The Effect of Age and Menopause on Intrinsic Functional Connectivity in Females and its Relation to Episodic Memory

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Abstract

There is growing evidence that age-related decreases in episodic memory arise at midlife, a time when females menopause. For some, menopause is associated with memory changes, raising the question of how reproductive aging interacts with chronological age effects on memory and brain function. In the current study we explored how age and menopause status related to whole-brain patterns of resting-state functional connectivity in a sample of 134 females, 20 to 60 years of age (N=64 pre-menopausal, N=33 peri-menopausal, N=37 post-menopausal) using multivariate behavioral partial least squares (PLS) connectivity analysis. We examined if the relationship between chronological age and whole-brain functional connectivity differed between pre-, peri- and post-menopausal participants, and if these connectivity patterns related to performance on a face-location source memory paradigm. When only menopausal status was considered, PLS connectivity results indicated meaningful differences in functional connectivity between peri- and post-menopausal women, with post-menopausal women having greater functional connectivity within and among the dorsal attention network, salience/ventral attention network, somato-motor network and visual network. They also displayed greater functional connectivity between the hippocampus and frontoparietal control, default, dorsal attention, and visual networks. However, linear regressions failed to predict source accuracy performance from this pattern of functional connectivity. This PLS also identified meaningful differences in pre-menopausal women compared to peri- and and post-menopausal women, with peri- and post-menopausal women displaying diminished functional connectivity within the frontoparietal control, default, dorsal attention, and salience networks as well as the hippocampus. Compared to pre-menopausal women, peri- and post-menopausal women also showed increased functional connectivity between the dorsal attention and defualt networks, between the hippocampus and dorsal attention

and salience/ventral attention networks, and greater functional connectivity within the visual network. Linear regressions were able to predict source accuracy performance from this pattern of functional connectivity. These functional connectivity differences between pre-menopausal women versus peri- and post-menopausal women were also captured in a PLS which only considered covarience in chronological age with functional connectivity among the entire sample (i.e. disregarding menopausal status). Again, linear regressions were able to predict source accuracy performance from this similar age-related pattern of functional connectivity identified in this second PLS. Therefore, menopause is associated with different patterns of resting state connectivity amongst visual, hippocampal and higher order attentional control networks. Only when comparing pre-menopausal women to peri- and post-menopausal women, differences in functional connectivity seem to relate to source accuracy performance.

Il est évident que le décline de mémoire épisodique lie au vieillissement commence à la quarantaine, un temps quand les femelles éprouvent la ménopause. Quelques femmes expérience les perturbations de mémoire pendent la ménopause, ceci soulève la question de comment le vieillissement reproductif interagit avec le vieillissement chronologique et cet effet sur le mémoire et la fonction du cerveau. Dans cette étude, nous avons examiné comment l'âge et l'état ménopause ont interagi aux motifs de connectivité fonctionnelle du cerveau entier pendent l'état de repos dans une échantillon de 134 femelles qui avaient entre 20 et 60 ans (N=64 pré-ménopauses, N=33 peri-ménopauses, et N=37 post-ménopauses) par utilisant l'analyse multivariée partiels moindres carres (PMC). Nous avons exploré si la relation entre l'age chronologique et la connectivité fonctionnelle du cerveau entier se différait entre les participants pré-ménopauses, peri-ménopauses, et post-ménopauses et si ces différences se rapportaient à leur performance sur un paradigme de visage-endroit mémoire. Quand seulement l'état ménopause

s'était considérée, les résultats de l'analyse PMC connectivité a indiqué qu'il y avait différences fiables de connectivité fonctionnelle entre les participants post-ménopauses et peri-ménopauses. Spécifiquement, les femmes post-ménopauses avaient connectivité augmentée entre et dedans les réseaux attentions dorsales, attentions saillences/ventrales, somato-motor and visuelles, comparé aux participants peri-ménopauses. Les participants post- ménopauses avaient aussi de la connectivité fonctionnelle augmentee entre l'hippocampes et les réseaux fronto-parietal contrôles, défauts, attentions dorsales, et visuelles. Néanmoins, les régressions linéaires ont échoué a prédire les performances des participants en utilisant ces motifs de connectivité fonctionnelle identifiée. Cet analyse PMC connectivité a aussi identifié un autre motif de connectivité fonctionnelle qui différait entre les participants pre-ménopauses et les participants peri- et post-ménopauses. Les participants peri- and post- ménopauses avaient de la connectivité fonctionnelle diminué dedans les réseaux fronto-parietal contrôles, défauts, attentions dorsales, et saillences/attentions ventrales, et aussi dedans l'hippocampe. Comparé aux participants pre-ménopauses, les participants peri- et post-ménopauses avaient aussi de la connectivité fonctionnelle augmentée entre les réseaux attentions dorsales et défauts, entre l'hippocampe et les reseaux attentions dorsales et saillences/attentions ventrales, et connectivité augmentée dedans le réseau visuel. Les régressions linéaires ont prédit les précisions sources des participants par ce motif de connectivité fonctionnelle. Ces différences en connectivité fonctionnel entre les participants pre-ménopauses et les participants peri- et post-ménopauses étaient restitué par un analyse PMC qui a considéré seulement la covariance entre l'âge chronologique et connectivité fonctionnelle pour l'échantillon entier (ignorant l'état ménopause). Les régressions linéaires ont prédit les précisions sources des participants par ce motif de connectivité fonctionnelle aussi. Donc, le ménopause se rapport aux motifs connectivités différents parmi le réseau visuel, les

réseaux d’ordre supérieur attentionnels et l’hippocampe. Seulement des que les femmes pre-menopauses étaient comparé aux femmes peri- et post-menopauses, les différences en connectivité fonctionnelle peux prédire leurs précisions sources.

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Contribution of Authors

Dr. Rajah supervised the project, provided guidance and feedback on study design and analyses, and developed the spatial source memory task. The primary author of the thesis, Sophia LoParco, contributed to study design, data collection, fMRI preprocessing, quality assurance, data analysis, and thesis writing. Additional assistance was provided by lab members involved in the larger project of Brain Health and Aging at Midlife and Menopause (BHAMM): Sricharana Rajagopal (fMRI preprocessing, quality control, analysis support), Alicia Duval (analysis support, data collection), Stamatoula Pasvanis (recruitment, data collection), Lina Khayyat (recruitment, data collection, fMRI quality control), Rosalie Young (recruitment, data collection), Jamie Snytte (fMRI quality control), and Arielle Crestol (data collection, guidance for thesis writing, and analysis support).

Introduction

Necessity for Considering Menopause and Midlife in Neurocognitive Aging

Episodic memory refers to memory for personally-experienced events, which has been conceptualized according to two dissociable cognitive process: item memory (recognition of familiar content, e.g. a previously seen face) and source memory (recollection of the context associated with the content that was remembered, e.g. which side of a screen a face was seen on) (Schacter et al., 1991). Studies have consistently demonstrated that both item and source memory generally decline in older adulthood (Cansino, 2009; Spaniol & Grady, 2012; Spencer & Raz, 1995). Previous studies using a face-location association paradigm in our lab have demonstrated that measurable declines in source memory, but not item memory, can be seen as early as midlife (Kwon et al., 2016). Additionally, our lab has also observed sex/gender-differences in the effect of age on neural activity supporting spatial context memory retrieval (Subramaniapillai et al., 2019). Together these findings underscore the importance of midlife as a time at which aging-related episodic memory changes emerge, and that there may be biological sex differences in the neural correlates of age-related memory decline.

Interestingly, midlife is a time when assigned female at birth (AFAB) individuals experience natural menopause, which on average occurs around 51 years of age (Soules et al., 2001). The stages of menopause include 1) pre-menopause: characterized by regular menstrual cyclicity, 2) perimenopause: characterized by irregular menstrual cyclicity (e.g., greater than 2 months without menstruation), and 3) post-menopause: characterized by a full 12 months without menstruation (i.e., amenorrhea) (Soules et al., 2001). There is recent work arguing that the neuroendocrine changes which occur during menopause may impact the neural underpinnings of some forms of episodic memory (Jacobs et al., 2016; Rentz et al., 2017; Taylor et al., 2019).

Behavioral and neuroimaging studies that have compared the performance of pre-, peri- and post-menopausal females have reported that on average post-menopausal females perform worse on associative memory tasks, i.e., remembering face-name associations, but not item recognition tasks. (Jacobs et al., 2016, 2017; Mosconi et al., 2018). It has been hypothesized that the associative memory decline observed in some menopausal females may be attributable to the menopause-related reductions in centrally circulating 17β-estradiol (Jacobs et al., 2016; Mosconi et al., 2018; Rettberg et al., 2014; Soules et al., 2001). This seems reasonable given that these cognitive processes are strongly supported by activity in the prefrontal cortex and hippocampus, which are two areas that have been shown to express estrogen receptors in humans, non-human primates, and rodents (Galea et al., 2017; Montague et al., 2008; Wang et al., 2010; Waters et al., 2011).

The neurocognitive aging literature on episodic memory decline in older adulthood, usually defined by the chronological age of individuals 65 years and older, typically has over-looked the sex-specific factor of reproductive aging and menopause at midlife in females (Chalfonte & Johnson, 1996; Craik, 1994; Schacter et al., 1991, 1994; Tulving, 1972,1984; Wegesin et al., 2002). The opposite can also be said for the literature on menopause, in that these studies typically ignore chronological aging effects by recruiting participants within a narrow age range (e.g., between 45-55 years of age). This begs the question of how the interaction between chronological and reproductive aging processes' might impact brain and cognitive health at midlife, especially given the observation that some females are more affected by menopause than others (Jacobs et al., 2016, 2017; Mosconi et al., 2018). The resulting knowledge would thus be very useful for discerning which individuals' cognition are more at risk of disruption at midlife and beyond.

Whole-Brain Resting-State Functional Connectivity Analysis

Episodic memory processes rely on the coordination of a distributed network of regions across the entire brain (Dickerson & Eichenbaum, 2010; Rugg & Vilberg, 2013). Capturing neural correlates of this higher-level cognitive process thus requires analysis of the whole brain rather than focusing on a single region or subset of regions. While a fuller picture of brain function allows for a more thorough investigation of wide-spread neurocognitive changes associated with chronological and reproductive aging, the high dimensionality of whole-brain imaging, specifically functional magnetic resonance imaging (fMRI), presents its own challenges for researchers wishing to interpret meaningful patterns of variance from such a large amount of data. Whole-brain resting-state functional connectivity analysis is a promising approach that has been developed as one solution to the high-dimensionality challenge, and it may offer critical insight into how chronological and reproductive aging reorganize individuals' intrinsic brain function. This method has become popular among researchers due to the fact that it can capture stable patterns of brain function among individuals which 1) are predictive of specific task-relevant activity (Cole et al., 2014; C. L. Grady et al., 2010; Hughes et al., 2020; Mennes et al., 2010; Shine et al., 2019), 2) distinguish between younger and older adults (Ferreira & Busatto, 2013; C. Grady et al., 2016; Spreng et al., 2016), and 3) can generate predictive models of disease spread along with the functional consequences of brain disease (Fornito et al., 2015; Mišić et al., 2015). Drawing from the well-established whole-brain functional connectivity methodology employed by the aging literature, we intend to apply this framework to not only investigate chronological aging, but also incorporate reproductive aging as a relevant, sex-specific factor for brain function at midlife. As such, the present study aims to characterize the independent and interactive impact of menopause and chronological age on resting-state functional connectivity

networks and its association to episodic memory performance in a large sample of healthy young and middle-aged females.

