magnetic resonance imaging (fMRI) studies, discourse comprehension involves the activation of large cortical networks. These different results could be explained by the experimental population characteristics. In our study, striatum involvement in discourse comprehension would likely be linked to DLB pathology. Future research involving functional imaging in prodromal DLB will be needed to go further in the understanding of the role of the striatum and striato-frontal processes underlying narrative comprehension.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

This research involving human participants were approved by the ethics committee: Comité de protection des Personnes Est IV Strasbourg (number: Eudract 2012-A00992-41/HUS5330). All participants provided written informed consent to this study.

Author contributions

AF, MJ, FB, and EF contributed to the conception and design of the study. AF, MJ, LL, PL, and MM organized the database. AF, MJ, and LL performed the statistical analysis. AF and MJ wrote the first draft of the manuscript. LL and FB wrote sections of the manuscript. AF, MJ, LL, and FB contributed to manuscript revision, read, and approved the submitted version. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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State-of-the-art review of the clinical research on menopause and hormone replacement therapy association with Parkinson's disease: What meta-analysis studies cannot tell us

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The menopause is a midlife endocrinological process that greatly affects women's central nervous system functions. Over the last 2 decades numerous clinical studies have addressed the influence of ovarian hormone decline on neurological disorders like Parkinson's disease and Alzheimer's disease. However, the findings in support of a role for age at menopause, type of menopause and hormone replacement therapy on Parkinson's disease onset and its core features show inconsistencies due to the heterogeneity in the study design. Here, we provide a unified overview of the clinical literature on the influence of menopause and ovarian hormones on Parkinson's disease. We highlight the possible sources of conflicting evidence and gather considerations for future observational clinical studies that aim to explore the neurological impact of menopause-related features in Parkinson's disease.

KEYWORDS

Parkinson's disease, menopause, hormone replacement therapy, risk, onset

Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder, affecting over 6 million people worldwide, and this number is projected to double over the next 20–30 years (Poewe et al., 2017; GBD 2016 Neurology Collaborators, 2018). The disease is characterized primarily by the loss of dopaminergic neurons and presence of Lewy bodies (α-synuclein accumulation within surviving neurons) in the substantia nigra

pars compacta (Poewe et al., 2017). The cardinal disease features consist in 4 main motor symptoms: bradykinesia, postural instability, rigidity, and tremor at rest (Poewe et al., 2017; Armstrong and Okun, 2020).

Evidence of a role for sex dimorphism in PD has increased over the last decades, showing that women, compared to men, present a lower PD incidence and prevalence (Wooten, 2004; Bourque et al., 2009; Hirsch et al., 2016; Meoni et al., 2020; Turcano and Savica, 2020), later onset (Haaxma et al., 2007; Meoni et al., 2020), and better motor scores in the Unified PD Rating Scale (UPDRS; Meoni et al., 2020). The influence of biological sex and gonadal hormones on dopamine neurodegeneration (Xing et al., 2017), neuroinflammation (Villa et al., 2016) and oxidative stress (Chainy and Sahoo, 2020) could explain the apparent less susceptibility and milder progression of motor symptoms in women (Cerri et al., 2019). However, how hormones modify PD features in women during menopause and how this compares to men needs further elucidation.

To understand if gonadal hormones play a role in the sexually dimorphic clinical presentation and response to treatment in PD patients (Georgiev et al., 2017), many studies have shown a link with total lifetime exposure to circulating sex hormones (Gatto et al., 2014), reproductive life events that alter ovarian hormone levels, like menopause and pregnancy (Martignoni et al., 2003) and hormone targeted therapies such as use of oral contraceptives (Cartwright et al., 2016) and hormone replacement therapy (HRT; Wang et al., 2015). Altogether, the current clinical literature points toward a trend on a neuroprotective effect of estrogen in PD (Tsang and Ho, 2001); however, inconsistencies between findings have been reported. In this review, we aimed to expand the current understanding of the hormone-PD link observed in patients by discussing the current and most updated clinical evidence. Specifically, we focus on three associations: age at menopause-age at PD onset, type of menopause-PD risk, and HRT use-PD risk. Further, we discuss the possible sources of discrepancies among studies, which meta-analysis studies may not be able to highlight.

Criteria for literature selection

We exhaustively covered the clinical literature, published until July 2021, focusing on the association between menopause and HRT with PD risk using the following key words: Menopause, Early Menopause, Premature Menopause, Perimenopause, Hormone replacement therapy, estrogen, progesterone, AND/OR Parkinson's disease, Neurodegenerative diseases, in PubMed and MEDLINE databases. The articles included in the main analysis of this review are shown in Table 1. Articles that did not assess the risk of PD or had incomplete data were excluded from the main discussion. Only papers with reported adjusted odds or relative risk ratios (OR/RR) were included in the figures.

Age at menopause-age at PD onset and risk of PD

The lower prevalence of PD in women compared to men (Gillies et al., 2014; Picillo et al., 2017; Marras et al., 2018; Meoni et al., 2020) suggests that reproductive life milestones, such as pregnancy, age at menopause, and duration of fertile life may protect women from greater PD deterioration (Martignoni et al., 2002; Miller and Cronin-Golomb, 2010; Gillies et al., 2014), since these determine the cumulative lifetime exposure to endogenous estrogens (Gatto et al., 2014; Yoo et al., 2020). Average age at onset in PD is ~60–65 years old (Poewe et al., 2017). From a clinical perspective, the closest reproductive life event to PD onset is the menopause transition (perimenopause), a period of approximately 5 years affecting women of ~45–55 years old. Perimenopause is typified by erratic fluctuations in hormone levels (Brinton et al., 2015) during a life stage that coincides with the PD prodromal phase (~10–20 years before clinical symptoms emerge). This terminates in a post-menopause stage characterized by low levels of ovarian hormones, estrogen, and progesterone (Harlow et al., 2012). The age at menopause is defined as the age at which a woman has had amenorrhea for 12 months ending the menopausal transition (Harlow et al., 2012). A higher occurrence of PD in post-menopausal versus pre-menopausal women has been reported (Ragonese et al., 2004; Ragonese et al., 2006; Labandeira-Garcia et al., 2016; Lv et al., 2017; Picillo et al., 2017; Jurado-Coronel et al., 2018). Hence, a clear understanding of recommendations, follow-ups, and therapies is needed for physicians treating patients during the perimenopause transition. Nevertheless, current PD treatments follow a one-size-fits-all approach and do not take sex and menopausal stage into account. In the next paragraphs we will highlight the main literature in support of a positive association between menopause and PD risk and age at onset.

We found 16 observational studies that have assessed the association between menopause and PD in the last two decades. 10 case–control, 3 longitudinal cohort and 3 cross-sectional studies (Benedetti et al., 2001; Ragonese et al., 2004; Popat et al., 2005; Ragonese et al., 2006; Nicoletti et al., 2007; Rocca et al., 2008; Simon et al., 2009; Yadav et al., 2012; Cereda et al., 2013; Gatto et al., 2014; Greene et al., 2014; Liu et al., 2014; Nitkowska et al., 2014; Frentzel et al., 2017). 7 case–control studies analyzed the risk of PD in women with early age at menopause (Figure 1). Among these, Benedetti et al. (2001) and Ragonese et al. (2004) have reported higher odds of PD onset in women which reached menopause before 46 years-old. However, the adjusted multiple logistic regression models in these studies did not reach statistical significance. The recent work by Canonico et al. showed a significant association between age at menopause <50 years-old and risk of PD (Canonico et al., 2021). Another report by Nitkowska et al. (2014) showed that while early menopause occurred in only 16% of the control subjects, this number increased to 24% in the PD cohort. Other cohort studies have explored the relationship as well between age at menopause and PD risk. Liu et al. (2014) reported increased odds of PD onset in

TABLE 1 Observational studies on menopause and HRT and the association with risk of PD included in the main text.

