CONSIDERING BIOLOGICAL SEX IN NEUROLOGICAL RESEARCH

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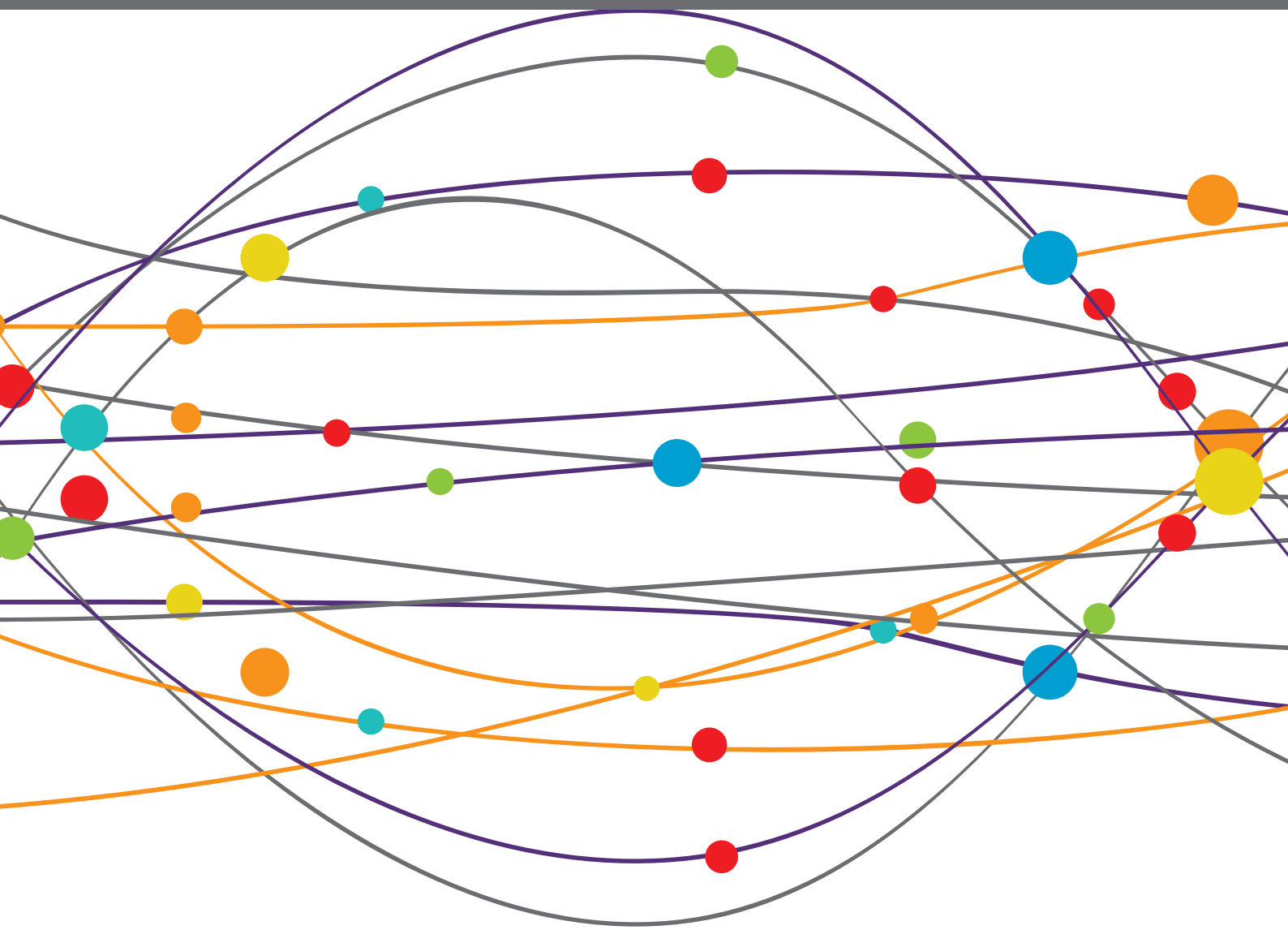

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CONSIDERING BIOLOGICAL SEX IN NEUROLOGICAL RESEARCH

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EDITORIAL
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Editorial: Considering Biological Sex in Neurological Research

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Keywords: male–female distinctiveness, sex, cognitive function, personalized medicine, gender

Editorial on the Research Topic

Considering Biological Sex in Neurological Research

Biological sex, gender, and gonadal hormone status have a substantial influence on the incidence, prevalence, presentation, treatment response, and outcome of diverse neurological/neuropsychological disorders and pathologies. Despite increasing recognition of the importance of these variables, demographic data collected from patients and participants in clinical trials and basic research do not routinely include information on gonadal hormone status (e.g., puberty, stage of menstrual cycle, menopause, or andropause) and results are often not stratified by sex or gonadal hormone status. In this special Issue, investigators and practitioners engaged in research and treatment of diverse neuropsychological issues in human subjects and animal models share their experimental results and observations regarding the impact of biological sex and gonadal hormones on cognitive performance in the context of diabetes, HIV, menopause, obesity and Parkinson's disease. Other contributions examine the role of biological sex and hormonal status in Huntington's disease, early-stage Alzheimer's disease, migraine, epilepsy, stroke, and traumatic brain injury.

Neurocognitive function in the context of prediabetes is addressed by Sundermann et al. who examined sex differences in how pre-diabetic status relates to trajectories of Alzheimer's-related clinical (verbal memory, executive function and language performance and dementia incidence) and biological (brain metabolism, hippocampal volume, cerebrospinal fluid p-tau$_{181}$/Aβ_{1-42} ratio) markers among cognitively normal and mildly cognitively impaired (MCI) older adults from the Alzheimer's Disease Neuroimaging Initiative ($N = 911$, 46% women). Whereas, pre-diabetes shows adverse effects on brain metabolism across sexes, only women with MCI showed associations between pre-diabetes and poorer executive function and language performance across time suggesting that women may be more susceptible to the negative effects of pre-diabetes on cognition. Using machine learning methods, *Rubin et al.* investigated how profiles of neurocognitive dysfunction among persons with HIV and their associated risk/protective factors differ by sex. Whereas, men with HIV showed an unimpaired profile and even a cognitively advantageous profile, women with HIV only showed impairment profiles that included global and domain-specific impairment. Meanwhile, the most discriminative risk/protective factors (e.g., reading level, age, and HIV disease characteristics) were similar across sexes. Results suggest that sex is a major contributor to the heterogeneity in cognitive impairment profiles in HIV.

In a study focusing on menopause, Maki and Thurston review the emerging literature on importance of examining menopausal symptoms, in addition to estradiol effects, as a mechanism contributing to cognitive and brain aging in women. The authors highlight the utility of objective measures of menopause symptoms, particularly vasomotor symptoms, in assessing associations with memory performance, brain structure and brain function.

Two papers describe the cognitive effects of gonadal hormone withdrawal and replacement in animal models: Zimmerman et al. examined cognitive performance and hippocampal volume in

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menopausal macaques fed a high-fat, high sugar diet and exposed to either immediate or delayed treatment with estradiol. The authors find beneficial effects of the delayed estradiol treatment on spatial memory and hippocampal volume relative to placebo. Conner et al. examined the effects of sex and gonadal hormones on episodic-like memory in a rat model of early (premotor) Parkinson's disease and report that male gonadectomy ameliorated memory deficits induced by the model in a domain-specific manner. The effect of orchiectomy was completely reversed by testosterone but estradiol had no effect, suggesting direct effects testosterone acting via androgen receptors.

Effects of sex and gonadal hormones on disease presentation are addressed in an opinion piece by Zielonka and Stawińska-Witoszyńska, where the importance of research in the underexplored topic of sex/gender differences in non-sex linked disorders is brought to light. As an example, they discuss the emerging evidence of sex/gender differences on the progression rate and clinical features pattern of Huntington's disease.

In a systematic review, Al-Hassany et al. surveyed existing findings regarding sex and gender differences in migraine and synthesized findings across domains of biological/pre-clinical, clinical, and population-level research. Important knowledge gaps are identified and priorities are set for further research in sex and gender differences in migraine and in therapeutic options.

The impact of sex and hormonal status on clinical management is addressed by Spiegel and Merius in the context of epilepsy, reviewing accumulating knowledge on the teratogenic effects of anti-seizure medications and the effects of gonadal hormones on the pharmacokinetic profile of these drugs and how it is translated into specific guidelines for the treatment of females before and after puberty and during pregnancy.

Two papers focus on sex differences in outcome following ischemic or traumatic injury to the brain: Scrutinio et al. address sex differences in long-term mortality and functional outcome after severe stroke. In this British cohort of 1,316 subjects (mean age 72, 44% women), they find lower stroke—related mortality in women relative to men but no advantage in functional recovery. A mortality advantage in women over 50 (presumably post-menopausal) relative to men in the same age group is also described in a minireview of sex differences in traumatic brain injury (Biegon). Studies reviewed also suggest that young (presumably pre-menopausal) women do not share this advantage and actually tend to have a worse outcome following mild concussive injuries to the brain. This minireview also highlights the very slow adaptation of sex as a biological variable in the design and analysis of clinical trials: Between 1996 and 2020, the percentage of women in key clinical trials in TBI was consistently below 30% and only one study, published in 2014, stratified outcome by sex.

This collection of studies and reviews exemplifies the continuity of sex/gender differences across different types of neurological disease and the importance of accounting for these differences in research. These findings also illustrate how endocrine events throughout the lifespan (e.g., menstrual cycle, menopause, pregnancy) may influence these sex differences in disease outcomes. As the field shifts toward personalized medicine, findings such as these create a platform for future studies to advance the development of risk assessments and diagnostic and therapeutic strategies that are optimized and personalized by sex and hormonal status. Moreover, discovery of differences in disease outcomes by sex and gonadal hormone status can serve as a window into causal pathways of disease and therapeutic targets, thus, enhancing our overall understanding of disease and their treatment in all.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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ORIGINAL RESEARCH
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Sex Differences in Long-Term Mortality and Functional Outcome After Rehabilitation in Patients With Severe Stroke

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Objective: We sought to determine sex differences in outcomes in patients with severe stroke who had been admitted to inpatient rehabilitation.

Methods: We studied 1,316 patients aged 18 to 99 (mean 72) classified as case-mix groups 0108, 0109, and 0110 of the Medicare case-mix classification system. These groups encompass the most severe strokes. Three outcomes were analyzed: (1) 3-year mortality from admission to rehabilitation; (2) combined outcome of transfer to acute care or death within 90 days from admission to rehabilitation; (3) functional outcome, including proportional recovery in motor functioning and good functional outcome as defined by achievement of a Functional Independence Measure (FIM)-motor score ≥65 points at discharge. Multivariable regression analyses were used to assess sex-difference in each outcome between women and men. The covariates examined included age, marital status, comorbidities, time from stroke onset to rehabilitation admission <30 days, ischemic stroke, dysphagia, neglect, motor FIM score at admission, and cognitive FIM score at admission.

Results: Kaplan-Meier estimated 3-year mortality rate was 20.7% in women and 22.0% in men. The crude hazard ratio (HR) of death for women compared with men was 0.94 (95% CI 0.74–1.20). After adjustment for significant covariates, the HR of 3-year mortality was 0.73 (95% CIs 0.56–0.96; $p = 0.025$). Comorbidity, including diabetes, anemia, coronary artery disease, atrial fibrillation, and chronic obstructive pulmonary disease, significantly increased mortality risk by 49–88%. The incidence of the combined outcome was 8.3% in women and 8.4% in men. The crude HR of the combined end-point for women compared with men was 1.05 (95% CI 0.72–1.53). After adjustment for significant covariates, the HR was 0.95 (95% CIs 0.65–1.40; $p = 0.810$). Likewise, no significant difference in proportional recovery or in the rate of achievement of a good functional outcome between women and men was observed.

Conclusion: Among patients admitted to inpatient rehabilitation after severe stroke, women and men had comparable crude mortality rates at 3 years. After multivariable

adjustment, however, women had lower mortality risk. No sex-differences in the risk of being transferred to acute care or dying within 90 days from admission to rehabilitation or in responsiveness to rehabilitation were observed.

Keywords: sex, stroke, mortality, functional outcome, rehabilitation

INTRODUCTION

Stroke is a leading cause of death and disability worldwide (1). According to the most recent report from the Global Burden of Disease Stroke Collaboration, there were 1·03 million incident strokes in Western Europe and 0·81 in North America in 2016 (1). Despite a substantial decline in stroke mortality in recent decades, stroke is the second leading cause of cardiovascular death worldwide (2). Approximately 20–25% of stroke survivors present severe disability (3). Comorbidity is prevalent in stroke patients and affects both life expectancy and disability (4).

Understanding sex differences in epidemiology, pathophysiology, outcomes, and treatment effectiveness is important since could provide evidence for reducing potential sex disparities. Previous studies of sex differences in post-stroke outcomes provided conflicting findings (5). Two recently published systematic reviews from the International Stroke Outcomes Study (INSTRUCT) research group suggest that sex differences in mortality and functional outcomes are eliminated after adjustment for age, pre-stroke functional limitation, and stroke severity (6, 7). The higher mortality risk for women was even reversed after adjustment (6). However, as noted by the Authors, the variability in measures of stroke severity used in individual studies may have introduced some bias in adjusted estimates (6). Furthermore, Gall et al., considering patient-reported outcomes, showed that women had worse functional outcomes than men, which persisted after accounting for a range of covariates (8). However, the role of rehabilitation was yet not addressed in any of the above-mentioned studies. Despite the large number of studies on sex differences in stroke, only few data are available on the relative responsiveness of women and men to rehabilitation (6). Early rehabilitation is effective in fostering functional recovery and may positively affect mortality (9, 10). An association between functional gain achieved with rehabilitation and mortality risk also has been demonstrated (11, 12). Another aspect to consider is that most individual studies have been based on patient populations with prevalent mild or moderate stroke and the relevance of research findings to the critical population with severe stroke remains elusive. Severe stroke is associated with increased burden of mortality and disability, wider interindividual variation in responsiveness to rehabilitation, and higher healthcare and social costs compared with less severe strokes (12, 13). Better understanding of sex differences in this challenging patient population could provide new insightful information and opportunities to reduce potential sex disparities. To address this issue, we studied 1,316 patients classified as case-mix groups (CMGs) 0108, 0109, and 0110 of the Medicare case-mix classification system (14), which was specifically developed to account for "the level of severity of a given case" (15). Case-mix groups 0108, 0109, and 0110 encompass the most severe strokes.

MATERIALS AND METHODS

Participants

Patients were recruited from the specialized stroke rehabilitation units of the Maugeri inpatient rehabilitation facilities (IRFs) of Cassano Murge (Bari), Telese Terme (Benevento), and Montescano (Pavia) in Italy. Enrolment periods varied among the participating centers but ran from February 2002 to September 2016 overall. A total of 3,646 patients admitted for stroke rehabilitation were identified using a computer-generated list obtained from our administrative database and by reviewing electronic medical records. We included patients admitted to stroke rehabilitation units $\leq$90 days from stroke occurrence and classified as CMG 0108 (weighted Functional Independence Measure [wFIM] motor score <26.15 and age >84.5), 0109 (wFIM motor score >22.35 and <26.15, and age <84.5), or 0110 (wFIM motor score <22.35 and age <84.5) of the Medicare case-mix classification system (14). Patients classified as CMGs 0101 to 0107, admitted to rehabilitation >90 days from stroke occurrence, or discharged against medical advice were excluded. Of the 3,646 patients, 1,316 fulfilling the selection criteria were included in the study. **Figure 1** shows the flowchart of patient selection. The Medicare classification system distinguishes 10 CMGs for stroke rehabilitation. Patients are assigned into one of the 10 distinct CMGs, based on age, the sum of weighted ratings for 12 FIM-motor items (transfer to tub or shower item is excluded), and the sum of FIM cognitive ratings (14). The FIM is currently the most widely used measure to describe the degree of impairment in activities of daily living in clinical practice. The motor-FIM score consists of 13 items assessing four domains of function (self-care, sphincter control, transfers, and locomotion). The cognitive-FIM score consists of five items assessing two domains (communication and social cognition). Each item is scored on a 7-point Likert scale, from 1 (total dependence) to 7 (total independence).

The characteristics of the three participating stroke rehabilitation units have been described previously (11, 12). The participating rehabilitation units are certified ISO9001 Quality Management Systems for activities of rehabilitation and share common rehabilitation programs. The interdisciplinary stroke rehabilitation teams comprise the following professionals with expertise in stroke rehabilitation: neurologist, physiatrist, physiotherapist, occupational therapist, speech and language therapist, neuropsychologist, and nurse. Trained therapists recorded admission and discharge FIM scores, as part of our formal rehabilitation program. Individual rehabilitation programs were structured to provide as much scheduled

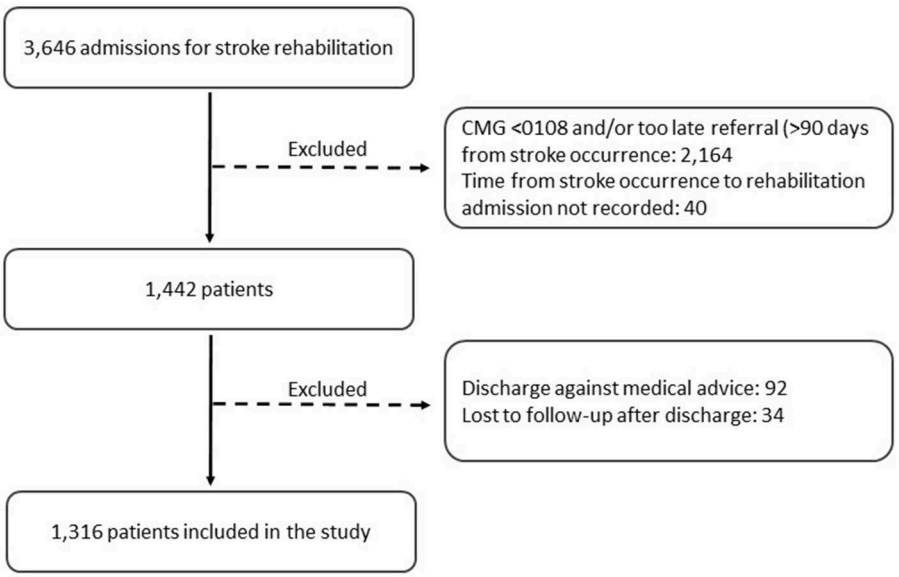


FIGURE 1 | Flowchart of patient selection.

rehabilitation therapy as possible, with the objective of providing therapy for 3 h per day for 5 days and for 1 h for 1 day of each week. Conformity to this standard is subject to periodic external audit by independent auditors of the Regional Health Agencies. The study was approved by our Institutional Review Board. Patients' data were deidentified.

Data Collection

All data were extracted from the electronic Hospital Information System networked between the participating centers. Vital status was ascertained by linking with the regional Health Information System.

Definitions

Coronary artery disease (CAD) was diagnosed based on a documented history of myocardial infarction, percutaneous coronary angioplasty, or coronary artery bypass grafting, or a previous hospitalization for CAD. Renal dysfunction was defined as estimated glomerular filtration rate <60 mL/min/1.73 m^2. Anemia was defined as hemoglobin <12 g/dL in women and <13 g/dL in men. Atrial fibrillation (AF) was diagnosed based on admission electrocardiogram. Chronic obstructive pulmonary disease (COPD) was diagnosed based on patient's medical records documenting a past diagnosis of COPD, chronic medication use for COPD, and/or previous hospitalizations for exacerbation of COPD. The Bedside Swallowing Assessment Scale, administered by a trained speech therapist, was used to diagnose dysphagia. If concerns regarding the safety and efficiency of swallow function emerged from the scale, a fiberoptic endoscopic evaluation of swallowing was performed. The Semi-Structured Scale for the Functional Evaluation of Hemi-inattention was used to diagnose personal neglect.

Outcomes

The following clinical and functional outcomes were analyzed: (1) all-cause mortality up to 3 years from admission to rehabilitation; (2) combined outcome of transfer to acute care or death within 90 days from admission to rehabilitation, whatever came first; (3) functional outcome. Two measures of functional outcome were used: (1) proportional recovery in motor functioning, as expressed by motor-FIM effectiveness, and (2) good functional outcome as defined by achievement of a FIM-motor score ≥65 points at discharge. Proportional recovery in motor functioning is calculated by the formula: (discharge motor-FIM score–admission motor-FIM score)/(maximum motor-FIM score–admission motor-FIM score) × 100 (16). Proportional recovery expresses the achieved proportion of available improvement in motor functioning (16). According to Stinear, "measuring proportional recovery enables the detection of treatment effects despite interindividual variability in absolute recoveries and outcomes" (17). To facilitate the interpretation of functional improvement, we also calculated the proportion of women and men who achieved a good functional outcome as defined by FIM-motor score ≥65 points at discharge. Based on Rasch analysis, patients with a score ≥65 "usually require either supervision or minimal assistance with mobility and self-care, indicating that the patient' physical care requirements for daily activities are minimal" (18).

Statistical Analysis

Data are reported as mean and standard deviation (SD) or median and interquartile range (IQR) for continuous variables or percentage for categorical variables. No variable was missing more than 0.1% of values. Rates of mortality were estimated by means of the Kaplan-Meier method and were compared between women and men using the log-rank test.

Covariates

The covariates examined included age (per 5-year increase above 65), marital status (married/not married), hypertension, diabetes, COPD, history of CAD, AF, anemia, renal dysfunction (estimated glomerular filtration rate <60 mL/min/1.73 m2), time from stroke onset to rehabilitation admission <30 days, ischemic stroke, dysphagia, neglect, motor FIM score at admission, and cognitive FIM score at admission. These variables were selected based on availability at time of presentation and prior studies showing an association with the outcomes of interest (4, 6, 11, 12, 17, 19–32) and were included in all analyses.

Three-Year All-Cause Mortality

Crude hazard ratio (HR) of death for women compared with men was estimated by univariable Cox regression analysis. Adjusted HR was estimated by multivariable backward stepwise Cox regression analysis ($p > 0.05$ to remove). Schoenfeld residuals after fitting Cox model were evaluated to test proportional-hazards assumption. Interactions between sex and covariates were estimated by using the likelihood ratio test.

In addition, since unmeasured potential confounding factors may affect hazard estimates, a sensitivity analysis was performed to explore the potential confounding effect of an unknown or unmeasured variable on the association of sex with 3-year survival (33). Hazard ratios for women vs. men adjusted for a hypothetical unmeasured binary variable with different distribution in the two sexes were estimated. The effect was quantified assuming a HR of 1.3, 1.4, and 1.5.

To explore the association between comorbidities and mortality, recursive-partitioning analysis (for censored survival data) was applied to cluster patients into risk subgroups according to comorbidities and to identify the combinations of comorbidities that were most influential for 3-year mortality, adjusting for age, sex, and type of stroke (ischemic or hemorrhagic) (34).

Combined Outcome

Crude and adjusted HRs of the combined outcome of transfer to acute care or death within 90 days from admission to rehabilitation for women compared with men were estimated as described above.

Functional Outcome

The association of baseline covariates with proportional recovery in motor functioning was assessed using beta regression. A multivariable analysis was performed to model the proportion of recovery on the basis of significant covariates and to estimate the effect of sex. Beta coefficients with standard error (SE) were reported. Crude odds ratio (OR) of good functional outcome for women compared with men was estimated by univariable logistic regression model. Adjusted OR was estimated by multivariable logistic regression analysis. These analyses were limited to the 1,209 patients who completed rehabilitation.

Sex, as main exposure variable, was included into all multivariable models regardless of significance level.

Finally, for each outcome, a full adjusted analysis was performed including all covariates.

TABLE 1 | Baseline characteristics stratified by sex.

	All (*N* = 1,316)	Women (*N* = 587)	Men (*N* = 729)
Demographics			
Age (years), mean (SD)	72 (12)	73 (11)	71 (11)
<65 years, *n* (%)	320 (24.3)	111 (18.9)	209 (28.7)
65 to 74 years, *n* (%)	376 (28.6)	164 (27.9)	212 (29.1)
≥75 years, *n* (%)	620 (47.1)	312 (53.2)	308 (42.2)
Marital status–married, *n* (%)	941 (71.5)	342 (58.3)	599 (82.2)
Comorbidities			
Hypertension, *n* (%)	954 (72.5)	441 (75.3)	513 (70.4)
Diabetes, *n* (%)	393 (29.9)	170 (29.0)	223 (30.6)
COPD, *n* (%)	189 (14.4)	80 (13.7)	109 (15.0)
CAD, *n* (%)	168 (12.8)	57 (9.7)	111 (15.2)
Atrial fibrillation, *n* (%)	344 (26.2)	203 (34.6)	141 (19.3)
Anemia (hemoglobin <13 g/dL in men, <12 g/dL in women), *n* (%)	465 (35.3)	194 (33.1)	271 (37.2)
Renal dysfunction (eGFR <60 mL/min/1.73 m^2), *n* (%)	233 (17.7)	118 (20.1)	115 (15.8)
Stroke-related characteristics			
CMG 108, *n* (%)	153 (11.6)	75 (12.8)	78 (10.7)
CMG 109, *n* (%)	123 (9.3)	51 (8.7)	72 (9.9)
CMG 110, *n* (%)	1,040 (79.0)	461 (78.5)	579 (79.4)
Time from stroke onset to rehabilitation admission (days), median (IQR)	23.7 (16.6)	23.2 (16.3)	24.1 (16.9)
Time from stroke onset to rehabilitation admission ≤30 days, *n* (%)	993 (75.5)	449 (76.5)	544 (74.6)
Ischemic stroke, *n* (%)	1,051 (79.9)	488 (83.1)	563 (77.2)
Hemorrhagic stroke, *n* (%)	265 (20.1)	99 (16.9)	166 (22.8)
Dysphagia, *n* (%)	277 (21.0)	120 (20.4)	157 (21.5)
Neglect, *n* (%)	187 (14.2)	87 (14.8)	100 (13.7)
Aphasia, *n* (%)	581 (44.1)	255 (43.4)	326 (44.7)
Site of impairment			
Right body, *n* (%)	663 (50.4)	291 (49.6)	372 (51.0)
Left body, *n* (%)	653 (49.5)	296 (50.4)	357 (49.0)
12-item motor-FIM score at admission, mean (SD)	17.4 (5.6)	17.3 (5.6)	17.4 (5.5)
Cognitive-FIM score at admission, mean (SD)	16.7 (9.3)	16.5 (9.3)	16.8 (9.2)
Length of stay (days), mean (SD)	54 (17)	54 (16)	54 (18)
Laboratory findings			
Blood urea nitrogen (mg/dl), mean (SD)	21 (11)	20 (11)	22 (10)
Serum creatinine (mg/dl), mean (SD)	0.89 (0.35)	0.79 (0.32)	0.96 (0.36)
eGFR (mL/min/1.73 m^2), mean (SD)	83 (26)	80 (25)	86 (26)
Serum sodium (mmol/l), mean (SD)	140.6 (4.1)	141.0 (4.0)	140.3 (4.1)
Serum sodium <135 mmol/l, *n* (%)	56 (4.2)	19 (3.2)	37 (5.1)
Hemoglobin (g/dl), mean (SD)	13.1 (1.8)	12.6 (1.6)	13.5 (1.8)
Total cholesterol (mg/dl), mean (SD)	163 (43)	176 (45)	153 (39)

N, denotes number; SD, standard deviation; COPD, chronic obstructive pulmonary disease; CAD, coronary artery disease; CMG, case-mix group; eGFR, estimated glomerular filtration rate.

All analyses were conducted using STATA software, version 14 (Stata-Corp LP, College Station, Tex).

RESULTS

Of the 1,316 patients included in the study, 587 (44.6%) were women and 729 (55.4%) men. **Table 1** shows baseline characteristics stratified by sex.

Three-Year Mortality

A total of 3,141 person-years of follow-up were examined during which 269 deaths (8.6 deaths/100 person-years) occurred. Median follow-up was 1,095 (IQR 668-1095) days. 79.3% of the survivors had a complete 3-year follow-up. Kaplan-Meier estimated 3-year mortality rate was 20.7% in women and 22.0% in men (**Figure 2**). Besides sex, age, marital status, diabetes, CAD, COPD, AF, anemia, dysphagia, neglect, and cognitive status were significantly associated with mortality risk at multivariable Cox analysis (**Supplementary Table 1**). There was no significant interaction between sex and any covariate with regard to 3-year mortality. **Table 2** shows crude and multivariable-adjusted HR of mortality for women compared with men. Female sex was associated with significantly decreased hazard for mortality compared with male sex. Estimates for sex remained virtually unchanged in fully adjusted models, including all covariates (**Table 2**).

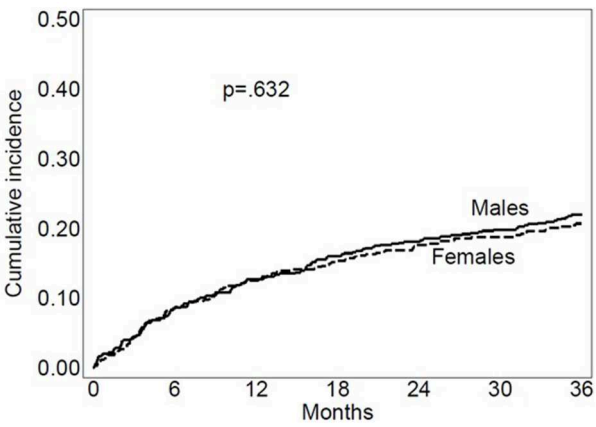


FIGURE 2 | Kaplan Meier mortality curves for women and men.

Sensitivity analysis showed that hazard estimate of 3-year mortality may be sensitive to unknown or unmeasured confounders. **Supplementary Table 2** shows HRs of 3-year mortality for women vs. men adjusted for a hypothetical unknown or unmeasured binary variable. As an example, an unmeasured binary confounder with a HR of 1.4 and a prevalence of 40% in men and 20% in women would raise the upper confidence limit of HR beyond 1.00.

Figure 3A depicts the results of recursive-partitioning analysis. The three highest risk subgroups included patients with concurrent anemia and AF (3-year mortality rate: 45.3%), anemia and CAD (3-year mortality rate: 41.8%), or atrial fibrillation and diabetes (3-year mortality rate: 39.5%). Overall, 239 (18.2%) patients were at high risk of death because of the combination of these comorbidities. These patients were grouped into a single high-risk category. The 357 (27.1%) patients without any comorbidity among diabetes, anemia, CAD, AF, and COPD were grouped into the low-risk category. The remaining 720 patients (54.7%) were grouped into an intermediate-risk category. There was no difference in the distribution of females and males across the three risk categories ($p = 0.145$) (**Figure 3B**). In comparison with the low-risk group, the adjusted HR of 3-year mortality for the high-risk category was 3.93 (95% CIs 2.64–5.84) and that for the intermediate-risk category 1.83 (95% CIs 1.26–2.66), regardless of age, sex, and type of stroke (**Supplementary Table 3**). **Figure 3C** shows Kaplan-Meier mortality curves for high-, intermediate- and low-risk categories.

Combined Outcome

The incidence of the combined outcome was 8.3% in women and 8.4% in men. At multivariable analysis, atrial fibrillation, dysphagia, anemia, and low cognitive FIM score were significantly associated with increased risk of the combined outcome (**Supplementary Table 4**). **Table 2** shows crude and multivariable-adjusted HR for the combined outcome for women compared with men. Since sex was not retained in multivariable analysis, it was forced into the multivariable model. After adjustment for significant covariates, female sex was not associated with risk of the combined outcome compared with male sex. Estimates for sex remained virtually unchanged in fully adjusted models, including all covariates (**Table 2**).

Functional Outcome

Mean proportional recovery in motor functioning achieved in women was statistically significantly lower than that

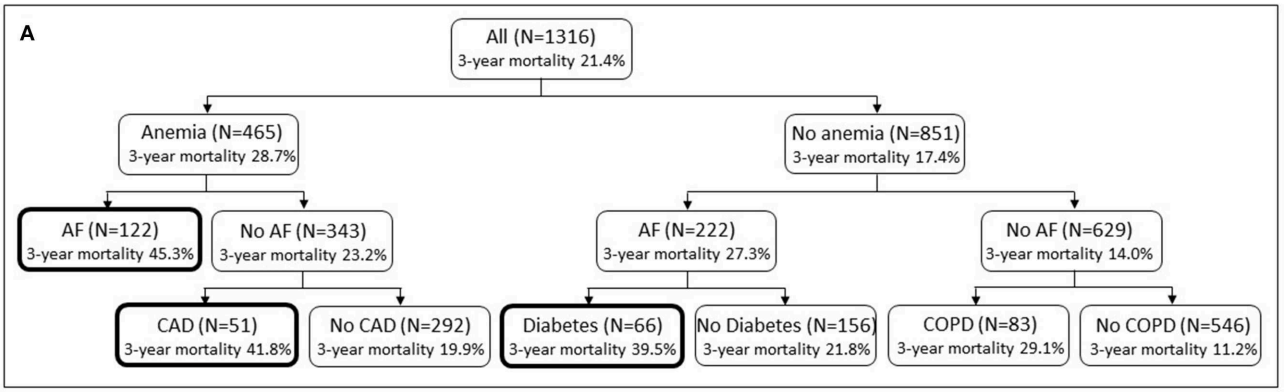
TABLE 2 | Crude and multivariable-adjusted estimates of the association of female sex with mortality and the combined outcome.

	N	Crude HR (95% CI)	*p*-value	Adjusted HR (95% CI)	*p*-value	Fully adjusted HR (95% CI)	*p*-value
Three-year mortality	1,316	0.94 (0.74–1.20)	0.632	0.73 (0.56–0.96)*	0.025	0.73 (0.56–0.95)	0.022
Combined outcome	1,316	1.05 (0.72–1.53)	0.789	0.95 (0.65–1.40)**	0.810	0.94 (0.62–0.96)	0.770

N, denotes the number of patients; HR, hazard ratio; CI, confidence interval.

**Adjusted for age, marital status, diabetes, coronary artery disease, chronic obstructive pulmonary disease, atrial fibrillation, anemia, dysphagia, neglect, and cognitive FIM score.*

***Adjusted for atrial fibrillation, dysphagia, anemia, and low cognitive FIM score.*



B

	Females (N=587)	Males (N=729)
Low risk, N (%)	146 (24.9)	211 (28.9)
Intermediate risk, N (%)	324 (55.2)	396 (54.3)
High risk, N (%)	117 (19.9)	122 (16.7)

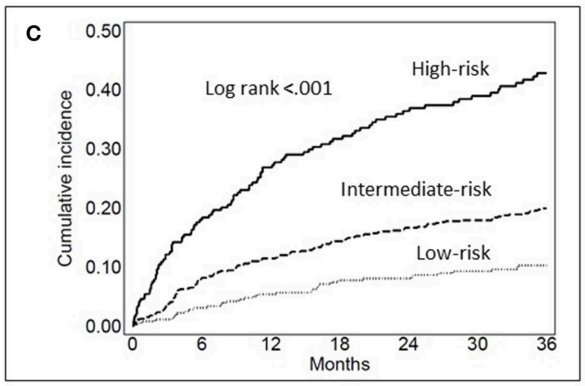


FIGURE 3 | Classification of patients into risk-of-death categories based on comorbidity. **(A)** Shows the results of recursive partitioning analysis. Bold lines denote the high-risk category. **(B)** Shows the distribution of females and males across the three risk categories. **(C)** Shows Kaplan Meier mortality curves for the high-, intermediate-, and low-risk categories.

achieved in men (31.4 ± 25.7 percent vs. 35.0 ± 24.8 percent; $p = 0.014$). At multivariable analysis, age, marital status, AF, time to rehabilitation admission from stroke onset <30 days, ischemic stroke, dysphagia, neglect, and admission motor- and cognitive-FIM scores were associated with proportional recovery (**Supplementary Table 5**). Since sex was not retained in multivariable analysis, it was forced into the multivariable model. After adjustment for significant covariates, no difference in proportional recovery between women and men was found (**Table 3**). The proportion of women and men who achieved a good functional outcome, defined by a FIM-motor score ≥65 points at discharge, was 15.4 and 16.4%, respectively. At multivariable logistic regression analysis, age, time to rehabilitation admission <30 days, ischemic stroke, dysphagia, neglect, motor and cognitive FIM scores were significantly associated with good functional outcome (**Supplementary Table 6**). **Table 3** shows crude and multivariable-adjusted HR for the combined outcome for women compared with men. Since sex was not retained in multivariable analysis, it was forced into the multivariable model. After adjustment for significant covariates, female sex was not associated with odds of good functional outcome. Estimates for sex remained virtually unchanged in fully adjusted models, including all covariates.

DISCUSSION

We investigated sex differences in outcomes in a large patient cohort with severe stroke who had been admitted to inpatient rehabilitation. There are three major findings of this study: (1) women in comparison with men were associated with a 27% lower adjusted 3-year risk of death. (2) Comorbidity had a statistically and clinically significant impact on mortality, regardless of age, sex and type of stroke. (3) No sex difference in the incidence of the combined outcome or in responsiveness to rehabilitation was observed.

Women and men had comparable crude mortality rates at 3 years. However, women had a 27% lower adjusted 3-year risk of death compared with men, possibly reflecting the female survival advantage until late in life in the general population (35). Female sex remained significantly associated with lower risk of death even after full adjustment. As discussed by Austed (35), mechanistic hypotheses to explain the female survival advantage focus on hormones, oxidative damage to DNA, and asymmetric inheritance of sex chromosomes. The finding of a lower adjusted mortality risk for women vs. men is consistent with the meta-analysis of Phan et al. (6), where a statistically significant 24% lower adjusted mortality rate ratio at 5 years for women compared with men was estimated. Our finding is also in

TABLE 3 | Crude and multivariable-adjusted estimates of the association of female sex with functional outcome.

	N	Crude β regression coefficient (SE)	*p*-value	Adjusted β regression coefficient (SE)	*p*-value	Fully adjusted β regression coefficient (SE)	*p*-value
Proportional recovery	1,209	−0.189 (0.059)	0.002	−0.035 (0.056) *	0.534	−0.045 (0.056)	0.422
		Crude OR (95% CI)		**Adjusted OR (95% CI)**		**Fully adjusted OR (95% CI)**	
Good functional outcome	1,209	0.92 (0.68–1.26)	0.613	1.11 (0.78–1.57) **	0.569	1.20 (0.84–1.72)	0.317

N, denotes the number of patients; SE, standard error; OR, odds ratio.

**Adjusted for age, marital status, atrial fibrillation, time to rehabilitation admission from stroke onset <30 days, ischemic stroke, dysphagia, neglect, and admission motor- and cognitive-FIM scores significant covariates.*

***Adjusted for age, time to rehabilitation admission <30 days, ischemic stroke, dysphagia, neglect, motor and cognitive FIM scores.*

line with the study of Bots et al. showing that mortality rate after stroke is higher among men than women across age groups until old age (19).

It has been suggested that stroke severity dominates risk for poor outcome in patients with severe stroke (36). Our data indicate that, in this critical subset of patients, comorbidities are significantly associated poor long-term survival. Consistent with previous studies (20–22), diabetes, CAD, COPD, AF, and anemia were independently associated with increased mortality, regardless of age, sex, and type of stroke. In comparison with the patient subgroup without any of these comorbidities, the high-risk subgroup had a nearly four-fold time increased risk of death within 3 years. Atrial fibrillation and anemia also doubled the risk of transfer to acute care and death within 90 days from rehabilitation admission. These findings are of particular interest because comorbidities are amenable to interventions. Recently, the American Stroke Association recommended that the focus of post-acute care should be on maximizing recovery, reducing mortality, and preventing recurrent strokes and cardiovascular events (37). Reasonably, tailoring management and secondary prevention according to comorbidities would result in better outcomes. Because of insufficient evidence, however, guidelines fail to provide guidance for care of stroke patients with comorbidity (38). Further research addressing care of patients with comorbidity is needed.

No differences in the incidence of the combined outcome of transfer from the rehabilitation setting to acute care or 90-day case fatality between women and men was observed. Likewise, the extent of functional recovery did not differ between women and men, even after multivariable adjustment. It should however be noted that a large proportion of interindividual variability in functional outcome remains unexplained. In a retrospective analysis of the Uniform Data System for Medical Rehabilitation data set, the proportion of functional recovery explained by a predictive model incorporating age, admission FIM motor score, and walking distance was as low as 10.7% (23). Identifying stroke recovery biomarkers could allow enhancing the ability to explain interindividual differences in post-stroke outcomes. Two recent meta-analyses showed that genetic variants and the severity of white matter hyperintensities, as assessed by magnetic resonance imaging or computed tomography at the time of stroke, are associated with functional outcome after ischemic stroke (39, 40). In another study, a panel of five biomarkers covering distinct pathophysiological pathways provided incremental prognostic information beyond that provided by a clinical model in predicting major disability and mortality after stroke (41).

Taken together, our data are in line with the sex mortality-morbidity paradox that women have lower mortality rates from most causes of death, but more years lived with disability (35).

The terms sex and gender have often been used interchangeably in studies that investigated differences in disease outcomes between men and women. However, sex and gender are conceptually distinct. While sex refers to biological and physiological characteristics, gender refers to "psychological, social, and cultural factors that shape attitudes, behaviors, and knowledge" (42). Sex and gender are both important determinants of health and response to interventions (42, 43). Thus, integrating sex- and gender-based analysis can lead to improved research methodology and improved assessment of differences in disease outcomes (42, 43). As an example, using a binary gender index (masculinity vs. femininity), Pelletier et al. found that feminine traits of personality were associated with adverse outcomes in young patients with acute coronary syndromes, regardless of sex (44). Potential pathways by which gender might affect post-stroke rehabilitation outcomes include social isolation, socioeconomic status, education, marital status, poorer pre-stroke function, level of anxiety, depression, and interaction with rehabilitation team and the doctor. Because of the complex and multidimensional nature of gender and the lack of standardized methods of analysis, however, operationalizing the intersection of gender and sex into scientific research remains a very challenging task (42, 43).

Limitations

Our study has strengths and limitations. To our knowledge, this is the first study specifically addressing sex differences in the critical population of patients with severe stroke. This study adds to previous knowledge by highlighting the impact of comorbidity on long-term mortality and by showing the absence of sex differences in responsiveness to rehabilitation in patients with severe stroke admitted to post-acute rehabilitation. Several limitations should be mentioned. We used hospital-based data

that may be prone to selection bias. However, although patients with severe stroke are less likely to be referred to inpatient rehabilitation facilities than those with mild/moderate stroke, access to rehabilitation is similar for women and men (6, 45). Since women with stroke are in general older than men and oldest old patients are less likely to undergo inpatient rehabilitation, an age-related selection bias may have occurred in our study. We used a stepwise approach based on statistical significance to select significant covariates. As reviewed by Talbot and Massamba (46), stepwise methods may overestimate exposure effects and underestimate statistical uncertainty. However, as recommended by Talbot and Massamba (46), we also reported the results from the fully adjusted models. Estimates for sex remained virtually unchanged in fully adjusted models. The retrospective design of the study did not allow accounting for other possible confounders not recorded in our data set. Sensitivity analysis showed that hazard estimates might be sensitive to unknown or unmeasured confounders, such as premorbid functional status. Poor pre-stroke functional status is more prevalent among women than in men and has generally been recognized as a predictor of worse outcomes in stroke survivors. In the meta-analysis of Phan et al., partial adjustment for pre-stroke disability alone attenuated the adverse effect of female sex on 5-year mortality by 55% (6). Thus, it is likely that adjustment for pre-stroke disability in our study would have resulted in even lower adjusted mortality risk for women compared with men. Moreover, some of the included covariates in the multivariable models could be intermediates on the causal pathway between exposure and outcomes, rather than confounders. As noted by Schisterman et al. (47), with adjustment for an intermediate variable in multivariable modeling, the observed association between the exposure and outcome will be a null-biased estimate of the total causal effect. This limitation should be taken into account in the interpretation of our findings. We did not examine the prognostic role of neuroimaging, which could provide incremental prognostic information over clinical and functional variables (40). Finally, we could not assess the causes of death. However, death certificates may lack accuracy (48).

CONCLUSION

In conclusion, women and men had comparable crude mortality rates at 3 years. After multivariable adjustment, however, women had lower mortality risk, probably reflecting the higher longevity of women. Comorbidity significantly affected the likelihood of survival, regardless of age, sex and type of stroke. No sex-differences in the risk of being transferred to acute care or dying within 90 days from admission to rehabilitation or in responsiveness to rehabilitation were observed.

DATA AVAILABILITY STATEMENT

The datasets generated for this study can be found upon request to the corresponding author of the article.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

DS designed the study, interpreted the data, drafted the initial manuscript and revised the manuscript. PB drafted the initial manuscript and revised the manuscript. PG conducted data analysis, prepared the figure, and revised the manuscript. BL interpreted the data and revised the manuscript. RT designed the study, interpreted the data, and revised the manuscript. All authors have contributed to manuscript revision, read, and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2020.00084/full#supplementary-material

REFERENCES

1. GBD 2016 Stroke Collaborators. Global, regional, and national burden of stroke, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet Neurol*. (2019) 18:439–58. doi: 10.1016/S1474-4422(19)30034-1
2. Katan M, Luft A. Global burden of stroke. *Semin Neurol*. (2018) 38:208–11. doi: 10.1055/s-0038-1649503
3. Xian Y, Thomas L, Liang L, Federspiel JJ, Webb LE, Bushnell CD, et al. Unexplained variation for hospitals' use of inpatient rehabilitation and skilled nursing facilities after an acute ischemic stroke. *Stroke*. (2017) 48:2836–42. doi: 10.1161/STROKEAHA.117.016904
4. Corraini P, Szépligeti SK, Henderson VW, Ording AG, Horváth-Puhó E, Sørensen HT. Comorbidity and the increased mortality after hospitalization for stroke: a population-based cohort study. *J Thromb Haemost*. (2018) 16:242–52. doi: 10.1111/jth.13908
5. Reeves MJ, Bushnell CD, Howard G, Gargano JW, Duncan PW, Lynch G, et al. Sex differences in stroke: epidemiology, clinical presentation, medical care, and outcomes. *Lancet Neurol*. (2008) 7:915–26. doi: 10.1016/S1474-4422(08)70193-5
6. Phan HT, Blizzard CL, Reeves MJ, Thrift AG, Cadilhac D, Sturm J, et al. Sex differences in long-term mortality after stroke in the INSTRUCT (INternational STRoke oUtComes sTudy): a meta-analysis of individual participant data. *Circ Cardiovasc Qual Outcomes*. (2017) 10:e003436. doi: 10.1161/CIRCOUTCOMES.116.003436

7. Phan HT, Blizzard CL, Reeves MJ, Thrift AG, Cadilhac DA, Sturm J, et al. Factors contributing to sex differences in functional outcomes and participation after stroke. *Neurology*. (2018) 90:e1945–53. doi: 10.1212/WNL.0000000000005602
8. Gall S, Phan H, Madsen TE, Reeves M, Rist P, Jimenez M, et al. Focused update of sex differences in patient reported outcome measures after stroke. *Stroke*. (2018) 49:531–5. doi: 10.1161/STROKEAHA.117.018417
9. Hsieh CY, Huang HC, Wu DP, Li CY, Chiu MJ, Sung SF. Effect of rehabilitation intensity on mortality risk after stroke. *Arch Phys Med Rehabil*. (2018) 99:1042–8. doi: 10.1016/j.apmr.2017.10.011
10. Chen CM, Yang YH, Chang CH, Chen PC. Effects of transferring to the rehabilitation ward on long-term mortality rate of first-time stroke survivors: a population-based study. *Arch Phys Med Rehabil*. (2017) 98:2399–407. doi: 10.1016/j.apmr.2017.03.020
11. Scrutinio D, Monitillo V, Guida P, Nardulli R, Multari V, Monitillo F, et al. Functional gain after inpatient stroke rehabilitation: correlates and impact on long-term Survival. *Stroke*. (2015) 46:2976–80. doi: 10.1161/STROKEAHA.115.010440
12. Scrutinio D, Guida P, Lanzillo B, Ferretti C, Loverre A, Montrone N, et al. Rehabilitation outcomes of patients with severe disability poststroke. *Arch Phys Med Rehabil*. (2019) 100:520–9. doi: 10.1016/j.apmr.2018.06.023
13. Xu XM, Vestesson E, Paley L, Desikan A, Wonderling D, Hoffman A, et al. The economic burden of stroke care in England, Wales and Northern Ireland: using a national stroke register to estimate and report patient-level health economic outcomes in stroke. *Eur Stroke J*. (2018) 3:82–91. doi: 10.1177/2396987317746516
14. Centers for Medicare & Medicaid Services (CMS), HHS. Medicare program; inpatient rehabilitation facility prospective payment system for FY 2006. Final rule. *Fed Regist*. (2005) 70:47879–8006. Available online at: https://www.govinfo.gov/content/pkg/FR-2005-08-15/pdf/05-15419.pdf
15. Centers for Medicare & Medicaid Services. 42 CFR Parts 412 and 413[CMS−1069–F]. Medicare program; prospective payment system for inpatient rehabilitation facilities. *Federal Register*. (2001) 66:41316–430. Available online at: https://www.govinfo.gov/content/pkg/FR-2001-08-07/pdf/01-19313.pdf
16. Koh GC, Chen CH, Petrella R, Thind A. Rehabilitation impact indices and their independent predictors: a systematic review. *BMJ Open*. (2013) 3:e003483. doi: 10.1136/bmjopen-2013-003483
17. Stinear CM. Prediction of motor recovery after stroke: advances in biomarkers. *Lancet Neurol*. (2017) 16:826–36. doi: 10.1016/S1474-4422(17)30283-1
18. Brock KA, Vale SJ, Cotton SM. The effect of the introduction of a case-mix-based funding model of rehabilitation for severe stroke: an Australian experience. *Arch Phys Med Rehabil*. (2007) 88:827–32. doi: 10.1016/j.apmr.2007.04.001
19. Bots SH, Peters SAE, Woodward M. Sex differences in coronary heart disease and stroke mortality: a global assessment of the effect of ageing between 1980 and 2010. *BMJ Glob Health*. (2017) 2:e000298. doi: 10.1136/bmjgh-2017–000298
20. Echouffo-Tcheugui JB, Xu H, Matsouaka RA, Xian Y, Schwamm LH, Smith EE, et al. Diabetes and long-term outcomes of ischaemic stroke: findings from get with the guidelines-stroke. *Eur Heart J*. (2018) 39:2376–86. doi: 10.1093/eurheartj/ehy036
21. Rønning OM, Stavem K. Predictors of mortality following acute stroke: a cohort study with 12 years of follow-up. *J Stroke Cerebrovasc Dis*. (2012) 21:369–72. doi: 10.1212/wnl.44.4.626
22. Li Z, Zhou T, Li Y, Chen P, Chen L. Anemia increases the mortality risk in patients with stroke: a meta-analysis of cohort studies. *Sci Rep*. (2016) 6:26636. doi: 10.1038/srep26636
23. Brown AW, Therneau TM, Schultz BA, Niewczyk PM, Granger CV. Measure of functional independence dominates discharge outcome prediction after inpatient rehabilitation for stroke. *Stroke*. (2015) 46:1038–44. doi: 10.1161/STROKEAHA.114.007392
24. Saposnik G, Kapral MK, Liu Y, Hall R, O'Donnell M, Raptis S, et al. IScore: a risk score to predict death early after hospitalization for an acute ischemic stroke. *Circulation*. (2011) 123:739–49. doi: 10.1161/CIRCULATIONAHA.110.983353
25. Wang IK, Liu CH, Yen TH, Jeng JS, Sung SF, Huang PH, et al. Renal function is associated with 1-month and 1-year mortality in patients with ischemic stroke. *Atherosclerosis*. (2018) 269:288–93. doi: 10.1016/j.atherosclerosis.2017.11.029
26. Goulart AC, Fernandes TG, Santos IS, Alencar AP, Bensenor IM, Lotufo PA. Predictors of long-term survival among first-ever ischemic and hemorrhagic stroke in a Brazilian stroke cohort. *BMC Neurol*. (2013) 13:51. doi: 10.1186/1471–2377-13–51
27. Rutten-Jacobs LC, Arntz RM, Maaijwee NA, Schoonderwaldt HC, Dorresteijn LD, van Dijk EJ, et al. Long-term mortality after stroke among adults aged 18 to 50 years. *JAMA*. (2013) 309:1136–44. doi: 10.1001/jama.2013.842
28. AboAlSamh DK, Abulaban AA, Khatri IA, Al-Khathaami AM. Renal dysfunction as a predictor of acute stroke outcomes. *Neurosciences*. (2017) 22:320–4. doi: 10.17712/nsj.2017.4.20170185
29. Dehlendorff C, Andersen KK, Olsen TS. Sex disparities in stroke: women have more severe strokes but better survival than men. *J Am Heart Assoc*. (2015) 4:e001967. doi: 10.1161/JAHA.115.001967
30. Brønnum-Hansen H, Davidsen M, Thorvaldsen P, Danish MONICA Study Group. Long-term survival and causes of death after stroke. *Stroke*. (2001) 32:2131–6. doi: 10.1161/hs0901.094253
31. Slot KB, Berge E, Dorman P, Lewis S, Dennis M, Sandercock P, et al. Impact of functional status at six months on long term survival in patients with ischaemic stroke: prospective cohort studies. *BMJ*. (2008) 336:376–9. doi: 10.1136/bmj.39456.688333.BE
32. Meyer MJ, Pereira S, McClure A, Teasell R, Thind A, Koval J, et al. A systematic review of studies reporting multivariable models to predict functional outcomes after post-stroke inpatient rehabilitation. *Disabil Rehabil*. (2015) 37:1316–23. doi: 10.3109/09638288.2014.963706
33. Lin DY, Psaty BM, Kronmal RA. Assessing the sensitivity of regression results to unmeasured confounders in observational studies. *Biometrics*. (1998) 54:948–63.
34. Ang KK, Harris J, Wheeler R, Weber R, Rosenthal DI, Nguyen-Tân PF, et al. Human papillomavirus and survival of patients with oropharyngeal cancer. *N Engl J Med*. (2010) 363:24–35. doi: 10.1056/NEJMoa0912217
35. Austad SN, Fischer KE. Sex differences in lifespan. *Cell Metab*. (2016) 23:1022–33. doi: 10.1016/j.cmet.2016.05.019
36. Lisabeth LD, Reeves MJ, Baek J, Skolarus LE, Brown DL, Zahuranec DB, et al. Factors influencing sex differences in poststroke functional outcome. *Stroke*. (2015) 46:860–3. doi: 10.1161/STROKEAHA.114.007985
37. Adeoye O, Nyström KV, Yavagal DR, Luciano J, Nogueira RG, Zorowitz RD, et al. Recommendations for the establishment of stroke systems of care: a 2019 update: a policy statement from the American Stroke Association. *Stroke*. (2019) 50:e187–210. doi: 10.1161/STR.0000000000000173
38. Nelson ML, Grudniewicz A, Albadry S. Applying clinical practice guidelines to the complex patient: insights for practice and policy from stroke rehabilitation. *Healthc Q*. (2016) 19:38–43. doi: 10.12927/hcq.2016.24697
39. Söderholm M, Pedersen A, Lorentzen E, Stanne TM, Bevan S, Olsson M, et al. Genome-wide association meta-analysis of functional outcome after ischemic stroke. *Neurology*. (2019) 92:e1271–83. doi: 10.1212/WNL.0000000000007138
40. Georgakis MK, Duering M, Wardlaw JM, Dichgans M. WMH and long-term outcomes in ischemic stroke: a systematic review and meta-analysis. *Neurology*. (2019) 92:e1298–308. doi: 10.1212/WNL.0000000000007142
41. Zhong C, Zhu Z, Wang A, Xu T, Bu X, Peng H, et al. Multiple biomarkers covering distinct pathways for predicting outcomes after ischemic stroke. *Neurology*. (2019) 92:e295–304. doi: 10.1212/WNL.0000000000006717
42. Tannenbaum C, Ellis RP, Eyssel F, Zou J, Schiebinger L. Sex and gender analysis improves science and engineering. *Nature*. (2019) 575:137–46. doi: 10.1038/s41586-019-1657-6
43. Rich-Edwards JW, Kaiser UB, Chen GL, Manson JE, Goldstein JM. Sex and gender differences research design for basic, clinical, and population studies: essentials for investigators. *Endocr Rev*. (2018) 39:424–39. doi: 10.1210/er.2017-00246
44. Pelletier R, Khan NA, Cox J, Daskalopoulou SS, Eisenberg MJ, Bacon SL, et al. Sex versus gender-related characteristics: which predicts outcome after acute coronary syndrome in the young. *J Am Coll Cardiol*. (2016) 67:127–35. doi: 10.1016/j.jacc.2015.10.067

45. Phan HT, Gall SL, Blizzard CL, Lannin NA, Thrift AG, Anderson CS, et al. Sex differences in care and long-term mortality after stroke: Australian stroke clinical registry. *J Womens Health.* (2019) 28:712–20. doi: 10.1089/jwh.2018.7171
46. Talbot D, Massamba VK. A descriptive review of variable selection methods in four epidemiologic journals: there is still room for improvement. *Eur J Epidemiol.* (2019) 34:725–30. doi: 10.1007/s10654-019-00529-y
47. Schisterman EF, Cole SR, Platt RW. Overadjustment bias and unnecessary adjustment in epidemiologic studies. *Epidemiology*. (2009) 20:488–95. doi: 10.1097/EDE.0b013e3181a819a1
48. Mieno MN, Tanaka N, Arai T, Kawahara T, Kuchiba A, Ishikawa S, Sawabe M. Accuracy of death certificates and assessment of factors for misclassification of underlying cause of death. *J Epidemiol*. (2016) 26:191–8. doi: 10.2188/jea.JE20150010

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Principles of Epilepsy Management for Women in Their Reproductive Years

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In the United States, there are over one million women with epilepsy (WWE) in their childbearing years. Pregnancy can be challenging for this population. A number of international registries have documented that children born to these women are at increased risk for major congenital malformations (MCM), lower intelligence quotient scores and neurodevelopmental disorders, when the mother is managed on antiseizure medications (ASMs). To prevent poor neonatal outcomes for this population, safe and thoughtful management strategies are necessary. We propose to divide these management strategies into five principles. These include (I) choosing suitable ASMs for the patient's seizure type, (II) choosing an ASM with the least teratogenic and cognitive side effects, (III) dosing at the lowest possible effective dosage, (IV) selecting the best ASM regimen as promptly as possible, even before a woman has her first menses, and (V) supplementing these patients with folic acid in order to try to enhance cognition and reduce neural tube defects.

Keywords: epilepsy, seizures, antiseizure medications (ASMs), women with epilepsy (WWE), reproductive years, teratogenic effects AEDs, major congenital malformations (MCM), neurocongnitive development

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INTRODUCTION

In the United States, there are over one million WWE in their childbearing years (1). Because of the reproductive potential of these women their management can often differ from males and post-menopausal women.

Management of seizures is traditionally guided by the classification of seizures as focal or generalized in onset. Thankfully, there are ASMs that can treat seizures in each classification. That selection is then narrowed down further in WWE based on the teratogenicity potential of these ASMs that is available from the various pregnancy registries. These registries include the North American Pregnancy Registry, The UK & Ireland Epilepsy and Pregnancy Register, EURAP Registry (includes 44 countries all around the world) and the Australian Registry.

Along with an increase of MCM some ASMs can also lead to lower intelligence quotient scores, and neurodevelopmental disorders (1). Unintended pregnancies further complicate this risk as they often lead to inadequate or delayed initiation of prenatal care and an increased risk for fetal exposure teratogenic substances such as alcohol and nicotine (2). In 2011, there were 45 unintended pregnancies for every 1,000 women aged 15–44 years (3). Similar rates are reflected worldwide in other developed countries, but are substantially higher in developing countries at 65 unintended pregnancies for every 1,000 women age 15–44 years (4). It is thus evident that WWE in their reproductive years require different management strategies to improve their healthcare outcomes as well as the health of their potential offspring.

THE FIVE PRINCIPLES

Principle I: Choosing the Best ASMs for the Patient's Seizure Type

Clarifying the type of seizure each patient experiences guides the practitioner in selecting the appropriate ASMs. ASMs are generally categorized as broad spectrum and narrow spectrum. Broad spectrum ASMs are defined as agents that can be effective for both focal and generalized onset seizure types. Narrow spectrum ASMs, on the other hand, are traditionally only used in patients whose seizures arise from a specific focus or foci.

Broad spectrum ASMs include valproic acid (VPA), lamotrigine, topiramate and levetiracetam. Some ASMs such as clobazam and rufinamide are FDA-approved for only certain types of generalized seizures but are frequently used "off label" as broad spectrum agents for all generalized seizure and focal seizure types. In addition, other ASMs such as brivaracetam, felbamate, zonisamide, and lacosamide are FDA approved to only treat focal seizures but are often used off label as broad spectrum ASMs for all generalized seizure types.

There are, however, narrow spectrum ASMs that can in fact worsen certain types of generalized seizures and are thus used to treat mostly focal seizures. These ASMs include carbamazepine, oxcarbazepine, phenytoin, pregabalin, and gabapentin. The focal seizures these ASMs treat can range from focal aware seizures, focal seizures with impaired awareness as well as focal to bilateral tonic clonic seizures (5).

Once the type of seizure is identified the practitioner can then narrow down the ASM list to the ones most suitable for the patient's seizure type.

Principle II: Choosing an ASM or ASMs With the Least Teratogenic and Cognitive Side Effects

We now have a variety of ASMs we can prescribe regardless of the seizure type the patient has. For WWE in the reproductive age group, the practitioner needs to further narrow the list of ASMs that are most appropriate in this patient population based on the ones that have the lowest rates of MCM. MCM are structural abnormalities that usually require surgical, medical, and cosmetic services (i.e., cleft lip, cleft palate, malformed limbs, neural tube defects, and cardiac abnormalities).

Since the 1990s birth outcomes of children born to WWE have been closely monitored through different pregnancy registries. Despite differences in methodology, the registries have generally reported similar findings and have all noted that exposure to VPA poses the greatest risk for MCMs. They have also shown that both lamotrigine and levetiracetam have a relatively low potential for MCMs. These findings have led to a marked difference in the way we now prescribe ASMs to WWE in the reproductive age group, with lamotrigine and levetiracetam being the most prescribed ASMs in many countries across the world (6, 7).

Monotherapy vs. Polytherapy

Polytherapy has been shown to increase the risk for major congenital malformation, however recent studies are proving this depends upon specific ASM combinations. The UK & Ireland Epilepsy and Pregnancy Register revealed highest MCM rates when levetiracetam was combined with VPA or carbamazepine (6.90%; 95% CI 1.91–21.96% and 9.38%; 95% CI 4.37–18.89%, respectively) and the lowest risk when combined with lamotrigine (1.77%; 95% CI 0.49–6.22%) (8). Similarly, EURAP data revealed highest MCM rates with ASM combinations that included VPA (9.1%; 95% CI 3.4–19.0% with lamotrigine and 15.4%; 95% CI 6.5–29.3% with carbamazepine), but not when carbamazepine was combined with all other non-VPAASMs (2.5%; 95% CI 1.1–4.36%) (9). Ultimately, avoiding polytherapy especially in combinations that include VPA is strongly recommended when possible.

Neurocognitive Considerations

While pregnancy registries focus on MCM, there has been growing evidence for the adverse effects of ASMs on neurocognitive development. Poorer cognitive ability has been proven with *in utero* exposure to specific ASMs. Children exposed to ASMs (monotherapy lacosamide, carbamazepine, lamotrigine, other, and polytherapy) had statistically poorer scores for overall development in comparison to children not exposed to ASMs ($p < 0.001$) (10). Differences in overall developmental ability were observed in children exposed to monotherapy VPA *in utero* when compared to the control group ($p < 0.001$). In addition, *in utero* exposure to VPA showed statistically more children below average range (score <84) for overall early development in comparison to control group (8%, $p < 0.001$). Similar results on neurocognitive development have been found in other studies where VPA and lamotrigine led to a statistically significant increased risk of having abnormal emotional and behavioral development (11). Conversely, carbamazepine was not associated with increased risk of emotional or behavioral development. Other neurodevelopmental finding showed increased risk of autism spectrum disorders and significantly reduced IQ scores with VPA in comparison to other ASMs (12–14).

Principle III: Dosing to Reduce Complications

Pregnancy registries' outcomes have not only guided us about which ASMs are considered the safest to prescribe for WWE in their reproductive years but have also shed light on ASM dosing in this population.

Dose-dependent risks were observed in the UK & Ireland Epilepsy and Pregnancy Register and the EURAP Registry with a higher risk of MCM at the higher ASM dosages (15). This is particularly true for women taking an ASM such as VPA (>1,000 mg/day in the first trimester, **Table 1**) (16, 18, 22). Higher rates of MCM were observed between low dose and high dose VPA and low dose and high dose carbamazepine, but not markedly different for low and high doses of lamotrigine (**Table 1**) (16). More recently, a Cochrane systematic review also supported dose-dependent major malformation risk for carbamazepine (>700 mg/d), lamotrigine (>325 mg/d), phenobarbital (>80 mg/d), and VPA>650 mg/d) (23, 24). Higher doses of VPA (preconception dose of >900 mg) were also associated with poorer overall developmental scores ($p < 0.001$) (10).

TABLE 1 | Major congeniital malformation rates from the UK & Ireland Epilepsy and Pregnancy Register, EURAP, Australian Pregnancy Register, and North American Antiepileptic Drug Pregnancy Registry.

Registry	MCM rate following antiepileptic drug exposure				
	Valproate	**Carbamazepine**	**Lamotrigine**	**Levetiracetam**	**Topiramate**
UK & Ireland Epilepsy and Pregnancy Register (8, 16, 17)	Dose: 0–≤600 mg 24/476 5.0% CI (3.4–7.4%)	Dose: 0–≤500 mg 14/721 1.9% CI (1.2–3.2%)	Dose: 0–≤200 mg 24/1,143 2.1% CI (1.4–3.1%)	2/304 0.7% CI (0.2–2.5%)	3/70 04.8% CI (1.7–13.3%)
	Dose: >600–≤1,000 mg 26/426 6.1% CI (4.2–8.8%)	Dose: >500–≤1,000 mg 20/739 2.7% CI (1.8–4.1%)	Dose: >200–≤400 mg 16/665 2.4% CI (1.5–4.0%)		
	Dose: >1,000 mg 31/297 10.4% CI (7.4–14.4%)	Dose: >1,000 mg 9/170 5.3% CI (2.7–9.5%)	Dose: >400 mg 9/276 3.4% CI (1.9–6.5%)		
EURAP (7, 18)	Dose: ≤650 mg/day 38/600 6.3% CI (4.5–8.6%)	Dose: ≤700 mg/day 58/1,276 4.5% CI (3.5–5.8)	Dose: ≤35 mg/day 46/1,870 2.5% CI (1.8–3.3%)	Dose: 250–4,000 mg/day 17/599 2.8% CI (1.7–4.5%)	Dose: 25–500 mg/day 6/152 3.9% CI (1.5–8.4%)
	Dose: >650–≤1,450 mg/day 75/666 11.3% CI (9.0–13.9%)	Dose: >700 mg/day 49/681 7.2% CI (5.4–9.4%)	Dose: >325 mg/day 28/644 4.3% CI (2.9–6.2%)		
	Dose: >1,450 mg/day 29/115 25.2% CI (17.6–34.2%)				
Australian Pregnancy Register (19)	43/290 14.8% CI (2.11–12.95%)	24/409 5.9% CI (0.8–5.33%)	20/406 4.9% CI (0.66–4.55%)	5/139 3.6% CI (0.37–4.29%)	1/53 1.9% CI (0.09–5.96%)
North American Antiepileptic Drug Pregnancy Registry (20)	30/323 9.3% CI (6.4–13.0%)	31/1,033 3.0% CI (2.1–4.2%)	31/1,562 2.0% CI (1.4–2.8%)	11/450 2.4% CI (1.2–4.3%)	15/359 4.2% CI (2.4–6.8%)

Table adaptation obtained from Elsevier, Kinney and Craig (21).
CI, 95% Confidence interval.

Principle IV: Promptly Selecting the Best ASM Regimen

The rate of unintended pregnancies is not only high in the general population but also in WWE. Thus, promptly selecting the best ASM regimen (based on the above principles) when a woman is nearing the reproductive years is very important. Herzog et al. found that of the 437 women who reported getting pregnant after seizure onset, 78.9% of them reported having at least one unintended pregnancy (25). Sadly, by the time a woman misses her first period after conception, primary neural tube formation (which occurs in the first 4 weeks of gestation) has already taken place and potential neural tube damage may be irreversible.

Additionally, changing medications while the patient is pregnant exposes the patient and her fetus to the unknown effectiveness of the new ASM, thereby, placing the woman at risk of having seizures during pregnancy. Epileptic seizures were found to be associated with a 1.36-fold increased risk for low birth weight infants, 1.63-fold increased risk for preterm delivery, and 1.37-fold increased risk for small-for-gestational-age infants in a nationwide population-based study for 1,016 Taiwanese women with epilepsy (26). Moreover, the effects of generalized tonic-clonic seizures during pregnancy are particularly worrisome as they can lead to fetal asphyxia, fetal bradycardia, reduced uterine contractions, direct injury (both to the mother and fetus), and fetal demise.

Principle V: Supplement All WWE in the Reproductive Age Group With Folic Acid

Folic Acid exposure has been shown to prevent neural tube defects in the general population (27, 28). Given that ASMs such as VPA can interfere with neural tube development it has become standard of care among epileptologists, to provide relatively high dosing of folic acid in the range of 2–5 mg to mitigate those effects. Despite this common practice, it is important to note that it has not been proven, thus far, that folic acid prevents neural tube defects in women taking ASMs (28–30). It is possible that the neural tube deficits that are linked to ASMs are due to mechanisms that do not involve folic acid metabolism (28, 29, 31).