The resting-state functional connectivity (RSFC) fMRI literature examines the statistical dependency of spontaneous blood-oxygen level dependent (BOLD) signal oscillations between discrete regions of the brain in the absence of experimental stimuli (Sporns et al., 2004). Complementary to the task-based fMRI literature, this method has been argued to capture intrinsic patterns of neural activity which may underly the evoked activity observed in the context of experimental task stimuli (Cabral et al., 2011). The justification for this argument is that the external task stimuli evoke neural communication and information flow across the brain to give rise to complex cognitive processes and this depends largely on the state of co-oscillatory neural activity dynamics intrinsic to the brain (Breakspear, 2017; Cole et al., 2014; Deco et al., 2011; Shine et al., 2019). Thus, task-related brain states seem to emerge from individuals' baseline resting-state network organization as defined by neural activity during their 'resting-state'. Collections of brain regions which show a high degree of co-oscillatory BOLD activity have been referred to as resting-state networks by this field (Deco et al., 2011; Power et al., 2011; Yeo et al., 2011). Network science terms have been employed to describe brain region members of these resting-state networks as nodes and their functional connectivity with other nodes as edges. The topology of network models can be conceptualized as how nodes are collectively organized by their edges with each other (Fornito et al., 2013). The neuroscientific meaning of topology in the context of RSFC would thus be the patterns of high/low co-oscillatory BOLD activity among regions of the brain. A study conducted by Cole and colleagues (Cole et al., 2014) demonstrated the functional relevance of resting-state networks by deriving functional connectivity matrices using the Power Atlas (Power et al., 2011) in two independent datasets containing resting-state

fMRI images and task-based fMRI images from 64 tasks in 15 participants (Cole et al., 2010) and 7 tasks in 118 participants from the Human Connectome Project (Barch et al., 2013). They showed a very high degree of correspondence (r=.90) between RSFC matrices and a combined multitask-based FC matrix for each dataset. These results suggest the relevance of the resting-state for functional brain states observed in the context of cognitive tasks. RSFC has been widely used to characterize age-related changes in brain function over the past two decades (for reviews see: Ferreira & Busatto, 2013; Jockwitz & Caspers, 2021), and emerging patterns of age-associated topological changes in resting-state networks will be discussed below.

Resting-State Functional Connectivity and Aging

One of the most robustly studied resting-state networks by aging researchers is the default mode network (Ferreira & Busatto, 2013). The default mode network (DMN) comprises the medial prefrontal cortex, the inferior parietal lobule, the hippocampus and the posterior cingulate cortex/retrosplenialcortex/precuneus (Buckner et al., 2008; Raichle et al., 2001). This network of regions gets its name due to observations that it is primarily activated at rest (in a task-free setting) and deactivated during task performance (Greicius et al., 2003), though this functional attribution of the DMN is still contentious as parts of it have been argued to play a role in tasks that tap into autobiographical memory processes or self-referential thinking (Spreng et al., 2010; Spreng & Grady, 2010; Vatansever et al., 2015). Nonetheless, reduced connectivity among regions of the DMN has repeatedly been observed in older adulthood (Esposito et al., 2008; C. L. Grady et al., 2010; Spreng et al., 2016; Tomasi & Volkow, 2012).

While the DMN seems to be the most robustly affected by age among the canonical resting-state networks (Ferreira & Busatto, 2013), reduced connectivity within its comprised

regions seems to be only part of the larger pattern of topological changes that occur in late life. Dedifferentiation is a term used to describe loss of neural functional specificity often seen in fMRI studies on aging (Cabeza et al., 2018). Work looking at functional dedifferentiation in the context of resting-state networks has begun to establish consistent evidence that resting-state networks display an age-related reduction of functional connectivity among regions within their respective networks as well as increased connectivity between regions typically ascribed to other networks (Chan et al., 2014; Geerligs et al., 2015; Goh, 2011; Koen et al., 2020; Ng et al., 2016; Setton et al., 2021; Spreng et al., 2016; Zonneveld et al., 2019). For example, Spreng and colleagues (2016) looked at differences between young and older adults' intrinsic connectivity of the DMN as well as the dorsal attention network (DAN) and saw that within-network connectivity was reduced for each of these networks but connectivity between these two networks was increased in older compared to younger adults at rest (Spreng et al., 2016).

Chan and colleagues in their 2014 publication took a graph theoretic approach to capturing dedifferentiated topological changes associated with aging. In a sample of adults ranging in age from 20 to 89 years of age they used a RSFC boundary mapping technique (Wig et al., 2014) to define ROIs which were then labelled as belonging to specific resting-state networks according to the canonical atlas developed by Power and colleagues (Power et al., 2011). With these network-defined nodes, they calculated a node-wise measure they referred as system segregation which broadly is a standardized difference of a node's functional connectivity to members within its own network minus its functional connectivity to members of other networks. Thus, the greater a node's system segregation the more functional connectivity it has within its own network compared to other networks. Overall, they reported mean node-wise system segregation was negatively correlated with greater age. This trend was linear among only

sensorimotor networks (e.g., visual, somatosensory/motor, and auditory networks), but quadratic among hetero-modal "association" networks, such as the DMN, ventral attention network, salience network, and fronto-parietal control network. Interestingly, the point of inflection for this quadratic relationship between mean system segregation of association networks and age was at midlife (roughly at 50 years of age). While the authors did not disaggregate this effect by sex, it prompts further inquiry when considering that the average age of menopause is 51 and 72% of their cohort between the age of 50-64 was female. The authors also showed that this system segregation measure was sensitive to individual differences in cognitive performance. Specifically, they residualized participants' composite performance on number of episodic memory tasks by age to show that independent of age, system segregation among associative networks was positively correlated to performance. This result was replicated in a longitudinal study shortly after by Ng and colleagues (Ng et al., 2016) who showed that system segregation between the fronto-parietal control network and DMN declined in a sample of 78 healthy older adults over the course of four years.

Zonneveld and colleagues (Zonneveld et al., 2019) investigated this effect for the anterior and posterior portions of the DMN independently in a large sample of middle- to older- aged adults consisting of 2878 participants total from the population-based Rotterdam Study. They found interesting dissociable patterns of age-related changes in RSFC topology among either portion of the DMN, such that the anterior portion displayed increased connectivity with the fronto-parietal control network while the posterior portion displayed increased connectivity with the dorsal attention network. Such findings have prompted further consideration for the heterogenous age-related topological variance of canonical resting-state subnetworks. Setton and colleagues (Setton et al., 2021) also investigated dedifferentiated resting-state network

architecture in older adulthood using resting-state data from 181 younger adults (age 20-34) and 120 older adults (age 60-89). When looking at sub-networks of the DMN, fronto-parietal control, and dorsal attention networks as defined by the 17-network Yeo atlas (Yeo et al., 2011), the authors used partial least squares (PLS) to identify latent variables of reduced functional connectivity within each subnetwork and greater functional connectivity among all subnetworks and this was particularly pronounced for dorsal attention A subnetwork (comprising superior parietal lobule), fronto-parietal control C subnetwork (comprising the precuneus and posterior cingulate), and DMN A subnetwork (comprising the inferior parietal lobule, dorsal prefrontal cortex, precuneus, posterior cingulate, and medial prefrontal cortex). This shifted pattern of higher-level subnetwork architecture observed in older adults negatively correlated with executive function test scores, but, interestingly, did not significantly correspond to episodic memory performance. The discrepancy of behavioral associations with dedifferentiated connectivity patterns between Setton and colleagues and Chan and colleagues could be due to the different implications of respective age-related network topological differences for specific cognitive processes. However, this discrepancy could just as likely be due to the different analytical choices in network definition and atlases used by the different groups.

To summarize, the literature on rsFC and aging has established consistent findings of dedifferentiated network topology in older adulthood, which seems relevant to episodic memory and other cognitive functions. The work described above provides a useful framework which the present study employs to address unanswered questions regarding the neural changes thought to occur at midlife.

Menopause and Neurocognition

Evidence from epidemiological surveys has indicated that many females experience disturbances in their cognition (i.e., "brain fog") in peri- as well as early post-menopause, and this is further corroborated by neuropsychological studies showing a significant effect of menopausal status on associative memory performance (Epperson et al., 2013; Rentz et al., 2017; Taylor et al., 2019). The menopausal transition occurs over several years with the average age of menopause being around 51 years of age and it is characterized by significant declines in ovarian hormones such as 17β-estradiol and progesterone as well as increases in follicular stimulating hormone (FSH) (Soules et al., 2001). Perimenopause is roughly a two-year portion of the menopausal transition which is characterized by irregular fluctuations in 17β-estradiol and two or more skipped cycles (Soules et al., 2001). Post-menopause is defined once an extended interval of amenorrhea (lack of menstruation) persists for at least 12 months. 17β-estradiol ceases to fluctuate at post-menopause and rather steadily declines. This depletion of 17β-estradiol is argued to be the primary mediator of menopausal impact on neurocognitive health at midlife and beyond, given its role in bioenergetic and neuromodulatory processes in the brain (Adams et al., 2001, 2002; Arevalo et al., 2015; Epperson et al., 2012; Girard et al., 2017; E. Jacobs & D'Esposito, 2011; E. G. Jacobs et al., 2017; Nejat & Chervenak, 2010; Östlund et al., 2003; Pritschet et al., 2020; Rettberg et al., 2014; Sheppard et al., 2018; Waters et al., 2011; Wu et al., 2011). The scientific literature on menopause-associated functional brain changes at midlife is extremely sparse (de Lange et al., 2021; Taylor et al., 2019, 2021). While no study to our knowledge has directly investigated whole-brain resting-state functional connectivity differences in females across the pre-, peri-, and postmenopausal stages of reproductive aging, there are studies conducted by our collaborators that have investigated differences in task-based fMRI neural activity based on reproductive stage

at midlife (E. G. Jacobs et al., 2016, 2017). In their 2016 study, Jacobs and colleagues investigated episodic memory in a sample of 32 pre-, 29 peri-, and 31 postmenopausal females (age range 46-55). The authors demonstrated that reproductive stage over chronological age predicted activity in the left hippocampus during verbal memory encoding, where pre- and peri-menopausal females had greater BOLD activity compared to postmenopausal females. Additionally, the authors investigated functional connectivity during encoding using a seed-based approach and found that postmenopausal females had greater bilateral hippocampal functional connectivity which was negatively correlated with performance and endogenous 17β-estradiol levels in all females. Lastly this study showed that when postmenopausal females's performance on an out-of-scanner face-name associative memory task was tertile split, higher performers compared to middle/low performers' neural activity was more closely resemblant of pre-/peri-menopausal females's, specifically they had lower bilateral hippocampal functional connectivity. Estrogenic depletion over the menopausal transition has been argued as a major factor in these observed neural and behavioral changes. There are likely many relevant sociocultural and environmental factors which also contribute to these findings, as womanhood is a social construct which encompasses many environmental factors in addition to reproductive hormones. There is a sizeable literature on estrogen's role in the brain and its cognitive relevance. The knowledge gained from this literature and how it contributes to our understanding of menopausal relevance for brain function will be discussed below.