Study	Type of study	PD sample size	Reproductive factors assessment type	Type of menopause	Multivariate/ Matched adjustment	HRT	HRT duration
Ascherio et al., 2003	Cohort	154	Medical record Self-reported	Natural Hysterectomy ≤1 oophorectomy Bilateral oophorectomy	(1), (3), (4), (7), (8), (9), (10)	HRT	<5 years ≥5 years
Baldereschi et al., 2003	Cohort	113	In-person interview	N/A	(1), (3), (5), (13)	ERT	N/A
Benedetti et al., 2001	Case–control	72	Medical record	Natural Surgical Hysterectomy only Bilateral oophorectomy	(5), (7)	ERT	< 6 months ≥ 6 months
Canonico et al., 2021	Case–control	130	Medical record In-person interview	Natural Hysterectomy Bilateral oophorectomy	(1), (3), (4), (5), (7), (9), (13), history of head trauma.	HRT	N/A
Cereda et al., 2013	Cross-sectional	497	Self-reported In-person interview	Natural Surgical	(1), (3), (5), (6), (12), (13) diabetes, hypertension, NSAID use, sedentary lifestyle, birth cohort and regular menses and the clinical features of PD.	HRT	>6 months
Currie et al., 2004	Case–control	68	Self-reported In-person interview	Natural	(1)	ERT	N/A
Frentzel et al., 2017	Cross-sectional	54	Medical record Self-reported	Natural	N/A	HRT	N/A
Gatto et al., 2014	Case–control	228	Self-reported	Natural Hysterectomy ≤1 oophorectomy Oophorectomy	(1), (2), (3)	HRT ERT	N/A
Greene et al., 2014	Case–control	743	Medical record Telephone interview	Natural Bilateral oophorectomy	(1), (3), (4), (5), (14), (15), (16)	HRT	≥ 5 years
Kim et al., 2021	Cohort	2,313	Medical records	N/A	(1), (2), diabetes, hypertension, cardiovascular disease, chronic kidney disease.	HRT	≤1 year 1–3 years 4–6 years >6 years
Kusters et al., 2021	Case–control	805	Medical record In-person interview	Natural Bilateral oophorectomy	(1), (3), (4), (5), (6), (7), (8)	HRT	N/A
Liu et al., 2014	Cohort	410	Self-reported	Natural Hysterectomy ≤1 oophorectomy	(1), (2), (3), (4), (7), (8), (10)	HRT ERT ERT + PRT	1–9 years ≥10 years
Lundin et al., 2014	Case–control	137	Medical records	N/A	(2), (3), (4), (5), (15),	HRT ERT ERT + PRT	≥ 2 years
Martignoni et al., 2003	Case–control	150	In-person interview	Natural Surgical	N/A	HRT	N/A
Nicoletti et al., 2011	Case–control	200	Self-reported	Natural Hysterectomy	(1), (3), (4), (15)	HRT	≥ 6 months
Nitkowska et al., 2014	Case–control	76	Medical record	Natural Surgical	N/A	N/A	N/A
Popat et al., 2005	Case–control	178	Medical record In-person interview	Natural Hysterectomy Hysterectomy ≥1 oophorectomy	(1), (3), (7), (8), (12)	HRT	1–10 years > 10 years
Ragonese et al., 2004	Case–control	131	Self-reported	Natural Surgical	(1), (3), (4), (5), (9)	ERT	≥ 6 months
Ragonese et al., 2006	Cross-sectional	145	Medical record	Natural Surgical	(3), (5), (8)	N/A	N/A
Rocca et al., 2008	Cohort	79	Medical record Self-reported In-person interview	Hysterectomy ≤1 oophorectomy Bilateral oophorectomy	(1), (5)	ERT	N/A
Rugbjerg et al., 2013	Cohort	77	Self-reported	Natural Hysterectomy Oophorectomy	(1)	HRT	N/A

(Continued)

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TABLE 1 (Continued)

Study	Type of study	PD sample size	Reproductive factors assessment type	Type of menopause	Multivariate/ Matched adjustment	HRT	HRT duration
Simon et al., 2009	Cohort	244	Self-reported	Natural Hysterectomy ≤1 oophorectomy Hysterectomy >1 oophorectomy	(1), (3), (4)	ERT PRT ERT + PRT	< 5 years ≥ 5 years
Yadav et al., 2012	Case–control	81	In-person interview	Natural Surgical	(1)	ERT	N/A
Yoo et al., 2020	Cohort	varies	Medical record, Self-reported	Natural Surgical (excluding hysterectomy +/− oophorectomy)	(3), (7), (9), (11), exercise, hypertension, diabetes, cancer, dyslipidemia.	HRT	< 2 years, 2–5 years, ≥ 5 years

Matching or multivariate adjustments were (1) age, (2) race, (3) smoking, (4) coffee/caffeine, (5) education, (6) positive familial history, (7) age at menopause, (8) type of menopause, (9) alcohol, (10) oral contraceptives, (11) parity, (12) respondent type, (13) pesticide-use, (14) degree of urbanization, (15) family PD history, (16) age at first symptom. N/A, not available; HRT, hormone replacement therapy; ERT, estrogen replacement therapy; PRT, progesterone replacement therapy.

women with early menopause (<45 years-old). Similarly, Simon et al. (2009) showed a trend toward a decreased risk of PD in women with menopause after 45 years-old, nevertheless these studies did not reach significance. Interestingly, in the cohort study from Rocca et al. (2008), a prominently higher and significant risk of PD was reported in women with premature menopause (<38 years-old) compared to women with early menopause (38–45 years old; Figure 1).

In accordance with case–control reports, cohort studies do not always reach statistical significance. Potential points of discrepancy between studies may be the unstandardized research criteria used to classify patients, regression adjustment criteria, and formal representation of the analyses. As an example, the cohort study by Rocca et al. (2008) reported that menopause occurring before 38 years-old is an independent risk factor for PD; however the study analyzed the risk only in women with history of oophorectomy, in contrast to the other 2 mentioned cohort studies by Liu et al. (2014) and Simon et al. (2009), which included patients with natural and surgical menopause. Although these 2 studies were similar (Simon et al., 2009; Liu et al., 2014), the regression analyses were adjusted for different covariates leading to a difficult comparison. Additional support for a significant association between late age at menopause and decrease risk of PD comes from a recent work by Yoo et al., although the data was expressed as a Hazard Ratio rather than OR/RR and patients with history of hysterectomy were excluded from the study (Yoo et al., 2020).

Interestingly, to reduce certain biases common in observational studies, a recent work by Kusters et al. proposed the use of Mendelian randomization (MR) analyses to address the association of menopause age and PD risk (Kusters et al., 2021). The authors applied a MR to identify genetic variants linked to menopause and PD and used the 8 identified single nucleotide polymorphisms as an instrumental variable to demonstrate a significant inversed association between menopause age and risk of PD (Kusters et al., 2021). This suggests that non modifiable factors, such as genetic variants, in concomitance with the menopause might influence the risk of PD.

One case–control (Yadav et al., 2012), and 3 cross-sectional (Ragonese et al., 2006; Cereda et al., 2013; Frentzel et al., 2017) studies have explored the linear association between age at menopause and age at PD onset. The cross-sectional studies conducted by Yadav et al. (2012) and Frentzel et al. (2017) reported a significant positive correlation. Yadav et al. showed a positive correlation between age at menopause and age at PD onset ($R = 0.55$, $p = 0.001$), analyzing age-matched PD and healthy females in their case–control study (Yadav et al., 2012). Whereas, Frentzel et al. (2017) analyzed age-matched PD females and males, reporting also a positive correlation between age at menopause and age at PD onset (beta = 0.370, $p < 0.01$, adjusted $R^2 = 0.121$). Likewise, Ragonese et al. (2006) (beta = 0.25, SE = 0.15, $p = 0.003$) and Cereda et al. (2013) (Coeff. = 13.03, SE =5.62, $p = 0.021$). Despite the heterogeneity in sample size and inclusion criteria of these 4 studies, the authors analyzed the association between age

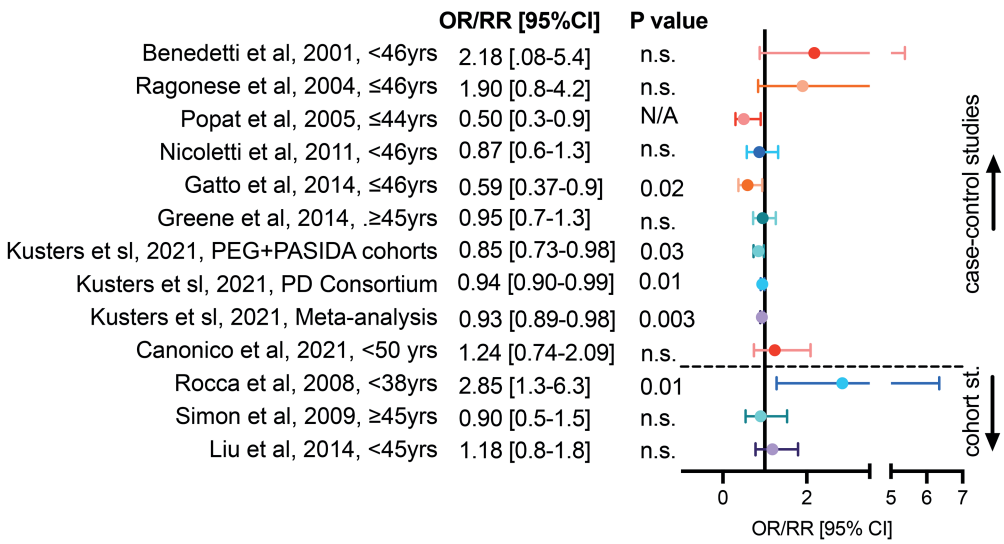

FIGURE 1
Age at menopause and risk of PD onset. Case–control studies in the upper section and cohort studies in the lower section of the forest plot are represented. For each study, figure reports adjusted OR/RR and 95% CI with level of statistical significance (*p* value). OR=odds ratio, RR=relative risk, 95% CI=95% confidence of interval.

at PD onset and age at menopause using numerical variables instead of binary categorizations, which led to more consistent and statistically significant results.

Type of menopause and risk of PD

To better understand how changes in levels of endogenous estrogen in menopausal women are associated with PD, previous works have looked at risk of PD in women with a history of surgical menopause. These studies suggested that abrupt decline of estrogen in women undergoing hysterectomy and uni/bilateral oophorectomy, commonly termed surgical menopause, may lead to a higher risk of PD, compared to women that experience a natural gradual change in estrogen levels during the menopause transition. However, the current evidence on this matter is in part inconsistent. In fact, it has been suggested that perimenopause acts as a neurological transition period, rendering the brain particularly susceptible to neurodegeneration (Brinton et al., 2015). Thus, the debate on whether the risk of PD neurodegeneration is triggered by the abrupt and complete loss of ovarian hormones, in the surgical menopause, or by erratic hormonal fluctuations during a critical window of time, such as the perimenopause, is still very active.