Recent literature, however, has shown that folic acid may be beneficial in reducing the risk of autistic traits, enhancing children's IQ, and language development if the mother has taken folic acid for 4 weeks pre-gestation and post-conception (32–34). What is not clear, is the exact dosage of folic acid that is needed to improve cognitive outcome. Best cognitive outcomes were observed in children of women taking at least 0.4 mg/day of folic acid in the NEAD study and at least 1 mg/day in the Norwegian

Mother and Child Cohort Study (32, 33). Since the data set is rather limited, we still support the use of about 4 mg of folic acid in patients who are taking ASMs that impair folic acid absorption (such as phenytoin, carbamazepine, and phenobarbital, as these can cause a deficiency of folic acid by interfering with the way it is absorbed). Patients taking VPA or who have a history of neural tube defects in their family should also be supplemented with about 4 mg of folic acid. For patients taking other form of ASMs we typically support the use of 2 mg/day of folic acid, until more literature is available on the least amount of folic acid that can enhance cognition.

DISCUSSION

Pregnancy registries have largely contributed to ASM management in WWE through the evidence of MCM risks. This has been further expanded by the growing evidence of cognitive, behavioral, and emotional effects of *in utero* ASM exposure provided by studies such as the NEAD study and the Norwegian Mother and Child Cohort Study.

Prescribing practices documented in the North American, EURAP, and Australian Registries have shown drastic changes over the last 5–10 years, with lamotrigine and levetiracetam now being the most prescribed ASMs. Recent data from the EURAP registry has shown that in fact these new practices have led to a statically significant reduction in MCM worldwide (7). With this change in practice, other impacts need to be considered and discussed with patients regarding children exposed to ASMs *in utero* such as lamotrigine, even though they may have a relatively low MCM rates (i.e., abnormal emotional and behavioral development) (11, 18).

It is important to note that there are some patients, particularly those with generalized forms of epilepsy such as Juvenile Myoclonic Epilepsy or Absence Epilepsy, in whom ASMs such as lamotrigine and levetiracetam may not be as effective in controlling seizures as VPA (35, 36). If the patient's seizures are not controlled by less teratogenic ASMs and VPA needs to be used, it is important to find the lowest effective dosage of this ASM to reduce the chances of MCMs as well as cognitive and behavioral deficits.

Even if a woman expresses no desire to become pregnant, all efforts should be made to change the ASM to one with less teratogenic potential to account for unintended pregnancies. It is also recommended that WWE in the reproductive age group take folic acid on a daily basis, particularly if they are sexually active, as this vitamin has been shown to reduce neural tube defects in the general population and enhance cognition in children exposed to ASMs *in utero*. Further research is needed to better understand the dosages of folic acid that provide the maximal benefit. In addition, there are a growing number of ASMs which were introduced to the market after the year 2000 that have unknown teratogenic and cognitive affects. These newer ASMs should be used with caution for WWE until more information is available.

Broadly, epilepsy management is complicated without even considering the sex differences between males and females. In treating WWE, the goal is to reduce the chances of MCM and enhance cognitive development in the fetus who is exposed to ASMs (1, 23).

AUTHOR CONTRIBUTIONS

RS: article conception and writing of the manuscript. HM: writing of the manuscript.

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REFERENCES

1. Sazgar M. Treatment of Women With Epilepsy. *Continuum.* (2019) 25:408–30. doi: 10.1212/con.0000000000000713
2. Finer LB, Zolna MR. Unintended pregnancy in the United States: incidence and disparities, 2006. *Contraception.* (2011) 84:478–85. doi: 10.1016/j.contraception.2011.07.013
3. Finer LB, Zolna MR. Declines in unintended pregnancy in the United States, 2008-2011. *N Engl J Med.* (2016) 374:843–52. doi: 10.1056/NEJMsa1506575
4. Bearak J, Popinchalk A, Alkema L, Sedgh G. Global, regional, and subregional trends in unintended pregnancy and its outcomes from 1990 to 2014: estimates from a Bayesian hierarchical model. *Lancet Glob Health.* (2018) 6:e380–9. doi: 10.1016/S2214-109X(18)30029-9
5. Fisher RS, Cross JH, French JA, Higurashi N, Hirsch E, Jansen FE, et al. Operational classification of seizure types by the International League Against Epilepsy: position paper of the ILAE Commission for Classification and Terminology. *Epilepsia.* (2017) 58:522–30. doi: 10.1111/epi.13670
6. Vajda FJ, O'Brien TJ, Graham J, Lander CM, Eadie MJ. The Australian Register of antiepileptic drugs in pregnancy: changes over time in the epileptic population. *J Clin Neurosci.* (2014) 21:1478–82. doi: 10.1016/j.jocn.2013.11.049
7. Tomson T, Battino D, Bonizzoni E, Craig J, Lindhout D, Perucca E, et al. Declining malformation rates with changed antiepileptic drug prescribing: an observational study. *Neurology.* (2019) 93:e831–40. doi: 10.1212/WNL.0000000000008001
8. Mawhinney E, Craig J, Morrow J, Russell A, Smithson WH, Parsons L, et al. Levetiracetam in pregnancy: results from the UK & Ireland epilepsy and pregnancy registers. *Neurology.* (2013) 80:400–5. doi: 10.1212/WNL.0b013e31827f0874
9. Holmes LB, Mittendorf R, Shen A, Smith CR, Hernandez-Diaz S. Fetal effects of anticonvulsant polytherapies: different risks from different drug combinations. *Arch Neurol.* (2011) 68:1275–81. doi: 10.1001/archneurol.2011.133
10. Bromley RL, Mawer G, Love J, Kelly J, Purdy L, McEwan L, et al. Early cognitive development in children born to women with epilepsy: a prospective report. *Epilepsia.* (2010) 51:2058–65. doi: 10.1111/j.1528-1167.2010.02668.x
11. Richards N, Reith D, Stitely M, Smith A. Developmental outcomes at age four following maternal antiepileptic drug use. *Epilepsy Behav.* (2019) 93:73–9. doi: 10.1016/j.yebeh.2019.01.018
12. Meador KJ, Baker GA, Browning N, Cohen MJ, Bromley RL, Clayton-Smith J, et al. Fetal antiepileptic drug exposure and cognitive outcomes at age 6 years (NEAD study): a prospective observational study. *Lancet Neurol.* (2013) 12:244–52. doi: 10.1016/S1474-4422(12)70323-X

13. Bromley RL, Mawer GE, Briggs M, Cheyne C, Clayton-Smith J, García-Fiñana M, et al. The prevalence of neurodevelopmental disorders in children prenatally exposed to antiepileptic drugs. *J Neurol Neurosurg Psychiatry*. (2013) 84:637–43. doi: 10.1136/jnnp-2012-304270
14. Veiby G, Daltveit AK, Schjølberg S, Stoltenberg C, Øyen AS, Vollset SE, et al. Exposure to antiepileptic drugs *in utero* and child development: a prospective population-based study. *Epilepsia*. (2013) 54:1462–72. doi: 10.1111/epi.12226
15. Brent RL. Environmental causes of human congenital malformations: the pediatrician's role in dealing with these complex clinical problems caused by a multiplicity of environmental and genetic factors. *Pediatrics*. (2004) 113(4 Suppl):957–68.
16. Campbell E, Kennedy F, Russell A, Smithson WH, Parsons L, Morrison PJ, et al. Malformation risks of antiepileptic drug monotherapies in pregnancy: updated results from the UK & Ireland Epilepsy and Pregnancy Registers. *J Neurol Neurosurg Psychiatry*. (2014) 85:1029–34. doi: 10.1136/jnnp-2013-306318
17. Hunt S, Russell A, Smithson WH, Parsons L, Robertson I, Waddell R, et al. Topiramate in pregnancy: preliminary experience from the UK Epilepsy and Pregnancy Register. *Neurology*. (2008) 71:272–6. doi: 10.1212/01.wnl.0000318293.28278.33
18. Tomson T, Battino D, Bonizzoni E, Craig J, Lindhout D, Perucca E, et al. Comparative risk of major congenital malformations with eight different antiepileptic drugs: a prospective cohort study of the EURAP registry. *Lancet Neurol*. (2018) 17:530–8. doi: 10.1016/s1474-4422(18)30107-8
19. Vajda FJE, Graham JE, Hitchcock AA, Lander CM, O'Brien TJ, Eadie MJ. Antiepileptic drugs and foetal malformation: analysis of 20 years of data in a pregnancy register. *Seizure*. (2019) 65:6–11. doi: 10.1016/j.seizure.2018.12.006
20. Hernández-Díaz S, Smith CR, Shen A, Mittendorf R, Hauser WA, Yerby M, et al. Comparative safety of antiepileptic drugs during pregnancy. *Neurology*. (2012) 78:1692–9. doi: 10.1212/WNL.0b013e3182574f39
21. Kinney MO, Craig JJ. Pregnancy and epilepsy; meeting the challenges over the last 25 years: the rise of the pregnancy registries. *Seizure*. (2017) 44:162–8. doi: 10.1016/j.seizure.2016.10.004
22. Wyszynski DF, Nambisan M, Surve T, Alsdorf RM, Smith CR, Holmes LB, et al. Increased rate of major malformations in offspring exposed to valproate during pregnancy. *Neurology*. (2005) 64:961. doi: 10.1212/01.WNL.0000154516.43630.C5
23. Weston J, Bromley R, Jackson CF, Adab N, Clayton-Smith J, Greenhalgh J, et al. Monotherapy treatment of epilepsy in pregnancy: congenital malformation outcomes in the child. *Cochrane Database Syst Rev*. (2016) 11:Cd010224. doi: 10.1002/14651858.CD010224.pub2
24. Andrade C. Major congenital malformations associated with exposure to antiepileptic drugs during pregnancy. *J Clin Psychiatry*. (2018) 79:e1–e4. doi: 10.4088/JCP.18f12449
25. Herzog AG, Mandle HB, Cahill KE, Fowler KM, Hauser WA. Predictors of unintended pregnancy in women with epilepsy. *Neurology*. (2017) 88:728–33. doi: 10.1212/WNL.0000000000003637
26. Chen YH, Chiou HY, Lin HC, Lin HL. Affect of seizures during gestation on pregnancy outcomes in women with epilepsy. *Arch Neurol*. (2009) 66:979–84. doi: 10.1001/archneurol.2009.142
27. Centers for Disease Control and Prevention. Spina bifida and anencephaly before and after folic acid mandate–United States, 1995-1996 and 1999-2000. *MMWR Morb Mortal Wkly Rep*. (2004) 53:362–5.
28. MRC Vitamin Study Research Group. Prevention of neural tube defects: results of the Medical Research Council Vitamin Study. *Lancet*. (1991) 338:131–7. doi: 10.1016/0140-6736(91)90133-A
29. Czeizel AE, Dudás I. Prevention of the first occurrence of neural-tube defects by periconceptional vitamin supplementation. *N Engl J Med*. (1992) 327:1832–5. doi: 10.1056/NEJM199212243272602
30. Ban L, Fleming KM, Doyle P, Smeeth L, Hubbard RB, Fiaschi L, et al. Congenital anomalies in children of mothers taking antiepileptic drugs with and without periconceptional high dose folic acid use: A Population-Based Cohort Study. *PLoS ONE*. (2015) 10:e0131130. doi: 10.1371/journal.pone.0131130
31. Morrow JI, Hunt SJ, Russell AJ, Smithson WH, Parsons L, Robertson I, et al. Folic acid use and major congenital malformations in offspring of women with epilepsy: a prospective study from the UK Epilepsy and Pregnancy Register. *J Neurol Neurosurg Psychiatry*. (2009) 80:506–11. doi: 10.1136/jnnp.2008.156109
32. Husebye ESN, Gilhus NE, Riedel B, Spigset O, Daltveit AK, Bjørk MH. Verbal abilities in children of mothers with epilepsy: association to maternal folate status. *Neurology*. (2018) 91:e811–821. doi: 10.1212/WNL.0000000000006073
33. Bjørk M, Riedel B, Spigset O, Veiby G, Kolstad E, Daltveit AK, et al. Association of folic acid supplementation during pregnancy with the risk of autistic traits in children exposed to antiepileptic drugs *in utero*. *JAMA Neurol*. (2018) 75:160–8. doi: 10.1001/jamaneurol.2017.3897
34. Meador KJ, Pennell PB, May RC, Brown CA, Baker G, Bromley R, et al. Effects of periconceptional folate on cognition in children of women with epilepsy: NEAD study. *Neurology*. (2020) 94:e729–40. doi: 10.1212/WNL.0000000000008757
35. Cerulli Irelli E, Morano A, Cocchi E, Casciato S, Fanella M, Albini M, et al. Doing without valproate in women of childbearing potential with idiopathic generalized epilepsy: implications on seizure outcome. *Epilepsia*. (2020) 61:107–14. doi: 10.1111/epi.16407
36. Silvennoinen K, de Lange N, Zagaglia S, Balestrini S, Androsova G, Wassenaar M, et al. Comparative effectiveness of antiepileptic drugs in juvenile myoclonic epilepsy. *Epilepsia Open*. (2019) 4:420–30. doi: 10.1002/epi4.12349

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Longitudinal Effects of Immediate and Delayed Estradiol on Cognitive Performance in a Spatial Maze and Hippocampal Volume in Menopausal Macaques Under an Obesogenic Diet

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The consumption of a diet high in fat and refined sugars has several health risks, including the development of cognitive decline and neurodegeneration. For women, menopause carries additional health risks that may interact with a high-fat diet in negative ways. Some symptoms of menopause, including cognitive impairments, can be modulated by hormone replacement therapy (HRT), but the hormonal formulation and the timing of the treatment relative to the onset of menopause are critical factors determining its efficacy. Little is known about how obesogenic, high-fat, high-sugar diets interact with HRT in menopause to affect cognition and neurodegeneration. Given the high prevalence of the consumption of an obesogenic Western-style diet, understanding how the effects of HRT are modulated by an obesogenic diet is critical for developing optimized therapeutic strategies for peri- and post-menopausal women. In this study, we investigated by magnetic resonance imaging (MRI) the effects of either immediate or delayed estradiol hormone therapy on cognition and neuroanatomy following ovo-hysterectomy (OvH) of aged, female rhesus macaques on an obesogenic diet. The macaques were followed for 2.5 years after ovo-hysterectomy, with four time points at which anatomical MRIs were acquired. Analysis of hippocampal volumes revealed an interaction between time point and treatment; hippocampal volumes in the delayed estrogen group, but not the immediate estrogen group, increased over time compared to those in untreated controls. Performance on a hippocampal-dependent spatial maze task showed improved performance in estrogen treated animals compared to OvH macaques given placebo. These results indicate that HRT may contribute to beneficial cognitive outcomes after menopause under an obesogenic diet.

Keywords: menopause, hormone replacement therapy, obesogenic diet, neurodegeneration, aging

INTRODUCTION

Menopause is associated with a cessation of the production of oocytes, as well as a decline in the production of endogenous estrogens and progesterone (1). This can result in a variety of physiological changes, including adverse changes to mood and cognitive effects such as effects on memory (1, 2). Given current average lifespan, most women will live upwards of one third of their lives in a post-menopausal state (3, 4). Hormone replacement therapy (HRT) was prescribed to alleviate the adverse symptoms of menopause for several decades. However, the publication of the findings from the Women's Health Initiative (WHI) in 2002 led to a decrease in HRT usage (3, 5). The results from WHIMS, the cognitive arm of the WHI, indicated that HRT (estrogens with or without progesterone) actually increased the risk of dementia and led to worse cognitive outcomes than post-menopausal women taking placebo (6). However, since the publication of the WHI results, it has become increasingly clear that the length of the period between the menopausal transition and the start of HRT, the critical period hypothesis, is an important factor in determining the outcome of treatment with longer delays being associated with less beneficial HRT outcomes. Participants were on average 10–12 years into menopause at the beginning of the WHI study (3, 7). Systematic reviews on the effects of HRT have yielded mixed results (8–11). This seems due in large part to the heterogeneity between HRT studies, highlighting the importance of investigating specific modulating factors.

Diet is known to be a critical factor in determining health outcomes. Specifically, a diet high in saturated fatty acids (SFAs) and refined sugars, has been shown to contribute substantially to the obesity epidemic as well as to the development of cognitive impairment and dementia (12, 13). This Western-style diet (WSD) has also been specifically associated with impairments in spatial learning and memory (12). Recent estimates indicate that 71.6% of American adults are overweight or obese, and that most Americans are eating more than the recommended amount of dietary fat and refined sugars (14, 15). It is important to note that most of the animal studies conducted to date investigating the effects of a WSD on cognition involved only male animals, highlighting the need to investigate how a WSD impacts health and cognition in female animals. Little is known about how WSDs interact with hormone replacement therapy in menopause. There is some evidence indicating that the effectiveness of HRT on behavioral and cognitive performance is modulated by an obesogenic diet in macaques (16) as well as in rodent models (17), but the mechanisms that may contribute to this diet-HRT interaction on cognition are not known.

The close phylogenetic relationship between non-human primates (NHPs) and humans is reflected in the similarities in neuroanatomy, cognition, and reproductive features in the two groups. Specifically, for work on reproductive senescence, NHPs provide several advantages over rodents as model animals. In both NHPs and humans, reproductive senescence in driven by the depletion of the follicular pool (18, 19). In contrast, rodent reproductive senescence is driven by dysregulation of the hypothalamic-pituitary-ovarian (HPO) axis, resulting in mature viable follicles that are present throughout the lifespan (4). This is in marked contrast to the complete depletion of ovarian follicles seen in primates after reproductive senescence (4). The shared mechanism of reproductive senescence between NHPs and humans, in addition to the relatively long lifespan of NHPs compared to rodents, makes NHP studies especially valuable for determining the effects of HRT on physiological systems in humans. Reproductive senescence in rhesus macaques has been characterized more extensively than in any other NHP (20). The current study involved aged animals rather than surgically menopausal young animals, thus making the results of this work more relevant to peri- and post-menopausal women.

There are many similarities between functional and anatomical correlates of aging in macaques and humans that make rhesus monkeys a valuable post-menopausal model to study cognitive aging. Cognitively, both species show particular patterns of impairment with age-related changes in executive functions, spatiotemporal memory, and recognition memory during healthy aging in the absence of dementia (21–23). In addition to these overall age-related cognitive declines, additional menopause-related cognitive impairments have been observed in both human and non-human primates after accounting for the effects of age (21, 24).

The gross anatomical patterns of aging share both similarities and differences between species. In the frontal cortex, decreases in the volumes of area 46 (part of the dorsolateral prefrontal cortex) and the anterior cingulate cortex have been observed in rhesus macaques using MRI (22). In the delayed nonmatching-to-sample (DNMS) visual recognition memory task, lower volume in both area 46 and the anterior cingulate cortex predicted worse performance on the DNMS task (23). These patterns overlap with gross volumetric declines in prefrontal areas that accompany aging in humans (25, 26).

In humans, hippocampal volume seems to be particularly susceptible to aging, even in the absence of pathology (27, 28). In contrast, hippocampal volumes have not been shown to decrease with age in rhesus monkeys (22, 29). Interestingly, Shamy et al. demonstrated that hippocampal volume predicted acquisition of a delayed response spatiotemporal task in aged rhesus macaques despite observing no significant age-related changes in hippocampal volume (22). Indeed, there are changes in hippocampal structure which predict cognitive performance. Hara et al. observed that, independent of age, synaptic density in the outer molecular layer of the dentate gyrus was lower in peri/post-menopausal rhesus macaques than premenopausal macaques (30). Further, the synaptic density in this area predicted performance on a recognition memory task (30).

The goal of the current study was to investigate the role of treatment timing as well as diet on the effects of HRT on spatial cognition and pertinent brain volumes. We investigated the time required to acquisition of a spatial maze task in aged, postmenopausal rhesus macaques treated with estrogen compared to untreated macaques kept under an obesogenic diet. Further, we analyzed how the volumes of the hippocampus, prefrontal cortex, amygdala, and motor cortex changed over the course of 2.5 years in these same monkeys,

who were either treated with HRT immediately after surgically-induced menopause, with HRT after a 2-year delay, or with a placebo control.

METHODS

Subjects

The animals and experimental treatment paradigm are as described previously by Coleman et al. (16). Briefly, aged female rhesus macaques of 17 years old or older were socially housed in groups of 2–4 animals throughout the study in indoor pens (∼3.7 × 2.1 × 2.1 m) and provided with enrichment such as toys and foraging devices. Animals were maintained under fixed photoperiods comprising 12 h of light and 12 h of darkness per day (12L:12D). The activity patterns of these animals have been previously described (31, 32). Monkeys were maintained on a standard laboratory chow (Lab Diet, Inc., St. Louis, MO) before being switched to a WSD at the start of the study (TAD; Lab Diet). Macronutrient composition of the diets is listed in **Table 1**. Water was provided *ad libitum*. Monkeys were fed the WSD for ∼6 weeks before being ovohysterectomized (OvH). The intended length of the study was 3 years; however, after 2.5 years it was necessary to terminate the study due to the manifestation of age-related pathologies.

Surgery

Monkeys were OvH via a laparotomy procedure by surgical personnel at the Oregon National Primate Research Center (ONPRC). Animals were removed from the group, sedated with ketamine (10 mg/kg) and transported to the surgical site. Animals were returned to their social group after the procedure.

Treatment

The macaques received either placebo for 30 months, estrogen immediately upon hysterectomy for 30 months, or placebo for 24 months followed by delayed estrogen treatment for an additional 6 months. Estrogen treatment was administered via the implantation of Silastic capsules subcutaneously into the periscapular region. The implants were intended to achieve serum estrogen concentrations between 70 and 100 pg/ml. Placebo-treated animals were implanted with an empty Silastic capsule. Serum estrogen levels were measured every 2 months to ensure that serum estrogen levels stayed within the desired range.

Spatial Maze Training

The dependent variable for performance on the spatial maze was trials to criterion. Performance was codified as shown in **Table 2**. Criterion was defined as reaching a behavioral score of 6. Only animals that achieved a score of 6 or above during the course of training were included in the analysis. Spatial maze training took place before the start of hormone treatment in the delayed estrogen group, thus for data analysis, the placebo and delayed estrogen groups were combined into the control group. Out of 8 animals in the estrogen group, 5 reached criterion. Out of 15 animals in the combined control group, 9 reached criterion. Macaques were allowed to search a series of boxes for treats which consisted of M&M's®, craisins®, dried mango, or marshmallows. Treats were initially placed in various locations on the floor of the testing room, then placed on the floor close to a box, then on top of the box, then on the lip of the entry hole of the box, and finally, hidden inside the box.

TABLE 1 | Macronutrient profile of standard chow and the WSD.

	Fat (%)	Carbohydrates (sugars)	Protein (%)
Standard Chow	13	69% (6%)	18
Western-style diet	36	44% (18.5%)	18

Magnetic Resonance Imaging

MRI Data Acquisition

Images were acquired at four time points throughout the study: (1) at baseline (before treatment); (2) after 1 year; (3) after 2 years; and (4) after 2.5 years. For each imaging session, the subject was anesthetized using ketamine (15 g/kg) and transferred to the ONPRC MRI Core Facility. The subject was then intubated and continually anesthetized with 1–1.5% isoflurane for the duration of the imaging (≤ 2 h). Animals were physiologically monitored during scans for well-being.

Images were acquired *in vivo* using a Siemens Tim Trio whole body 3T system (Erlangen, Germany) with a 15-channel quadrature knee coil. For each subject at each time point, four T_1-weighted magnetization-prepared rapid acquisition gradient echo (MPRAGE) images were acquired with the following parameters: TR = 2500 ms, TE = 3.86 ms, TI = 1100 ms, Flip angle = 12 deg, Voxel size = 0.5 × 0.5 × 0.5 mm^3, FOV = 128 × 128 mm^2, Matrix size = 256 × 256.

MRI Analysis

Preprocessing of the T_1-weighted MPRAGE images was implemented for all imaging sessions. At the beginning, four T_1-weighted images collected for the same subject at the same time point were merged as the final anatomical image after motion correction and intensity bias correction. The motion correction was implemented by the rigid-body alignment with the first acquired MPRAGE image using "antsRegistrationSyN.sh" tool from Advanced Normalization Tools (ANTS) software (33). After motion correction, the

TABLE 2 | Behavioral scoring criteria for the spatial maze task.

Score	Performance Indicator
0	Failure to eat or pick up any treats
1	Picking up treats within the room
2	Picking up treats from below the box
3	Reaching to top of box and removing treat
4	Reaching to lip of box and removing treat (large hole)
5	Reaching to lip of box and removing treat (small hole)
6	Reaching into box and removing treat
7	Reaching into box and removing treat more than once consecutively
8	Reaching into box and removing treat more than once consecutively without searching any other boxes prior to reaching treat

intensity bias field of each image was corrected using a B-spline approximation routine and hierarchical optimization scheme with the "N4BiasFieldCorrection" tool also in ANTS (34). Then, all four corrected images were averaged as the final image for structural analysis. Next, the baseline merged T1-weighted images were B-spline nonlinearly registered to a head image from the INIA19 template (35) using "antsRegistrationSyN.sh." Using the resulting transformation parameters, the INIA19 brain mask was reversely mapped to each subject's original space to generate the brain mask using a nearest neighbor interpolation method. For the following time points, the brain masks were generated using the same method but updated references with the corresponding merged image of the same subject at the previous time point. Based on brain masks, skull-stripping of all merged MPRAGE images was achieved and the process of intensity bias correction for all brain images was repeated using the obtained brain masks to limit the correction region to improve correction quality.

After the preprocessing of all MPRAGE images, all baseline brain images were nonlinearly registered to the INIA19 brain template image. According to the transformation parameters, the label map of the INIA19 template, i.e. NeuroMaps, was inversely mapped to each individual original space using nearest neighbor interpolation method. Subsequently, brain image from each subject's next imaging session was registered to their previous imaging session, and then the inversed mapping of the label maps from the previous imaging session using the resulting registration parameters were implemented to generate the label maps for the next imaging session. All volumetric analysis was based on the acquired label maps for each subject.

Because of sample size of this study, we defined four well-resolved regions-of-interest (ROIs) from the smaller parcellations included in the NeuroMaps labels. A list of the included structures for each of the ROIs in presented in **Table 3**. **Figure 1** shows the ROI boundaries in the glass brain of an

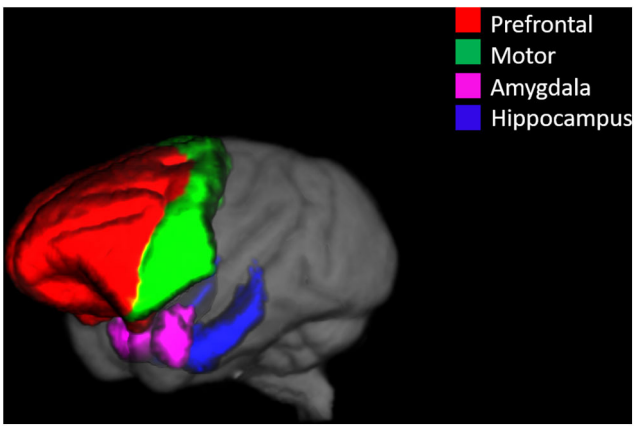

FIGURE 1 | An example glass brain of an individual monkey with the predefined ROIs. The prefrontal ROI is highlighted red, the motor ROI is highlighted green, the amygdala ROI is highlighted magenta, and the hippocampal ROI is highlighted blue.

TABLE 3 | NeuroMaps labels included in ROI structures.

Hippocampus	Prefrontal	Motor	Amygdala
Perforant path	Anterior cingulate gyrus	Precentral gyrus	Lateral amygdalar nucleus
Oriens layer of the hippocampus	Frontal white matter		Accessory basal nucleus of the amygdala
CA fields	Superior frontal gyrus		Basal nucleus of the amygdala
Granular layer of the dentate gyrus	Middle frontal gyrus		Claustral amygdalar area
Molecular layer of the hippocampus	Inferior frontal gyrus		Periamygdalar area
Pyramidal cell layer of the hippocampus	Fronto-orbital gyrus (macaque)		Amygdalar island
Molecular layer of the dentate gyrus	Lateral orbital gyrus		Paralaminar nucleus of the amygdala
Parasubicular area	Straight gyrus		Amygdalohippocampal area
Uncinate gyrus	Medial orbital gyrus		Hippocampal-amygdaloid transition area
Prosubiculum			Amygdala
Subiculum			Central amygdalar nucleus
Presubiculum			Medial amygdalar nucleus
Stratum pyramidale of the CA1 field			Anterior amygdalar area
Stratum pyramidale of the CA3 field			Cortical amygdalar nucleus
Stratum pyramidale of the CA2 field			Amygdala—not otherwise specified
Hilus of the dentate gyrus			
Hippocampal sulcus			
Entorhinal sulcus			
CA1 field			
CA2 field			
Fascia dentata			

Both left and right labels were included for all bilateral structures.

example subject. All of the ROI boundaries were defined *a priori* before any statistical analysis of the volumes. Hippocampal and prefrontal ROIs were defined specifically because of their hypothesized role in spatial maze performance. The amygdala ROI was defined because a previous study on these monkeys (16) observed treatment differences in anxiety-type behaviors which we hypothesized could both affect performance on the task and could manifest anatomically as differential relative amygdala sizes. A motor ROI was defined as a region that we did not expect to be substantially involved in treatment effects on cognitive performance to serve as an anatomical control for the other anatomical analyses. In order to correct for overall head size, the ROIs were normalized by the total brain volume, and these relative volumes were used for all subsequent analysis.

Statistical Analysis

All statistical analyses were completed using the computing environment *R* (version 3.6.0; R Development Core Team, 2019). Significance for all tests performed was defined as an alpha value of ≤ 0.05. *R* packages used for the analysis and presentation of data included: car (3.0.6), dplyr (0.8.1), effects (4.1.3), ggplot2 (3.1.1), ggpubr (0.2), ggsignif (0.6.0), lme4 (1.1.21), lmerTest (3.1.0), psych (1.8.12), and xlsx (0.6.1) (36–44). The *R* code for the analysis is provided in the **Supplementary Materials**.

Performance on the spatial maze was assessed using two sample *t*-tests to compare the means of trials to criterion between rhesus monkeys given immediate estrogen and the OvH controls. Because trials to criterion may be expected to be count-distributed, we also tested these group differences using a Poisson model. Since the OvH + Delayed E group had still not received estrogen at the time of training in the study, the subjects belonging to this group were combined with the OvH control group for the comparison of performance on the spatial maze training. Because of the temporal proximity to the spatial maze training, relative volumes calculated from the second imaging session (1-year after OvH) were used to predict trials to criterion. Pearson correlations were used to test the relationships between cognitive task variables and the four selected ROI volumes. An overall treatment difference in the number of training days was tested using a one-way ANOVA.

The longitudinal effects of the treatments on each ROI volume were modeled using a linear mixed effect model:

$$Relative\ Volume \sim Year * Treatment + (1|Subject)$$

Where random effect intercepts are estimated for the subject, and fixed effects are estimated for the slopes for the year, the treatment factor, and their interaction. *T*-tests and *p*-values were calculated using the Satterthwaite's degrees of freedom method. Since the OvH + Delayed E group did not receive HRT until after 2 years, an additional one-way ANOVA was conducted to assess if there were any group differences on hippocampal volume for the final imaging session only.

RESULTS

Spatial Maze

Of the 23 monkeys in the study, only 14 of the monkeys reached criterion on the spatial maze training. Levene's test for homogeneity of variance showed that the variances for trials to criterion were not significantly different between the OvH + immediate E and OvH control groups $F_{(1, 12)} = 0.195$, $p = 0.667$. As illustrated in **Figure 2**, estrogen treated animals ($M = 2.00$, $SD = 1.41$, $n = 5$ animals) compared to OvH animals given placebo only ($M = 4.89$, $SD = 1.69$, $n = 9$), had significantly fewer trials to criterion, $t_{(12)} = -3.23$, $p = 0.00724$. Using a Poisson model, similar results were obtained, $z = -2.55$, $p = 0.0107$.

While there was a significant effect of treatment on the performance during the training of the spatial maze task, there was no relationship between the brain volumes in our chosen ROIs and the spatial maze trials to criterion, (Amygdala: $r_{(12)} = -0.476$, $p = 0.0851$, Hippocampus: $r_{(12)} = -0.244$, $p = 0.401$, Prefrontal: $r_{(12)} = -0.0284$, $p = 0.923$, Motor: $r_{(12)} = 0.217$, $p = 0.455$).

Over the course of the spatial maze training, individual monkeys received varying total number of days of training. As shown in **Figure 3A**, this variation in days trained positively correlated with the relative hippocampal volumes measured in the imaging session following the spatial maze training, $r_{(21)} = 0.432$, $p = 0.0396$. At later imaging sessions, this correlation no longer reached significance, but still showed a trend at 2 years after treatment, $r_{(17)} = 0.420$, $p = 0.0732$, and 2.5 years after treatment, $r_{(14)} = 0.456$, $p = 0.0756$. None of the other chosen ROIs showed a significant correlation with the total number of days of training at the imaging session following training (Amygdala: $r_{(21)} = 0.190$, $p = 0.386$, Prefrontal: $r_{(21)} = -0.0151$, $p = 0.945$, Motor: $r_{(21)} = -0.383$, $p = 0.0712$).

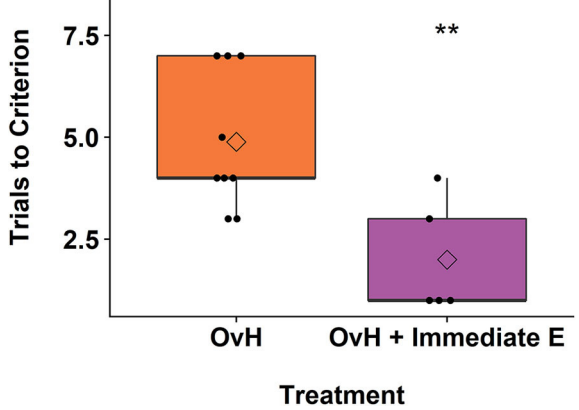

FIGURE 2 | A box plots demonstrating the difference between the OvH and OvH + Immediate E groups in trials to criterion on the spatial maze task. The circles show the scores of individual animals, while the diamonds within the box plots represent the group means. $^{**}p < 0.01$.

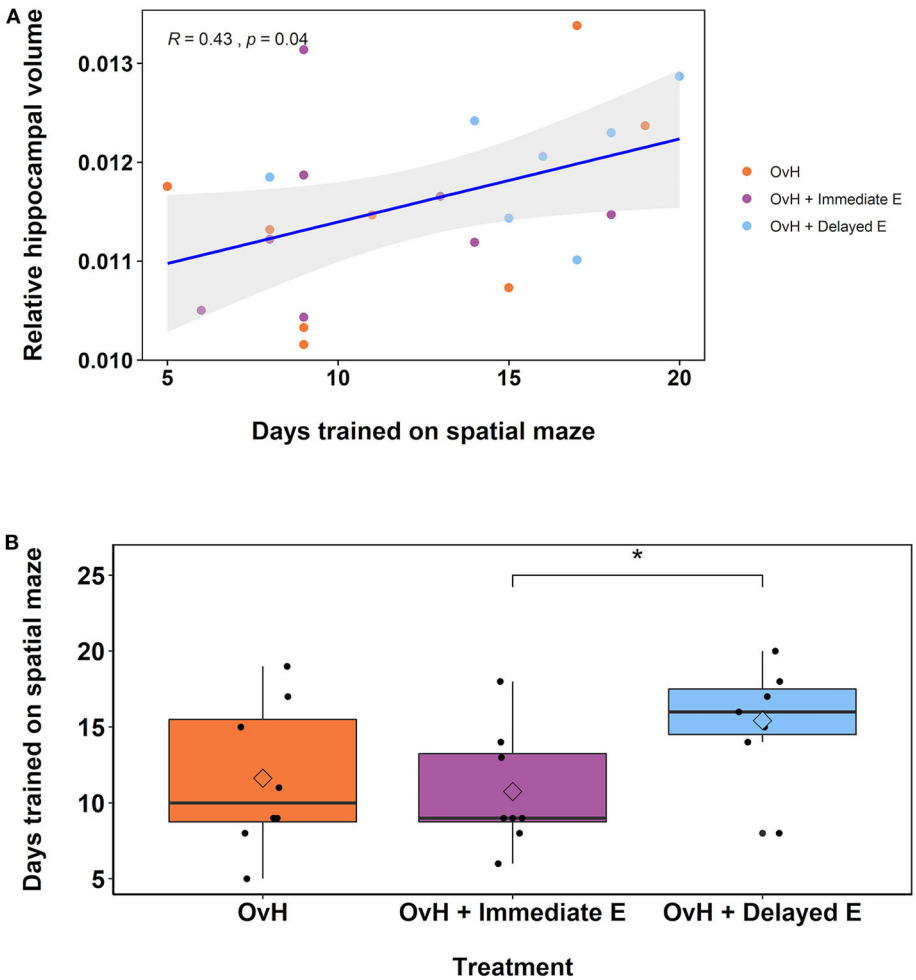

FIGURE 3 | (A) There was a significant correlation between the relative hippocampal volumes from the second imaging session and the total number of days trained on the spatial maze task. The animals belonging to the different experimental groups are indicated in distinct colors. **(B)** Box plots demonstrating the difference between the OvH, OvH + Immediate E, and OvH + Delayed E groups with regard to the total number of days trained on the spatial maze task. The circles illustrate the scores of individual animals, while the diamonds within the box plots represent the group means. *$p < 0.05$ (uncorrected t-test). means. *$p < 0.05$ (uncorrected t-test).

Given the observation that the number of days of training on the spatial maze correlated with the hippocampal volumes, particularly at the 1-year imaging session, we assessed whether there was a difference in the total number of training days by treatment group. As shown in **Figure 3B**, there was a non-significant, but suggestive effect of treatment group on the total number of days trained, $F_{(2, 20)} = 2.51$, $p = 0.106$. Welch independent sample t-tests showed a difference between the OvH + Immediate E and OvH + Delayed E groups, $t_{(12.817)} = -2.337$, $p = 0.0364$, but not between the OvH and the OvH + Delayed E groups, $t_{(12.880)} = -1.693$, $p = 0.115$, or the OvH and the OvH + Immediate E groups, $t_{(13.389)} = 0.396$, $p = 0.698$.

Treatment Effects on Brain Volumes

In addition to assessing the relationship between cognitive performance and regional brain volumes, the longitudinal effects of the treatments on brain volume were also modeled. We modeled the time*group fixed effects for predicting each of the regional volumes. A significant fixed effect was observed where increased relative hippocampal volume over time was predicted only in the OvH + Delayed E group ($p = 0.0374$, **Figure 4**, **Table 4**). An ANOVA on the final imaging session data, after the OvH + Delayed E group received HRT, did not show overall group differences, $F_{(2, 13)} = 2.106$, $p = 0.161$.

Additionally, time alone significantly predicted increases in both the relative prefrontal ($p = 0.00301$; **Table 5**) and relative motor ($p = 0.00290$; **Table 6**) volumes. No fixed effects in this model predicted the relative amygdala volumes, although there was a trend, whereby in the OvH + Delayed E group the relative amygdala volumes increased over time (**Table 7**).

DISCUSSION

In this study, the effects of estradiol treatment on spatial maze performance in aged OvH macaques on an obesogenic diet were

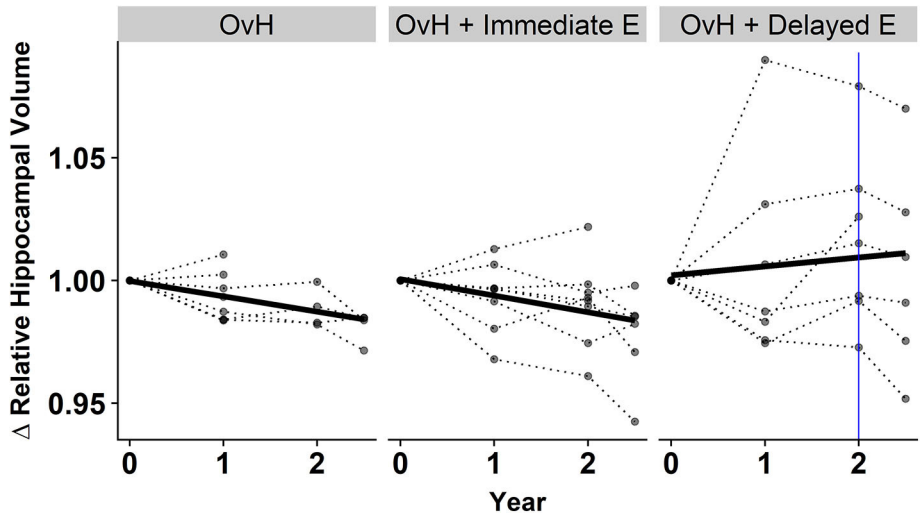

FIGURE 4 | There was a treatment x year interaction for predicting the relative hippocampal volumes from the results of the linear mixed effect model. The relative hippocampal volumes are presented as changes from the baseline hippocampal volumes for each subject. Individual animal trajectories are shown by connecting time points with dotted lines. Each treatment group is showed as its own panel. The blue line in the OvH + Delayed E group shows the time point where HRT began to be administered.

TABLE 4 | Linear mixed-effect model results predicting relative hippocampus volumes.

Fixed effects	Estimate	Standard Error	df	*t* value	Pr (>|t|)
(Intercept)	1.152e-02	3.203e-04	2.058e+01	35.956	<2e-16***
Year	−6.899e-05	3.949e-05	5.527e+01	−1.747	0.0862
OvH + Immediate E	−8.818e-06	4.529e-04	2.056e+01	−0.019	0.9847
OvH + Delayed E	4.266e-04	4.688e-04	2.056e+01	0.910	0.3734
Year: OvH + Immediate E	−3.476e-06	5.062e-05	5.515e+01	−0.069	0.9455
Year: OvH + Delayed E	1.097e-04	5.143e-05	5.514e+01	2.133	**0.0374***

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

TABLE 5 | Linear mixed-effect model results predicting relative prefrontal volumes.

Fixed effects	Estimate	Standard Error	df	*t* value	Pr (>|t|)
(Intercept)	1.110e-01	1.424e-03	2.143e+01	77.970	<2e-16***
Year	7.887e-04	2.542e-04	5.575e+01	3.102	0.00301**
OvH + Immediate E	2.846e-03	2.013e-03	2.140e+01	1.414	0.17178
OvH + Delayed E	1.978e-03	2.084e-03	2.139e+01	0.949	0.35317
Year: OvH + Immediate E	−1.222e-04	3.261e-04	5.551e+01	−0.375	0.70933
Year: OvH + Delayed E	−3.954e-04	3.313e-04	5.548e+01	−1.193	0.23782

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

TABLE 6 | Linear mixed-effect model results predicting relative motor volumes.

Fixed effects	Estimate	Standard Error	df	*t* value	Pr (>|t|)
(Intercept)	4.350e-02	9.830e-04	2.067e+01	44.249	<2e-16***
Year	3.844e-04	1.233e-04	5.535e+01	3.116	0.0029**
OvH + Immediate E	7.622e-04	1.390e-03	2.065e+01	0.548	0.5893
OvH + Delayed E	−3.535e-04	1.439e-03	2.065e+01	−0.246	0.8083
Year: OvH + Immediate E	−1.864e-04	1.581e-04	5.523e+01	−1.179	0.2434
Year: OvH + Delayed E	1.433e-04	1.606e-04	5.522e+01	0.892	0.3764

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

TABLE 7 | Linear mixed-effect model results predicting relative amygdala volumes.

Fixed effects	Estimate	Standard Error	df	*t* value	Pr (>|t|)
(Intercept)	8.971e-03	2.665e-04	2.052e+01	33.665	<2e-16***
Year	4.996e-05	3.073e-05	5.525e+01	1.626	0.1096
OvH + Immediate E	−1.623e-04	3.768e-04	2.051e+01	−0.431	0.6712
OvH + Delayed E	−1.586e-04	3.900e-04	2.051e+01	−0.407	0.6884
Year: OvH + Immediate E	−3.933e-05	3.938e-05	5.515e+01	−0.999	0.3223
Year: OvH + Delayed E	7.805e-05	4.001e-05	5.514e+01	1.950	0.0562

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

investigated. Additionally, the effects of immediate vs. delayed estrogen treatment on brain anatomy were assessed. Treatment with estradiol improved performance on spatial maze training, as measured by trials to criterion. In addition, average hippocampal volume increased over time in the delayed estrogen group, but not in the immediate estrogen or placebo groups. There was no effect of hormone treatment on volume in any other brain region investigated.

The finding that estrogen treatment improved acquisition efficiency of the spatial task in OvH macaques is supported by a body of previous literature in both rodent and monkey models, indicating that estradiol treatment improves spatial cognition in females deprived of endogenous estrogens (45–50). In aged surgically menopausal rhesus macaques, improvements in aspects of spatial working memory were reported (49, 51). However, while in the rodent literature largely improvements in spatial cognition with estrogen supplementation as well as during the high-estrogen phases of the estrus cycle have been reported [for a review see Korol and Pisani (46)], the monkey literature is more equivocal. In some studies, impaired spatial performance at high estrogen points in the cycle as well as with the maintenance of ovarian hormones in old age is reported (52, 53). This could be due to methodological differences between studies, as well as to the relative small sample sizes typical of nonhuman primate studies where groups consisting of 4–6 individuals are not uncommon.

The current study was performed in aged female macaques, making this model of menopause relevant to human health. Younger macaques have been shown to differ from older macaques in their cognitive changes after surgical menopause, as well as in changes associated with exogenous hormone supplementation after surgical menopause, highlighting the importance of using an age-appropriate model (48, 53).

Recently, Coleman et al. (16) reported on the effects of both delayed and immediate estrogen replacement on behavior performance compared to placebo-controls after OvH in the same aged rhesus macaques under obesogenic diets involved in the present study (16). Their results demonstrated that macaques under an obesogenic diet exhibited increased sedentary and anxiety-like behaviors, but that immediate estrogen replacement promoted activity and ameliorated anxiety-like behavior. In contrast, delayed estrogen (∼2 years after surgical menopause) showed equivocal results, highlighting that hormone replacement therapy starting shortly after the cessation of endogenous production of estrogens is more likely to yield health benefits.

The group by session interaction observed in predicting hippocampal volume was somewhat unexpected. The delayed estrogen group showed increasing hippocampal volumes over time compared to the other groups, even though the beneficial effects of HRT would be expected to be stronger with immediate hormone replacement after OvH. A closer look at the individual trajectories in hippocampal volumes (**Figure 4**) suggests some possible reasons underlying this effect. First, it appears that the volumetric increases between baseline and the year 1 imaging sessions drive the effect of an overall increase over the 2.5 years. However, this increase in volume precedes any HRT in the delayed treatment group. This highlights the discrepancy between the patterns observed between the delayed estrogen group and the OvH control, since neither group had received any estrogen. An analysis of group differences at only the last imaging time point, after the delayed estrogen group had received HRT, showed no overall group differences in relative hippocampal volume, adding further support to the idea that something other than the HRT was driving the difference in hippocampal volumes. This study provides some suggestive evidence that the difference might de due to the amount of training on the task given to the monkeys in the different groups. There was an unexpected suggestive, but non-significant, effect of treatment group on the total number of days the monkeys were trained on the task. There was also a significant relationship between hippocampal volume and the number of days of training on the spatial maze task. Taken together, these results suggest that the difference between the trajectories of hippocampal volumetric change may have more to do with the amount of time spent training on the task than the hormone replacement treatment.

While there was a correlation between the number of days of training and the hippocampal volume, there was no relationship between hippocampal volume and the actual performance on the task. This is an intriguing contrast, suggesting that *changes* in hippocampal volume over time might be more reflective of cognitive training, whereas *current* hippocampal volume might be more predictive of cognitive performance. For example, Shamy and colleagues demonstrated that hippocampal volume predicted acquisition of a delayed response spatiotemporal task, despite finding no evidence of age-related changes in hippocampal volumes (22). Hippocampal volumes have been shown to be particularly plastic to environmental factors over relatively short time periods. For example, in a recent study done

on eight expeditioners to a constrained and isolated environment in Antarctica, substantial average decreases in volume of about 7.2% were observed after a 14 month period (54). It is possible that the enrichment from a hippocampally-mediated task could act on hippocampal volumes over the training period and even offset or reverse potential declines from being housed in pens with smaller social units.

Rapid changes in hippocampal volume on the same time scale as those observed here have also been reported in previous animal studies. In mice, over the course of the 4–6 day estrus cycle, changes in hippocampal volume of 2–3%, which are in the range of the changes seen in the present study, have been observed (55). In humans, aerobic exercise increases hippocampal volume transiently after only 6 weeks of the intervention (56). Importantly, both studies found rapid changes in hippocampal volume in adult animals, indicating that adult hippocampal plasticity is sufficient to measurably change the volume of the hippocampus. Additionally, spatial learning can increase the size of the adult hippocampus, as illustrated by Wollett and Maguire's studies in London taxi drivers (57). The changes in hippocampal volume seen in our study could have been driven by the learning aspect of training on the spatial maze, or alternatively by the exercise inherent in the spatial maze training protocol.

Estrogen has known effects on weight due to its actions on food intake suppression (58–60). Additionally, adiposity has been linked to cognitive impairment, especially during aging (61). Thus, body weight could account for some of the cognitive changes seen in estrogen replacement regimens like those described here. These effects are difficult to separate, especially in small samples, and future studies should consider the effects of these modulating factors.

Some limitations of the present study include the generalizability of surgical menopause to natural menopause (however, older animals were used to approximate the equivalent age of clinical menopause), the lack of a non-obesogenic group of animals for comparison, as well as the relative small sample size typical for NHP studies (62). Some previous work has indicated that in humans as well as rodent models, surgical menopause results in different cognitive outcomes, often more adverse, than cognitive outcomes seen in natural or gradual menopause (63, 64). This is likely due to the fact that abrupt loss of hormones seems to be more deleterious for cognition than the more gradual loss seen in natural menopause. However, while natural menopause (as measured by serum estradiol and luteinizing hormone levels, as well as ovarian histology) has been observed in rhesus macaques, it was found in only a subset of studied animals and occurred in their late twenties, which is beyond the average lifespan of this species (65). Thus, surgical menopause in aged macaques is arguably the best alternative. The fact that all of the monkeys were old and under an obesogenic diet precludes the possibility of investigating diet-driven effects on anatomy and make it very difficult to observe any age-related declines in volumes. In addition, larger ROIs were chosen for analysis in order to increase the size of the volumes to look at possible changes and reduce comparisons. However, by choosing just a few ROIs that are relatively large, it is possible that more subtle differences from the smaller, functionally distinct regions that compose the larger regions and/or other brain regions might have been missed.

In summary, this study provides evidence that immediate estrogen replacement after menopause, under an obesogenic diet, contributes to improved performance on the acquisition of a spatial task. Further, this study highlights the potential importance of the course and type of training in predicting gross measures of hippocampal anatomy. This is a particularly critical point, since the training itself and not the performance, was related to hippocampal volume in the present study. Thus, future research on brain health parameters should consider equalizing training time instead of training to a criterion, controlling for the timing of the training, and investigating baseline differences in brain volumes if possible. While the research presented here shows a promising effect of immediate HRT on ameliorating menopause-related cognitive decline under and obesogenic diet, more research is needed to determine the mechanisms driving this effect and its interactions with biological changes resulting from consuming a high-fat, high-sugar diet.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The animal study was reviewed and approved by OHSU IACUC committee.

AUTHOR CONTRIBUTIONS

JR directed, designed, and oversaw the performed analyses and edited the manuscript. CB and HU obtained funding, designed, and directed the overall study. SK directed and designed the MR imaging of the primates in this study. CK directed and oversaw the preprocessing of the anatomical images. ZL implemented the preprocessing of the images. PK conducted statistical analyses for the spatial maze task and shared the writing of the first draft of the manuscript with BZ. BZ designed and created the ROIs for each monkey, conducted the statistical analyses for the anatomical data, shared the writing of the manuscript with PK, and generated the figures. All authors contributed to the editing of the manuscript, reviewed, and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2020.00539/full#supplementary-material

REFERENCES

1. Takahashi TA, Johnson KM. Menopause. *Med Clin North Am.* (2015) 99:521–34. doi: 10.1016/j.mcna.2015.01.006
2. Monteleone P, Mascagni G, Giannini A, Genazzani AR, Simoncini T. Symptoms of menopause - Global prevalence, physiology and implications. *Nat Rev Endocrinol.* (2018) 14:199–215. doi: 10.1038/nrendo.2017.180
3. McCarrey AC, Resnick SM. Postmenopausal hormone therapy and cognition. *Horm Behav.* (2015) 74:167–72. doi: 10.1016/j.yhbeh.2015.04.018
4. Koebele S V., Bimonte-Nelson HA. Modeling menopause: the utility of rodents in translational behavioral endocrinology research. *Maturitas.* (2016) 87:5–17. doi: 10.1016/j.maturitas.2016.01.015
5. Ettinger B, Wang SM, Leslie RS, Patel BV, Boulware MJ, Mann ME, et al. Evolution of postmenopausal hormone therapy between 2002 and 2009. *Menopause N Y N.* (2012) 19:610–5. doi: 10.1097/gme.0b013e31823a3e5d
6. Coker LH, Espeland MA, Rapp SR, Legault C, Resnick SM, Hogan P, et al. Postmenopausal hormone therapy and cognitive outcomes: the women's health initiative memory study (WHIMS). *J Ster Biochem Mol Biol.* (2010) 118:304–10. doi: 10.1016/j.jsbmb.2009.11.007
7. Harman SM, Naftolin F, Brinton EA, Judelson DR. Is the estrogen controversy over? Deconstructing the Women's Health Initiative study: a critical evaluation of the evidence. *Ann N Y Acad Sci.* (2005) 1052:43–56. doi: 10.1196/annals.1347.004
8. Maki PM, Sundermann E. Hormone therapy and cognitive function. *Hum Reprod Update.* (2009) 15:667–81. doi: 10.1093/humupd/dmp022
9. Leblanc ES, Janowsky J, Chan BKS, Nelson HD. Hormone replacement therapy and cognition: Systematic review and meta-analysis. *J Am Med Assoc.* (2001) 285:1489–99. doi: 10.1001/jama.285.11.1489
10. Lethaby A, Hogervorst E, Richards M, Yesufu A, Yaffe K. Hormone replacement therapy for cognitive function in postmenopausal women. *Cochrane Database Syst Rev.* (2008) 1:CD003122. doi: 10.1002/14651858.CD003122.pub2
11. Low LF, Anstey KJ. Hormone replacement therapy and cognitive performance in postmenopausal women - a review by cognitive domain. *Neurosci Biobehav Rev.* (2006) 30:66–84. doi: 10.1016/j.neubiorev.2005.05.003
12. Freeman LR, Haley-Zitlin V, Rosenberger DS, Granholm A-C. Damaging effects of a high-fat diet to the brain and cognition: a review of proposed mechanisms. *Nutr Neurosci.* (2014) 17:241–51. doi: 10.1179/1476830513Y.0000000092
13. Smith E, Hay P, Campbell L, Trollor JN. A review of the association between obesity and cognitive function across the lifespan: implications for novel approaches to prevention and treatment. *Obes Rev.* (2011) 12:740–55. doi: 10.1111/j.1467-789X.2011.00920.x
14. Hales CM, Fryar CD, Carroll MD, Freedman DS, Ogden CL. Trends in obesity and severe obesity prevalence in US youth and adults by sex and age, 2007-2008 to 2015-2016. *JAMA.* (2018) 319:1723–5. doi: 10.1001/jama.2018.3060
15. Ritchie H, Roser M. *Diet Compositions*. Our World Data (2017). Available online at: https://ourworldindata.org/diet-compositions
16. Coleman K, Robertson ND, Maier A, Bethea CL. Effects of immediate or delayed estradiol on behavior in old menopausal macaques on obesogenic diet. *J Obes.* (2018) 2018:e1810275. doi: 10.1155/2018/1810275
17. Wada T, Sameshima A, Yonezawa R, Morita M, Sawakawa K, Tsuneki H, et al. Impact of central and peripheral estrogen treatment on anxiety and depression phenotypes in a mouse model of postmenopausal obesity. *PLoS ONE.* (2018) 13:e209859. doi: 10.1371/journal.pone.0209859
18. de Jesús Rovirosa-Hernández M, María, González MH, Guevara-Pérez MÁ, García-Orduña F, de los Ángeles Aguilar-Tirado A, et al. "Menopause in Nonhuman Primates: A Comparative Study with Humans," In: *A Multidisciplinary Look at Menopause*. IntechOpen, (2017). 2017:25. doi: 10.5772/intechopen.69657
19. Edson MA, Nagaraja AK, Matzuk MM. The mammalian ovary from genesis to revelation. *Endocr Rev.* (2009) 30:624–712. doi: 10.1210/er.2009-0012
20. Colman RJ. Non-human primates as a model for aging. *Biochim Biophys Acta - Mol Basis Dis.* (2018) 1864:2733–41. doi: 10.1016/j.bbadis.2017.07.008
21. Hara Y, Rapp PR, Morrison JH. Neuronal and morphological bases of cognitive decline in aged rhesus monkeys. *Age.* (2012) 34:1051–73. doi: 10.1007/s11357-011-9278-5
22. Shamy JL, Habeck C, Hof PR, Amaral DG, Fong SG, Buonocore MH, et al. Volumetric correlates of spatiotemporal working and recognition memory impairment in aged rhesus monkeys. *Cereb Cortex.* (2011) 21:1559–73. doi: 10.1093/cercor/bhq210
23. Herndon JG, Moss MB, Rosene DL, Killiany RJ. Patterns of cognitive decline in aged rhesus monkeys. *Behav Brain Res.* (1997) 87:25–34. doi: 10.1016/S0166-4328(96)02256-5
24. Roberts JA, Gilardi KVK, Lasley B, Rapp PR. Reproductive senescence predicts cognitive decline in aged female monkeys. *NeuroReport.* (1997) 8:2047–51.
25. Raz N, Gunning FM, Head D, Dupuis JH, McQuain J, Briggs SD, et al. Selective aging of the human cerebral cortex observed in vivo: differential vulnerability of the prefrontal gray matter. *Cereb Cortex.* (1997) 7:268–82. doi: 10.1093/cercor/7.3.268
26. Raz N, Lindenberger U, Rodrigue KM, Kennedy KM, Head D, Williamson A, et al. Regional brain changes in aging healthy adults: general trends, individual differences and modifiers. *Cereb Cortex N Y N.* (2005). 15:1676–89. doi: 10.1093/cercor/bhi044
27. Raz N, Rodrigue KM, Head D, Kennedy KM, Acker JD. Differential aging of the medial temporal lobe: a study of a five-year change. *Neurology.* (2004) 62:433–8. doi: 10.1212/01.wnl.0000106466.09835.46
28. Raz N, Ghisletta P, Rodrigue KM, Kennedy KM, Lindenberger U. Trajectories of brain aging in middle-aged and older adults: regional and individual differences. *Neuroimage.* (2010) 51:501–11. doi: 10.1016/j.neuroimage.2010.03.020
29. Shamy JLT, Buonocore MH, Makaron LM, Amaral DG, Barnes CA, Rapp PR. Hippocampal volume is preserved and fails to predict recognition memory impairment in aged rhesus monkeys (Macaca mulatta). *Neurobiol Aging.* (2006) 27:1405–15. doi: 10.1016/j.neurobiolaging.2005.07.019
30. Hara Y, Park CS, Janssen WGM, Roberts MT, Morrison JH, Rapp PR. Synaptic correlates of memory and menopause in the hippocampal dentate gyrus in rhesus monkeys. *Neurobiol Aging.* (2012) 33:421.e17-421.e28. doi: 10.1016/j.neurobiolaging.2010.09.014
31. Purnell JQ, Urbanski HF, Kievit P, Roberts CT, Bethea CL. Estradiol replacement timing and obesogenic diet effects on body composition and metabolism in postmenopausal macaques. *Endocrinology.* (2019) 160:899–914. doi: 10.1210/en.2018-00884
32. Urbanski HF, Mueller K, Bethea CL. Effect of an obesogenic diet on circadian activity and serum hormones in old monkeys. *Endocr Connect.* (2017) 6:380–3. doi: 10.1530/EC-17-0108
33. Avants BB, Epstein CL, Grossman M, Gee JC. Symmetric diffeomorphic image registration with cross-correlation: evaluating automated labeling of

elderly and neurodegenerative brain. *Med Image Anal.* (2008) 12:26–41. doi: 10.1016/j.media.2007.06.004
34. Tustison NJ, Avants BB, Cook PA, Zheng Y, Egan A, Yushkevich PA, et al. N4ITK: improved N3 bias correction. *IEEE Trans Med Imaging.* (2010) 29:1310–20. doi: 10.1109/TMI.2010.2046908
35. Rohlfing T, Kroenke CD, Sullivan EV, Dubach MF, Bowden DM, Grant KA, et al. The INIA19 template and neuromaps atlas for primate brain image parcellation and spatial normalization. *Front Neuroinformatics.* (2012) 6:27. doi: 10.3389/fninf.2012.00027
36. Wickham H, François R, Henry L, Müller K. *Dplyr: A Grammar of Data Manipulation.* (2019).
37. Fox J, Weisberg S. *An R Companion to Applied Regression*. Third Sage (2019).
38. Wickham H. *Ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York (2016).
39. Kassambara A. *Ggpubr: "ggplot2" Based Publication Ready Plots.* (2018).
40. Ahlmann-Eltze C. *Ggsignif: Significance Brackets for "Ggplot2."* (2019).
41. Bates D, Mächler M, Bolker B, Walker S. Fitting linear mixed-effects models using lme4. *J Stat Softw.* (2015) 67:1–48. doi: 10.18637/jss.v067.i01
42. Kuznetsova A, Brockhoff PB, Christensen RHB. lmerTest package: Tests in linear mixed effects models. *J Stat Softw.* (2017) 82:1–26. doi: 10.18637/jss.v082.i13
43. Revelle W. *Psych: Procedures for Psychological, Psychometric, and Personality Research*. Northwestern University (2018)
44. Dragulescu AA, Arendt C. *Xlsx: Read, Write, Format Excel 2007 and Excel 97/2000/XP/2003 Files.* (2018).
45. Markham JA, Pych JC, Juraska JM. Ovarian hormone replacement to aged ovariectomized female rats benefits acquisition of the Morris water maze. *Horm Behav.* (2002) 42:284–93. doi: 10.1006/hbeh.2002.1819
46. Korol DL, Pisani SL. Estrogens and cognition: Friends or foes?: An evaluation of the opposing effects of estrogens on learning and memory. *Horm Behav.* (2015) 74:105–15. doi: 10.1016/J.YHBEH.2015.06.017
47. Kundu P, Korol DL, Bandara S, Monaikul S, Ondera CE, Helferich WG, et al. Licorice root components mimic estrogens in an object location task but not an object recognition task. *Horm Behav.* (2018) 103:2. doi: 10.1016/j.yhbeh.2018.06.002
48. Kohama SG, Renner L, Landauer N, Weiss AR, Urbanski HF, Park B, et al. Effect of ovarian hormone therapy on cognition in the aged female rhesus macaque. *J Neurosci.* (2016) 36:10416–24. doi: 10.1523/JNEUROSCI.0909-16.2016
49. Rapp PR, Morrison JH, Roberts JA. Cyclic estrogen replacement improves cognitive function in aged ovariectomized rhesus monkeys. *J Neurosci.* (2003) 23:5708–14. doi: 10.1523/jneurosci.23-13-05708.2003
50. Voytko M Lou, Tinkler GP, Browne C, Tobin JR. Neuroprotective effects of estrogen therapy for cognitive and neurobiological profiles of monkey models of menopause. *Am J Primatol.* (2009) 71:794–801. doi: 10.1002/ajp.20705
51. Lacreuse A, Wilson ME, Herndon JG. Estradiol, but not raloxifene, improves aspects of spatial working memory in aged ovariectomized rhesus monkeys. *Neurobiol Aging.* (2002) 23:589–600. doi: 10.1016/S0197-4580(02)00002-7
52. Lacreuse A, Verreault M, Herndon JG. Fluctuations in spatial recognition memory across the menstrual cycle in female rhesus monkeys. *Psychoneuroendocrinology.* (2001) 26:623–39. doi: 10.1016/S0306-4530(01)00017-8
53. Lacreuse A. Effects of ovarian hormones on cognitive function in nonhuman primates. *Neuroscience.* (2006) 138:859–67. doi: 10.1016/j.neuroscience.2005.09.006
54. Stahn AC, Gunga H-C, Kohlberg E, Gallinat J, Dinges DF, Kühn S. Brain changes in response to long Antarctic expeditions. *N Engl J Med.* (2019) 381:2273–5. doi: 10.1056/NEJMc1904905
55. Qiu LR, Germann J, Spring S, Alm C, Vousden DA, Palmert MR, et al. Hippocampal volumes differ across the mouse estrous cycle, can change within 24 hours, and associate with cognitive strategies. *NeuroImage.* (2013) 83:593–8. doi: 10.1016/j.neuroimage.2013.06.074
56. Thomas AG, Dennis A, Rawlings NB, Stagg CJ, Matthews L, Morris M, et al. Multi-modal characterization of rapid anterior hippocampal volume increase associated with aerobic exercise. *NeuroImage.* (2016) 131:162–70. doi: 10.1016/j.neuroimage.2015.10.090
57. Woollett K, Maguire EA. Acquiring "the knowledge" of London's layout drives structural brain changes. *Curr Biol.* (2011) 21:2109–14. doi: 10.1016/j.cub.2011.11.018
58. Czaja JA. Ovarian influences on primate food intake: assessment of progesterone actions. *Physiol Behav.* (1978) 21:923–8. doi: 10.1016/0031-9384(78)90167-1
59. Rosenblatt H, Dyrenfurth I, Ferin M, vande Wiele RL. Food intake and the menstrual cycle in rhesus monkeys. *Physiol Behav.* (1980) 24:447–9. doi: 10.1016/0031-9384(80)90234-6
60. Wilson ME, Moore CJ, Ethun KF, Johnson ZP. Understanding the control of ingestive behavior in primates. *Horm Behav.* (2014) 66:86–94. doi: 10.1016/j.yhbeh.2014.04.005
61. Naderali EK, Ratcliffe SH, Dale MC. Obesity and Alzheimer's disease: a link between body weight and cognitive function in old age. *Am J Alzheimers Dis Other Demen.* (2009) 24:445–9. doi: 10.1177/1533317509348208
62. Carvalho C, Gaspar A, Knight A, Vicente L. Ethical and scientific pitfalls concerning laboratory research with non-human primates, and possible solutions. *Animals.* (2019) 9:9010012. doi: 10.3390/ani9010012
63. Henderson VW, Sherwin BB. Surgical versus natural menopause. *Menopause.* (2007) 14(Suppl. 1):572–9. doi: 10.1097/gme.0b013e31803df49c
64. Acosta JI, Mayer L, Talboom JS, Tsang CW, Smith CJ, Enders CK, et al. Transitional versus surgical menopause in a rodent model: Etiology of ovarian hormone loss impacts memory and the acetylcholine system. *Endocrinology.* (2009) 150:4248–59. doi: 10.1210/en.2008-1802
65. Walker ML, Herndon JG. Menopause in nonhuman primates? *Biol Reprod.* (2008) 79:398–406. doi: 10.1095/biolreprod.108.068536

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OPINION
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Gender Differences in Non-sex Linked Disorders: Insights From Huntington's Disease

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INTRODUCTION

Gender plays a role in the prevalence and natural course of several disorders. It is apparent in neurodegenerative diseases like Parkinson's disease more prevalent in men, Alzheimer's disease more prevalent in women, or Lewy body dementia more prevalent in men. Rarely, however, recently more often, in autosomally conditioned diseases, gender differences are being identified (1–21).

Huntington's disease (HD) as a rare neurodegenerative (recently reported peripheral tissue involvement), incurable—therefore still displaying natural course—disorder with an autosomal dominant pattern of inheritance with full penetrance in most cases (22). Therefore it was not explored for gender differences for many years. Gender was considered in HD, however, with respect to a parent of an HD patient, as it was observed that disease inherited from a father resulted in symptoms anticipation, namely earlier onset and faster progression than when inherited from the mother (23–25). It was later explained by a higher probability for elongation of the causative HD mutation during spermatogenesis, than during oogenesis (26, 27). Very quickly after identification of a causative mutation in 1993, it was observed that there is a negative correlation between the number of CAG repeats in the causative gene (expansion of causative mutation) and onset age, namely larger expansion of CAG repetitive sequence in the HD gene resulted in earlier HD symptoms onset (28). The rate of HD progression was explored early after the causative gene identification. Interesting results were observed in small groups (29–31), but lack of proper tools for progression measurement resulted in a lack of any results indicating a correlation between CAG repeats expansion and rate of the disease progression (32–35). First important (36) and deciding findings confirming that the rate of HD progression is dependent on CAG repeats number were described in 2008 in a large HD cohort study (37). In the same year, another small study indicated gender differences in HD patients (38).

SEX PLAYS A ROLE

The finding described in the research paper published in 2008 (38) came rather as a surprise during the study's data analysis (38). First, the study was based on just 41 HD patients: 24 women (38). A statistically significant correlation between the number of CAG repeats and scores in Unified Huntington's Rating Scale (UHDRS) subscales—motor, cognitive, functional, independence and total functional capacity (TFC)—were identified in women but not in men. Moreover, time from onset correlated with scores in above listed UHDRS subscales in women only. These findings provided insight allowing further investigation to study gender differences in HD.