Rationale for Current Study, Objectives, and Hypotheses

Rationale

Given the sparsity of literature on menopausal relevance in brain health at midlife and into older adulthood, the current study will contribute new knowledge regarding the impact of menopause

on intrinsic functional brain network organization in females at midlife. Importantly, this knowledge will delineate the effects of reproductive and chronological aging on resting-state functional connectivity networks while also testing the cognitive relevance of identified patterns through the inclusion of behavioral data in multivariate analyses. These results will provide predictions for future work investigating relevant cognitive domains susceptible to impairment at midlife, as well as underscore the importance of considering sex/gender relevant factors in studies of neurocognitive aging.

Objectives

The current study aims to use resting-state functional connectivity analysis in tandem with multivariate partial least squares (PLS) analysis in a sample of healthy young and middle-aged adult females to identify 1) differences in resting-state functional connectivity among cohorts separated by menopausal status. 2) the correlation between chronological age and resting-state functional connectivity when menopausal status is disregarded. 3) the effect of menopausal status on correlations between participants' chronological age and whole-brain, resting-state functional connectivity. Post-hoc linear regressions will then predict participants' subsequent episodic memory performance from their 'brain-scores', representing the degree to which they display the patterns of resting-state functional connectivity identified in the above PLS analyses. Episodic memory will be assessed using a spatial context memory task which was selected based on previous findings in our laboratory showing that impaired performance occurs at midlife (Kwon et al., 2016).

Hypotheses

We expect to see reproductive and chronological age-related patterns of dedifferentiated connectivity among 7 *a priori* defined resting-state networks from the Schaefer 2018 centroid

atlas (Schaefer et al., 2018). Additionally, we will include hippocampal ROIs as established by Damoiseaux and colleagues (Damoiseaux et al., 2016) due to its well documented role in episodic memory processes and rich density of estrogen receptors. We expect to see a reorganization of hippocampal regions functional connectivity among attentional and default mode networks corresponding to menopausal status as well as chronological age at midlife. Specific hypotheses will thus be outlined below.

Hypothesis 1: The first hypothesis will be addressed in a mean-centered PLS which will identify reliable differences in functional connectivity between groups of participants separated into cohorts by reproductive status using the STRAW criteria (Soules et al., 2001). These groups will consist of 3 cohorts: young and middle-aged pre-menopausal adults, middle-aged peri-menopausal adults, and middle-aged post-menopausal adults. Across the default mode network (DMN), dorsal attention network (DAN), and fronto-parietal control network (FPN), we expect to see a pattern of dedifferentiation (reduction of within-network functional connectivity and increase of between-network functional connectivity) in the post-menopausal cohort relative to pre- and peri menopausal cohorts (Chan et al., 2014; Cole et al., 2014; Setton et al., 2021). We also expect to see increased functional connectivity among ROIs of the hippocampus for post- compared to pre- and peri-menopausal participants (E. G. Jacobs et al., 2016).

Hypothesis 2: In a second PLS, all participants will be considered in a single group (i.e., menopausal status will be disregarded). Participants' chronological age will instead be considered as a continuous variable of interest here. This type of PLS, often referred to as a behavioral-PLS (BPLS), will identify maximal patterns of covariance between participants' chronological age and their functional connectivity data. Patterns of functional connectivity identified to correlate

with age here should again resemble dedifferentiation findings from the aging literature, especially for the DMN, DAN, and FPN.

Hypothesis 3: For the final PLS, participants will again be grouped by reproductive status using the STRAW criteria (Soules et al., 2001) to distinguish pre-, peri-, and postmenopausal participants from each other. In this PLS, participants' chronological age will be included as a variable of interest to be correlated with functional connectivity data within each group. This BPLS will identify maximal patters of covariance between age and connectivity that differ among groups. We predict that postmenopausal participants in contrast to pre-/peri-menopausal participants will display age-related dedifferentiated patterns of functional connectivity specific to the DMN and attention networks (dorsal and fronto-parietal control). This dedifferentiated pattern of functional connectivity will positively correlate with age in the postmenopausal group only (Chan et al., 2014; Zonneveld et al., 2019).

Methods

Participants

Participants in this experiment come from a sample of adult females (20-60 years of age). Each participants' reproductive status was assessed using the Stages of Reproductive Aging Workshop (STRAW) criteria which include self-report questionnaires and serum measures of estradiol, follicular stimulating hormone (FSH), and luteinizing hormone (Soules et al., 2001). Recruitment was conducted using web-based advertisements and posters in the Montreal metropolitan area. To be eligible for participation participants had to be in good health by not indicating any of the following in their medical history: total hysterectomy, bilateral oophorectomy, cataract, glaucoma, untreated age- related maculopathy, risk factors for cardiovascular disease such as uncontrolled hypertension or untreated high cholesterol, diabetes, history of estrogen-related cancers, neurological diseases or insult, any psychiatric disorder, claustrophobia, prior serious head injury, history of alcoholism, currently drinking >14 units of alcohol/week, or currently smoking >40 cigarettes per day. Participants also had to have received a high school diploma, adhere to the magnetic resonance imaging (MRI) safety requirements, and consent to giving blood samples. All participants provided informed consent before participating in the study and they received $40 immediately after their first session and $60 immediately after their second session as compensation. This study was approved by the Douglas Mental Health University Institute Research Ethics Board. Participants completed two sessions on separate visits: an initial behavioral session (Session 1) and a second MRI session (Session 2). After exclusions specified at each step outlined below, the current sample of participants with usable data for analyses stands at 134. Sixty-four of these are young and middle-aged pre-menopausal, 33 are middle-aged peri-menopausal and 37 are middle-aged postmenopausal.

Procedure

Online Screening Questionnaire: An initial 20-minute online screening questionnaire was administered to individuals interested in participating in the study. This screening questionnaire included questions regarding basic demographic information such as age, gender, medical history, reproductive history, and education. Participants who seemed cognitively, physically, and psychologically healthy were contacted for participation in the study.

Session 1: When participants arrived at the Brain Imaging Center of the Douglas Mental Health University Institute, they agreed to two consent forms regarding participation in the study and providing blood samples. After which, participants completed a neuropsychological battery of assessments and a mock-scanner procedure for exposure to the MRI scanner environment and familiarization with the in-scanner spatial context memory task. This session lasted approximately two and a half hours. Only participants who met eligibility on relevant neuropsychological assessments were invited to participate in session two. Eligibility relevant to this study is as follows:

The Mini-International Neuropsychiatric Interview (M.I.N.I; Sheehan et al., 1998) is used to identify psychiatric disorders. One point is obtained when all diagnostic criteria are met for a single disorder. Participants were excluded from the study dependent on positive modules.

The Edinburgh Inventory (Oldfield, 1971) is a measure of handedness. Participants indicate whether they use their left, right, or both hands to perform tasks. Participants who exclusively use their right hand for all tasks receive a score of 100%. While participants will not be excluded if they are left-handed, scores were noted for each individual and reported within groups.

The Beck Depression Inventory II (BDI-II; Beck, Steer, & Brown; 1996) is a 21-item self- report questionnaire for depressive symptoms from the previous two weeks. Out of a total score of 63, a score above 13 suggests mild depression, a score above 20 suggests moderate depression, and a score above 29 suggests severe depression. Participants with a score above 12 were excluded from this study.

The Beck Anxiety Inventory (BAI; Beck, Epstein, Brown, & Steer, 1988) is a 21-item self-report questionnaire for anxiety symptoms during the last month. Out of a total score of 63, a score above 21 suggests moderate anxiety and a score above 36 suggests potential cause for concern. Participants with a score above 21 were excluded from this study.

The Mini-Mental State Exam (MMSE; Cockrell & Folstein, 1988) is a measure of general cognitive functioning. This tool is used to evaluate mental status and cognitive impairment in the elderly population. The assessment consists of 11 sections assessing diverse cognitive abilities. Out of a total score of 30, participants with a score below 27 were excluded from the study.

The California Verbal Learning Test II (CVLT-II; Delis et al., 2000) is a measure of verbal episodic memory. This test includes measures for short-term and long-term recall for both cued and free recall. The CVLT is used as a measure for exclusion. The cut-off scores for the CVLT are varied dependent on age and education level (Norman, Evans, Miller, & Heaton, 2000).

The National Adult Reading Test (NART; (Strauss et al., 2006)/French NART (fNART; (Mackinnon & Mulligan, 2005) is used as an estimate of premorbid intelligence. Predicted full-scale IQ is derived from equations and calibrated against the Wechsler Adult Intelligence Scale full-scale IQ (WAIS-IV FSIQ; English version) or WAIS-R verbal (French version; Nelson, 1982; Mackinnon and Mulligan, 2005; Wechsler, 2008). Participants with scores exceeding 2.5 standard deviations from the mean after adjusting for age and education were excluded.

Menopausal Status Categorization: Participants' menopausal statuses were categorized according to the STRAW-10 criteria (Soules et al., 2001). Specifically, participants self-reported details of their reproductive cycles. Those who reported consistently regular menstrual cyclicity were categorized as pre-menopausal. Those who reported variable menstrual cyclicity, +/- 7 days variation in menstruation or greater than 60 days without menstruation (i.e. amenorrhea) were categorized as perimenopausal. Participants who reported a full year or more of amenorrhea were categorized as post-menopausal.

Session 2: Those who met our inclusion criteria and agreed to continue participation returned for their second session on a later date. The second session consisted of a pregnancy test, a sequence of MRI scans and a blood draw taken within an hour of scanning by a certified nurse to assess hormonal levels for STRAW categorization. Participants underwent a T1-weighted structural MRI scan, and 10 minutes of resting-state functional MRI (rsfMRI) scans, which were conducted with eyes open, while participants looked at a central white fixation cross on a black background.