Among the clinical studies that have investigated the role of menopause in PD, case control studies by Benedetti et al. (2001) and Ragonese et al. (2004) have found opposite association between risk of PD onset and surgical menopause, although the type of surgical menopause was not specified. Interestingly, the risk of PD onset seems to be linked to the type of surgical menopause performed. In Canonico et al. a significant association between bilateral oophorectomy, but not hysterectomy, and risk of PD was found (Canonico et al., 2021). The prevalence of bilateral oophorectomy in controls was 9% in comparison to nearly 25% in PD cases. Furthermore, hysterectomy performed before 45 years-old increases the risk of PD onset, as reported by Nicoletti et al. (2011) and Popat et al. (2005) and in the cohort study by Rugbjerg et al. (2013). However, the findings were not significant in adjusted models. Divergent results have been reported regarding hysterectomy combined with unilateral oophorectomy. While some reports (Ascherio et al., 2003; Simon et al., 2009; Gatto et al., 2014; Liu et al., 2014) indicate no association with the risk of PD onset, studies by Benedetti et al. (2001) and Rocca et al. (2008), reported a significant risk of PD onset (up to three-fold higher). Instead, hysterectomy combined with bilateral oophorectomy has shown a protective effect in most case control and cohort studies (Ascherio et al., 2003; Popat et al., 2005; Simon et al., 2009; Rugbjerg et al., 2013; Gatto et al., 2014; Greene et al., 2014), with the exception of Rocca et al. (2008). Altogether, these studies point towards an increased risk of PD onset when hysterectomy is combined with unilateral oophorectomy and, at younger age (Figures 2A,B).

Despite the studies mentioned above suggest an increased risk of PD in women who underwent surgical menopause, compared to those who experienced a physiological menopause, the evidence about type of menopause (i.e., natural vs. surgical) and its relation to the risk of PD onset remains conflicting. The source of inconsistent findings between these studies might be related to

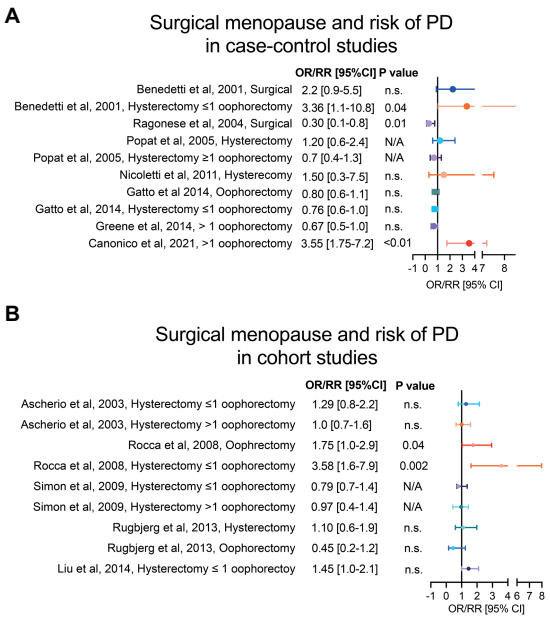

FIGURE 2
Type of menopause and risk of PD. Panel **(A)** shows case–control studies and panel **(B)** cohort studies that have analysed the risk of PD in women with surgical menopause compared to women with natural menopause. For each study, we reported the adjusted OR/RR and 95% CI with level of statistical significance (p value). OR=odds ratio, RR=relative risk, 95% CI=95% confidence of interval.

important confounding factors. Particularly, the underlying condition that prompts the surgical indication of hysterectomy and/or oophorectomy, and the medical management of the different type of menopause. The most common indications for hysterectomy are leiomyoma and abnormal uterine bleeding, known to be deeply related to progesterone and estrogen abnormalities (Vilos et al., 2015; Jewson et al., 2020). Hence, even though both ovaries are preserved in this surgical procedure, this is preceded by hormonal dysfunctions (Torrealday et al., 2017). When bilateral oophorectomy is performed, with or without uterus resection, women commonly receive preventive exogenous gonadal hormones (Domchek and Rebbeck, 2007). This may be a confounding factor when evaluating the association between bilateral oophorectomy and risk of PD onset and may explain the protective trend observed in some studies. Regarding the increased risk found for hysterectomy combined with unilateral oophorectomy, previous evidence has shown ovarian failure in the contralateral ovary following unilateral oophorectomy (Farquhar et al., 2005), paralleled by a loss of blood supply to the remaining ovary due to the uterus resection (Ahn et al., 2002). Furthermore, although higher risk of early ovarian failure has been reported in patients with history of unilateral oophorectomy (Rosendahl et al., 2017), most of these women do not receive HRT (Read et al., 2010). Finally, it's worth mentioning that a possible age-dependent effect, found in linear trend analyses of age at surgical menopause and risk of PD onset (Rocca et al., 2008), adds more variability to the mentioned findings. This suggests that age stratification should be analyzed in more depth in future studies.

Hormone replacement therapy and risk of PD

The molecular weight of endogenous gonadal hormones allows easy diffusion across the blood–brain barrier (Diotel et al., 2018). Likewise for exogenous steroids (Cipolla et al., 2009), although their preventive or detrimental potential on the neurons remains unclear (Simpkins et al., 2005). Exogenous steroids, also known as HRT, are commonly prescribed to women to relieve menopausal symptoms (Valdes and Bajaj, 2020). Conventional HRT includes both estrogen and progesterone hormones with various formulations and exerting different specificity of effects on the gonadal-brain axis (Schipper, 2016; Del Rio et al., 2018).

Several studies have explored the role of HRT on the risk of PD onset. A trend toward an increased risk of PD onset in women that received HRT, without distinction of formulation type, was observed in case–control and cohort studies (Ascherio et al., 2003; Martignoni et al., 2003; Popat et al., 2005; Nicoletti et al., 2007; Simon et al., 2009; Rugbjerg et al., 2013; Greene et al., 2014; Liu et al., 2014), as shown in Figures 3A,B. However, only in the study from Gatto et al. (2014) the results reached statistical significance in adjusted models. A more detailed assessment of the HRT formulations evidences a modest increased risk of PD in users of estrogen replacement therapy (ERT) in 2 cohort studies (Simon et al., 2009; Liu et al., 2014) in contrast to 6 case–control studies (Benedetti et al., 2001; Baldereschi et al., 2003; Currie et al., 2004; Ragonese et al., 2004; Gatto et al., 2014; Lundin et al., 2014) that showed a trend for a protective or no effect of ERT. The discrepancies between cohort and case–control studies may be explained by lack of analyses regarding the age at therapy initiation, type or stage of menopause, as well as the ERT subtype. Importantly, the combination of ERT with progesterone or progesterone-like replacement therapy (PRT) in Liu et al. (2014) and Lundin et al. (2014) showed a significant increased risk of PD onset. Moreover, Simon et al. (2009) reported higher odds of PD risk in a small sample of women receiving PRT alone compared to women receiving ERT alone or combined therapy.

In support of this, a recent retrospective analysis by Kim et al. showed that sources of discrepancy in the effect of HRT on different neurodegenerative disorders, including PD, may be related to route and duration of HRT administration (Kim et al., 2021), whereas a significantly reduced relative risk (RR) of PD was reported in women taking oral, but not transdermal, therapy. Additionally, this work supports the importance of including large sample sizes in this type of studies (Kim et al., 2021).

Regarding the ERT findings, it is important to highlight that there are two common formulations: the esterified estrogens and the conjugated estrogens. The esterified estrogens are predominantly estrone, whereas conjugated estrogens are a

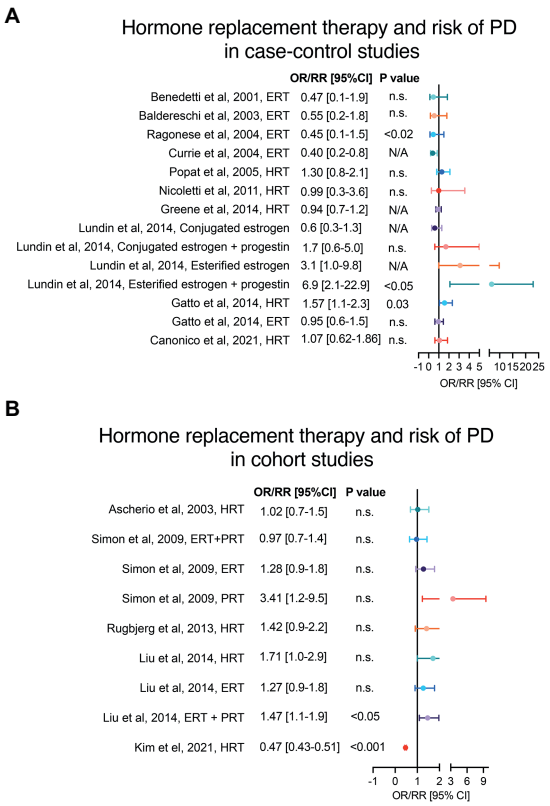

FIGURE 3
Hormone replacement therapy and risk of PD. Panels **(A,B)** show case–control and cohort studies, respectively, that have analysed the risk of PD in women with history of HRT. Adjusted OR/RR and 95% CI with level of statistical significance (*p* value). OR=odds ratio, RR=relative risk, 95% CI=95% confidence of interval, HRT=hormone replacement therapy, ERT=estrogen replacement therapy, PRT=progesterone replacement therapy.