In 2013, a large cohort analysis based on 1,267 HD affected individuals was performed (39). This study based on data collected in REGISTRY, an Observational Study of the European Huntington's

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Disease Network (EHDN) (40) population, and was aimed to identify gender differences in several HD features including differences in the rate of the disease progression based on following annual visits when patients were assessed in UHDRS subscales. The study was controlled for several environmental factors. The most important finding of this study was identification of a significant gender difference in the rate of HD progression controlled among other things for disease burden (calculated variable incorporating CAG repeats number in larger allele and age): DB = (CAG number in the larger allele – 35.5) × age in years (41). Disease burden reflects the stage of brain pathology in HD and includes the factor responsible for 70% of the variability in HD onset, namely CAG repeats number in mutated *HTT* allele (42, 43). Both genders did not differ for the disease burden or for onset age, but the progression rate in women was faster. Other gender differences were identified in cross-sectional analysis for years of education (men studied longer), presence of depression (in women more often), history of depression (in women more often), alcohol abuse (more often in men), and cigarette smoking (more often in men). To explain the observed difference in the rate of HD progression, the longitudinal analysis was controlled for several variables like disease duration, years of education, presence of depression, depressive episodes in the past, history of psychotic disturbances, history of obsessive-compulsive disorders, history of suicidal ideation, smoking, alcohol abuse, and drug usage. These variables were used as confounders in the longitudinal analysis did not clarify the inter-gender differences in progression rate. The above-described study provided solid evidence to confirm the presence of gender differences in HD clinical picture and what is more important in rate of HD progression.

Above mentioned findings were confirmed and explored in more detail in 2018 in another study, which aimed at a gender effect in particular symptomatic domains of HD and their contribution to functional abilities and quality of life (44). The clinical picture of HD is formed by three major symptomatic domains, namely motor symptoms (e.g., clumsiness, chorea, dystonia), cognitive impairment (subcortical dementia), and behavioral disturbances (e.g., depression, apathy, irritability, and aggression) (22). All of these domains contribute to functional abilities of affected individuals but the impact of a particular domain for progression of functional disability was not clearly explained in previously published studies (45–48). Considering the study (39), it was important to evaluate also gender role in this contribution. The study (44) based on 2,191 HD affected individuals (1,080 women), REGISTRY study's participants, annually examined in UHDRS for several years, was controlled for the same factors as previously described study. In women significantly stronger correlations between all symptomatic spheres and HD progression rate was observed, motor domain contributed the most, followed by cognitive and behavioral; moreover, motor symptoms were responsible for more variability in functional abilities in women than in men, while cognitive symptoms had an opposite contribution (more variability in men than in women). This means that motor symptoms were the strongest contributors to functional abilities in both genders, particularly in women, although cognitive symptoms are more important in men for functional abilities than in women.

One year after the previously described research paper was published, another cohort study delivered surprising results (49). Here it was observed in a huge cohort of 67 millions of Americans performed between 2003 and 2016 that HD has a significantly higher prevalence in women estimated on 7.05 per 100,000 than in men, 6.91 per 100,000. This result may suggest a more severe HD pathologic process in women.

There is also evidence based on pre-clinical settings where in animal HD models gender differences were identified. The first important research paper was published in 2007; in the murine model, it was observed that a lower level of extracellular ascorbate in the striatum in males reflected a more severe phenotype than in females (50). In 2008 another study suggested the neuroprotective effect of 17b-estradiol in females in rat HD model (51). In 2016 in BACHD mouse model of Huntington's disease, it was found that circadian activity levels, rhythm precision, and behavioral fragmentation are more severe in males (52). Finally, in 2019 more severe deficits in neuroprotective nitric oxide synthase activity in the HD cortex and striatum were observed mostly in Q175 males of HD mouse model (53). In contrast to human studies, animal research results indicate a more severe picture in males.

DISCUSSION

Above mentioned studies indicate presence of gender differences in HD. The limitation factor of the finding (39, 44) is a lack of inclusion of two important, however difficult to be assessed variables, namely concomitant disorders and medications. There is still a question whether differences in these two variables could explain gender differences reported so far (39, 44). Depression was considered as a confounder in both studies but did not explain differences in HD progression rate between genders. The prevalence of non-psychiatric concomitant disorders does not differ between genders as reported in recently published paper (54). It seems that both genders are not treated differently in HD but this should be explored in detail.

Also animal studies, confirming gender differences, bring contrary results of gender burden, displaying their ample limitations in explanation of pathologic processes behind observed differences (50–53). The findings in animal models suggest their imperfection rather (55). Hormonal disturbances observed in an animal model (51) and in HD patients (56, 57) could be supportive to explain the phenomenon, but a cause of the gender differences in HD seems to be more complex and require future studies in larger HD cohorts.

A recently published study on huntingtin's or *HTT* gene role in neurodevelopment in boys and girls (58) showed that in girls a longer CAG sequence in larger allele (still in normal range) correlated with thicker cortex and better cognition when in boys this impact was weaker being restricted mainly to lower putamen/cerebellum volume ratio in boys with higher CAG repeats number (58). This observation could suggest that also

HTT gene mutation in women could exert stronger impact than in men.

The gender differences were identified in other neurodegenerative movement disorders caused by a dynamic mutation (CAG repeats expansion, similar to this causing HD, so called because number of CAG repeats may change during meiosis, or during mitosis) located in autosomal genes, namely in spinocerebellar ataxia (SCA) type 3 and 6 (13). In women, the progression of non-ataxia motor symptoms was faster than in men in those diseases. This effect in SCA type 3 was confirmed in a follow up study on the same European cohort (14). Moreover, faster progression in women with SCA 2 and 3 was reported in other studies (15, 16). This is consistent with findings in HD, suggesting that gender differences could be related to specific dynamic mutation mechanism, making it different than other monogenetic disorders.

Moreover, gender differences were described in various *CACNA1A* gene mutations, e.g., SCA 6 as an example of trinucleotides extension, episodic ataxia type 2 (EA2) in case of loss-of-function, and familial hemiplegic migraine-1 (FHM1) an example of gain-of-function missense mutation. In women, EA2 and FHM1 phenotypes were present when in men with the same mutation not (17). Interestingly also in *CACNA1S* gene mutation, which is another channel disorder, namely hypokalemic periodic paralysis, gender differences based on reduced penetrance in women and full in men were identified (18).

Gender differences in monogenetic autosomal neurological diseases do not always result in worsened progression or more severe clinical picture in one gender. This was confirmed by observation in Neurofibromatosis Type 1 (NF1). In a study conducted across girls and boys using "MOXO test," which is being used for patients with ADHD, it was found that while the boys performed better than girls in attention and timing, they exhibited worse scores for impulsivity and hyperactivity. The observed difference does not comply with findings in general ADHD population, therefore a contribution of *NF1* gene mutation is likely (12). Gender differences in monogenetic neurologic disorders can be also race dependent as in the case of facioscapulohumeral muscular dystrophy type 1 (FSHD). The mutation in this disease similarly to this in HD is located in chromosome 4 and is related to nucleic acid length but in contrast refers to another place on the chromosome and results not from extension but from the contraction of D4Z4 repeats number. In a Korean study, it was identified that women are more seriously affected than men (1) when in studies based on European cohorts, men were more seriously affected (2–5). It was controlled for age and D4Z4 repeats number (1). In another neuromuscular monogenetic disease, namely Charcot–Marie–Tooth type 1A, women present a more severe phenotype and earlier onset age (6–9). It seems that gender differences, apart from those observed in SCA, in monogenetic neurological diseases do not reflect differences described in HD.

In monogenetic non-neurologic diseases, the more severe picture was described in men with autosomal dominant polycystic kidney disease (19, 20). In thalassemia major bone mass reduction was more prevalent and more severe in men. This finding was however accompanied by another one that women were more vulnerable for bones mass loss when hypogonadism co-existed, therefore, hormonal contribution seems to play an important role in observed differences (21). Hormonal factors partially explain more prevalent clinical manifestation of acute intermittent porphyria in women, in which the acute attacks occur rarely before puberty and its frequency and severity decline after menopause (10). Lower level of estrogens in postmenopausal women has been suggested to explain also more severe atherosclerosis in women affected by familial hypercholesterolemia (11).

Sex differences in common movement disorders were nicely summarized in a review published earlier this year (59). In movement's disorders, diseases' severity fluctuations related to menstrual cycle in women were reported in female patients with Parkinson's disease (60–62) and dystonia (63). It shed light on the effect of estrogens, indicating potential worsening in post-menopausal women (64). Future research in HD should, therefore, consider clinical differences between pre- and post-menopausal women included these on HRT to elucidate this effect in HD.

Currently, it is clear that there are gender differences in non-sex-linked genetic disorders. They are not well-understood; therefore, they ought to be investigated further, as they could shed light on disease mechanisms and pathogenesis. In diseases where gender differences were identified, the modeling, design, and interpretation of observational studies and clinical trials should be performed with respect to the gender of participants.

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All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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REFERENCES

1. Park HJ, Hong JM, Lee JH, Lee HS, Shin HY, Kim SM, et al. Low D4Z4 copy number and gender difference in Korean patients with facioscapulohumeral muscular dystrophy type 1. *Neuromuscul Disord.* (2015) 25:859–64. doi: 10.1016/j.nmd.2015.08.004
2. Ricci G, Scionti I, Sera F, Govi M, D'Amico R, Frambolli I, et al. Large scale genotype-phenotypeanalyses indicate that novel prognostic tools are required for families with facioscapulohumeral muscular dystrophy. *Brain.* (2013) 136:3408–17. doi: 10.1093/brain/awt226
3. Sakellariou P, Kekou K, Fryssira H, Sofocleous C, Manta P, Panousopoulou A, et al. Mutation spectrum and phenotypic manifestation in FSHD Greek patients. *Neuromuscul Disord.* (2012) 22:339–49. doi: 10.1016/j.nmd.2011.11.001
4. Tonini MM, Passos-Bueno MR, Cerqueira A, Matioli SR, Pavanello R, Zatz M. Asymptomatic carriers and gender differences in

facioscapulohumeral muscular dystrophy (FSHD). *Neuromuscul Disord.* (2004) 14:33–8. doi: 10.1016/j.nmd.2003.07.001
5. Zatz M, Marie SK, Cerqueira A, Vainzof M, Pavanello RC, Passos-Bueno MR. The facioscapulohumeral muscular dystrophy (FSHD1) gene affects males more severely and more frequently than females. *Am J Med Genet.* (1998) 77:155–61. doi: 10.1002/(SICI)1096-8628(19980501)77:2<155::AID-AJMG9>3.0.CO;2-R
6. Padua L, Aprile I, Cavallaro T, Commodari I, La Torre G, Pareyson D, et al. Variables influencing quality of life and disability in Charcot Marie Tooth (CMT) patients: Italian multicentre study. *Neurol Sci.* (2006) 27:417–23. doi: 10.1007/s10072-006-0722-8
7. Padua L, Shy ME, Aprile I, Cavallaro T, Pareyson D, Quattrone A, et al. Correlation between clinical/neurophysiological findings and quality of life in Charcot-Marie-Tooth type 1A. *J Peripher Nerv Syst.* (2008) 13:64–70. doi: 10.1111/j.1529-8027.2008.00159.x
8. Vinci P, Serrao M, Millul A, Deidda A, De Santis F, Capici S, et al. Quality of life in patients with Charcot-Marie-Tooth disease. *Neurology.* (2005) 65:922–4. doi: 10.1212/01.wnl.0000176062.44360.49
9. Colomban C, Micallef J, Lefebvre MN, Dubourg O, Gonnaud PM, Stojkovic T, et al. Clinical spectrum and gender differences in a large cohort of Charcot-Marie-Tooth type 1A patients. *J Neurol Sci.* (2014) 336:155–60. doi: 10.1016/j.jns.2013.10.029
10. Schuurmans MM, Schneider-Yin X, Rüfenacht UB, Schnyder C, Minder CE, Puy H, et al. Influence of age and gender on the clinical expression of acute intermittent porphyria based on molecular study of porphobilinogen deaminase gene among Swiss patients. *Mol Med.* (2001) 7:535–42. doi: 10.1007/BF03401859
11. Mattina A, Giammanco A, Giral P, Rosenbaum D, Carrié A, Cluzel P, et al. Polyvascular subclinical atherosclerosis in familial hypercholesterolemia: the role of cholesterol burden and gender. *Nutr Metab Cardiovasc Dis.* (2019) 29:1068–76. doi: 10.1016/j.numecd.2019.06.015
12. Cohen R, Halevy A, Aharon S, Shuper A. Attention deficit hyperactivity disorder in neurofibromatosis type 1: evaluation with a continuous performance test. *J Clin Neurol.* (2018) 14:153–7. doi: 10.3988/jcn.2018.14.2.153
13. Jacobi H, Bauer P, Giunti P, Labrum R, Sweeney MG, Charles P, et al. The natural history of spinocerebellar ataxia type 1, 2, 3, and 6: a 2-year follow-up study. *Neurology.* (2011) 77:1035–41. doi: 10.1212/WNL.0b013e31822e7ca0
14. Jacobi H, du Montcel ST, Bauer P, Giunti P, Cook A, Labrum R, et al. Long-term disease progression in spinocerebellar ataxia types 1, 2, 3, and 6: a longitudinal cohort study. *Lancet Neurol.* (2015) 14:1101–8. doi: 10.1016/S1474-4422(15)00202-1
15. Klockgether T, Lüdtke R, Kramer B, Abele M, Bürk K, Schöls L, et al. The natural history of degenerative ataxia: a retrospective study in 466 patients. *Brain.* (1998) 121:589–600. doi: 10.1093/brain/121.4.589
16. França MC Jr, D'Abreu A, Nucci A, Cendes F, Lopes-Cendes I. Progression of ataxia in patients with Machado-Joseph disease. *Mov Disord.* (2009) 24:1387–90. doi: 10.1002/mds.22627
17. Pradotto L, Mencarelli M, Bigoni M, Milesi A, Di Blasio A, Mauro A. Episodic ataxia and SCA6 within the same family due to the D302N CACNA1A gene mutation. *J Neurol Sci.* (2016) 371:81–4. doi: 10.1016/j.jns.2016.10.029
18. Li F, Li QQ, Tan ZX, Zhang SY, Liu J, Zhao EY, et al. A novel mutation in CACNA1S gene associated with hypokalemic periodic paralysis which has a gender difference in the penetrance. *J Mol Neurosci.* (2012) 46:378–83. doi: 10.1007/s12031-011-9596-1
19. Ishikawa I, Maeda K, Nakai S, Kawaguchi Y. Gender difference in the mean age at the induction of hemodialysis in patients with autosomal dominant polycystic kidney disease. *Am J Kidney Dis.* (2000) 35:1072–5. doi: 10.1016/S0272-6386(00)70042-4
20. Gretz N, Ceccherini I, Kränzlin B, Klöting I, Devoto M, Rohmeiss P, et al. Gender-dependent disease severity in autosomal polycystic kidney disease of rats. *Kidney Int.* (1995) 48:496–500. doi: 10.1038/ki.1995.319
21. Kyriakou A, Savva SC, Savvides I, Pangalou E, Ioannou YS, Christou S, et al. Gender differences in the prevalence and severity of bone disease in thalassaemia. *Pediatr Endocrinol.* (2008) 6(Suppl 1):116–22.
22. Walker FO. Huntington's disease. *Lancet.* (2007) 369:218–28. doi: 10.1016/S0140-6736(07)60111-1
23. Telenius H, Kremer HP, Theilmann J, Andrew SE, Almqvist E, Anvret M, et al. Molecular analysis of juvenile Huntington disease: the major influence on (CAG)n repeat length is the sex of the affected parent. *Hum Mol Genet.* (1993) 2:1535–40. doi: 10.1093/hmg/2.10.1535
24. Trottier Y, Biancalana V, Mandel JL. Instability of CAG repeats in Huntington's disease: relation to parental transmission and age of onset. *J Med Genet.* (1994) 31:377–82. doi: 10.1136/jmg.31.5.377
25. Nørremølle A, Sørensen SA, Fenger K, Hasholt L. Correlation between magnitude of CAG repeat length alterations and length of the paternal repeat in paternally inherited Huntington's disease. *Clin Genet.* (1995) 47:113–7. doi: 10.1111/j.1399-0004.1995.tb03941.x
26. Aziz NA, van Belzen MJ, Coops ID, Belfroid RD, Roos RA. Parent-of-origin differences of mutant HTT CAG repeat instability in Huntington's disease. *Eur J Med Genet.* (2011) 54:413–8. doi: 10.1016/j.ejmg.2011.04.002
27. Simard O, Grégoire MC, Arguin M, Brazeau MA, Leduc F, Marois I, et al. Instability of trinucleotidic repeats during chromatin remodeling in spermatids. *Hum Mutat.* (2014) 35:1280–4. doi: 10.1002/humu.22637
28. Duyao M, Ambrose C, Myers R, Novelletto A, Persichetti F, Frontali M, et al. Trinucleotide repeat length instability and age of onset in Huntington's disease. *Nat Genet.* (1993) 4:387–92. doi: 10.1038/ng0893-387
29. Illarioshkin SN, Igarashi S, Onodera O, Igarashi S, Onodera O, Markova ED, et al. Trinucleotide repeat length and rate of progression of Huntington's disease. *Ann Neurol.* (1994) 36:630–5. doi: 10.1002/ana.410360412
30. Brandt J, Bylsma FW, Gross R, Stine OC, Ranen N, Ross CA. Trinucleotide repeat length and clinical progression in Huntington's disease. *Neurology.* (1996) 46:527–31. doi: 10.1212/WNL.46.2.527
31. Zielonka D, de Mezer M, Niezgoda A, Reperowicz K, Krzyzosiak W, Kozubski W. Clinical picture of patients with Huntington's disease in relation to the number of trinucleotide CAG repeats in IT-15 gene. *Neurol Neurochir Pol.* (2002) 36:903–9.
32. Claes S, Van Zand K, Legius E, Dom R, Malfroid M, Baro F, et al. Correlations between triplet repeat expansion and clinical features in Huntington's disease. *Arch Neurol.* (1995) 52:749–53. doi: 10.1001/archneur.1995.00540320021009
33. Ashizawa T, Wong LJ, Richards CS, Caskey CT, Jankovic J. CAG repeat size and clinical presentation in Huntington's disease. *Neurology.* (1994) 44:1137–43. doi: 10.1212/WNL.44.6.1137
34. Kieburtz K, MacDonald M, Shih C, Feigin A, Steinberg K, Bordwell K, et al. Trinucleotide repeat length and progression of illness in Huntington's disease. *J Med Genet.* (1994) 31:872–4. doi: 10.1136/jmg.31.11.872
35. Kremer B, Clark CM, Almqvist EW, Raymond LA, Graf P, Jacova C, et al. Influence of lamotrigine on progression of early Huntington disease: a randomized clinical trial. *Neurology.* (1999) 53:1000–11. doi: 10.1212/WNL.53.5.1000
36. Rosenblatt A, Liang KY, Zhou H, Abbott MH, Gourley LM, Margolis RL, et al. The association of CAG repeat length with clinical progression in Huntington disease. *Neurology.* (2006) 66:1016–20. doi: 10.1212/01.wnl.0000204230.16619.d9
37. Ravina B, Romer M, Constantinescu R, Biglan K, Brocht A, Kieburtz K, et al. The relationship between CAG repeat length and clinical progression in Huntington's disease. *Mov Disord.* (2008) 23:1223–7. doi: 10.1002/mds.21988
38. Zielonka D, Niezgoda A, Olejniczak M, Krzyzosiak W, Marcinkowski J, Kozubski W. Gender differences in the CAG repeats and clinical picture correlations in Huntington's disease. *Ceska Slovenska Neurol Neurochirurg.* (2008) 71:688–94.
39. Zielonka D, Marinus J, Roos RA, De Michele G, Di Donato S, Putter H, et al. The influence of gender on phenotype and disease progression in patients with Huntington's disease. *Parkinsonism Relat Disord.* (2013) 19:192–7. doi: 10.1016/j.parkreldis.2012.09.012
40. Handley O, van Walsem M, Juni P, Bachoud-Levi AC, Bentivoglio AR, Bonelli RM, et al. Study protocol of registry e version 2.0-European Huntington's disease network (EHDN). *Hygeia Public Health.* (2011) 46:115–82.
41. Penney JB Jr, Vonsattel JP, MacDonald ME, Gusella JF, Myers RH. CAG repeat number governs the development rate of pathology in Huntington's disease. *Ann Neurol.* (1997) 41:689–92. doi: 10.1002/ana.410410521
42. Brinkman RR, Mezei MM, Theilmann J, Almqvist E, Hayden MR. The likelihood of being affected with Huntington disease by a particular age, for a specific CAG size. *Am J Hum Genet.* (1997) 60:1202–10.

43. Djousse L, Knowlton B, Hayden M, Almqvist EW, Brinkman R, Ross C, et al. Interaction of normal and expanded CAG repeat sizes influences age at onset of Huntington disease. *Am J Med Genet.* (2003) 119A:279–82. doi: 10.1002/ajmg.a.20190
44. Zielonka D, Ren M, De Michele G, Roos RA, Squitieri F, Bentivoglio AR, et al. The contribution of gender differences in motor, behavioral and cognitive features to functional capacity, independence and quality of life in patients with Huntington's disease. *Parkinsonism Relat Disord.* (2018) 49:42–7. doi: 10.1016/j.parkreldis.2018.01.006
45. Marder K, Zhao H, Myers RH, Cudkowicz M, Kayson E, Kieburtz K, et al. Rate of functional decline in Huntington's disease. Huntington Study Group. *Neurology.* (2000) 54:452–8. doi: 10.1212/WNL.54.2.452
46. Peavy GM, Jacobson MW, Goldstein JL, Hamilton JM, Kane A, et al. Cognitive and functional decline in Huntington's disease: dementia criteria revisited. *Mov Disord.* (2010) 25:1163–9. doi: 10.1002/mds.22953
47. Bylsma FW, Rothlind J, Hall MR, Folstein SE, Brandt J. Assessment of adaptive functioning in Huntington's disease. *Mov Disord.* (1993) 8:183–90. doi: 10.1002/mds.870080212
48. Mahant N, McCusker EA, Byth K, Graham S, Huntington Study G. Huntington's disease: clinical correlates of disability and progression. *Neurology.* (2003) 61:1085–92. doi: 10.1212/01.WNL.0000086373.32347.16
49. Bruzelius E, Scarpa J, Zhao Y, Basu S, Faghmous JH, Baum A. Huntington's disease in the United States: variation by demographic and socioeconomic factors. *Mov Disord.* (2019) 34:858–65. doi: 10.1002/mds.27653
50. Dorner JL, Miller BR, Barton SJ, Brock TJ, Rebec GV. Sex differences in behavior and striatal ascorbate release in the 140 CAG knock-in mouse model of Huntington's disease. *Behav Brain Res.* (2007) 178:90–7. doi: 10.1016/j.bbr.2006.12.004
51. Bode FJ, Stephan M, Suhling H, Pabst R, Straub RH, Raber KA, et al. Sex differences in a transgenic rat model of Huntington's disease: decreased 17β-estradiol levels correlate with reduced numbers of DARPP32+ neurons in males. *Hum Mol Genet.* (2008) 17:2595–609. doi: 10.1093/hmg/ddn159
52. Kuljis DA, Gad L, Loh DH, MacDowell Kaswan Z, Hitchcock ON, Ghiani CA, et al. Sex differences in circadian dysfunction in the BACHD mouse model of Huntington"s disease. *PLoS One.* (2016) 11:e0147583. doi: 10.1371/journal.pone.0147583
53. Padovan-Neto FE, Jurkowski L, Murray C, Stutzmann GE, Kwan M, Ghavami A, et al. Age- and sex-related changes in cortical and striatal nitric oxide synthase in the Q175 mouse model of Huntington's disease. *Nitric Oxide.* (2019) 83:40–50. doi: 10.1016/j.niox.2018.12.002
54. Zielonka D, Witkowski G, Puch EA, Lesniczak M, Mazur-Michalek I, Isalan M, et al. Prevalence of non-psychiatric comorbidities in pre-symptomatic and symptomatic Huntington's disease gene carriers in Poland. *Front Med.* (2020) 7:79. doi: 10.3389/fmed.2020.00079
55. Rangel-Barajas C, Rebec GV. Overview of Huntington's disease models: neuropathological, molecular, behavioral differences. *Curr Protoc Neurosci.* (2018) 83:e47. doi: 10.1002/cpns.47
56. Markianos M, Panas M, Kalfakis N, Vassilopoulos D. Plasma testosterone in male patients with Huntington's disease: relations to severity of illness and dementia. *Ann Neurol.* (2005) 57:520–5. doi: 10.1002/ana.20428
57. Markianos M, Panas M, Kalfakis N, Vassilopoulos D. Plasma testosterone, dehydroepiandrosterone sulfate, and cortisol in female patients with Huntington's disease. *Neuro Endocrinol Lett.* (2007) 28:199–203.
58. Lee JK, Ding Y, Conrad AL, Cattaneo E, Epping E, Mathews K, et al. Sex-specific effects of the Huntington gene on normal neurodevelopment. *J Neurosci Res.* (2017) 95:398–408. doi: 10.1002/jnr.23980
59. Meoni S, Macerollo A, Moro E. Sex differences in movement disorders. *Nat Rev Neurol.* (2020) 16:84–96. doi: 10.1038/s41582-019-0294-x
60. Horstink MW, Strijks E, Dluzen DE. Estrogen and Parkinson's disease. *Adv Neurol.* (2003) 91:107–14.
61. Quinn NP, Marsden CD. Menstrual- related fluctuations in Parkinson's disease. *Mov Disord.* (1986) 1:85–7. doi: 10.1002/mds.870010112
62. Sandyk R. Estrogens and the pathophysiology of Parkinson's disease. *Int J Neurosci.* (1989) 45:119–22. doi: 10.3109/00207458908986223
63. Gwinn-Hardy KA, Adler CH, Weaver AL, Fish NM, Newman SJ. Effect of hormone variations and other factors on symptom severity in women with dystonia. *Mayo Clin Proc.* (2000) 75:235–40. doi: 10.4065/75.3.235
64. Tsang KL, Ho SL, Lo SK. Estrogen improves motor disability in Parkinsonian postmenopausal women with motor fluctuations. *Neurology.* (2000) 54:2292–8. doi: 10.1212/WNL.54.12.2292

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Biological Sex and Sex Hormone Impacts on Deficits in Episodic-Like Memory in a Rat Model of Early, Pre-motor Stages of Parkinson's Disease

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Episodic memory deficits are among the earliest appearing and most commonly occurring examples of cognitive impairment in Parkinson's disease (PD). These enduring features can also predict a clinical course of rapid motor decline, significant cognitive deterioration, and the development of PD-related dementia. The lack of effective means to treat these deficits underscores the need to better understand their neurobiological bases. The prominent sex differences that characterize episodic memory in health, aging and in schizophrenia and Alzheimer's disease suggest that neuroendocrine factors may also influence episodic memory dysfunction in PD. However, while sex differences have been well-documented for many facets of PD, sex differences in, and sex hormone influences on associated episodic memory impairments have been less extensively studied and have never been examined in preclinical PD models. Accordingly, we paired bilateral neostriatal 6-hydroxydopamine (6-OHDA) lesions with behavioral testing using the What-Where-When Episodic-Like Memory (ELM) Task in adult rats to first determine whether episodic-like memory is impaired in this model. We further compared outcomes in gonadally intact female and male subjects, and in male rats that had undergone gonadectomy—with and without hormone replacement, to determine whether biological sex and/or sex hormones influenced the expression of dopamine lesioned-induced memory deficits. These studies showed that 6-OHDA lesions profoundly impaired recall for all memory domains in male and female rats. They also showed that in males, circulating gonadal hormones powerfully modulated the negative impacts of 6-OHDA lesions on What, Where, and When discriminations in domain-specific ways. Specifically, the absence of androgens was shown to fully attenuate 6-OHDA lesion-induced deficits in ELM for "Where" and to partially protect against lesion-induced deficits in ELM for "What." In sum, these findings show that 6-OHDA lesions in rats recapitulate the vulnerability of episodic memory seen in early PD. Together with similar evidence recently obtained for spatial working memory, the present findings also showed that diminished androgen levels provide powerful, highly selective protections against the harmful effects that 6-OHDA lesions have on memory functions in male rats.

Keywords: 6-OHDA, androgen, estrogen, dopamine, mild cognitive impairment, neostriatum

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INTRODUCTION

Parkinson's Disease (PD) is a complex neurodegenerative disorder that is characterized by motor signs such as bradykinesia and non-motor symptoms that include deficits in sensory processing, sleep disturbance, and cognitive impairment (1–4). Parkinson's disease is also characterized by sex differences in many of its features (5–9). For example, the incidence and prevalence of PD are both higher in males than in females (10–13). Male PD patients also tend to experience earlier disease onset (14, 15) and more rapid declines in motor function (12). By understanding how gonadal hormones influence these and other disease processes, critical discoveries that could lead to improved, perhaps sex-specific ways to more effectively treat them. This may be especially important for the cognitive impairments associated with PD. These include deficits in executive, mnemonic and/or visuospatial function (4, 16–20) and are present in up to 40% of patients at or before the onset of motor signs (20, 21). Because these signs are also largely resistant to (19, 20, 22) or exacerbated by (23–25) available therapeutics, the also have a cumulative prevalence of more than 70% over the course of illness (20, 26). Moreover, these enduring, progressively worsening facets of disease (17–20) are frequently described by patients and caregivers as significantly disabling and negatively interfering with activities and quality of daily life (27, 28). This brings into sharp focus the need to better understand the factors that mediate and/or modulate the vulnerability of these complex processes in PD. As described below, these factors include biological sex and sex hormones, which are examined here in specific contexts of episodic memory deficits in PD.

Memory disturbances are among the earliest and most frequently reported cognitive deficits in PD (4, 16, 18, 29). For example, roughly 20% of newly diagnosed PD patients present with memory complaints and/or have demonstrable memory impairments at the time of clinical diagnosis (30). One domain of memory that is especially at risk, however, is episodic memory, i.e., the integrated recall of information about the time, place and nature of previously experienced events (31). Thus, deficits in episodic memory are reported among PD patients as often, or even more frequently than executive dysfunction (21, 32, 33). Further, the presence of episodic memory deficits has been shown to correlate with or predict certain clinical features including accelerated rates of motor and cognitive decline (34, 35), and higher risk for developing PD-related dementia (26, 36, 37)– a major cause of hospitalization, institutionalization and death among PD patients (38–41). These characteristics suggest that resolving the factors that render episodic memory vulnerable in PD may be uniquely important in identifying biomarkers and/or biological targets with predictive, preventive, and/or therapeutic value. Accordingly, imaging and autopsy studies in PD patients have been used to probe for brain changes that predict and/or correlate with the severity of episodic memory impairment. These studies have identified thinning or decreased volume in hippocampus and in medial temporal and frontal lobe cortices (42–44) as well as increased total volumes of white matter hyperintense lesions in the frontal and temporal lobes (45) as promising candidates. Although less is known about the physiological processes that render episodic memory vulnerable in PD, there are reasons to suspect that biological sex and/or sex hormones play important roles. First, episodic memory is characterized by lifelong sex differences in healthy humans (46–49). Further, sex differences also mark the incidence and severity of episodic memory deficits seen during cognitive aging (47, 50, 51) in schizophrenia (52, 53) and in Alzheimer's disease (54–57). Finally, although less studied than motor features, there is clear evidence for sex differences in cognitive impairment in PD (5, 6, 58, 59) including data showing that episodic memory deficits are more common and worsen more rapidly in males (60) and respond better to multimodal exercise interventions in females (61, 62). To date, however, there have been no studies that take advantage of preclinical animal models to more precisely determine whether and how biological sex and sex hormones influence episodic memory in PD. We recently validated the use of the What Where When Episodic Like Memory task (63) in adult male and female rats for modeling human sex differences in episodic memory function, and in adult male rats that were gonadectomized or gonadectomized and supplemented with testosterone propionate or estradiol for identifying hormone impacts on this memory form (64). Here we used this same task and these same animal groups, but added sham and partial, bilateral neostriatal 6-hydroxydomapine (6-OHDA) lesions to probe for sex differences in and sex hormone impacts on episodic-like memory function in a rodent model of early, premotor stages of PD (65, 66).

MATERIALS AND METHODS

Animal Subjects

A total of 22 female and 55 male Sprague-Dawley rats were used (Taconic Farms, Germantown, New York, USA). All subjects were between 2 and 2.5 months of age at the beginning of the experiment, and were 3–3.5 months old at the time of behavioral testing. All rats received bilateral neostriatal injections of either 6-hydroxydopamine (6-OHDA) or vehicle (below). The females were gonadally intact. Among the males, 22 were gonadally intact, 11 were gonadectomized (GDX), 11 were GDX and supplemented with testosterone propionate (GDX+TP), and 11 were GDX and supplemented with 17β-estradiol (GDX+E). Rats were pair-housed by sex in standard-sized tub cages (Lab Products, Inc., Seaford, DE, USA) under a 12-h non-reversed light-dark cycle with food (Purina PMI Lab Diet: ProLab RMH 3000) and water available ad libitum. The cages and water bottles were made from bisphenol–free plastic (Zyfone) and ground corncob bedding (Bed O' Cobs, The Anderson Inc., Maumee, Ohio, USA) was used. All procedures involving rats were approved by the Institutional Animal Care and Use Committee at Stony Brook University and were performed in accordance with the U.S. Public Health Service Guide for Care and Use of Laboratory Animals to minimize their discomfort.

Surgeries

Surgical procedures were performed under aseptic conditions using inhalation of isoflurane (1% in oxygen) as anesthesia

and subcutaneous injection of buprenorphine (0.03 mg/kg) or ketorolac (3 mg/kg) for post-operative analgesia.

Gonadectomy

For this procedure, a midline incision was made to the scrotum, the vas deferens were bilaterally ligated with silk suture, and the testes were removed. For hormone-supplemented rats, pellets (Innovative Research of America, Sarasota, Florida) were implanted at the surgical site; the pellets used were designed to release either 25 pg of 17β -estradiol per milliliter of blood per day or 3–4 ng of testosterone propionate per milliliter of blood per day. These pellets have been shown in previous investigations to maintain plasma estradiol and testosterone levels within physiological ranges (67–69). Two weeks after this procedure, rats received 6-OHDA lesions (below).

6-OHDA and SHAM Lesions

Thirty minutes prior to surgery, all rats were injected with desipramine hydrochloride (20 mg/kg, intraperitoneal). Next, rats were anesthetized (isoflurane) and placed in a stereotaxic frame. A midline incision was made to expose the skull surface and burr holes were drilled bilaterally at coordinates targeting the middle one/third of the left and right rostral caudate nucleus (AP: +0.5 mm, ML: ±3.0 mm, relative to Bregma). A glass micropipette containing either 6-hydroxydopamine (6-OHDA) (6 μg/μL) dissolved in ascorbic saline (de-ionized water containing 0.9% NaCl and 0.1% ascorbic acid), or ascorbic saline alone (SHAM), was lowered 5.8 mm below the dura. As the pipette was withdrawn, solution was intermittently ejected at roughly 2–5 min intervals (Nanoject, Drummond Scientific, Broomall, PA, infusion rate ∼0.15 μl/min) at 11 evenly-spaced dorsoventral depths located between 5.8 and 3.8 mm below the dura; the total injected volume was 2 μl/hemisphere. Following the last injection, the micropipette was kept in place for an additional 10 min before being slowly withdrawn. Two weeks later, rats began a regimen of handling, habituation, and behavioral testing that culminated to testing on the What-Where-When (WWWhen) ELM task 10 days after that (24 days after lesion surgery).

Behavioral Testing

Testing took place during rats' subjective night (lights on), between the hours of 9:30 am. and 3:00 pm. By conducting studies during these earlier hours of rats' subjective nights, testing times within and across female subjects avoided the potential for overlap with the precipitous decreases in estrogen, the rapid increases in follicle stimulating hormone, and the rapid increases and subsequent sharp declines in progesterone and luteinizing hormone that begin toward the end of rats' subjective nights and continue into the subjective day (70). Testing was conducted in a core facility consisting of a central holding room and 5 adjacent, sound attenuated testing rooms. The testing arena used was an open circular platform that was 0.61 m in diameter and located 0.91 m above the floor (padded) near the center of a 3 m by 3 m testing room with fixed, high contrast visual cues on three walls. The platform surface was covered by black vinyl to provide grip and allow the platform to be wiped clean with 70% ethanol between trials. A webcam (LogiTech) was suspended two feet above the arena to record trials.

Prior to WWWhen testing, rats completed testing on 2 or 3 unrelated, non-incentivized, pseudorandom presented single-trial paradigms (Home Base Formation and Novel Object Preference or Object-in-Place Preference Testing). Each task used a different testing arena and different objects (where applicable), but the same holding and testing rooms, and the same cylindrical start box used for WWWhen testing. After a 3–5 day break, rats received an additional habituation session during which they were allowed to freely explore the WWWhen testing platform with no objects present for 10 min. Testing on the WWWhen task took place the following day.

On testing day, rats were given two 5 min sample trials (S1, S2) and one 5 min test trial (TT); all trials were separated by 50 min inter-trial intervals that rats spent in home cages in the central holding room. Trials began by placing rats in the opaque start cylinder (0.2 m across, 0.3 m high) at the center of the arena. After a 10 s delay, the cylinder was lifted away and rats were allowed to freely explore. During the sample trials (S1, S2), rats were presented with one of two quadruplicate sets of novel objects that were similar in overall size, were made of the same material (plastic or glass), but differed in shape, color and/or surface texture. The object sets used for S1 vs. S2 trials were pseudo-randomly assigned to subjects within all groups. During the S1 trial, objects were arranged in a triangular array and for the S2 trial they were placed to form the corners of a square. During the test trial (TT), two objects from S2 (recent familiar, RF) and two objects from S1 (old familiar, OF) were presented in square S2 configuration. Accordingly, one of the S1 objects appeared in its original location (old familiar stationary, OFS) and was presented in a new location (old familiar displaced, OFD). Both recent familiar objects occupied original (stationary) positions (see **Figure 1**).

Health and Hormone Status

The weights and general health of all subjects were tracked before and after gonadectomy and/or 6-OHDA or SHAM lesion surgery. In all subjects, body mass progressively increased and there were no signs of dehydration. The estrous cycles of female rats were also tracked using vaginal lavage. Cytological samples were collected via saline flush on the day of lesion or sham lesion surgery, on the day of WWWhen testing, and every 1–3 days in between. On testing day, samples were collected prior to animals' acclimation to the central holding room in the testing suite. For males, the effectiveness of hormone manipulations was assessed post-mortem via dissection and weighing of the androgen-sensitive bulbospongiosis muscles (BSM).

Euthanasia

Following behavioral testing, rats were euthanized by transcardial perfusion. First, rats were lightly anesthetized using inhalation of isoflurane (1% in oxygen) and were then injected intraperitoneally with a ketamine (150 mg/kg), xylazine (15 mg/kg) mixture to induce deep anesthesia. After verifying the absence of deep reflexes, rats were perfused, first with phosphate buffered saline (PBS, ∼100 mls), and then with 4%

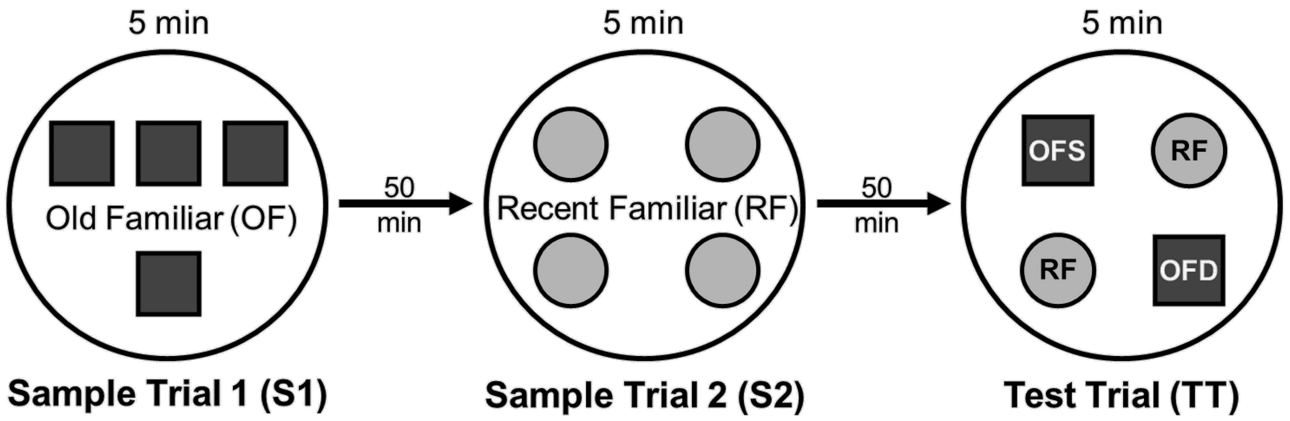


FIGURE 1 | Schematic of the experimental protocol for the What-Where-When Episodic-Like Memory Task (63). Rats explore two sets of novel quadruplicate objects during a first and second sample trial (S1 and S2, respectively). This is followed by a test trial (TT) where two Recent Familiar (RF) objects from S2 are presented in their original positions and two Old Familiar (OF) objects from S1 are present, with one placed in its original position [Old Familiar Stationary (OFS)] and one that has been displaced from its original location [Old Familiar Displaced (OFD)]. Each trial is 5 min in duration and separated by an inter-trial interval of 50 min.

paraformaldehyde in 0.1M PB, pH 6.5 (flow rate 30 ml/min) for 5 min followed by 4% paraformaldehyde in 0.1M borate buffer, pH 9.5 (flow rate 35 ml/min) for 15 min.

Tissue Processing and Histology

Immediately after perfusion, the BSM complex in male subjects were dissected and weighed. However, for 4 MALE SHAM and 4 MALE 6-OHDA rats, BSM weights were not recovered. The brains were also removed from all subjects. These were post-fixed in 4% paraformaldehyde in 0.1M borate buffer for 24 h (4°C) and were then cryoprotected by immersion in 0.1M phosphate buffer (PB) containing 30% sucrose (2–5 days, 4°C). Next, the brains were rapidly frozen in powdered dry ice and serially sectioned in the coronal plane on a freezing microtome (40 μm). A one-in-six series of sections spanning the rostrocaudal extent of the caudate nucleus was processed for immunohistochemistry using antibodies against the dopamine-synthesizing enzyme, tyrosine hydroxylase (TH). Briefly, sections were rinsed in 0.1M PB, incubated in 0.1M PB containing 1% H_2O_2 (30 min), and were then rinsed again in 0.1M PB prior to an antigen retrieval step involving a 20 min incubation in sodium citrate buffer, pH 8.5 at 80°C. After rinses in tris buffered saline (TBS), pH 7.4, the sections were placed in TBS, pH 7.4 containing 10% normal swine serum (NSS) for 2 h, and then in primary antisera (anti-TH monoclonal antibody; Chemicon International Inc, Temecula, CA; MAB318, diluted 1:1000 in TBS containing 1% NSS) for 4 days (4°C). Following further rinses in TBS, pH 7.4, the sections were incubated overnight in biotinylated secondary antibody (Vector Laboratories, Burlingame, CA, USA, diluted 1:100 in TBS containing 1% NSS, 4°C). Next, sections were rinsed in TBS, pH 7.4 and incubated in avidin-complexed horseradish peroxidase (ABC, Vector Laboratories, 5 h, room temperature). Finally, the sections were rinsed first in TBS pH 7.4 then in TB, pH 7.6 before being reacted using 3–3′ diaminobenzidine as chromagen and 1% H_2O_2 as catalyst. The immunoreacted sections were then slide mounted and sealed under coverslips using Permount (Electron Microscopy Science, Hartfield PA, USA).

Data Analyses

Evaluation of Estrous Cycle

Samples from vaginal lavage (saline) were cytologically evaluated using low power light microscopy and differential interference contrast illumination. Females were determined to be in estrus when samples had an abundance of cornified, anucleated epithelial cells; in diestrus when samples showed a predominance of leukocytes; and in proestrus when samples were largely comprised of round, nucleated epithelial cells (70).

Efficacy of GDX and Hormone Replacement

The extents to which GDX and hormone replacement modulated circulating hormone levels were assessed by comparing weights of the androgen-sensitive bulbospongiosis muscles across male groups.

Evaluations of 6-OHDA Lesions

A Zeiss Axioskop outfitted with an Infinity 3 Lumenera digital camera was used to collect low-power brightfield light microscopic images of TH-immunoreacted sections. These images were imported into ImageJ (Open Source, 1.52a) and separate, calibrated outlines were drawn around the entire caudate nucleus (excluding nucleus accumbens) and around the lesioned zones, defined as areas within the caudate where TH-immunostaining fell to background levels. The areas subtended by both sets of outlines were used along with section thickness and numbers of sections per case to calculate total caudate and total lesion volumes on per hemisphere, per subject bases. Lesion symmetries were defined as the ratio of the larger compared to the smaller lesion volume, regardless of whether it was in the left or right hemisphere.

Behavioral Analyses

Behavioral scoring was completed by trained observers who were blind to animal condition using event capture (Behavioral Observation Research Interactive Software—BORIS, 7.0.4, Open Source) and tracking software (Tracker 4.62, Open Source). For all trials, scored behaviors were defined and measured as follows:

Latency to Investigate Objects: time (seconds) between the start of the trial (removal of the start box) and the first investigatory contact with an object.
Grooming: time (seconds) spent licking or preening any part of the body.
Rearing: time (seconds) spent making upward/vertical motion either with forepaws in contact with an object (without vibrissae or snout in contact with the object) or while free-standing.
Ledge Investigation: time (seconds) spent at and actively investigating the ledge/edge of the arena or looking out into the surrounding testing room environment.
Ambulation: time (seconds) spent engaged in forward motion, calculated from changes in position magnitudes measured in digitized tracks of rats' movement across the arena surface on per frame bases (Tracker 4.62).
Stationary: time (seconds) spent sitting in one location away from the arena edge, and not engaging in grooming or object exploration behaviors.
Object Exploration: time (seconds) spent in physical contact with objects, actively using vibrissae or snout to investigate, with or without rearing.
Discrimination Indices (DI): calculated during Test Trials based on time (seconds) spent exploring objects designated as recent familiar (RF), old familiar (OF), old familiar stationary (OFS), or old familiar displaced (OFD):

$$\textit{WHAT DI} = \left[\textit{average OF} - \textit{average RF}\right] \div \left[\textit{average OF} + \textit{average RF}\right]$$
$$\textit{WHERE DI} = \left[\textit{OFD} - \textit{OFS}\right] \div \left[\textit{OFD} + \textit{OFS}\right]$$
$$\textit{WHEN DI} = \left[\textit{OFD} - \textit{average RF}\right] \div \left[\textit{OFD} + \textit{average RF}\right]$$

Statistics

Statistical comparisons were used to evaluate behavioral endpoints for (1) sex differences [i.e., contrasts of gonadally intact sham-operated females (FEM SHAM) and males (MALE SHAM)], (2) sex differences in sensitivity to 6-OHDA lesions [i.e., contrasts of FEM SHAM and MALE SHAM with gonadally intact 6-OHDA lesioned females (FEM 6-OHDA) and males (MALE 6-OHDA)], and (3) sex hormone modulation of sensitivity to 6-OHDA lesions in males [i.e., contrasts of MALE SHAM and MALE 6-OHDA with the 6-OHDA lesioned GDX and hormone replacement cohorts (GDX 6-OHDA, GDX+TP 6-OHDA, GDX+E 6-OHDA)]. Due to small and uneven sample sizes, no attempts were made to statistically assess effects of estrous cycle stage among females.

All statistical analyses were performed using IBM SPSS, Version 25 (SPSS, Inc., Chicago, IL, USA). Data sets were first evaluated using descriptive statistics and tests for homogeneity of variance (Levine's F-test). From there, one-way analyses of variance (ANOVA) were used to compare BSM weights, lesion size, and lesion symmetry across groups. Repeated-measures ANOVAs were used to compare: all measures of object exploration, including DIs across groups; all measures of Non-Object Exploration (Other) behaviors within and across groups; and to evaluate within-groups differences in Other Behaviors across trials. For these comparisons, Mauchly's test for sphericity of the covariance matrix was applied and degrees of freedom were corrected as necessary using the Huyhn-Feldt epsilon. Allowed *post-hoc* tests used the Fisher's Protected Least Significant Difference (PLSD); for comparisons of two groups, a $p < 0.05$ level was accepted as significant; for comparisons of multiple groups, significance was determined using a Bonferroni corrected alpha ($p < 0.0491$ for comparisons of the 4 gonadally intact female and male groups and $p < 0.0489$ for comparisons of the 5 male groups). Robustness of object discriminations was assessed within groups for Test Trials using one-sample t-tests to identify DIs as significantly different from zero. Lastly, regression analyses were used within groups to evaluate 6-OHDA-induced lesion size as a function of BSM weight and to evaluate task performance metrics as functions of 6-OHDA lesions.

RESULTS

Estrous Cycle in Females and Effectiveness of Hormone Manipulations in Males

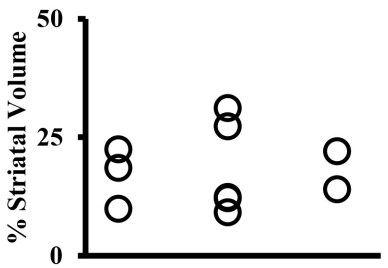
Vaginal lavage samples obtained from female subjects (FEM SHAM, FEM 6-OHDA) showed that on the day of SHAM or 6-OHDA lesion surgery, 3 females from the FEM SHAM group were in estrus, 6 were in diestrus, and 2 were in proestrus, whereas among the FEM 6-OHDA group, 3 females were in estrus, 5 were in diestrus, and 2 were in proestrus at the time surgery was performed (**Table 1**). All subjects were also found to have resumed and retained a regular 4-day estrous cycle after surgery. On the day of WWWhen testing, 5 females from the FEM SHAM group were in estrus, 5 were in diestrus, and 1 was in proestrus; from the FEM 6-OHDA group, 3 females were in estrus, 4 were in diestrus, and 3 were in proestrus (**Table 1**).

The efficacies of GDX and of GDX with hormone replacements were verified in expected group differences in the weights of the androgen-sensitive bulbospongiosis muscles (BSM). Specifically, average BSM weights were larger in the gonadally intact groups (MALE SHAM, MALE-6-OHDA) and in the GDX+TP 6-OHDA rats compared to muscle mass in the GDX 6-OHDA and GDX+E 6-OHDA groups (**Table 2**). One-way ANOVAs confirmed that there were significant main effects of Group for BSM mass [$F_{(4,41)} = 94.634$, $p < 0.0001$]. Follow-up *post-hoc* comparisons further confirmed that BSM weights in GDX 6-OHDA and GDX+E 6-OHDA rats were similar to each other and were significantly lower than BSM mass in the gonadally intact (MALE SHAM, MALE 6-OHDA) and GDX+TP 6-OHDA groups (all $p < 0.0001$).

TABLE 1 | Above: Table showing the numbers of sham-operated (SHAM) and 6-hydroxydopamine (6-OHDA) lesioned female rats identified by vaginal cytology as being in estrus (EST), diestrus (DI), or proestrus (PRO) on the day that SHAM or 6-OHDA lesions were made (Day of Surgery) and on the day of testing for the What-Where-When Episodic-like memory task (Day of Testing).

Females	EST	DI	PRO
SHAM			
Day of lesion surgery	3	6	2
Day of testing	5	5	1
6-OHDA			
Day of lesion surgery	3	5	2
Day of testing	3	4	3
LESION SIZE			

6-OHDA lesion size for females in estrus (n = 3), diestrus (n = 5), or proestrus (n = 2) on the day of surgery



Below: Scatter plots showing the volumes of 6-OHDA lesions, expressed as percent of total caudate nucleus volume, for female rats that were identified as being in EST, DI, or PRO on the day of surgery.

TABLE 2 | Group mean weights of the androgen-sensitive bulbospongiosis (BSM) mass [in grams ± standard error of the mean (SEM)] for gonadally intact sham-operated (SHAM) and 6-hydroxydopamine lesioned (6-OHDA) males and for the males that were gonadectomized (GDX), GDX and supplemented with testosterone propionate, or GDX supplemented with 17β-estradiol and 6-OHDA lesioned (GDX 6-OHDA, GDX+TP 6-OHDA, GDX+E 6-OHDA, respectively).

MALES	MEAN BSM (g) ± SEM
SHAM	1.67 ± 0.05
6-OHDA	1.64 ± 0.05
GDX 6-OHDA	0.53 ± 0.03* ** ***
GDX+TP 6-OHDA	0.96 ± 0.08
GDX+E 6-OHDA	0.51 ± 0.03* ** ***

One-way analyses of variance identified significant main effects of Group on BSM weight; the allowed post-hoc comparisons confirmed that BSM weights of the GDX 6-OHDA and GDX+E 6-OHDA males were similar to each other and were significantly different (lower) than muscle weights for the gonadally intact MALE SHAM (*), gonadally intact MALE 6-OHDA (**), and GDX+TP 6-OHDA groups (***) (all $p < 0.0001$).

6-OHDA and SHAM Lesions

Light microscopic evaluations of TH-immunoreacted coronal sections showed that SHAM lesions had no discernible effects on staining intensity or striatal integrity in either male or female rats (**Figure 2A**). In contrast, injections of 6-OHDA produced discrete, bilateral zones of markedly diminished TH-immunostaining in all lesioned groups (**Figures 2B–F**). These sites were cylindrically shaped, about 0.5–1 mm in diameter, and were centered on the middle third of the caudate at roughly mid-septal levels. These sites also tended to be symmetrical (**Figures 2B–F**) and, on average, occupied 17–24% of the left, right, and total caudate nucleus (neostriatal) volumes in all groups (**Figure 2G**). There were, however, a small number of subjects in each group where left/right hemispheric differences in lesion volume exceeded 20%. There rats were all carefully assessed for evidence of circling or turning bias during WWWhen testing; this identified 2 rats (1 FEM 6-OHDA, 1 GDX 6-OHDA) where strong left-right turning biases and tight circling were noted, leading both to be removed from the study. Among the remaining subjects, statistical comparisons (one-way ANOVAs) confirmed that there were no significant or near significant main effects of Group on measures of lesion volume or lesion symmetry for either gonadally intact females vs. males or among the four male groups. Further, although not statistically assessed, there were no observed differences in lesion volume for females that were in estrus, diestrus, or proestrus on the day of lesion surgery (**Table 1**). Regression analyses that compared BSM weights to 6-OHDA-induced striatal lesion sizes in males were also found to be mainly non-significant ($R^2 = 0.04$–0.19); the only significant relationship identified between BSM weight and lesion size was in the MALE 6-OHDA group ($R^2 = 0.752$, $p = 0.011$), where the BSM dataset was incomplete.

What-Where-When Performance

Non-object Exploration (Other) Behaviors (S1, S2, TT)

Latency to explore objects and times spent grooming, rearing, investigating the arena ledge, ambulating, and remaining stationary were all assessed during both sample trials (S1, S2) and during the test trial (TT). These data showed that rats in most groups spent similar amounts of trial times engaged in these discrete activities, and showed similar systematic increases or decreases in certain behaviors across trials (**Figure 3**). Specifically, rats in all groups initiated exploration of objects within about 1 second during S1 and by TT were waiting for closer to 5 s to begin interacting with objects (**Figure 3**, top panel). Rats in all groups also spent minimal times grooming (6–20 s) and small but gradually increasing amounts of time rearing from S1 (1–4 s, **Figure 3A**) to TT (4–15 s, **Figure 3C**). More time was spent investigating the arena ledge, ambulating, or remaining stationary. However, while rats in most groups were stationary for roughly 90–120 s of all trial times, during S1 (but not other trials, **Figure 3C**) the FEM SHAM were stationary for on average <60 s (**Figure 3A**). Conversely, for ambulation, rats in most groups engaged in locomotion for more than 60 s during S1 (**Figure 3A**), but by the TT only engaged in locomotion for about 45 s (**Figure 3C**). In contrast, the FEM SHAM group initially spent 90 s or more ambulating during S1 (**Figure 3A**) before decreasing locomotion to scores that were similar to the other groups in S2 and TT (**Figures 3B,C**). Similarly, for ledge investigation, most groups spent 60 s or less of trial times (S1, S2, TT) at the arena edge (**Figure 3**). However, the FEM SHAM group again engaged in more ledge investigation ∼90 s, during S1 and S2 (**Figures 3A,B**). By the TT, however, all groups were spending roughly 60 s in investigating the arena's ledge (**Figure 3C**). Within-trials repeated-measures ANOVAs confirmed that there were significant main effects of Behavior for all trials and for both sets of group contrasts [gonadally intact females

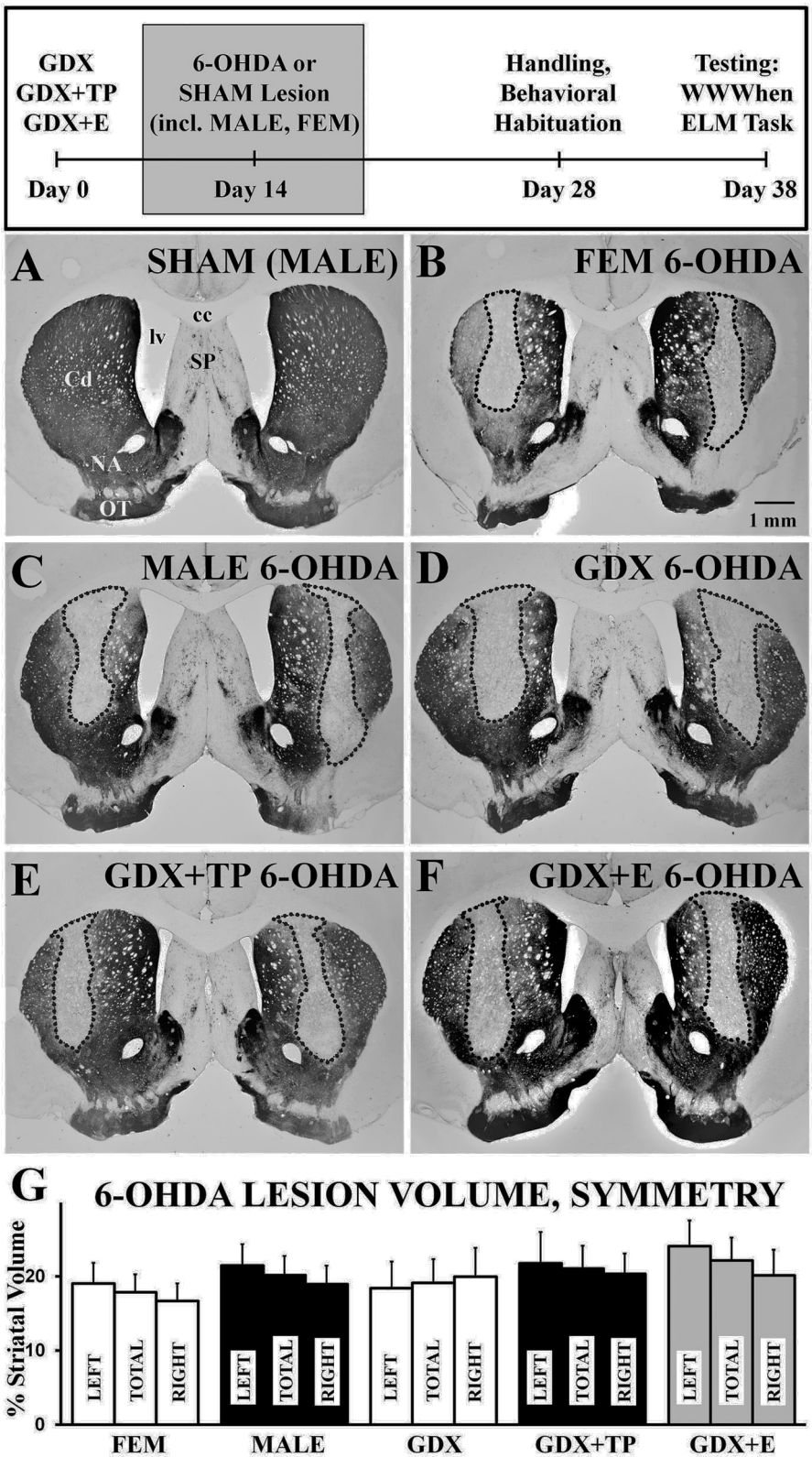


FIGURE 2 | Representative low-power, brightfield light microscopic images of coronal sections through septal levels of the caudate nucleus (Cd) that are immunoreacted for tyrosine-hydroxylase (TH) **(A–G)**. The section from a gonadally intact sham-operated male (**A**, SHAM) shows dense labeling throughout the

(Continued)

FIGURE 2 | caudate. Sections from a 6-hydroxydopamine (6-OHDA) lesioned female (**B**, FEM 6-OHDA), a 6-OHDA lesioned male (**C**, MALE 6-OHDA) and from 6-OHDA lesioned rats that were gonadectomized (**D**, GDX 6-OHDA), GDX and supplemented with testosterone propionate (**E**, GDX+TP 6-OHDA), or GDX and supplemented with 17β-estradiol (**F**, GDX+E 6-OHDA) all show discrete bilateral zones of diminished TH-immunostaining (outlined). The experimental timeline showing when lesions and sham lesion were made is shown above, and bar graphs **(G)** and bar graphs showing group average sizes and symmetries of 6-OHDA lesions, expressed as percentages of the left (LEFT), right (RIGHT), and total (TOTAL) caudate nucleus volumes ± standard error of the mean, are shown below for all groups below. cc, corpus callosum; NA, nucleus accumbens; SP, septal nucleus; lv, lateral ventricle; OT, olfactory tubercle. Scale bar in **B** = 1 mm.

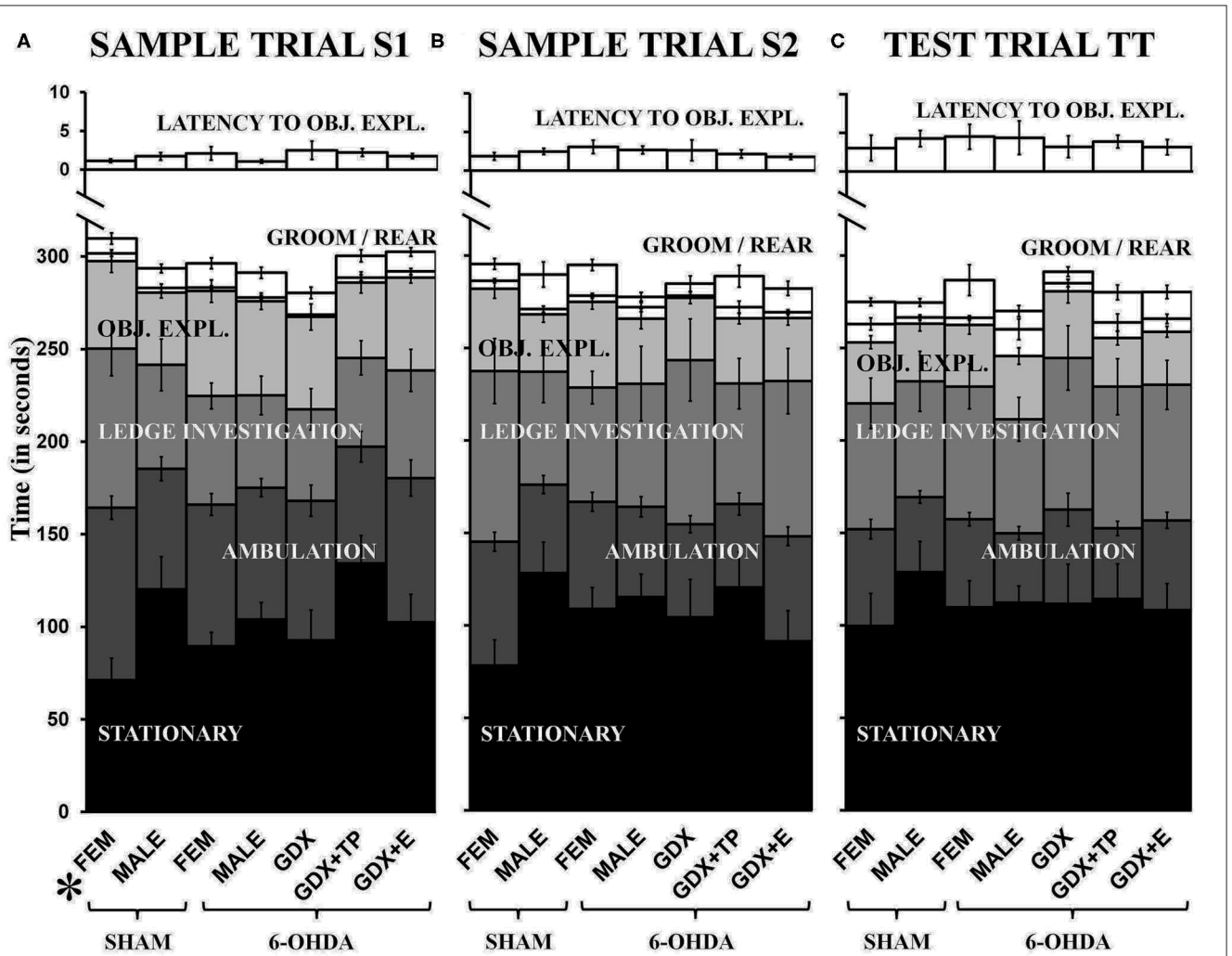


FIGURE 3 | Stacked bar graphs showing average amounts of times in seconds, ± standard error of the mean, that rats spent stationary (black), ambulating (dark gray), engaged in ledge investigation (gray), object exploration (OBJ. EXPL., light gray), and grooming or rearing (GROOM/REAR) (white) during sample trial S1 **(A)**, sample trial S2 **(B)**, and the test trial TT **(C)**. The average latency (in seconds) from trial start to exploration of a first object (LATENCY TO OBJ.EXPL.) is also shown in white above on different time scale. Comparisons from left to right show that with the exceptions of higher levels of S1 ambulation and lower levels of S1 stationary behavior in gonadally intact sham lesioned females (SHAM, FEM), rats in all other groups [gonadally intact, sham lesioned males (SHAM, MALE), MALE and FEM rats that were 6-hydroxydopamine-lesioned (6-OHDA), and 6-OHDA male rats that were gonadectomized (GDX), GDX and supplemented with testosterone propionate (GDX+TP), or GDX and supplemented with 17β-estradiol (GDX+E)] apportioned trial times similarly. The *post-hoc* comparisons performed after the identification of significant main effects of Group and significant interactions between Behavior and Group for MALE/FEMALE comparisons for S1 showed that these effects were driven by significantly increased ambulation and decreased stationary behavior in SHAM operated FEM compared to SHAM operated MALES ($p = 0.002$, 0.012).

vs. males: $F_{(1.47-1.60,\ 57.32-62.23)} = 71.14–103.37$, $p < 0.0001$; all male groups: $F_{(1.39-1.59,\ 67.86-77.91)} = 73.74–119.38$, $p < 0.0001$]. Significant main effects of Group and significant interactions between Behavior and Group were also identified for S1 for in the gonadally intact female vs. male contrast [Group: $F_{(3,\ 39)} = 3.04$, $p = 0.04$; Behavior x Group: $F_{(4.78,\ 62.13)} = 2.50$, $p = 0.042$]; allowed *post-hoc* comparisons confirmed that these main effects were driven by significantly higher levels of ambulation and significantly lower levels of stationary behaviors in the FEM SHAM compared to MALE SHAM group

during the S1 trial ($p = 0.002$ and 0.007, respectively). Repeated-measures ANOVAs that separately compared behaviors across trials and groups identified significant main effects of Trial for latency to explore objects, rearing, ambulation, and stationary behavior in the gonadally intact female vs. male groups contrast [$F_{(1.40-2.00,\ 54.42-78.00)} = 3.57$–$71.84$, $p = 0.000$–0.007] and for latency to explore objects, rearing, ambulation, and ledge investigation among the male groups [$F_{(1.41-2.00,\ 69.25-98.00)} = 7.06$–$49.02$, $p = 0.000$–0.005]; no significant or near significant main effects of Group and no significant or near significant interactions between Group and Trial were identified for either groups contrast. Finally, regression analyses that assessed within groups differences in discrete Other Behaviors as functions of 6-OHDA lesion sizes were overwhelmingly non-significant ($R^2 = 0.00$–0.34). Across all behaviors, trials, and groups, only two significant relationships were identified. These were positive correlations in the GDX 6-OHDA group between 6-OHDA lesion size and stationary behavior during S2, and between lesion size and latency to explore objects during TT ($R^2 = 0.41$, $p = 0.047$, and $R^2 = 0.45$, $p = 0.039$, respectively).

Object Exploration: Total Object Exploration (S1, S2, TT)

Rats in all groups spent similar total amounts of time exploring objects within trials and progressively less time exploring objects from S1 to S2 to TT trials (**Figure 3**). Specifically, all cohorts explored objects for, on average, nearly 60 s during S1 (**Figure 3A**), for 30–45 s in S2 (**Figure 3B**), and for 25–35 s in TT (**Figure 3C**). Separate one-way ANOVAs that compared these times within trials for the two contrasts (gonadally intact female vs. male groups and among all male groups) found no significant or near significant main effects of Group for Total Object Exploration for any trial. Across-trials comparisons (repeated-measures ANOVAs) for groups contrasts further identified significant main effects of Trial [gonadally intact female vs. male groups: $F_{(2,78)} = 21.42$, $p < 0.0001$; all male groups: $F_{(1.90,\ 92.86)} = 35.19$, $p < 0.0001$] but no significant or near significant main effects of Group and no significant or near significant interactions between Trial and Group for either comparison. Regression analyses that assessed total object exploration as a function of lesion size in the 6-OHDA groups were predominantly non-significant ($R^2 = 0.000$–0.25). However, a significant positive relationship between lesion size and total object exploration was found for the GDX+E cohort for the TT ($R^2 = 0.49$, $p = 0.017$). Finally, comparisons were also made in which the data were stratified by the order in which quadruplicate object sets were presented (Object Order). These within-groups, across-trials repeated-measures ANOVAs found no significant or near significant main effects of Object Order and no significant or near significant interactions between Object Order and Trial for total object exploration for any group.