MRI Acquisition

Whole-brain imaging was conducted on a Siemens 3T Prisma-fit scanner. Participants lay supine in the scanner with a 32-channel head coil. Pads were used to stabilize participants heads and earplugs were given to participants to reduce the noise generated by the scanner. Resting-state stimuli (fixation cross) were back projected onto a screen in the scanner and participants looked at a mirror mounted to the head coil to view. For participants requiring corrected visual acuity, plastic optical corrective glasses were provided. All participants were first scanned with a T1-weighted structural MRI (TR=2300ms, TE=2.36ms, voxel-size=1×1×1mm, FOV = 256 mm2,

time of acquisition = 5 min 3 s). Next, resting-state blood-oxygen-level-dependent (BOLD) functional MRI scans were obtained with T2*-weighted gradient-echo echo-planar image (EPI) pulse sequence over one run consisting of 296 timepoints (37 slices, 4mm slice thickness, TR=2000ms, TE=30ms, voxel-size=4×4×4mm, FOV = 256 mm2, flip angle=90 degrees, phase-encoding direction= A/P, time of acquisition = 10 min).

Preprocessing

Preprocessing is performed using fMRIPrep 20.2.0 (Esteban, Markiewicz, et al. (2018); Esteban, Blair, et al. (2018); RRID:SCR_016216), which is based on Nipype 1.5.1 (Gorgolewski et al. (2011); Gorgolewski et al. (2018); RRID:SCR_002502).

Anatomical data preprocessing: A total of 1 T1-weighted (T1w) images for each participant was corrected for intensity non-uniformity (INU) with N4BiasFieldCorrection (Tustison et al. 2010), distributed with ANTs 2.3.3 (Avants et al. 2008, RRID:SCR_004757), and used as T1w-reference throughout the workflow. The T1w-reference was then skull-stripped with a Nipype implementation of the antsBrainExtraction.sh workflow (from ANTs), using OASIS30ANTs as target template. Brain tissue segmentation of cerebrospinal fluid (CSF), white-matter (WM) and gray-matter (GM) was performed on the brain-extracted T1w using fast (FSL 5.0.9, RRID:SCR_002823, Zhang, Brady, and Smith 2001). Brain surfaces were reconstructed using recon-all (FreeSurfer 6.0.1, RRID:SCR_001847, Dale, Fischl, and Sereno 1999), and the brain mask estimated previously was refined with a custom variation of the method to reconcile ANTs-derived and FreeSurfer-derived segmentations of the cortical gray-matter of Mindboggle (RRID:SCR_002438, Klein et al. 2017). Volume-based spatial normalization to two standard spaces (MNI152NLin2009cAsym, MNI152NLin6Asym) was performed through nonlinear

registration with antsRegistration (ANTs 2.3.3), using brain-extracted versions of both T1w reference and the T1w template. The following templates were selected for spatial normalization: ICBM 152 Nonlinear Asymmetrical template version 2009c [Fonov et al. (2009), RRID:SCR_008796; TemplateFlow ID: MNI152NLin2009cAsym], FSL's MNI ICBM 152 non-linear 6th Generation Asymmetric Average Brain Stereotaxic Registration Model [Evans et al. (2012), RRID:SCR_002823; TemplateFlow ID: MNI152NLin6Asym].

Functional data preprocessing

For each of the rsfMRI scans the following preprocessing was performed. First, a reference volume and its skull-stripped version were generated using a custom methodology of fMRIPrep. A B0-nonuniformity map (or fieldmap) was estimated based on a phase-difference map calculated with a dual-echo GRE (gradient-recall echo) sequence, processed with a custom workflow of SDCFlows inspired by the epidewarp.fsl script and further improvements in HCP Pipelines (Glasser et al. 2013). The fieldmap was then co-registered to the target EPI (echo-planar imaging) reference run and converted to a displacements field map (amenable to registration tools such as ANTs) with FSL's fugue and other SDCflows tools. Based on the estimated susceptibility distortion, a corrected EPI (echo-planar imaging) reference was calculated for a more accurate co-registration with the anatomical reference. The BOLD reference was then co-registered to the T1w reference using bbregister (FreeSurfer) which implements boundary-based registration (Greve and Fischl 2009). Co-registration was configured with six degrees of freedom. Head-motion parameters with respect to the BOLD reference (transformation matrices, and six corresponding rotation and translation parameters) are estimated before any spatiotemporal filtering using mcflirt (FSL 5.0.9, Jenkinson et al. 2002).

The BOLD time-series (including slice-timing correction when applied) were resampled onto their original, native space by applying a single, composite transform to correct for head-motion and susceptibility distortions. These resampled BOLD time-series will be referred to as preprocessed BOLD in original space, or just preprocessed BOLD. The BOLD time-series were resampled into standard space, generating a preprocessed BOLD run in MNI152NLin2009cAsym space. First, a reference volume and its skull-stripped version were generated using a custom methodology of fMRIPrep. Automatic removal of motion artifacts using independent component analysis (ICA-AROMA, Pruim et al. 2015) was performed on the preprocessed BOLD on MNI space time-series after removal of non-steady state volumes and spatial smoothing with an isotropic, Gaussian kernel of 6mm FWHM (full-width half-maximum). Corresponding "non-aggressively" denoised runs were produced after such smoothing. Additionally, the "aggressive" noise-regressors were collected and placed in the corresponding confounds file. Several confounding time-series were calculated based on the preprocessed BOLD: framewise displacement (FD), DVARS and three region-wise global signals. FD was computed using two formulations following Power (absolute sum of relative motions, Power et al. (2014)) and Jenkinson (relative root mean square displacement between affines, Jenkinson et al. (2002)). FD and DVARS are calculated for each functional run, both using their implementations in Nipype (following the definitions by Power et al. 2014). The three global signals are extracted within the CSF, the WM, and the whole-brain masks. Additionally, a set of physiological regressors were extracted to allow for component-based noise correction (CompCor, Behzadi et al. 2007). Principal components are estimated after high-pass filtering the preprocessed BOLD time-series (using a discrete cosine filter with 128s cut-off) for the two CompCor variants: temporal (tCompCor) and anatomical (aCompCor). tCompCor components are then calculated from the

top 2% variable voxels within the brain mask. For aCompCor, three probabilistic masks (CSF, WM and combined CSF+WM) are generated in anatomical space. The implementation differs from that of Behzadi et al. in that instead of eroding the masks by 2 pixels on BOLD space, the aCompCor masks are subtracted a mask of pixels that likely contain a volume fraction of GM. This mask is obtained by dilating a GM mask extracted from the FreeSurfer's aseg segmentation, and it ensures components are not extracted from voxels containing a minimal fraction of GM. Finally, these masks are resampled into BOLD space and binarized by thresholding at 0.99 (as in the original implementation). Components are also calculated separately within the WM and CSF masks. For each CompCor decomposition, the k components with the largest singular values are retained, such that the retained components' time series are sufficient to explain 50 percent of variance across the nuisance mask (CSF, WM, combined, or temporal). The remaining components are dropped from consideration. The head-motion estimates calculated in the correction step were also placed within the corresponding confounds file. The confound time series derived from head motion estimates and global signals were expanded with the inclusion of temporal derivatives and quadratic terms for each (Satterthwaite et al. 2013). Frames that exceeded a threshold of 0.5 mm FD or 1.5 standardised DVARS were annotated as motion outliers. All resamplings can be performed with a single interpolation step by composing all the pertinent transformations (i.e. head-motion transform matrices, susceptibility distortion correction when available, and co-registrations to anatomical and output spaces). Gridded (volumetric) resamplings were performed using antsApplyTransforms (ANTs), configured with Lanczos interpolation to minimize the smoothing effects of other kernels (Lanczos 1964). Non-gridded (surface) resamplings were performed using mri_vol2surf (FreeSurfer).

Functional Data Post-processing

After initial preprocessing by fMRIPrep, two independent raters in our laboratory visually examined the anatomical and functional raw and preprocessed images to determine if any structural or motion related abnormalities were present and unaccounted for by fMRIPrep's standard preprocessing procedures. Only one participant was removed at this step due to gross anatomical abnormality that was not fully accounted for by fMRIPrep's normalization procedure. After this visual quality control step, participants' images normalized to ICBM 152 Nonlinear Asymmetrical template version 2009c [Fonov et al. (2009), RRID:SCR_008796; TemplateFlow ID: MNI152NLin2009cAsym] were selected to go through a final custom denoising pipeline programmed using python's Nilearn package (http://nilearn.github.io) which consists of brain extraction via each individual's fMRIPrep generated brain mask, removal of the first five volumes, spatial smoothing using a 6 mm FWHM isotropic Gaussian kernel, confound time-series regression, and 0.003-0.08 Hz bandpass filter in that order. Specific confound time-series selected from the fMRIPrep generated outputs were the time-series of independent components identified by ICA-AROMA as likely related to motion. These components were identified for each participant independently (Pruim et al. 2015). This procedure was selected based off of the recommendations from a recent evaluation conducted by Parkes and colleagues (Parkes et al., 2018).

Functional Connectivity Analysis

The mean BOLD signal time-series are extracted from 200 spherical ROIs defined in the Schaefer 2018 atlas (Schaefer et al., 2018) from each participants rsfMRI scan. Additionally, 4 hippocampal ROIs were include from coordinates provided by Damoiseaux and colleagues

(Damoiseaux et al., 2016). Each ROI had a radius of 3mm. After each participant's time-series was created, high-motion volumes were then removed (i.e., scrubbed) before correlation matrix creation. This scrubbing procedure removed data-points from time-series which corresponded to a high degree of motion during scanning. This was defined as time-points during which motion exceeded our *a priori* defined thresholds of either .5 mm framewise displacement or 2 standard deviations from the mean global BOLD signal for the run. Additionally, one volume preceding and one volume following motion outlier time-points were removed from time-series. At this step, three participants were excluded for having less than 180 volumes (6 minutes) of resting-state data remaining. After this scrubbing procedure, whole-brain functional connectivity estimates were calculated as the Pearson's correlation between each of the 204 ROI's time-series using python's Nilearn package (http://nilearn.github.io). This yielded functional connectivity matrices for each individual in our sample which could then be used in partial least squares connectivity analysis.