mixture of more biologically active estrogens (like 17b-estradiol) with greater affinity for estrogen receptors than estrone (Lundin et al., 2014); nevertheless, these two formulations share the same FDA indications (Harper-Harrison and Shanahan, 2020). Lundin et al. (2014) showed that the trend toward an increased or decreased risk of PD onset is inverse depending on whether esterified or conjugated formulations are administered. Another interesting observation in the Benedetti et al. (2001) study was the opposite ERT contribution to PD in women depending on the menopausal type (i.e., natural vs. surgical). This association was supported by a similar observation in the study by Popat et al. (2005) in which women with natural menopause that received HRT had lower odds of developing PD, while women with oophorectomy plus hysterectomy on HRT had higher risk of PD onset. Additionally, studies on dementia and Alzheimer's disease, like the one by Whitmer et al. (2011), support the "window of opportunity hypothesis" that the use of HRT in midlife (before or during early menopause) only may be neuroprotective, whereas HRT initiation in late life could have deleterious effects and worsen the neurodegenerative processes (Marras and Saunders-Pullman, 2014; Kim and Brinton, 2021). Thus, we suggest that a stratification analysis of age at HRT initiation may clarify discrepancies seen in previous clinical observational studies and, moreover, could shed lights on possible age/time-dependent mechanisms of hormones in the central nervous system.

Although the publication of the Women's Health Initiative data in 2002 supported that HRT increases the risk of stroke and breast cancer (Rossouw et al., 2002), a recent national survey study reported that 37% of women are current or former HRT users (Gass et al., 2015). Therefore, a rigorous assessment of the HRT doses and different formulations in regard to the type of menopause, age of menopause, duration of HRT, medical indication of HRT, and other factors that can interact with HRT, such as caffeine consumption (Kim et al., 2017), is needed to improve recommendations for women in menopause. Even more, evidence from some of the mentioned observational studies regarding HRT suggests that estrogens may not be the only gonadal hormones capable of affecting the course of PD. Hence, the role of progesterone in influencing the central nervous system directly, through its neuronal receptors, or indirectly, through its action on the peripheral systems, requires further elucidation (Bourque et al., 2019; Cardia et al., 2019; Jarras et al., 2020; Kim et al., 2021).

In Lundin et al. the type of progestin used was the synthetic progesterone formulation medroxy-progesterone acetate (MPA), whereas this was not specified in Liu et al. and Simon et al (Simon et al., 2009; Liu et al., 2014; Lundin et al., 2014). Pre-clinical cell and animal models have shown progesterone to be neuroprotective, but not MPA (Singh and Su, 2013). Preclinical data suggest that progesterone may be neuroprotective in PD by increasing dopaminergic neurotransmission, exerting anti-inflammatory activity, and modulating several other neurotransmitter systems (including glutamatergic, GABAergic, norepinephrine, serotonin, and acetylcholine; Callier et al., 2001; Kritzer et al., 2003; Casas et al., 2013; Barth et al., 2015; Litim et al., 2017). Differently from endogenous progesterone, MPA is detrimental to neurons as it can induce glutamate toxicity and counteract the neuroprotective and neurotrophic effects of 17beta-estradiol (E2; Nilsen et al., 2006; Singh and Su, 2013). This is of particular importance as MPA is often the progestin used in HRT (Kim et al., 2021) and could therefore explain the trend towards increased risk of PD observed in the 3 abovementioned studies (Simon et al., 2009; Liu et al., 2014; Lundin et al., 2014).

Future perspectives and conclusions

Throughout this review, we have highlighted that within the same type of studies the conflicting evidence underlines the different methods of data collection, patient's classification, and regression models. Similarly, in different types of observational studies the discrepancies may relate to bias in the population inclusion criteria and sample size. Our work emphasizes the importance of considering a uniform standard criterion to adjust

regression models with a consistent statistical and clinical judgment. We believe that despite some inconsistent results, the current findings support a role for menopause on the risk of PD onset. This is an exciting research field for scientists working in basic, pre-clinical and clinical sciences aiming to elucidate the underlying mechanisms in PD and promoting better strategies to manage menopausal patients accordingly to their risk profile.

The effect of gonadal steroids on the brain dopamine system has been the subject of numerous pre-clinical publications in the past several decades. Pre-clinical studies have thus far led the way to elucidate the effect of gonadal steroids more consistently on dopamine containing neurons in wild type and parkinsonian animals, especially in toxin-based rodent models of PD (Dluzen et al., 1996; Miller et al., 1998; Grandbois et al., 2000; Quinlan et al., 2013; Gillies et al., 2014; Smith and Dahodwala, 2014; Almey et al., 2015; Rodriguez-Perez et al., 2015; Almey et al., 2016; Labandeira-Garcia et al., 2016; Jurado-Coronel et al., 2018). Several works support the hypothesis that menopause may constitute a triggering risk factor, which interaction with other risk factors and other possible pathological processes may modify the onset of PD. For instance, pre-clinical studies using rotenone and MPTP toxin-induced animal models of PD showed that ovariectomy abolishes the neuroprotective advantage observed in the substantia nigra and striatum of females as compared to males with PD. Conversely, treatment with estrogen reduces dopaminergic neurodegeneration in the substantia nigra and restores dopaminergic transmission (Disshon and Dluzen, 2000; Mitra et al., 2015; Shen et al., 2017; Makav and Eroglu, 2021). Similarly, clinical studies are starting to elucidate the combined effect of menopause with other PD risk factors. Among postmenopausal women, sleep disturbances were associated with approximately 10–30% increased PD risk after ∼16 years follow-up; although prospective cohort studies that include both men and women of diverse backgrounds are required to confirm these findings (Beydoun et al., 2022).

Other nuances important to mention include the notion that, as suggested by the considerable number of studies reviewed in this article, the physiological changes and pathological mechanisms involved in PD neurodegeneration may adapt distinctively at different stages of the menopause process. Moreover, recent studies have approached the concept that perimenopause does not equal the simple loss of estrogen, but that it represents a period of gonadal endocrine imbalance and neurological transition during which the nigro-striatal circuit is more susceptible to PD neurodegeneration (Brinton et al., 2015).

Author contributions

SU, SM, RM, and TM: conceptualization, methodology, resources, investigation, writing—draft preparation, review, and editing. RM and TM: funding acquisition. RM: project administration. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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Association of cerebral spinal fluid copper imbalance in amyotrophic lateral sclerosis

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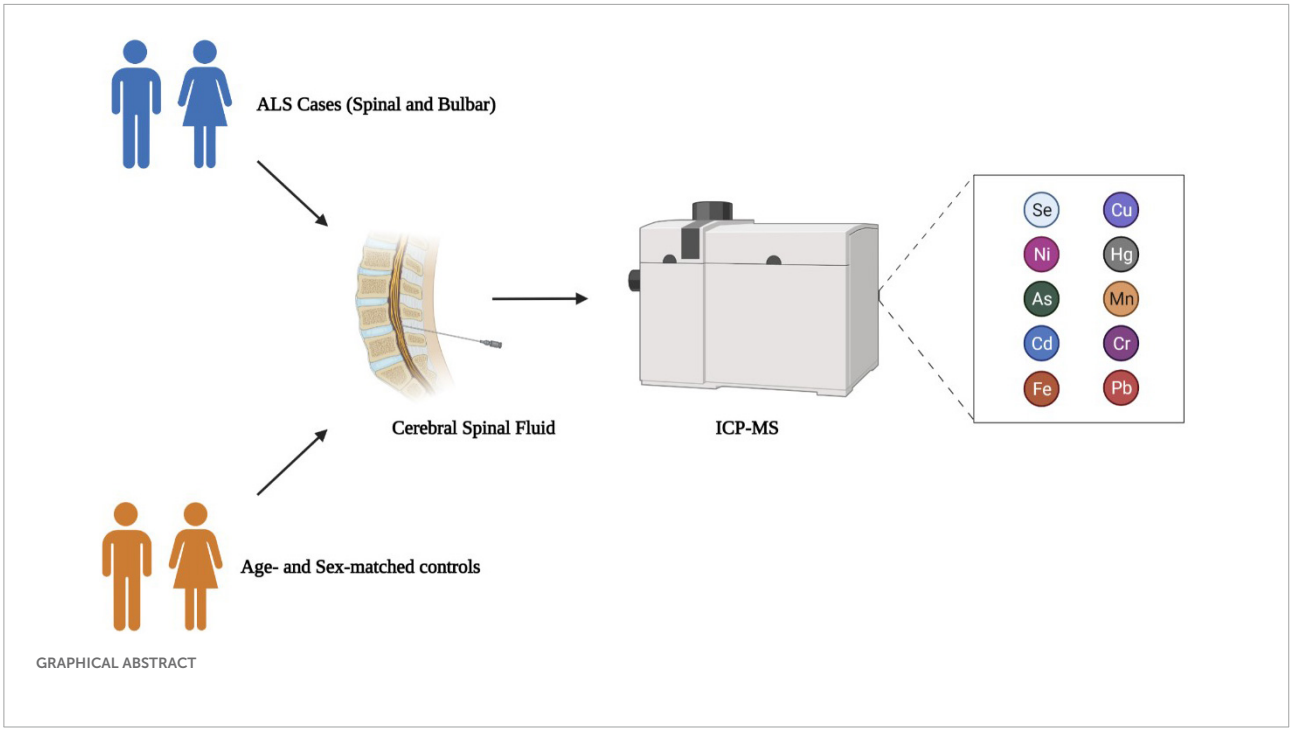
A plethora of environmental risk factors has been persistently implicated in the pathogenesis of amyotrophic lateral sclerosis (ALS), including metal/metalloids. This study aimed to examine potential associations between cerebral spinal fluid (CSF) metal/metalloids and ALS risks. CSF concentrations of copper (Cu), nickel (Ni), mercury (Hg), arsenic (As), manganese (Mn), and iron (Fe) in ALS (spinal- and bulbar-onset) patients and controls were measured using inductively coupled plasma mass spectrometry (ICP-MS). Results from this study revealed marked differences between control, spinal-onset, and bulbar-onset groups. We report that Cu levels were lower in the ALS and spinal-onset groups compared to the control group. Ni level were higher in the spinal-onset group compared to the control and bulbar-onset groups. In addition, associations between CSF metal/metalloid levels with disease severity, sex, and serum triglycerides were also examined to broach the potential relevance of neurotoxic metal/metalloids in ALS disease heterogeneity.