Object Exploration: Individual Object Exploration (S1, S2)

Analyses of rats' investigations of individual objects showed that in both of the sample trials (S1, S2) rats in all groups divided exploration times more or less evenly and spent ∼10–20 s exploring each object in S1 (**Table 3A**) and 5–15 s exploring each object in S2 (**Table 3B**). Within-groups repeated-measures ANOVAs identified isolated cases where main effects of Individual Object were significant: for FEM SHAM [$F_{(2.50,\ 24.98)} = 12.45$, $p < 0.0001$] and GDX 6-OHDA [$F_{(3,27)} = 5.53$, $p = 0.004$] in S1; for GDX 6-OHDA in S2 [$F_{(3,27)} = 4.50$, $p = 0.011$]. The objects and exploration times that drove these main effects were identified in follow-up pair-wise comparisons and are shown in bold in gray-shaded cells in **Tables 3A,B**.

The durations of individual instances or bouts of object exploration were also evaluated (**Table 3**). This measure was similar and similarly brief for all groups (<2 s) in both sample trials (**Table 3**). Within-groups comparisons (repeated-measures ANOVAs) generally found that main effects of Object Exploration Duration were non-significant. However, a significant main effect of Object Exploration Duration was identified for the MALE 6-OHDA group, albeit only for S1 ($F_{(3,30)} = 5.50$, $p = 0.004$). Follow-up pair-wise comparisons identified the object-specific measure of exploration duration that drove this main effect, which is shown in bold in a gray-shaded cell in **Table 3A**. Finally, regression analyses showed that 6-OHDA lesion size was most often not a significant predictor of either mean exploration times for individual objects ($R^2 = 0.000$–0.253) or mean durations of individual bouts of object exploration ($R^2 = 0.000$–0.38) during S1 or S2. The single exception was a significant positive relationship identified for GDX+TP group between larger lesion size and longer mean exploration times for individual objects during S2 ($R^2 = 0.42$, $p = 0.031$).

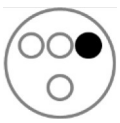
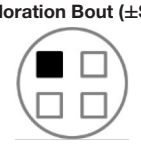
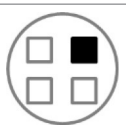
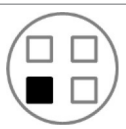
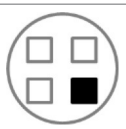
Object Exploration: Discrimination Indices (TT)

During test trials, rats' observation times were unevenly distributed among the 2 "old familiar" (OF) and 2 "recent familiar" (RF) objects present. Discrimination Indices (DI) calculated from these differences were compared across groups using repeated-measures ANOVAs. These identified: significant main effects of Group for comparisons of gonadally intact female and male groups [$F_{(3,39)} = 19.51$, $p < 0.0001$] and for comparisons of all male groups: [$F_{(4,49)} = 9.31$, $p < 0.0001$]; significant main effects of Discrimination among the males [$F_{(1.22,\ 59.99)} = 14.07$, $p < 0.001$]; and a significant interaction between Discrimination and Group for the gonadally intact female vs. male comparisons [$F_{(3.67,\ 47.70)} = 3.04$, $p = 0.003$]. The allowed *post-hoc* comparisons (Bonferroni-corrected pair-wise comparisons) are presented along with additional analyses separately below for discriminations of "What," "Where," and "When."

What' discrimination

Average "What" DIs calculated for gonadally intact FEM SHAM and MALE SHAM rats were 0.40 and 0.33, respectively. Their preferential investigation of "old familiar" (S1) vs. "recent familiar" (S2) objects contrasted sharply with the average DIs calculated for FEM 6-OHDA and MALE-6-OHDA groups, which were −0.03 and −0.08, respectively (**Figure 4A**). *Post-hoc* comparisons (Bonferroni corrected alpha 0.0491) confirmed

TABLE 3 | Average times (in seconds) of exploration of individual objects during sample trial S1 **(A)** and sample trial S2 **(B)**.

Object	Key	FEM SHAM	FEM 6-OHDA	MALE SHAM	MALE 6-OHDA	GDX 6-OHDA	GDX+TP 6-OHDA	GDX+E 6-OHDA
(A) SAMPLE TRIAL S1: Total Individual Object Exploration (±SEM)								
Duration of Exploration Bout (±SEM)								
Object 1		7.25 ± 1.35 0.90 ± 0.16	13.69 ± 4.27 1.33 ± 0.30	8.58 ± 1.51 0.99 ± 0.15	10.61 ± 1.78 0.84 ± 0.07	14.43 ± 2.46 1.07 ± 0.08	10.50 ± 2.38 1.09 ± 0.12	11.60 ± 2.13 1.06 ± 0.14
Object 2		9.95 ± 1.67 1.06 ± 0.12	14.52 ± 1.95 0.87 ± 0.10	11.42 ± 1.09 1.03 ± 0.19	13.05 ± 1.86 0.76 ± 0.08	11.13 ± 1.25 0.86 ± 0.05	14.07 ± 3.37 0.93 ± 0.12	15.68 ± 2.29 0.91 ± 0.05
Object 3		10.05 ± 2.30 1.09 ± 0.11	12.93 ± 2.65 1.01 ± 0.11	9.26 ± 1.76 1.07 ± 0.11	10.69 ± 1.99 0.91 ± 0.11	**8.42 ± 1.80** 0.98 ± 0.11	7.64 ± 0.87 1.10 ± 0.11	10.79 ± 0.99 1.00 ± 0.09
Object 4		**20.08 ± 2.66** 1.22 ± 0.11	15.45 ± 3.42 0.99 ± 0.13	9.76 ± 1.35 1.14 ± 0.09	16.49 ± 3.13 **1.16 ± 0.12**	**18.48 ± 2.83** 1.14 ± 0.12	12.03 ± 3.11 0.96 ± 0.07	12.13 ± 2.04 0.91 ± 0.09
(B) SAMPLE TRIAL S2: Total Individual Object Exploration (±SEM)								
Duration of Exploration Bout (±SEM)								
Object 1		8.06 ± 1.77 1.06 ± 0.15	11.28 ± 2.35 1.05 ± 0.14	9.53 ± 2.70 0.88 ± 0.11	7.86 ± 1.86 0.95 ± 0.10	7.25 ± 1.56 0.89 ± 0.11	7.90 ± 1.73 0.93 ± 0.11	5.90 ± 1.44 0.93 ± 0.13
Object 2		8.96 ± 1.64 1.17 ± 0.18	12.83 ± 2.17 1.20 ± 0.20	5.39 ± 1.27 0.95 ± 0.18	5.53 ± 1.45 0.74 ± 0.11	**4.59 ± 1.16** 0.97 ± 0.13	9.55 ± 3.38 1.25 ± 0.15	8.91 ± 2.65 1.07 ± 0.19
Object 3		13.63 ± 2.12 1.08 ± 0.08	10.68 ± 2.00 1.10 ± 0.13	8.48 ± 1.07 0.98 ± 0.09	10.61 ± 1.98 0.91 ± 0.22	9.78 ± 1.49 0.97 ± 0.08	9.93 ± 2.08 0.88 ± 0.12	7.75 ± 1.63 0.77 ± 0.13
Object 4		13.77 ± 2.63 1.16 ± 0.12	11.44 ± 2.07 1.10 ± 0.13	7.65 ± 2.72 1.27 ± 0.09	11.21 ± 3.25 1.03 ± 0.15	12.10 ± 1.91 1.37 ± 0.23	7.82 ± 1.49 1.08 ± 0.15	11.48 ± 1.56 1.05 ± 0.07

Schematics shown on the left show the relative position/identity of the individual object assessed; the values located in the adjacent cells show average total exploration times [in sec, ± standard error of the mean (SEM)] (top) and average durations of separate bouts of exploration (±SEM) (bottom) for that object for gonadally intact sham-operated females (FEM SHAM) and males (MALE SHAM), for gonadally intact 6-hydroxydopamine lesioned (6-OHDA) females (FEM 6-OHDA) and males (MALE 6-OHDA), and for 6-OHDA male rats that were gonadectomized (GDX), GDX and supplemented with testosterone propionate, or GDX and supplemented with 17β-estradiol (GDX 6-OHDA, GDX+TP 6-OHDA, and GDX+E 6-OHDA). Within-groups comparisons (repeated-measures analyses of variance) of total times spent exploring individual objects identified significant main effects of Object for FEM SHAM and GDX 6-OHDA groups in S1 and for GDX 6-OHDA rats in S2. Significant main effects of Object for measures of durations of individual bouts of object exploration were also found for the MALE 6-OHDA group in S1. The individual objects driving these main effects identified in follow-up pair-wise post-hoc comparisons are shown in bold, in gray-shaded cells.

that "What" DIs were similar for the FEM SHAM and MALE SHAM groups; were similar for FEM 6-OHDA and MALE 6-OHDA groups; but were significantly lower for MALE 6-OHDA and FEM 6-OHDA rats vs. MALE SHAM and FEM SHAM groups (all $p < 0.0001$). Group differences in the robustness of discrimination were further supported in one-sample t-tests showing that "What" DIs were significantly >0 for FEM SHAM [$t_{(1,10)} = 9.55, p < 0.0001$] and MALE SHAM rats [$t_{(1,10)} = 4.23$, $p = 0.002$], but were not significantly different from zero for the FEM-6-OHDA or MALE 6-OHDA groups. Finally, regression analyses showed that for both sexes, the negative impacts of 6-OHDA lesions on "What" discrimination were not significantly predicted by individual differences in lesion size (FEM 6-OHDA: R^2 =0.20; MALE 6-OHDA: $R^2 = 0.08$, see **Figure 4D**).

Analyses of data from the male groups showed that "What" DIs in GDX+TP 6-OHDA rats were low (−0.06) and were

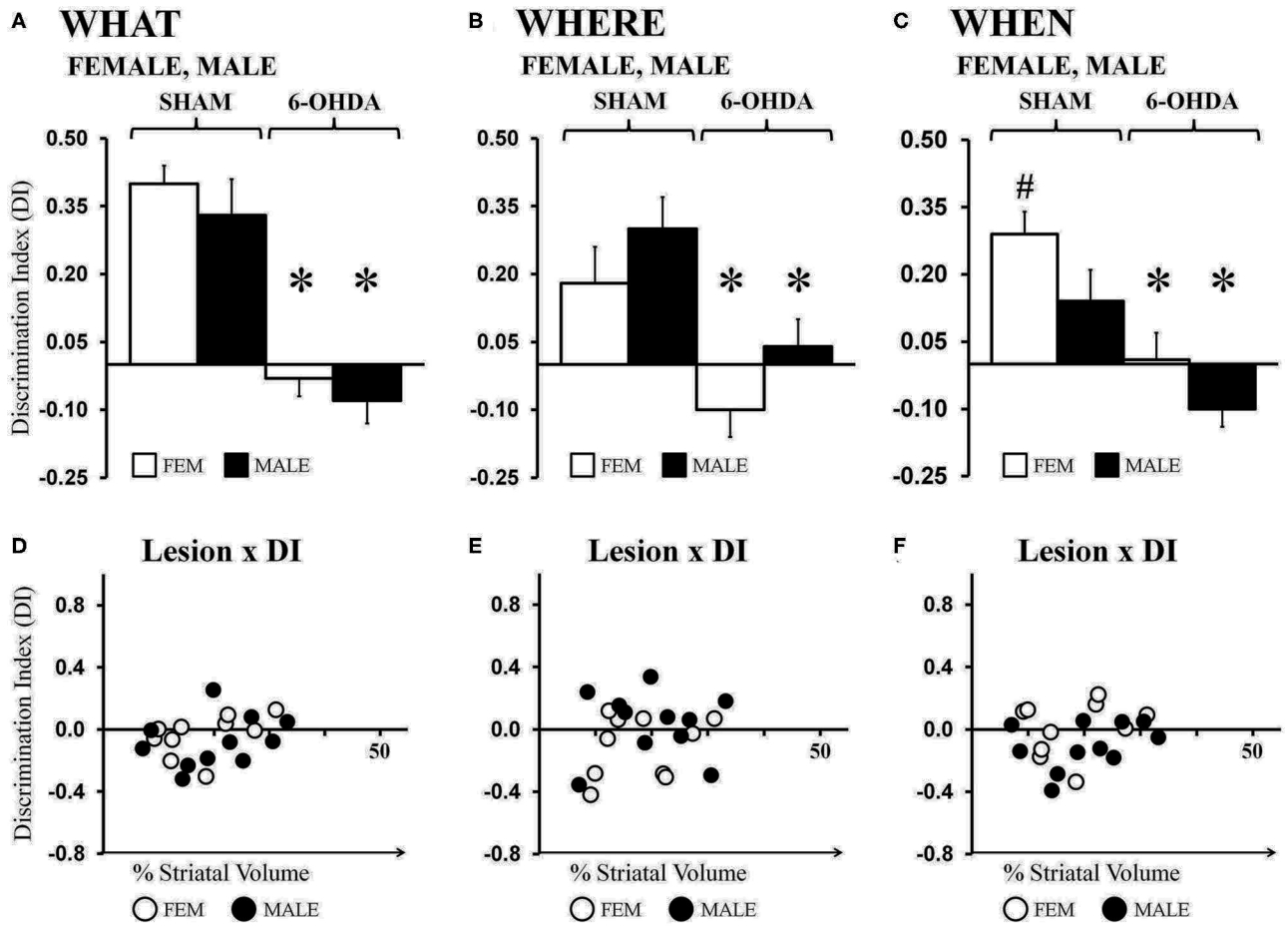


FIGURE 4 | Bar graphs showing average "What" **(A)**, "Where" **(B)**, and "When" **(C)** discrimination indices (DIs) calculated from object exploration in Test Trials for gonadally intact sham-operated (SHAM) and 6-hydroxydopamine lesioned (6-OHDA) female (FEM, white) and male (MALE, black) rats. DIs for all domains were robust for FEM SHAM and MALE SHAM; trends for MALE over FEM advantage for "Where," and FEM over MALE advantage for "When" were also seen. In contrast, DIs for all domains were impaired in FEM 6-OHDA and MALE 6-OHDA groups. Results from *post-hoc* comparisons are shown as follows: significant differences in DIs between the SHAM and 6-OHDA lesioned female and male rats (*); near significant differences in "When" discrimination between the SHAM-lesioned FEM and MALE groups ($p = 0.053$, #). Scatter plots of "What" **(D)**, "Where" **(E)**, and "When" **(F)** DIs expressed as functions of the size (percent of total striatal volume) of 6-OHDA lesions for individual gonadally intact FEM (white circles) and MALE (black circles) rats show no significant or consistent relationships between the two.

similar to those of the gonadally intact MALE 6-OHDA group. In contrast, DIs calculated for the GDX 6-OHDA and GDX+E 6-OHDA groups were 0.16 and 0.13, respectively. These values were lower than those observed for MALE SHAMs but higher than DIs calculated for the MALE 6-OHDA cohort (**Figure 5A**). *Post-hoc* comparisons (Bonferroni-corrected alpha = 0.0489) confirmed that "What" DIs in MALE 6-OHDA and GDX+TP 6-OHDA rats were similar to each other and one-sample *t*-tests showed that for both groups, these values were not significantly different from zero. Additionally, these analyses showed that "What" DIs for the GDX 6-OHDA and GDX+E 6-OHDA rats were similar to each other, but were significantly greater than DIs in the MALE 6-OHDA and GDX+TP 6-OHDA groups ($p = 0.007$–0.025), and significantly or near significantly lower than the "What" DIs of MALE SHAMs (GDX 6-OHDA: $p = 0.05$; GDX+E 6-OHDA: $p = 0.02$). One-sample *t*-tests showed that DIs for each group were significantly different from zero [GDX 6-OHDA: $t_{(1,9)} = 2.72$, $p = 0.024$; GDX+E 6-OHDA: $t_{(1,10)} = 2.477$, $p = 0.033$]. Regression analyses found no significant relationships between lesion sizes and the degrees of impairment or relative sparing observed for "What" discrimination for any of the 6-OHDA-lesioned groups [GDX 6-OHDA: $R^2 = 0.079$; GDX+TP 6-OHDA: $R^2 = 0.163$; GDX+E 6-OHDA: $R^2 = 0.002$] (**Figure 5D**).

"Where" discrimination

Average "Where" DIs calculated for FEM SHAM and MALE SHAM rats were 0.18 and 0.30, respectively. In contrast, strong preference for displaced vs. stationary "old familiar" objects was not seen in the FEM 6-OHDA or MALE 6-OHDA groups; these two "Where" DIs were −0.10 and 0.04, respectively (**Figure 4B**). *Post-hoc* comparisons (Bonferroni-corrected alpha = 0.0491)

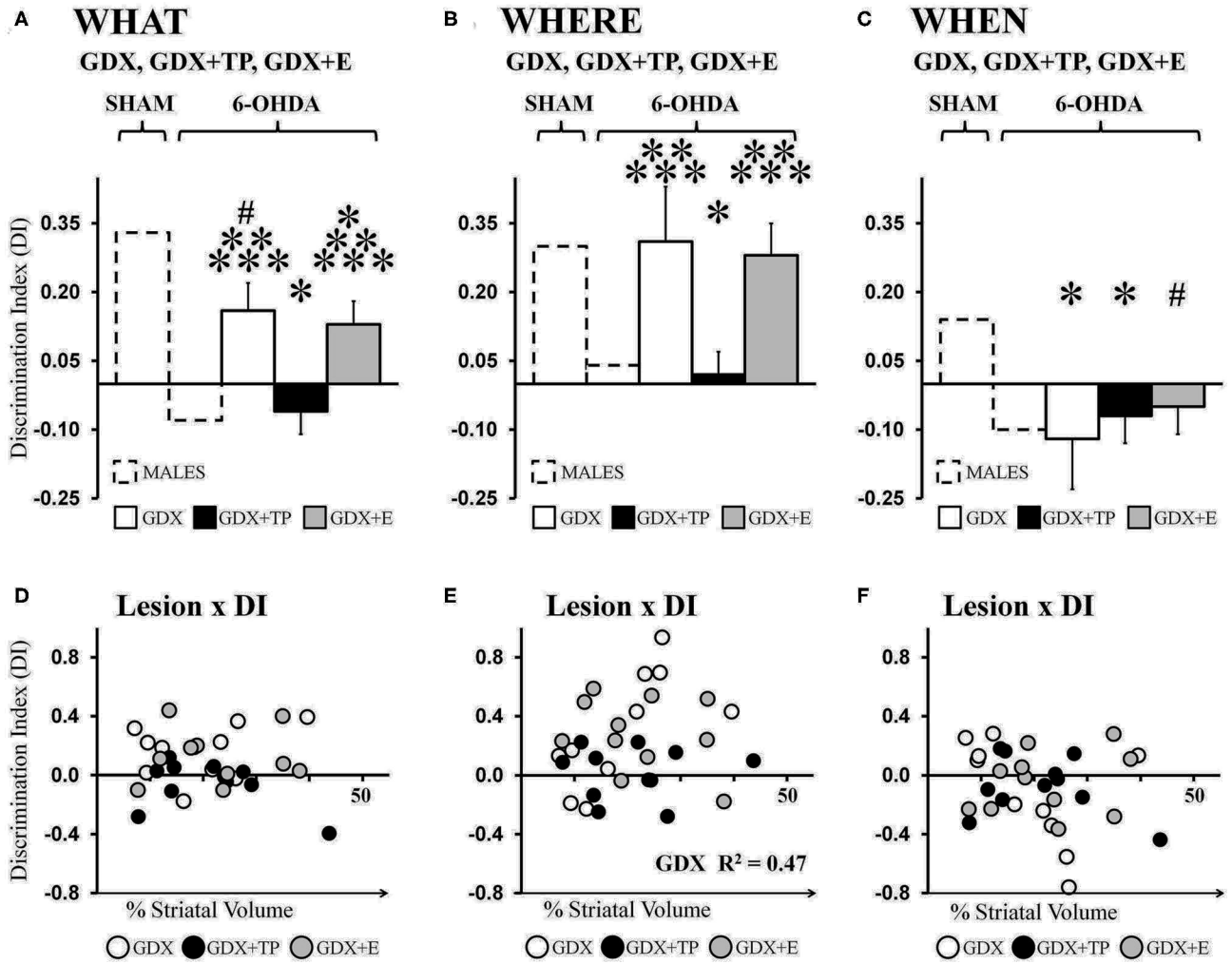


FIGURE 5 | Bar graphs showing average "What" **(A)**, "Where" **(B)**, and "When" **(C)** discrimination indices (DIs) calculated from object exploration in Test Trials for 6-hydroxydopamine (6-OHDA) lesioned male rats that were gonadectomized (GDX, white), that were GDX and supplemented with testosterone propionate (black), or GDX and supplemented with 17β-estradiol 6-OHDA (gray); data from gonadally intact SHAM and 6-OHDA lesioned MALES are shown for reference (dashed outline). The GDX+TP cohort was profoundly impaired for "What" **(A)**, "Where" **(B)**, and "When"**(C)** discriminations. In contrast, DI's for the GDX and GDX+E groups were moderate for "What," robust for "Where," and poor for "When." Results from allowed *post-hoc* comparisons of DI that included all of the male groups are shown as follows: significantly different than MALE SHAM (*); near significantly different than MALE SHAM (#); significantly different than MALE 6-OHDA (**); significantly different than GDX+TP 6-OHDA groups (***).Scatter plots below of "What" **(D)**, "Where" **(E)**, and "When" **(F)** DIs as functions of the size (percent of total striatal volume) of 6-OHDA lesions for individual GDX (white circles), GDX+TP (black circles), and GDX+E (gray circles) 6-OHDA rats show no consistent relationships between the two for "What" or "When" DI. For "Where" DI, a significant positive correlation was found between larger lesions and better "Where" DI for GDX 6-OHDA rats; the R^2-value is shown on the plot **(E)**.

showed that although the "Where" DI in the MALE SHAM group was noticeably greater than that of the FEMALE SHAM rats, and in the MALE-6-OHDA group compared to the FEM 6-OHDA subjects, these difference did not reach statistical significance. However, *post-hoc* testing did confirm that differences in DI's between the SHAM and 6-OHDA groups were significant for both sexes (females, $p = 0.007$; males, $p = 0.009$). One-sample *t*-tests further showed that "Where" DIs were significantly >0 for FEM SHAM [$t_{(1,10)} = 2.39$, $p = 0.038$] and MALE SHAM [$t_{(1,10)} = 4.07$, $p = 0.002$] rats but were not significantly different from zero for the FEM 6-OHDA or MALE 6-OHDA cohorts. Regression analyses also showed that for both sexes, the degrees to which "Where" discriminations were negatively impacted were not significantly predicted by differences in 6-OHDA lesion sizes (FEM 6-OHDA: R^2 =0.07; MALE 6-OHDA: R^2 = 0.00, see **Figure 4E**).

Average "Where" DIs calculated for 6-OHDA lesioned GDX, GDX+TP, and GDX+E rats were 0.16, −0.06, and 0.13, respectively (**Figure 5B**). These data indicate preferential investigation of the displaced vs. the stationary "old familiar" object in GDX 6-OHDA and GDX+E 6-OHDA groups but not in GDX+TP 6-OHDA rats. *Post-hoc* comparisons (Bonferroni-corrected alpha of 0.0489) confirmed that DIs were similar in MALE 6-OHDA and GDX+TP 6-OHDA rats; were similar in the

MALE SHAM, GDX 6-OHDA, and GDX+E 6-OHDA groups; and were significantly different (lower) in MALE 6-OHDA and GDX+TP 6-OHDA rats compared to the MALE SHAM, GDX 6-OHDA, and GDX+E 6-OHDA groups (p = 0.013–0.032). Group differences in the robustness of "Where" DIs were also reflected in one-sample t-tests showing that, like MALE SHAMS, the DIs of GDX 6-OHDA, and GDX+E 6-OHDA rats were significantly >0 [GDX 6-OHDA: $t_{(1,9)} = 2.50$, $p = 0.034$; GDX+E 6-OHDA: $t_{(1,10)} = 3.79$, $p = 0.004$] but that, like MALE 6-OHDA rats, DIs from the GDX+TP 6-OHDA groups were not significantly different from zero. Lastly, regression analyses (**Figure 5E**) found little evidence for significant relationships between 6-OHDA lesion size and degrees to which "Where" DI was impaired (GDX+TP 6-OHDA: $R^2 = 0.00$) or spared (GDX+E 6-OHDA: $R^2 = 0.09$). The only significant relationship found was a positive correlation between larger lesion size and greater/better 'Where' discrimination in the GDX 6-OHDA group ($R^2 = 0.467$, $p = 0.029$).

"When" discrimination

Average 'When' DIs calculated for FEM SHAM and MALE SHAM rats (0.29 and 0.14, respectively) indicated preferential investigation of the stationary "old familiar" object vs. the average investigation of the two "recent familiar" objects in both sexes that was consistently stronger in the FEM SHAM group. In contrast, average "When" DIs for FEM 6-OHDA and MALE 6-OHDA rats were similar to each other and much lower than those of sham-operated rats (0.01 and −0.10, respectively) (**Figure 4C**). *Post-hoc* comparisons (Bonferroni-corrected alpha of 0.0491) of "When" DIs identified the DIs of the FEM SHAM group as nearly significantly different (greater) than those of MALE SHAM rats ($p = 0.053$); the DIs of the FEM 6-OHDA and MALE 6-OHDA groups as similar to each other; and the DIs for the FEM SHAM vs. FEM 6-OHDA groups and for the MALE SHAM vs. MALE 6-OHDA groups as significantly different ($p = 0.001$, $p = 0.003$, respectively). One-sample t-tests also showed that "When" DIs: were significantly or near significantly >0 for FEM SHAM [$t_{(1,10)} = 5.49$, $p < 0.0001$] and MALE SHAM [$t_{(1,10)} = 2.09$, $p = 0.064$] groups; were not significantly different from zero for the FEM 6-OHDA group; and were significantly lower than zero for MALE 6-OHDA rats [$t_{(1,10)} = 2.33$, $p = 0.042$]. Regression analyses also showed that 6-OHDA lesion size did not significantly predict the magnitude of "When" discrimination deficits in either sex (FEM 6-OHDA: $R^2 = 0.04$; MALE 6-OHDA: $R^2 = 0.11$, see **Figure 4F**).

Average "When" DIs calculated for GDX 6-OHDA, GDX+TP 6-OHDA, and GDX+E 6-OHDA rats (−0.12, −0.07, and −0.05, respectively) indicated poor discrimination in all three groups (**Figure 5C**). Allowed *post-hoc* comparisons (Bonferroni-corrected alpha = 0.0489) showed that "When" DIs in these groups were similar to each other, were similar to DIs in the MALE 6-OHDA group, but were significantly or nearly significantly different from DIs in the MALE SHAM group (GDX 6-OHDA: p = 0.016; GDX+TP 6-OHDA: $p = 0.042$; GDX+E 6-OHDA, $p = 0.064$). One-sample t-tests further showed that "When" DIs for GDX 6-OHDA, GDX+TP 6-OHDA, and GDX+E 6-OHDA groups were not significantly different from zero. Finally, regression analyses confirmed that "When" DI values were not significantly predicted by the size of 6-OHDA-induced striatal lesions in any of these groups (GDX 6-OHDA: $R^2 = 0.21$; GDX+TP 6-OHDA: $R^2 = 0.10$; GDX+E 6-OHDA: $R^2 = 0.06$, see **Figure 5F**).

DISCUSSION

Memory impairments, including those involving episodic memory, present in roughly 20% of PD patients at or before the onset of motor deficits (30) and afflict more than 50% of patients over the course of illness (20, 26). Episodic memory impairments in particular can also predict a more rapid and more severe decline in motor and memory function (34, 35) and signal a greater probability of developing PD-related dementia (26, 36, 37). These clinical characteristics combine with the overall resistance of cognitive and mnemonic disturbances in PD to available therapeutics (19, 20, 22) to bring urgency to resolving questions about the neural underpinnings of episodic memory dysfunction in PD. While imaging studies have begun to identify structural brain changes that correlate with and in some cases predict the onset of episodic memory deficits in PD (42–44), less is known about the physiological factors that render episodic memory vulnerable in this disease. These factors could serve as biomarkers that prompt early, possibly preventive intervention and facilitate planning for long-term clinical care. These factors could also represent novel therapeutic targets that address unmet clinical needs for more effective treatment of the cognitive and mnemonic deficits in PD (19, 20, 22). This study was stimulated by findings suggesting that biological sex and/or sex hormones are among the factors that influence episodic memory function in PD (60–62) and used a preclinical PD model to investigate this further. Specifically, partial, bilateral neostriatal 6-OHDA dopamine lesions in female and male rats were paired with classical methods of hormone monitoring and manipulation and with behavioral testing using the WWWhen Episodic-Like Memory task. These studies confirmed and extended recent evidence for rats as suitable models for human sex differences in episodic memory (64). Thus, in keeping with sex differences described in human episodic memory for temporal and spatial information (71–79), non-significant trends were seen indicating that FEM SHAM rats outperformed MALE SHAM rats in "When" discrimination and gonadally intact that MALE SHAM rats tended to outperform FEM SHAM rats in "Where" discrimination. Further, these studies identified striking, negative impacts of 6-OHDA dopamine lesions on ELM function for all domains in gonadally intact rats of both sex. Finally, the data showed that ELM deficits in 6-OHDA lesioned male rats were strongly influenced by circulating hormone levels in domain specific ways. Specifically, similar to the gonadally intact males, 6-OHDA lesions in GDX+TP rats significantly impaired discriminations of "What," "Where," and "When." However, in the 6-OHDA-lesioned GDX and GDX+E groups, "When" discrimination was fully impaired, "What" discrimination was partially disrupted, and "Where" discrimination remained fully intact. As discussed below, these patterns of memory impairment and sparing map to ELM domains recently identified as

differentially sensitive to circulating estrogens and/or androgens (64) and offer a second, powerful example where the normally harmful effects of androgen depletion prove beneficial for memory function in 6-OHDA lesioned rats (66). First, however, the strategies used to minimize potential confounds from non-mnemonic influences on the ELM data are considered.

Experimental Design and Data Analyses Minimize Non-mnemonic Confounds

Key variables examined in this study included biological sex and monitored and manipulated sex hormone levels. These variables require that study design and data interpretation be informed by known sex differences in key behaviors, and by the broad range of metabolic and motivational states that gonadal hormones modulate in sex-specific ways. These include prominent sex differences in and sex hormone impacts on, sensitivity to positive and negative reinforcement (80–88). Thus, while there are several options for laboratory testing of ELM in rodents (89–93), the WWWhen task was selected for its leveraging of rats' innate preference for novelty and spontaneous investigations of novel objects encountered in the environment (63, 92). In fact, this task shares many of the same benefits recently espoused for single trial object recognition tasks in investigating neuroendocrine influences on learning and memory (94). In addition to mitigating potential confounds associated with reward contingencies, the WWWhen task is also minimally stressful and thus reduces the potential for bias arising from sex-specific impacts of stress on cognition and affect in rats (95–97). Studies in rat showing that stress sex-specifically impacts dopamine physiology in brain regions including the neostriatum (98), and studies in humans showing negative impacts of stress on episodic memory performance (99–103) further reinforce the importance of adopting testing procedures in animal subjects that minimize this variable.

This study also included variables of partial, bilateral neostriatal 6-OHDA lesions. This brings additional possibility for sex- and sex hormone-specific caveats. For example, studies in rats and mice have shown that the extent of and/or susceptibility to the effects of toxin-induced dopamine lesions is greater in males than in females (104–107); is greater for females in diestrus compared to proestrus (108–110); is greater in gonadally intact compared to GDX males (104, 111); and is greater in GDX males supplemented with the non-aromatizable androgen, dihydrotestosterone, compared to GDX males treated with estradiol (104, 105, 112). Moreover, these differences have been shown to be especially to exclusively robust for moderately sized, partial 6-OHDA lesions (6, 104). For these reasons, rigorous quantitative evaluations were made in this study of 6-OHDA lesion volume and lesion symmetry in all subjects and groups. As in a previous study using a similar lesioning protocol (66), there was some inter-subject variance in lesion measures. However, these tended to be small. Further, there were no obvious correlations between lesion size and estrous cycle stage at the time of surgery among the female subjects; there were no significant or near significant correlations between lesion size and hormone status at the time of surgery in males; there were no significant or near significant group differences in 6-OHDA lesion size or symmetry; and other than a small number of isolated cases involving non-mnemonic behaviors, the only significant correlation found between lesion size and ELM was a single positive association between larger lesion size and improved "Where" discrimination in GDX 6-OHDA rats. This adds to arguments for the behavioral sparing in this group as being highly unlikely to be due to smaller lesion sizes.

Finally, this study used lesion strategies developed intentionally to model early stages of PD and to yield motor deficits that are minimal or absent (65, 113–117). Nonetheless, multiple behaviors were evaluated during both the Sample and Test trials in addition to object exploration/discrimination, to confirm that 6-OHDA lesioned rats had the same abilities to ambulate, navigate, and explore as SHAM lesioned controls. Importantly, there were no group differences among the 6-OHDA or SHAM groups in ambulation, rearing, grooming, arena ledge investigation, object exploration, or stationary behavior for any trial. Rather, the 6-OHDA and SHAM groups apportioned and modified their engagement in all major activities—including object exploration (below), similarly both within and across trials.

ELM in a Preclinical Model of PD: Validation of Sham-Lesioned Controls

Partial unilateral or partial bilateral nigrostriatal dopamine lesions in rats and mice have been shown to elicit measurable changes in active avoidance, working memory, reference memory, object recognition, and/or other cognitive domains that model those that are at risk in early and pre-motor stages of PD (66, 115, 118–123). To our knowledge, however, the present study is the first to ask whether episodic memory deficits, which are also at risk in PD (21, 32, 33), are induced in a preclinical dopamine lesion PD model (partial bilateral neostriatal 6-OHDA lesions). As a critical control, we included cohorts of gonadally intact male and female rats that were sham lesioned, i.e., bilaterally injected with acidified vehicle. Evaluations of the injected neostriatal regions revealed no evidence of local tissue disruption or obvious change (increases or decreases) in the intensity of TH-immunostaining compared to adjacent, un-injected neostriatal zones. Nonetheless, both SHAM groups were systematically probed for possible effects on an array of task-related motor, exploratory, and other behaviors. These analyses revealed sex differences that were consistent with the increased activity/ambulation that has been reported for female compared to male rats in Novel Open Field testing (124, 125). Specifically, the FEM SHAM rats engaged in significantly more active behavior (ambulation, ledge investigation) and displayed significantly less inactivity (stationary behavior) than the MALE SHAM rats. Comparisons with published data from this lab, where WWWhen testing was carried out in un-operated, gonadally intact male and female rats (64), also showed similarities in both the proportions of trial times that SHAM vs. un-operated rats allotted to major task-related behaviors, and in calculated discrimination indices. Thus, for the latter DIs in SHAM and un-operated control male and female rats alike were

some 1.2–2.4 times greater for "What" discrimination compared to discriminations of "Where" and "When" (64).

Sex differences in DIs observed for the MALE SHAM and FEM SHAM rats in this study were non-significant. However, trends in the data were observed that were similar to those identified in the two prior studies of ELM in rats that included biological sex as a covariate. For example, in keeping with findings for human males as outperforming females in tests of episodic memory requiring visuospatial recall (75, 76, 79), all rat studies including the present showed better "Where" discrimination in gonadally intact male compared to gonadally intact female subjects (64, 126). The present study also revealed a trend for better discrimination of "When" in FEM SHAM compared to MALE SHAM rats. This potentially aligns with findings showing that women perform better than men in temporal ordering tasks and in estimating temporal features of remembered events (71–74, 77, 78). However, despite the robust evidence that supports superior performance of women in tests of episodic memory requiring recall of pictures and objects (79, 127), no rodent studies to date– including the present, have identified corresponding significant or non-significant trends for female over male differences in rats' discriminations of "What." While the presence or absence of sex differences could be related to the different cells and circuits that process what, where and when ELM domains [see (128)], they may also be explained by the likelihood that discriminations based on multiple object features ("What") are more easily made than those based on more constrained dimensions of "Where" or "When" (94, 129, 130). Accordingly, it may be necessary to increase the mnemonic demands of the WWWhen task (e.g., lengthen inter-trial intervals or reduce the number of distinguishing dimensions for sample objects) in order to reveal the full extent to which human patterns of domain-specific sex differences in episodic memory are recapitulated in rats.

ELM in a Preclinical Model of PD: Sex Differences

Sex differences characterize the incidence and prevalence of PD (10–13) and differentiate many of its motor, autonomic and affective disturbances (6, 12, 131–133). Although less intensively studied, consensus findings also link male gender to increased risk for developing PD-related cognitive dysfunction and dementia (5, 6, 58–60, 134). Episodic memory deficits have also been shown to be more common, and to worsen more rapidly in males (60) and to respond better to multimodal exercise interventions in females (61, 62). Thus, similar to what has been more firmly established for aging, Alzheimer's disease, and schizophrenia (50–55, 57), there are reasons to suspect that biological sex and sex hormones also influence episodic memory dysfunction in PD in potentially therapeutic ways. One objective of this study was to determine the utility of a preclinical, 6-OHDA lesion rat model of PD to more rigorously evaluate these influences. Using the WWWhen task and well-validated sham-lesioned controls (above), we showed that partial, bilateral neostriatal 6-OHDA lesions indeed induced significant, highly robust ELM deficits in both male and female rats. Specifically, rats of both sexes were profoundly impaired in discriminating among the Test trial objects based on domains of "What," "Where," and "When." However, mindful of data from this lab and others showing that 6-OHDA lesions can induce potentially confounding perseveration, difficulty in disengaging from stimuli, and delays in initiating behavior (66, 135) we also evaluated 6-OHDA and SHAM rats for latency to initiate object exploration, total object exploration times, total amounts of time spent exploring individual objects, and mean durations of single bouts of object exploration across groups. Due perhaps to the small arena size and the proximity of objects to the central start position, there were no indications that 6-OHDA lesioned rats (of any group) hesitated at the starts of Sample or Test trials. Further, and possibly related to the relatively short distances that separated sample objects, there was no evidence that any of the 6-OHDA lesioned groups engaged in prolonged or perseverative explorations of individual objects. These findings reinforce conclusions that differential object investigations observed during Test trials aptly reflected rats' ELM and further identify the WWWhen task and the testing apparatus used as suitable for evaluating episodic memory dysfunction in 6-OHDA lesioned rats. However, in contrast to the need for increased mnemonic demands to resolve sex differences in ELM in gonadally intact animals, any sex differences that may have been present among the MALE 6-OHDA and FEM 6-OHDA rats were obscured by the extremely low levels of discrimination seen in both groups. Thus, shorter inter-trial intervals may be needed to avoid basement effects and determine whether a more severe PD-related memory phenotype in males that is predicted in the human literature (5, 6, 59, 60) is borne out in MALE 6-OHDA compared to FEM 6-OHDA rats. It may also be useful to evaluate ELM in 6-OHDA lesioned male and female rats sooner and/or at several intervals after the induction of chemical lesions. Although inarguably abrupt compared to the progression of dopamine loss in PD, the strategy used here of injecting 6-OHDA among the axon terminal fields of nigrostriatal DA neurons has been shown to produce a more protracted time course of dopamine depletion compared to 6-OHDA injections targeting the medial forebrain bundle or substantia nigra (113). Thus, while not without caveats, this model might be useful for exploring and better understanding the sex differences in onset and/or rates of memory decline that are seen in PD (5, 6, 59, 60). Genetic rodent models of PD such as PINK1 knockout rats, which have been shown to undergo progressive nigrostriatal dopamine loss (136, 137), may also be well- and perhaps better-suited for this purpose.

ELM in a Preclinical Model of PD: Hormone Impacts in Males

We recently demonstrated the utility of pairing 6-OHDA lesions with classical hormone manipulations in male rats as important, previously untested means of modeling and exploring hormone impacts on cognitive dysfunction in PD. Specifically, we used Barnes maze testing to evaluate and compare spatial working memory, learning strategy, and other higher order functions in 6-OHDA-lesioned male rats that were either gonadally intact, gonadectomized (GDX), or GDX and supplemented with testosterone or estradiol (66). These analyses were informed by earlier work showing that the

principal measures of Barnes maze performance assessed are impaired by GDX and are attenuated in GDX rats supplemented with testosterone but not estradiol i.e., are androgen-sensitive (138). What was found was that these androgen-sensitive elements of behavior (spatial working memory, other frontal lobe operations) were profoundly impaired by 6-OHDA lesions—but only in animal groups where circulating androgen levels were physiologic, i.e., only in male 6-OHDA lesioned gonadally intact and GDX+TP rats. These findings seem consistent with recent studies showing that the motor consequences of similar experimental dopamine lesions are also dependent on and/or exacerbated by circulating androgens in males (139, 140). However, we also found that in 6-OHDA lesioned GDX and GDX+E rats, Barnes maze performance rivaled that of un-lesioned, hormonally intact controls (66). Thus, in these two groups, neither the profound Barnes maze deficits that are normally induced by androgen depletion (138) nor those that are produced in control and GDX+TP by 6-OHDA lesions were present (66). As discussed below, data from the present study suggest that similarly intriguing and perhaps therapeutically relevant relationships also exist between experimental dopamine lesions, circulating androgens, and processes of ELM.

Recent studies from this lab carried out in un-lesioned rats showed that GDX in male rats profoundly impairs ELM for "What", "Where", and "When" discriminations (64). However, these studies also showed that GDX-induced deficits were rescued by estrogen and/or testosterone replacement in highly selective, domain specific ways (64). Specifically, GDX-induced deficits in "What" discrimination were fully attenuated by TP and were partially rescued by E, thus indicating a requirement for both estrogen and androgen signaling. In contrast, GDX-induced deficits in "Where" discrimination were fully attenuated by TP and were unaffected by E, thus indicating androgen-sensitivity and estrogen-insensitivity. Finally, GDX-induced deficits in "When" discrimination were fully and equally attenuated by both TP and E, indicating their estrogen-sensitivity and androgen-insensitivity (64). The present assessment of 6-OHDA lesion impacts on these same ELM domains, in these same groups (gonadally intact, GDX, GDX+E, GDX+TP), revealed profound lesion-induced deficits for "What," "Where," and "When" discrimination, albeit only in the MALE 6-OHDA and GDX+TP 6-OHDA groups. In contrast, in the GDX 6-OHDA and GDX+E 6-OHDA rats, there was selective sparing of 6-OHDA-induced deficits that was highly specific for androgen-sensitive discrimination domains. Thus, in both GDX 6-OHDA and GDX+E 6-OHDA rats, discrimination of "Where" was fully spared, discrimination of "What" was partially spared, and discrimination of "When" was fully vulnerable to 6-OHDA lesion-induced deficits. It is important to repeat that neither impairments nor sparing of ELM discriminations were related to differences in 6-OHDA lesion sizes. Rather, as previously seen for Barnes maze testing (66), the data were dependent on circulating androgen levels. Specifically, physiological levels of androgen, which normally support these and other cognitive functions (64, 81, 141–145), render these processes vulnerable to dysfunction induced by nigrostriatal dopamine depletion. The data also support the corollary that androgen depletion, which is normally harmful to cognition and memory (64, 138, 146, 147), protects these domains from dysregulation and dysfunction induced by experimental 6-OHDA lesions. Because the WWWhen task concurrently measures discriminations that are explicitly estrogen-sensitive ("When"), uniquely androgen-sensitive ("Where"), and requiring of both ("What") (64), especially strong arguments can be made for androgens, and not estrogens, as conferring behavioral vulnerability and protection, and for the targeted behaviors as being androgen, and not estrogen-sensitive. One explanation for how this occurs could lie in off-setting impacts on prefrontal dopamine levels. Specifically, nigrostriatal 6-OHDA lesions have been shown to diminish dopamine levels in prefrontal and cingulate cortices (148, 149) whereas GDX has been shown to selectively increase dopamine levels in these cortical regions in an androgen-dependent, estrogen-insensitive manner (67, 150, 151). Thus, given the well-established inverted U-shaped function that defines the relationship between dopamine levels and prefrontal cortical functions (152), it is possible that the combination of neostriatal 6-OHDA lesions and GDX or GDX+E yield prefrontal dopamine levels that are more functionally optimal compared to rats that are 6-OHDA-lesioned (hypodopaminergic) or hormonally manipulated (hyperdopaminergic) alone. In MALE 6-OHDA and GDX+TP 6-OHDA rats, there are no hormone-related, dopamine-facilitating influences present to balance the prefrontal hypodopaminergia induced by 6-OHDA lesions, thus, predictably, leading to cognitive impairment (152–155). However, while prior studies linked androgen regulation/dysregulation to frontal lobe functions (66) the present studies extend this relationship to processes of ELM. This opens the possibility for androgen-mediated actions and mechanisms also targeting additional brain regions and neurotransmitters systems that along with the frontal lobe are critical for mediating this form of memory. These additional targets could include medial temporal lobe structures such as the entorhinal cortices, the hippocampal formation, and the septo-hippocampal cholinergic systems. All have been inexorably linked to episodic and other types of memory functions in healthy humans (156, 157), are strongly linked to the disturbances in episodic and other memory processes in PD (44, 158, 159) and are highly sensitive to androgens in rats (160–162). Data from rodent studies further underscore the need to evaluate and compare androgen impacts on lateral and medial entorhinal cortices and on CA1 and CA3 hippocampal subfields. Like gonadal hormones, these loci have been shown to play striking, domain-specific roles in what, where, and where elements of episodic memory [e.g., (128, 163–169)]. Finally, there are intriguing data showing pivotal roles for hippocampal area CA3 (89) and for functional interactions between medial prefrontal cortex and the CA1 and CA3 subfields (170–172) in the integration of "What," "Where," and/or "When" information into cohesive episodic memories. Identifying the hormone sensitivities of these important sites, circuits, and mnemonic processes, however, will require studies that not only combine hormone manipulations with site specific and disconnection lesion strategies but that also use an alternate version of the

WWWhen task that incorporates recent and old familiar objects that are displaced into the test trial (173). Unlike the version of the WWWhen task used here, the test trial configuration of this task allows for explicit measurement of interactions between memory for object location and temporal ordering (128). While data from this lab have shown that hormone modulation of memory functions tapped in the version of the WWWhen task used here (64) differs from hormone sensitivities identified for memory processes engaged in preference tasks based on object features (174) or location (175) alone, testing on the alternative WWWhen task will be important in more definitively tying hormone impacts to the integrative aspects of ELM.

CONCLUSIONS AND FUTURE DIRECTIONS

Both the motor signs and the non-motor symptoms of PD are distinguished by prominent sex differences (5, 7–9, 12, 58, 59). This study asked how gonadal hormones influence the cognitive impairments of PD in hopes of discovering new ways to more effectively combat these largely treatment-resistant aspects of the disease. Focusing specifically on processes of episodic memory, we found that this memory form is highly susceptible to impairment caused by a 6-OHDA dopamine lesion model of PD in male and female rats. However, in male rats, we also found that 6-OHDA-induced impairments in discrimination domains previously identified as androgen-sensitive (64) could be strongly attenuated by reducing circulating androgen levels. Understanding the basis for these potentially therapeutic actions may be especially pressing to resolve, given the prevalence of low testosterone levels in PD patients (176) and current practices of using hormone replacement therapies to elevate them (177). With the present identification of 6-OHDA lesions and WWWhen ELM testing in rats as a validated experimental framework, suitable means are now at hand for deeply investigating these actions and uncovering their neurobiological bases.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/**Supplementary Material**.

ETHICS STATEMENT

The animal study was reviewed and approved by Institutional Animal Care and Use Committee at Stony Brook University.

AUTHOR CONTRIBUTIONS

MC is a Ph.D. candidate who helped develop the experiments and was responsible for behavioral testing, for data analysis and archiving, for figure preparation, and for contributing to manuscript writing and editing. DJ is an undergraduate student who assisted with animal handling, behavioral analyses, data archiving, and manuscript editing. BA is a Professor in Stony Brook University's Department of Psychology who assisted in developing the experiments, provided guidance for behavioral testing and analysis, and contributed to manuscript editing. MK is a Professor in Stony Brook University's Department of Neurobiology and Behavior who developed the experiments, provided oversight for all aspects of the study, assisted with behavioral testing and histology, and contributed to manuscript writing and editing. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2020.00942/full#supplementary-material

REFERENCES

1. Jankovic J. Parkinson's disease: clinical features and diagnosis. *J Neurol Neurosurg Psychiatry.* (2008) 79:368–76. doi: 10.1136/jnnp.2007.131045
2. Sveinbjornsdottir S. The clinical symptoms of Parkinson's disease. *J Neurochem.* (2016) 139:318–24. doi: 10.1111/jnc.13691
3. Del Rey NL-G, Quiroga-Varela A, Garbayo E, Carballo-Carbajal I, Fernández-Santiago R, Monje MHG, et al. Advances in Parkinson's disease: 200 years later. *Front Neuroanat.* (2018) 12:113. doi: 10.3389/fnana.2018.00113
4. Papagno C, Trojano L. Cognitive and behavioral disorders in Parkinson's disease: an update. I: cognitive impairments. *Neurol Sci.* (2018) 39:215–23. doi: 10.1007/s10072-017-3154-8
5. Miller IN, Cronin-Golomb A. Gender differences in Parkinson's disease: clinical characteristics and cognition. *Move Disord.* (2010) 25:2695–703. doi: 10.1002/mds.23388
6. Gillies GE, Pienaar IS, Vohra S, Qamhawi Z. Sex differences in Parkinson's disease. *Front Neuroendocrinol.* (2014) 35:370–84. doi: 10.1016/j.yfrne.2014.02.002
7. Jurado-Coronel JC, Cabezas R, Ávila Rodríguez MF, Echeverria V, García-Segura LM, Barreto GE. Sex differences in Parkinson's disease: features on

clinical symptoms, treatment outcome, sexual hormones and genetics. *Front Neuroendocrinol.* (2018) 50:18–30. doi: 10.1016/j.yfrne.2017.09.002
8. Ullah MF, Ahmad A, Bhat SH, Abu-Duhier FM, Barreto GE, Ashraf GM. Impact of sex differences and gender specificity on behavioral characteristics and pathophysiology of neurodegenerative disorders. *Neurosci Biobehav Rev.* (2019) 102:95–105. doi: 10.1016/j.neubiorev.2019.04.003
9. Meoni S, Macerollo A, Moro E. Sex differences in movement disorders. *Nat Rev Neurol.* (2020) 16:84–96. doi: 10.1038/s41582-019-0294-x
10. Baldereschi M, Di Carlo A, Rocca WA, Vanni P, Maggi S, Perissinotto E, et al. Parkinson's disease and parkinsonism in a longitudinal study: two-fold higher incidence in men. *Neurology.* (2000) 55:1358–63. doi: 10.1212/WNL.55.9.1358
11. Shulman LM, Bhat V. Gender disparities in Parkinson's disease. *Expert Rev Neurother.* (2006) 6:407–16. doi: 10.1586/14737175.6.3.407
12. Haaxma CA, Bloem BR, Borm GF, Oyen WJG, Leenders KL, Eshuis S, et al. Gender differences in Parkinson's disease. *J Neurol Neurosurg Psychiatry.* (2007) 78:819–24. doi: 10.1136/jnnp.2006.103788
13. Taylor KSM, Cook JA, Counsell CE. Heterogeneity in male to female risk for Parkinson's disease. *J Neurol Neurosurg Psychiatry.* (2007) 78:905–6. doi: 10.1136/jnnp.2006.104695
14. Alves G, Muller B, Herlofson K, HogenEsch I, Telstad W, Aarsland D, et al. Incidence of Parkinson's disease in Norway: the Norwegian ParkWest study. *J Neurol Neurosurg Psychiatry.* (2009) 80:851–7. doi: 10.1136/jnnp.2008.168211
15. Martinez-Martin P, Falup Pecurariu C, Odin P, van Hilten JJ, Antonini A, Rojo-Abuin JM, et al. Gender-related differences in the burden of non-motor symptoms in Parkinson's disease. *J Neurol.* (2012) 259:1639–47. doi: 10.1007/s00415-011-6392-3
16. Pfeiffer HCV, Løkkegaard A, Zoetmulder M, Friberg L, Werdelin L. Cognitive impairment in early-stage non-demented Parkinson's disease patients. *Acta Neurol Scand.* (2014) 129:307–18. doi: 10.1111/ane.12189
17. Cosgrove J, Alty JE, Jamieson S. Cognitive impairment in Parkinson's disease. *Postgraduate Med J.* (2015) 91:212–20. doi: 10.1136/postgradmedj-2015-133247
18. Aarsland D, Creese B, Politis M, Chaudhuri KR, Ffytche DH, Weintraub D. Cognitive decline in Parkinson disease. *Nat Rev Neurol.* (2017) 13:217–31. doi: 10.1038/nrneurol.2017.27
19. Goldman JG, Vernaleo BA, Camicioli R, Dahodwala N, Dobkin RD, et al. Cognitive impairment in Parkinson's disease: a report from a multidisciplinary symposium on unmet needs and future directions to maintain cognitive health. *NPJ Parkinson's Dis.* (2018) 4:19. doi: 10.1038/s41531-018-0055-3
20. Fang C, Lv L, Mao S, Dong H, Liu B. Cognition deficits in Parkinson's disease: mechanisms and treatment. *Parkinson's Dis.* (2020) 2020:2076942. doi: 10.1155/2020/2076942
21. Aarsland D, Kurz MW. The epidemiology of dementia associated with Parkinson disease. *J Neurol Sci.* (2010) 289:18–22. doi: 10.1016/j.jns.2009.08.034
22. Rowe J, Hughes L, Ghosh B, Eckstein D, Williams-Gray C, Fallon S, et al. Parkinson's disease and dopaminergic therapy—differential effects on movement, reward and cognition. *Brain.* (2008) 131:2094–105. doi: 10.1093/brain/awn112
23. Cools R. Enhanced or impaired cognitive function in Parkinson's disease as a function of dopaminergic medication and task demands. *Cerebral Cortex.* (2001) 11:1136–43. doi: 10.1093/cercor/11.12.1136
24. Czernecki V. Motivation, reward, and Parkinson's disease: influence of dopatherapy. *Neuropsychologia.* (2002) 40:2257–67. doi: 10.1016/S0028-3932(02)00108-2
25. Abboud H, Floden D, Thompson NR, Genc G, Oravivattanakul S, Alsallom F, et al. Impact of mild cognitive impairment on outcome following deep brain stimulation surgery for Parkinson's disease. *Parkinsonism Related Disord.* (2015) 21, 249–253. doi: 10.1016/j.parkreldis.2014.12.018
26. Aarsland D, Andersen K, Larsen JP, Lolk A. Prevalence and characteristics of dementia in Parkinson disease. *Arch Neurol.* (2003) 60:387. doi: 10.1001/archneur.60.3.387
27. Schrag A. What contributes to quality of life in patients with Parkinson's disease? *J Neurol Neurosurg Psychiatry.* (2000) 69:308–12. doi: 10.1136/jnnp.69.3.308
28. Elgh E, Domellã M, Linder J, Edstrãm M, Stenlund H, Forsgren L. Cognitive function in early Parkinson's disease: a population-based study. *Eur J Neurol.* (2009) 16:1278–84. doi: 10.1111/j.1468-1331.2009.02707.x
29. Pagonabarraga J, Kulisevsky J. Cognitive impairment and dementia in Parkinson's disease. *Neurobiol Dis.* (2012) 46:590–6. doi: 10.1016/j.nbd.2012.03.029
30. Breen KC, Drutyte G. Non-motor symptoms of Parkinson's disease: the patient's perspective. *J Neural Trans.* (2013) 120:531–5. doi: 10.1007/s00702-012-0928-2
31. Tulving E. Episodic memory: from mind to brain. *Ann Rev Psychol.* (2002) 53:1–25. doi: 10.1146/annurev.psych.53.100901.135114
32. Yarnall AJ, Breen DP, Duncan GW, Khoo TK, Coleman SY, Firbank MJ, et al. Characterizing mild cognitive impairment in incident Parkinson disease: The ICICLE-PD study. *Neurology.* (2014) 82:308–16. doi: 10.1212/WNL.0000000000000066
33. Weintraub D, Simuni T, Caspell-Garcia C, Coffey C, Lasch S, Siderowf A, et al. Cognitive performance and neuropsychiatric symptoms in early, untreated Parkinson's disease. *Move Disord.* (2015) 30:919–27. doi: 10.1002/mds.26170
34. Muslimović D, Post B, Speelman JD, Schmand B. Cognitive profile of patients with newly diagnosed Parkinson disease. *Neurology.* (2005) 65:1239–45. doi: 10.1212/01.wnl.0000180516.69442.95
35. Broeders M, De Bie R, Velseboer DC, Speelman JD, Muslimovic D, Schmand B. Evolution of mild cognitive impairment in Parkinson disease. *Neurology.* (2013) 81:346–52. doi: 10.1212/WNL.0b013e31829c5c86
36. Emre M, Aarsland D, Brown R, Burn DJ, Duyckaerts C, Mizuno Y, et al. Clinical diagnostic criteria for dementia associated with Parkinson's disease. *Move Disord.* (2007) 22:1689–707. doi: 10.1002/mds.21507
37. Robbins TW, Cools R. Cognitive deficits in Parkinson's disease: a cognitive neuroscience perspective. *Move Disord.* (2014) 29:597–607. doi: 10.1002/mds.25853
38. Aarsland D, Larsen JP, Tandberg E, Laake K. Predictors of nursing home placement in Parkinson's disease: a population-based, prospective study. *J Am Geriatr Soc.* (2000) 48:938–42. doi: 10.1111/j.1532-5415.2000.tb06891.x
39. Hughes TA, Ross HF, Mindham RHS, Spokes EGS. Mortality in Parkinson's disease and its association with dementia and depression. *Acta Neurol Scand.* (2004) 110:118–23. doi: 10.1111/j.1600-0404.2004.00292.x
40. Halliday G, Hely M, Reid W, Morris J. The progression of pathology in longitudinally followed patients with Parkinson's disease. *Acta Neuropathol.* (2008) 115:409–15. doi: 10.1007/s00401-008-0344-8
41. Hely MA, Morris JGL, Reid WGJ, Trafficante R. Sydney multicenter study of Parkinson's disease: non- L -dopa–responsive problems dominate at 15 years. *Move Disord.* (2005) 20:190–9. doi: 10.1002/mds.20324
42. Filoteo JV, Reed JD, Litvan I, Harrington DL. Volumetric correlates of cognitive functioning in nondemented patients with Parkinson's disease. *Mov Disord.* (2014) 29:360–7. doi: 10.1002/mds.25633
43. Pirogovsky-Turk E, Filoteo JV, Litvan I, Harrington DL. Structural MRI correlates of episodic memory processes in Parkinson's disease without mild cognitive impairment. *J Parkinsons Dis.* (2015) 5:971–81. doi: 10.3233/JPD-150652
44. La C, Linortner P, Bernstein JD, Cruadhlaoich MAIU, Fenesy M, Deutsch GK, et al. Hippocampal CA1 subfield predicts episodic memory impairment in Parkinson's disease. *NeuroImage Clin.* (2019) 23:101824. doi: 10.1016/j.nicl.2019.101824
45. Dunet V, Fartaria MJ, Deverdun J, Le Bars E, Maury F, Castelnovo G, et al. Episodic memory decline in Parkinson' s disease: relation with white matter hyperintense lesions and influence of quantification method. *Brain Imaging Behav.* (2018) 13:810–8. doi: 10.1007/s11682-018-9909-x
46. Herlitz A, Reuterskiöld L, Lovén J, Thilers PP, Rehnman J. Cognitive sex differences are not magnified as a function of age, sex hormones, or puberty development during early adolescence. *Dev Neuropsychol.* (2013) 38:167–79. doi: 10.1080/87565641.2012.759580
47. Pauls F, Petermann F, Lepach AC. Gender differences in episodic memory and visual working memory including the effects of age. *Memory.* (2013) 21:857–74. doi: 10.1080/09658211.2013.765892
48. Gur RE, Gur RC. Sex differences in brain and behavior in adolescence: findings from the Philadelphia neurodevelopmental cohort. *Neurosci Biobehav Rev.* (2016) 70:159–70. doi: 10.1016/j.neubiorev.2016.07.035

49. Asperholm M, Nagar S, Dekhtyar S, Herlitz A. The magnitude of sex differences in verbal episodic memory increases with social progress: Data from 54 countries across 40 years. *PLoS ONE.* (2019) 14:e0214945. doi: 10.1371/journal.pone.0214945
50. Ferreira L, Ferreira Santos-Galduróz R, Ferri CP, Fernandes Galduróz JC. Rate of cognitive decline in relation to sex after 60 years-of-age: a systematic review. *Geriatrics Gerontol Int.* (2014) 14:23–31. doi: 10.1111/ggi.12093
51. McCarrey AC, An Y, Kitner-Triolo MH, Ferrucci L, Resnick SM. Sex differences in cognitive trajectories in clinically normal older adults. *Psychol Aging.* (2016) 31:166–75. doi: 10.1037/pag0000070
52. Gur Ruben GR. Memory in health and schizophrenia. *Dialogues Clin Neurosci.* (2013) 15:399–410. Available online at: https://www.dialoguescns.org/contents-15-4/dialoguesclinneurosci-15-399/
53. Mendrek A, Mancini-Marïe A. Sex/gender differences in the brain and cognition in schizophrenia. *Neurosci Biobehav Rev.* (2016) 67:57–78. doi: 10.1016/j.neubiorev.2015.10.013
54. Henderson V. Estrogens, episodic memory, and Alzheimer's disease: a critical update. *Semin Reproduc Med.* (2009a) 27:283–93. doi: 10.1055/s-0029-1216281
55. Irvine K, Laws KR, Gale TM, Kondel TK. Greater cognitive deterioration in women than men with Alzheimer's disease: a meta analysis. *J Clin Exp Neuropsychol.* (2012) 34:989–98. doi: 10.1080/13803395.2012.712676
56. Laws KR, Irvine K, Gale TM. Sex differences in cognitive impairment in Alzheimer's disease. *World J Psychiatry.* (2016) 6:54–65. doi: 10.5498/wjp.v6.i1.54
57. Laws KR, Irvine K, Gale TM. Sex differences in Alzheimer's disease. *Curr Opinn Psychiatry.* (2018) 31:133–9. doi: 10.1097/YCO.0000000000000401
58. Heller J, Dogan I, Schulz JB, Reetz K. Evidence for gender differences in cognition, emotion and quality of life in Parkinson's disease? *Aging Dis.* (2014) 5:63–75. doi: 10.14336/AD.2014.050063
59. Picillo M, Nicoletti A, Fetoni V, Garavaglia B, Barone P, Pellecchia MT. The relevance of gender in Parkinson's disease: a review. *J Neurol.* (2017) 264:1583–607. doi: 10.1007/s00415-016-8384-9
60. Cholerton B, Johnson CO, Fish B, Quinn JF, Chung KA, Peterson-Hiller AL, et al. Sex differences in progression to mild cognitive impairment and dementia in Parkinson's disease. *Parkinsonism Related Disord.* (2018) 50:29–36. doi: 10.1016/j.parkreldis.2018.02.007
61. Gobbi LTB, Barbieri FA, Vitorio R, Pereira MP, Teixeira-Arroyo C. Effects of a multimodal exercise program on clinical, functional mobility and cognitive parameters of idiopathic Parkinson's disease patients. *Diagnostics Rehabil Parkinson's Dis.* (2011) 339–52. doi: 10.5772/17693
62. Teixeira-Arroyo C, Rinaldi NM, Batistela RA, Barbieri FA, Vitório R, Gobbi LTB. Exercise and cognitive functions in Parkinson's disease: gender differences and disease severity. *Motriz: Revista de Educação Física.* (2014) 20:461–9. doi: 10.1590/S1980-65742014000400014
63. Dere E, Huston J, De Souza Silva MA. Integrated memory for objects, places, and temporal order: evidence for episodic-like memory in mice. *Neurobiol Learn Mem.* (2005b) 84:214–21. doi: 10.1016/j.nlm.2005.07.002
64. Conner MC, Adeyemi OM, Anderson BJ, Kritzer MF. Domain-specific contributions of biological sex and sex hormones to what, where and when components of episodic-like memory in adult rats. *Eur J Neurosci.* (2020) 52:2705–23. doi: 10.1111/ejn.14676
65. Branchi I, D'Andrea I, Armida M, Carnevale D, Ajmone-Cat MA, Pèzzola A, et al. Striatal 6-OHDA lesion in mice: Investigating early neurochemical changes underlying Parkinson's disease. *Behav Brain Res.* (2010) 208:137–43. doi: 10.1016/j.bbr.2009.11.020
66. Betancourt E, Wachtel J, Michaelos M, Haggerty M, Conforti J, Kritzer MF. The impact of biological sex and sex hormones on cognition in a rat model of early, pre-motor Parkinson's disease. *Neuroscience.* (2017) 345:297–314. doi: 10.1016/j.neuroscience.2016.05.041
67. Adler A, Vescovo P, Robinson JK, Kritzer MF. Gonadectomy in adult life increases tyrosine hydroxylase immunoreactivity in the prefrontal cortex and decreases open field activity in male rats. *Neuroscience.* (1999) 89:939–54. doi: 10.1016/S0306-4522(98)00341-8
68. Kritzer MF. Effects of acute and chronic gonadectomy on the catecholamine innervation of the cerebral cortex in adult male rats: insensitivity of axons immunoreactive for dopamine-β-hydroxylase to gonadal steroids, and differential sensitivity of axons immunoreactive. *J Compar Neurol.* (2000) 427:617–33. doi: 10.1002/1096-9861(20001127)427:4<617::AID-CNE9>3.0.CO;2-#
69. Turvin JC, Messer WS, Kritzer MF. On again, off again effects of gonadectomy on the acoustic startle reflex in adult male rats. *Physiol Behav.* (2007) 90:473–82. doi: 10.1016/j.physbeh.2006.10.010
70. Marcondes FK, Bianchi FJ, Tanno AP. Determination of the estrous cycle phases of rats: some helpful considerations. *Braz J Biol.* (2002) 62:609–14. doi: 10.1590/S1519-69842002000400008
71. Friedman A, Pines A. Sex differences in gender-related childhood memories. *Sex Roles.* (1991) 25:25–32. doi: 10.1007/BF00289314
72. Skowronski JJ, Betz AL, Thompson CP, Shannon L. Social memory in everyday life: recall of self-events and other-events. *J Personal Soc Psychol.* (1991) 60:831. doi: 10.1037/0022-3514.60.6.831
73. Herz RS, Cupchik GC. An experimental characterization of odor-evoked memories in humans. *Chem Senses.* (1992) 17:519–28. doi: 10.1093/chemse/17.5.519
74. Seidlitz L, Diener E. Sex differences in the recall of affective experiences. *J Personal Soc Psychol.* (1998) 74:262. doi: 10.1037/0022-3514.74.1.262
75. Lewin C, Wolgers G, Herlitz A. Sex differences favoring women in verbal but not in visuospatial episodic memory. *Neuropsychology.* (2001) 15:165–73. doi: 10.1037/0894-4105.15.2.165
76. Herlitz A, Yonker JE. Sex differences in episodic memory: the influence of intelligence. *J Clin Exp Neuropsychol.* (2002) 24:107–14. doi: 10.1076/jcen.24.1.107.970
77. Pillemer D, Wink P, DiDonato T, Sanborn R. Gender differences in autobiographical memory styles of older adults. *Memory.* (2003) 11:525–32. doi: 10.1080/09658210244000117
78. Pohl RF, Bender M, Lachmann G. Autobiographical memory and social skills of men and women. *Appl Cogn Psychol.* (2005) 19:745–59. doi: 10.1002/acp.1104
79. Asperholm M, Hogman N, Rafi J, Herlitz A. What did you do yesterday? A meta-analysis of sex differences in episodic memory Psychol Bull. (2019) 145:785–821. doi: 10.1037/bul0000197
80. Van Haaren F, Van Hest A, Heinsbroek RPW. Behavioral differences between male and female rats: effects of gonadal hormones on learning and memory. *Neurosci Biobehav Rev.* (1990) 14:23–33. doi: 10.1016/S0149-7634(05)80157-5
81. Luine VN, Beck KD, Bowman RE, Frankfurt M, Maclusky NJ. Chronic stress and neural function: accounting for sex and age. *J Neuroendocrinol.* (2007) 19:743–51. doi: 10.1111/j.1365-2826.2007.01594.x
82. Luine VN. Sex steroids and cognitive function. *J Neuroendocrinol.* (2008) 20:866–72. doi: 10.1111/j.1365-2826.2008.01710.x
83. Osborne DM, Edinger K, Frye CA. Chronic administration of androgens with actions at estrogen receptor beta have anti-anxiety and cognitive-enhancing effects in male rats. *Age.* (2009) 31:191–8. doi: 10.1007/s11357-009-9114-3
84. Becker JB, Chartoff E. Sex differences in neural mechanisms mediating reward and addiction. *Neuropsychopharmacology.* (2019) 44:166–83. doi: 10.1038/s41386-018-0125-6
85. Chowdhury TG, Wallin-Miller KG, Rear AA, Park J, Diaz V, Simon NW, et al. Sex differences in reward- and punishment-guided actions. *Cogn Affect Behav Neurosci.* (2019) 19:1404–17. doi: 10.3758/s13415-019-00736-w
86. Heck AL, Handa RJ. Sex differences in the hypothalamic–pituitary–adrenal axis' response to stress: an important role for gonadal hormones. *Neuropsychopharmacology.* (2019) 44:45–58. doi: 10.1038/s41386-018-0167-9
87. Rincón-Cortés M, Herman JP, Lupien S, Maguire J, Shansky RM. Stress: influence of sex, reproductive status and gender. *Neurobiology Stress.* (2019) 10:100155. doi: 10.1016/j.ynstr.2019.100155
88. Luine V, Bowman R, Serrano P. Sex differences in acute stress effects on spatial memory and hippocampal synaptic neurochemicals. *Understand Stress Work.* (2017) 52:52–6. doi: 10.1016/B978-0-12-802175-0.00032-2
89. Li J-S, Chao Y-S. Electrolytic lesions of dorsal CA3 impair episodic-like memory in rats. *Neurobiol Learn Memory.* (2008) 8:192–8. doi: 10.1016/j.nlm.2007.06.006