PLS Analyses

To address our hypotheses, partial least squares (PLS) analysis was conducted using the open source PLSGUI software (https://www.rotman-baycrest.on.ca/index.php?section=345) in MATLAB version 8.3.0 (R2014a; Mathworks, Inc., Natick, MA). PLS analysis is a multivariate statistical analysis technique frequently used to test correlations between high-dimensional neuroimaging datasets and behavioral or experimental measures which are deemed reliable through a bootstrapping method (McIntosh et al., 1996). Scripts created in our laboratory (https://github.com/Charana22/pls_connectivity) were used to apply PLS to connectivity data. Three PLS analyses were conducted to address our hypotheses. The first PLS was a mean-

centered PLS which identified functional connectivity values which reliably differed between our pre-specified groups of interest based on menopausal status. The second two PLS analyses related patterns of reliable edgewise covariation with our variables of interest (i.e., age) in a manner similar to the behavioral PLS method (B-PLS) used for task-based fMRI data. The first B-PLS addressed functional connectivity which covaried with participants' normalized, chronological age amongst the entire sample as a single group. The second B-PLS also addressed functional connectivity which covaried with participants' normalized, chronological age. However, in this second B-PLS, participants were grouped by menopausal status as in the mean-centered PLS stated above so that differences in age-related functional connectivity covariance could be assessed between groups.

Participants' connectivity matrices were stacked in a single matrix where each column corresponded to a single edge between two ROIs (i.e., edge), each row corresponded to a single participant in our sample, and the values corresponded to Pearson's r value for each participant's edge. A separate matrix included variables of interest where each row corresponded to a participant in the same order as the first matrix, and each column corresponded to a variable of interest (e.g., age). The participants' data were stacked according to group (young and middle-aged pre-menopausal, middle-aged peri-menopausal, and middle-aged postmenopausal) for both the connectivity data matrix and behavioral variables of interest matrix. The behavioral matrix was transposed and cross correlated with the connectivity data matrix to produce a combined correlation matrix which was then decomposed via singular value decomposition. Orthogonal latent variables (LVs) were created by projecting the original matrices onto their saliences so that each LV comprised of a singular value representing the amount of covariance accounted for by that LV, a single matrix with values corresponding to all edges' weighted contribution, and a

correlation profile for each behavioral variable of interest's association to the matrix of edgewise contribution. Bootstrapping was performed to establish the stability of each edge's weighted contribution. Standard error for each edge was calculated from 500 bootstrap tests and a bootstrap ratio (BSR) was estimated by dividing the edge's weighted contribution by its standard error. The top 5% of edges' BSRs were retained as the most reliable and included for visualization of results.

Results

Participants

A total of 279 female participants between the ages of 20 and 60 were initially enrolled in the study. Of these, 129 either withdrew or were excluded based off the testing criteria listed above. Of the remaining 150 females, 16 participants were excluded due to either technical issues with the MRI scanner, issues with the testing protocol due to tardiness, poor quality resting-state scan images (either due to excessive motion or abnormalities in image acquisition for example loss of considerable BOLD signal in areas of interest), or indeterminate menopausal status. After these exclusions, 134 participants with sufficient quality resting-state fMRI scans were included for PLS connectivity analyses used to address hypotheses regarding age and menopausal effects on functional connectivity. These participants were divided into three groups based on menopausal status using STRAW-10 for PLS analyses addressing hypotheses 1 and 3 (pre-, peri-, and post-menopausal). From the 134 participants, 76.1% were Caucasian, 3.0% Latin American, 2.2% Black, 1.5% South Asian, 2.9% Chinese, 2.2% aboriginal, .7% Caucasian- Southeast Asian, 1.5% Caucasian-Latin American, and 0.7% Arab. Ethnic data was missing for the remaining 9% of females. Details on the demographics for these groups of participants can be found in Table 1. T-tests for significant differences in demographics among cohorts were conducted in R version 4.1.0.

Table 1: Participant Demographics by Menopausal Status

	PRE-MENOPAUSAL	PERI-MENOPAUSAL	POST-MENOPAUSAL	TOTAL
N	64	33	37	134
AGE	35.45(9.67)*	50.40(4.03)*	55.77(3.02)*	44.74(11.57)
EDUCATION	16.18(2.12)	15.97(1.91)	15.8(2.41)	16.02(2.15)
SOURCE ACCURACY EASY	0.63(0.21) †‡	0.50(0.21) †	0.45(0.21) ‡	0.55(0.22)
SOURCE ACCURACY HARD	0.44(0.20) †‡	0.32(0.17) †	0.31(0.15) ‡	0.37(0.19)
MOCA	27.89(1.78) ‡	27.55(1.56) †	26.46(2.43) †‡	27.4(2.02)
BDI-II	4.22(4.02)	5.00(4.66)	5.16(5.31)	4.67(4.55)
BAI	5.25(5.06)	4.64(4.46)	3.59(3.95)	4.64(4.65)
HANDEDNESS (%RIGHT)	78(44)	75(39)	85(32)	79(40)
ENGLISH	33	48	27	35
FRENCH	52	30	51	46
OTHER	16	21	22	18

Note: Mean(standard deviation). MoCA= Montreal Cognitive Assessment; BDI-II= Beck Depression Inventory II; BAI= Beck Anxiety Inventory; Handedness= Edinburgh Handedness Inventory; English=% English Speakers; French=% French Speakers; Other=% Other Language. *, † ,‡ are used to denote significant differences ($p<0.05$) among different groups.

RS-fMRI Results

The first PLS connectivity analysis was a mean-centered PLS conducted to address hypothesis 1, which predicted dedifferentiated patterns of whole-brain resting-state functional connectivity by menopausal status. This PLS identified two significant latent variables (LV1, $p=0.002$, LV2, $p=0.04$) of which LV1 explained 62.1% of the covariance between menopausal groups and functional connectivity and LV2 explained 37.9% of the covariance between menopausal groups and functional connectivity (see Figure 1). LV1 characterized functional connectivity that was reliably diminished amongst peri-menopausal participants and reliably greater amongst post-menopausal participants (Fig. 1A). In contrast, LV2 characterized functional connectivity that was reliably greater amongst pre-menopausal participants and reliably diminished amongst peri- and post-menopausal participants. Peri- and post-menopausal functional connectivity was not significantly different between the two groups for LV2.

Density plots were constructed by thresholding edges with the top 5% BSR for positive negative edges separately. These edges were then grouped by networks and a density score was calculated to represent the number of edges remaining after thresholding as a function of total possible edges between two networks (see Fig. 1B and 1C for positive and negative density plots respectively). These plots help visualize major patterns of reliable functional connectivity between networks or within a network (along the diagonal of the matrix) and mitigates the

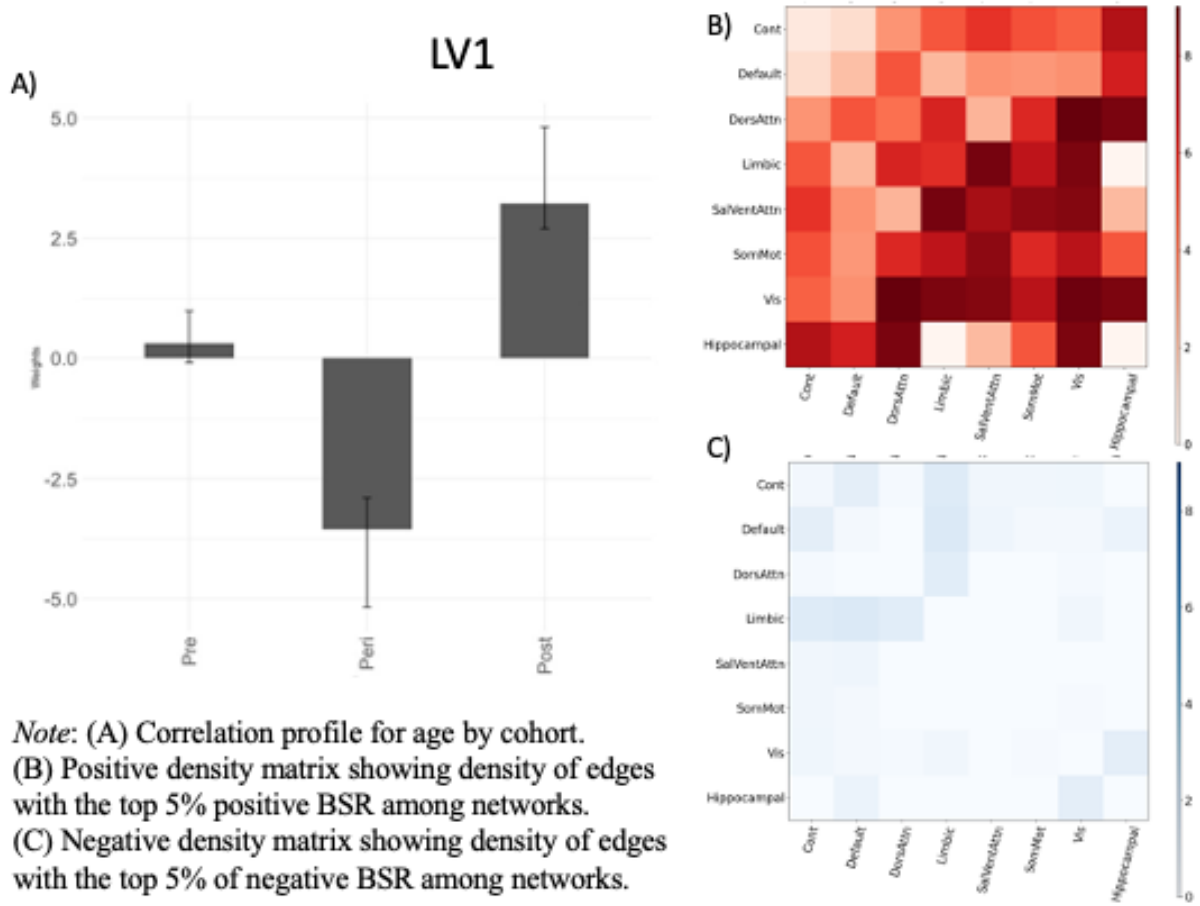
advantage that larger networks with a greater number of ROIs have for potentially contributing to significant edges. The creation of these plots was done in python version 3.9 using functions from the networkx version 2.2 module library (for documentation, see https://networkx.org/documentation/stable/) as well as matplotlib version 3.5.1 module library (for documentation, see https://matplotlib.org/3.5.1/index.html).

Figure 1B shows identified patterns of functional connectivity among networks which was diminished in peri- compared to post-menopausal participants. This connectivity was surprisingly wide-spread and included a large portion of edges within the dorsal attention, limbic, salience/ventral attention, somato-motor, and visual networks as well as between each of these networks, however less so between the salience/ventral attention and dorsal attention networks. Connectivity between regions of the hippocampus and visual, somato-motor, salience/ventral attention, dorsal attention, default and fronto-parietal control networks was also identified to be considerably greater in post- compared to peri-menopausal participants. This was also true for connectivity between the fronto-parietal control network and all other networks except for the default mode network. Lastly, connectivity between the default mode network and dorsal attention network was greater for post- compared to peri-menopausal participants. In contrast, connectivity identified to be greater in peri- compared to post-menopausal participants (Fig. 1C) was extremely sparse. Thus, post-menopausal functional connectivity is distinguished from peri-menopausal functional connectivity, and this seems to be marked by wide-spread increases in functional connectivity both within and between resting-state networks rather than a dedifferentiated pattern as hypothesized.