KEYWORDS

ALS, copper, metal/metalloids, CSF, neurodegenerative

Introduction

Amyotrophic lateral sclerosis (ALS), also referred to as "Lou Gehrig's disease," is a debilitating neurodegenerative disease with an incredibly intricate etiology. ALS manifestation is theorized as a stepwise process that involves both susceptible variants and environmental triggers (Al-Chalabi and Hardiman, 2013; Paez-Colasante et al., 2015; Cook and Petrucelli, 2019). The multifaceted pathogenic mechanisms coupled with genetic variability and complex environmental exposure may be important reasons for the complex biological heterogeneity of ALS, as well as why many preclinical ALS treatment trials fail in phase 3. Notably, a proportion of ALS patients (~10%) show Mendelian inheritance while the majority (~90%) of cases occur sporadically with no familial history. Moreover, at least 30 genes have been correlated with ALS.



Namely, C9orf72, TARDBP, SOD1, and FUS account for 70% of familial ALS cases in European populations (Chiò et al., 2014). However, unlike schizophrenia patients who show a number of common variants, ALS pathogenesis is primarily based on rare variants (van Rheenen et al., 2016). Large population-based analyses have suggested that certain risk variants have higher susceptibility for disease manifestation when coupled with environmental risk factors (Hardiman et al., 2017). Early case-control studies conducted in Guam and Japan suggested that exposure to cyanotoxins is correlated with high ALS susceptibility (Bradley et al., 2013). Other risk factors include military service, metals/metalloids, pesticides and insecticides, electromagnetic fields, physical activity, head injury, glutamate toxicity, and smoking (Talbott et al., 2016). At the molecular level, impairment in RNA metabolism, protein homeostasis, neuroinflammation and mitochondrial dysfunction have all been evidenced in ALS pathogenesis (Higgins et al., 2003; Wang et al., 2011; Brites and Vaz, 2014; Magrané et al., 2014; Sama et al., 2014; Conicella et al., 2016; Hardiman et al., 2017). It is important to note that dysregulations of the above cellular processes in ALS are likely to interact and culminate leading to disruptions in the broader mechanistic network, and that it is unlikely that any one of the dysregulated mechanisms is singularly responsible for ALS pathogenesis. The same should be considered when investigating the roles of environmental risk factors. However, the extent and order of event for each of these factors on disease contribution remains unclear.

Metal/metalloid imbalance has been implicated in various human diseases including cancer, cardiovascular disease, and neurodegenerative diseases. Correlation between heavy and trace metals such as lead (Pb), mercury (Hg), Chromium (Cr), arsenic (As), cadmium (Cd), aluminum (Al), manganese (Mn), magnesium (Mg), selenium (Se), nickel (Ni), copper (Cu), and zinc (Zn) have been investigated in a number of ALS studies (Al-Chalabi and Hardiman, 2013; Bocca et al., 2015; Dickerson et al., 2020; Farace et al., 2020). However due to varied methods, medium, populations studied, and heterogeneity of the disease, there exist large variations between studies making replication of results difficult. To date, no study has found causative links between metal/metalloid imbalance and ALS onset. For instance, onset of ALS can be clinically classified as either spinal, muscle weakness starting in the limbs, or bulbar, symptoms characterized by difficulties in swallowing and speech. Progression rate of bulbar onset ALS tend to be faster than spinal onset, and is considered the more severe variant. Males are more likely to develop spinal onset, while females have increased likelihood of developing bulbar onset (Logroscino et al., 2010). Men (1:350) also have higher lifetime risks than women (1:400) (Colombrita et al., 2009; Chang et al., 2012; van Es et al., 2017). Current prognosis for survival is 2–5 years after initial diagnosis (Miller et al., 2009; Brown and Al-Chalabi, 2017; Hulisz, 2018). Bulbar onset, lower ALS Functional Rating Scale (ALSFRS-R) score, and older patients often show lower survival rate (Kiernan et al., 2011). Additionally, ALS incidence differs by geographical region and ethnicity. Currently, most epidemiological studies have been based on European populations, which have an incidence rate of 2–3 cases per 100,000 individuals (Logroscino and Piccininni, 2019).

Notably, regions with relatively more homogenous populations such as Scotland and Ireland, incidence rates are particularly high (2.6/100,000 individuals). On the contrary, East and South Asia have shown lower incidence rates (0.7–0.8/100,000 individuals), although there is still a lack of epidemiological studies for individual countries in these regions (Logroscino et al., 2010; Joensen, 2012; Chiò et al., 2013; Marin et al., 2014). In addition to incidence, survival rate also varies greatly by geography. Specifically, European populations have been evidenced to have shorter (24 months) survival time than Central Asian populations (48 months) (Marin et al., 2016). Representational studies from a diverse range of geographical regions are necessary for deeper understanding the roles of genetics and metal/metalloid imbalance on ALS disease pathogenesis.

In this study, we aim to assess potential differences in levels of heavy and trace metals/metalloids found in the cerebral spinal fluid (CSF) of ALS patients and corresponding controls. Specifically, we examined levels of copper (Cu), nickel (Ni), mercury (Hg), arsenic (As), manganese (Mn), and iron (Fe) using ICP-MS in 29 ALS patients and 9 controls from a cohort based in Shaanxi, China (Northwestern region). Findings from this study reveal onset- and sex-dependent metal dyshomeostasis in ALS patient CSF as well as potential mixture effects.

Subjects and methods

Study population

Twenty-nine sporadic cases diagnosed with definite ALS according to the revised El Escorial criteria and 9 age- and sex-matched controls recruited from the First Affiliated Hospital of Xi'an Jiaotong University (China) were included in this observational study. The control group consist individuals with non-neurodegenerative diseases such as headache and lower pack pain. As part of routine hospital visit, baseline demographic information, location of onset, ALSFRS-r score, smoking, drinking and exposure history, and laboratory test results were collected. All demographic and clinical information were collected by medical professionals. All participants provided informed consent prior to the procedures. This study was approved by the Institutional Ethical Committee of Xi'an Jiaotong University.

Cerebral spinal fluid collection

CSF samples were obtained by lumbar pucture in the L3/L4 or L4/L5 interspace and collected into trace element free polypropylene tubes in 1 mL aliquots. All procedures were conducted at the First Affiliated Hospital of Xi'an Jiaotong University. Samples were gently mixed and centrifuged at 2,000*g* at 4°C for 10 min to eliminate insoluble materials and cells. Immediately after, samples were deep frozen and stored at −80°C until further analysis at Xi'an Jiaotong University iHarbor Research Center. Samples were thawed on ice prior to analysis.

Metal/metalloid analysis

Aliquots of 200 μL CSF samples were diluted 10 folds with 65% Nitric Acid (Sinpharm Chemical Reagent Co., Ltd) and 31% hydrogen peroxide. Samples were dissolved on heat block (AS ONE, CHP-250DF, Japan) at 150°C. All metals [selenium (Se), copper (Cu), nickel (Ni), mercury (Hg), arsenic (As), manganese (Mn), cadmium (Cd), chromium (Cr), iron (Fe), and lead (Pb)] were quantified using Inductively Coupled Plasma-Mass Spectrometer (ICP-MS, PerkinElmer, NexION® 350D, USA) in Nebulizer Gas Flow STD/KED (Instrumental parameters: Nebulizer Gas Flow 0.85 L/min, Auxiliary Gas Flow1.45 L/min, Mass range: 1∼260 a.m.u, Dark noise <0.2, Sensitivity: >105 cps/ppb 115 In, Long term stability: <4%, Precise of isotopic ratio: <0.1%). All blank samples were analyzed concurrently as the collected samples to ensure accuracy. The limit of quantification (LOQ) for each element were calculated and expressed as μg/L for all the metals 12.5 Selenium (Se), 0.475 Copper (Cu), 0.1 Nickel (Ni), 0.05 (Hg), 0.175 Arsenic (As), 0.55 Manganese (Mn), 0.01 (Cd), 4.8 (Cr), 21.475 (Fe), 1.225 (Pb). Because measurements for Se, Cd, Cr, and Pb were below the LOQ, results for these metal/metalloids have been omitted.