90. Tronche C, Lestage P, Louis C, Carrie I, Béracochéa D. Pharmacological modulation of contextual "episodic-like" memory in aged mice. *Behav Brain Res.* (2010) 215:255–60. doi: 10.1016/j.bbr.2010.04.009
91. Crystal JD, Smith AE. Binding of episodic memories in the rat. *Curr Biol.* (2014) 24:2957–61. doi: 10.1016/j.cub.2014.10.074
92. Binder S, Dere E, Zlomuzica A. A critical appraisal of the what-where-when episodic-like memory test in rodents: achievements, caveats and future directions. *Prog Neurobiol.* (2015) 130:71–85. doi: 10.1016/j.pneurobio.2015.04.002
93. Panoz-Brown D, Panoz-Brown D, Corbin HE, Dalecki SJ, Gentry M, Brotheridge S, et al. Rats remember items in context using episodic memory. *Curr Biol.* (2016) 26:2821–6. doi: 10.1016/j.cub.2016.08.023
94. Luine V. Recognition memory tasks in neuroendocrine research. *Behav Brain Res.* (2015) 285:158–64. doi: 10.1016/j.bbr.2014.04.032
95. Shansky RM, Lipps J. Stress-induced cognitive dysfunction: hormone-neurotransmitter interactions in the prefrontal cortex. *Front Hum Neurosci.* (2013) 7:123. doi: 10.3389/fnhum.2013.00123
96. Kokras N, Dalla C. Preclinical sex differences in depression and antidepressant response: Implications for clinical research. *J Neurosci Res.* (2017) 95:731–6. doi: 10.1002/jnr.23861
97. Luine V, Gomez J, Beck K, Bowman R. Sex differences in chronic stress effects on cognition in rodents. *Pharmacol Biochem Behav.* (2017) 152:13–9. doi: 10.1016/j.pbb.2016.08.005
98. Dalla C, Antoniou K, Kokras N, Drossopoulou G, Papathanasiou G, Bekris S, et al. Sex differences in the effects of two stress paradigms on dopaminergic neurotransmission. *Physiol Behav.* (2008) 93:595–605. doi: 10.1016/j.physbeh.2007.10.020
99. Piefke M, Weiss PH, Markowitsch HJ, Fink GR. Gender differences in the functional neuroanatomy of emotional episodic autobiographical memory. *Hum Brain Mapp.* (2005) 24:313–24. doi: 10.1002/hbm.20092
100. Andreano JM, Cahill L. Sex influences on the neurobiology of learning and memory. *Learn Mem.* (2009) 16:248–66. doi: 10.1101/lm.918309
101. Gagnon SA, Wagner AD. Acute stress and episodic memory retrieval: neurobiological mechanisms and behavioral consequences. *Ann N Y Acad Sci.* (2016) 1369:55–75. doi: 10.1111/nyas.12996
102. Merz CJ. Contribution of stress and sex hormones to memory encoding. *Psychoneuroendocrinology.* (2017) 82:51–8. doi: 10.1016/j.psyneuen.2017.05.002
103. Merz CJ, Wolf OT. Sex differences in stress effects on emotional learning. *J Neurosci Res.* (2017) 95:93–105. doi: 10.1002/jnr.23811
104. Murray HE, Pillai AV, McArthur SR, Razvi N, Datla KP, Dexter DT, et al. Dose- and sex-dependent effects of the neurotoxin 6-hydroxydopamine on the nigrostriatal dopaminergic pathway of adult rats: differential actions of estrogen in males and females. *Neuroscience.* (2003) 116:213–22. doi: 10.1016/S0306-4522(02)00578-X
105. Gillies GE, McArthur, S. Independent influences of sex steroids of systemic and central origin in a rat model of Parkinson's disease: a contribution to sex-specific neuroprotection by estrogens. *Hormones Behav.* (2010) 57:23–34. doi: 10.1016/j.yhbeh.2009.06.002
106. Bourque M. Male/female differences in neuroprotection and neuromodulation of brain dopamine. *Front Endocrinol.* (2011) 2:35. doi: 10.3389/fendo.2011.00035
107. Litim N, Morissette M, Di Paolo T. Neuroactive gonadal drugs for neuroprotection in male and female models of Parkinson's disease. *Neurosc Biobehav Rev.* (2016) 67:79–88. doi: 10.1016/j.neubiorev.2015.09.024
108. Yu L, Liao PC. Sexual differences and estrous cycle in methamphetamine-induced dopamine and serotonin depletions in the striatum of mice. *J Neural Trans.* (2000) 107:419–27. doi: 10.1007/s007020070084
109. Datla KP, Murray HE, Pillai AV, Gillies GE, Dexter DT. Differences in dopaminergic neuroprotective effects of estrogen during estrous cycle. *Neuroreport.* (2003) 14:47–50. doi: 10.1097/00001756-200301200-00009
110. Dluzen DE, Horstink MWIM. Estrogen as neuroprotectant of nigrostriatal dopaminergic system: laboratory and clinical studies. *Endocrine.* (2003) 21:67–76. doi: 10.1385/ENDO:21:1:67
111. Bourque M, Morissette M, Cote M, Soulet D, Di Paolo T. Implication of GPER1 in neuroprotection in a mouse model of Parkinson's disease. *Neurobiol Aging.* (2013) 34:887–901. doi: 10.1016/j.neurobiolaging.2012.05.022
112. Al-Sweidi S, Morissette M, Bourque M, Di Paolo T. Estrogen receptors and gonadal steroids in vulnerability and protection of dopamine neurons in a mouse model of Parkinson's disease. *Neuropharmacology.* (2011) 61:583–91. doi: 10.1016/j.neuropharm.2011.04.031
113. Simola N, Morelli M, Carta AR. The 6-Hydroxydopamine model of parkinson's disease. *Neurotoxi Res.* (2007) 11:151–67. doi: 10.1007/BF03033565
114. Blandini F, Armentero M-T, Martignoni E. The 6-hydroxydopamine model: News from the past. *Parkinsonism Related Disord.* (2008) 14:S124–9. doi: 10.1016/j.parkreldis.2008.04.015
115. Tadaiesky MT, Dombrowski PA, Figueiredo CP, Cargnin-Ferreira E, Da Cunha C, Takahashi RN. Emotional, cognitive and neurochemical alterations in a premotor stage model of Parkinson's disease. *Neuroscience.* (2008) 156:830–40. doi: 10.1016/j.neuroscience.2008.08.035
116. Francardo V, Recchia A, Popovic N, Andersson D, Nissbrandt H, Cenci MA. Impact of the lesion procedure on the profiles of motor impairment and molecular responsiveness to L-DOPA in the 6-hydroxydopamine mouse model of Parkinson's disease. *Neurobiol Dis.* (2011) 42:327–40. doi: 10.1016/j.nbd.2011.01.024
117. McDowell K, Chesselet M-F. Animal models of the non-motor features of Parkinson's disease. *Neurobiol Dis.* (2012) 46:597–606. doi: 10.1016/j.nbd.2011.12.040
118. Miyoshi E, Wietzikoski S, Camplessei M, Silveira R, Takahashi RN, Da Cunha C. Impaired learning in a spatial working memory version and in a cued version of the water maze in rats with MPTP-induced mesencephalic dopaminergic lesions. *Brain Res Bulletin* (2002) 58:41–7. doi: 10.1016/S0361-9230(02)00754-2
119. Ferro MM, Bellissimo MI, Anselmo-Franci JA, Angellucci MEM, Canteras NS, Da Cunha C. Comparison of bilaterally 6-OHDA- and MPTP-lesioned rats as models of the early phase of Parkinson's disease: histological, neurochemical, motor and memory alterations. *J Neurosci Methods.* (2005) 148:78–87. doi: 10.1016/j.jneumeth.2005.04.005
120. Ho Y-J, Ho S-C, Pawlak CR, Yeh K-Y. Effects of d-cycloserine on MPTP-induced behavioral and neurological changes: potential for treatment of Parkinson's disease dementia. *Behav Brain Res.* (2011) 219:280–90. doi: 10.1016/j.bbr.2011.01.028
121. Moreira CG, Barbiero JK, Ariza D, Dombrowski PA, Sabioni P, Bortolanza M, et al. Behavioral, neurochemical and histological alterations promoted by bilateral intranigral rotenone administration: a new approach for an old neurotoxin. *Neurotox Res.* (2012) 21:291–301. doi: 10.1007/s12640-011-9278-3
122. Li X, Redus L, Chen C, Martinez PA, Strong R, Li S, et al. Cognitive dysfunction precedes the onset of motor symptoms in the mitopark mouse model of Parkinson's disease. *PLoS ONE.* (2013) 8:e71341. doi: 10.1371/journal.pone.0071341
123. Nezhadi A, Esmaeili-Mahani S, Sheibani V, Shabani M, Darvishzadeh F. Neurosteroid allopregnanolone attenuates motor disability and prevents the changes of neurexin 1 and postsynaptic density protein 95 expression in the striatum of 6-OHDA-induced rats' model of Parkinson's disease. *Biomed Pharmacother.* (2017) 88:1188–97. doi: 10.1016/j.biopha.2017.01.159
124. Koos Slob A, Huizer T, Van Der Werff Ten Bosch JJ. Ontogeny of sex differences in open-field ambulation in the rat. *Physiol Behav.* (1986) 37:313–5. doi: 10.1016/0031-9384(86)90239-8
125. Scholl JL, Afzal A, Fox LC, Watt MJ, Forster GL. Sex differences in anxiety-like behaviors in rats. *Physiol Behav.* (2019) 211:112670. doi: 10.1016/j.physbeh.2019.112670
126. Rossetti MF, Varayoud J, Andreoli MF, Stoker C, Luque EH, Ramos JG. Sex- and age-associated differences in episodic-like memory and transcriptional regulation of hippocampal steroidogenic enzymes in rats. *Mol Cell Endocrinol.* (2018) 470:208–18. doi: 10.1016/j.mce.2017.11.001
127. Herlitz A, Airaksinen E, Nordstrom E. Sex differences in episodic memory: the impact of verbal and visuospatial ability. *Neuropsychology.* (1999) 13:590–7. doi: 10.1037/0894-4105.13.4.590
128. Chao OY, De Souza Silva MA, Yang Y-M, Huston JP. The medial prefrontal cortex - hippocampus circuit that integrates information of object, place and time to construct episodic memory in rodents: behavioral, anatomical

and neurochemical properties. *Neurosci Biobehav Rev.* (2020) 113:373–407. doi: 10.1016/j.neubiorev.2020.04.007
129. Ennaceur A, Michalikova S, Bradford A, Ahmed S. Detailed analysis of the behavior of Lister and Wistar rats in anxiety, object recognition and object location tasks. *Behav Brain Res.* (2005) 159:247–66. doi: 10.1016/j.bbr.2004.11.006
130. Inagaki T, Gautreaux C, Luine V. Acute estrogen treatment facilitates recognition memory consolidation and alters monoamine levels in memory-related brain areas. *Horm Behav.* (2010) 58:415–26. doi: 10.1016/j.yhbeh.2010.05.013
131. Cabezas R, Ávila M, Gonzalez J, El-Bachá RS, Báez, E, Garcã-A-Segura LM, et al. Astrocytic modulation of blood brain barrier: perspectives on Parkinson's disease. *Front Cell Neurosci.* (2014) 8:211. doi: 10.3389/fncel.2014.00211
132. Colombo D, Abbruzzese G, Antonini A, Barone P, Bellia G, Franconi F, et al. The "gender factor" in wearing-off among patients with Parkinson's disease: a post hoc analysis of DEEP study. *Sci World J.* (2015) 2015:787451. doi: 10.1155/2015/787451
133. Cerri S, Mus L, Blandini F. Parkinson's disease in women and men: what's the difference? *J Parkinson's Dis.* (2019) 9:501–15. doi: 10.3233/JPD-191683
134. Nicoletti A, Vasta R, Mostile G, Nicoletti G, Arabia G, Iliceto G, et al. Gender effect on non-motor symptoms in Parkinson's disease: are men more at risk? *Parkinsonism Relat Disord.* (2016) 35:69–74. doi: 10.1016/j.parkreldis.2016.12.008
135. Amalric M, Moukhles H, Nieoullon A, Daszuta A. Complex deficits on reaction time performance following bilateral intrastriatal 6-OHDA infusion in the rat. *Eur J Neurosci.* (1995) 7:972–80. doi: 10.1111/j.1460-9568.1995.tb01085.x
136. Dave KD, De Silva S, Sheth NP, Ramboz S, Beck MJ, Quang C, et al. Phenotypic characterization of recessive gene knockout rat models of Parkinson's disease. *Neurobiol Dis.* (2014) 70:190–203. doi: 10.1016/j.nbd.2014.06.009
137. Villeneuve LM, Purnell PR, Boska MD, Fox HS. Early expression of Parkinson's disease-related mitochondrial abnormalities in PINK1 knockout rats. *Mol Neurobiol.* (2016) 53:171–86. doi: 10.1007/s12035-014-8927-y
138. Locklear MN, Kritzer MF. Assessment of the effects of sex and sex hormones on spatial cognition in adult rats using the Barnes maze. *Horm Behav.* (2014) 66:298–308. doi: 10.1016/j.yhbeh.2014.06.006
139. Cunningham RL, Macheda T, Watts LT, Poteet E, Singh M, Roberts JL, et al. Androgens exacerbate motor asymmetry in male rats with unilateral 6-hydroxydopamine lesion. *Hormones Behav.* (2011) 60:617–24. doi: 10.1016/j.yhbeh.2011.08.012
140. Litim N, Bourque M, Al Sweidi S, Morissette M, Di Paolo T. The 5α-reductase inhibitor dutasteride but not finasteride protects dopamine neurons in the MPTP mouse model of Parkinson's disease. *Neuropharmacology.* (2015) 97:86–94. doi: 10.1016/j.neuropharm.2015.05.015
141. Ceccarelli I, Scaramuzzino A, Aloisi AM. Effects of gonadal hormones and persistent pain on non-spatial working memory in male and female rats. *Behav Brain Res.* (2001) 123:65–76. doi: 10.1016/S0166-4328(01)00195-4
142. Cherrier MM, Asthana S, Plymate S, Baker L, Matsumoto A, Peskind E, et al. Testosterone supplementation improves spatial and verbal memory in healthy older men. *Neurology.* (2001) 57:80–8. doi: 10.1212/WNL.57.1.80
143. Janowsky J. Thinking with your gonads: testosterone and cognition. *Trends Cogn Sci.* (2006) 10:77–82. doi: 10.1016/j.tics.2005.12.010
144. Hawley WR, Grissom EM, Martin RC, Halmos MB, Bart CLS, Dohanich GP. Testosterone modulates spatial recognition memory in male rats. *Horm Behav.* (2013) 63:559–65. doi: 10.1016/j.yhbeh.2013.02.007
145. Locklear MN, Cohen AB, Jone A, Kritze MF. Sex differences distinguish intracortical glutamate receptor-mediated regulation of extracellular dopamine levels in the prefrontal cortex of adult rats. *Cerebral Cortex.* (2014) 26:599–610. doi: 10.1093/cercor/bhu222
146. Daniel JM, Winsauer PJ, Moerschbaecher JM. Castration in rats impairs performance during acquisition of a working memory task and exacerbates deficits in working memory produced by scopolamine and mecamylamine. *Psychopharmacology.* (2003) 170:294–300. doi: 10.1007/s00213-003-1537-4
147. Sandstrom NJ, Kim JH, Wasserman MA. Testosterone modulates performance on a spatial working memory task in male rats. *Horm Behav.* (2006) 50:18–26. doi: 10.1016/j.yhbeh.2005.09.008
148. Smith A. Effect of bilateral 6-Hydroxydopamine lesions of the medial forebrain bundle on reaction time. *Neuropsychopharmacology.* (2002) 26:756–64. doi: 10.1016/S0893-133X(01)00420-1
149. Debeir T, Ginestet L, François C, Laurens S, Martel J-C, Chopin P, et al. Effect of intrastriatal 6-OHDA lesion on dopaminergic innervation of the rat cortex and globus pallidus. *Exp Neurol.* (2005) 193:444–54. doi: 10.1016/j.expneurol.2005.01.007
150. Kritzer MF, Brewer A, Montalmant F, Davenport M, Robinson JK. Effects of gonadectomy on performance in operant tasks measuring prefrontal cortical function in adult male rats. *Horm Behav.* (2007) 51:183–94. doi: 10.1016/j.yhbeh.2006.07.005
151. Aubele T, Kritzer MF. Androgen influence on prefrontal dopamine systems in adult male rats: localization of cognate intracellular receptors in medial prefrontal projections to the ventral tegmental area and effects of gonadectomy and hormone replacement on glutamate-stimulated. *Cerebral Cortex.* (2012) 22:1799–812. doi: 10.1093/cercor/bhr258
152. Williams GV, Castner, SA. Under the curve: critical issues for elucidating D1 receptor function in working memory. *Neuroscience.* (2006) 139:263–76. doi: 10.1016/j.neuroscience.2005.09.028
153. Floresco SB, Magyar O. Mesocortical dopamine modulation of executive functions: beyond working memory. *Psychopharmacology.* (2006) 188:567–85. doi: 10.1007/s00213-006-0404-5
154. Cools R, D'Esposito M. Inverted-U–shaped dopamine actions on human working memory and cognitive control. *Biolog Psychiatry.* (2011) 69:e113–25. doi: 10.1016/j.biopsych.2011.03.028
155. Ott T, Nieder A. Dopamine and cognitive control in prefrontal cortex. *Trends Cogn Sci.* (2019) 23:213–34. doi: 10.1016/j.tics.2018.12.006
156. Dickerson BC, Eichenbaum H. The episodic memory system: neurocircuitry and disorders. *Neuropsychopharmacology.* (2010) 35:86–104. doi: 10.1038/npp.2009.126
157. Pause BM, Zlomuzica A, Kinugawa K, Mariani J, Pietrowsky R, et al. Perspectives on episodic-like and episodic memory. *Front Behav Neurosci.* (2013) 7:33. doi: 10.3389/fnbeh.2013.00033
158. Kehagia AA, Barker RA, Robbins TW. Cognitive impairment in Parkinson's disease: the dual syndrome hypothesis. *Neurodegener Dis.* (2013) 11:79–92. doi: 10.1159/000341998
159. Das T, Hwang JJ, Poston KL. Episodic recognition memory and the hippocampus in Parkinson's disease: a review. *Cortex.* (2019) 113:191–209. doi: 10.1016/j.cortex.2018.11.021
160. Nakamura N, Fujita H, Kawata M. Effects of gonadectomy on immunoreactivity for choline acetyltransferase in the cortex, hippocampus, and basal forebrain of adult male rats. *Neuroscience.* (2002) 109:473–85. doi: 10.1016/S0306-4522(01)00513-9
161. Mitsushima D. Sex differences in the septo-hippocampal cholinergic system in rats: behavioral consequences. In: *Biological Basis of Sex Differences in Psychopharmacology.* Berlin, Heidelberg: Springer. (2010). p. 57–71. doi: 10.1007/7854_2010_95
162. Atwi S, McMahon D, Scharfman H, Maclusky NJ. Androgen modulation of hippocampal structure and function. *Neuroscientist.* (2016) 22:46–60. doi: 10.1177/1073858414558065
163. Eichenbaum H, Yonelinas AP, Ranganath C. The medial temporal lobe and recognition memory. *Ann Rev Neurosci.* (2007) 30:123–52. doi: 10.1146/annurev.neuro.30.051606.094328
164. Deshmukh SS, Knierim JJ. Representation of non-spatial and spatial information in the lateral entorhinal cortex. *Front Behav Neurosci.* (2011) 5:69. doi: 10.3389/fnbeh.2011.00069
165. Barbosa FF, de Oliveira Pontes IM, Ribeiro S, Ribeiro AM, Silva RH. Differential roles of the dorsal hippocampal regions in the acquisition of spatial and temporal aspects of episodic-like memory. *Behav Brain Res.* (2012) 232:269–77. doi: 10.1016/j.bbr.2012.04.022
166. Castilla-Ortega E, Pedraza C, Chun J, Fonseca FRD, Estivill-Torrús G, Santín LJ. Hippocampal c-Fos activation in normal and LPA1-null mice after two object recognition tasks with different memory demands. *Behav Brain Res.* (2012) 232:400–5. doi: 10.1016/j.bbr.2012.04.018

167. Barbosa FF, Santos JR, Meurer YSR, Macêdo PT, Ferreira LMS, Pontes IMO, et al. Differential cortical c-Fos and Zif-268 expression after object and spatial memory processing in a standard or episodic-like object recognition task. *Front Behav Neurosci.* (2013) 7:112. doi: 10.3389/fnbeh.2013.00112
168. Castilla-Ortega E, Rosell-Valle C, Pedraza C, de Fonseca FR, Estivill-Torrús G, Santín LJ. Voluntary exercise followed by chronic stress strikingly increases mature adult-born hippocampal neurons and prevents stress-induced deficits in 'what–when–where'memory. *Neurobiol Learn Mem.* (2014) 109:62–73. doi: 10.1016/j.nlm.2013.12.001
169. Beer Z, Vavra P, Atucha E, Rentzing K, Heinze H-J, Sauvage M. The memory for time and space differentially engages the proximal and distal parts of the hippocampal subfields CA1 and CA3. *PLoS Biol.* (2018) 16:e2006100. doi: 10.1371/journal.pbio.2006100
170. Barker GRI, Evuarherhe O, Warburton EC. Object-in-place associative recognition memory depends on glutamate receptor neurotransmission within two defined hippocampal-cortical circuits: a critical role for AMPA and NMDA receptors in the hippocampus, perirhinal, and prefrontal cortices. *Cereb Cortex.* (2015) 25:472–81. doi: 10.1093/cercor/bht245
171. De Souza Silva MA, Huston JP, Wang AL, Petri D, Chao OYH. Evidence for a specific integrative mechanism for episodic memory mediated by AMPA/kainate receptors in a circuit involving medial prefrontal cortex and hippocampal CA3 region. *Cereb Cortex*. (2015) 6:3000–9. doi: 10.1093/cercor/bhv112
172. Chao OY, Nikolaus S, Lira Brandao M, Huston JP, de Souza Silva MA. Interaction between the medial prefrontal cortex and hippocampal CA1 area is essential for episodic-like memory in rats. *Neurobiol Learn Mem.* (2017) 141:72–7. doi: 10.1016/j.nlm.2017.03.019
173. Kartteke E, Desouzasilva M, Huston J, Dere E. Wistar rats show episodic-like memory for unique experiences. *Neurobiol Learn Memory.* (2006) 85:173–82. doi: 10.1016/j.nlm.2005.10.002
174. Aubele T, Kaufman R, Montalmant F, Kritzer MF. Effects of gonadectomy and hormone replacement on a spontaneous novel object recognition task in adult male rats. *Hormones Behav.* (2008) 54:244–52. doi: 10.1016/j.yhbeh.2008.04.001
175. McConnell SE, Alla J, Wheat E, Romeo RD, McEwen B, Thornton JE. The role of testicular hormones and luteinizing hormone in spatial memory in adult male rats. *Horm Behav.* (2012) 61:479–86. doi: 10.1016/j.yhbeh.2012.01.003
176. Okun MS. Refractory nonmotor symptoms in male patients with Parkinson disease due to testosterone deficiency. *Arch Neurol.* (2002) 59:807. doi: 10.1001/archneur.59.5.807
177. Okun MS, Fernandez HH, Rodriguez RL, Romrell J, Suelter M, Munson S, et al. Testosterone therapy in men with Parkinson disease. *Arch Neurol.* (2006) 63:729. doi: 10.1001/archneur.63.5.729

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Menopause and Brain Health: Hormonal Changes Are Only Part of the Story

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Most studies of menopause and brain aging have focused on the role of the sex steroid hormone, estradiol, as a key mechanisms contributing to cognitive and brain aging in women. An emerging literature demonstrates that beyond endogenous estradiol levels, menopausal symptoms, particularly vasomotor symptoms (VMS), are also key determinants of menopause-related changes in cognition and brain function. Critically, that literature shows the importance of using objective techniques to identify associations of VMS with memory performance, brain structure, and brain function. While self-report measures are important patient-centered outcomes in women's health research, objective measures of VMS typically relate more strongly to indices of cognitive and brain health. Currently, it is premature to make a causal claim about VMS and memory dysfunction, but initial findings raise the possibility that women with VMS might experience an improvement in cognition with VMS treatment. More generally, these findings underscore the utility of investigating female-specific risk factors for cognitive decline.

Keywords: menopause, cognition, vasomotor, brain, neuroimaging, cardiovascular

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INTRODUCTION

The burgeoning field of research in menopause and brain health has its roots in the discovery, now more than 30 years ago, by Catherine Woolley in the laboratory of Bruce McEwen that the structure and function of the hippocampus is influenced by changes in physiological levels of the sex steroid hormone, estradiol (1, 2). This foundational discovery propelled the growth of basic science research on the role of sex steroid hormones in brain function and brain structure, establishing a protective role in both rodent and non-human models. While there is a clear role for research on the role of sex steroid hormones in brain aging, comparatively little work has been conducted on the role of menopausal symptoms in brain aging. The hallmark symptom of the menopause is vasomotor symptoms (VMS), hot flashes and night sweats. These symptoms persist for many women well beyond the final menstrual period, into periods of risk for dementia, when levels of estradiol have plateaued (3). The continuity of VMS beyond the menopausal transition raises important questions about their potential role in cognition and brain health. This focus is important not only mechanistically but also clinically. If VMS contribute to cognitive dysfunction in women, any effective treatment for VMS regardless of whether the treatment is hormonal, non-hormonal, or lifestyle, could potentially confer cognitive benefit.

In this review, we present an overview of VMS in relation to cognition and brain function at midlife and beyond. We consider the methodologies used to measure VMS and the importance of using objective VMS measures in research studies. We draw on observational studies and clinical trials to show initial evidence of a relationship between VMS and memory dysfunction—the cognitive domain that appears most sensitive to female reproductive factors (4). We consider the mechanisms by which VMS can influence memory, drawing on our neuroimaging studies and on studies showing linkages to other risk factors for cognitive aging, particularly cardiovascular disease, and sleep dysfunction. We conclude that emerging evidence suggests a role for VMS in cognitive aging in women.

VMS EPIDEMIOLOGY

VMS are the classic symptom of the menopause transition. VMS are experienced by most midlife women at some point during the menopause transition (5). For a third of women, VMS are frequent or severe. Whereas, VMS have long been assumed to be an incidental midlife symptom, more recent research has brought a wealth new knowledge about this prevalent midlife experience that has challenged long-held assumptions, elucidating their epidemiology and implications for women's health and functioning.

Whereas, VMS were once thought to be time-delimited events isolated to a few years around the onset on the postmenopause, recent data indicate that VMS persist for an average of 7–10 years for moderate-severe or frequent VMS, and much longer for milder symptoms (3, 6). Major cohort studies from the United States (US) and around the globe have further indicated that not all women follow the same trajectories of VMS over the transition (7, 8). For example, in the United States, 18% of women have VMS primarily when they are still cycling early in the transition, 29% of women have VMS have VMS primarily postmenopausally once their cycles have stopped, 27% of women have few or no VMS, and 25% of women have VMS that persist from early in the transition through the later postmenopausal years (7).

A growing body of research also underscores the pronounced racial/ethnic differences in VMS. In the United States, African American women have the most frequent, persistent, and bothersome VMS of any racial/ethnic groups, with over 80% of African American women reporting VMS at some point during the transition (9, 10). Approximately 50–70% of non-Hispanic White, Asian (Japanese, Chinese), and Hispanic report VMS during the menopause, with slightly (non-significantly) lower rates among Asian women. Similarly, in an Australian sample, Asian women have lower rates of VMS than other groups, and in an international consortium of menopause studies, Japanese women were less likely to report severe VMS than were Caucasian women (11, 12). Further, independent of race/ethnicity, less educated women and women in lower socioeconomic positions have more VMS than their more educated and affluent counterparts in both the United States (5) and in the United Kingdom (8).

VMS can have a profound impact on women's lives. They are a consistent predictor of depressed mood, sleep problems, and poorer quality of life during the menopause transition across social, emotional, and physical domains of functioning (13–15). In addition to these important implications for quality of life and functioning, recent data have also linked VMS to key indices of physical and neurocognitive health.

VMS PHYSIOLOGY

Insights into the physiology of VMS has expanded greatly in recent years with the accumulation of clinical and basic science studies elucidating the critical role of hypothalamic KNDy (kisspeptin, neurokinin B and dynorphin) neurons (16). These neurons are located in the arcuate nucleus (infundibular nucleus in humans) of the hypothalamus, and play a critical role in the hypothalamic-pituitary-gonadal (HPG) axis. They are hypothesized to function as the gonadotropin releasing hormone (GnRH) pulse generator. In that role, KNDy neurons are thought to control the release of follicle stimulating hormone (FSH) and luteinizing hormone (LH), which stimulate ovarian production of estradiol. In turn, estradiol acts on KNDy neurons through negative feedback. As levels of estrogen decrease at menopause, KNDy neurons undergo hypertrophy (enlargement) and are reversed with estrogen supplementation (17). With respect to thermoregulatory function, KNDy neurons project to the median preoptic nucleus (MnPO) of the hypothalamus. There they bind to neurokinin 3 receptors (NK_3R) which in turn project to heat dissipation effectors. The ligand for NK_3R receptors is the endogenous neuropeptide, neurokinin B (NKB). As levels of estrogen decline, levels of NKB rise and activate NK_3R receptors in the MnPO. This overactivation results in rapid heat dissipation response that women experience symptomatically as VMS. Targeting this mechanism, pharmacologic antagonists of NK_3R are a new line of therapeutics to treat VMS in women, and lower VMS frequency and intensity more rapidly than conventional menopausal hormone therapy (HT) (18).

It has been known for quite some time that while declines in estradiol in the menopausal transition set the general stage for VMS and are therefore a necessary factor, changes in estradiol are not a sufficient proximal cause of individual VMS events (19). All women transition through the menopause but not all women have VMS. From a mechanistic perspective, the view that excessive NK3 signaling by NKB in the MnPO causes VMS accounts for the necessary role of estrogen withdrawal and for findings of a temporal, but not causal relationship of VMS and pulses of LH (16). However, other physiologic systems have been linked to VMS including the autonomic nervous system (particularly vagal withdrawal) (20, 21), the thermoregulatory system (22), and the hypothalamic pituitary adrenal axis (more fully elucidated below). Thus, significant advances have been made, yet further research is required to fully understand the underlying physiology of VMS, which is turn can advance the science on VMS and brain health.

VMS MEASUREMENT: WHAT OBJECTIVE TECHNIQUES TELL US THAT SELF-REPORT DOES NOT

VMS are measured in several ways. Self-report approaches include questionnaires that ask women to recall their VMS from weeks, months, or years prior. In other work, diaries to report VMS are employed, and are completed at the end of the day, upon waking, or at the time of the VMS (23). However, these self-report measures have important limitations. It is well-established that the self-report of physical symptoms is subject to memory, mood, and other reporting processes (24–27). Important, VMS can also be assessed physiologically. The most well-established physiologic measure of VMS is sternal skin conductance (28). Early work supported the validity of this approach, which has been used in multiple subsequent observational studies and even clinical trials (29, 30). These physiologic measures are particularly important to understanding the etiology of the VMS and their relationships to key health indices apart from the influence of psychological, memory, and other reporting factors.

Psychological, sleep quality, and other non-VMS factors have a well-established impact on reporting of VMS. Typically, 62–72% of reported VMS are associated with an objective VMS, and 47–70% of objective VMS accompanied by a subjective report (31), with estimates varying by factors including whether the VMS occurred during sleep or wake, mood at the time of reporting, and the quality of the prior night's sleep. For example, more negative mood was associated with increased reporting of VMS not detected physiologically (31). Women with more negative mood also appraise their VMS to be more bothersome, apart from the occurrence of the VMS (32). Notably, physiologic VMS do not show placebo responses (in contrast to the 30% placebo response with diary-reported VMS (28, 30). Such evidence validates the physiologic VMS measures, as a "true" VMS measure that would not be expected to change with a placebo intervention. Further, physiologic VMS are particularly important when assessing nocturnal VMS. Accurately reporting VMS during sleep can be challenging and influenced by the quality of the sleep itself. For example, several studies with self-report and physiologic VMS measures show that reporting of VMS upon waking is influenced by the quality of sleep the prior night (27, 33). Some research indicates that reports of nighttime VMS recalled upon waking may be more related to sleep quality than to the occurrence of VMS themselves (33).

Finally, use of physiologic VMS measures is particularly important when investigating the links between VMS and cognition. Poorer memory performance may be associated with less accurate recall of VMS, thereby introducing a significant bias into the study when using self-reported VMS measures only. Despite this methodological limitation of self-report VMS, most of the literature relating VMS to memory performance relies on self-report measures of VMS. Ambulatory VMS monitors allow for real-time self-report of VMS events and therefore do not rely on recall for self-report VMS. In this way, studies using ambulatory monitors allow for the investigation of both self-report and physiologic VMS in relation to memory, without the influence of recall bias.

VMS AND MEMORY

In addition to VMS, cognitive complaints and objective cognitive difficulties are common in the menopausal transition. For example, among 12,425 healthy women 40–55 years of age in a baseline study of the SWAN, 39% of women complained of forgetfulness (34). Among those women, complaints of forgetfulness (yes/no) varied by menopausal status; compared to the premenopause, the odds of forgetfulness increased by 44% in the early perimenopause, 43% in the late perimenopause, 27% in the postmenopause, and 27% odds in surgical menopause (34). In the Seattle Midlife Women's Health Study, predictors of memory complaints included age, hot flashes, anxiety, depressed mood, perceived stress, perceived health and history of sexual abuse (35). Subjective memory complaints relate to objective cognitive difficulties in midlife women. In the Rochester Investigation of Cognition and Menopause (RICAM), memory complaints on a standardized questionnaire, the Memory Function Questionnaire, were associated with decreased encoding on a verbal memory test and with working memory (36) and in later work with working memory and attention/concentration (37). In our own work, subjective ratings of current memory were associated with performance on a measure of delayed verbal memory (38). Large-scale prospective studies of healthy women followed across the menopausal transition demonstrate subtle but reliable changes in verbal learning and memory, as well as processing speed (39, 40). Notably, initial evidence from these studies indicate that performance declines in the perimenopause but appears to normalize in the postmenopause (39, 40). If this pattern of change in memory across the menopausal transition is valid, it is difficult to envisage how declines in estradiol alone can account for changes in memory, as memory appears to bounce back while declines in estradiol persist.

A growing body of evidence suggests that menopausal symptoms, particularly VMS, may account in part for changes in memory in midlife women. Importantly, subjective VMS generally are unrelated to memory performance, including a study of 6-year longitudinal cohort study of 1,903 midlife women in SWAN (41). The studies showing evidence of a relationship between VMS and memory performance relied on the use of the ambulatory monitors (42–44). The first evidence emerged from a cross-sectional investigation of women with moderate-to-severe VMS (43). Women in that study wore ambulatory VMS monitors, recorded subjective VMS on that monitor and/or on a diary (useful for sleep VMS), and performed a battery of neuropsychological tests. A higher frequency of physiologic VMS but not subjective VMS, particularly during sleep, were associated with lower performance on a verbal memory test. Self-reported sleep, age, race, and mood did not account for those relationships. The association between VMS and worse memory was recently replicated in breast cancer survivors, again

with associations evident only in physiologic VMS (42). In breast cancer survivors, effects were not differentially stronger during sleep. Importantly, these associations were independent of actigraphy-based measures of sleep, mood and other factors (42).

The findings from these cross-sectional studies of physiologic VMS and memory difficulties, raised the possibility that treating VMS may result in improvements in memory. To address that question, we examined changes in memory performance and physiological VMS in a pilot randomized, sham-control study of stellate ganglion blockade (SGB) (44). SGB is an anesthesia procedure used in pain medicine and involves administration of a local anesthetic to the C6 ganglion. Consistent with findings from open-label trials (45–48), SGB reduced moderate-to-severe VMS and reduced physiologic VMS compared to sham (30). While SGB did not significantly improve memory, the magnitude of improvement in physiologic VMS was significantly associated with the magnitude of improvement in memory performance (44). Those findings suggested a linear relationship between improvements in VMS frequency and improvements in memory, and raise the possibility that other hormonal and non-hormonal interventions for VMS may confer memory benefits.

VMS AND BRAIN HEALTH

In a series of neuroimaging studies in midlife women, physiological VMS but not subjective VMS, were associated with brain health. In MsHeart, the frequency of physiologic VMS was positively associated with increases in white matter hyperintensities (49). White matter hyperintensities (WMH) are risk factors for AD and vascular dementia. Whether VMS are causally related to WMH is unknown, but the pathophysiological basis of this association is likely to involve cardiovascular disease (CVD) risk factors, as CVD risk factors are linked to both VMS and WMH (50).

In addition to brain structure, physiologic VMS are associated with alterations in brain function measured using functional magnetic resonance imaging (fMRI), including resting state functional connectivity (51) and blood-oxygen-level dependent (BOLD) fMRI during performance of a verbal memory task (52). In Ms. Heart, we investigated the relationship of VMS with alterations in the default mode network (DMN) during the resting state (52). The DMN is an organized network in the brain that is active during rest and suppressed during tasks requiring attention to the external world. The DMN includes regions along the anterior and posterior midline, the lateral parietal cortex, prefrontal cortex, and medial temporal lobe, including the hippocampus (53). In Ms. Heart, the frequency of VMS was associated with alterations in this network at rest as measured with fMRI (51). The pattern of alterations was characterized by hyperconnectivity of the hippocampus with multiple frontal regions, and this hyperconnectivity was especially pronounced for sleep VMS. Importantly, these associations persisted after controlling for age, race, education, and sleep. Hyperconnectivity in the DMN has been viewed as reflecting a fundamental response to neurological disruption (54), often viewed as compensatory in nature. As in the memory studies, physiologic VMS but not reported VMS were associated with alterations in the DMN, underscoring the importance of objective methods for VMS ascertainment.

In a recent fMRI study, we examined the association between VMS and brain activation during performance of a verbal memory task, including word encoding and word recognition conditions (52). Physiologic VMS but not reported VMS were associated with decreased memory and with alterations in brain activity during the memory task. During word encoding, more frequent physiologic VMS were positively associated with activation in the left orbitofrontal cortex, left middle frontal gyrus/superior frontal gyrus, right superior frontal gyrus, and right parahippocampus. During recognition, more frequent VMS were positively associated with activation in bilateral middle frontal and superior frontal regions and bilateral hippocampus/parahippocampus. Overall, these data suggest that VMS-related declines in memory may be due to alterations in the function of the hippocampus, parahippocampus, and multiple regions of the prefrontal cortex.

Together, the two fMRI studies present a consistent pattern that drives ongoing work in this area. The areas of the DMN associated with physiologic VMS—the hippocampus, medial frontal and orbitofrontal cortex—overlapped considerably with the brain areas associated with VMS in the memory study. In both cases, the patterns were of increased connectivity or activity, consistent with a compensatory response. Neither study found an association between brain function and subjective VMS.

VMS AND BRAIN HEALTH PUTATIVE MECHANISMS

Cardiovascular

VMS have been linked to multiple indicators of CVD risk. In both cross-sectional and longitudinal studies, self-reported VMS have been associated with a more adverse CVD risk factor profile, including high blood pressure and hypertension risk, higher lipids (total cholesterol, LDL cholesterol, triglycerides, ApoB), a more insulin resistant profile, diabetes risk, and in some studies, a more pro-inflammatory or pro-coagulant profile (55). Controlling for traditional CVD risk factors, both self-reported. In the National Institutes of Health-funded MsHeart study, physiologically assessed presence or frequency of VMS have also been linked to poorer endothelial function, particularly for younger midlife women (56, 57). In MsHeart and other studies, more frequent self-reported and physiologically assessed VMS have been associated with greater carotid atherosclerosis (intima media thickness and plaque) (58–60). Other work suggests that reported VMS may also be associated with increased risk of future clinical CVD events (e.g., myocardial infarction, heart failure, stroke) and CVD mortality, which may depend on the timing of the VMS (61, 62). These associations between VMS and CVD risk generally are not explained by endogenous estradiol levels when these measurements are available. Notably, poorer cardiovascular health (e.g., adverse CVD risk factors, subclinical and clinical CVD) is a well-established risk factor for dementia (63–66). Thus,

CVD risk should be considered as one mechanism linking VMS to poorer cognitive health and dementia risk.

Cortisol and Blood Flow

VMS are associated with elevated levels of cortisol, a steroid hormone that is released by the adrenal glands in response to physical and psychological stress and activation of the hypothalamic-pituitary-adrenal (HPA) axis (67–69). Cortisol levels increase 20 min after a VMS, as measured objectively by changes in finger skin temperature and skin resistance (67). Increased cortisol may be one factor contributing to cognitive difficulties in midlife women with VMS. Elevated cortisol has been linked to decreases in memory and executive functioning, particularly in women (70–73). Levels of glucocorticoids at both high and low levels, in an inverted-U dose response curve, are associated with suboptimal performance on learning and memory retrieval tasks (74). Other work demonstrates that VMS frequently cause a reduction in blood flow to the brain (∼5% reduction) (75).

Sleep

In addition to VMS, sleep problems are common during the menopause transition, with up to half of midlife women reporting sleep problems (14). In longitudinal cohort studies, reported VMS are one of the most consistent predictors and robust of reported sleep problems during the menopause transition (76). Although findings employing objective measures of sleep and VMS initially produced somewhat mixed findings, several recent studies using objective measures of both VMS and sleep have further supported the importance of VMS to sleep (77). For example, we found a 5-fold odds of actigraphy-detected wakening with an objective VMS than during times without objective VMS; these wake episodes were observed irrespective of whether the woman reported the VMS or even knew she was having VMS (78). This poor sleep can have implications for women's brain health. We have found that women with greater objectively-detected wake after sleep onset had increased WMH even after considering multiple covariates including VMS (79). Sleep disturbance negatively affects cognitive function during healthy aging (80) and is associated with an increased risk of Alzheimer's disease (81). VMS may therefore negatively impact cognition and brain health through disrupted sleep quality.

DISCUSSION

An emerging body of work indicates that VMS may be an important determinant of cognition at midlife and beyond. This association emerged with the use of objective techniques for measuring VMS, and was not evident with self-report. About 70% of women will experience VMS and it is now recognized that this hallmark menopausal symptom persists in many women for more than a decade after the final menstrual period. While the focus on estradiol and brain health is clearly justified on the basis of a wealth of basic science studies, this emerging body of work supports the need to expand the focus beyond hormonal effects in menopause to menopausal symptoms. This focus is all the more important because of the possibility that VMS might be a modifiable risk factor for memory dysfunction.

Initial findings from a pilot study raise the possibility that women with VMS might experience an improvement in cognition once treated. It is clear that currently no strong claim can be made about a causal relationship between VMS and memory decline, but at minimum VMS may help to identify women at risk for memory decline at midlife.

At the systems level, our ongoing work in MsBrain funded by the National Institute on Aging explores relationships between physiologic VMS and brain health across a range of neuroimaging outcomes, including fMRI measures of verbal memory, resting state, and emotion perception; WMH; diffusion tensor imaging; and brain volume in a large sample of women who also contribute neuropsychological assessments, actigraphy-based measures of sleep, measures of subclinical CVD and CVD risk factors, hormone measures, and a wide range of sociodemographic and clinical variables. Findings from that study will not only provide insights into the reliability of initial findings in MsHeart, but will also allow for a more comprehensive assessment of the complex array of factors that link VMS to cognitive health. Ultimately, this line of research will contribute to a growing literature identifying female-specific risk factors for cognitive aging.

AUTHOR CONTRIBUTIONS

PM and RT each contributed to the writing of this review. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Woolley CS, Gould E, Frankfurt M, McEwen BS. Naturally occurring fluctuation in dendritic spine density on adult hippocampal pyramidal neurons. *J Neurosci.* (1990) 10:4035–9. doi: 10.1523/JNEUROSCI.10-12-04035.1990
2. Woolley CS, McEwen BS. Estradiol mediates fluctuation in hippocampal synapse density during the estrous cycle in the adult rat. *J Neurosci.* (1992) 12:2549–54. doi: 10.1523/JNEUROSCI.12-07-02549.1992
3. Avis NE, Crawford SL, Greendale G, Bromberger JT, Everson-Rose SA, Gold EB, et al. Study of Women's Health Across the, duration of menopausal vasomotor symptoms over the menopause transition. *JAMA Intern Med.* (2015) 175:531–9. doi: 10.1001/jamainternmed.2014.8063
4. Maki PM. Verbal memory and menopause. *Maturitas.* (2015) 82:288–90. doi: 10.1016/j.maturitas.2015.07.023
5. Gold EB, Colvin A, Avis N, Bromberger J, Greendale GA, Powell L, et al. Longitudinal analysis of the association between vasomotor symptoms and race/ethnicity across the menopausal transition: study of

women's health across the nation. *Am J Public Health.* (2006) 96:1226–35. doi: 10.2105/AJPH.2005.066936
6. Freeman EW, Sammel MD, Lin H, Liu Z, Gracia CR. Duration of menopausal hot flushes and associated risk factors. *Obstet Gynecol.* (2011) 117:1095–4. doi: 10.1097/AOG.0b013e318214f0de
7. Tepper PG, Brooks MM, Randolph JF Jr, Crawford SL, El Khoudary SR, Gold EB, et al. Characterizing the trajectories of vasomotor symptoms across the menopausal transition. *Menopause.* (2016) 23:1067–74. doi: 10.1097/GME.0000000000000676
8. Mishra GD, Kuh D. Health symptoms during midlife in relation to menopausal transition: British prospective cohort study. *BMJ.* (2012) 344:e402. doi: 10.1136/bmj.e402
9. Thurston RC, Joffe H. Vasomotor symptoms and menopause: findings from the study of Women's Health across the Nation. *Obstet Gynecol Clin North Am.* (2011) 38:489–501. doi: 10.1016/j.ogc.2011.05.006
10. Freeman EW, Grisso JA, Berlin J, Sammel M, Garcia-Espana B, Hollander L. Symptom reports from a cohort of African American and white women in the late reproductive years. *Menopause.* (2001) 8:33–42. doi: 10.1097/00042192-200101000-00007
11. Mishra G, Lee C, Brown W, Dobson A. Menopausal transitions, symptoms and country of birth: the Australian Longitudinal Study on Women's Health. *Aust N Z J Public Health.* (2002) 26:563–70. doi: 10.1111/j.1467-842X.2002.tb00367.x
12. Chung HF, Pandeya N, Dobson AJ, Kuh D, Brunner EJ, Crawford SL, et al. The role of sleep difficulties in the vasomotor menopausal symptoms and depressed mood relationships: an international pooled analysis of eight studies in the InterLACE consortium. *Psychol Med.* (2018) 48:2550–61. doi: 10.1017/S0033291718000168
13. Avis NE, Colvin A, Bromberger JT, Hess R, Matthews KA, Ory M, et al. Change in health-related quality of life over the menopausal transition in a multiethnic cohort of middle-aged women: Study of Women's Health Across the Nation. *Menopause.* (2009) 16:860–9. doi: 10.1097/gme.0b013e3181a3cdaf
14. Kravitz HM, Joffe H. Sleep during the perimenopause: a SWAN story. *Obstetr Gynecol Clin North Am.* (2011) 38:567–86. doi: 10.1016/j.ogc.2011.06.002
15. Bromberger JT, Kravitz HM, Chang YF, Cyranowski JM, Brown C, Matthews KA. Major depression during and after the menopausal transition: Study of Women's Health Across the Nation (SWAN). *Psychol Med.* (2011) 41:1879–88. doi: 10.1017/S003329171100016X
16. Rance NE, Dacks PA, Mittelman-Smith MA, Romanovsky AA, Krajewski-Hall SJ. Modulation of body temperature and LH secretion by hypothalamic KNDy (kisspeptin, neurokinin B and dynorphin) neurons: a novel hypothesis on the mechanism of hot flushes. *Front Neuroendocrinol.* (2013) 34:211–27. doi: 10.1016/j.yfrne.2013.07.003
17. Sandoval-Guzman T, Stalcup ST, Krajewski SJ, Voytko ML, Rance NE. Effects of ovariectomy on the neuroendocrine axes regulating reproduction and energy balance in young cynomolgus macaques. *J Neuroendocrinol.* (2004) 16:146–53. doi: 10.1111/j.0953-8194.2004.01143.x
18. Prague JK, Roberts RE, Comninos AN, Clarke S, Jayasena CN, Nash Z, et al. Neurokinin 3 receptor antagonism as a novel treatment for menopausal hot flushes: a phase 2, randomised, double-blind, placebo-controlled trial. *Lancet.* (2017) 389:1809–20. doi: 10.1016/S0140-6736(17)30823-1
19. Freedman RR. Biochemical, metabolic, and vascular mechanisms in menopausal hot flashes. *Fertil Steril.* (1998) 70:332–7. doi: 10.1016/S0015-0282(98)00137-X
20. de Zambotti M, Colrain IM, Sassoon SA, Nicholas CL, Trinder J, Baker FC. Vagal withdrawal during hot flashes occurring in undisturbed sleep. *Menopause.* (2013) 20:1147–53. doi: 10.1097/GME.0b013e31828aa344
21. Thurston RC, Matthews KA, Chang Y, Santoro N, Barinas-Mitchell E, von Kanel R, et al. Changes in heart rate variability during vasomotor symptoms among midlife women. *Menopause.* (2016) 23:499–505. doi: 10.1097/GME.0000000000000586
22. Freedman RR, Krell W. Reduced thermoregulatory null zone in postmenopausal women with hot flashes. *Am J Obstet Gynecol.* (1999) 181:66–70. doi: 10.1016/S0002-9378(99)70437-0
23. Fisher WI, Thurston RC. Hot flashes: phenomenology and measurement. In: Sievert LL, Brown DE, editors. *Biological Measures of Human Experience Across the Lifespan: Making Visible the Invisible.* Cham: Springer (2016). p. 233-54. doi: 10.1007/978-3-319-44103-0_11
24. Erskine A, Morley S, Pearce S. Memory for pain: a review. *Pain.* (1990) 41:255–65. doi: 10.1016/0304-3959(90)90002-U
25. Stone AA, Broderick JE, Schwartz JE, Shiffman S, Litcher-Kelly L, Calvanese P. Intensive momentary reporting of pain with an electronic diary: reactivity, compliance, patient satisfaction. *Pain.* (2003) 104:343–51. doi: 10.1016/S0304-3959(03)00040-X
26. Cohen S, Doyle WJ, Skoner DP, Fireman P, Gwaltney JM Jr, Newsom JT. State and trait negative affect as predictors of objective and subjective symptoms of respiratory viral infections. *J Pers Soc Psychol.* (1995) 68:159. doi: 10.1037/0022-3514.68.1.159
27. Fu P, Matthews KA, Thurston RC. How well do different measurement modalities estimate the number of vasomotor symptoms? Findings from the study of women's health across the nation FLASHES study. *Menopause.* (2014) 21:124–30. doi: 10.1097/GME.0b013e318295a3b9
28. Carpenter JS, Andrykowski MA, Freedman RR, Munn R. Feasibility and psychometrics of an ambulatory hot flash monitoring device. *Menopause.* (1999) 6:209–15. doi: 10.1097/00042192-199906030-00006
29. Carpenter JS, Storniolo AM, Johns S, Monahan PO, Azzouz F, Elam JL, et al. Randomized, double-blind, placebo-controlled crossover trials of venlafaxine for hot flashes after breast cancer. *Oncologist.* (2007) 12:124–35. doi: 10.1634/theoncologist.12-1-124
30. Walega DR, Rubin LH, Banuvar S, Shulman LP, Maki PM. Effects of stellate ganglion block on vasomotor symptoms: findings from a randomized controlled clinical trial in postmenopausal women. *Menopause.* (2014) 21:807–14. doi: 10.1097/GME.0000000000000194
31. Thurston RC, Blumenthal JA, Babyak MA, Sherwood A. Emotional antecedents of hot flashes during daily life. *Psychosom Med.* (2005) 67:137–46. doi: 10.1097/01.psy.0000149255.04806.07
32. Thurston RC, Bromberger JT, Joffe H, Avis NE, Hess R, Crandall CJ, et al. Beyond frequency: who is most bothered by vasomotor symptoms? *Menopause.* (2008) 15:841–7. doi: 10.1097/gme.0b013e318168f09b
33. Thurston RC, Blumenthal JA, Babyak MA, Sherwood A. Association between hot flashes, sleep complaints, and psychological functioning among healthy menopausal women. *Int J Behav Med.* (2006) 13:163–72. doi: 10.1207/s15327558ijbm1302_8
34. Gold EB, Sternfeld B, Kelsey JL, Brown C, Mouton C, Reame N, et al. Relation of demographic and lifestyle factors to symptoms in a multi-racial/ethnic population of women 40-55 years of age. *Am J Epidemiol.* (2000) 152:463–73. doi: 10.1093/aje/152.5.463
35. Mitchell ES, Woods NF. Cognitive symptoms during the menopausal transition and early postmenopause. *Climacteric.* (2011) 14:252–61. doi: 10.3109/13697137.2010.516848
36. Weber M, Mapstone M. Memory complaints and memory performance in the menopausal transition. *Menopause.* (2009) 16:694–700. doi: 10.1097/gme.0b013e318196a0c9
37. Weber MT, Mapstone M, Staskiewicz J, Maki PM. Reconciling subjective memory complaints with objective memory performance in the menopausal transition. *Menopause.* (2012) 19:735–41. doi: 10.1097/gme.0b013e318241fd22
38. Drogos LL, Rubin LH, Geller SE, Banuvar S, Shulman LP, Maki PM. Objective cognitive performance is related to subjective memory complaints in midlife women with moderate to severe vasomotor symptoms. *Menopause.* (2013) 20:1236–42. doi: 10.1097/GME.0b013e318291f5a6
39. Greendale GA, Huang MH, Wight RG, Seeman T, Luetters C, Avis NE, et al. Effects of the menopause transition and hormone use on cognitive performance in midlife women. *Neurology.* (2009) 72:1850–7. doi: 10.1212/WNL.0b013e3181a71193
40. Epperson CN, Sammel MD, Freeman EW. Menopause effects on verbal memory: findings from a longitudinal community cohort. *J Clin Endocrinol Metab.* (2013) 98:3829–38. doi: 10.1210/jc.2013-1808
41. Greendale GA, Wight RG, Huang MH, Avis N, Gold EB, Joffe H, et al. Menopause-associated symptoms and cognitive performance: results from the study of women's health across the nation. *Am J Epidemiol.* (2010) 171:1214–24. doi: 10.1093/aje/kwq067
42. Fogel J, Rubin LH, Kilic E, Walega DR, Maki PM. Physiologic vasomotor symptoms are associated with verbal memory dysfunction in breast cancer survivors. *Menopause.* (2020). doi: 10.1097/GME.0000000000001608. [Epub ahead of print].

43. Maki PM, Drogos LL, Rubin LH, Banuvar S, Shulman LP, Geller SE. Objective hot flashes are negatively related to verbal memory performance in midlife women. *Menopause.* (2008) 15:848–56. doi: 10.1097/gme.0b013e31816d815e
44. Maki PM, Rubin LH, Savarese A, Drogos L, Shulman LP, Banuvar S, et al. Stellate ganglion blockade and verbal memory in midlife women: evidence from a randomized trial. *Maturitas.* (2016) 92:123–9. doi: 10.1016/j.maturitas.2016.07.009
45. Lipov E, Lipov S, Stark JT. Stellate ganglion blockade provides relief from menopausal hot flashes: a case report series. *J Womens Health.* (2005) 14:737–41. doi: 10.1089/jwh.2005.14.737
46. Lipov EG, Joshi JR, Sanders S, Wilcox K, Lipov S, Xie H, et al. Effects of stellate-ganglion block on hot flushes and night awakenings in survivors of breast cancer: a pilot study. *Lancet Oncol.* (2008) 9:523–32. doi: 10.1016/S1470-2045(08)70131-1
47. Haest K, Kumar A, Van Calster B, Leunen K, Smeets A, Amant F, et al. Stellate ganglion block for the management of hot flashes and sleep disturbances in breast cancer survivors: an uncontrolled experimental study with 24 weeks of follow-up. *Ann Oncol.* (2012) 23:1449–54. doi: 10.1093/annonc/mdr478
48. van Gastel P, Kallewaard JW, van der Zanden M, de Boer H. Stellate-ganglion block as a treatment for severe postmenopausal flushing. *Climacteric.* (2013) 16:41–7. doi: 10.3109/13697137.2012.709889
49. Thurston RC, Aizenstein HJ, Derby CA, Sejdic E, Maki PM. Menopausal hot flashes and white matter hyperintensities. *Menopause.* (2016) 23:27–32. doi: 10.1097/GME.0000000000000481
50. Debette S, Schilling S, Duperron MG, Larsson SC, Markus HS. Clinical significance of magnetic resonance imaging markers of vascular brain injury: a systematic review and meta-analysis. *JAMA Neurol.* (2019) 76:81–94. doi: 10.1001/jamaneurol.2018.3122
51. Thurston RC, Maki PM, Derby CA, Sejdic E, Aizenstein HJ. Menopausal hot flashes and the default mode network. *Fertil Steril.* (2015) 103:1572–8.e1. doi: 10.1016/j.fertnstert.2015.03.008
52. Maki PM, Wu M, Rubin LH, Fornelli D, Drogos LL, Geller S, et al. Hot flashes are associated with altered brain function during a memory task. *Menopause.* (2020) 27:269–77. doi: 10.1097/GME.0000000000001467
53. Buckner RL, Andrews-Hanna JR, Schacter DL. The brain's default network: anatomy, function, and relevance to disease. *Ann N Y Acad Sci.* (2008) 1124:1–38. doi: 10.1196/annals.1440.011
54. Hillary FG, Roman CA, Venkatesan U, Rajtmajer SM, Bajo R, Castellanos ND. Hyperconnectivity is a fundamental response to neurological disruption. *Neuropsychology.* (2015) 29:59–75. doi: 10.1037/neu0000110
55. Thurston RC. Vasomotor symptoms: natural history, physiology, and links with cardiovascular health. *Climacteric.* (2018) 21:96–100. doi: 10.1080/13697137.2018.1430131
56. Thurston RC, Chang Y, Barinas-Mitchell E, Jennings JR, von Kanel R, Landsittel DP, et al. Physiologically assessed hot flashes and endothelial function among midlife women. *Menopause.* (2017) 24:886–93. doi: 10.1097/GME.0000000000000857
57. Thurston RC, Sutton-Tyrrell K, Everson-Rose SA, Hess R, Matthews KA. Hot flashes and subclinical cardiovascular disease: findings from the study of women's health across the nation heart study. *Circulation.* (2008) 118:1234–40. doi: 10.1161/CIRCULATIONAHA.108.776823
58. Thurston RC, Chang Y, Barinas-Mitchell E, Jennings JR, Landsittel DP, Santoro N, et al. Menopausal hot flashes and carotid intima media thickness among midlife women. *Stroke.* (2016) 47:2910–5. doi: 10.1161/STROKEAHA.116.014674
59. Thurston RC, Sutton-Tyrrell K, Everson-Rose SA, Hess R, Powell LH, Matthews KA. Hot flashes and carotid intima media thickness among midlife women. *Menopause.* (2011) 18:352–8. doi: 10.1097/gme.0b013e3181fa27fd
60. Ozkaya E, Cakir E, Kara F, Okuyan E, Cakir C, Ustun G, et al. Impact of hot flashes and night sweats on carotid intima-media thickness and bone mineral density among postmenopausal women. *Int J Gynaecol Obstet.* (2011) 113:235–8. doi: 10.1016/j.ijgo.2010.12.020
61. Thurston RC, Johnson BD, Shufelt CL, Braunstein GD, Berga SL, Stanczyk FZ, et al. Menopausal symptoms and cardiovascular disease mortality in the Women's Ischemia Syndrome Evaluation (WISE). *Menopause.* (2017) 24:126–32. doi: 10.1097/GME.0000000000000731
62. Szmuilowicz ED, Manson JE, Rossouw JE, Howard BV, Margolis KL, Greep NC, et al. Vasomotor symptoms and cardiovascular events in postmenopausal women. *Menopause.* (2011) 18:603–10. doi: 10.1097/gme.0b013e3182014849
63. Zeki Al Hazzouri, Vittinghoff E, Sidney S, Reis JP, Jacobs DR Jr, et al. Intima-media thickness and cognitive function in stroke-free middle-aged adults: findings from the coronary artery risk development in young adults study. *Stroke.* (2015) 46:2190–6. doi: 10.1161/STROKEAHA.115.008994
64. Kivipelto M, Helkala EL, Laakso MP, Hanninen T, Hallikainen M, Alhainen K, et al. Midlife vascular risk factors and Alzheimer's disease in later life: longitudinal, population based study. *BMJ.* (2001) 322:1447–51. doi: 10.1136/bmj.322.7300.1447
65. Whitmer RA, Sidney S, Selby J, Johnston SC, Yaffe K. Midlife cardiovascular risk factors and risk of dementia in late life. *Neurology.* (2005) 64:277–81. doi: 10.1212/01.WNL.0000149519.47454.F2
66. Romero JR, Beiser A, Seshadri S, Benjamin EJ, Polak JF, Vasan RS, et al. Carotid artery atherosclerosis, MRI indices of brain ischemia, aging, and cognitive impairment: the Framingham study. *Stroke.* (2009) 40:1590–6. doi: 10.1161/STROKEAHA.108.535245
67. Meldrum DR, Defazio JD, Erlik Y, Lu JK, Wolfsen AF, Carlson HE, et al. Pituitary hormones during the menopausal hot flash. *Obstet Gynecol.* (1984) 64:752–6.
68. Woods NF, Carr MC, Tao EY, Taylor HJ, Mitchell ES. Increased urinary cortisol levels during the menopausal transition. *Menopause.* (2006) 13:212–21. doi: 10.1097/01.gme.0000198490.57242.2e
69. Gibson CJ, Thurston RC, Matthews KA. Cortisol dysregulation is associated with daily diary-reported hot flashes among midlife women. *Clin Endocrinol.* (2016) 85:645–51. doi: 10.1111/cen.13076
70. Greendale GA, Kritz-Silverstein D, Seeman T, Barrett-Connor E. Higher basal cortisol predicts verbal memory loss in postmenopausal women: Rancho Bernardo study. *J Am Geriatr Soc.* (2000) 48:1655–8. doi: 10.1111/j.1532-5415.2000.tb03878.x
71. Seeman TE, McEwen BS, Singer BH, Albert MS, Rowe JW. Increase in urinary cortisol excretion and memory declines: MacArthur studies of successful aging. *J Clin Endocrinol Metab.* (1997) 82:2458–65. doi: 10.1210/jc.82.8.2458
72. Newcomer JW, Selke G, Melson AK, Hershey T, Craft S, Richards K, et al. Decreased memory performance in healthy humans induced by stress-level cortisol treatment. *Arch Gen Psychiatry.* (1999) 56:527–33. doi: 10.1001/archpsyc.56.6.527
73. McCormick CM, Lewis E, Somley B, Kahan TA. Individual differences in cortisol levels and performance on a test of executive function in men and women. *Physiol Behav.* (2007) 91:87–94. doi: 10.1016/j.physbeh.2007.01.020
74. Lupien SJ, Gaudreau S, Tchiteya BM, Maheu F, Sharma S, Nair NP, et al. Stress-induced declarative memory impairment in healthy elderly subjects: relationship to cortisol reactivity. *J Clin Endocrinol Metab.* (1997) 82:2070–5. doi: 10.1210/jc.82.7.2070
75. Lucas RA, Ganio MS, Pearson J, Crandall CG. Brain blood flow and cardiovascular responses to hot flashes in postmenopausal women. *Menopause.* (2013) 20:299–304. doi: 10.1097/gme.0b013e31826e45f0
76. Kravitz HM, Zhao X, Bromberger JT, Gold EB, Hall MH, Matthews KA, et al. Sleep disturbance during the menopausal transition in a multi-ethnic community sample of women. *Sleep.* (2008) 31:979–90.
77. de Zambotti M, Colrain IM, Javitz HS, Baker FC. Magnitude of the impact of hot flashes on sleep in perimenopausal women. *Fertil Steril.* (2014) 102:1708–15.e1. doi: 10.1016/j.fertnstert.2014.08.016
78. Thurston RC, Chang Y, Buysse DJ, Hall MH, Matthews KA. Hot flashes and awakenings among midlife women. *Sleep*. (2019) 42:zsz131. doi: 10.1093/sleep/zsz131
79. Thurston RC, Wu M, Aizenstein HJ, Chang Y, Mitchell EB, Derby CA, et al. Sleep characteristics and white matter hyperintensities among midlife women. *Sleep*. (2019) 43:zsz298. doi: 10.1093/sleep/zsz298
80. Scullin MK, Bliwise DL. Sleep, cognition, and normal aging: integrating a half century of multidisciplinary research. *Perspect Psychol Sci.* (2015) 10:97–137. doi: 10.1177/1745691614556680

81. Bubu OM, Brannick M, Mortimer J, Umasabor-Bubu O, Sebastiao YV, Wen Y, et al. Sleep cognitive impairment, and alzheimer's disease: a systematic review and meta-analysis. *Sleep*. (2017) 40:zsw032. doi: 10.1093/sleep/zsw032

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frontiers in Neurology

SYSTEMATIC REVIEW
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Giving Researchers a Headache – Sex and Gender Differences in Migraine

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Migraine is a common neurovascular disorder affecting ∼15% of the general population. Ranking second in the list of years lived with disability (YLD), people living with migraine are greatly impacted by this especially burdensome primary headache disorder. In ∼30% of individuals with migraine, transient neurological symptoms occur (migraine aura) that further increase migraine burden. However, migraine burden is differential with respect to sex. Though one-year prevalences in childhood are similar, starting with puberty, migraine incidence increases at a much higher rate in females than males. Thus, migraine over the life course occurs in women three to four times more often than in men. Attacks are also more severe in women, leading to greater disability and a longer recovery period. The sex disparity in migraine is believed to be partly mediated through fluctuations in ovarian steroid hormones, especially estrogen and progesterone, although the exact mechanisms are not yet completely understood. The release of the neuropeptide calcitonin gene-related peptide (CGRP), followed by activation of the trigeminovascular system, is thought to play a key role in the migraine pathophysiology. Given the burden of migraine and its disproportionate distribution, the underlying cause(s) for the observed differences between sexes in the incidence, frequency, and intensity of migraine attacks must be better understood. Relevant biological as well as behavioral differences must be taken into account. To evaluate the scope of the existing knowledge on the issue of biological sex as well as gender differences in migraine, we conducted a systematized review of the currently available research. The review seeks to harmonize existing knowledge on the topic across the domains of biological/preclinical, clinical, and population-level research, which are traditionally synthesized and interpreted in isolation. Ultimately, we identify knowledge gaps and set priorities for further interdisciplinary and informed research on sex and gender differences as well as gender-specific therapies in migraine.

Keywords: migraine, headache, aura, review (article), primary headache, sex, gender

INTRODUCTION

Migraine is a common neurovascular disorder in the general population (1), estimated to affect 1.3 billion [95% uncertainty interval (UI) 1.2–1.4] people, corresponding to a global all-age point prevalence of 18.0% (2).

Migraine attack severity and frequency can vary over time and lead to different degrees of disability (3). Thus, globally, migraine accounts for 47.2 million (95% UI 30.0–68.7) years of life lived with disability (YLDs) (2). It ranks second in YLD among all causes of disability defined by the Global Burden of Disease study (GBD), and its burden is not localized to specific GBD regions but is globally distributed (4).

Migraine has a transient nature and is described by the International Classification of Headache Disorders (ICHD) as a primary headache disorder with recurrent unilateral headaches. Two major types of migraine are distinguished: migraine with aura and migraine without aura. In both types, the headache attacks last 4–72 h, are pulsating, and can be accompanied by nausea, photophobia, and/or phonophobia. Migraine with aura additionally presents with unilateral and fully reversible central nervous system symptoms (1).

The prevalence of migraine in women is higher than in men (2), and sex hormones are believed to play a key role in this discrepancy (5). After all, in females of childbearing age, migraine accounts for 11.2% of total YLDs (4). Sex hormones, especially fluctuations of estrogen and progesterone, are believed to impact the pathogenesis of migraine (6). Brain magnetic resonance imaging (MRI) studies have confirmed structural as well as functional differences between males and females with migraine (7).

Animal studies have further confirmed differences with respect to sex hormones implicated in migraine between male and female rats (8, 9), however, the majority of animal studies have been performed in male animals (5, 10). In contrast, male participants in clinical and population-based research studies on migraine are largely lacking, and differences with respect to sex and gender in people suffering from migraine have yet to be rigorously addressed (11).

Despite considerable research activity in the field of migraine over the last two decades (12), we still know little about the underlying mechanisms of the development of migraine (13).

Previous reviews have provided extensive narrative overviews of the available evidence regarding sex differences in migraine, considering both preclinical/biological as well as clinical aspects (11, 14, 15). However, a systematized search of the literature, to our knowledge, has not yet been conducted. This review aims to synthesize new evidence on sex and gender differences in migraine (published within the last 5 years). Using a systematized review approach to synthesize findings from different research fields, we attempted to provide a transparent and complete overview of recent relevant literature.

Furthermore, we aimed to identify areas of particular promise for future exploration and use interdisciplinary, translational knowledge to fill gaps in the individual subdomains of migraine research. Based on this comprehensive overview, we provide researchers with recommendations for further research in this field.

METHODS

We conducted a systematized review of the scientific literature concerning gender- and sex aspects of migraine in adults. We searched four databases: Embase, Medline (Ovid), Web-of-Science (Core Collection) and Google Scholar for relevant articles published between 01-01-2015 and 18-12-2019. Since we identified multiple earlier narrative reviews on the topic, we focused on the synthesis of newly published findings in an effort to avoid unnecessary overlap with previous reviews and to build directly upon these findings. Only scientific articles (including reviews) presenting sex- and/or gender-specific findings in original migraine research were considered for inclusion. We excluded articles limited to estimating well-described incidence and prevalence differences of migraine in men and women in various settings. We further excluded articles that were not focused on sex- or gender- specific aspects but had restricted study populations composed of only one specific sex or gender group. An exception was made for articles describing studies pertaining to women's biological health (e.g., menstruation, pregnancy and menopause), as new findings in this area are crucial to facilitate a better understanding of these sex differences.