The second latent variable of this mean-centered PLS identified connectivity patterns which distinguished pre-menopausal participants from both peri- and post-menopausal

participants (Fig. 2A). The positive density plot depicted in figure 2B is comprised of edges which had reliably greater functional connectivity in pre- compared to peri- and post-menopausal groups. Specifically, functional connectivity within the fronto-parietal control, default, dorsal attention, and salience/ventral attention networks as well as the hippocampus was most notably diminished in peri- and post-menopausal groups compared to the pre-menopausal group.

Fig 1: Mean-Centered PLS Results – LV1

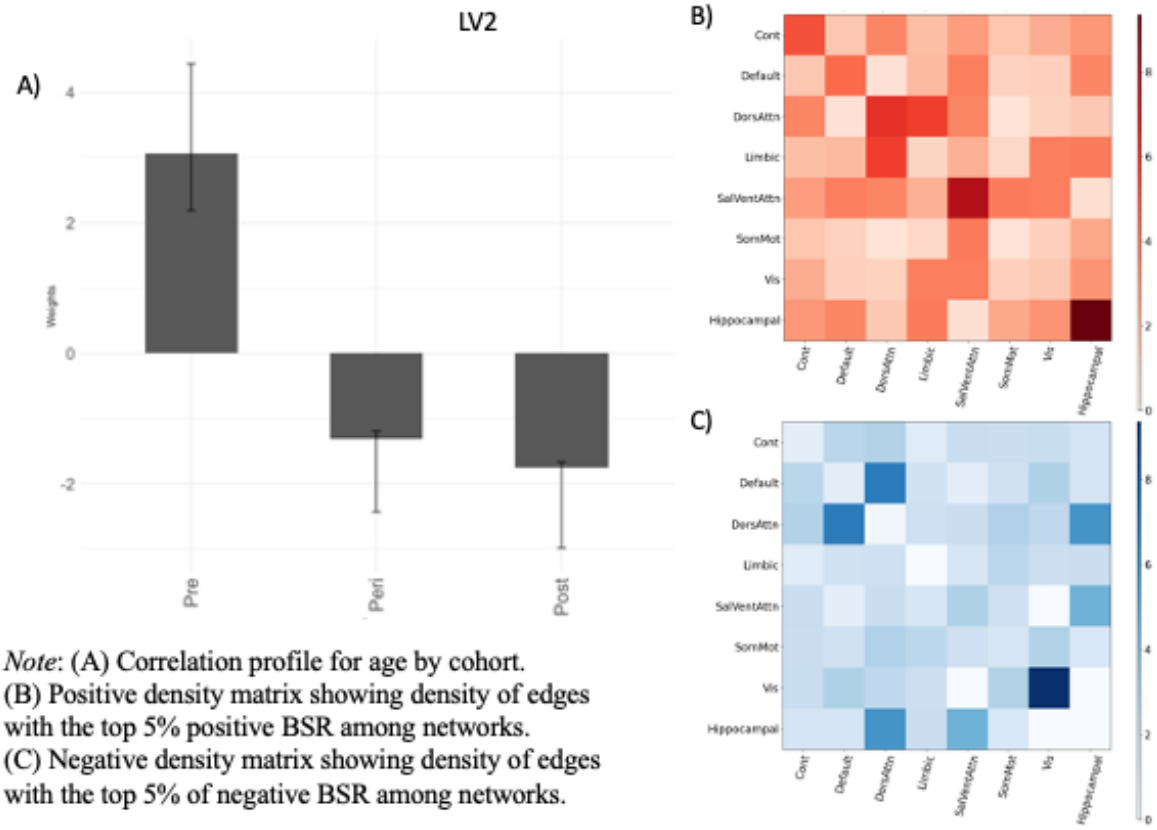


Note: (A) Correlation profile for age by cohort. (B) Positive density matrix showing density of edges with the top 5% positive BSR among networks. (C) Negative density matrix showing density of edges with the top 5% of negative BSR among networks.

This was also true for functional connectivity between the limbic and dorsal attention networks, the fronto-parietal control and dorsal attention networks, as well as between the hippocampus and fronto-parietal control, default, limbic, somato-motor, and visual networks. In contrast,

connectivity which was greater for peri- and post- compared to pre-menopausal participants is shown in the negative density plot of Figure 2C and includes reliable density of edges within the visual network as well as between the default and dorsal attention networks, and lastly, between the hippocampus and dorsal attention and salience/ventral attention networks. Overall the pattern identified in LV2 seems to align with hypothesis 1 in that the fronto-parietal control, default, and dorsal attention networks display diminished within-network functional connectivity while at the same time greater between network connectivity was identified amongst the dorsal attention network and default network as well as the hippocampus.

Fig 2: Mean-Centered PLS Results – LV2

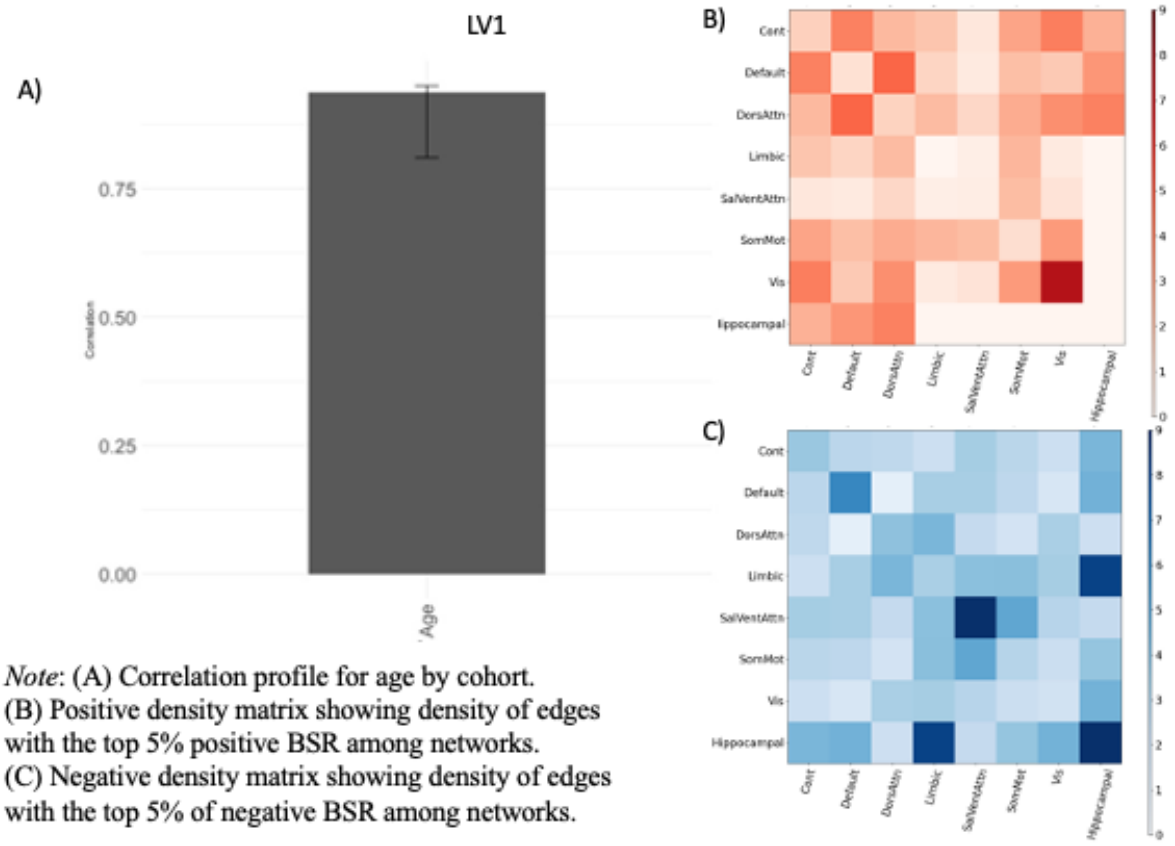


Note: (A) Correlation profile for age by cohort.
(B) Positive density matrix showing density of edges with the top 5% positive BSR among networks.
(C) Negative density matrix showing density of edges with the top 5% of negative BSR among networks.

The second PLS connectivity analysis conducted was a behavioral PLS (B-PLS) which included participants' normalized age as a continuous variable of interest to be correlated with functional connectivity for the entire sample (Fig. 3). This was conducted to address hypothesis 2, which predicted age-related patterns of dedifferentiation to be identified for higher-level networks (fronto-parietal control, default, and dorsal attention) when menopausal status was ignored. One significant latent variable was identified for this B-PLS (LV1, $p=0.037$) and accounted for 100% of covariance between age and functional connectivity due to it being the only latent variable identified by B-PLS.

Patterns of functional connectivity from LV1 which positively correlated with age are encompassed in the positive density matrix below (Fig. 3B). This included a reliable density of edges whose functional connectivity displayed age-related increases within the visual network as well as between default network and fronto-parietal control network, dorsal attention network, and hippocampus. The hippocampus also displayed increased functional connectivity between the fronto-parietal control and dorsal attention networks. Lastly, a considerable amount of age-related increases in functional connectivity was identified between the visual network and fronto-parietal control, dorsal attention, and somato-motor networks.