Serum triglyceride quantification

Blood tests were performed at the First Affiliated Hospital of Xi'an Jiaotong University during patients' first visit to the hospital (after experiencing first symptoms). Fresh blood samples (after overnight fasting) were tested for serum triglyceride.

Statistical analysis

Statistical analyses were performed using the GraphPad Prism 8.0 software and R (ggplot2). All demographic information was presented as frequencies along with percentages. Comparative analyses of each metal/metalloid between groups were carried out using non-parametric pair-wise Mann–Whitney tests, p-value <0.05 was considered statistically significant. Results are presented as medians, 25th, and 75th interquartile ranges (IQR).

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Results

Baseline demographic data

A total of 38 participants were included in this study, 29 ALS cases, and 9 controls. As shown in Table 1, 4 females (44.44%) and 5 males (55.56%) were included in the control group. For the ALS group, 16 were female (55.17%) and 13 were male (44.83%). The median age was 59.1 years (51–73) and 55.63 years (42–69) for the control and ALS groups, respectively. The average ALSFRS-R scores for the ALS patients was 41.73 (21–48). We further divided the ALS group into spinal (n = 20) and bulbar (n = 9) onset groups. In the spinal onset group, 9 cases were female (45%) and 11 were male (55%). In the bulbar onset group, there was a higher percentage of female cases (77.78%, n = 7) compared to male cases (22.22%, n = 2). The median ages for the spinal and bulbar onset groups were 55.7 (42–69) and 55.5 (44–67) years, respectively. The average ALSFRS-R score for the spinal group was 40.9 (21–47) and 43.4 (34–48) for the bulbar group.

Cerebral spinal fluid metal/metalloid levels

ICP-MS was used to measure levels of Cu, Ni, Hg, As, Mn, and Fe in CSF of ALS patients and corresponding controls. Data were analyzed at multiple levels to thoroughly examine variation in metal/metalloid levels based on disease onset, progression, as well as sex and potential synergistic effects. First, we analyzed differences in metal levels between ALS patients and corresponding controls. Interestingly, contrary to previous findings, none of the metals in our study were found significantly different between control and ALS groups except for Cu, which was found lower in the ALS group (Cu = 80.12 μg/L, p = 0.05) compared to the control group (Cu = 129.7 μg/L) (Table 2). Noted, confounding factors including age, smoking, drinking, education level, and BMI showed no significant effects. Copper deficiency in ALS patients has also been reported in previous studies (Weihl and Lopate, 2006; Barros et al., 2018). Second, we divided the ALS group into bulbar and spinal onset groups to examine potential differences in metal levels between different types of ALS onset (Figure 1). As shown in Table 3, Cu levels were significantly lower in the spinal group (Cu = 78.11 μg/L, p = 0.04) compared to the control group (Cu = 129.7 μg/L). However, there was no noteworthy difference in Cu levels between bulbar onset and control groups. These results suggest that low Cu levels found in the ALS group may be predominantly contributed by spinal onset patients. In addition, Ni levels were significantly higher in the spinal group (Ni = 4.21 μg/L) compared to both control (Ni = 2.23 μg/L, p = 0.02) and bulbar (Ni = 3.30 μg/L, p = 0.03) groups. These results indicate potential differences in metal levels between bulbar and spinal onset patients, and that low Cu and high Ni levels may be associated with spinal onset. Third, we analyzed potential sex-dependent differences in CSF metal levels by dividing ALS cases into male and female groups. As shown in Table 4, there were no significant differences in CSF metal levels between female and male ALS patients. This suggests that sex may not be a confounding factor in CSF metal/metalloid levels for ALS patients. Fourth, to examine potential correlations between metal levels and ALS disease severity, we performed Pearson correlation analysis, and found that CSF metals were not correlated with disease severity (Table 5). Lastly, we conducted correlational assessment to examine potential associations between metals. As shown in Figure 2A, positive and significant correlations were found for the following metals: Cu/Ni, As/Cu, As/Ni, Fe/Cu, Fe/Ni, and Fe/As. To confirm whether these correlations are specific to ALS cases, we also conducted a correlation analyses for the control group. As shown in Figure 2B, significant correlations were found for As/Cu. This suggests correlations between As and Cu may not be specific to ALS patients. Correlations between Cu/Ni, As/Ni, Fe/Cu, Fe/Ni, and Fe/As in ALS patients may be interesting to investigate for future studies. These results are interesting as many of the metals listed such as Mn, Hg, and As were not found significantly different in the above analyses,

TABLE 1 Baseline demographic characteristics for ALS cases and controls.

	Control		ALS	
	Non-ALS (N = 9)	Total (N = 29)	Spinal onset (N = 20)	Bulbar onset (N = 9)
Sex				
Females	4 (44.44%)	16 (55.17%)	9 (45%)	7 (77.78%)
Males	5 (55.56%)	13 (44.83%)	11 (55%)	2 (22.22%)
Median age (years)	59.1 (51–73)	55.63 (42–69)	55.7 (42–69)	55.5 (44–67)
Average ALSFRSR	–	41.73 (21–48)	40.9 (21–47)	43.4 (34–48)

TABLE 2 CSF metal/metalloid levels (median, 25th, and 75th percentile) for ALS cases and controls.

Metal/metalloid (μg/L)	Control N = 9 Median (IQR)	ALS N = 29 Median (IQR)	*P-value*
Cu*	129.70 (95.55–258.7)	80.12 (70.25–138.5)	0.05
Ni	2.23 (1.84–3.73)	3.66 (3.10–5.52)	0.07
Hg	0.27 (0.10–0.76)	0.26 (0.16–0.38)	0.61
As	0.90 (0.59–1.55)	0.70 (0.58–1.24)	0.52
Mn	2.07 (1.39–2.85)	1.75 (1.43–2.72)	0.51
Fe	283.0 (197.8–371.9)	201.3 (157.1–296.0)	0.19

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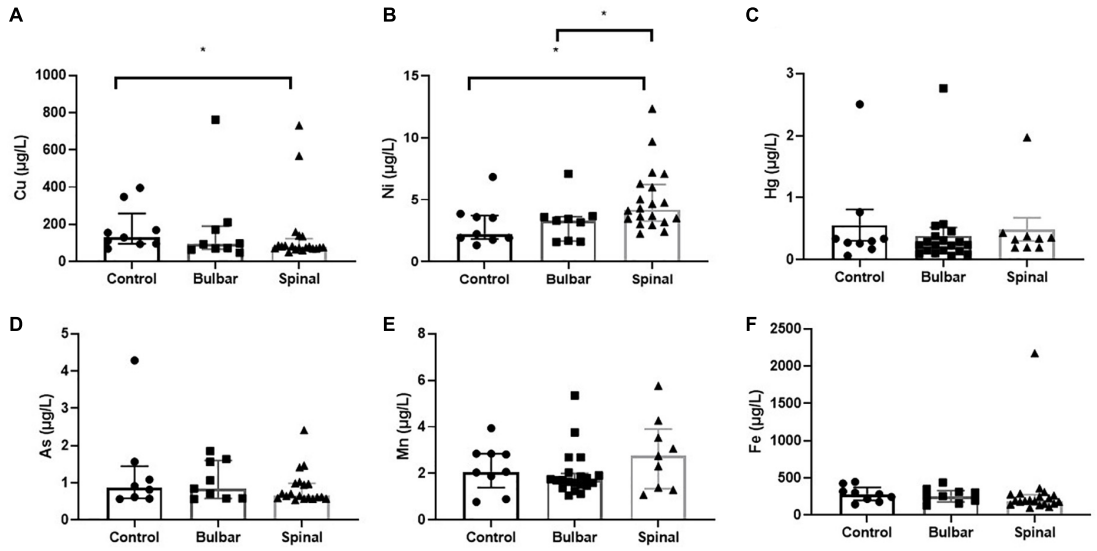

FIGURE 1
The scatterplots illustrate CSF Cu **(A)**, Ni **(B)**, Hg **(C)**, As **(D)**, Mn **(E)**, and Fe **(F)** levels found in control, bulbar-, and spinal-onset ALS patients. Data presented indicate median and IQR. *$p < 0.05$.

TABLE 3 Distribution of CSF metal/metalloid levels (median, 25th, and 75th percentile) by disease onset.