Additionally, since this review focuses on migraine in adults, we did not include articles exclusively reporting findings on children or adolescents (aged <18 years), except for articles concerning menstruation or menarche. Moreover, studies investigating consequences and comorbidities of migraine, as well as those assessing efficacy and effectiveness of specific treatments and therapies were not included. We further restricted study eligibility to include articles available in English and as full-text manuscripts. We also excluded case reports and case series, book chapters, news articles, conference abstracts or other contributions, and dissertations.

The search strings used in the different databases included known synonyms of migraine and sex/gender terminology to maximize the sensitivity of the search. We used the following terms to conduct the literature search: "migraine," "primary headache," "sex characteristics," "sex factors," "gender identity," "hormone,*" "intersex,*" "gender,*" "transgender,*" "hermaphrod,*" "female,*" "woman,*" "women," "male," "man," "men," "sex," "sexual," "sexes," and "dimorphism." The specific search strings can be found in the **Supplementary**.

We conducted the search on December 18th, 2019. Manuscripts were excluded during full-text screening for the following reasons: articles limited to prevalence, no gender-specific aspects, articles limited to one sex/gender or not reporting differences/similarities, averaging of effects across sex/gender groups (e.g., through matching on sex/gender), studies focused on comorbidities, testing of specific migraine treatments, no full-text accessible, text was part of a book chapter, and previously unidentified duplicates. After full-text screening, included articles were categorized into five domains including: basic science, epidemiology, clinical science, genetics and neuroimaging, for synthesis.

Contrary to previous works, we placed a particular emphasis on including (trans)gender and sex aspects - hormonal, behavioral, cultural as well as psychological traits - of migraine,

which may provide important biological mechanistic insights. So far, a strong focus has been placed on sex differences, however, we believe that gender aspects might also play an important role in explaining the uneven distribution of migraine. Thus, we included both sex and gender aspects in this review. In this review, we define "sex" as referring to biological differences, concerning hormones, chromosomes, and sex organs, whereas we consider "gender" to refer to a social construct, encompassing enacted roles and behaviors, which are shaped by cultures and can change over time (16, 17). We believe both sex and gender are important determinants of health (18).

We acknowledge that modern definitions of sex and gender go beyond the binary in an effort to include individuals such as those identifying as intersex, transgender, genderfluid or genderqueer. Although such non-binary definitions are largely absent in the migraine literature, they have been captured as comprehensively as possible in this review. In our approach, we sought to identify important areas with some scientific consensus about sex and gender differences in migraine research.

RESULTS

Running the search strategy resulted in 1,901 unique retrieved articles after deduplication. Two reviewers (LAH and JH) independently screened the titles and abstracts of all retrieved articles for inclusion based on the prespecified eligibility criteria. After the first screening, there was initial agreement on inclusion of 67 articles and an additional 113 articles after discussion. For 15 articles, no consensus was found, and a third independent reviewer (JLR) was consulted to determine which of these were eligible. In total, 120 articles were eligible for full-text screening. After the second screening stage, 79 out of the 120 articles were ultimately selected for inclusion in the review. **Figure 1** shows the flowchart of the review process, which was modified from PRISMA 2009 Flow Diagram (19).

Epidemiology

In 2017, the global point prevalence of migraine was estimated to be 18.1% (95% UI 16.8–19.4), affecting ∼1.3 billion (95% UI 1.2–1.4) people globally (2). Migraine is reported to be two-to-three times more prevalent in women than in men (11, 14) and have a 3- to 4-fold prevalence in women after puberty compared to men (20). This equates to a point prevalence of 22.6% (95% UI 21.1–24.3) in females and 13.5% (95% UI 12.5–14.5) in males in 2017 (2). One-year migraine attack prevalence estimates indicate active migraine in 18% of females and 6% of males globally, whereas the cumulative incidences over the lifetime are 43% and 18% respectively (11, 14, 21). The prevalence ratio between males and females does not stay constant but varies with age (11, 14, 22). The largest 1-year prevalence ratio between sexes can be observed at 30.2 years of age (11, 23).

There is a tendency of earlier onset of migraine in boys (22). The infantile migraine prevalence is similar for boys and girls, with both showing an increase beginning during puberty (11). However, this increase is steeper in women (11). According to the 2017 data, the migraine point prevalence peaks for both sexes between ages 35–39 reaching 34.5% (95% UI 29.9–39.3) and 20.3% (95% UI 17.5–27.2), respectively (2). Tonini suggests that active migraine prevalence follows a bimodal pattern with peaks around the age of 35 years and 50 years (20). However, the latest GBD data from 2017 do not show this second peak in this age group (2). In women, active migraine tends to decrease after menopausal transition (11, 14). In contrast with prevalent migraine, the peak in migraine incidence can already be observed during the age range of 20–24 years in women and 15–19 years in men (2, 11).

The global burden of migraine is indicated by the high rate of 618.4 (95% UI 392.5–898.8) years lived with disability (YLDs) per 100,000 people (2). From the latest reports in 2016, migraine ranked second among diseases leading to YLDs globally and accounted for 5.6% of YLDs globally (4). Migraine is described as particularly burdensome among middle-aged women (4). In general, migraine accounts for 6.6% (95% UI 4.6–8.7) of YLDs in women and 4.4% (95% UI 3.0–6.1) in men (2). A cross-sectional survey using a time trade-off approach found that men attribute lower health state utilities to migraine conditions compared to women (24). Especially from a life course perspective, these utilities were found to be significantly different between males and females (24). In this study, health state utilities were determined by how much of their remaining lifetime participants would be willing to sacrifice for a migraine free health state (24).

Migraine studies clearly demonstrate the impact of the disease on everyday life. A cross-sectional survey about family, relationship, career, educational, and financial aspects in migraine patients showed that overall, males and females were similarly affected in many areas (25). Gender differences were observed in that men indicated to worry more about migraine compromising their careers than women. However, migraine appeared to actually have a greater impact on women's careers compared to men's (25). Moreover, compared to men, women more often indicated they would have better overall health and less stress without migraine (25). The cross-sectional study from Brazil showed that both men and women with migraine reported more psychologically demanding jobs, less freedom in decision making in their jobs, and less social support at work (26). Especially among women, work conflicting with family and leisure was significantly associated with migraine activity (26). Furthermore, Hammond et al. found differences in associations between migraine and marital status, with men having higher odds of migraine in common-law relationships. Among older women, divorced or separated females had the highest odds of having migraine (27). Differences were also found in sexual orientation; gay and bisexual men aged 45–85 had a 50% higher odds of ever having migraine, whereas lesbian and bisexual women in the same age range had a 23% lower odds compared to heterosexual men and women, respectively (27). It was hypothesized that the underlying factor for the observed increased odds among gay and bisexual men was minority stress (27), however, to our knowledge, this has not yet been formally investigated. Also in terms of ethnicity, higher odds for migraine prevalence were observed in older non-white people compared to older white people in both sexes (27).

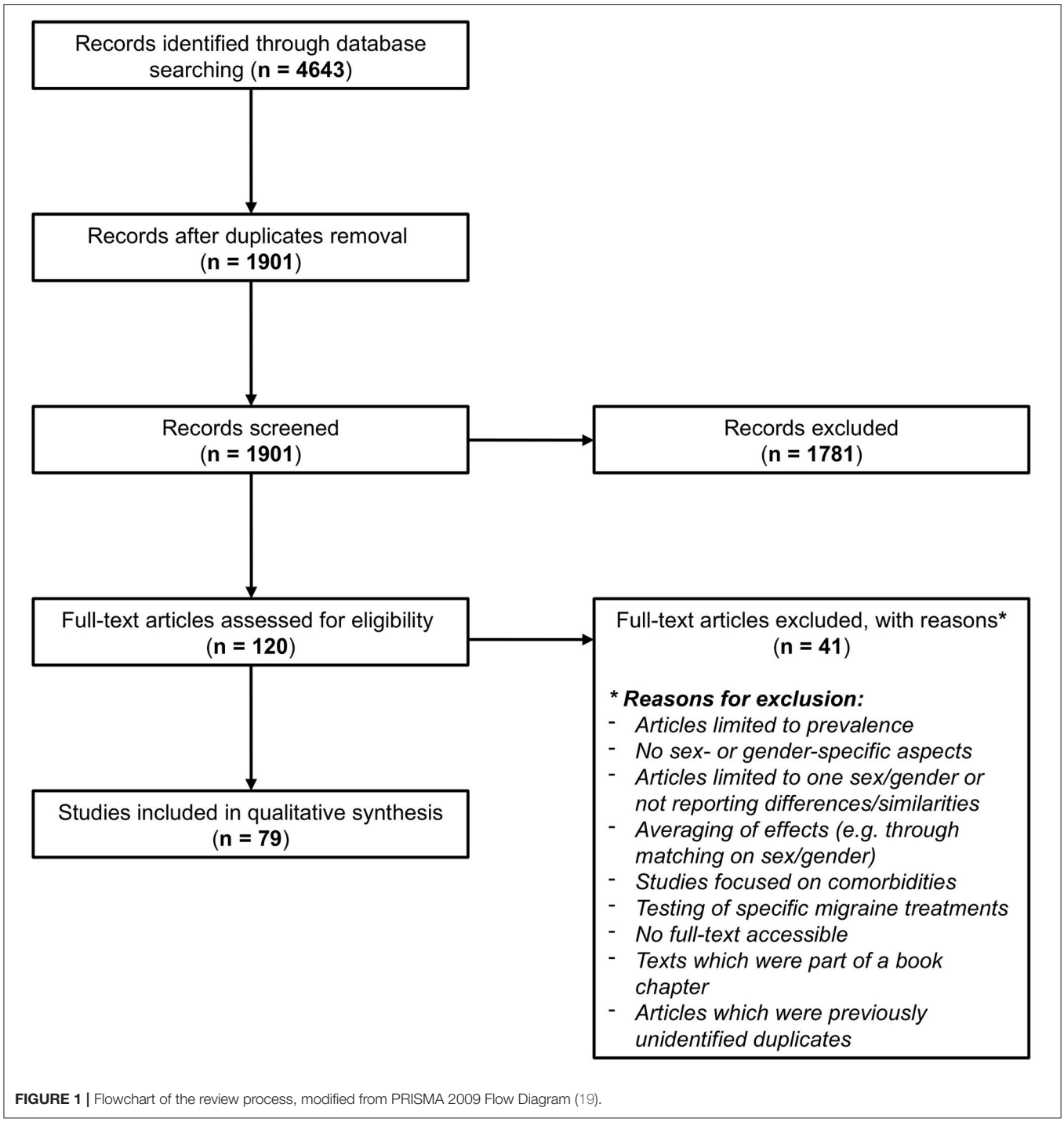

FIGURE 1 | Flowchart of the review process, modified from PRISMA 2009 Flow Diagram (19).

Conflicting results were found concerning education and income. In a recent cross-sectional study, education and social support were not associated with migraine (27). However, in another cross sectional analysis of the European Health Interview Survey for Spain, it was found that migraine was more prevalent in both men and women with lower education and income levels (28). Among older women, a social gradient was observed, with a 3% (95% CI 0.96–1.01) decrease in the odds of migraine with increasing social status, whereas in men aged 45–85 years, migraine was associated with economic status (27).

Certain kinds of physical activity were found to be associated with lower odds of having migraine, but only among older women (27). Accordingly, another study found that both females and males reporting low levels of physical activity had a higher odds of having migraine (29). Additionally, hypertension and consumption of light alcoholic beverages showed an association

with migraine; however, consumption of strong alcoholic beverages was not found to be associated with migraine (29). Furthermore, associations between migraine and a history of head trauma as well as family history of headache were found in both women and men (29).

In general, migraine is characterized by a pattern of migraine attacks, remission, and relapse, with men tending to have longer remission periods than women (11, 20). Furthermore, headache attack duration is reported to be longer in women experiencing migraine attacks (20). Attack frequency and intensity seem to be similar in males and females, but severe headaches persist longer over the lifetime of women and are reported to be more bothersome (20). Symptoms including photo- and/or phonophobia, nausea, vomiting, and skin allodynia occur more often in women with migraine attacks than in men (20).

It remains unclear whether biological sex plays an important role in the transition from episodic to chronic migraine (11). In the Chronic Migraine Epidemiology and Outcomes (CaMEO) study, no major differences were observed, however, after adjusting for demographics, comorbidities, and headache features, the odds for such a transition was 43% higher for men compared to women (30).

Concerning the subtypes, migraine without aura is more common in both sexes (11). The remission rate appears unaffected by migraine subtype, with a cohort study showing that 46% were free of active migraine by the age of 50 (11, 31).

Among women, there is another differentiation of migraine subtypes based on menstruation, which distinguishes pure menstrual migraine, menstrually related migraine and non-menstrual migraine (32).

A cohort study by Scher et al. (30) found that 56.5% of males and 68.6% of females identified individual trigger factors for their migraine. While triggers including "hunger/skipping meals, thirst/dehydration, flashing/bright lights, extended screen exposure, neck strain, change in sleep patterns, caffeine [or lack of it], certain foods, [and] changes in weather/temperature" were more often reported by women, men reported common triggers to include "strong odors/scents and stress/stressful times" (30). Furthermore, in women, the time around menses, before and after onset, was often reported as a trigger factor (32).

Basic Science and Preclinical Research

Sex Aspects

As the pathophysiological mechanism of migraine is complicated and involves a variety of interrelated neurological and vascular processes, we highlight the most important developments in basic research related to sex differences. It is important to realize that several aspects involved in the pathophysiology of migraine may interact with each other, and thus the topics below are tightly interrelated.

Central mechanisms

Previous research has shown that migraine auras might be caused by cortical spreading depression (CSD), which is a propagating wave of initial depolarization of neuronal and glial cells followed by prolonged depression (33, 34). Although not all migraine auras are followed or accompanied by headache pain (typical aura without headache), trigeminovascular activation induced by CSD may be, at least in part, responsible for the migrainous headache. Ovarian hormones have been linked to CSD susceptibility (10, 14, 15, 35, 36).

Low serotonin (5-HT) levels and reduced brain serotonin synthesis have also been linked to migraine (37). Serotonin (5-HT) is synthesized from tryptophan, which is transformed into 5-hydroxytryptophan (5-HTP) via the enzyme tryptophan hydroxylase (TPH). Estrogen is able to influence enzymes at different stages of the metabolism of tryptophan. Chauvel et al. explored the interrelations between serotonin, cortical excitability, and sex hormones in female and male rats. Their findings confirm that: (a) elevated estrogen levels increase cortical excitability, while estrogen withdrawal decreases CSD and normalizes it, (b) 5-HTP decreases the occurrence of CSD, but only in the presence of ovarian hormones and (c) in oophorectomized rats that received estradiol replacement, increased CSD was observed, which decreased after estradiol withdrawal (38).

The observed results from animal models might offer an explanation for the clinically observed association between attacks with aura and stable, high estrogen levels (e.g., during pregnancy or intake of the combined contraceptive pill). Following this line of reasoning, it seems feasible that the majority of menstrually related migraine attacks, due to estrogen withdrawal, tend to occur without aura (38).

Familial hemiplegic migraine. Other studies reported results from animal models of familial hemiplegic migraine type 2 (FHM2). FHM2 is a rare subtype of migraine with aura caused by mutations in the ATP1A2 gene, which encodes the α2 subunit of the Na+/K+ pump (39). This altered ion homeostasis leads to a disturbance of the sodium homeostasis (40).

An included article supported the hypothesis that estrogen plays an important role in the glutamate system in the brain (and blood). The introduction of a new α2Na+/K+-ATPase knock-in (KI) mouse model ($\alpha2^{+/G301R}$) showed that male mutated mice have a prolonged recovery phase after induction of CSD compared to wild type mice. Also, a link between the female sex hormone cycle and the glutamate system was established. Female $\alpha2^{+/G301R}$ mice showed female-specific behaviors, including hypolocomotion and reduced motor skill learning/coordination. These effects were rescued after treatment with Depoprovera, a progestin-only contraceptive, which leads to stabilized and low estrogen levels. Lastly, it was observed that the uptake function of glutamate was affected and that glutamate levels were increased in lysates from various female brain regions in mice (41).

Krost et al. also experimented with the aforementioned $\alpha2^{+/G301R}$ mouse model. The experiments showed increased susceptibility to CSD in $\alpha2^{+/G301R}$ mice; however, the results did not show sex-based effect of this increased susceptibility in adult and aged female mice compared to female mice. Nevertheless, these results suggest that this effect might be present during a restricted period of the menstrual cycle. The authors also found a decreased susceptibility to CSD after menopause in female mice

but not with age in male mice, indicating an age-associated shift toward CSD (42).

We recommend a prior review by Dehghani and Karatas (43) concerning the use of FMH mouse models for more details on the study of sex differences in pathophysiological (including neurophysiological and behavioral) mechanisms involved in migraine.

Nociception-related proteins. Studies have demonstrated that estradiol receptors are located in trigeminal nociceptors. Therefore, the binding of estradiol might lead to the activation of extracellular signal-regulated kinase (ERK) and an increase of nociception (44).

To study the role of estrogen in the pathogenesis of migraine, Vermeer et al. utilized a multibehavioral model of migraine in rats and investigated responses to the exposure of estrogen. As compared to vehicle treatment, estradiol treatment led to a statistically significant decrease in locomotor activity as well as significant light and noise avoidance, allodynia-associated behaviors, and an enhanced acoustic startle. Moreover, estradiol treatment led to an increased expression of genes associated with estradiol signaling (expression of estrogen receptors), inflammation, vasodilation, and endogenous cannabinoid metabolism. Lastly, this treatment led to the activation of the nociception-related ERK (45).

Guo et al. investigated the role of the expression of proteins involved in the transmission of nociceptive signals, including brain-derived neurotrophic factor (BDNF) and its receptor, tropomyosin receptor kinases (TrkB), as well as ERK and its downstream target, cAMP-responsive element binding protein (CREB), in migraine by using injections of nitroglycerin to provoke migraine in rats. A positive relationship between the BDNF/TrkB and ERK/CREB pathways and the contribution of estrogen was observed. Indeed, female ovariectomized rats showed a significant decrease in the expression of BDNF, TrkB, p-CREB, and p-ERK in migraine attacks and intervals compared to rats with intact ovaries. However, the administration of estrogen recovered the expression in these ovariectomized rats. Moreover, researchers observed higher serum levels of BDNF in female than in male rats during migraine attacks (46).

Peripheral mechanisms

Dysregulation of neuropeptides. Animal studies have provided important insights into the influences of calcitonin gene-related peptide (CGRP) in migraine. This neuropeptide is thought to play a pivotal role in the pathophysiology of migraine, as it contributes to trigeminal nerve hypersensitivity and photosensitivity (47, 48). Avona et al. recently reported a female-specific response to dural administration of CGRP in a rodent migraine model assessing cutaneous periorbital hypersensitivity (49). Specifically, female rats and mice responded to lower doses of dural CGRP than their male counterparts. The authors concluded that female sex hormones have the ability to increase vasodilation in response to CGRP; however, the anatomical and physiological mechanisms explaining this difference in response have yet to be elucidated (49).

Pro- and anti-inflammatory mediators. Compelling evidence has been published in support of the theory that migraine is associated with a sterile inflammation of the dura, leading to increased pro-inflammatory cytokines and chemokines in plasma and cerebrospinal fluid during attacks (50–52). McIlvried et al. hypothesized that stress, being a triggering factor in migraine, induces a shift in the balance of pro- and anti-inflammatory mediator expression in dural lymphoid cells toward those that trigger a migraine attack. Moreover, they hypothesized that this effect is larger in females and that it is (partly) dependent on sympathetic postganglionic innervation of the dura. The authors tested their hypothesis in adult male and female rats. The results confirmed their hypothesis, as a sex difference was observed in (a) the increase in pro-inflammatory mediators, (b) decrease in anti-inflammatory mediators, and (c) expression of some inflammatory mediators. Moreover, (d) sympathetic postganglionic innervation only influenced the stress-induced increase of pro-inflammatory mediators in females. This article offers possibilities for different therapeutic targets in males and females (53).

We also recommend the review by Loewendrof et al. for a detailed overview of the role of mast cells, which are part of the innate immune system, and sex hormones in migraine (54).

TRP channels. Other structures implicated in various sensorial functions and in the pathophysiology of migraine include the non-selective cation channels Transient Receptor Potential (TRP) channels (55). Our search strategy included a review which describes the role of gonadal hormones in the activation, modulation, and regulation of the main thermoTRP channels in migraine (56).

We additionally point readers to previously published reviews from 2017 and 2018 focused on sex differences of migraine in animal models for more detailed discussions (11, 14, 15). Lastly, we recommend a review by Loewendorf et al. for a comprehensive and short summary of the literature concerning the mechanisms central sensitization, channelopathy, and sodium homeostasis in migraine (54).

Gender Aspects

Obviously, gender aspects in laboratory animal studies are hard to describe and might, if present at all, be mainly related to behavioral aspects. However, investigation of sex differences in animal behavior e.g., (41, 45), could be possibly interpreted as studies on gender aspects. Indeed, the way pain is experienced can be thought of as a behavioral aspect, although also sex components definitely influence pain perception. Whereas it is generally accepted in humans that pain is experienced differently in men and women, understanding the motivation behind certain behavioral traits in animals is impossible. Descriptions of behaviors that reflect pain in animal studies include avoidance responses and tending to the site of pain. It is worth mentioning that behavioral aspects in animal studies are consistent with human studies. Indeed, lower pain thresholds and an increased need for opioids have been shown in females vs. males (54).

Translational Science: "From Bench to Bedside"

Some articles combined basic research with research in the clinical setting involving (migraine) patients, although they generally described sex differences rather than gender differences. These articles mainly aimed to describe the pathophysiology of migraine.

A study by Karkhaneh et al. determined the effect of 17β-estradiol on the expression and activity of genes involved in the process of neurogenic inflammation. The authors studied the regulation of CGRP expression, inducible nitric oxide synthase (iNOS) activity, and NO and interleukin-1beta (IL-1β) release in females with pure menstrual migraine and age-and sex-matched healthy individuals. Cultured peripheral blood mononuclear cells from these participants were treated with 17β-estradiol both, at physiological and pharmacological doses. The pharmacological dose caused a significant increase in mRNA expression of CGRP in both groups. In contrast, the physiological dose caused a significant decrease in mRNA expression of CRP, CGRP protein levels, IL-1β release, NO production and iNOS activity only in females with pure menstrual migraine (57).

In a review article, Labastida-Ramírez et al. describe sex differences of CGRP in migraine. This review highlights the profound effects varying ovarian hormones have on the trigeminovascular system in both animal and human migraine preclinical research models (58).

Ibrahimi et al. measured dermal blood flow after topical forearm application of capsaicin (the active component of chili peppers) to study endogenous release of CGRP among a group of migraine sufferers and individuals without migraine. Unlike males, females without migraine showed changes in the CGRP-dependent dermal blood flow response, which was also elevated during menstruation compared to the late-secretory phase. Females with migraine also had higher dermal blood flow responses during both menstruation and the late-secretory phase compared to females without migraine, suggesting an interrelationship between the menstrual cycle, migraine, and vascular effects mediated by CGRP (59).

Additionally, a comparable human model was developed for application of capsaicin on forehead skin, which is a trigeminal nerve–innervated dermatome, in order to study the effects of the menstrual cycle. The underlying mechanism includes activation of a TRP (TRPV1) channel by capsaicin, thereby enhancing the release of CGRP. The authors did not detect changes in trigeminovascular reactivity during the cycle in patients with menstrually related migraine in contrast to females without migraine (60).

Clinical Science

Our search strategy retrieved a variety of original articles and reviews containing clinical evidence on the relationship between migraine, sex, and gender. As these results cover an extensive range of themes and research questions, we have classified them into different categories for the sex aspect of this review: sex hormones, reproductive events (occurring in women), biomarkers, and additional topics - which might be related to the pathophysiology of migraine. Clinical articles concerning gender aspects have been described separately for lifestyle factors, although it should be kept in mind that our two main themes - sex and gender - contain overlapping aspects.

Sex Aspects

Sex hormones

The "estrogen withdrawal hypothesis" has been expanded by several articles we included. Earlier studies consistently showed a withdrawal of estrogen in the late luteal (or premenstrual) phase, whereas the association between migraine and withdrawal of estrogen in the follicular (or periovulatory) phase has been more debated (61–64).

Pavlović et al. provide a possible explanation for the latter and show the influences of the menstrual cycle phase and timing (65). They compared the daily sex hormone levels and within-women rates of change between females with a self-reported history of migraine and controls aged 42–52 years. This study showed significant differences in the decline of urinary estrogen in the late luteal phase; individuals with migraine showed a faster decline. However, no differences in the rate of decline in the periovulatory phase and no significant differences in the peak or mean daily levels of estrogen were observed between individuals with migraine and controls. Lastly, they found that in the migraine group, the rate of decline in estrogen does not discriminate cycles with and without an acute headache. Therefore, the authors hypothesize that rapid estrogen withdrawal is not a direct trigger of migraine, but rather an endogenous characteristic and a marker for neuroendocrine vulnerability in females with migraine due to a disruption of the trigeminovascular system. Also, as progesterone has modulatory effects on estrogen in migraine, its rising levels may counteract the effects of periovulatory estrogen decline (65).

Few studies were solely performed in a male study population or attempted to investigate the role of sex hormones in this underrepresented group. It is not clear whether sex hormones modulate migraine susceptibility (risk and activity) in men as they do in women. Moreover, the role of testosterone in migraine is described in fewer studies than estrogen or progesterone.

A prospective study compared the levels and ratio of sex hormone plasma (17β-estradiol and free testosterone) in a group of medication-free, non-obese men with episodic migraine (18–74 years) to males without migraine group-matched for age and body mass index (BMI). In this study, males with migraine exhibited elevated estradiol levels, both absolute and relative to free testosterone, and showed clinical evidence of functional androgen deficiency (66). A pilot study investigated total serum testosterone levels in males aged 26–51 years with chronic migraine. Men with chronic migraine had lower total testosterone levels compared to an age-matched normative population, suggesting abnormalities in the regulation of the hypothalamus-pituitary-gonadal (HPG) axis (67). These abnormalities were then confirmed by Li et al. considering the levels of hormones. They found that levels of progesterone in males and females with migraine in the postmenopausal phase were lower compared to healthy controls. Also, they found significantly higher levels of gonadotropin-releasing hormone (GNRH) in males with migraine, in the follicular phase as well as

the luteal phase in females with migraine, and in postmenopausal females with migraine compared to controls (68).

As all three studies were limited by their small sample sizes, larger studies are needed to replicate these findings and to investigate whether hormone fluctuations (as well as testosterone supplementation) might be associated with changes in migraine activity and frequency.

Lastly, supporting the hypothesis that migraine should be seen as a consequence of a loss of neurohormonal and metabolic integrity instead of a primary disorder, Dzugan et al. reported successfully treating difficult-to-treat males ($n = 3$) and females ($n = 27$) with migraine and a mean age of 46.4 years. Participants received therapy with bio-identical hormones to restore hormonal levels. As part of the restoration of the integrity between various systems (including the HPG axis) in order to manage migraine, they postulate that serum levels of all basic hormones (pregnenolone, dehydroepiandrosterone sulfate (DHEA), total testosterone, total estrogen, and progesterone) should be near to optimal with physiological cycle (69).

We direct readers to an article by Delaruelle et al. for a review covering studies investigating the influence of female and male sex hormones on primary headaches, including migraine published between 1997 and 2017 (70).

Reproductive milestones

Sex disparities in migraine have been also explained by the occurrence of reproductive life events, which are linked to changes in hormone levels. We sorted the included articles according to these events. Moreover, for an additional and similar overview of these sex-specific characteristics in individuals with migraine for an enhanced understanding of the impact of hormones, we recommend the reviews written by Maleki et al., Allais et al., Todd et al., Pakalnis et al. as well as the overview given by Bronet et al. (22, 71–74). We further recommend the review by Hipolito Rodrigues, which emphasizes the menopausal transition (75).

Puberty. Little data are available concerning risk factors for the onset of migraine in pediatric populations. Therefore, the association between pubertal timing and migraine was investigated in a recently published article. Researchers found that being a woman with an early age of menarche (early puberty) in a mainly Caucasian population was associated with a 7% lower odds of migraine at early adulthood - although pathophysiological links remain to be investigated (76).

Menstruation. The International Classification of Headache Disorders (ICHD) subdivides menstrual migraine (MM) (with and without aura) into pure menstrual migraine and menstrually related migraine, both with and without aura (1). Evidence regarding the association between migraine and menses has been further built upon in this review.

A relationship between self-reported migraine and menses has been reported in almost 60% of women (77). Previous studies consistently found that MM attacks have a longer duration, are more challenging to treat and are more impairing (78–82). These findings have been confirmed by a more recent study of Pavlović et al., as data from the American Migraine Prevalence and Prevention (AMPP) Study showed that self-reported migrainous headache related to menses impacted women to a larger extent. Also, women with pure menstrual migraine were more impaired due to the attacks, while women with menstrually related migraine had the overall highest burden (77). Allodynia, nausea, vomiting, and phonophobia related to the migraine attacks have been reported to be more frequent in females with MM as well (83).

The influence of hormonal changes on the development of migraine without and with aura is reflected in the results of a cross-sectional study by Taha et al. Indeed, among females with migraine, progesterone levels were significantly higher among those without aura in both phases of the menstrual cycle (follicular and luteal phase). Estradiol levels showed almost similar effects in the phases of the cycle. In the luteal phase, this hormone was significantly higher among females with migraine without aura, while in the follicular phase, it was significantly higher among females with mild migraine without aura. Moreover, a significant increase of prolactin was associated with an increase in the severity of migraine with and without aura (84). Interestingly, in the aforementioned translational study by Ibrahimi et al. (60), relatively low mean estradiol levels were detected during days 19–21 of the menstrual cycle of the patients with menstrually related migraine (60).

Conflict in the literature exists concerning pain intensity and associated symptoms as a result of the fact that these studies relied on the women's diagnosis of MM (in a clinical setting) and possibly suffered from (self-)selection bias (62, 78, 79, 81). Therefore, a prospective study by Vetvik et al. used records of headache diaries from women with and without a diagnosis of MM without aura according to the ICHD criteria. They compared the clinical characteristics of MM and non-MM without aura attacks in a random sample of women aged 30–34 years with and without MM based on diary assessment. Results of this study showed that in a representative sample of females fulfilling the ICHD criteria for MM without aura, MM without aura attacks had a significantly longer duration and were more often accompanied by severe nausea than non-MM attacks without aura. However, in women with migraine without aura who did not fulfill the ICHD criteria for MM (no diary-confirmed diagnosis of MM), no significant differences between menstrual and non-menstrual attacks were found (85).

Moreover, an exploratory study showed that chronic (15 or more days/month for more than 3 months) (1) rather than episodic migraine might be driving the associations with menstrual-cycle disorders in general and dysmenorrhea specifically (86).

Regarding other menstruation-related conditions, our search strategy retrieved two original articles about endometriosis with complementary findings. As endometriosis shares similar characteristics in terms of its clinical manifestations, epidemiology, and pathogenesis with migraine, it has been shown that migrainous headache was more frequent in the women with endometriosis than in women without endometriosis as well as in infertile women. Authors of both studies, therefore, suggest that females with migraine should be screened for endometriosis

criteria and vice versa, allowing for more individualized treatment (87, 88).

Pregnancy and breastfeeding. The association between menstruation and migraine seems to be noticeable during pregnancy. Indeed, the large, population-based Akershus Birth Cohort (ABC) Study in Norway showed that pregnant women with self-reported MM report higher headache intensity during early pregnancy and directly postpartum compared to women without self-reported MM. Both groups showed a significant improvement during the second half of their pregnancies and directly postpartum. Moreover, the investigators report that hormonal and menstruation-related factors (premenstrual syndrome, age at menarche, and the use of hormonal contraception before pregnancy) showed no association with headache intensity, except for irregular cycles (89).

The influence of events during pregnancy, including fetal growth, on the development of migraine during adulthood has also been investigated. Data from the Norwegian population-based Nord-Trøndelag Health Study (HUNT 3) showed significant effect modification by sex in the association between being born very small for gestational age and the odds of migraine development later in life. This effect was only observed in males, probably due to differences between both sexes in adaptations of the fetal-placental unit. Altered activity of neurotransmitters and/or changes in the brain structure and connectivity might also be an explanation (90).

Several reviews have been published investigating the (beneficial) effects of pregnancy on migraine and migraine on pregnancy, as well as the influence on breastfeeding (91, 92). Moreover, Parikh describes the expected course of migraine during pregnancy and the post-partum period as well as considerations for preventive and abortive medications, although the latter topic goes beyond the scope of this review (93).

Menopause. Migraine shows variability in the clinical presentations in the menopausal period, as migraine attacks might improve, worsen or remain unchanged during this transition (75). The AMPP study also explored the influence of the perimenopausal status on the frequency of migraine attacks in women aged 35–65 years (94). A 1.4-fold increased risk for high-frequency headache was found during the perimenopause as compared to the premenopause. This effect might be confounded or mediated by medication overuse or depression (94). We recommend a systematic review by Ripa et al. for a more thorough overview of the articles documenting the relationship between migraine and menopause (95).

Additional topics

In our search, we also found a case-control study intended to build further upon the hypothesized role of iron in migraine, which plays an essential role in the synthesis of serotonin, dopamine and norepinephrine (96). Accumulation of iron in the brain, specifically in the deep brain nuclei and the periaqueductal gray matter, has been shown to be related to the migraine neurobiology - supported by earlier neuroimaging evidence (97–100). Results of this study indicated a relationship between hemoglobin, ferritin, as well as iron-deficiency anemia in subjects suffering from migraine - mainly women and girls. These findings suggest that treatment for iron-deficiency anemia or iron supplementation might be a beneficial preventive method for patients suffering from migraine coinciding with iron deficiency anemia. Indeed, both migraine and iron-deficiency (induced by heavy menstrual flow) are more common in young females (96).

Another study investigating event-related potential among individuals with migraine without aura in the interictal period showed that females had more severe abnormalities in visual neurocognitive processing under attentional conditions compared to males (101).

Gender Aspects

Migraine characteristics

Besides differences in the presentation of migraine between sexes, differences between the actual experience or manifestations of migraine have been previously described, which may be more related to gender.

A large, longitudinal, internet-based panel study showed that women reported more frequent attacks and were more likely to be disabled by their attacks than men (102). According to this cross-sectional survey, women were significantly more likely to report that the pain was unilateral and of pounding, pulsating, or throbbing in nature. Also, symptoms of nausea, photophobia, phonophobia, osmophobia, and cutaneous ictal allodynia had been reported significantly more often in women (102). Additionally, median headache duration of both migraine with and without aura seems to be longer in women than in men (103). An age-dependent variation of migraine associated symptoms (nausea, photophobia, and phonophobia) were only observed in females with migraine with and without aura, as significant changes in their frequency were mainly seen after the age of 30 (103).

Stress and lifestyle

A synergistic relationship between female sex and high levels of daily stress on risk of migraine headaches has been described. Females exposed to stress seem to have a higher 1-year prevalence of migraine, indicating an interplay between biological, sex-related factors and environmental stress in the progression of migraine (104).

Lifestyle factors also appear to play a role in migraine. An inverse relationship between migraine and dietary sodium intake was described by Pogoda et al. This relationship was most obvious in females with a lower BMI, while in men, the relationship did not differ by BMI after confounding adjustment (105).

Genetics and Biomarkers

There are indications that genetic factors may explain observed phenotypic sex differences in migraine (20). In their review, Vetvik and MacGregor discuss that commonly cited genetic explanations for these differences, such as the disease being autosomal dominant in women and autosomal recessive in men, or migraine being a direct consequence of an inherited variant, might be too simplistic (11). They emphasize that migraine is

polygenetic, and it is also likely that differences observed between sexes are also influenced by environmental factors.

At present, a total of 38 genomic variants have been implicated in migraine risk (106). Only one of these 38 susceptible loci is located on the X-Chromosome (11).

A link between migraine attack frequency and family history of migraine was found in males in a cross-sectional study, suggesting a certain genetic predisposition (107). Furthermore, an investigation of 12 common migraine risk loci did not find differences in risks between males or females and across different migraine subtypes (migraine with and without aura), or clinic- vs. population settings (108).

Another genotyping study, focusing specifically on the PRDM16 rs2651899 variant, found a significant difference in this variant for migraine without aura as well as the female subgroup compared to controls without migraine in all investigated models (106). The TRPM8 rs10166942 variant showed an association with migraine in male subjects and with migraine with aura, but not in females (106). However, this study had an imbalance between male and female participants and a rather small sample size ($n = 300$) for a genotyping study.

A study investigating the relationship between the progesterone receptor gene and migraine found that an PGR polymorphism was not directly connected to predisposition to migraine, however, it led to a later onset of migraine supposedly through reduced neuronal excitability in the brain (109). However, this finding was only observed in females due to the small number of male participants in the study (109).

Sazci et al. report that previous studies showed an association between methylenetetrahydrofolate reductase (MTHFR) variants C677T (rs1801133), A1298C (rs1801131) and migraine (110). Furthermore, the nicotinamide-N-methyltransferase (NNMT) variant rs694539, expressed in the brain and other nervous tissue as a cytoplasmic enzyme, was associated with migraine presence (110). The implicated MTHFR variants and the NNMT variant have been linked to higher plasma homocysteine levels, which, in turn, have been associated with migraine risk (110). However, the association between the NNMT variant and migraine was only found in women, with 4.3 times the odds for women with an AA genotype and the same magnitude of protective effect in individuals with the G allele (110).

Another genotyping study in a North Indian population (n = 500) showed a relationship between the rs10156191T variant and the odds of migraine presence in females (OR = 1.46) as well as with the odds of having migraine without aura (OR = 1.21) (111). However, due to methodological inconsistencies, the accuracy of these results is not clear. Still, these findings support previous hypotheses that diamine oxidase (DAO) increases the risk of migraine, especially in women (112). Moreover, the results suggest that the T allele in the same genetic variant has a protective effect against migraine in men (111). Both the rs2052129 and rs10156191 variants belong to the DAO gene, which is associated with high histamine levels, which, have been implicated in migraine pathophysiology (111). The presence of the investigated genetic variants is thought to lead to a reduction of DAO activity (111). Similar findings were retrieved from Caucasian Spanish (112) as well as North Indian (111) populations.

Fang et al. investigated the involvement of two specific genetic variants (rs12134493 and rs2078371), belonging to the tetraspanin 2 (TSPAN2) gene, in a Han Chinese population (113). They concluded that rs2078371 could be a potential risk factor for migraine susceptibility, especially in women and individuals with migraine without aura, which is similar to results found previously in Western populations (113). However, the biological mechanism of how TSPAN2 variation impacts migraine needs further exploration (113).

Another genotyping study (114) concentrated on twelve genetic variants previously reported to be related to migraine or the metabolism of sex hormones. A variant of rs2229741 was observed more in women with migraine compared to controls, suggesting a protective association for migraine (114). Furthermore, the GG genotype of the rs726281 variant, which is part of the estrogen receptor 1 (ESR1), lowers the susceptibility for migraine in a subgroup of women with menstrual related migraine (114). These results were retrieved from a Turkish population with a rather small sample size ($n = 284$). A meta-analysis also found that people with polymorphisms in two specific exons of the ESR1 (4 325C>G, 8 594G>A) are more susceptible to migraine, at least in a caucasian population (115).

In a small genome-wide association study, with 268 cases and 142 controls, an association between menstrual migraine and rs2506142 variant, which is located near the neuropilin 1 gene (NRP1), was found (116). The authors conclude that NRP1 might play a role in the etiology of menstrual migraine. NRP1 is involved in neuronal development processes in the nervous and vascular system, as well as in menstruation (116). It has been shown that activity of NRP1 is increased at the same time estrogen levels drop in the proliferative phase (116). Moreover, Sazci et al. report about a relation between that genetic variant in the SYNE1 and TNF genes and menstrual migraine (110).

So far, findings suggest that there might be a certain overall genetic predisposition for migraine presence. However, there are no clear differences in the association pattern between sexes. Some studies only showed significant associations of identified single genetic variants or genetic risk scores for one sex, mainly females. But due to the imbalances in sex distribution and partly small sample size of some studies, these findings should be interpreted with great caution. It is suspected that additional endogenous or exogenous factors may explain this difference (11). In the comparison between male and female migraine cases, it seems more likely that migraine has a stronger genetic basis among men than women (108). Still, Nyholt et al. report that differences in genetic risks in the subgroups are outweighed by similarities, suggesting that further investigation is needed. Thus, epigenetics came into the focus of attention, concentrating on interactions between different genetic predispositions and environmental influences (20).

There has also been research activity in the field of biomarkers, however, not very recently. In a review by Tietjen

and Khubchandani (117) articles investigating the connection between various vascular biomarkers and migraine were identified (129 citations). It was reported that some studies found elevated CRP levels in female subgroups with migraine; however, the results about CRP and migraine were very inconsistent. For other biomarkers, either no sex difference was found, such as in adiponectin levels, or no sex differences were mentioned at all. If results referred to women only, the study was conducted in an exclusively female study population, thus no conclusion about sex differences could be made (117).

Neuroimaging

The study of brain imaging contributes to an enhanced understanding of the pathophysiology of migraine, as functional, chemical, and structural differences between males and females with migraine have been observed (20, 71). Surprisingly, our search strategy only retrieved reviews, but no original articles. The review by Maleki and Androulakis explains neuroimaging patterns that distinguish females from males in migraine, also in relation to sex-related influences. Moreover, the authors mention the influence of neuroimaging in the light of some earlier described specific life events, including the perimenopause, estradiol decline and puberal phase (71). In addition, Chong et al. provide an overview of studies concerning sex difference published in the period between January 2012 and 2016 (118).

Previous functional MRI studies have shown that the posterior insular cortex and the precuneus are thicker in females compared to males with migraine (7). Also, a smaller volume of the parahippocampal gyrus was observed in these males. Moreover, female sex has been related to lower gray matter in the right dorsolateral prefrontal cortex (119).

These imaging studies also seem to explain the attribution of gender aspects in migraine, as they show gender-related differences in the involvement of regions which govern the emotional aspects of pain. Indeed, the possible involvement of the hippocampus, though not yet established, might explain gender (and sex) differences in behavioral responses to the stress and explain differences in migraine attack (20).

As highlighted by Schroeder et al. and Pavlovic et al., the majority of the neuroimaging studies have included mainly females with migraine (14, 15). Lastly, there exist very limited neuroimaging studies with considerably small sample sizes that have examined sex-related differences in migraine (71).

DISCUSSION

In this review, we systematically searched literature exploring sex and gender differences in migraine in the areas of epidemiology, basic science, clinical science, genetics, and neuroimaging and have summarized the findings.

Migraine is a highly prevalent and burdensome disease, particularly among women. Still, the disease is responsible for a great share of disability in both sexes. Distributional differences in gender-related lifestyle factors such as marital status, employment, and social support have also been observed among persons suffering from migraine. While many biological factors have been hypothesized to play a role in the sex difference, particularly sex hormones or sex hormonal fluctuations, a clear understanding of why migraine is different between women and men remains illusive. Many studies from which we extracted results lacked a clear operationalization of sex and gender, even when sex and/or gender aspects were the main focus of the research. Further complicating the evidence synthesis, the majority of studies presenting prevalence do not consistently use either lifetime prevalence or 1-year attack prevalence, making them difficult to compare. Other studies lack any specification of a time frame, which is indispensable for a correct interpretation of the results.

From a pathophysiological point of view, the influence of sex differences related to hormone levels on a variety of mechanisms (including the role of CGRP, cortical spreading depression, and various anti- and proinflammatory mediators) involved in migraine have primarily been studied in animals and often in isolation, as no experimental model of migraine covers all aspects of this disorder. This may be a limitation, since these processes are interrelated and are known to influence each other. Little research regarding gender roles can be conducted on a basic sciences level; however, some important behavior-related aspects have been explored. Translational studies on the topic include human studies exploring trigeminovascular reactivity in a non-invasive manner.

The majority of articles identified in this review consisted of clinical studies and reviews. Clearly, sex hormones (including estrogen, progesterone and testosterone) contribute prominently to the observed sex-related differences. Specifically, changes in the migraine phenotype (including the amount of attacks and intensity) attributable to female-specific reproductive milestones highlight the profound effects of these sex aspects, with migraine becoming more prevalent in women starting in puberty, peaking in their thirties, and steeply declining after menopause. The clinical studies indicate that gender-related aspects are also important to consider, as males and females might have different pain perceptions and experiences. Reporting bias in clinical studies should not be overlooked, as the evidence indicates males more often underreport their symptoms, which likely contributes to a continued underrepresentation in clinical (and epidemiological) studies.

In the genetics literature, specific variants have been found to be associated with certain migraine subtypes, such as migraine with aura in both sexes or menstrual migraine in women, as well as the presence of migraine in females. Certain genetic variants (rs2651899, rs694539, rs10156191, rs2078371, rs2506142) appear to make individuals more susceptible to ever having migraine. Though genetics may provide useful mechanistic insights, the individual studies acknowledge that genetic aspects alone do not provide a full explanation for the sex differences observed in this multifactorial disease. In addition, as men were underrepresented in these studies and some had small sample sizes, their conclusions should be interpreted with caution.

Surprisingly, in the field of research about biomarkers and migraine, beyond the articles included in one review from 2015 (117), we did not identify any new articles in this area. Lastly, we only found reviews and no original articles on neuroimaging

describing sex- and gender related differences, highlighting another gap in the existing literature.

Strengths and Limitations

This review has several strengths. First, unlike several narrative reviews on this topic, we developed an extensive, systematic search strategy and employed a rigorous inclusion and screening in an effort to systematically identify all recent relevant articles. We hope the thorough description of the searching steps and access to the developed search strings contribute to the transparency and reproducibility of our method. We synthesized newly published evidence in the context of the main findings of previously published review articles in an effort to provide broad topical coverage while minimizing unnecessary redundancy. Furthermore, we made an effort to go beyond biological sex aspects and also include gender aspects (behaviors, roles, etc.) where available. In an attempt to convey the distinction between both concepts, in our synthesis, we attempted to consistently operationalize sex as biological differences and gender as social construct that is shaped by cultures and varies over time. This approach stands in contrast to previous reviews and much of the original literature, in which the terms "sex" and "gender" are often used interchangeably.

Some limitations should be considered in the interpretation of our results. First, this review is limited by the articles we found through our search strategy. To limit extensive overlap with previous reviews and focus on the newest literature (2015-present), older studies were summarized more broadly based on existing reviews (11, 14, 15, 20, 22, 43, 54, 56, 58, 70–75, 91–93, 95, 117, 118). Second, we excluded articles investigating the effects of migraine therapies and treatments to focus on sex- and gender-differences in migraine as a disease, itself, rather than sex-specific differences related to individual treatment strategies, adherence, and migraine management. We acknowledge there are important (behavioral) differences between males and females with respect to treatment, but these are outside the scope of the present review (102, 120). Robbins and Bernat did not identify articles on treatment efficacy stratified by sex, illustrating that this topic also needs more attention in the field of migraine treatment research (121). Sex and gender differences in migraine comorbidities were also outside the scope of our review.

Finally, we did not provide a rigorous quality assessment of the included articles, though we made an effort to caution against over-interpretation, especially where the evidence was particularly thin. Given these aforementioned limitations, we do not claim that this review is exhaustive in terms of findings in the field of sex and gender differences in migraine, however, we believe it is quite comprehensive given its scope.

Recommendations

Taking the above-mentioned gaps into account, we encourage further studies on sex and gender aspects in migraine across all relevant basic, clinical and population science domains and advocate for clearer operationalization of both terms - sex and gender - in future work.

Second, we observed that most studies did not contain a primary objective to identify sex- and/or gender differences. These differences were rather stated as secondary findings, implying that some studies may not have been sufficiently powered to detect differences between sex or gender strata. We advocate for *a priori* consideration of relevant research questions pertaining to sex and/or gender and investigation of sex and/or gender as a potential effect measure modifier. Furthermore, many studies matched participants with and without migraine by sex (or gender) to answer the study's primary research question, which makes inferences about the relationship between sex (or gender) and migraine impossible by design. This highlights a large methodological issue pervasive in the included literature: authors often matched for sex and provided averaged estimates, even though sex and gender differences are well-known. Therefore, a point of improvement for future studies is to avoid averaging of the effect size across of both sexes and/or genders, or to at least additionally provide stratified estimates.

Third, we echo the comments of other authors (11, 117) encouraging further research to include diverse study populations. In our review, we were explicit in our aims to identify new findings in transgender or intersex people and included these terms in the search strategy. To date, we found only two studies (122, 123) that investigated migraine in transgender people. This highlights sex and gender diversity as an insufficiently studied area in migraine research.

Fourth, basic science studies with a more representative distribution of sexes are warranted. Indeed, female animals are often underrepresented in animal studies. Although it may be justified to constrain the experimental animal population to one sex (or a specific age) for some research questions, it is a common misunderstanding that the inclusion of only male animals will lead to more reproducible results with less variation. While results of animal experiments in females may be affected by their estrous cycle and may thus be subject to intraindividual variation over time, experiments in male animals may likewise exhibit such variations for other reasons such as differences between dominant and subordinate males when housed together (124). Although most included basic science articles used a balanced distribution of males and females, there is still considerable room for improvement. Similar issues also arise in human studies, where only a few studies investigated migraine in males. As mentioned in the review by Schroeder et al. (15), societal differences and stigma surrounding this "feminized" disease should not be underestimated.

Fifth, we noticed potential for improvement in methodological quality and reporting across the included articles. In the first stage of the screening process, we were forced to exclude several articles due to methodological issues. Furthermore, in the presentation of the results, it was often difficult to discern whether the described methods were actually (and correctly) applied. Many articles reported conclusions that did not align with their stated aims, were overstated, or did not reasonably reflect their results.

Sixth, we encourage translational research of sex and gender across the three pillars - basic, clinical, and epidemiological studies - of the broad field of medicine. Although we identified existing translational research ("from benchside to bedside"), no articles assessed translational aspects from benchside to clinic

or population level. Perhaps unsurprisingly, gender aspects were more often thematized in epidemiological studies, whereas sex aspects were addressed more frequently in basic science. Clinical articles showed fluidity of these domains and contained both aspects. Although the study of gender aspects in basic science might be challenging, behavioral animal studies might be an interesting area for further exploration.

Lastly, we recommend the adoption of a more holistic and interdisciplinary approach to understanding sex and gender differences in migraine across the different domains. Genetics and biomarkers alone have not provided a conclusive answer in explaining the observed sex and gender differences, and further work is needed to explore possible important intersections between genetics, pathophysiology, behavior and environment.

CONCLUSION

A thorough understanding of sex and gender differences in migraine provides important insights into the pathophysiological processes involved in migraine as well as implications on a population level. Though research into these aspects in the domains of epidemiology, basic science, clinical research, genetics, and neuroimaging continues, several observed sex and gender differences remain unexplored. Therefore, future studies in migraine research should prioritize sex and gender aspects, consider using consistent definitions of these concepts, and employ suitable methods to explore these relevant differences instead of controlling for them.

AUTHOR CONTRIBUTIONS

LA-H and JH created the search strategy, conducted the search, screening, selection of the articles, and drafted the manuscript. TK, AM, and MP contributed with consultation on specific topics (according to their expertise) as well as synthesis of results and their interpretation. JR provided project supervision and support throughout the project by coordinating the search strategy, reviewing the articles, and drafting of the manuscript. All authors provided critical comments on the manuscript draft and approved the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

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REFERENCES

1. Headache Classification Committee of the International Headache Society (IHS). The International classification of headache disorders, 3rd edition. *Cephalalgia.* (2018) 38:629–808. doi: 10.1177/0333102417738202
2. Institute for Health Metrics and Evaluation. *GBD Results Tool.* IHME, University of Washington Seattle (WA) (2020). Available online at: http://ghdx.healthdata.org/gbd-results-tool (accessed March 19, 2020).
3. Buse DC, Loder EW, Gorman JA, Stewart WF, Reed ML, Fanning KM, et al. Sex differences in the prevalence, symptoms, and associated features of migraine, probable migraine and other severe headache: results of the American migraine prevalence and prevention (AMPP) study. *Headache.* (2013) 53:1278–99. doi: 10.1111/head.12150
4. GBD 2016 Headache Collaborators. Global, regional, and national burden of migraine and tension-type headache, 1990-2016: a systematic analysis for the Global burden of disease study 2016. *Lancet Neurol.* (2018) 17:954–76. doi: 10.1016/S1474-4422(18)30322-3
5. Peterlin BL, Gupta S, Ward TN, Macgregor A. Sex matters: evaluating sex and gender in migraine and headache research. *Headache.* (2011) 51:839–42. doi: 10.1111/j.1526-4610.2011.01900.x
6. Sacco S, Ricci S, Degan D, Carolei A. Migraine in women: the role of hormones and their impact on vascular diseases. *J Headache Pain.* (2012) 13:177–89. doi: 10.1007/s10194-012-0424-y
7. Maleki N, Linnman C, Brawn J, Burstein R, Becerra L, Borsook D. Her versus his migraine: multiple sex differences in brain function and structure. *Brain.* (2012) 135:2546–59. doi: 10.1093/brain/aws175
8. Greco R, Tassorelli C, Mangione AS, Smeraldi A, Allena M, Sandrini G, et al. Effect of sex and estrogens on neuronal activation in an animal model of migraine. *Headache.* (2013) 53:288–96. doi: 10.1111/j.1526-4610.2012.02249.x
9. Boes T, Levy D. Influence of sex, estrous cycle, and estrogen on intracranial dural mast cells. *Cephalalgia.* (2012) 32:924–31. doi: 10.1177/0333102412454947
10. Bolay H, Berman NEJ, Akcali D. Sex-related differences in animal models of migraine headache. *Headache.* (2011) 51:891–904. doi: 10.1111/j.1526-4610.2011.01903.x
11. Vetvik KG, MacGregor EA. Sex differences in the epidemiology, clinical features, and pathophysiology of migraine. *Lancet Neurol.* (2017) 16:76–87. doi: 10.1016/S1474-4422(16)30293-9
12. Puledda F, Messina R, Goadsby PJ. An update on migraine: current understanding and future directions. *J Neurol.* (2017) 264:2031–9. doi: 10.1007/s00415-017-8434-y
13. Jacobs B, Dussor G. Neurovascular contributions to migraine: Moving beyond vasodilation. *Neuroscience.* (2016) 338:130–44. doi: 10.1016/j.neuroscience.2016.06.012
14. Pavlovic JM, Akcali D, Bolay H, Bernstein C, Maleki N. Sex-related influences in migraine. *J Neurosci Res.* (2017) 95:587–93. doi: 10.1002/jnr.23903

15. Schroeder RA, Brandes J, Buse DC, Calhoun A, Eikermann-Haerter K, Golden K, et al. Sex and gender differences in migraine—evaluating knowledge gaps. *J Womens Health.* (2018) 27:965–73. doi: 10.1089/jwh.2018.7274
16. National Institute of Health. *Sex & Gender | Office of Research on Women's Health. National Institute of Health*. (2020). Available online at: https://orwh.od.nih.gov/sex-gender (accessed March 6, 2020).
17. World Health Organization. *Gender: Definitions*. (2020). Available online at: http://www.euro.who.int/en/health-topics/health-determinants/gender/gender-definitions (accessed March 27, 2020).
18. Johnson JL, Greaves L, Repta R. *Better Science with Sex and Gender: a Primer for Health Research*. Vancouver, BC: Women's Health Research Network (2007).
19. Moher D, Liberati A, Tetzlaff J, Altman DG, PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med.* (2009) 6:e1000097. doi: 10.1371/journal.pmed.1000097
20. Tonini MC. Gender differences in migraine. *Neurol Sci.* (2018) 39:77–8. doi: 10.1007/s10072-018-3378-2
21. Stewart WF, Wood C, Reed ML, Roy J, Lipton RB, AMPP Advisory Group. Cumulative lifetime migraine incidence in women and men. *Cephalalgia*. (2008). 28:1170–8. doi: 10.1111/j.1468-2982.2008.01666.x
22. Broner SW, Bobker S, Klebanoff L. Migraine in women. *Semin Neurol.* (2017) 37:601–10. doi: 10.1055/s-0037-1607393
23. Victor TW, Hu X, Campbell JC, Buse DC, Lipton RB. Migraine prevalence by age and sex in the United States: a life-span study. *Cephalalgia.* (2010) 30:1065–72. doi: 10.1177/0333102409355601
24. Rencz F, Brodszky V, Péntek M, Bereczki D, Gulácsi L. Health state utilities for migraine based on attack frequency: a time trade-off study. *Neurol Sci.* (2015) 36:197–202. doi: 10.1007/s10072-014-1920-4
25. Buse DC, Fanning KM, Reed ML, Murray S, Dumas PK, Adams AM, et al. Life with migraine: effects on relationships, career, and finances from the chronic migraine epidemiology and outcomes (CaMEO) study. *Headache.* (2019) 59:1286–99. doi: 10.1111/head.13613
26. Griep RH, Toivanen S, Santos IS, Rotenberg L, Juvanhol LL, Goulart AC, et al. Work-family conflict, lack of time for personal care and leisure, and job strain in migraine: results of the Brazilian longitudinal study of adult health (ELSA-Brasil). *Am J Ind Med.* (2016) 59:987–1000. doi: 10.1002/ajim.22620
27. Hammond NG, Stinchcombe A. Health behaviors and social determinants of migraine in a canadian population-based sample of adults aged 45-85 years: findings from the CLSA. *Headache.* (2019) 59:1547–64. doi: 10.1111/head.13610
28. Jiménez-Trujillo I, López-de-Andrés A, Del Barrio JL, Hernández-Barrera V, Valero-de-Bernabé M, Jiménez-García R. Gender differences in the prevalence and characteristics of pain in spain: report from a population-based study. *Pain Med.* (2019) 20:2349–59. doi: 10.1093/pm/pnz004
29. Lebedeva ER, Kobzeva NR, Gilev DV, Olesen J. Factors associated with primary headache according to diagnosis, sex, and social group. *Headache.* (2016) 56:341–56. doi: 10.1111/head.12757
30. Scher AI, Wang SJ, Katsarava Z, Buse DC, Fanning KM, Adams AM, et al. Epidemiology of migraine in men: results from the chronic migraine epidemiology and outcomes (CaMEO) study. *Cephalalgia.* (2019) 39:296–305. doi: 10.1177/0333102418786266
31. Bille B. A 40-year follow-up of school children with migraine. *Cephalalgia.* (1997) 17:488–91, 487. doi: 10.1046/j.1468-2982.1997.1704488.x
32. Pavlović JM. Evaluation and management of migraine in midlife women. *Menopause.* (2018) 25:927–9. doi: 10.1097/GME.0000000000001104
33. Lauritzen M. Pathophysiology of the migraine aura. The spreading depression theory. *Brain.* (1994) 117:199–210. doi: 10.1093/brain/117.1.199
34. Smith JM, Bradley DP, James MF, Huang CL-H. Physiological studies of cortical spreading depression. *Biol Rev Camb Philos Soc.* (2006) 81:457–81. doi: 10.1111/j.1469-185X.2006.tb00214.x
35. Brennan KC, Romero Reyes M, López Valdés HE, Arnold AP, Charles AC. Reduced threshold for cortical spreading depression in female mice. *Ann Neurol.* (2007) 61:603–6. doi: 10.1002/ana.21138
36. Bolay H, Moskowitz MA. The emerging importance of cortical spreading depression in migraine headache. *Revue Neurologique.* (2005) 161:655–7. doi: 10.1016/S0035-3787(05)85108-2
37. Hamel E. Serotonin and migraine: biology and clinical implications. *Cephalalgia.* (2007) 27:1293–300. doi: 10.1111/j.1468-2982.2007.01476.x
38. Chauvel V, Multon S, Schoenen J. Estrogen-dependent effects of 5-hydroxytryptophan on cortical spreading depression in rat: modelling the serotonin-ovarian hormone interaction in migraine aura. *Cephalalgia.* (2018) 38:427–36. doi: 10.1177/0333102417690891
39. de Fusco M, Marconi R, Silvestri L, Atorino L, Rampoldi L, Morgante L, et al. Haploinsufficiency of ATP1A2 encoding the Na+/K+ pump α2 subunit associated with familial hemiplegic migraine type 2. *Nat Genet.* (2003) 33:192–6. doi: 10.1038/ng1081
40. Gritz SM, Radcliffe RA. Genetic effects of ATP1A2 in familial hemiplegic migraine type II and animal models. *Hum Genomics.* (2013) 7:8. doi: 10.1186/1479-7364-7-8
41. Bøttger P, Glerup S, Gesslein B, Illarionova NB, Isaksen TJ, Heuck A, et al. Glutamate-system defects behind psychiatric manifestations in a familial hemiplegic migraine type 2 disease-mutation mouse model. *Sci Rep.* (2016) 6:22047. doi: 10.1038/srep22047
42. Kros L, Lykke-Hartmann K, Khodakhah K. Increased susceptibility to cortical spreading depression and epileptiform activity in a mouse model for FHM2. *Sci Rep.* (2018) 8:16959. doi: 10.1038/s41598-018-35285-8
43. Dehghani A, Karatas H. Mouse models of familial hemiplegic migraine for studying migraine pathophysiology. *Curr Neuropharmacol.* (2019) 17:961–73. doi: 10.2174/1570159X17666190513085013
44. Liverman CS, Brown JW, Sandhir R, Klein RM, McCarson K, Berman NEJ. Oestrogen increases nociception through ERK activation in the trigeminal ganglion: evidence for a peripheral mechanism of allodynia. *Cephalalgia.* (2009) 29:520–31. doi: 10.1111/j.1468-2982.2008.01755.x
45. Vermeer LMM, Gregory E, Winter MK, McCarson KE, Berman NEJ. Behavioral effects and mechanisms of migraine pathogenesis following estradiol exposure in a multibehavioral model of migraine in rat. *Exp Neurol.* (2015) 263:8–16. doi: 10.1016/j.expneurol.2014.09.011
46. Guo JQ, Deng HH, Bo X, Yang XS. Involvement of BDNF/TrkB and ERK/CREB axes in nitroglycerin-induced rat migraine and effects of estrogen on these signals in the migraine. *Biol Open.* (2017) 6:8–16. doi: 10.1242/bio.021022
47. Eftekhari S, Warfvinge K, Blixt FW, Edvinsson L. Differentiation of nerve fibers storing CGRP and CGRP receptors in the peripheral trigeminovascular system. *J Pain.* (2013) 14:1289–303. doi: 10.1016/j.jpain.2013.03.010
48. Edvinsson L. Role of cgrp in migraine. *Handb Exp Pharmacol.* (2019) 255:121–30. doi: 10.1007/164_2018_201
49. Avona A, Burgos-Ega C, Burton MD, Akopian AN, Price TJ, Dussor G. Dural calcitonin gene-related peptide produces female-specific responses in rodent migraine models. *J Neurosci.* (2019) 39:4323–31. doi: 10.1523/JNEUROSCI.0364-19.2019
50. Waeber C, Moskowitz MA. Migraine as an inflammatory disorder. *Neurology.* (2005) 64(10 Suppl. 2):S9–15. doi: 10.1212/WNL.64.10_suppl_2.S9
51. Perini F, D'Andrea G, Galloni E, Pignatelli F, Billo G, Alba S, et al. Plasma cytokine levels in migraineurs and controls. *Headache.* (2005) 45:926–31. doi: 10.1111/j.1526-4610.2005.05135.x
52. Fidan I, Yüksel S, Ymir T, Irkeç C, Aksakal FN. The importance of cytokines, chemokines and nitric oxide in pathophysiology of migraine. *J Neuroimmunol.* (2006) 171:184–8. doi: 10.1016/j.jneuroim.2005.10.005
53. McIlvried LA, Borghesi LA, Gold MS. Sex-, stress-, and sympathetic post-ganglionic neuron-dependent changes in the expression of pro- and anti-inflammatory mediators in rat dural immune cells. *Headache.* (2015) 55:943–57. doi: 10.1111/head.12596
54. Loewendorf AI, Matynia A, Saribekyan H, Gross N, Csete M, Harrington M. Roads less traveled: Sexual dimorphism and mast cell contributions to migraine pathology. *Front Immunol.* (2016) 7:140. doi: 10.3389/fimmu.2016.00140
55. Clapham DE. TRP channels as cellular sensors. *Nature.* (2003) 426:517–24. doi: 10.1038/nature02196

56. Artero-Morales M, Gonzalez-Rodriguez S, Ferrer-Montiel A. TRP channels as potential targets for sex-related differences in migraine pain. *Front mol biosci.* (2018) 5:73. doi: 10.3389/fmolb.2018.00073
57. Karkhaneh A, Ansari M, Emamgholipour S, Rafiee MH. The effect of 17β-estradiol on gene expression of calcitonin gene-related peptide and some pro-inflammatory mediators in peripheral blood mononuclear cells from patients with pure menstrual migraine. *Iran J Basic Med Sci.* (2015) 18:894–901.
58. Labastida-Ramírez A, Rubio-Beltrán E, Villalón CM, Maassen Van Den Brink A. Gender aspects of CGRP in migraine. *Cephalalgia.* (2019) 39:435–44. doi: 10.1177/0333102417739584
59. Ibrahimi K, Vermeersch S, Frederiks P, Geldhof V, Draulans C, Buntinx L, et al. The influence of migraine and female hormones on capsaicin-induced dermal blood flow. *Cephalalgia.* (2017) 37:1164–72. doi: 10.1177/0333102416668659
60. Ibrahimi K, van Oosterhout WP, van Dorp W, Danser AH, Garrelds IM, Kushner SA, et al. Reduced trigeminovascular cyclicity in patients with menstrually related migraine. *Neurology.* (2015) 84:125–31. doi: 10.1212/WNL.0000000000001142
61. Johannes CB, Linet MS, Stewart WF, Celentano DD, Lipton RB, Szklo M. Relationship of headache to phase of the menstrual cycle among young women: a daily diary study. *Neurology.* (1995) 45:1076–82. doi: 10.1212/WNL.45.6.1076
62. Stewart WF, Lipton RB, Chee E, Sawyer J, Silberstein SD. Menstrual cycle and headache in a population sample of migraineurs. *Neurology.* (2000) 55:1517–23. doi: 10.1212/WNL.55.10.1517
63. MacGregor EA, Hackshaw A. Prevalence of migraine on each day of the natural menstrual cycle. *Neurology.* (2004) 63:351–3. doi: 10.1212/01.WNL.0000133134.68143.2E
64. Kibler JL, Rhudy JL, Penzien DB, Rains JC, Meeks GR, Bennett W, et al. Hormones, menstrual distress, and migraine across the phases of the menstrual cycle. *Headache.* (2005) 45:1181–9. doi: 10.1111/j.1526-4610.2005.00241.x
65. Pavlović JM, Allshouse AA, Santoro NF, Crawford SL, Thurston RC, Neal-Perry GS, et al. Sex hormones in women with and without migraine. *Neurology.* (2016) 87:49–56. doi: 10.1212/WNL.0000000000002798
66. van Oosterhout WPJ, Schoonman GG, van Zwet EW, Dekkers OM, Terwindt GM, Maassen van Den Brink A, et al. Female sex hormones in men with migraine. *Neurology.* (2018) 91:e374–81. doi: 10.1212/WNL.0000000000005855
67. Shields LBE, Seifert T, Shelton BJ, Plato BM. Testosterone levels in men with chronic migraine. *Neurol Int.* (2019) 11:8079. doi: 10.4081/ni.2019.8079
68. Li W, Diao X, Chen C, Li C, Zhang Y, Li Y. Changes in hormones of the hypothalamic-pituitary-gonadal axis in migraine patients. *J Clin Neurosci.* (2018) 50:165–71. doi: 10.1016/j.jocn.2017.11.011
69. Dzugan SA, Dzugan KS. Is migraine a consequence of a loss of neurohormonal and metabolic integrity? A new hypothesis. *Neuro Endocrinol Lett.* (2015) 36:421–9.
70. Delaruelle Z, Ivanova TA, Khan S, Negro A, Ornello R, Raffaelli B, et al. Male and female sex hormones in primary headaches. *J Headache Pain.* (2018) 19:117. doi: 10.1186/s10194-018-0922-7
71. Maleki N, Androulakis XM. Is there any MRI pattern that discriminates female from male migraine patients? *Front Neurol.* (2019) 10:961. doi: 10.3389/fneur.2019.00961
72. Allais G, Chiarle G, Sinigaglia S, Airola G, Schiapparelli P, Benedetto C. Estrogen, migraine, and vascular risk. *Neurol Sci.* (2018) 39:11–20. doi: 10.1007/s10072-018-3333-2
73. Todd C, Lagman-Bartolome AM, Lay C. Women and migraine: the role of hormones. *Curr Neurol Neurosci Rep.* (2018) 18:42. doi: 10.1007/s11910-018-0845-3
74. Pakalnis A. Migraine and hormones. *Semin Pediatr Neurol.* (2016) 23:92–4. doi: 10.1016/j.spen.2016.01.005
75. Hipolito Rodrigues MA, Maitrot-Mantelet L, Plu-Bureau G, Gompel A. Migraine, hormones and the menopausal transition. *Climacteric.* (2018) 21:256–66. doi: 10.1080/13697137.2018.1439914
76. Maleki N, Kurth T, Field AE. Age at menarche and risk of developing migraine or non-migraine headaches by young adulthood: a prospective cohort study. *Cephalalgia.* (2017) 37:1257–63. doi: 10.1177/0333102416677999
77. Pavlović JM, Stewart WF, Bruce CA, Gorman JA, Sun H, Buse DC, et al. Burden of migraine related to menses: results from the AMPP study. *J Headache Pain.* (2015) 16:24. doi: 10.1186/s10194-015-0503-y
78. Pinkerman B, Holroyd K. Menstrual and nonmenstrual migraines differ in women with menstrually-related migraine. *Cephalalgia.* (2010) 30:1187–94. doi: 10.1177/0333102409359315
79. Granella F, Sances G, Allais G, Nappi RE, Tirelli A, Benedetto C, et al. Characteristics of menstrual and nonmenstrual attacks in women with menstrually related migraine referred to headache centres. *Cephalalgia.* (2004) 24:707–16. doi: 10.1111/j.1468-2982.2004.00741.x
80. MacGregor EA, Frith A, Ellis J, Aspinall L, Hackshaw A. Incidence of migraine relative to menstrual cycle phases of rising and falling estrogen. *Neurology.* (2006) 67:2154–8. doi: 10.1212/01.wnl.0000233888.18228.19
81. MacGregor EA, Victor TW, Hu X, Xiang Q, Puenpatom RA, Chen W, et al. Characteristics of menstrual vs nonmenstrual migraine: a post hoc, within-woman analysis of the usual-care phase of a nonrandomized menstrual migraine clinical trial. *Headache.* (2010) 50:528–38. doi: 10.1111/j.1526-4610.2010.01625.x
82. Couturier EGM, Bomhof MAM, Neven AK, van Duijn NP. Menstrual migraine in a representative Dutch population sample: prevalence, disability and treatment. *Cephalalgia.* (2003) 23:302–8. doi: 10.1046/j.1468-2982.2003.00516.x
83. Güven B, Güven H, Çomoglu S. Clinical characteristics of menstrually related and non-menstrual migraine. *Acta Neurol Belg.* (2017) 117:671–6. doi: 10.1007/s13760-017-0802-y
84. Taha MA, Mohammed EA. Assessment of hormonal changes in female patients with migraine. *Indian J Public Health Res Dev.* (2019) 10:568–73. doi: 10.5958/0976-5506.2019.01335.4
85. Vetvik KG, Benth JS, MacGregor EA, Lundqvist C, Russell MB. Menstrual versus non-menstrual attacks of migraine without aura in women with and without menstrual migraine. *Cephalalgia.* (2015) 35:1261–8. doi: 10.1177/0333102415575723
86. Spierings ELH, Padamsee A. Menstrual-cycle and menstruation disorders in episodic vs chronic migraine: an exploratory study. *Pain Med.* (2015) 16:1426–32. doi: 10.1111/pme.12788
87. Maitrot-Mantelet L, Hugon-Rodin J, Vatel M, Marcellin L, Santulli P, Chapron C, et al. Migraine in relation with endometriosis phenotypes: results from a French case-control study. *Cephalalgia.* (2019) 40:606–13. doi: 10.1177/0333102419893965
88. Karamustafaoglu Balci B, Kabakci Z, Guzey DY, Avci B, Guler M, Attar E. Association between endometriosis, headache, and migraine. *J Endometriosis Pelvic Pain Disord.* (2019) 11:19–24. doi: 10.1177/2284026518818975
89. Petrovski BÉ, Vetvik KG, Lundqvist C, Eberhard-Gran M. Characteristics of menstrual versus non-menstrual migraine during pregnancy: a longitudinal population-based study. *J Headache Pain.* (2018) 19:27. doi: 10.1186/s10194-018-0853-3
90. Børte S, Winsvold BS, Stensland SO, Småstuen MC, Zwart JA. The effect of foetal growth restriction on the development of migraine and tension-type headache in adulthood. The HUNT study. *PLoS ONE.* (2017) 12:e0175908. doi: 10.1371/journal.pone.0175908
91. Afridi SK. Current concepts in migraine and their relevance to pregnancy. *Obstetric Med.* (2018) 11:154–9. doi: 10.1177/1753495X18769170
92. Allais G, Chiarle G, Sinigaglia S, Mana O, Benedetto C. Migraine during pregnancy and in the puerperium. *Neurol Sci.* (2019) 40(Suppl. 1):81–91. doi: 10.1007/s10072-019-03792-9
93. Parikh SK. Unique populations with episodic migraine: pregnant and lactating women. *Curr Pain Headache Rep.* (2018) 22:80. doi: 10.1007/s11916-018-0737-x
94. Martin VT, Pavlovic J, Fanning KM, Buse DC, Reed ML, Lipton RB. Perimenopause and menopause are associated with high frequency headache in women with migraine: results of the American migraine prevalence and prevention study. *Headache.* (2016) 56:292–305. doi: 10.1111/head.12763
95. Ripa P, Ornello R, Degan D, Tiseo C, Stewart J, Pistoia F, et al. Migraine in menopausal women: a systematic review. *Int J Womens Health.* (2015) 7:773–82. doi: 10.2147/IJWH.S70073