Fig3: B-PLS Single Group – LV1



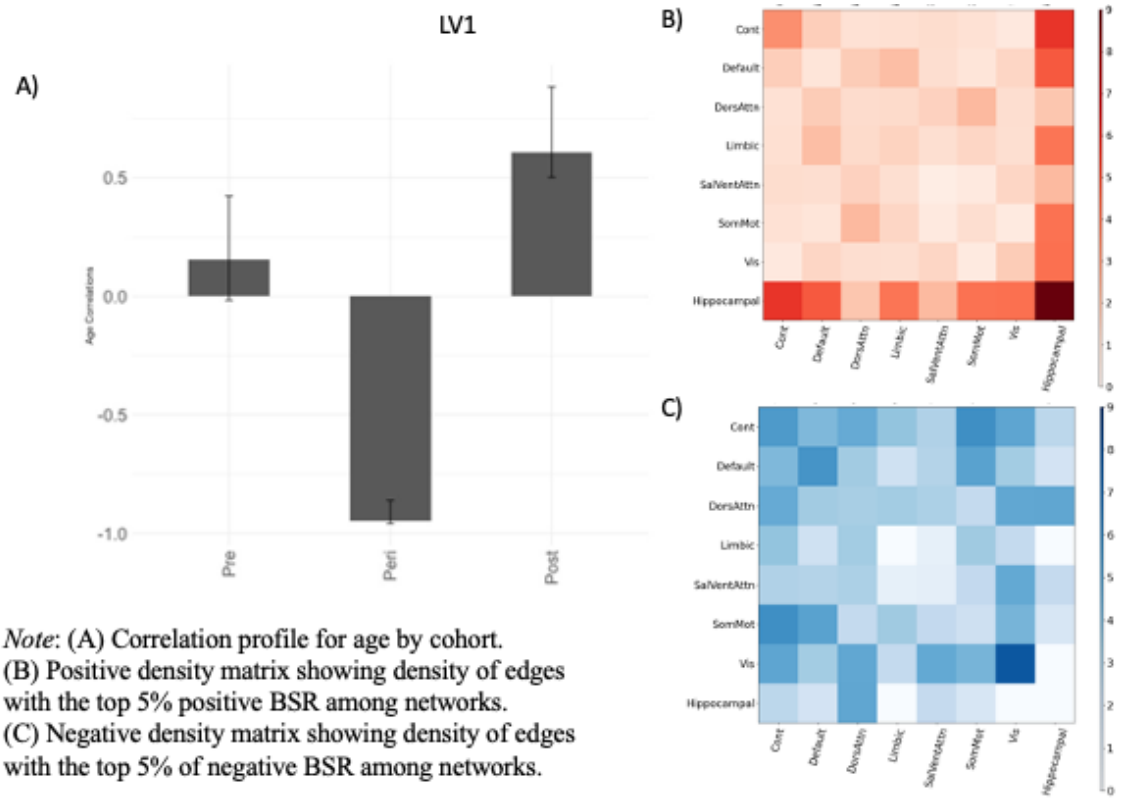
Note: (A) Correlation profile for age by cohort. (B) Positive density matrix showing density of edges with the top 5% positive BSR among networks. (C) Negative density matrix showing density of edges with the top 5% of negative BSR among networks.

This latent variable also captured patterns of functional connectivity which had age-related decreases amongst our entire sample (Fig. 3C). A considerable density of edges with age-related decreases in their functional connectivity was observed within the default and salience/ventral attention networks as well as the hippocampus. Age-related decreases in functional connectivity was also identified between the hippocampus and limbic network as well as the fronto-parietal control, default, somato-motor and visual networks, but to a lesser extent. Overall, the results of this B-PLS somewhat align with hypothesis 2, in that the default mode network seems to most notably show a dedifferentiated pattern of functional connectivity associated with age. Specifically, functional connectivity within the default mode network displayed age-related

decreases while functional connectivity between nodes of the default mode network and fronto-parietal control and dorsal attention networks displayed age-related increases.

The third and final PLS conducted was also a B-PLS similar to the B-PLS discussed previously. This B-PLS also included participants' normalized age as a variable of interest to be correlated with functional connectivity, however, unlike the previous B-PLS, this analysis considered participants' menopausal status (Fig. 4). As such, this B-PLS addressed hypothesis 3 which predicted age-related patterns of dedifferentiated functional connectivity for the default mode and higher attentional networks (fronto-parietal, dorsal) that are specific to the post-menopausal group only. This analysis identified one latent variable that was only on the cusp of significance (LV1, p=0.062) and accounted for 42.9% of covariance between group differences in age-related functional connectivity.

Figure 4: B-PLS For Age Among the Three Groups – LV1



Note: (A) Correlation profile for age by cohort. (B) Positive density matrix showing density of edges with the top 5% positive BSR among networks. (C) Negative density matrix showing density of edges with the top 5% of negative BSR among networks.

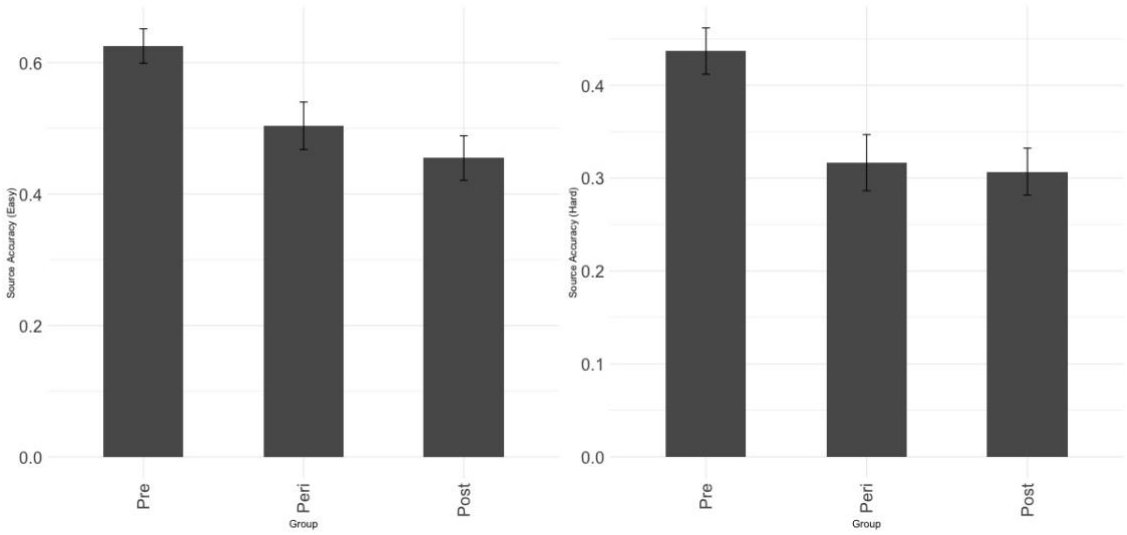
Post-hoc Behavioral Results

After their resting-state scan, participants completed a face-location memory task in the scanner. During this task, participants were shown a series of faces which appeared in one of four quadrants of the screen and were instructed to remember which quadrant they saw the face. After a delay, participants were then presented these same faces along with new faces in the center of the screen. They were instructed to respond in one of six ways: if the face was an old face and they remembered the quadrant in which they had previously seen it, they were instructed to indicate the quadrant (i.e., bottom left, bottom right, top left, or top right); if they recognized the face, but could not remember the quadrant, they were instructed to indicate the face was familiar; if the face was unfamiliar they were to indicate the face was new. This task consisted of an easy as well as a hard condition. For the easy condition, participants were shown six initial faces during the encoding phase, while for the hard condition they were shown twelve faces. Source retrieval accuracy was calculated for their performance by taking the ratio of all responses in which they correctly indicated the quadrant of an old face over the sum of responses in which they either indicated old faces as just familiar (i.e., recognition), misattributed the quadrant of an old face (i.e., source misattribution), or indicated an old face was new. Performance on the subsequent face-location source memory task was assessed for group differences in source retrieval accuracy among pre-, peri-, and post-menopausal groups in two ANOVA analyses for the easy as well as the hard conditions of the task separately. The first ANOVA identified a significant effect of menopausal status on source accuracy for the easy condition ($F_{(2,131)} = 8.86$, $p=0.0002$), see Figure 5A. Tukey post-hoc tests for this ANOVA revealed that pre-menopausal group had significantly greater source accuracy (mean=0.63, SE=0.03) compared to the perimenopausal group (mean=0.50, SE=0.03, p=0.02) as well as the post-menopausal group

(mean=0.45 , SE= 0.03, p=0.002) for the easy condition. Peri- and post-menopausal groups did not significantly differ from each other (p=0.58). The ANOVA conducted for the hard condition of the task (Figure 5B) also identified a significant effect of menopausal status ($F_{(2,131)} = 7.96$, p=0.0005). Tukey post-hoc tests for this ANOVA revealed that the pre-menopausal group had significantly greater source accuracy (mean=0.47, SE=0.02) compared to the perimenopausal group (mean=0.32, SE=0.03, p=0.007) as well as the post-menopausal group (mean=0.31, SE=0.03, p=0.002). Peri- and post-menopausal groups did not significantly differ from each other (p=0.97).

Figure 5: Bar Graphs Showing Menopausal Group Differences in Spatial Source Accuracy

A) **B)**



Note: Error Bars represent Standard Error of the Mean.

Post-hoc Brain-Behavior Associations

Linear regressions were run to predict participants' spatial source memory retrieval accuracy on the subsequent face-location memory paradigm from the degree to which their functional connectivity data weighed into or aligned with the age/menopause related patterns of connectivity identified by significant latent variables of the PLS analyses described above. Because each individual participant receives a weight, otherwise known as a 'brain score', these weights can be used to predict individual measures from how strongly their functional connectivity data resembles the identified pattern for a given latent variable. Before these regressions were conducted, three participants who did not have any correct source retrieval events were removed from analyses. In total, six regressions were conducted: three regressions with normalized source retrieval accuracy on the easy version of the task as the dependent variable and three regressions with normalized source retrieval accuracy on the hard version of the task as the dependent variable. Brain scores for the three significant latent variables were the only predictors included in the models. We hypothesized that participants' weighted contribution to age/menopausal related functional connectivity patterns would predict poorer episodic memory performance. Finally, a Bonferroni correction was applied for all six of these regressions such that $p<0.008$.

The first two regressions, models 1 and 2, corresponded to LV1 from the mean-centered PLS which distinguished peri- from post-menopausal functional connectivity (Fig.1). In these regressions, brain scores were not a significant predictor of performance for either the easy (β=-0.02, SE=0.02, t=-1.0, p=0.32) or the hard versions of the task (β=0.01, SE=0.02, t=0.05, p=0.61).

The second two regressions, models 3 and 4, corresponded to LV2 from the mean-centered PLS which distinguished pre- from peri- and post-menopausal functional connectivity. In these regressions, brain scores significantly predicted performance for both the easy (β=0.11, SE=0.03, t=3.31, p=0.001) and the hard versions of the task (β=0.10, SE=0.03, t=3.12, p=0.002). Note that because brain scores were positive for pre-menopausal participants and negative for peri- and post-menopausal participants the positive beta values indicate a pre-menopausal brain scores were predictive of better normalized spatial source retrieval accuracy and conversely peri- and post-menopausal brain scores were predictive of poorer normalized spatial source retrieval accuracy.

The third and final two regressions, models 5 and 6, corresponded to LV1 from the B-PLS which identified patterns of functional connectivity associated with greater age across the entire sample as a single group (irrespective to menopausal status). In these models, brain scores significantly predicted performance for both the easy (β=-0.09, SE=0.03, t=-2.87, p=0.005) and the hard versions of the task (β=-0.10, SE=0.03, t=-3.03, p=0.003).