Metal/metalloid (µg/L)	Control $N = 9$ Median (IQR)	Spinal onset $N = 20$ Median (IQR)	Bulbar onset $N = 9$ Median (IQR)
Cu*	129.7 (95.55–258.7)	78.11 (70.71–124.1)	93.37 (66.78–191.7)
Ni*	2.23 (1.84–3.73)	4.21 (3.27–6.23)	3.30 (1.65–3.64)
Hg	0.30 (0.22–0.55)	0.34 (0.20–0.41)	0.23 (0.14–0.38)
As	0.90 (0.59–1.55)	0.67 (0.59–1.01)	0.84 (0.57–1.59)
Mn	2.07 (1.39–2.85)	2.76 (1.34–3.91)	1.71 (1.48–2.01)
Fe	283.0 (197.8–371.9)	193.2 (151.5–274.3)	254.5 (177.6–330.2)

*Mann–Whitney test shows significant difference between Spinal and Control groups for Cu ($p = 0.04$) and Ni ($p = 0.02$), as well as between Bulbar and Spinal groups for Ni ($p = 0.03$).

although synergistic or mixture effects between metals have also been reported in other ALS studies (Figueroa-Romero et al., 2020).

Correlation of cerebral spinal fluid metal/metalloids with serum triglyceride

Further analyses were conducted to examine potential correlation between CSF metal/metalloid levels with clinical features in ALS patients (Table 6). Routine laboratory test results included data on serum glucose, cholesterol, triglyceride, T3/T4 levels, etc. Interestingly, these clinical biomarkers were not found correlationed with CSF metal/metalloids levels. In addition, while triglyceride levels did not correlate with disease severity, the spinal group showed significantly higher levels than the bulbar group (Figure 3). In addition, we did not find significant correlation between ALSFR-S and triglyceride levels. Follow-up studies with additional triglyceride and ALSFRS-R data can be used to examine change in ALSFR-S versus change in triglyceride levels. This may reveal more about the potential relationship between tryglyceride and ALS disease progression. While serum triglyceride is mainly used as a biomarker for metabolic and cardiovascular disease, recent studies have indicated potential links to neurodegenerative diseases (Nägga et al., 2018; Bernath et al., 2020; Liu et al., 2020). Our findings suggest that while serum triglyceride did not correlate with any of the CSF metal/metalloids, it may be associated with the more severe form of ALS.

Discussion

Main findings from this study suggest that CSF metal/metalloid levels not only differed between controls

TABLE 4 Distribution of CSF metal/metalloid levels (median, 25th, and 75th percentile) by sex for ALS cases.

Metal/metalloid (μg/L)	Female $N = 16$ Median (IQR)	Male $N = 13$ Median (IQR)	*P-value*
Cu	78.11 (67.27–169.2)	81.96 (70.25–90.40)	0.68
Ni	3.69 (3.04–6.52)	3.51 (3.10–5.52)	0.68
Hg	0.29 (0.09–0.44)	0.19 (0.09–0.32)	0.45
As	0.97 (0.64–1.52)	0.61 (0.57–0.70)	0.11
Mn	1.74 (0.54–2.88)	1.67 (0.99–3.62)	0.63
Fe	266.2 (161.7–311.1)	185.6 (157.1–246.9)	0.12

TABLE 5 Association between CSF metal/metalloid levels and ALSFRS-R scores.

Metal/metalloid	Correlation coefficients	*P-value*
Cu	0.05	0.77
Ni	−0.02	0.91
Hg	−0.13	0.56
As	0.04	0.83
Mn	0.30	0.20
Fe	0.03	0.87

and ALS patients, but also between ALS patients with different forms of onset. Unlike some of the previous studies which have reported significant differences in various metals between control and ALS patients, our results indicate that only copper was significantly lower in the ALS group compared to the control group. Moreover, copper levels were especially low in spinal-onset patients. This result is in line with a previous study which showed lower CSF copper levels in spinal patients compared to bulbar patients (Patti et al., 2020). Copper imbalance has been reported in several neurodegenerative diseases including ALS, Alzheimer's Disease, Menkes Disease, and Parkinson's Disease (Telianidis et al., 2013; Chang and Hahn, 2017; Hilton et al., 2020; Qin et al., 2022). Currently, both high and low levels of copper have been correlated with ALS (Kapaki et al., 1997; Hozumi et al., 2011; Roos et al., 2013; Peters et al., 2016; Cicero et al., 2017; Qin et al., 2022). In ALS patient CSF samples, high copper levels have been reported by two research groups (Hozumi et al., 2011; Patti et al., 2020). On the other hand, a study led by Kapaki et al. demonstrated low copper levels in ALS patient CSF (Kapaki et al., 1997). Despite a lack of consensus between studies, which may be due to differences in participant characteristics (age, ethnicity, etc.), it seems that an imbalance in CSF copper levels may be correlated with ALS pathogenesis. Copper is an essential trace element that can cross the blood-brain barrier (BBB) via cerebral capillaries, which are mostly covered by astrocytes (Choi et al., 2009). In fact, concentrations of copper ions in the CNS (80 μM) are found higher than that of the blood (16 μM), muscle (10 μM), and lung (30 μM) (Hamilton et al., 1972). In the CNS, copper can be found in all parts of the brain and can function to promote neurotransmission, synaptic activities, free radical detoxification, and mitochondrial respiration (Gaier et al., 2013; Gil-Bea et al., 2017; Giampietro et al., 2018; Qin et al., 2022). Copper ions are incorporated into the cell via copper transporter 1 (CTR1) and divalent metal transporter 1 (DMT1) membrane proteins (Kuo et al., 2001; Lee et al., 2001; Arredondo et al., 2003). Once inside the cell, copper levels are closely regulated by efflux and influx pumps such as ATP7A (Tokuda and Furukawa, 2016). SOD1 is known to have high affinity for copper ions; changes in its expression level have been evidenced to influence copper levels in the spinal cord (Li et al., 2006; Lelie et al., 2011). However, copper imbalance has not been reported in SOD1-ALS cases, suggesting that ALS-related copper imbalance may be regulated via other trafficking systems. For example, various copper-requiring proteins and enzymes such as P-type ATPase, and cytochrome c oxidase receive copper ions via specific chaperones including HAH1, COX17, and CCS (Petris et al., 1996; Wong et al., 2000; Takahashi et al., 2002; Hamza et al., 2003). Notably, changes in ATP7A and CTR1 levels have also been found to affect copper accumulation inside the cell (Tokuda et al., 2013). In addition, mitochondrial copper has also been suggested to play a role in the pathophysiology of ALS, as mitochondrial dysfunction and metabolic defects represent important hallmarks for ALS motor neuron degeneration (Kong and Xu, 1998; Wiedemann et al., 2002; Muyderman and Chen, 2014; Carrì et al., 2017). Although no direct evidence of copper-induced mitochondrial dysfunction has been reported in the development of ALS, changes in certain copper-dependent enzymes such as PARK7, COX1, and COX2 have been shown to trigger neuronal death through regulation of mitochondrial function (Fujita et al., 1996; Borthwick et al., 1999; Wang et al., 2016). At the moment, there is no established consensus on whether high or low levels of copper is responsible for ALS, rather it seems that the imbalance of copper levels may be the key as both copper deficiency and accumulation can have compromising effects on normal cellular functions. Future studies cross-linking copper levels in the human body and potential changes in copper trafficking systems may provide further insight.

Next, while our findings indicate that there is no significant difference in CSF nickel levels between control and ALS groups. However, spinal-onset patients showed comparably higher nickel levels than both the control and bulbar groups. Nickel is considered an irritant, but also an essential and carcinogenic metal, known to accumulate in neuronal tissues, promote oxidative stress and mitochondrial damage, as well as inhibit neurotransmission (Saito et al., 2016; Song et al., 2017; Ijomone et al., 2018; Andrew et al., 2022). Previous reports have indicated that nickel is capable of stimulating the production of serum nitric oxide, which can interact with mitochondrial superoxide to form reactive peroxynitrite, thereby regulate neurotransmission (Cruz et al., 2004; Calabrese et al., 2010;

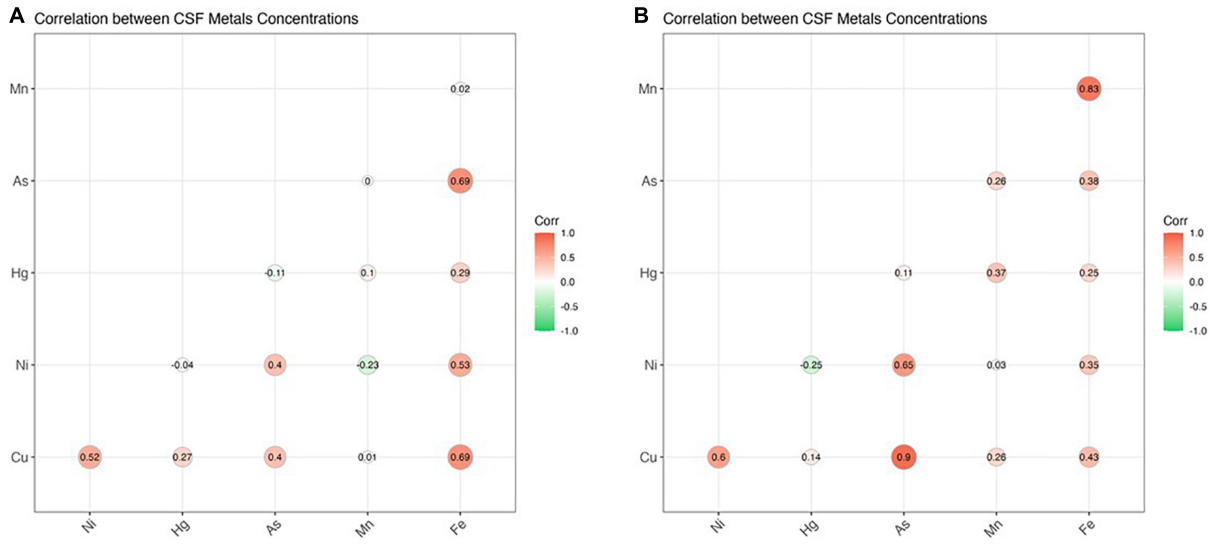

FIGURE 2
The figure illustrates correlation coefficients for CSF metal/metalloids in ALS cases **(A)** and healthy controls **(B)**. Significant correlations ($p < 0.05$) were found for the following metals: Cu/Ni, Cu/As, Ni/As, Fe/Cu, Fe/Ni, and Fe/As (ALS) as well as Cu/As (control). Thresholds of significance are as follows: 0.85 for healthy controls and for 0.3 for ALS cases.