96. Tayyebi A, Poursadeghfard M, Nazeri M, Pousadeghfard T. Is there any correlation between migraine attacks and iron deficiency anemia? A case-control study. *Int J Hematol Oncol Stem Cell Res.* (2019) 13:164–71. doi: 10.18502/ijhoscr.v13i3.1276
97. Tepper SJ, Lowe MJ, Beall E, Phillips MD, Liu K, Stillman MJ, et al. Iron deposition in pain-regulatory nuclei in episodic migraine and chronic daily headache by MRI. *Headache.* (2012) 52:236–43. doi: 10.1111/j.1526-4610.2011.02056.x
98. Welch KMA. Iron in the migraine brain; a resilient hypothesis. *Cephalalgia.* (2009) 29:283–5. doi: 10.1111/j.1468-2982.2008.01725.x
99. Kruit MC, Launer LJ, Overbosch J, van Buchem MA, Ferrari MD. Iron accumulation in deep brain nuclei in migraine: a population-based magnetic resonance imaging study. *Cephalalgia.* (2009) 29:351–9. doi: 10.1111/j.1468-2982.2008.01723.x
100. Welch KM, Nagesh V, Aurora SK, Gelman N. Periaqueductal gray matter dysfunction in migraine: cause or the burden of illness? *Headache.* (2001) 41:629–37. doi: 10.1046/j.1526-4610.2001.041007629.x
101. Guo Y, Xu S, Nie S, Han M, Zhang Y, Chen J, et al. Female versus male migraine: an event-related potential study of visual neurocognitive processing. *J Headache Pain.* (2019) 20:38. doi: 10.1186/s10194-019-0995-y
102. Lipton RB, Munjal S, Alam A, Buse DC, Fanning KM, Reed ML, et al. Migraine in America symptoms and treatment (MAST) study: baseline study methods, treatment patterns, and gender differences. *Headache.* (2018) 58:1408–26. doi: 10.1111/head.13407
103. Bolay H, Ozge A, Saginc P, Orekici G, Uludüz D, Yalln O, et al. Gender influences headache characteristics with increasing age in migraine patients. *Cephalalgia.* (2015) 35:792–800. doi: 10.1177/0333102414559735
104. Slatculescu AM, Chen Y. Synergism beten female gender and high levels of daily stress associated with migraine headacwehes in Ontario, Canada. *Neuroepidemiology.* (2018) 51:183–9. doi: 10.1159/000492503
105. Pogoda JM, Gross NB, Arakaki X, Fonteh AN, Cowan RP, Harrington MG. Severe headache or migraine history is inversely correlated with dietary sodium intake: NHANES 1999-2004. *Headache.* (2016) 56:688–98. doi: 10.1111/head.12792
106. Kaur S, Ali A, Ahmad U, Pandey AK, Singh B. rs2651899 variant is associated with risk for migraine without aura from North Indian population. *Mol Biol Rep.* (2019) 46:1247–55. doi: 10.1007/s11033-019-04593-1
107. Pelzer N, Louter MA, van Zwet EW, Nyholt DR, Ferrari MD, van den Maagdenberg AMJM, et al. Linking migraine frequency with family history of migraine. *Cephalalgia.* (2019) 39:229–36. doi: 10.1177/0333102418783295
108. Nyholt DR, Anttila V, Winsvold BS, Kurth T, Stefansson H, Kallela M, et al. Concordance of genetic risk across migraine subgroups: impact on current and future genetic association studies. *Cephalalgia.* (2015) 35:489–99. doi: 10.1177/0333102414547784
109. Palmirotta R, Barbanti P, Ialongo C, de Marchis ML, Alessandroni J, Egeo G, et al. Progesterone receptor gene (PROGINS) polymorphism correlates with late onset of migraine. *DNA Cell Biol.* (2015) 34:208–12. doi: 10.1089/dna.2014.2534
110. Sazci A, Sazci G, Sazci B, Ergul E, Idrisoglu HA. Nicotinamide-N-Methyltransferase gene rs694539 variant and migraine risk. *J Headache Pain.* (2016) 17:93. doi: 10.1186/s10194-016-0688-8
111. Kaur S, Ali A, Siahbalaei Y, Ahmad U, Nargis F, Pandey AK, et al. Association of Diamine oxidase (DAO) variants with the risk for migraine from North Indian population. *Meta Gene.* (2019) 24:100619. doi: 10.1016/j.mgene.2019.100619
112. García-Martín E, Martínez C, Serrador M, Alonso-Navarro H, Ayuso P, Navacerrada F, et al. Diamine oxidase rs10156191 and rs2052129 variants are associated with the risk for migraine. *Headache.* (2015) 55:276–86. doi: 10.1111/head.12493
113. Fang J, Yuan X, An X, Qu H, Wang C, Hong G, et al. Involvement of the tetraspanin 2 (TSPAN2) gene in migraine: a case-control study in Han Chinese. *Front Neurol.* (2018) 9:714. doi: 10.3389/fneur.2018.00714
114. CoSkun S, Yucel Y, Çim A, Cengiz B, Oztuzcu S, Varol S, et al. Contribution of polymorphisms in ESR1, ESR2, FSHR, CYP19A1, SHBG, and NRIP1 genes to migraine susceptibility in Turkish population. *J Genet.* (2016) 95:131–40. doi: 10.1007/s12041-016-0625-2
115. Li L, Liu R, Dong Z, Wang X, Yu S. Impact of ESR1 gene polymorphisms on migraine susceptibility: a meta-analysis. *Medicine.* (2015) 94:e0976. doi: 10.1097/MD.0000000000000976
116. Pollock CE, Sutherland HG, Maher BH, Lea RA, Haupt LM, Frith A, et al. The NRP1 migraine risk variant shows evidence of association with menstrual migraine. *J Headache Pain.* (2018) 19:31. doi: 10.1186/s10194-018-0857-z
117. Tietjen GE, Khubchandani J. Vascular biomarkers in migraine. *Cephalalgia.* (2015) 35:95–117. doi: 10.1177/0333102414544976
118. Chong CD, Schwedt TJ, Dodick DW. Migraine: what imaging reveals. *Curr Neurol Neurosci Rep.* (2016) 16:64. doi: 10.1007/s11910-016-0662-5
119. Dai Z, Zhong J, Xiao P, Zhu Y, Chen F, Pan P, et al. Gray matter correlates of migraine and gender effect: a meta-analysis of voxel-based morphometry studies. *Neuroscience.* (2015) 299:88–96. doi: 10.1016/j.neuroscience.2015.04.066
120. Brusa P, Allais G, Rolando S, Baratta F, Giaccone M, Bussone G, et al. Migraine attacks in the pharmacy: a gender subanalysis on treatment preferences. *Neurol Sci.* (2015) 36:93–5. doi: 10.1007/s10072-015-2156-7
121. Robbins NM, Bernat JL. Minority representation in migraine treatment trials. *Headache.* (2017) 57:525–33. doi: 10.1111/head.13018
122. Pringsheim T, Gooren L. Migraine prevalence in male to female transsexuals on hormone therapy. *Neurology.* (2004) 63:593–4. doi: 10.1212/01.WNL.0000130338.62037.CC
123. Aloisi AM, Bachiocco V, Costantino A, Stefani R, Ceccarelli I, Bertaccini A, et al. Cross-sex hormone administration changes pain in transsexual women and men. *Pain.* (2007) 132(Suppl. 1):S60–7. doi: 10.1016/j.pain.2007.02.006
124. Shansky RM. Are hormones a "female problem" for animal research? *Science.* (2019) 364:825–6. doi: 10.1126/science.aaw7570

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Sex Differences in the Patterns and Predictors of Cognitive Function in HIV

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Despite advancements in antiretroviral therapy, mild cognitive deficits persist in nearly half of people with HIV (PWH). The profile of impairment in HIV is highly variable with deficits observed in a range of cognitive domains. Despite evidence of greater cognitive impairment among women with HIV (WWH) vs. men with HIV (MWH), it is unclear how MWH and WWH differ in the type of cognitive impairment and in risk factors associated with cognitive impairment profiles. In a large and well-characterized sample of PWH, we used machine learning to identify profiles of cognitive functioning and their associated factors overall and within sex. Participants included 1,666 PWH (201 WWH; 1,465 MMH) from the HIV Neurobehavioral Research Program who completed a neuropsychological test battery at their baseline visits. Using demographically-adjusted T-scores from 13 test outcomes assessing motor skills, executive functioning, attention/working memory, episodic learning and memory, verbal fluency, and processing speed, we used Kohonen self-organizing maps to identify patterns of high-dimensional data by mapping participants to similar nodes based on T-scores (MCLUST R package). Random forest models were used to determine how sociodemographic (e.g., age, education), clinical (e.g., depressive symptoms, substance use disorder), and biological (e.g., HIV disease characteristics) factors differentially related to membership within a cognitive profile. All analyses were repeated within sex. Three cognitive profiles were identified overall and within each sex. Overall and within MWH, there were unimpaired and global weakness profiles. The third profile in the total sample demonstrated relatively weak auditory attention whereas in MWH showed relative strengths in attention and processing speed. Conversely, there was no unimpaired profile among WWH. Rather, WWH demonstrated separate profiles reflecting weakness in motor skills, a relative weakness in learning and delayed recall, and global weaknesses with spared recognition memory. Despite different cognitive profiles by sex, the most discriminative factors were similar between men and women and included reading level (cognitive reserve), current and nadir CD4 count, plasma HIV viral load, duration of HIV disease, age, depressive symptoms, and race/ethnicity. Findings fill a knowledge gap concerning sex differences in cognitive impairment in PWH and inform personalized risk reduction and therapeutic strategies.

Keywords: HIV, sex differences, cognition, women, neuropsychology, cognitive profiles

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INTRODUCTION

The Human Immunodeficiency Virus (HIV) enters the central nervous system (CNS) within days of initial infection (1), in many cases leading to neurological, cognitive, and behavioral complications. Cognitive deficits are a common feature of HIV/AIDS. While the incidence of HIV-associated dementia has considerably decreased in the era of modern ART suppressing viral replication, mild cognitive deficits with no change in everyday function persist in 24% [95% confidence interval (CI) = 20.3–26.8] of people with HIV (PWH) and mild cognitive deficits with mildly decreased everyday function persist in about 13.3% (95% CI = 10.6–16.3) of PWH (2). Although executive function and memory deficits are most common in PWH in the post-ART era, the characterization of cognitive impairment in HIV is highly variable with deficits observed in a range of cognitive domains (3). Previous studies using statistical clustering techniques have identified differing profiles of cognitive function among PWH with some profiles resembling global impairment across domains while other profiles resemble more domain-specific impairment, particularly in the domains of episodic memory and executive function (4–7). Similarly, there is also substantial variability in the risk factors associated with cognitive deficits among PWH that range from biological (e.g., $CD4^+$ T-cell count, HIV viral load, comorbid health conditions), demographic (e.g., age, sex, race/ethnicity) to psychosocial factors (e.g., low education, depression, substance use/dependence). The persistence of cognitive impairment in the era of modern ART among PWH and the variability in the profiles and risk factors associated with cognitive impairment suggests that non-HIV factors associated with aging, comorbid conditions (e.g., cardiovascular disease) and psychosocial risk factors (e.g., poverty, poor education) likely contribute to cognitive impairment given the high prevalence of these factors among PWH (8, 9). With this in mind, we propose looking beyond the construct of HIV-associated neurocognitive disorders (HAND) to identify the underlying pathophysiology linked to cognitive impairment as HAND requires other comorbidities to be ruled out as primary contributing factors.

Biological sex is an important determinant of cognitive impairment among PWH. In a recent literature review of sex differences in cognitive impairment among PWH (10), seven cross-sectional (11–17) and one longitudinal analysis (18) identified sex differences on global measures of cognitive impairment among PWH. Additionally, six cross-sectional (13–15, 17, 19, 20) and one longitudinal analysis (21) also reported sex differences in domain-specific cognitive performance. The strongest available evidence of adequately-powered studies indicates that WWH show greater deficits than MWH in the domains of learning and memory followed by speed of information processing and motor functioning, with inconsistent findings in executive functioning (17, 21).

The greater vulnerability of WWH to cognitive impairment may reflect sociodemographic differences between men and women with HIV. WWH tend to have a higher prevalence of psychosocial risk factors including poverty, low literacy levels, low educational attainment, substance abuse, poor mental health, and barriers to health care services (10, 22) as compared to MWH. These psychosocial risk factors may have biological effects on the brain that lead to reduced cognitive reserve among WWH (23, 24) as evidenced by findings of greater susceptibility of cognitive function to the effects of mental health factors (e.g., depression) among WWH vs. MWH (25). Additionally, biological factors such as sex steroid hormones (e.g., estrogen, testosterone) and female-specific hormonal milieus (e.g., pregnancy, menstrual cycle, menopause transition) may contribute to sex differences in cognitive test performance in PWH. However, it remains unclear how MWH and WWH may differ in the patterns of cognitive impairment and risk factors associated with these patterns of cognitive impairment. Previous reports of impairment profiles among PWH have identified them in combined samples of men and women (4–7), masking possible sex-specific patterns of cognitive impairment among PWH. Furthermore, although a number of studies reported sex differences in the presence and pattern of cognitive impairment (14, 16, 17) and greater cognitive decline compared to MWH (18), only one study (17) was adequately powered to address meaningful sex difference in global cognitive function (10). A well-powered examination of the patterns and determinants of cognitive impairment by sex, that also controls for other demographic differences between WWH and MWH (e.g., age, education, race/ethnicity), can help to clarify the contribution of sex to heterogeneity in cognitive impairment among PWH. Such an examination could also clarify the related psychosocial vs. biological factors and, thereby, optimize risk assessments and intervention strategies in both sexes.

Leveraging comprehensive neuropsychological (NP) data from the large-scale cohort of the HIV Neurobehavioral Research Program (HNRP) at the University of California-San Diego, we used novel machine learning methods to identify differing profiles of cognitive function in PWH and to evaluate how these profiles differ between women and men in sex-stratified analyses. Rather, than using traditional cognitive domain scores, we used each of the NP test outcomes given that prior studies indicate that the correlation of NP test scores does not map to traditional domain scores in PWH. Furthermore, we determined how sociodemographic (e.g., age, education, race/ethnicity), clinical (e.g., functional status, depression, substance use disorders) and biological (e.g., measures of HIV disease severity, ART use, cardiovascular comorbid conditions, Hepatitis C co-infection) factors related to cognitive profiles within women and men. Based on previous studies among PWH (4–6), we hypothesized that the machine learning approach would identify distinct subgroups of individuals with normal cognitive function, global cognitive impairment, and domain-specific cognitive impairment. We further hypothesized that groups with domain-specific cognitive impairment would differ by sex, with WWH showing more consistent memory and processing speed impairment than MWH. Finally, we expected that similar sociodemographic/clinical/biological determinants would distinguish cognitive profiles (e.g., age, education, race, HIV viral load) among WWH and MWH; however, in line with previous research (25), we expected that depressive symptoms would be more strongly

associated with cognitive impairment profiles among WWH than MWH.

MATERIALS AND METHODS

Participants

Participants included 1,666 PWH (201 WWH; 1,465 MWH) enrolled in various NIH-funded research studies at the University of California, San Diego's HNRP, https://hnrp.hivresearch.ucsd.edu/. Study assessment details have been published elsewhere (3). The UCSD Institutional Review Board approved the studies. Participants provided written informed consent and were compensated for their participation. Exclusion criteria for the parent studies included history of non-HIV-related neurological, medical, or psychiatric disorders that affect brain functioning (e.g., seizure, stroke, psychosis), learning disabilities, and a first language that was not English. Inclusion in the current study required completion of neuropsychological and neuromedical evaluations at the baseline study visit. Exclusion criteria for the current study included a positive urine toxicology test for illicit drugs (excluding marijuana) or Breathalyzer test for alcohol on the day of clinic visit on the day of study visit.

NP Test Evaluation

NP test performance was assessed through a comprehensive, standardized, battery of tests that measure seven domains of cognition, including complex motor skills, executive function, attention/working memory, episodic learning, episodic memory (delayed recall and recognition), verbal fluency, and information processing speed. Motor skills were assessed by the Grooved Pegboard (GPEG) Dominant and Non-dominant Hand tests (26). Executive functioning was assessed by the Trail Making Test (TMT)-Part B (27) and the Stroop Color and Word Test interference score (28). Attention/working memory was assessed by the Paced Auditory Serial Addition Task (PASAT-50) (29, 30). Episodic learning was assessed by the Total Learning scores of the Hopkins Verbal Learning Test-Revised (HVLT-R) (31) and the Brief Visuospatial Memory Test-Revised (BVMT-R) (32). Episodic memory was assessed by the Delayed Recall and Recognition scores of the HVLT-R and BVMT-R. Verbal Fluency was assessed by the "FAS" Letter Fluency test (33). Information processing speed was assessed by the WAIS-III Digit Symbol Test (34), the TMT-Part A, and the Stroop Color and Word Test color naming score. Raw test scores were transformed into age-, education-, sex-, and race/ethnicity-adjusted T-scores based on normative samples of HIV-uninfected persons (35, 36). The use of demographically-adjusted T-scores are intended to control for these demographic effects as they occur in the general population.

Factors Associated With NP Profiles

We examined sociodemographic, clinical, and biological factors associated with cognitive impairment in the literature and available with enough participants to be adequately powered in analyses. Sociodemographic factors included age, years of education, and race/ethnicity. Although these factors were used to create the T-scores, there can still be remaining demographic associations with cognition within clinical populations such as PWH. For example, there is considerable interest in the possibility of abnormal cognitive aging PWH; also, in general, older PWH tend to have had their infections longer, may have had longer periods without benefit of suppressive ART, and more history of worse immunosuppression. Clinical factors included functional status as indicated by the number of daily activities with decreased independence from the Instrumental Activities of Daily Living questionnaire (IADL) from the modified version of the Lawton and Brody Activities of Daily Living Questionnaire (37), reading level (a proxy for cognitive reserve) based on the Wide Range Achievement Test-4 Reading subtest (WRAT-4 Reading) (38), self-reported depressive symptoms on the Beck Depression Inventory versions I (BDI-I) or II (BDI-II) (39), and diagnosis of lifetime and current major depressive disorder (MDD) as well as lifetime alcohol, cannabis, or other (i.e., amphetamine, cocaine, hallucinogen, inhalant, sedative, opioid, and PCP) substance use disorder based on the Composite International Diagnostic Interview using DSM–IV criteria (CIDI version 2.1) (40). Biological factors included HIV disease variables such as current $CD4^+$ T-cell count, lowest $CD4^+$ T-cell count ever recorded (nadir CD4), plasma HIV viral load, estimated duration of HIV disease, current use of ART, current use of anticholinergic-based medications (e.g., urinary incontinence and chronic obstructive pulmonary disease medications), Hepatitis C co-infection, and the cardiovascular comorbid conditions of hypertension, hyperlipidemia, and diabetes.

Statistical Analyses

All 13 NP tests were used to find groups of similar cognitive profiles within each participant subset (MWH, WWH) and in the total sample using a pipeline that consisted of dimension reduction with Kohonen self-organizing maps (SOM) followed by clustering to identify profiles based on those reduced dimensions. SOM was implemented using the Kohonen package in R (41). SOM is an unsupervised machine learning technique used to identify patterns in high-dimensional data (numerous variables) by producing a two-dimensional representation consisting of multiple nodes where each node is a group of one or more individuals with similar cognitive profiles and the location of the nodes within the 2-D representation is also a metric of similarity. Unlike probabilistic models, each individual can only be assigned to one node. The SOM grid consisted of a 10×10 hexagonal grid of nodes and the number of clusters for the final profiling was selected by looping over models created from 3 to 20 clusters and selecting the number that had the best fit based on entropy. Similar nodes were then clustered (grouped together) using the MClust package (42). MClust is an R Software package used for model-based clustering using finite normal mixture modeling that provides functions for parameter estimation via the Expectation-Maximization algorithm with an assortment of covariance structures which vary in distribution (spherical, diagonal, or ellipsoidal), volumes (equal or variable), shape (equal of variable), and orientation (equal or variable, only for ellipsoidal distribution). This program identifies the best model based on entropy (a model fit statistic). Once the clustering of the nodes was completed, cluster profiles were assigned to the

individuals associated with that node. By using SOM and MClust in sequence, we were able to achieve fine-tuned clustering based on patterns of performance in cognitive testing.

Factors predicting profile membership between each impaired and unimpaired profile in the overall sample and within each group (MWH, WWH) were explored by creating a predictive Random Forest (RF) model using the Caret (43) package in R and then extracting variable importance (44). RF is an ensemble machine learning model based on classification trees that results in powerful prediction models based on non-linear combinations of subsets of input variables. Prior to model creation, the Synthetic Minority Over-sampling Technique (SMOTE) with the DMwR (45) package was used to control for bias due to any imbalance in the number of cases. RF models were created using internal validation using a 10-fold resampling method repeated 5 times. Pre-processing before RF creation involved removing variables as predictors if they had low variance or if they had >50% missing data. Any missing data in the remaining variables was imputed using the Multivariate Imputation by Chained Equations (46) (MICE) package in R using random forest imputations. ROC confidence intervals were calculated using the pROC package in R with 2,000 stratified bootstrap replicates (95% CI). Variable importance of all variables included in the RF models was used as the outcome metric of the predictive power of each variable. Variable importance is a scaled number [0–100] that indicates how important that variable is to the final predicted outcome in that model. For each tree in the RF model, the out-of-bag portion of the data is recorded and repeated after permuting each predictor variable. The difference between the accuracy with and without each variable is averaged over all trees and then normalized by the standard error. For visualization, all variables were plotted by relative variable importance, and attention was given to the top 10 variables in each profile. Variable importance indicates how much that variable contributes to overall prediction accuracy, but as RF is non-linear model it does not indicate directionality.

While the analysis pipeline and packages used along with the parameter inputs are stated above, we have added our code into a **Supplementary Material** to facilitate rigor and reproducibility.

RESULTS

Participants

Table 1 provides sociodemographic, behavioral, and clinical factors for 1,666 PWH (1,465 men; 201 women). On average, participants were 41.8 years of age [standard deviation (SD) = 9.8] with 13.3 years of education (SD = 2.7). Fifty-eight percent were White and 18% Black. Mental health comorbidities were common. Forty-eight percent had a lifetime, and 19% had a current, diagnosis of MDD. With respect to HIV-related clinical characteristics, 60% were on combination ART and 42% were virally suppressed. Compared to MWH, WWH were less educated, had lower WRAT-4 scores, were less likely to be white, and had a shorter duration of HIV disease (P's $<$ 0.05). Additionally, WWH reported more IADL dependence and were more likely to have HCV co-infection compared to MWH (P's $<$ 0.05).

Table 2 provides NP test performance for the total sample and by sex. In the total sample, average performance on BVMT-R delayed recall, HVLT-R (total learning, delayed recall, recognition), GPEG-non-dominant, and PASAT had T-scores <45 or 0.5 standard deviations from the general population mean (T-score of 50). WWH performed worse than MWH on BVMT-R total learning on average ($P = 0.01$). However, WWH performed better on the recognition measures of the BVMT-R and HVLT-R (P's < 0.01). This was also the case examining percent impairment using a T-score cutoff of 40 (P's < 0.001).

Identification of Cognitive Profiles in the Total Sample

Profiles where the mean T-score on all cognitive outcomes was >45 and <55 were considered an "unimpaired average" profile. To describe the profiles, tests where the average T-scores of all participants in that cluster were <45 were considered weaknesses, and those where the average was <40 were considered impaired. An average >55 was considered a relative strength in the context of other domains being in the average range (>45 and <55).

Profiling of the 1,666 PWH resulted in three total groups using an ellipsoidal multivariate mixture model with equal orientation with an entropy of 0.982 (**Figure 1A**).

- **Profile 1 ($n = 618$): *Unimpaired*** indicated by the average T-score for all NP outcomes falling into the normal/average range between 45 and 55.
- **Profile 2 ($n = 461$): *Relatively weak auditory attention and episodic memory*** indicated by weaknesses in HVLT-R (learning and delayed recall), Letter Fluency, and PASAT.
- **Profile 3 ($n = 587$): *Global weaknesses*** indicated by average T-scores in the impaired range on all BVMT-R outcomes, HVLT-R learning and delayed recall, GPEG-non-dominant, and PASAT as well as weaknesses on TMT-Part B, Letter Fluency, GPEG-dominant, and Digit Symbol.

Figure 2A provides the percent impairment on each task within each of the profiles. **Supplementary Table 1** also provides T-scores and percent impairment on each task within each of the profiles for reference.

Identification of Cognitive Profiles in MWH and WWH Separately

Profiling of the 1,465 MWH also resulted in three groups using an ellipsoidal multivariate mixture model with equal orientation with an entropy of 0.993 (**Figure 1B**).

- **Profile 1 ($n = 753$): *Unimpaired*** indicated by the average T-score for all NP outcomes falling into the normal range between 45 and 55.
- **Profile 2 ($n = 286$): *Unimpaired with relative strength in attention and processing speed*** indicated by relative strengths (T-scores above 55) on TMT-Part A&B and Digit Symbol compared to Profile 1. Similar to Profile 1, all other NP outcomes fell into the normal range between 45 and 55.
- **Profile 3 ($n = 426$): *Global weaknesses*** indicated by impairment on all BVMT-R, HVLT-R and GPEG outcomes

TABLE 1 | Demographic, behavioral, and clinical characteristics in the total sample of people with HIV and by sex.

		Sex		
	Total (***N* = 1,666**)	**Men** (***n* = 1,465**)	**Women** (***n* = 201**)	
	***n* (%)**	***n* (%)**	***n* (%)**	***P*-value**
Age, M (SD)	41.7 (9.8)	41.9 (9.8)	40.7 (9.6)	0.09
Years of education, M (SD)	13.3 (2.7)	13.5 (2.7)	12.0 (2.5)	<0.001
WRAT-4, M (SD)	99.1 (13.5)	99.8 (13.3)	93.5 (13.2)	<0.001
Race				<0.001
White	971 (58)	890 (61)	81 (40)	
Black	314 (19)	259 (18)	55 (28)	
Hispanic	299 (18)	246 (17)	53 (26)	
Other	82 (5)	70 (4)	12 (6)	
IADL dependence	2.5 (2.9)	2.4 (2.8)	2.9 (3.1)	0.03
BDI-II	13.5 (10.4)	13.5 (10.4)	13.5 (9.9)	0.99
DSM-IV (CIDI) diagnoses				
MDD				
Current	224 (19)	203 (19)	21 (18)	0.94
Lifetime	572 (48)	510 (48)	572 (48)	0.24
Alcohol (current or lifetime*)	72 (6)	66 (6)	6 (5)	0.84
Cannabis (current or lifetime*)	63 (5)	60 (6)	3 (3)	0.25
Substance use (current or lifetime*)	691 (73)	627 (73)	64 (71)	0.74
Anticholinergic medications	422 (25)	367 (25)	55 (27)	0.53
Hypertension	334 (21)	298 (21)	36 (18)	37
Hyperlipidemia	219 (14)	193 (14)	26 (13)	0.9
Diabetes	82 (5)	70 (5)	12 (6)	0.64
HCV	317 (20)	259 (18)	58 (29)	<0.001
Log plasma viral load, M (SD)	3.1 (1.3)	3.1 (1.3)	3.1 (1.2)	0.83
CD4 count, M (SD)				
Current	429.0 (296.9)	426.9 (295.8)	443.7 (305.3)	0.46
Nadir	229.9 (219.9)	250.4 (231.8)	232.4 (221.4)	0.21
Duration of HIV disease, M (SD)	9.1 (7.8)	9.3 (8.0)	7.0 (5.6)	0.005
AIDS diagnosis	969 (58)	854 (59)	115 (57)	0.07
On ART	966 (60)	857 (60)	109 (56)	0.3

*M, mean; SD, standard deviation; ART, antiretroviral therapy; WRAT-4, Wide Range Achievement Test; IADL, Instrumental Activities of Daily Living; BDI, Beck depression inventory; CIDI, Composite International Diagnostic Interview; HCV, Hepatitis C. *Current or Lifetime Use disorder (abuse or dependence).*

and weaknesses on TMT-Part A&B, Letter Fluency, and Digit Symbol.

Profiling of the 201 WWH also resulted in three groups using an ellipsoidal multivariate mixture model with equal orientation with an entropy of 0.989 (**Figure 1C**).

- **Profile 1 (*n* = 64): *Weakness in motor function*** indicated by the average T-score falling into the normal/average range (>45) or above average (>55) on all tests except for GPEG.
- **Profile 2 (*n* = 67): *Relative weaknesses in learning and memory*** indicated by weaknesses on learning and delayed recall on the BVMT-R and HVLT-R with the average T-scores for the other outcomes falling in the normal range (>45).
- **Profile 3 (*n* = 70): *Global weaknesses with spared verbal recognition*** indicated by average T-scores in the impaired range on learning and delayed recall on the BVMT-R and HVLT-R, GPEG, PASAT, and Digit Symbol, and weaknesses on BVMT-R recognition, TMT, and Letter Fluency. Notably, average recognition on the HVLT-R was in the normal range (M = 47.1, SD = 14.5).

Figure 2B provides the percent impairment on each task within each of the profiles within MWH and **Figure 2C** in WWH. **Supplementary Tables 2**, **3** also provide T-scores and percent impairment on each task within each of the profiles for reference.

TABLE 2 | Neuropsychological test performance in the total sample of people with HIV and by sex.

		Sex		
	Total	**Men**	**Women**	
	(*N* = 1,666)	**(*n* = 1,465)**	**(*n* = 201)**	***P*-value**
T-scores	**M (SD)**	**M (SD)**	**M (SD)**	
BVMT-R				
Total learning	45.4 (10.0)	45.6 (10.1)	43.84 (9.6)	0.01
Delayed recall	42.2 (11.4)	45.2 (11.5)	45.6 (11.1)	0.6
Recognition	45.8 (13.7)	45.5 (13.7)	48.3 (13.1)	0.006
HVLT-R				
Total learning	42.6 (11.6)	42.5 (11.7)	43.0 (11.1)	0.56
Delayed recall	43.2 (11.7)	43.3 (11.8)	42.6 (10.8)	0.41
Recognition	44.4 (13.8)	43.6 (13.6)	50.5 (13.3)	<0.001
Grooved pegboard				
Dominant	45.4 (11.9)	45.4 (11.8)	45.4 (12.5)	0.98
Non-dominant	44.7 (11.3)	44.7 (11.3)	44.4 (11.6)	0.7
Trail making test				
Part A	48.3 (11.9)	48.2 (11.9)	49.1 (11.7)	0.31
Part B	46.3 (11.7)	46.1 (11.7)	47.4 (11.4)	0.14
Letter fluency	47.0 (11.5)	47.0 (11.2)	46.9 (13.2)	0.96
PASAT 50	44.7 (11.6)	44.7 (11.6)	44.9 (11.8)	0.79
Digit symbol test	47.2 (11.2)	47.3 (11.3)	46.9 (10.7)	0.67
PERCENT IMPAIRMENT (40 CUTPOINT)	***N* (%)**	***n* (%)**	***n* (%)**	
BVMT-R				
Total learning	429 (29)	499 (30)	70 (34)	0.13
Delayed recall	483 (33)	546 (33)	63 (31)	0.70
Recognition	434 (30)	487 (29)	53 (26)	0.38
HVLT-R				
Total learning	595 (41)	670 (40)	75 (37)	0.41
Delayed recall	564 (38)	641 (38)	77 (38)	1.00
Recognition	476 (33)	510 (31)	34 (17)	<0.001
Grooved pegboard				
Dominant	470 (32)	536 (32)	66 (33)	0.89
Non-dominant	461 (31)	523 (31)	62 (31)	0.92
Trail making test				
Part A	297 (20)	341 (20)	44 (22)	0.66
Part B	410 (28)	457 (27)	47 (23)	0.20
Letter fluency	342 (23)	396 (24)	54 (27)	0.31
PASAT 50	508 (35)	580 (35)	72 (36)	0.81
Digit symbol test	378 (26)	430 (26)	52 (26)	1.00

M, mean; SD, standard deviation; BVMT-R, Benton Visual Retention Test-Revised; HVLT-R, Hopkins Verbal Learning Test-Revised; PASAT, Paced Auditory Serial Addition Test.

Predictors of Cognitive Profiles in the Total Sample

In RF models, the top 10 variables distinguishing each of the impaired profiles—*Relatively weak auditory attention and episodic memory* [Profile 2; receiver operating curve (ROC) = 0.94] and *Global weaknesses* (Profile 3; ROC = 0.95)—from the unimpaired profile (Profile 1) were the same and included: WRAT-4, age, duration of HIV disease, nadir CD4 counts, education, BDI-II, IADL dependence, log plasma viral load, and race/ethnicity (**Figure 3A**). In each case, the impaired profiles or those with weaknesses (2 and 3) had lower WRAT-4 and higher BDI-II scores than the unimpaired profile (Profile 1) (**Table 3**). The *Relatively weak auditory attention and episodic memory* profile (Profile 2) group also was less educated, more

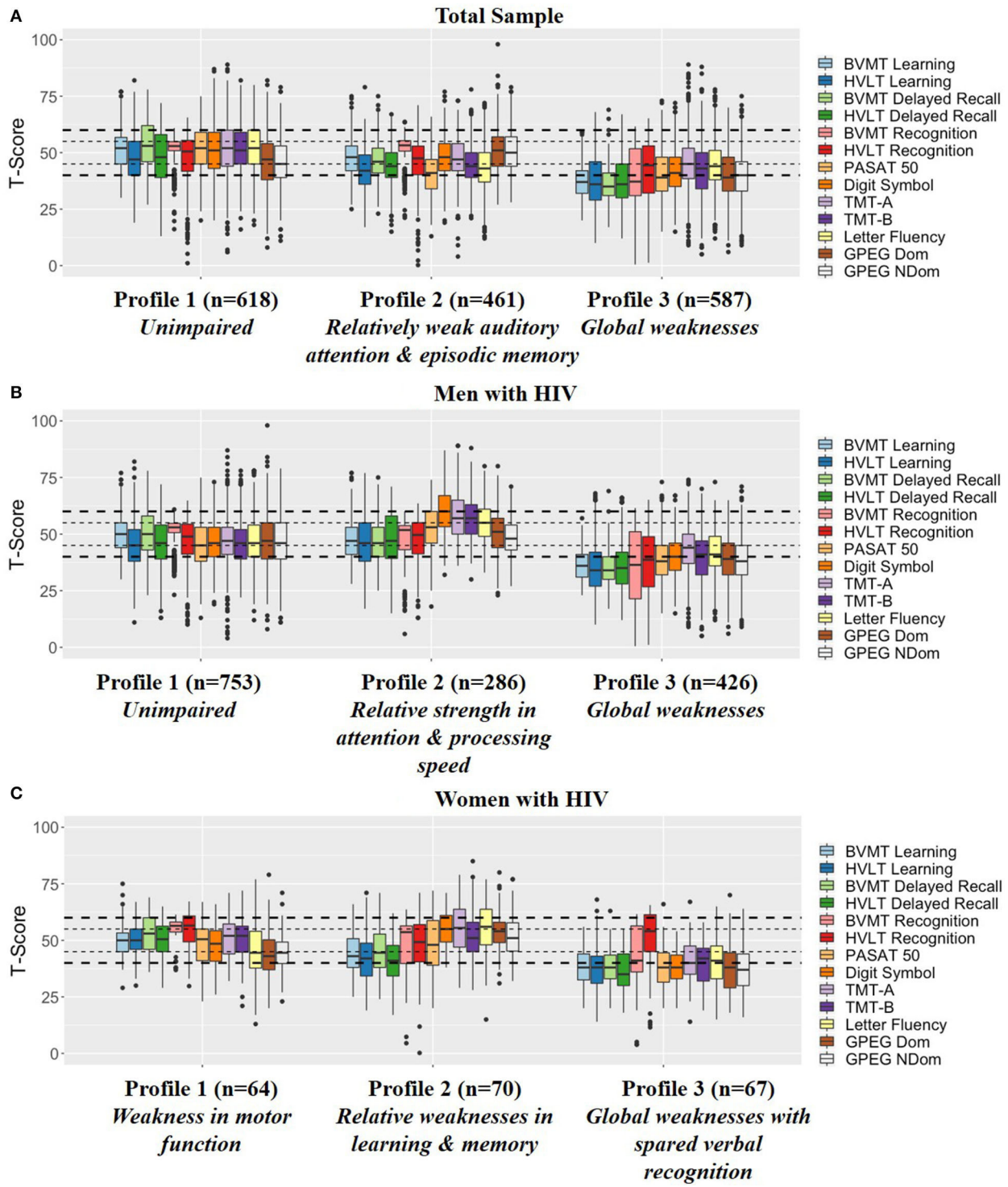

FIGURE 1 | Profiling results in the **(A)** Total sample, **(B)** Men with HIV, and **(C)** Women with HIV. The small dotted line indicates a half of a standard deviation above and below the mean whereas the large dotted line indicates a full standard deviation above and below the mean. BVMT, Brief Visuospatial Memory Test-Revised; HVLT, Hopkins Verbal Learning Test Revised; PASAT, Paced Auditory Serial Addition Task; TMT, Trail Making Test; GPEG, Grooved pegboard.

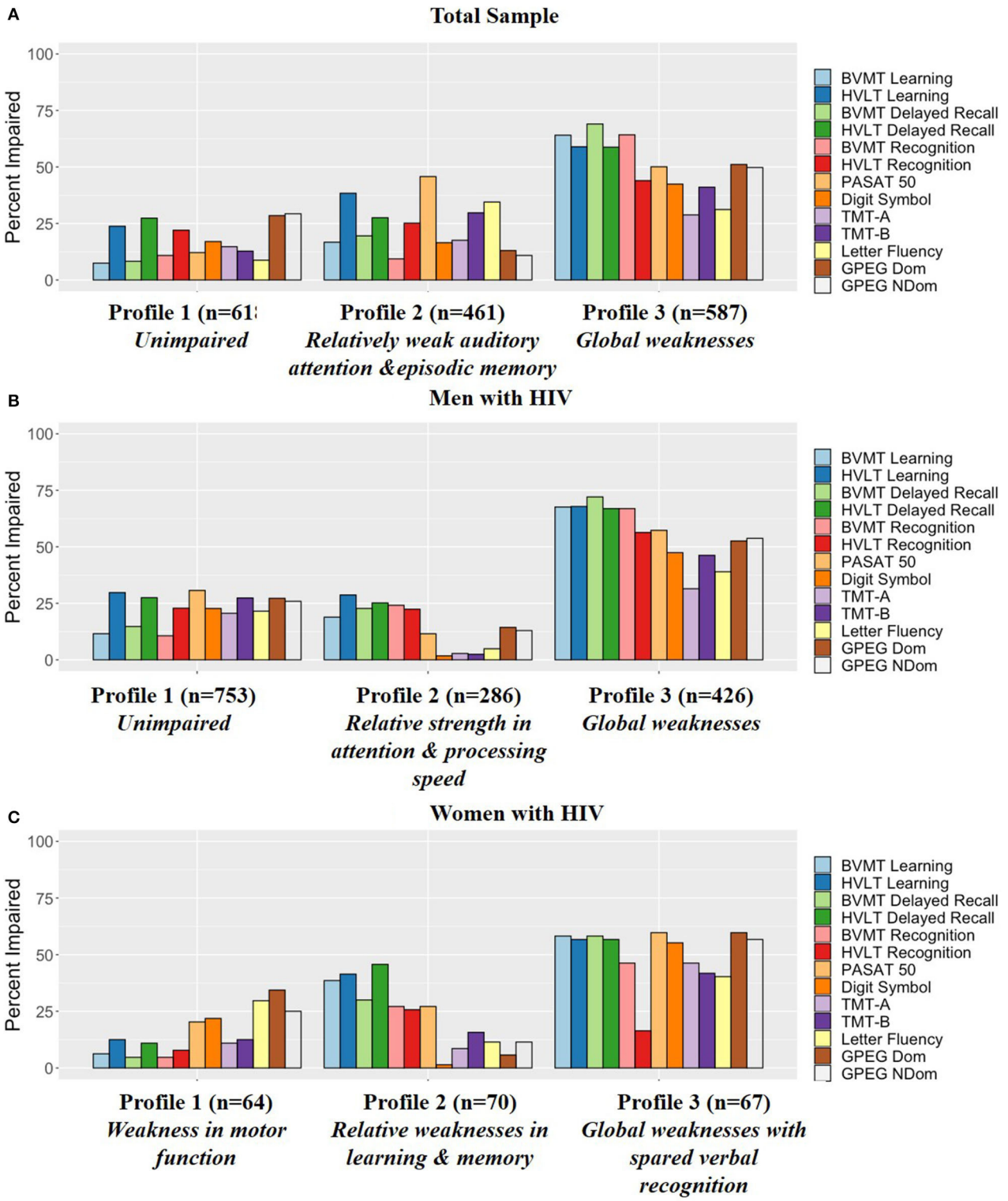

FIGURE 2 | Percent impairment (T-score cutoff of 40) for each profile in the **(A)** Total sample, **(B)** Men with HIV, and **(C)** Women with HIV. BVMT, Brief Visuospatial Memory Test-Revised; HVLT, Hopkins Verbal Learning Test Revised; PASAT, Paced Auditory Serial Addition Task; TMT, Trail Making Test; GPEG, Grooved pegboard.

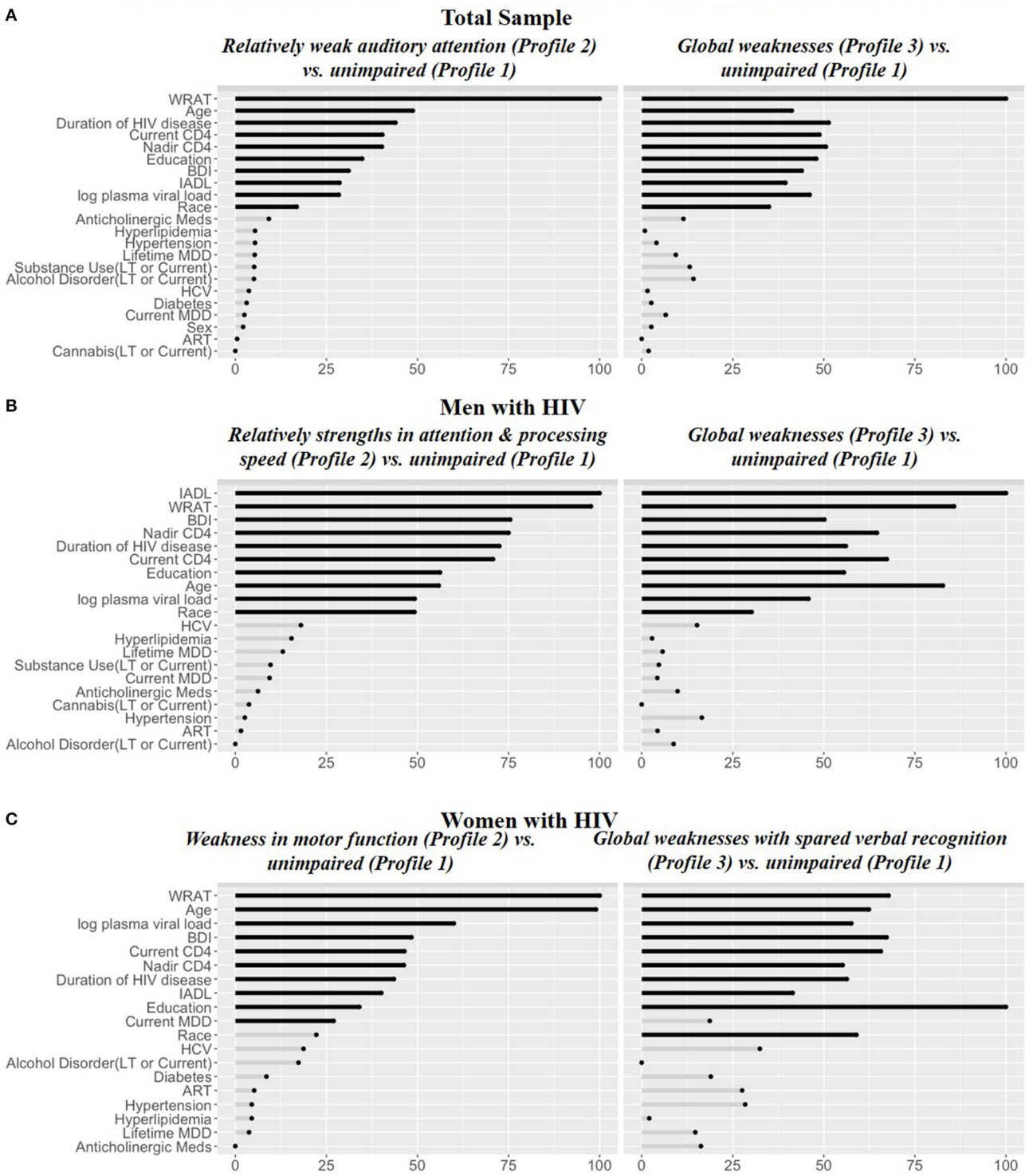

FIGURE 3 | Random forest variable importance from the models for **(A)** Total sample, **(B)** Men with HIV, and **(C)** Women with HIV. IADL, Instrumental Activities of Daily Living; WRAT, Wide Range Achievement Test-4 Reading subtest; BDI, Beck Depression Inventory; LT, lifetime; HCV, Hepatitis C co-infectious; MDD, major depressive disorder.

TABLE 3 | Demographic, behavioral, and clinical characteristics in the total sample of people with HIV by cognitive profile.

	Profile 1	Profile 2	Profile 3	
	Unimpaired (*n* = 618) *n* (%)	**Relatively weak auditory attention and episodic memory (*n* = 461) *n* (%)**	**Global weaknesses (*n* = 587) *n* (%)**	***P*-value**
Age, M (SD)	41.2 (9.1)	39.4 (9.4)	44.2(10.2)	<0.001
Male	544 (88)	401 (87)	520 (89)	0.73
Years of education, M (SD)	13.5 (2.5)	12.9 (2.7)	13.5 (2.8)	<0.001
WRAT-4, M (SD)	102.2 (11.9)	96.5 (13.6)	97.6 (14.2)	<0.001
Race				<0.001
White	387 (63)	202 (44)	382 (65)	
Black	119 (19)	104 (23)	91 (15)	
Hispanic	76 (12)	132 (29)	91 (15)	
Other	36 (6)	23 (5)	23 (4)	
IADL dependence	2.2 (2.7)	2.2 (2.7)	3.0 (3.1)	<0.001
BDI-II	12.4 (9.9)	13.4 (10.8)	14.7(10.4)	<0.001
DSM-IV (CIDI) diagnoses				
MDD				
Current	67 (17)	59 (16)	98 (22)	0.06
Lifetime	198 (51)	154 (43)	220 (50)	0.05
Alcohol (current or lifetime*)	19 (5)	25 (7)	28 (6)	0.47
Cannabis (current or lifetime*)	23 (6)	21 (6)	19 (4)	0.52
Substance use (current or lifetime*)	232 (76)	208 (72)	251 (71)	0.41
Anticholinergic medication	127 (21)	125 (27)	170 (29)	0.002
Hypertension	123 (21)	71 (16)	140 (25)	0.002
Hyperlipidemia	82 (14)	44 (10)	93 (16)	0.008
Diabetes	38 (6)	14 (3)	30 (5)	0.05
HCV	111 (19)	81 (18)	125 (22)	0.18
Log plasma viral load, M (SD)	3.2 (1)	3.0 (1.3)	3.1 (1.4)	0.05
CD4 count, M (SD)				
Current	434.6 (283.1)	449.9 (305.6)	406 (303.5)	0.05
Nadir	245.1 (214.7)	259.8 (251.2)	197.5 (198.3)	<0.001
Duration of HIV disease, M (SD)	8.9 (7.8)	7.9 (7.2)	10.2 (8.2)	<0.001
AIDS diagnosis	331 (54)	253 (55)	385 (66)	<0.001
On ART	322 (54)	275 (61)	369 (65)	<0.001

*ART, antiretroviral therapy; WRAT-4, Wide Range Achievement Test-4th edition; IADL, Instrumental Activities of Daily Living; BDI, Beck depression inventory; CIDI, Composite International Diagnostic Interview; HCV, Hepatitis C; *Current or Lifetime Use disorder (abuse or dependence).*

likely to be Hispanic, and had a shorter duration of HIV disease compared to the unimpaired profile (Profile 1). However, the *Global Weaknesses* profile (Profile 3) was older, had more IADL dependence, a longer duration of HIV disease, and lower current and nadir CD4 counts as compared to the unimpaired profile.

Predictors of Cognitive Profiles in MWH and WWH Separately

MWH

In RF models in MWH, the top 10 variables distinguishing the *Global weaknesses* profile (Profile 3) and *Relative strength in attention and processing speed* (Profile 2) from the unimpaired profile (Profile 1) (ROC = 0.95 and ROC = 0.92, respectively) were the same and included: IADL dependence, WRAT-4, BDI-II, nadir CD4 count, duration of HIV disease, current CD4 count, education, age, log plasma viral load, and race (**Figure 3B**). The *Global weaknesses* profile (Profile 3) was older, had higher IADL dependence, and BDI-II scores, longer duration of HIV disease, and lower current and nadir CD4 counts compared to the unimpaired profile (Profile 1) (**Table 4**). The *Relative strength in attention and processing speed* (Profile 2) was more likely to be White, had higher WRAT-4 and lower BDI-II scores, had higher current and nadir CD4 counts,

TABLE 4 | Demographic, behavioral, and clinical characteristics in the sample of men with HIV by cognitive profile.

	Profile 1	Profile 2	Profile 3	
	Unimpaired (*n* = 753) *n* (%)	**Relative strength in attention and processing speed (*n* = 286) *n* (%)**	**Global weaknesses (*n* = 426) *n* (%)**	***P*-value**
Age, M (SD)	40.6 (9.1)	41.9 (9.8)	44.3(10.6)	<0.001
Years of education, M (SD)	13.4 (2.52)	13.7 (2.6)	13.6 (2.9)	0.245
WRAT-4, M (SD)	99.7 (12.8)	104.1 (11.8)	96.8 (14.5)	<0.001
Race				0.01
White	432 (57)	188 (66)	270 (63)	
Black	146 (19)	48 (17)	65 (15)	
Hispanic	139 (19)	31 (11)	76 (18)	
Other	36 (5)	19 (6)	15 (4)	
IADL complaints	2.2 (2.7)	1.4 (1.9)	3.4 (3.3)	<0.001
BDI	13.1 (10.4)	10.5 (9.)	16.2 (10.7)	<0.001
CIDI diagnoses				
MDD				
Current	105 (19)	21 (10)	77 (24)	<0.001
Lifetime	259 (47)	92 (45)	159 (49)	0.66
Alcohol (current or lifetime*)	40 (7)	3 (1)	23 (7)	0.009
Cannabis (current or lifetime*)	36 (7)	8 (4)	16 (5)	0.31
Substance use (current or lifetime*)	321 (74)	121 (73)	183 (72)	0.90
Anticholinergic medication	195 (26)	48 (17)	124 (29)	<0.001
Hypertension	140 (19)	51 (19)	107 (26.)	0.01
Hyperlipidemia	96 (13)	40 (15)	57 (14)	0.82
Diabetes	40 (5)	8 (3)	22 (5)	0.22
HCV	125 (17)	46 (17)	88 (21)	0.14
Log plasma viral load, M (SD)	3.2 (1.3)	2.9 (1.3)	3.2 (1.5)	0.01
CD4 count, M (SD)				
Current	432.4 (301.1)	479.0 (272.8)	381.4 (295.0)	<0.001
Nadir	238.6 (232.8)	268.2 (209.8)	188.8 (196.1)	<0.001
Duration of HIV disease, M (SD)	8.9 (7.8)	8.5 (7.4)	10.4 (8.6)	0.01
AIDS diagnosis	440 (56)	113 (47)	281 (66)	<0.001
On ART	426 (58)	166 (59)	265 (64)	0.10

*ART, antiretroviral therapy; WRAT-4, Wide Range Achievement Test; IADL, Instrumental Activities of Daily Living; BDI, Beck depression inventory; CIDI, Composite International Diagnostic Interview; HCV, Hepatitis C; *Current or Lifetime Use disorder (abuse or dependence).*

and lower log plasma viral loads than the unimpaired profile (Profile 1).

WWH

In RF models in WWH, nine out of the top 10 variables distinguishing the *Relative weaknesses in learning and memory* profile (Profile 2) and *Global weakness with spared verbal recognition* (Profile 3) from the profile only demonstrating *Weakness in motor function* (Profile 1; ROC = 0.95 and ROC = 0.90, respectively) were the same and included: WRAT-4, age, log plasma viral load, BDI-II, current and nadir CD4 count, duration of HIV infection, IADL dependence, and education (**Figure 3C**). The only unique variable distinguishing the profile demonstrating *Relative weaknesses in learning and memory* (Profile 2) from the profile demonstrating *Weakness in motor function* (Profile 1) was race. However, the unique variable distinguishing the profile demonstrating *Global weaknesses with spared verbal recognition* (Profile 3) from the profile demonstrating *Weakness in motor function* (Profile 1) was a current diagnosis of MDD. The *Relative weaknesses in learning and memory* profile (Profile 2) was older, less likely to be Hispanic, more likely to be non-Hispanic White, and had lower BDI-II scores compared the profile demonstrating *Weakness in motor function* (Profile 1) (**Table 5**). However, the *Global weaknesses with spared verbal recognition* profile (Profile 3) was more likely to be non-Hispanic White, have lower WRAT-4 scores, had more IADL dependence and higher BDI-II scores, was more likely to have a current diagnosis of MDD, have higher log plasma viral loads, lower current and nadir CD4 counts, and a shorter duration of HIV disease

TABLE 5 | Demographic, behavioral, and clinical characteristics in the sample of women with HIV by cognitive profile.

	Profile 1	Profile 2	Profile 3	
	Weakness in motor function	Relative weaknesses in learning and memory	Global weaknesses with spared verbal recognition	*P*-value
	(*n* = 64)	(*n* = 70)	(*n* = 67)	
	n (%)	*n* (%)	*n* (%)	
Age, M (SD)	37.4 (8.5)	42.4 (9.9)	41.9 (9.8)	0.005
Years of education, M (SD)	11.5 (2.5)	12.6 (2.4)	11.9 (2.6)	0.03
WRAT-4, M (SD)	96.6 (9.8)	95.5 (13.9)	88.9 (13.5)	0.003
Race				0.007
White	21 (33)	32 (46)	28 (42)	
Black	16 (25)	26 (37)	13 (19)	
Hispanic	25 (39)	8 (11)	20 (30)	
Other	2 (3)	4 (6)	6 (9)	
IADL dependence	2.7 (3.0)	2.2 (2.7)	3.8 (3.5)	0.01
BDI-II	13.7 (9.5)	10.9 (9.2)	16.0 (10.5)	0.01
DSM-IV (CIDI) diagnoses				
MDD				
Current	5 (14)	2 (5)	14 (34)	0.003
Lifetime	17 (47)	17 (45)	28 (68)	0.06
Alcohol (current or lifetime*)	3 (8)	2 (5)	1 (2)	0.51
Cannabis (current or lifetime*)	1 (3)	2 (5)	0 (0)	0.34
Substance use (current or lifetime*)	22 (81)	21 (66)	21 (67)	0.36
Anticholinergic medication	16 (25)	18 (26)	21 (31)	0.66
Hypertension	8 (12)	16 (23)	12 (18)	0.28
Hyperlipidemia	5 (8)	9 (13)	12 (18)	0.21
Diabetes	5 (8)	2 (3)	58	0.4
HCV	22 (34)	18 (26)	18 (27)	0.53
Log plasma viral load, M (SD)	3.1 (0.9)	3.1 (1.2)	3.2 (1.3)	0.72
CD4 count, M (SD)				
Current	448.4 (282.9)	516.5 (309.6)	358.2 (304.7)	0.01
Nadir	298.6 (269.1)	273.1 (232.0)	181.3 (173.5)	0.009
Duration of HIV disease, M (SD)	7.3 (5.4)	7.41 (6.1)	6.5 (5.3)	0.76
AIDS diagnosis	32 (50)	36 (51)	47 (70)	0.03
On ART	34 (55)	36 (52)	39 (61)	0.58

*ART, antiretroviral therapy; WRAT-4, Wide Range Achievement Test; IADL, Instrumental Activities of Daily Living; BDI, Beck depression inventory; CIDI, Composite International Diagnostic Interview; HCV, Hepatitis C; *Current or Lifetime Use disorder (abuse or dependence).*

compared to the profile demonstrating *Weakness in motor function* (Profile 1).

DISCUSSION

In this large-scale study using a novel pipeline combination of machine learning methods, we provide further evidence in support of heterogeneity in cognitive function among PWH. Our results do not negate the heterogeneity in cognitive function in HIV-uninfected individuals but rather highlights the heterogeneity among PWH that can often be masked by a dichotomous HAND categorization. In the total sample, we identified an unimpaired profile, a profile of relatively weak auditory attention and episodic memory, and a global weakness profile. As expected, given the relative sample sizes, the cognitive patterns in the total sample were in greater alignment with those found among MWH compared to WWH. Similar to results in the overall sample, we identified an unimpaired profile and a global weakness profile in MWH; however, unlike the overall sample and inconsistent with hypotheses of domain-specific cognitive impairment profiles in both MWH and WWH, MWH demonstrated a profile with relative strengths in attention and processing speed. Conversely, there were no unimpaired, cognitive strength or global weakness profiles among WWH. Rather, as hypothesized WWH demonstrated cognitive profiles reflecting a global weakness (with spared verbal recognition) and domain-specific impairment including a weakness in learning and memory and motor skills. These findings suggest that sex and the sociodemographic factors associated with female sex within the HIV-infected population

contribute to the heterogeneity in cognitive function among PWH. Studies examining cognitive function in combined samples of men and women may mask important sex differences in cognitive functioning among PWH, particularly in male-dominant samples such as the current sample. These sex differences in cognitive profiles among PWH may result from biological sex differences and/or the psychosocial factors that tend to characterize WWH more than MWH (e.g., low education, poverty). Biological sex differences include those seen in the general population such as sex steroid hormones (e.g., estrogen, progesterone, testosterone), female-specific reproductive events (e.g., parity, reproductive span, hormone therapies) and genetic factors or previously-reported sex differences specifically in HIV disease characteristics unmeasured herein (e.g., size of viral reservoirs, CD4 cell count at seroconversion) (47, 48). Regardless of the underlying mechanism, characterizing these sex differences in cognitive functioning among PWH can provide inroads to identifying mechanisms of cognitive dysfunction and optimizing risk assessments and diagnostic and therapeutic strategies for each sex.

A notable sex difference in profiles was the lack of the unimpaired or cognitive strength profile among WWH that was observed among MWH. Our cognitive profile analyses are in line with prior studies that suggests that WWH are often but not always, more likely to demonstrate cognitive deficits than MWH (10). Our analysis suggests that the impairment manifests more often as domain-specific impairment (i.e., learning, memory, motor) in women than in men that may not be revealed in a more cross-domain summary measure like GDS or global T-scores. This female vulnerability to cognitive deficits is thought to reflect sociodemographic differences whereby low education and socioeconomic status and their associated psychosocial risk factors (e.g., depression, poverty, early-life trauma, barriers to health care, co-infections) are more prevalent among WWH vs. MWH (10, 22, 49). These psychosocial risk factors can have adverse effects on the brain that lower cognitive reserve (23, 24, 50, 51), suggesting that interventions geared toward addressing these psychosocial factors should be a priority for WWH and/or for women who are at increased risk of HIV. In support of these studies, Sundermann et al. (17) found that the higher rates of cognitive impairment in WWH vs. MWH were eliminated after adjusting for the lower reading level (i.e., WRAT-4 score) that characterized WWH compared to MWH. Biological differences may also contribute to sex differences in the pattern and magnitude of cognitive impairment in PWH including disease characteristics, brain structure/function, sex steroid hormones and female-specific hormonal milieus (e.g., pregnancy, menstrual cycle, menopause transition). There is also evidence to suggest that WWH may be more cognitively susceptible than MWH to the effects of mental health factors (25).

As mentioned, only women demonstrated more domain-specific cognitive profiles including weakness in motor functioning and relative weakness in learning and memory. Similarly, previous studies report that learning, memory, and motor functioning are among the domains in which cognitive impairment is more common among WWH vs. MWH (10) and these differences persisted after adjusting for HIV RNA and CD4 counts (21). These sex differences in domain-specific impairment may reflect psychosocial factors (e.g., cognitive reserve, mental health), biological factors (sex steroid hormones, genetic), or interactions among them. Although women in general demonstrate relative advantages in verbal memory and fine motor function compared men (52–57) likely due, at-least in part, to the effects of estrogen on the developing brain and the neuroprotective effects of circulating estradiol (58–60), the menopause transition has been associated with declines in verbal memory and motor function (61–63). The mean age of women in our study was 41 (SD = 9.6; 33% >45 years of age) suggesting that a portion of women may be experiencing cognitive deficits associated with reproductive aging. Germane to the learning/memory impairment in WWH, women are more vulnerable to the negative effects of stress hormones on hippocampal-dependent tests compared to men (64). This finding may be particularly relevant to the current sample considering the high prevalence of psychosocial stressors among WWH including childhood trauma and domestic violence (65).

Unlike MWH, WWH demonstrated a global impairment profile with spared verbal recognition. Consistently, previous findings regarding memory impairment among PWH found this impairment to be more dependent on frontal and subcortical structures with relatively normal memory retention but impaired memory retrieval (recall but not recognition deficits) (66–68). Even in the female-specific profile of relative weakness in learning and memory, recognition was less impaired compared to learning and recall. We can only speculate as to why the sparing of recognition in the global impairment profile was specific to WWH and to verbal vs. visual memory. It is possible that, in the context of cognitive impairment in HIV, the female advantage in verbal memory may be most salient for the least cognitively-taxing memory component, recognition performance, and this advantage is not fully adjusted for in our demographically-corrected T-scores.

Despite the heterogeneity in cognitive profiles by sex, the sociodemographic/clinical/biological factors associated with these cognitive profiles were similar for MWH and WWH suggesting that, although the same factors confer increased vulnerability to cognitive dysfunction, the adverse effects of these factors impact brain function differently in men and women. In both MWH and WWH, WRAT-4 had the greatest discriminative value of profile class followed by HIV disease variables (e.g., CD4 count, viral load and estimated duration of HIV disease), depressive symptoms, age, race/ethnicity and years of education. WRAT-4 scores have been consistently identified as an important determinant of cognitive function among PWH, with lower WRAT-4 scores conferring risk for cognitive impairment (17, 69). WRAT-4 performance may be particularly salient in this population, given that reading level may reflect education quality, above and beyond years of education, especially in lower socioeconomic populations because of the many factors impacting education quality (e.g., ability to attend school, economic disadvantages in schools within low SES districts) (69). Additionally, reading level is associated with health outcomes including hospitalizations and outpatient doctor visits (70) and, thus, may be a proxy

for biopsychosocial factors underlying general health (e.g., socioeconomic status, self-efficacy).

HIV disease variables were also strong determinants of cognitive profiles in both men and women. Aside from some instances of a shorter duration of HIV disease relating to more cognitive impairment in WWH and in the total sample, the more biologically-based HIV disease variables were associated with cognitive impairment in the expected direction; higher current and nadir CD4 count and lower viral load were protective against cognitive impairment. It is curious that the global weakness with spared verbal recognition profile in women was associated with more severe HIV-related variables (i.e., higher viral loads, lower current, and nadir CD4 counts) yet with shorter duration of HIV infection. We speculate that the shorter HIV infection in WWH may reflect CNS effects of untreated and/or early-course HIV infection. Alternatively, the self-reported shorter duration of infection may not have been accurate, to the extent that WWH lived longer with untested/undetected infections. Findings are consistent with a wealth of literature relating proxies of HIV disease burden and severity to cognitive function (71–73) and suggests that, even in the era of effective ART when viral suppression is common, HIV disease burden can have adverse effects on the brain possibly due to poor penetration of ARTs into the CNS, ART resistance, poor medication adherence (74), and/or the establishment of viral reservoirs in the CNS reservoir (75, 76).

In line with hypotheses of mental health factors relating to cognitive impairment profiles more strongly in women, current diagnosis of MDD was a predictor of cognitive profiles only among WWH. Although the prevalence of a current or lifetime diagnosis of MDD did not differ between WWH and MWH, MDD was an important risk factor of demonstrating *Global weaknesses with spared verbal recognition* (Profile 2) compared to the profile demonstrating only *Weakness in motor function* (Profile 1). This finding aligns with our work demonstrating that MDD may have a greater impact in women compared to men (25). Our work indicates that HIV comorbid with depression affects certain cognitive domains including cognitive control, and that these effects are largest in women. Specifically, WWH with elevated depressive symptoms had 5 times the odds of impairment on Stroop Trial 3, a measure of behavioral inhibition, compared to HIV-uninfected depressed women, and 3 times the odds of impairment on that test compared to depressed MWH. In a recent meta-analysis, small to moderate deficits in declarative memory and cognitive control were documented not only in individuals with current MDD but also in individuals with remitted MDD, leading to the conclusion that these deficits occur independently of episodes of low mood in individuals with "active" MDD (77). Together these lines of work suggest that MDD would exacerbate (or co-occur with factors that cause) cognitive difficulties in PWH, particularly in the cognitive domains of declarative memory and cognitive control in WWH.