Discussion

The central aim of this study was to assess how chronological and reproductive aging affect intrinsic functional organization of the brain at rest and the extent to which this is relevant to performance on a subsequent face-location source memory task. By employing whole-brain resting-state functional connectivity we were able to assess the differences in large-scale brain networks according to menopausal status and chronological age in a sample of 134 adult females between the ages of 20 and 60 years old. Our hypotheses were motivated by the aging literature on resting-state networks. Specifically, we hypothesized that patterns of dedifferentiated functional connectivity among higher-level attention networks and the default mode network would emerge at midlife, and this would in part be explained by menopausal status rather than just chronological age. We also hypothesized that this pattern of dedifferentiation would predict spatial source retrieval accuracy on a subsequent face-location task. The results partially support our hypotheses, however the patterns of connectivity among resting-state networks related to chronological and reproductive aging seem to indicate a more complex pattern of functional connectivity which is not completely explained by dedifferentiation. Furthermore, while some of the identified patterns were able to predict source accuracy performance in our sample, not all the identified patterns were related to behavior. Nonetheless these findings do indicate menopausal as well as chronological aging relevance for resting-state functional connectivity networks and episodic memory at midlife.

The first PLS analysis conducted was a mean-centered PLS which was intended to capture between-group differences in functional connectivity according to pre-, peri-, and post-menopausal status. This PLS resulted in two significant latent variables (LV1, LV2, see Figures 1 & 2). LV1 explained a larger percentage of the covariance in functional connectivity among

groups and specifically identified patterns of functional connectivity which differed between peri- and post-menopausal participants. In contrast, LV2 captured functional connectivity differences between pre-menopausal participants and both peri- and post-menopausal participants who did not display significant differences between each other for this pattern. The functional connectivity patterns captured by LV1 and LV2 were starkly different from each other.

LV1 showed wide-spread, heightened functional connectivity in the post-menopausal group among the fronto-parietal control network, dorsal attention, limbic, salience/ventral attention, somato-motor, and visual networks but to a minimal extent between the dorsal and salience/ventral attention networks. All of these networks, except for the fronto-parietal control network, also showed greater functional connectivity within themselves post-menopause. The default mode network only showed heightened functional connectivity between the dorsal attention network and hippocampus. Lastly, the hippocampus also displayed increased functional connectivity between the fronto-parietal control, dorsal attention, visual, somato-motor, and to a minimal extent the salience/ventral attention networks in post-menopausal participants compared to peri-menopausal participants. There was almost no functional connectivity for LV1 that was diminished in post- versus peri-menopausal participants. This result was somewhat surprising in that it does not seem to indicate dedifferentiation per se due to the fact that functional connectivity within networks was also increased. As this is the first study to our knowledge which has examined large-scale resting-state functional connectivity networks across the whole brain according to menopausal status, it is somewhat difficult to contextualize this result. However, this latent variable offers insight into differences in large-scale functional networks at midlife associated with reproductive aging, which have been largely overlooked by aging research (Taylor et al., 2019). Post-hoc linear regressions could not predict spatial source

accuracy performance from participants' brain scores for this latent variable, which suggests that the differences may not be relevant to that episodic process. This lack of association between functional connectivity and spatial source memory performance may also be indicative of heterogeneity within the post-menopausal group. This would be in-line with observations from studies which show that not all early post-menopausal participants exhibit differences in brain function during tasks associated with verbal episodic memory decline (Jacobs et al., 2016; Rentz et al., 2017).

LV2 from this mean-centered PLS captured differences in functional connectivity between pre-menopausal group and the peri- and post-menopausal groups who did not significantly differ from each other (see Figure 2). More specifically, functional connectivity was diminished within the fronto-parietal control, default, dorsal attention, salience/ventral attention networks and the hippocampus for the peri- and post-menopausal groups compared to the pre-menopausal group. There were notable functional connectivity increases between the default mode network and dorsal attention network, between the hippocampus and dorsal and salience/ventral attention networks, and finally within the visual network. This pattern mirrors findings in the aging literature by Spreng and colleagues (2016) study showing dedifferentiation of the default mode and dorsal attention networks in older compared to younger adults (Spreng et al., 2016). This suggests that the dedifferentiation of the default mode and dorsal attention networks seen in older adulthood may emerge at midlife, potentially during perimenopause and persist through post-menopause into older adulthood. We found hippocampal regions had greater functional connectivity with the dorsal attention network in peri- and post-menopausal groups. This result is seemingly counterintuitive given that Spreng and colleagues (2016) observed diminished medial temporal lobe (MTL) connectivity with the default mode as well as the dorsal

attention network. This could be an effect specific to menopausal status and midlife which is not sustained through older adulthood, however it just may as well be due to different methods of defining hippocampal regions of interest. Contrary to LV1, this pattern of functional connectivity identified by LV2 was predictive of poorer performance in peri- and post-menopausal participants as evident by our linear regression analysis. As such, it is more likely that these functional connectivity patterns are indicative of dedifferentiation rather than compensation (Cabeza et al., 2018; Koen et al., 2020). A notable caveat of this finding is the age differences between pre- peri- and post-menopausal groups in our sample. The pre-menopausal group included young adults and had a mean age of 35 years old while the peri- and post-menopausal groups had mean ages of 50 and 55 respectively. As such, LV2 which distinguished functional connectivity of pre- from peri- and post-menopausal groups likely encompasses chronological age differences between young and middle-aged participants as well as menopausal status differences.

The results from our B-PLS (see Figure 3), which investigated how chronological age as a continuous variable correlates with functional connectivity among the entire sample as one group (i.e. disregarding menopausal status), help to contextualize the mean-centered PLS results and especially LV2 discussed above. This PLS identified a single significant latent variable (LV1) that incorporated functional connectivity associated with chronological age in our sample. The most prominent age-related increases were found for connectivity within the visual network as well as connectivity between the default mode network and fronto-parietal control and dorsal attention networks. Notable age-related decreases in functional connectivity included connectivity within the default mode network, salience/ventral attention network, as well as within nodes of the hippocampus. This dedifferentiated pattern of functional connectivity for the

default mode network with the fronto-parietal control and dorsal attention networks is consistent with the literature on aging (C. Grady et al., 2016; Setton et al., 2021; Spreng et al., 2016) and demonstrates that this pattern can be seen as early as midlife. Interestingly, greater functional connectivity between the default mode network and fronto-parietal network was not captured by either LV1 or LV2 of the mean-centered PLS described above. This may suggest that the between network increases in connectivity typically seen for the DMN and FPCN are predominantly impacted by chronological age processes rather than menopausal status. Additionally, this age-related LV1 did not capture substantial connectivity decreases with the fronto-parietal control and dorsal attention networks which was seen for LV2 of the mean-centered PLS distinguishing peri- and post-menopausal groups from the pre-menopausal group. This could indicate that decreases within these two networks may emerge at peri-menopause and thus be primarily a menopausal effect. Participants brain scores from this latent variable were predictive of spatial source accuracy in post-hoc linear regressions which suggests that this age-related pattern of functional connectivity is relevant to this specific domain of episodic memory.

The final B-PLS compared the relationship between chronological age and functional connectivity as a function of menopausal status. This B-PLS identified a single latent variable (LV1) that was trending towards significance ($p=0.06$) (see Figure 4). This LV1 captured connectivity which showed an inverse relationship with age for the peri- and post-menopausal groups only. The most prominent connectivity which decreases with age in the peri-menopausal group and also increases with age in the post-menopausal group was connectivity within the hippocampus. This is complementary to Jacobs and colleagues' task-based finding that post-menopausal connectivity was greater within the hippocampus (E. G. Jacobs et al., 2016). It also contextualizes the findings from LV2 of the mean-centered PLS as well as LV1 of the single-

group B-PLS which together showed age and menopausal decreases for functional connectivity within the hippocampus. Connectivity between the hippocampus and both the fronto-parietal control network and the default mode network showed age-related decreases in the peri-menopausal group and age-related increases in the post-menopausal group. This was somewhat surprising for the default mode network as Spreng and colleagues had shown MTL decreases in functional connectivity with the default mode network when comparing older and younger adults (Spreng et al., 2016). However, dorsal attention network functional connectivity between the hippocampus showed the opposite age-related effect among these two groups. That is, functional connectivity between the dorsal attention network and the hippocampus increased with age for the peri-menopausal group and decreased with age for the post-menopausal group. This helps contextual discrepancies in our findings from the mean-centered LV2 which showed increased dorsal attention network functional connectivity for the peri- and post-menopausal groups compared to the pre-menopausal group. It could be the case that dorsal attention network connectivity with the hippocampus displays increases from young adulthood to midlife and decreases from midlife into older adulthood. This possibility highlights the valuable insight gained from LV1 of the final between-group B-PLS described here: that age-associated changes in large scale functional connectivity networks shifts across the peri- to post-menopausal stages of reproductive aging. Thus, reproductive and chronological aging seem to interact at midlife and their influence on brain function is not fully understood by analyses which disregard one or the other.

Limitations

As with all studies, this study has some limitations. The most prominent limitation is the sample size, especially for PLS analyses which compared between menopausal groups. While our group

sizes were sufficient for attaining reliable results, greater sample sizes will allow for cross-validation of these results to verify their generalizability. Additionally, greater recruitment of middle-aged pre-menopausal participants would allow for analyses which separated young from middle-aged pre-menopausal participants. This would result in four groups (young adult pre-, middle-aged pre-, peri-, and post-menopausal) which might be better suited in distinguishing chronological aging from menopausal effects.

Recruitment itself was limited by the COVID-19 pandemic which required intermittent halting of experimental testing involving human participants. Not only did COVID-19 influence the amount of testing that could occur from March of 2020 onward, it also likely had a profound impact on mental well-being and neurological health of participants.

Future Directions

This study was one of the first to look at large-scale resting-state network reorganization across the entire brain associated with reproductive and chronological aging. While it has captured a big picture of how intrinsic functional brain networks change according to these two factors at midlife, we did not include regions of the sub-cortex (e.g., the thalamus and basal ganglia) or the cerebellum which are thought to also play a major role in the canonical networks of the cortex. Inclusion of these regions could offer more insight to patterns of reorganized connectivity seen in the current study. Additionally, we only focused on prominent network-level interactions in these analyses. Further research could explore key effects at the edge-level or at the level of nodes, especially nodes derived to be hubs among large-scale networks. Lastly, behavioral analyses were somewhat minimal in this study, and further work could better understand the behavioral relevance of resting-state networks impacted by aging and menopause by comparing it to functional connectivity derived from task-based fMRI images.

Conclusions

In conclusion, the present study identified interactive and independent contributions of reproductive and chronological aging on functional connectivity and its relevance for episodic memory. These patterns were partially explained by our hypotheses predicting dedifferentiation of higher-level attentional networks and the default mode network. However, the functional reorganization of brain networks at midlife has shown to be more complex. Nonetheless, inclusion of both menopausal status and chronological age provided critical insights in that both these factors are relevant to brain health and function as well as cognition at midlife.

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