Hattiwale et al., 2013). In a case-control study based on ALS patients in Denmark, Dickerson et al. found that women occupationally exposed to nickel had higher adjusted odds of developing ALS (Dickerson et al., 2020). In another study based on examining metal biomarkers in teeth, ALS cases were found to have 1.65 times more nickel than controls, indicating potential correlation between childhood metal uptake and later adulthood-onset (Figueroa-Romero et al., 2020). Interestingly, high nickel levels in body fluids of AD patients have been positively correlated with alcohol consumption and hepatotoxicity, and that nickel chelation may have beneficial effects on inhibiting Aβ42 peptide aggregation (Ormerod et al., 1997; Benoit and Maier, 2021). While nickel chelation may be a promising therapeutic strategy for ALS, potential difference in nickel levels between spinal and bulbar onset patients warrant further evaluation.

This study also examined potential sex-dependent differences in CSF metal/metalloid levels in ALS patients. We report that there was no significant differences in CSF metal/metalloid levels between male and female ALS patients nor healthy controls. A previous study based on participants from Bangladesh reported difference in selenium concentrations based on gender, and that males exhibited higher selenium levels than females. Depending on the dose, selenium can either be nutritional and toxic for the human body. Probable link has been reported between selenium toxicity and increased ALS risks (Vinceti et al., 1997). In particular, evidence suggests that selenium accumulation can induce neuronal apoptosis, and promote the translocation of copper/zinc SOD1 into the mitochondria (Xiao et al., 2006; He and Cui, 2021; Wandt et al., 2021). However, as with many metals, controversial results regarding selenium levels in ALS patients have also been reported. In particular, reduced blood selenium levels were found correlated with ALS in two other studies (Moriwaka et al., 1993; Peters et al., 2016).

In addition to onset- and sex-dependent analyses, we also examined potential associations between metals in ALS patients. In particular, correlations between Cu/Ni, As/Ni, Fe/Cu, Fe/Ni, and Fe/As were found specifically for ALS patients. Further investigation into the correlation between Fe and other CSF metal/metalloids may be interesting as it was found to be positively correlated with all tested metals except for Hg and Mn. Many of these metals such as mercury, arsenic, and manganese were not individually associated with either ALS onset or disease severity, although the results may be an indication of potential mixture effects between metals. Previous research has also indicated that exposure to metal mixtures may be positively correlated with disease outcome in ALS mouse model (Figueroa-Romero et al., 2020).

Over accumulation of triglycerides can be an important risk factor for cardiovascular disease. In the brain, imbalance of lipids or dyslipidemia can disrupt normal synaptic functions, membrane trafficking and protein activities (Blasco et al., 2017). Increased triglyceride level has been shown to be positively correlated AD risks (Nägga et al., 2018; Bernath et al., 2020). Yet, correlations between serum triglyceride levels and ALS risks have so far been inconsistent (Dorst et al., 2011; Blasco et al., 2017; Mariosa et al., 2017; Liu et al., 2020). High serum triglyceride levels were found correlated with prolonged life expectancy and better prognosis for ALS

TABLE 6 Association between CSF metal/metalloid levels and serum triglycerides for ALS cases.

Metal/metalloid	Correlation coefficients	*P-value*
Cu	−0.03	0.84
Ni	−0.11	0.58
Hg	0.34	0.13
As	−0.11	0.55
Mn	0.01	0.95
Fe	−0.30	0.12

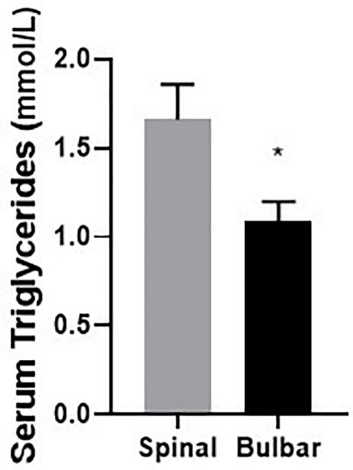

FIGURE 3
Serum triglyceride level is significantly higher in the spinal group compared to the bulbar group. Data presented for all ALS cases indicate mean and standard error. *$p < 0.05$.

patients in several studies (Dorst et al., 2011; Nakamura et al., 2022). However, the difference in serum triglyceride levels between ALS patients and healthy controls are still controversial. One study found that serum triglyceride levels are found lower in ALS patients than in controls (Blasco et al., 2017). However, another study reported no significant difference in serum triglyceride between ALS patients and healthy controls (Chiò et al., 2009). To the best of our knowledge, this is the first study to examine potential associations between CSF metal/metalloids and serum triglyceride levels in ALS patients. Our results indicate that CSF metal/metalloids were not correlated with serum triglyceride levels in ALS patients. Previous epidemiology studies have linked heavy metal exposure to dyslipidemia. In particular, mercuy, lead, arsenic, copper, nickel, and cadmium were reported to be positively associated with serum triglyceride levels (Buhari et al., 2020; Ma et al., 2020; Kim et al., 2022). In animal studies, cadmium has been evidenced to enhance triglyceride accumulation through reduction of lipoprotein lipase activity (Barański et al., 1983). Interestingly, serum triglyceride level did not correlate with ALS disease severity, although it was significantly higher in the spinal group compared to the bulbar group. Further confirmation is warranted, especially with the inclusion of a control group.

Results of this study is limited by the relatively small sample number and singularity in the type of sample used. Another major limitation of this study is that the measurements were only carried out once. We acknowledge that data interpretation can be limited by sample size and statistical imperfections. However, despite these limitations, this observational study presents valuable new information. Through the implementation of CSF samples, inclusion of stratified groups and analyses, we were able to gain insight into the potential association between CSF metal/metalloid levels with different forms of ALS onset, disease severity, sex, and serum triglycerides. Further validation is necessary with a larger participant pool and the incorporation of various sample types including urine, blood, and hair. In addition, it remains unclear whether these findings can be extended to other populations, although we provide new insights from a less studied geographical region. Lastly, future studies may also consider examining both genetic changes and metal exposure to gain further insight, including RNA-seq analyses to examine potential change in gene expression along with metal/metalloid levels.

Conclusion

Metal homeostasis in the CNS is critically important for normal cellular processes and brain function. Our study examined potential associations of six CSF metal/metalloids with onset- and sex-specific ALS risks. Main findings identified that CSF copper and nickel levels differed by form of ALS onset. We conclude that while little difference was found between control and ALS groups, our results highlight the association of CSF metal/metalloids with ALS onset, sex, and disease severity.

Data availability statement

The original contributions presented in this study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving human participants were reviewed and approved by Institutional Ethical Committee of Xi'an Jiaotong University. The patients/participants provided their written informed consent to participate in this study.

Author contributions

QC and JD conceived the study. PW, TW, XQ, RJ, RZ, JJ, FH, and XX performed the material preparation, data collection, and analysis. QC, PW, and JD wrote the manuscript. All authors provided comments on draft versions, read, and approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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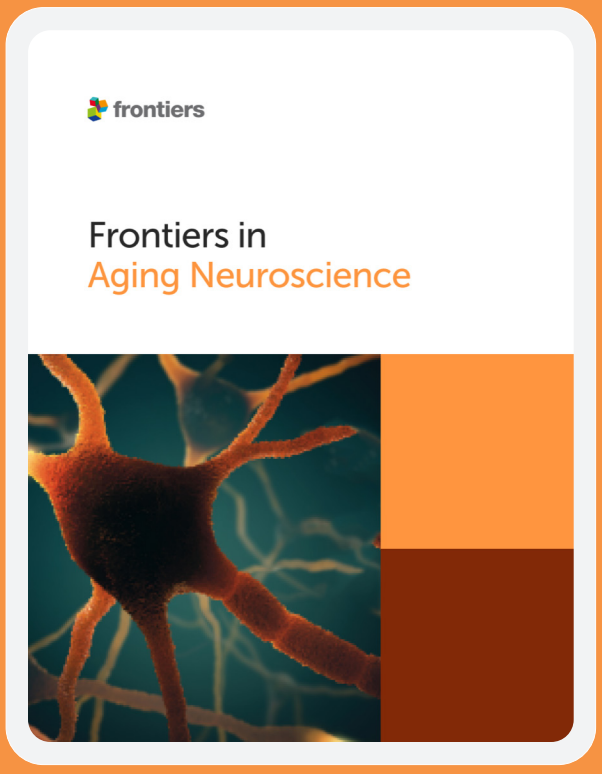

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