Our study has limitations. Although we were adequately powered within both WWH and MWH (10), the magnitude of power was discrepant by sex considering that women represented 20% of our sample. Larger-scale studies in WWH only are currently underway. The generalizability of our findings also warrant additional study as the profiles identified here may not represent the profiles among all PWH. Due to the unavailability of data, we were unable to explore certain psychosocial factors (e.g., early life trauma, perceived stress) as potential determinants of cognitive profiles. Our analyses were cross-sectional which allows us to identify determinants associated with cognitive profiles but precludes us from determining the temporal relationships between these factors and cognitive function. Although many of the related factors may be risk factors for cognitive impairment, reverse causality is possible with some of the factors resulting from cognitive impairment (e.g., depression, IADL). Additionally, interpretation of the machine learning results should be done with care as RF is an ensemble model that is inherently non-linear in nature. This means that the importance and predictive power of every variable is specified in the context of other variables. This can lead to situations where an important predictive variable in the RF model has no significant difference in the overall comparison but has dramatic differences when included with other variables in the model. As such, this model should be interpreted as hypothesis-generating and identifies variables in need of further investigation. Lastly, because our study was focused on sex differences in cognitive profiles within PWH, we did not include a HIV-seronegative comparison group. Thus, we cannot determine the degree to which HIV contributes to sex differences in cognitive profiles. However, the independent HIV-related predictors does suggest that HIV has a role. Despite these limitations, we selected RF over linear models such as lasso and ridge regression because RF models had more predictive power and higher accuracy in this data compared to the linear models, even linear models with tuning parameters such as ridge and lasso that can used for feature selection. The results from these models mirror the *P*-values for the univariate comparisons (see **Tables 1**–**5**), which is expected since analysis of variance and *t*-tests are also linear models. Moreover, RF models are more optimal for handling missing data, the inclusion of categorical predictor variables, and the use of categorical outcome measures which was the case in the present study. RF models also account for the complexity in the data that can arise from multicollinearity often seen in large feature sets.

In conclusion, our results also suggest that sex is a contributor to the heterogeneity in cognitive profiles among PWH and that cognitive findings from MWH or male-dominant samples cannot be wholly generalized to WWH. Whereas, MWH showed an unimpaired profile and even a cognitively advantageous profile, WWH only showed impairment profiles that included global and more domain-specific impairment, which supports previous findings of greater cognitive impairment in WWH than in MWH (10). Although the strongest determinants of cognitive profiles were similar in MWH and WWH including WRAT-4, HIV disease characteristics, age and depressive symptoms, the direction of these associations sometimes differed. This suggests that the effects of certain biological, clinical, or demographic factors on the brain and cognition may manifest differently in MWH and WWH and that sex may contribute to heterogeneity not only in cognitive profiles but in their determinants although studies with larger numbers of WWH are

needed to more definitively test these hypotheses. It is important to detect these differing cognitive profiles and their associated risk/protective factors as this information can help to identify differing mechanisms contributing to cognitive impairment and whether these mechanisms are related to HIV disease, neurotoxic effects of ART medications, and/or comorbidities that are highly prevalent among PWH (e.g., depression, substance abuse, hyperlipidemia). Given the longer lifespan of PWH in the era of effective antiretroviral therapy, cognitive profiling will also inform aging-related effects on cognition in the context of HIV and perhaps early clinical indicators of age-related neurodegenerative disease. By identifying cognitive profiles and their underlying mechanisms, we can ultimately improve our ability to treat by tailoring and directing intervention strategies to those most likely to benefit. Overall, our results stress the importance of considering sex differences in studies of the pathogenesis, clinical presentation, and treatment of cognitive dysfunction in HIV.

DATA AVAILABILITY STATEMENT

The data analyzed in this study is subject to the following licenses/restrictions: data from our study are available upon request. Persons with HIV are highly stigmatized, and even in the presence of strong de-identification, the risk of re-identification is real. Protecting persons with HIV remains our top priority. The Data Access Committee whom imposed these restrictions and to whom data requests should be made is the HNRP Data Resource Committee. Requests to access these datasets should be directed to hnrpresource@ucsd.edu.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Institutional Review Board of the University of California, San Diego. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

ES aggregated the data. LR, RD, and EP conducted statistical analyses. LR and ES have primary responsibility for final content and wrote the paper. All authors contributed to the project conception, design, manuscript editing, statistical review, and read and approved the final 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/fneur.2020.551921/full#supplementary-material

REFERENCES

1. Valcour V, Chalermchai T, Sailasuta N, Marovich M, Lerdlum S, Suttichom D, et al. Central nervous system viral invasion and inflammation during acute HIV infection. *J Infect Dis.* (2012) 206:275–82. doi: 10.1093/infdis/jis326
2. Wang Y, Liu M, Lu Q, Farrell M, Lappin JM, Shi J, et al. Global prevalence and burden of HIV-associated neurocognitive disorder: a meta-analysis. *Neurology.* (2020). doi: 10.1212/WNL.0000000000010752. [Epub ahead of print].
3. Heaton RK, Franklin DR, Ellis RJ, Mccutchan JA, Letendre SL, Leblanc S, et al. HIV-associated neurocognitive disorders before and during the era of combination antiretroviral therapy: differences in rates, nature, and predictors. *J Neurovirol.* (2011) 17:3–16. doi: 10.1007/s13365-010-0006-1
4. Dawes S, Suarez P, Casey CY, Cherner M, Marcotte TD, Letendre S, et al. Variable patterns of neuropsychological performance in HIV-1 infection. *J Clin Exp Neuropsychol.* (2008) 30:613–26. doi: 10.1080/13803390701565225
5. Gomez D, Power C, Gill MJ, Koenig N, Vega R, Fujiwara E. Empiric neurocognitive performance profile discovery and interpretation in HIV infection. *J Neurovirol.* (2018) 25:72–84. doi: 10.1007/s13365-018-0685-6

6. Pasipanodya EC, Montoya JL, Campbell LM, Hussain MA, Saloner R, Paolillo EM, et al. Metabolic risk factors as differential predictors of profiles of neurocognitive impairment among older HIV+ and HIV- adults: an observational study. *Arch Clin Neuropsychol.* (2019). doi: 10.1093/arclin/acz040. [Epub ahead of print].
7. May PE, Heithoff AJ, Wichman CS, Phatak VS, Moore DJ, Heaton RK, et al. Assessing cognitive functioning in people living with HIV (PLWH): factor analytic results from CHARTER and NNTC cohorts. *J Acquir Immune Defic Syndr.* (2020) 83:251–9. doi: 10.1097/QAI.0000000000002252
8. Nightingale S, Winston A, Letendre S, Michael BD, Mcarthur JC, Khoo S, et al. Controversies in HIV-associated neurocognitive disorders. *Lancet Neurol.* (2014) 13:1139–51. doi: 10.1016/S1474-4422(14)70137-1
9. Milanini B, Catella S, Perkovich B, Esmaeili-Firidouni P, Wendelken L, Paul R, et al. Psychiatric symptom burden in older people living with HIV with and without cognitive impairment: the UCSF HIV over 60 cohort study. *AIDS Care.* (2017) 29:1178–85. doi: 10.1080/09540121.2017.1281877
10. Rubin LH, Neigh GN, Sundermann EE, Xu Y, Scully EP, Maki PM. Sex Differences in neurocognitive function in adults with HIV: patterns, predictors, and mechanisms. *Curr Psychiatry Rep.* (2019) 21:94. doi: 10.1007/s11920-019-1089-x
11. Vassallo M, Durant J, Lebrun-Frenay C, Fabre R, Ticchioni M, Andersen S, et al. Virologically suppressed patients with asymptomatic and symptomatic HIV-associated neurocognitive disorders do not display the same pattern of immune activation. *HIV Med.* (2015) 16:431–40. doi: 10.1111/hiv.12246
12. Foca E, Magro P, Motta D, Compostella S, Casari S, Bonito A, et al. Screening for neurocognitive impairment in HIV-infected individuals at first contact after HIV diagnosis: the experience of a large clinical center in Northern Italy. *Int J Mol Sci.* (2016) 17:434. doi: 10.3390/ijms17040434
13. Kabuba N, Menon JA, Franklin DR Jr, Heaton RK, Hestad KA. HIV- and AIDS-associated neurocognitive functioning in Zambia - a perspective based on differences between the genders. *Neuropsychiatr Dis Treat.* (2016) 12:2021–8. doi: 10.2147/NDT.S105481
14. Royal W 3rd, Cherner M, Burdo TH, Umlauf A, Letendre SL, Jumare J, et al. Associations between cognition, gender and monocyte activation among HIV infected individuals in Nigeria. *PLoS ONE.* (2016) 11:e0147182. doi: 10.1371/journal.pone.0147182
15. Burlacu R, Umlauf A, Luca A, Gianella S, Radoi R, Ruta SM, et al. Sex-based differences in neurocognitive functioning in HIV-infected young adults. *AIDS.* (2018) 32:217–25. doi: 10.1097/QAD.0000000000001687
16. Gascon MRP, Vidal JE, Mazzaro YM, Smid J, Marcusso RMN, Capitao CG, et al. Neuropsychological assessment of 412 HIV-infected individuals in Sao Paulo, Brazil. *AIDS Patient Care STDS.* (2018) 32:1–8. doi: 10.1089/apc.2017.0202
17. Sundermann EE, Heaton RK, Pasipanodya E, Moore RC, Paolillo EW, Rubin LH, et al. Sex differences in HIV-associated cognitive impairment. *AIDS.* (2018) 32:2719–26. doi: 10.1097/QAD.0000000000002012
18. Heaton RK, Franklin DR Jr, Deutsch R, Letendre S, Ellis RJ, Casaletto K, et al. Neurocognitive change in the era of HIV combination antiretroviral therapy: the longitudinal CHARTER study. *Clin Infect Dis.* (2015) 60:473–80. doi: 10.1093/cid/ciu862
19. Behrman-Lay AM, Paul RH, Heaps-Woodruff J, Baker LM, Usher C, Ances BM. Human immunodeficiency virus has similar effects on brain volumetrics and cognition in males and females. *J Neurovirol.* (2016) 22:93–103. doi: 10.1007/s13365-015-0373-8
20. Do TC, Kerr SJ, Avihingsanon A, Suksawek S, Klungkang S, Channgam T, et al. HIV-associated cognitive performance and psychomotor impairment in a Thai cohort on long-term cART. *J Virus Erad.* (2018) 4:41–7. doi: 10.1016/S2055-6640(20)30243-0
21. Maki PM, Rubin LH, Springer G, Seaberg EC, Sacktor N, Miller EN, et al. Differences in cognitive function between women and men with HIV. *J Acquir Immune Defic Syndr.* (2018) 79:101–7. doi: 10.1097/QAI.0000000000001764
22. Maki PM, Martin-Thormeyer E. HIV, cognition and women. *Neuropsychol Rev.* (2009) 19:204–14. doi: 10.1007/s11065-009-9093-2
23. Singer M. AIDS and the health crisis of the U.S. urban poor; the perspective of critical medical anthropology. *Soc Sci Med.* (1994) 39:931–48. doi: 10.1016/0277-9536(94)90205-4
24. Tsai AC, Burns BF. Syndemics of psychosocial problems and HIV risk: a systematic review of empirical tests of the disease interaction concept. *Soc Sci Med.* (2015) 139:26–35. doi: 10.1016/j.socscimed.2015.06.024
25. Rubin LH, Springer G, Martin EM, Seaberg EC, Sacktor NC, Levine A, et al. Elevated depressive symptoms are a stronger predictor of executive dysfunction in HIV-infected women than men. *J Acquir Immune Defic Syndr.* (2019) 81:274–83. doi: 10.1097/QAI.0000000000002029
26. Klove H. *Clinical Neuropsychology*. New York, NY: Saunders (1963).
27. Reitan R, Wolfson D. *The Halstead-Reitan Neuropsychological Test Battery: Theory and Clinical Interpretation.* Tuscon, AZ: Neuropsychology Press (1985).
28. Golden CJ. *The Stroop Color and Word Test: A Manual for Clinical and Experimental Use.* Chicago, IL: Stoelting (1978).
29. Gronwall DMA. Paced auditory serial-addition task: a measure of recovery from concussion. *Perceptual Motor Skills.* (1977) 44:367–73. doi: 10.2466/pms.1977.44.2.367
30. Diehr MC, Heaton RK, Miller W, Grant I. The Paced Auditory Serial Addition Task (PASAT): norms for age, education, and ethnicity. *Assessment.* (1998) 5:375–87. doi: 10.1177/107319119800500407
31. Brandt J, Benedict R. *Hopkins Verbal Learning Test-Revised: Professional Manual*. Lake Magdalene, FL: Psychological Assessment Resources (2001).
32. Benedict RHB. *Brief Visuospatial Memory Test - Revised: Professional Manual*. Lutz, FL: Psychological Assessment Resources, Inc (1997).
33. Borkowski JG, Benton AL, Spreen O. Word fluency and brain damage. *Neuropsychologia.* (1967) 5:135–40. doi: 10.1016/0028-3932(67)90015-2
34. Wechsler D. *Wechsler Adult Intelligence Scale,* Vol. 4. San Antonio, TX: Pearson (2008).
35. Heaton RK, Miller SW, Taylor MJ, Grant I. *Revised Comprehensive Norms for an Expanded Halstead-Reitan Battery: Demographically Adjusted Neuropsychological Norms for African American and Caucasian Adults Scoring Program*. Odessa, FL: Psychological Assessment Resources (2004).
36. Norman MA, Moore DJ, Taylor M, Franklin D Jr, Cysique L, Ake C, et al. Demographically corrected norms for African Americans and Caucasians on the Hopkins Verbal Learning Test-Revised, Brief Visuospatial Memory Test-Revised, Stroop Color and Word Test, and Wisconsin Card Sorting Test 64-Card Version. *J Clin Exp Neuropsychol.* (2011) 33:793–804. doi: 10.1080/13803395.2011.559157
37. Lawton MP, Brody EM. Assessment of older people: self-maintaining and instrumental activities of daily living. *Gerontologist.* (1969) 9:179–86. doi: 10.1093/geront/9.3_Part_1.179
38. Wilkinson GS, Robertson GJ. *WRAT 4: Wide Range Achievement Test.* Lutz, FL: Psychological Assessment Resources (2006).
39. Beck AT, Steer RA, Brown GK. *Beck Depression Inventory-II*. San Antonio, TX: PsychCorp (1996).
40. Wittchen HU. Reliability and validity studies of the WHO–Composite International Diagnostic Interview (CIDI): a critical review. *J Psychiatr Res.* (1994) 28:57–84. doi: 10.1016/0022-3956(94)90036-1
41. Wehrens R, Buydens L. Self- and super-organizing maps in R: the kohonen package. *J Stat Softw.* (2007) 21:1–19. doi: 10.18637/jss.v021.i05
42. Fraley C, Raftery A, Scrucca L. *mclust: Normal Mixture Modeling for Model-Based Clustering, Classification, and Density Estimation* (2014).
43. Kuhn M. Building predictive models in R using the caret package. *J Stat Softw.* (2008) 28:1–26. doi: 10.18637/jss.v028.i05
44. Kuhn M. *Variable Importance Using the Caret Package* (2012).
45. Torgo L, Torgo M. *Package 'DMwR'*. Comprehensive R Archive Network (2013).
46. Van Buuren S, Groothuis-Oudshoorn K. mice: multivariate imputation by chained equations in R. *J Stat Softw.* (2011) 45:1–67. doi: 10.18637/jss.v045.i03
47. Hewitt RG, Parsa N, Gugino L. Women's health. The role of gender in HIV progression. *AIDS Read.* (2001) 11:29–33.
48. Griesbeck M, Scully E, Altfeld M. Sex and gender differences in HIV-1 infection. *Clin Sci.* (2016) 130:1435–51. doi: 10.1042/CS20160112
49. Maki PM, Rubin LH, Valcour V, Martin E, Crystal H, Young M, et al. Cognitive function in women with HIV: findings from the Women's Interagency HIV Study. *Neurology.* (2015) 84:231–40. doi: 10.1212/WNL.0000000000001151
50. Basso MR, Bornstein RA. Estimated premorbid intelligence mediates neurobehavioral change in individuals infected with HIV

across 12 months. *J Clin Exp Neuropsychol.* (2000) 22:208–18. doi: 10.1076/1380-3395(200004)22:2;1-1;FT208
51. Farinpour R, Miller EN, Satz P, Selnes OA, Cohen BA, Becker JT, et al. Psychosocial risk factors of HIV morbidity and mortality: findings from the Multicenter AIDS Cohort Study (MACS). *J Clin Exp Neuropsychol.* (2003) 25:654–70. doi: 10.1076/jcen.25.5.654.14577
52. Ruff RM, Parker SB. Gender- and age-specific changes in motor speed and eye-hand coordination in adults: normative values for the Finger Tapping and Grooved Pegboard Tests. *Perceptual Motor Skills.* (1983) 76:1219–30. doi: 10.2466/pms.1993.76.3c.1219
53. Bleecker ML, Bolla-Wilson K, Agnew J, Meyers DA. Age-related sex differences in verbal memory. *J Clin Psychol.* (1988) 44:403–11. doi: 10.1002/1097-4679(198805)44:3<403::AID-JCLP2270440315>3.0.CO;2-0
54. Kramer JH, Delis DC, Daniel M. Sex differences in verbal learning. *J Clin Psychol.* (1988) 44:907–15. doi: 10.1002/1097-4679(198811)44:6<907::AID-JCLP2270440610>3.0.CO;2-8
55. Kramer JH, Delis DC, Kaplan E, O'donnell L, Prifitera A. Developmental sex differences in verbal learning. *Neuropsychology.* (1997) 11:577–84. doi: 10.1037/0894-4105.11.4.577
56. Barrett-Connor E, Kritz-Silverstein D. Gender differences in cognitive function with age: the Rancho Bernardo study. *J Am Geriatr Soc.* (1999) 47:159–64. doi: 10.1111/j.1532-5415.1999.tb04573.x
57. Schmidt SL, Oliveira RM, Rocha FR, Abreu-Villaca Y. Influences of handedness and gender on the grooved pegboard test. *Brain Cogn.* (2000) 44:445–54. doi: 10.1006/brcg.1999.1204
58. Joffe H, Hall JE, Gruber S, Sarmiento IA, Cohen LS, Yurgelun-Todd D, et al. Estrogen therapy selectively enhances prefrontal cognitive processes: a randomized, double-blind, placebo-controlled study with functional magnetic resonance imaging in perimenopausal and recently postmenopausal women. *Menopause.* (2006) 13:411–22. doi: 10.1097/01.gme.0000189618.48774.7b
59. Arevalo MA, Azcoitia I, Garcia-Segura LM. The neuroprotective actions of oestradiol and oestrogen receptors. *Nat Rev Neurosci.* (2015) 16:17–29. doi: 10.1038/nrn3856
60. Engler-Chiurazzi EB, Brown CM, Povroznik JM, Simpkins JW. Estrogens as neuroprotectants: estrogenic actions in the context of cognitive aging and brain injury. *Prog Neurobiol.* (2017) 157:188–211. doi: 10.1016/j.pneurobio.2015.12.008
61. Epperson CN, Sammel MD, Freeman EW. Menopause effects on verbal memory: findings from a longitudinal community cohort. *J Clin Endocrinol Metab.* (2013) 98:3829–38. doi: 10.1210/jc.2013-1808
62. Weber MT, Rubin LH, Maki PM. Cognition in perimenopause: the effect of transition stage. *Menopause.* (2013) 20:511–7. doi: 10.1097/gme.0b013e31827655e5
63. Weber MT, Maki PM, Mcdermott MP. Cognition and mood in perimenopause: a systematic review and meta-analysis. *J Steroid Biochem Mol Biol.* (2014) 142:90–8. doi: 10.1016/j.jsbmb.2013.06.001
64. Seeman TE, Mcewen BS, Singer BH, Albert MS, Rowe JW. Increase in urinary cortisol excretion and memory declines: MacArthur studies of successful aging. *J Clin Endocrinol Metab.* (1997) 82:2458–65. doi: 10.1210/jc.82.8.2458
65. Cohen M, Deamant C, Barkan S, Richardson J, Young M, Holman S, et al. Domestic violence and childhood sexual abuse in HIV-infected women and women at risk for HIV. *Am J Public Health.* (2000) 90:560–5. doi: 10.2105/AJPH.90.4.560
66. Peavy G, Jacobs D, Salmon DP, Butters N, Delis DC, Taylor M, et al. Verbal memory performance of patients with human immunodeficiency virus infection: evidence of subcortical dysfunction. The HNRC Group. *J Clin Exp Neuropsychol.* (1994) 16:508–23. doi: 10.1080/01688639408402662
67. Becker JT, Caldararo R, Lopez OL, Dew MA, Dorst SK, Banks G. Qualitative features of the memory deficit associated with HIV infection and AIDS: cross-validation of a discriminant function classification scheme. *J Clin Exp Neuropsychol.* (1995) 17:134–42. doi: 10.1080/13803399508406588
68. White DA, Taylor MJ, Butters N, Mack C, Salmon DP, Peavy G, et al. Memory for verbal information in individuals with HIV-associated dementia complex. HNRC Group. *J Clin Exp Neuropsychol.* (1997) 19:357–66. doi: 10.1080/01688639708403864
69. Manly JJ, Touradji P, Tang MX, Stern Y. Literacy and memory decline among ethnically diverse elders. *J Clin Exp Neuropsychol.* (2003) 25:680–90. doi: 10.1076/jcen.25.5.680.14579
70. Baker DW, Parker RM, Williams MV, Clark WS. Health literacy and the risk of hospital admission. *J Gen Intern Med.* (1998) 13:791–8. doi: 10.1046/j.1525-1497.1998.00242.x
71. Stankoff B, Calvez V, Suarez S, Bossi P, Rosenblum O, Conquy L, et al. Plasma and cerebrospinal fluid human immunodeficiency virus type-1 (HIV-1) RNA levels in HIV-related cognitive impairment. *Eur J Neurol.* (1999) 6:669–75. doi: 10.1046/j.1468-1331.1999.660669.x
72. Valcour V, Yee P, Williams AE, Shiramizu B, Watters M, Selnes O, et al. Lowest ever CD4 lymphocyte count (CD4 nadir) as a predictor of current cognitive and neurological status in human immunodeficiency virus type 1 infection–The Hawaii Aging with HIV Cohort. *J Neurovirol.* (2006) 12:387–91. doi: 10.1080/13550280600915339
73. Ellis RJ, Badiee J, Vaida F, Letendre S, Heaton RK, Clifford D, et al. CD4 nadir is a predictor of HIV neurocognitive impairment in the era of combination antiretroviral therapy. *AIDS.* (2011) 25:1747–51. doi: 10.1097/QAD.0b013e32834a40cd
74. Cysique LA, Brew BJ. Neuropsychological functioning and antiretroviral treatment in HIV/AIDS: a review. *Neuropsychol Rev.* (2009) 19:169–85. doi: 10.1007/s11065-009-9092-3
75. Kramer-Hammerle S, Rothenaigner I, Wolff H, Bell JE, Brack-Werner R. Cells of the central nervous system as targets and reservoirs of the human immunodeficiency virus. *Virus Res.* (2005) 111:194–213. doi: 10.1016/j.virusres.2005.04.009
76. Spudich SS, Ances BM. Neurologic complications of HIV infection: highlights from the 2013 Conference on Retroviruses and Opportunistic Infections. *Top Antivir Med.* (2013) 21:100–8.
77. Rock PL, Roiser JP, Riedel WJ, Blackwell AD. Cognitive impairment in depression: a systematic review and meta-analysis. *Psychol Med.* (2014) 44:2029–40. doi: 10.1017/S0033291713002535

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Considering Biological Sex in Traumatic Brain Injury

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Published epidemiological studies of traumatic brain injury (TBI) of all severities consistently report higher incidence in men. Recent increases in the participation of women in sports and active military service as well as increasing awareness of the very large number of women who sustain but do not report TBI as a result of intimate partner violence (IPV) suggest that the number of women with TBI is significantly larger than previously believed. Women are also grossly under-represented in clinical and natural history studies of TBI, most of which include relatively small numbers of women, ignore the role of sex- and age-related gonadal hormone levels, and report conflicting results. The emerging picture from recent studies powered to detect effects of biological sex as well as age (as a surrogate of hormonal status) suggest young (i.e., premenopausal) women are more likely to die from TBI relative to men of the same age group, but this is reversed in the 6th and 7th decades of life, coinciding with postmenopausal status in women. New data from concussion studies in young male and female athletes extend this finding to mild TBI, since female athletes who sustained mild TBI are significantly more likely to report more symptoms than males. Studies including information on gonadal hormone status at the time of injury are still too scarce and small to draw reliable conclusions, so there is an urgent need to include biological sex and gonadal hormone status in the design and analysis of future studies of TBI.

Keywords: head trauma, concussion, men, women, sex differences

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INTRODUCTION

Traumatic brain injury is a major cause of death and disability, especially among young people, and a major public health problem throughout the world. Epidemiological studies of TBI, mostly relying on emergency department and hospitalization records, consistently report higher incidence in men (1–4), sometimes explained by the higher propensity of men to be involved in physical altercations, military service and contact sports. However, this contention may need to be revised in view of recent publications suggesting that millions of women are exposed to TBI or repeated concussions caused by intimate partner violence (IPV), which are often unreported and undetected (5, 6). Similarly, in most observational and clinical studies of TBI, women represent ∼30% or less of subjects (**Tables 1**, **2**). Consequently, the natural history, outcome and pathophysiology of TBI in women in general, and IPV victims in particular, have received little systematic investigation to date. Investigation of TBI and concussion outcome in women is also complicated by the fact that during their life time, women undergo massive and abrupt changes in gonadal hormone secretion at puberty and menopause, and are exposed to fluctuating levels of the same hormone across the menstrual cycle during their reproductive years.

Female and male gonadal hormones are known to exert many diverse effects on brain structure and function [reviewed in (39)] which are likely to modulate the brain response to trauma in multiple ways and may, in principle, offer sex-specific treatment targets. This is an important and timely question since with the increasing involvement of women in the military (40) and in sports, including contact sports such as Rugby (41); the number of women at risk for sustaining TBI or concussion is on the rise, while treatment algorithms are still based on the results of clinical studies with an overwhelming majority of men (70% or above), none of which resulted in an FDA approved treatment for the neurological sequelae of TBI; despite promising results from animal studies (**Table 1**). A comprehensive literature review of sex differences in human TBI and animal models has been published recently (42), with 73 papers demonstrating better TBI outcome in men relative to women, 41 papers which show the opposite (women better than men), 28 papers reporting no difference and 14 papers reporting mixed results. Obviously, these results are not conducive to understanding female-specific risks and attributes of TBI. The reasons for the discrepancy between human and animal studies have been addressed by us and others and are outside the scope of this minireview (42, 43).

Another emerging issue related to effects of biological sex on outcome of TBI is the recent recognition of the devastating long-term sequelae of repeated concussive TBIs (44), most notably chronic traumatic encephalopathy, a condition discovered and initially studied exclusively in male athletes (45).

Here we offer a concise critical review of emerging data on the effect of biological sex and hormonal status on TBI incidence and outcome, highlighting some possible mechanisms and identifying significant knowledge gaps which need to be filled in order to improve outcome of TBI.

TABLE 1 | Key clinical trials in TBI.

References	%women	Outcome	Analysis by Sex
Young et al. (7)	21	3-month GOS	No
Marshall et al. (8)	24	6-month GOS	No
Marmarou et al. (9)	29	3-month GOS	No
Morris et al. (10)	23	6-month GOS	No
Clifton et al. (11)	NR	6- month GOS	NR
Maas et al. (12)	18.6	6-month GOS	No
McCarthy et al. (13)	26	3-24 month GOS	No
Giacino et al. (14)	27	4-6 week DRS	No
Zafonte et a. (15)	25	3 months GOS	No
Skolnick et al. (16)	21	6-month GOS	No
Wright et al. (17)	26	6-month GOS-E	Yes (M > F)*
Nichol et al. (18)	16	6-month GOS-E	No
CRASH-3 collaborators (19)	19	4-week mortality	No
Rowell et al. (20)	26	6-month GOS-E	No

*GOS, Glasgow outcome scale; DRS, disability rating scale; GOS-E, Glasgow coma scale, extended. NR, not reported. M, men; F, women. M > F better outcome in men. *Trend, $p = 0.07$.*

Only phase III trials including > 300 subjects are included.

None of these studies was analyzed for a sex x age interaction.

TBI INCIDENCE AND PREVALENCE IN MEN AND WOMEN

Traumatic brain injury is a major public health concern and prominent cause of death and disability. Worldwide, in 2016, there were ∼27 million new cases of TBI with an age-adjusted incidence rate of 369 per 100,000—representing a 3.6% increase from 1990. In the same year, prevalence was 55.5 million individuals, representing an 8.4% increase from 1990 (46). In the US, TBI statistics published by the Centers for Disease Control and Prevention (1–3, 47) show that the combined rates for TBI-related emergency department (ED) visits, hospitalizations, and deaths in the United States have been on the rise and totaled 823.7 per 100,000 US population in 2010. Furthermore, an estimated cumulative 5.3 million individuals are living with a TBI-related disability in the United States. This represents a prevalence of ∼2% of the U.S. population (47).

Epidemiological studies consistently report higher incidence in men, such that the odds of sustaining a TBI are 2.22 times higher in men than in women (4). The reported TBI prevalence in the general population is 16.7% among males and 8.5% among females. Overall, males account for ∼59% of all reported TBI-related medical visits in the United States (48). This robust and consistent sex difference is sometimes explained by the higher propensity of men to be involved in physical altercations, military service, and contact sports (4). Sex differences in TBI incidence are modulated by age, and recent reports show that among the elderly (over 65), overall TBI incidence (49) and rates of ED visits for mild TBI were higher for women than for men (50). Similarly, rates of sports related injuries in young women seem to be equal or higher to those of men [e.g., (51–54), **Table 3**].

Importantly, studies relying on reported injuries and ED visits likely paint a distorted picture of the actual incidence of TBI, since they do not include TBIs suffered by female victims of intimate partner violence (IPV). IPV is a highly gendered behavior, such that the majority of perpetrators are men and the majority of victims are women, and TBIs suffered in this context are often unreported (79, 80). The Centers for Disease Control and Prevention (81) report that 32 million women in the United States have experienced IPV during their lifetime. Moreover, the National Intimate Partner and Sexual Violence Survey states that nearly one in four women in the United States have experienced severe physical violence (being hit, kicked, choked, beaten, burned, stabbed, or shot) during their lifetime by an intimate partner (82). Many of these violent attacks are likely to result in traumatic or anoxic brain injury, since it is common for abusers to target the victim's face, neck, and head (83, 84), with the prevalence of IPV-related TBI estimated as 60 to 90% (79, 85). A recent study found that more than 80% of IPV victims referred from homeless or domestic violence shelters sustained multiple TBIs, 84% had clinically significant symptoms, yet only 21% sought medical attention at the time of injury (85). This very low rate reflects the fact that many battered women

TABLE 2 | Representative observational studies of moderate and severe TBI biomarkers and outcomes.

References	N (%F)	Primary outcome	Analysis by	
			Sex	Sex X Age
Farin et al. (21)	957 (23)	ICP, Edema	Yes (M > F)	Yes (F < 50 worst)
King et al. (22)	159 (23)	12-month GOS	Yes (M = F)	No
Davis et al. (23)	13,247 (24)	In-Hospital mortality	Yes (M = F)	Yes (> 50F > M)
Corrigan et al. (24)	3,444 (28)	RTW	Yes (M > F)	Yes (> 55 F > M)
Berry et al. (25)	72,294 (30.8)	Mortality	Yes (F > M)	Yes (> 45 F > M)
Ottochian et al. (26)	1,807 (22)	Mortality	Yes (M > F)	Yes (> 55 M > F)
Yeung et al. (27)	(2979) 29	Mortality, edema	Yes (M = F, mortality M > F, edema)	NA*
Lavoie et al. (28)	175 (25)	Depression (PHQ-9)	Yes (M = F)	No
Walker et al. (29)	10,125 (27)	1-5 year GOS	Yes (M = F)	No
Puffer et al. (30)	1,169 (26)	GOS-E trajectory	No	No
Wilkins et al. (31)	304 (19)	6-24 month GOS-E	No	No
Stromberg et al. (32)	7,867 (25)	RTW	Yes (M = F)	No
Deng et al. (33)	264 (22)	ICP, edema, surgery	Yes (M = F, edema) (F > M, surgery)	No
Gruen et al. (34)	164 (25)	30 day mortality	No	No
Mellett et al. (35)	429 (22)	Mortality, GOS, NRS, DRS	No	No
Okonkwo et al. (36)	1,359 (32)	GFAP, GOS-E	No	No
Xu et al. (37)	1,206 (32)	CRP, GOS-E	No	No
Kerezoudis et al. (38)	2,508 (35)	Mortality	No	NA**

ICP, intracranial pressure; GOS, Glasgow outcome scale; RTW, return to work; GOS-E, Glasgow coma scale, extended; NRS, Neurobehavioral rating scale; DRS, disability rating scale, GFAP, Glial Fibrillary acidic protein; CRP, c-reactive protein.
*NA, not applicable, *Study only included young women **All participants were elderly.*

may never go to the Emergency Department or get treated by health care providers (86–89) resulting in underreporting and poor detection. Given the numbers of women over the age of 15 experiencing IPV, there could be more than 31,000,000 women who have received a traumatic brain injury (90) in the US today- a number which should radically change our perception of TBI demographics.

BIOLOGICAL SEX AND TBI OUTCOME

TBI outcome is highly variable: Moderate and severe injuries may result in death, persistent vegetative state, severe disability, moderate disability, or good recovery, which form the basis for the most commonly used TBI outcome scales—initially the 5 step Glasgow outcome scale, (GOS), more recently replaced by the 8 step extended GOS (GOS-E). Mild TBI and concussion, which actually account for the majority (∼75%) of TBI cases (2), rarely result in death or severe disability but are often associated with long term changes in cognition and behavior (91, 92), which can be assessed by scales such as the Rivermeade post-concussion questionnaire [RPQ, (22, 93)]. Early studies which established risk factors for poor outcome in TBI, such as advancing age (94) did not report analyses by sex.

The influence of biological sex on the outcome of TBI has been the subject of several analyses with contradictory results, possibly due to the relatively small number of women and girls in clinical studies, lack of information on hormonal status and the wide disparity in outcome measures used for the comparison, which included such disparate measures as return to work, bacteremia and mortality [(42); **Tables 1**, **2**]. Thus, studies comparing men and women without paying attention to hormonal status or age report no differences in outcome, better outcome in women or better outcome in men (27, 95–99). Probably for the same reason, the recognition of various risk factors for poor TBI outcome, including advanced age, is biased toward men, and this has not changes since age as a risk factor for poor TBI outcome was first reported by Teasdale et al. (94). Consequently the treatment guidelines for head injury [e.g., (100, 101)] are heavily influenced by findings in male patients. Differences between the sexes in the frequencies of risk factors and their effect on early and late outcome as measured by TBI-specific outcome scales (i.e., GOS and GOSE) have not been systematically investigated to date. Similarly, research on mild TBI and concussion, which actually account for the majority (∼75%) of TBI cases (2) in the general population is also plagued by small studies, disparate outcome measures and paucity of women (see **Table 3**). Understandably, mild TBI/concussion is more highly prevalent among athletes and the military (102, 103). While studies focusing on contact sports in which women are not represented (91, 104, 105) do not contribute to the question at hand, there is a recent explosion of publications on sex differences in incidence and outcome of sports related concussions in sports in which women do engage (**Table 3**). These studies include a higher proportion of women relative to clinical and observational studies moderate—severe

TABLE 3 | Representative studies of mild TBI and sports concussion incidence and outcomes.

References	N (%F)	Primary outcome	Analysis by	
			Sex	Sex and Age
Colvin et al. (55)	234 (60.3)	PCS, IMPACT	Yes (M > F)	NA
Preiss et al. (56)	260 (34)	PCS	Yes (M > F)	Yes (MinorF > adultF)
Bazarian et al. (57)	1425 (45.1)	RPQ, PCS	Yes (M > F)	Yes (F < 55 worst)
Covassin et al. (58)	296 (31.4)	IMPACT, PCS	Yes (M > F)	NA
Styrke et al. (59)	163 (31.8)	RPQ 3 years	Yes (M > F)	No
McMahon et al. (60)	375 (29.9)	GOS-E, BSI-18, RPQ	No	No
BluMfeld et al. (61)	1500 (40)	Incidence, Symptoms, duration	Yes (M > F)	No
Ma et al. (62)	108 (100)	Incidence	NA	NA
Albanese et al. (63)	53 (50.8)	PCS, ASI-3, DTS, NSI	Yes (M > F)	No
Brickell et al. (64)	172 (50)	NSI, PCL-C	Yes (M > F)	NA
Chandran et al. (65)	580 (51)	Incidence, duration	Yes (M > F)	NA
Harrold et al. (66)	426 (58)	SCAT3 and K-D	Yes (M > F)	No
MacDonald et al. (67)	94 (7)	5 year GOS-E	No	No
Mollayeva et al. (68)	94 (38)	Pain	Yes (M = F)	No
Roos et al. (51)	3825 (59)	Incidence	Yes (M > F)	No
Rosene et al. (52)	415 (30)	Incidence	Yes (M = F)	No
Bahraini et al. (69)	4012 (5.4)	NSI	Yes (M > F)	NA
Lippa et al. (70)	158 (50)	NSI, PTSD, AIS.	Yes (M > F)	NA
Terry et al. (71)	1265 (42)	Symptom duration	Yes (M > F)	No
Varriano et al. (72)	436 (42.6)	PCS rate	Yes (M > F)	Yes (Young > old)
Nelson et al. (73)	1154 (34.4)	GOS-E 3, BSI-18, RPQ	No	No
Yue et al. (74)	100 (29)	GOS-E	Yes (M > F)	NA
Combs et al. (75)	494 (44.7)	Graded symptom checklist	Yes (M > F)	NA
Kennedy et al. (76)	184 (10.3)	Depression	Yes (M > F)	NA
Putukian et al. (77)	1922 (33.8)	Incidence, duration	Yes (M > F)	NA
Spano et al. (78)	778 (11.4)	Incidence, Symptoms, duration	Yes (M > F)	No

PCS, post-concussion syndrome; IMPACT, Immediate Post-concussion Assessment and Cognitive Testing; RPQ, Rivermeade Post-concussion Questionnaire; BSI-18, Brief Symptom Inventory 18; Asl-3, Anxiety Sensitivity Index-3; DTS, Distress tolerance scale; NSI, Neurobehavioral Symptom Index; PCL-C, PTSD checklist, civilian version; SCAT-3, Sport Concussion Assessment Tool 3; K-D, King-Devick scale; GSC, Graded symptom checklist.
NA, not applicable, all participants were young.

TBI (**Tables 1**, **2**) and despite the wide range of sports included, from water polo through rugby to Jio-Jistsu, and the very large variation in outcome measures (**Table 3**), there appears to be a near consensus that women are more likely to receive concussions in sports and have a worse outcome. However, these studies cannot be generalized to the population at large since they typically include only young healthy subjects engaging in sports (**Table 3**).

Biological Sex, Age and TBI-Related Mortality

The picture is somewhat less confusing if we focus our attention on relatively large (N $\geq$ 1000 total) studies reporting "hard," completely objective outcome measures such as mortality and persistent vegetative state which segregate outcome by both age and sex. An early example is the community study published by Klauber et al. (95), which reported no effect of sex on mortality. However, upon perusal of the breakdown of mortality data by sex as well as age (in decades), The results show that between puberty and old age, there is no significant effect of age on mortality in women, while mortality in men shows a strong and highly significant association with increasing age, as would be expected from prior studies (94). This sex x age interaction on the outcome of TBI results in a reversal of a sex difference in TBI mortality, which occurs around age 50, thereby negating an overall effect of sex on outcome. Thus, between the ages of fifteen and fifty, men have a (small) mortality advantage over women in the same age groups, but this is reversed after age fifty, when men are significantly more likely to die relative to younger men or women in all age groups. This pattern emerged in the absence of sex differences in injury severity (95). Assuming age is a reasonable surrogate for hormonal status in the absence of actual data, age 15 and over may be considered to be post pubertal and women over 50 may be considered to be mostly post-menopausal. This age cutoff is commonly used to separate mostly pre- and mostly postmenopausal female patients because the majority of women reach menopause during the decade between 45 and 55 years of age (106, 107), whereas in men a continuous decline in testosterone levels is associated with ages >50 years (108).

In a subsequent study involving 25,300 emergency head-related admissions, it was found that women were more likely to die from all head injuries (OR = 1.3) with an even higher likelihood of death from violent head injuries (OR = 2.38). The authors also note that women 15 or older stayed in the hospital longer than men (109).

Davis et al. (23) published a study of a total of 13,437 patients (n = 3,178 females and 10,259 males) with moderate-to-severe TBI (head AIS > or = 3) from a county trauma registry. While overall mortality was similar in men and women, a separate analysis performed for premenopausal (< 50 years) vs. postmenopausal (> or = 50 years) patients, after stratification by decade of life, revealed no statistically significant difference in mortality of pre-menopausal females vs. males, though outcome was significantly better in postmenopausal females vs. males (OR 0.63, 95% CI) with similar rates of hypotension (Systolic blood pressure < 90 mm Hg), head Abbreviated Injury Score (AIS), and Injury Severity Score (ISS). Stratification by decade of life revealed the gender survival differential inflection point to occur between ages 40-49 (OR 1.06, 95% CI 0.66-1.71, $p = 0.798$) and ages 50-59 (OR 0.38, 95% CI 0.20-0.74, $p = 0.005$). The authors then conclude that endogenous female sex hormone production is not neuroprotective in human TBI. These results also dovetail with those of studies performed more recently (25, 27). Berry et al. (25) examined records of 72,294 moderate and severe injury patients from the National Trauma Database (2000–2005) and found that peri- and postmenopausal women (Age more than 45) demonstrated improved survival relative to men, but premenopausal women did not. The exception to this trend is the (relatively modestly sized) study by Ottochian et al. (26); which included 1,800 subjects (22% women) and reported a survival advantage for men over 55 relative to women over 55. The study by Yeung et al. (27), which included women under 45, reported no survival advantage in "hormonally active" women as compared to men in the same age range. Consequently, authors of both papers concur that estrogen does not appear to confer neuroprotection in women after TBI.

Sex Differences in Mild TBI/Concussion Outcome

Sex differences in concussion incidence and outcome were reviewed by Dick (110) who concluded that the literature supports higher incidence and worse outcome in women. A later review (111) opined the literature reviewed did not support this conclusion. **Table 3** features representative subsequent studies, a few of which stratified data by sex as well as age. Thus, in a 2010 study, Bazarian et al. (57) examined mTBI outcome in 1425 mTBI patients (45.1% female) presenting to an academic emergency department. Men were significantly less likely to be in a higher Post concussive symptoms (PCS) score category relative to women (OR = 0.62), and this association was more prominent during child-bearing years (between puberty and menopause) for females. The authors conclude that female sex is associated with significantly higher odds of poor outcome after mTBI, as measured by PCS score, after control for appropriate confounders. This conclusion resonates with the results of a 2009 study (56) reporting no sex difference in post-concussion symptoms among minors (presumably mostly pre-pubertal subjects) with worse outcome in adult women (**Table 3**). This study did not include women older than 50. This is common in many of the more recently published studies on concussion in men and women performed in athletes engaged in a variety of sports and in military personnel, and therefore including a relatively young population (**Table 3**). With this caveat in mind, there appears to be a near-consensus that across different sports, women are more likely than men to suffer sports-related concussions, report more symptoms, have a slower recovery and overall more negative outcome. The latter observations were also reported in an exquisitely designed study focusing on female service members (64), whereby women (N = 86) reported more symptoms despite having been matched with the male comparison group (N = 86) for TBI severity, mechanism of injury, bodily injury severity, days post-injury, age, number of deployments, theater where wounded, branch of service, and rank. A pilot publication on 100 subjects with mTBI (29% women) from the TRACK TBI study examined PTSD as an outcome and concluded that sex may interact with age for PTSD symptomatology, with females 30–39 y at highest risk (74). The authors conclude that prevention and rehabilitation/counseling strategies after mTBI should likely be tailored for age and sex. Rather disappointingly, larger studies published using TRACK-TBI data, while including a similar percentage of women, did not use sex as a grouping variable in the analysis of outcomes [(60, 73), **Table 3**].

Neurodegenerative Disease Following Single or Repeated TBI in Men and Women

Traumatic brain injury is believed to be an important risk factor for neurodegenerative diseases, such as Alzheimer's disease (AD) and chronic traumatic encephalopathy (CTE) (112, 113). Despite the fact that AD incidence and prevalence is significantly higher in women relative to men, and significant sex differences in the disease trajectory and response to treatment (114–116), there is no information on whether the AD risk associated with TBI is modulated by biological sex.

Chronic traumatic encephalopathy (CTE), a dementia-like syndrome which manifests at younger ages than AD, appears to be linked to repeated exposure to Mild TBIs/concussions rather than a single TBI and is associated with an anatomically distinct pattern of tau deposition in the absence of significant amyloid deposits (105, 117). This condition was initially characterized and subsequently studied exclusively in male athletes and military personnel (44, 45, 118–120). Consequently, there are no reports on TBI-related CTE in women since the modern definition of this entity. Tantalizingly, the only description of CTE-like brain pathology in a woman is a case study published by Roberts et al. (121) titled "Dementia in a punch-drunk wife," describing a woman who died following prolonged and severe IPV.

POSSIBLE MECHANISMS UNDERLYING SEX DIFFERENCES IN TBI OUTCOME

The mechanisms underlying the relatively poor outcome of young women with TBI and concussion are not known, though several suggestions have been made based on small studies in mild TBI. Thus, Albanese et al. (63) propose that higher anxiety sensitivity mediates gender differences in post concussive symptoms, and another study cites higher preinjury migraine rates in women as a reason for longer time to return to school and sports among concussed female athletes (71). Attempts to use a similar approach regarding depression yielded conflicting results (28, 76). Yue et al. (74), summarizing results from the TRACK-TBI pilot, make the general observation that the sex differences they observe "may be attributable to cortical maturation, biological response, social modifiers, and/or differential self-report" although suspected sex differences in the latter variable have not been consistent when examined in the athlete population (122, 123). In addition, Alsalaheen et al. (124) and Grafton et al. (125) invokes different strategies to stabilize the head in response to impulsive loads as a possible explanation for sex differences in concussion injury risk. Recent studies also suggest sex differences in biomechanics of concussion in sports (126). However, these studies usually report higher impacts in males and it is hard to see how these findings can explain the consistent findings of worse outcome in women.

Effects of Gonadal Steroid Levels

Both female and male gonadal hormones are known to exert multiple diverse effects on brain structure and function; which can be roughly divided into irreversible (organizational) effects during brain development and reversible (activational) effects after puberty [reviewed in (39)]. Numerous, though not all, animal studies suggested that female sex hormones improve brain injury outcome [Reviewed in (42, 127)]. These animal studies led to a series of clinical trials of progesterone in human TBI, however the pivotal phase III trials failed to provide any evidence of improvement in outcome [(16), **Table 1**]. This was the only human study in which gonadal steroid levels were manipulated through hormone administration in men and women. Interestingly, the studies were not designed to examine outcome by sex. In a study of the relationship between endogenous progesterone levels and menstrual cycle phase in women (128), the authors found that women injured during the luteal phase of their menstrual cycle, when progesterone concentration is high, had significantly lower General Health Ratings and higher RPQ somatic scores one month after injury than women injured during the follicular phase of their cycle, suggesting that high ambient levels of female gonadal steroids have a negative rather than a positive effect on mTBI outcome. In a similar vein, estradiol was identified as a "potent mortality marker," with strong relationships between increased serum E2 levels and elevated mortality risk after severe TBI reported by Wagner et al. (129). NB, the study populations was mostly male and results were not analyzed be sex.

Sex Differences in Brain Volume

Total intracranial volume has been shown to be an independent predictor of the effect of TBI on intelligence, in accordance with the cognitive reserve theory (130). In a similar vein, Ystad et al. (131) reported a highly significant correlation between hippocampal volume and performance of a verbal memory (CVLT) task, and Umile et al. (132) confirmed the vulnerability of the medial temporal lobe to mild TBI, which correlated with neuropsychological deficits. A recent study of cognitive outcome in TBI demonstrated a significant declines (relative to individual premorbid intelligence) in abstract reasoning as measured by Raven's progressive matrices–R (RPM-R) in moderate-severe as well as mild TBI (133). In this study, there was a highly significant correlation between the volume of the insula and deficits in RPM-R performance. These studies did not report data from women.

Sex differences in intracranial volume, brain size and regional size, are found from birth and are thought to reflect organizational effects of gonadal steroids which occur during fetal brain development. On average, men have a larger brain size than women as denoted by a higher intracranial volume (ICV) and total brain volume (∼8–15% larger volumes in men), higher tissue/region-specific volume (134), a greater amount of neurons, increased global cortical thickness and larger total cortical surface area relative to women (135–137). In a more recent article which compared 58 young women and 44 young men, Martinez et al. showed ICV and total brain volume were highly significantly smaller in women relative to men ($t = 8.22$, $p < 0.00005$ and $t = 7.61$, $p < 0.00005$, respectively). Importantly, the size of the sex difference in regional brain volumes diminishes with advancing age (138).

A similar sex X age interaction was observed in several regional studies: In an early study focusing on the corpus callosum (139), the cross sectional area of the corpus callosum and splenium was measured off a midsagittal MRI image from subjects with Alzhemier's disease (AD), age matched elderly controls, and young controls. Analysis of the healthy control data by sex and age shows reduction in callosal area with age in men which is not observed in women, resulting in a reversal of the sex difference seen in young controls (men > women) when the comparison is performed in elderly subjects (Women > men). In another study focusing on hippocampal volume, analysis of the healthy control population in the ADNI data base (115) showed that hippocampal volume (mean(STD) 7175 (886) mm^3 $N = 187$ women vs. 7539 (935) mm^3, $N = 192$ men) is slightly (5%) but significantly ($p < 0.0001$) higher in elderly men relative to women, while the sex difference in intracranial volume was 12.7% [mean 1423 cc in women and 1604 in men, (115)]. These results dovetail with long-standing evidence of earlier and steeper age-related declines in brain regional volumes in men relative to women (140–142) which has also been confirmed for the hippocampus. For example, in a study of hippocampal volume in early adulthood [39 men and 41 women, age 18–42 years, (143)], a significant negative correlation with age for both left and right hippocampus was found in men ($r = -0.47$ and -0.44, respectively) but not in women ($r = 0.01$ and 0.02, respectively). The volume decline in men appeared to

be linear, starting at the beginning of the third life decade and ∼1.5% per annum.

Investigations of moderate and severe TBI have demonstrated significant brain atrophy over the first year after injury in many brain regions, even those that are remote from direct injury, including the cingulate gyrus and the hippocampus (144–148). As in most other studies of TBI, the number of women included in these rather small studies did not support analysis of a sex x age interaction. To elaborate, the Schonberger (147) study included 74 men and 24 women, mostly under 50, and the Zhou study (148) examined 27 men and only 5 women.

Taken together, the sex x age interaction on TBI outcome and brain volumes described above supports the notion that women, possibly due to their smaller total and regional brain size (smaller brain reserve), have a worse outcome of moderate-severe as well as mild TBI/concussion compared to men; but this difference may be diminished or even reversed with advancing age since brain reserve is diminished at a steeper rate in men relative to women.

Sex and Age Differences in Brain Swelling

The well-documented effects of female gonadal hormones on fluid balance (149–151) and the high frequency of idiopathic intracranial hypertension in premenopausal female patients (152, 153) supports the likelihood of differences between the sexes in frequencies of brain swelling and intracranial hypertension following TBI, specifically in presumably premenopausal women (< 50 years of age). Brain swelling (edema) and the resultant increase in intracranial pressure are known risk factors for poor outcome in humans as well as in animal models of TBI (154–159). If TBI-related swelling and ICP time-dose are indeed influenced by sex and hormonal status, i.e., higher in young women than in post-menopausal women, this could be another contribution to the sex x age interaction on outcome described above.

The Tirilizad study was one of the first clinical studies in TBI to include outcome, CT and intracranial pressure data indicative of brain swelling in a study population large and diverse enough to enable statistically powered comparisons of brain swelling between young and >50 male and female patients with moderate/severe head injury (8, 21). Overall, female patients had a significantly greater frequency of brain swelling visualized on CT than male patients−35% compared with 24% ($p = 0.0008$). This increased frequency was characteristic of premenopausal women (< 51 years of age), who had a 38% rate of swelling compared with 24% among their male counterparts ($p = 0.002$), which did not change with age (21). The frequency in postmenopausal female patients (> 50 years of age) was comparable to the frequency in men. Subsequent analysis showed that the increased frequency of brain swelling in female patients was not due to higher injury severity or other confounders including advanced age, presence of subarachnoid hemorrhage (SAH), or systemic hypotension. Further analysis of the relationship between intracranial hypertension (defined as an ICP >20 mm Hg during >25% of the time it was monitored) and sex demonstrated a significantly greater frequency of intracranial hypertension among female compared with male patients (39% compared with 31%; $p < 0.03$). The sex-related difference in frequency was even more dramatic in the population < 50 years (40% compared with 30%; $p < 0.02$). The increased frequency of intracranial hypertension in women and girls was not due to increased injury severity. As was the case with brain edema, the difference in rates of intracranial hypertension between the sexes was most significant among the less severely injured patients (GCS scores of 7 or 8 [33% compared with 20%; $p < 0.02$]) (21).

The findings from the Tirilizad study were corroborated in a more recent international study of TBI, showing that brain edema was associated with female sex ($P = 0.02$), and the odds of brain edema in females were greater than for males in a cohort of young subjects recruited in Hong Kong (27). The second cohort included in this study, recruited in Australia, demonstrated a smaller sex difference in the same direction which did not reach statistical significance. This study recruited subjects in the age range 12–45 so that only premenopausal females were included.

SUMMARY AND CONCLUSIONS

Research conducted in the last couple of decades has significantly improved our understanding of the impact of biological sex on TBI incidence and outcome. However, some glaring still exist due to the slow and incomplete acceptance of the imperative to include women in TBI studies and report results stratified by sex, which need to be proactively addressed in the future.

Key Findings

- There is increasing recognition of the high prevalence of TBI among the tens of millions of women who live with domestic violence and fail to report- or seek medical attention for-their injuries.
- Recent studies of TBI outcome which include adequate numbers of women challenge the long held view (based on animal studies) that reproductive-age women, by virtue of high levels of estrogen and progesterone, are likely to have a better TBI outcome relative to men.
- Accumulating evidence shows that reproductively competent women (after puberty and before menopause) are at higher risk for poor outcome, while postmenopausal women fare better than men of similar age (>50 years old), whose outcome worsen with age.

Knowledge Gaps

- While recent findings suggest an important contribution of gonadal hormone levels to clinical outcome of TBI of all severities, these variables are not assessed or measured in the overwhelming majority of TBI studies.
- The safety and efficacy of old and new TBI interventions in women across the life span is unknown
- The importance of risk factors for poor outcome of TBI, established in mostly-male populations, is largely unknown in women

Next Steps

- Female subjects with TBI need to be proactively sought out and recruited from domestic violence shelters and agencies.

- Results of clinical and research studies on TBI need to be stratified by sex and gonadal hormone status.
- Female TBI victims need to be queried about their hormonal status, i.e., Pre- or post-pubertal, pre- or post-menopausal and if reproductively competent, estimated stage of menstrual cycle (last menstrual period).
- Both men and women can benefit from actual acute and repeated measurement of sex steroid levels, including androgens, estrogens, progesterone, in order to understand possible sex-specific impact of TBI on reproductive health and possibly provide new sex-sensitive guidelines and sex-specific hormone-based treatment targets.

AUTHOR CONTRIBUTIONS

AB conceived of and wrote the paper.

REFERENCES

1. Centers for Disease Control and Prevention. *Report to Congress on Mild Traumatic Brain Injury in the United States: Steps to Prevent a Serious Public Health Problem*. Atlanta, GA: National Center for Injury Prevention and Control. (2003). Available online: https://www.cdc.gov/traumaticbraininjury/pdf/mtbireport-a.pdf
2. Centers for Disease Control and Prevention. *Traumatic Brain Injury in the United States: Fact sheet*. (2014). www.cdc.gov/traumaticbraininjury/get_the_facts.html.
3. Centers for Disease Control and Prevention. *Surveillance Report of Traumatic Brain Injury-Related Emergency Department Visits, Hospitalizations, Deaths—United States. 2014*. Atlanta, GA. (2019). Available online at: https://stacks.cdc.gov/view/cdc/78062
4. Frost RB, Farrer TJ, Primosch M, Hedges DW. Prevalence of traumatic brain injury in the general adult population: a meta-analysis. *Neuroepidemiology*. (2013) 40:154–9. doi: 10.1159/000343275
5. Centers for Disease Control and Prevention. Adverse health conditions and health risk behaviors associated with intimate partner violence. *Morb Mortal Weekly Rep*. (2008) 57:113–7.
6. Monahan K, Goldfine A, Biegon A. Traumatic brain injuries in victims of intimate partner violence: an underappreciated source of neurological morbidity. *Fut Neurol.* (2017) 12:189–91. doi: 10.2217/fnl-2017-0026
7. Young B, Runge JW, Waxman KS, Harrington T, Wilberger J, Muizelaar JP, et al. Effects of pegorgotein on neurologic outcome of patients with severe head injury. *JAMA*. (1996) 276:583–5434.
8. Marshall LF, Maas AI, Marshall SB, Bricolo A, Fearnside M, Klauber MR, et al. A multicenter trial on the efficacy of using tirilazad mesylate in cases of head injury. *J Neurosurg.* (1998) 89:519–25. doi: 10.3171/jns.1998.89.4.0519
9. Marmarou A, Nichols J, Burgess J, Newell D, Troha J, Burnhame D, et al. Effects of the bradykinin antagonist Bradycor (deltibant, CP-1027) in severe traumatic brain injury: results of a multi-center, randomized, placebo-controlledtrial. American Brain Injury Consortium Study Group. *J. Neurotrauma.* (1999) 16:431–44. doi: 10.1089/neu.1999.16.431
10. Morris GF, Bullock R, Marshall SB, Marmarou A, Maas A, Marshall LF. Failure of the competitive N-methyl-D-aspartate antagonist Selfotel (CGS 19755) in the treatment of severe head injury: results of two-phase III clinical trials. The Selfotel Investigators. *J. Neurosurg.* (1999) 91:737–43. doi: 10.3171/jns.1999.91.5.0737
11. Clifton GL, Miller ER, Choi SC, Levin HS, McCauley S, Smith Jr KR, et al. Lack of effect of hypothermia in acute brain injury. *N Engl J Med.* (2001) 344:556–63. doi: 10.1056/NEJM200102223440803
12. Maas A, Murray G, Henney III H, Kassem N, Legrand V, Mangelus M, et al. Efficacy and safety of dexanabinol in severe traumatic brain injury: results of a phase III randomized, placebo-controlled, clinical trial. *Lancet Neurol*. (2005) 5:38–45. doi: 10.1016/S1474-4422(05)70253-2
13. McCarthy MC, Moncrief H, Sands JM, Markert RJ, Hall LC, Wenker IC, et al. Neurologic outcomes with cerebral oxygen monitoring in traumatic brain injury. *Surgery*. (2009) 146:585–91. doi: 10.1016/j.surg.2009.06.059
14. Giacino JT, Whyte J, Bagiella E, Kalmar K, Childs N, Khademi A, et al. OTR, Mercer W, Maurer-Karattup P, Dr,Rer.Nat., Sherer M. Placebo-controlled trial of amantadine for severe traumatic brain injury. *N Engl J Med.* (2012) 366:819–26. doi: 10.1056/NEJMoa1102609
15. Zafonte RD, Bagiella E, Ansel BM, Novack TA, Friedewald WT, Hesdorffer DC, et al. Effect of citicoline on functional and cognitive status among patients with traumatic brain injury: Citicoline Brain Injury Treatment Trial (COBRIT). *JAMA*. (2012) 308:1993–2000. doi: 10.1001/jama.2012.13256
16. Skolnick BE, Maas AI, Narayan RK, van der Hoop RG, MacAllister T, Ward JD, et al. A clinical trial of progesterone for severe traumatic brain injury. *N Engl J Med*. (2014) 371:2467–76. doi: 10.1056/NEJMoa1411090
17. Wright DW, Yeatts SD, Silbergleit R, Palesch YY, Hertzberg VS, Frankel M, et al. Very early administration of progesterone for acute traumatic brain injury. *N Engl J Med.* (2014) 371:2457–66. doi: 10.1056/NEJMoa1404304
18. Nichol A, French C, Little L, Haddad S, Presneill J, Arabi Y, et al. Erythropoietin in traumatic brain injury (EPO-TBI): a double-blind randomised controlled trial. *Lancet.* (2015) 386:2499–506. doi: 10.1016/S0140-6736(15)00386-4
19. CRASH-3 trial collaborators. Effects of tranexamic acid on death, disability, vascular occlusive events and other morbidities in patients with acute traumatic brain injury (CRASH-3): a randomised, placebo-controlled trial. *Lancet*. (2019) 394:1713–23. doi: 10.1016/S0140-6736(19)32233-0
20. Rowell SE, Meier EN, McKnight B, Kannas D, May S, Sheehan K, et al. Effect of out-of-hospital tranexamic acid vs placebo on 6-month functional neurologic outcomes in patients with moderate or severe traumatic brain injury. *JAMA*. (2020). 324:961–74. doi: 10.1001/jama.2020.8958
21. Farin A, Deutsch R, Biegon A, Marshall LF. Sex-related differences in patients with severe head injury: greater susceptibility to brain swelling in female patients 50 years of age and younger. *J Neurosurg.* (2003) 98:32–6. doi: 10.3171/jns.2003.98.1.0032
22. King NS, Crawford S, Wenden FJ, Moss NE, Wade DT. The Rivermead Post Concussion Symptoms Questionnaire: a measure of symptoms commonly experienced after head injury and its reliability. *J Neurol.* (1995) 242:587–92. doi: 10.1007/BF00868811
23. Davis DP, Douglas DJ, Smith W, Sise MJ, Vilke GM, Holbrook TL, et al. Traumatic brain injury outcomes in pre- and post-menopausal females versus age-matched males. *J Neurotrauma.* (2006) 23:140–8. doi: 10.1089/neu.2006.23.140
24. Corrigan JD, Lineberry LA, Komaroff E, Langlois JA, Selassie AW, Wood KD. Employment after traumatic brain injury: differences between men and women. *Arch Phys Med Rehabil.* (2007) 88:1400–9. doi: 10.1016/j.apmr.2007.08.006
25. Berry C, Ley EJ, Tillou A, Cryer G, Margulies D, R., et al. The effect of gender on patients with moderate to severe head injuries. *J Trauma.* (2009) 67:950–3. doi: 10.1097/TA.0b013e3181ba3354
26. Ottochian M, Salim A, Berry C, Chan LS, Wilson MT, Margulies DR. Severe traumatic brain injury: is there a gender difference in mortality? *Am J Surg.* (2009) 197:155–8. doi: 10.1016/j.amjsurg.2008.09.008
27. Yeung JHH, Mikocka-Walus AA, Cameron PA, Poon WS, Ho HF, Chang A, et al. Protection from traumatic brain injury in hormonally active women vs men of a similar age: a retrospective international study. *Arch Surg.* (2011) 146:436–42. doi: 10.1001/archsurg.2011.46
28. Lavoie S, Sechrist S, Quach N, Ehsanian R, Duong T, Gotlib IH, et al. Depression in men and women one year following traumatic brain injury (TBI): a TBI model systems study. *Front Psychol.* (2017) 8:634. doi: 10.3389/fpsyg.2017.00634
29. Walker WC, Stromberg KA, Marwitz JH, Sima AP, Agyemang AA, Graham KM, et al. Predicting long-term global outcome after traumatic brain injury: development of a practical prognostic tool using the traumatic brain

injury model systems national database. *J Neurotrauma.* (2018) 35:1587–95. doi: 10.1089/neu.2017.5359
30. Puffer RC, Yue JK, Mesley M, Billigen JB, Sharpless J, Fetzick AL, et al. Recovery trajectories and long-term outcomes in traumatic brain injury: a secondary analysis of the phase 3 citicoline brain injury treatment clinical trial. *World Neurosurg.* (2019) 125:e909–e15. doi: 10.1016/j.wneu.2019.01.207
31. Wilkins TE, Beers SR, Borrasso AJ, Brooks J, Mesley M, Puffer R, et al. Favorable functional recovery in severe traumatic brain injury survivors beyond six months. *J Neurotr.* (2019) 36:3158–63. doi: 10.1089/neu.2018.6153
32. Stromberg KA, Agyemang AA, Graham KM, Walker WC, Sima AP, Marwitz JH, et al. Using decision tree methodology to predict employment after moderate to severe traumatic brain injury. *J Head Trauma Rehab.* (2019) 34:E64–74. doi: 10.1097/HTR.0000000000000438
33. Deng H, Zusman BE, Nwachuku EL, Yue JK, Chang YF, Conley YP, et al. B-Cell lymphoma 2 (Bcl-2) gene is associated with intracranial hypertension after severe traumatic brain injury. *J Neurotrauma.* (2020). doi: 10.1089/neu.2020.7028
34. Gruen DS, Guyette FX, Brown JB, Okonkwo DO, Puccio AM, Campwala IK, et al. Association of prehospital plasma with survival in patients with traumatic brain injury: a secondary analysis of the PAMPer cluster randomized clinical trial. *JAMA Netw Open.* (2020) 3:e2016869. doi: 10.1001/jamanetworkopen.2020.16869
35. Mellett K, Ren D, Alexander S, Osier N, Beers SR, Okonkwo DO, et al. Genetic variation in the TP53 gene and patient outcomes following severe traumatic brain injury. *Biol Res Nurs.* (2020) 22:334–40. doi: 10.1177/1099800420912335
36. Okonkwo DO, Puffer RC, Puccio AM, Yuh EL, Yue JK, Diaz-Arrastia R, et al. Point-of-Care platform blood biomarker testing of glial fibrillary acidic protein versus S100 calcium-binding protein B for prediction of traumatic brain injuries: a transforming research and clinical knowledge in traumatic brain injury study. *J Neurotrauma.* (2020) 37:2460–7. doi: 10.1089/neu.2020.7140
37. Xu L, Yue JK, Korley FK, Puccio AM, Yuh EL, Sun X, et al. High-sensitivity C-reactive protein is a prognostic biomarker of 6- month disability after traumatic brain injury: results from the TRACK-TBI study. *J Neurotrauma.* (2020) 71:1–41. doi: 10.1089/neu.2020.7177
38. Kerezoudis P, Goyal A, Puffer RC, Parney IF, Meyer FB, Bydon M. Morbidity and mortality in elderly patients undergoing evacuation of acute traumatic subdural hematoma. *Neurosurg Focus.* (2020) 49:E22. doi: 10.3171/2020.7.FOCUS20439
39. Marrocco J, McEwen BS. Sex in the brain: hormones and sex differences. *Dialogues Clin Neurosci.* (2016) 18:373–83. doi: 10.31887/DCNS.2016.18.4/jmarrocco
40. Street AE, Vogt D, Dutra L. A new generation of women veterans: stressors faced by women deployed to Iraq and Afghanistan. *Clin Psychol Rev.* (2009) 29:685–94. doi: 10.1016/j.cpr.2009.08.007
41. World Rugby. *More Women Are Playing Rugby Than Ever Before.* (2015). Available online at: https://www.world.rugby/news/60275
42. Gupte R, Brooks W, Vukas R, Pierce J, Harris J. Sex differences in traumatic brain injury: what we know and what we should know. *J Neurotrauma.* (2019) 36:3063–91. doi: 10.1089/neu.2018.6171
43. Narayan RK, Michel ME, Ansell B, Baethmann A, Biegon A, Bracken MB, et al. Clinical trials in head injury. *J Neurotrauma.* (2002) 19:503–57. doi: 10.1089/089771502753754037
44. Montenigro PH, Alosco ML, Martin BM, Daneshvar DH, Mez J, Chaisson CE, et al. Cumulative head impact exposure predicts later-life depression, apathy, executive dysfunction, and cognitive impairment in former high school and college football players. *J Neurotrauma.* (2017) 34:328–40. doi: 10.1089/neu.2016.4413
45. Mez J, Daneshvar DH, Kiernan PT, Abdolmohammadi B, Alvarez VE, Huber BR, et al. Clinicopathological evaluation of chronic traumatic encephalopathy in players of American football. *JAMA*. (2017) 318:360–370. doi: 10.1001/jama.2017.8334
46. Global Burden of Disease 2016 Traumatic Brain, I.njury, Spinal Cord. C.ollaborators. Global, regional, and national burden of traumatic brain injury and spinal cord injury, 1990-2016: A systematic analysis for the Global Burden of Disease Study 2016. *Lancet*. (2019) 18:56–87. doi: 10.1016/S1474-4422(18)30415-0
47. Centers for Disease Control and Prevention. *Report to Congress on Traumatic Brain Injury in the United States: Epidemiology and Rehabilitation*. Atlanta, GA. (2015). Available online at: https://www.cdc.gov/traumaticbraininjury/pdf/TBI_Report_to_Congress_Epi_and_Rehab-a.pdf
48. Faul M, Xu L, Wald MM, Coronado VG. *Traumatic Brain in the United States: Emergency Department Visits, Hospitalizations and Deaths, 2002–2006*. Centers for Disease Control and Prevention, National Center for Injury Control and Prevention. (2010). https://www.cdc.gov/traumaticbraininjury/pdf/blue_book.pdf
49. Gardner RC, Dams-O'Connor K, Morrissey MR, Manley GT. Geriatric traumatic brain injury: epidemiology, outcomes, knowledge gaps, future directions. *J Neurotrauma.* (2018) 35:889–906. doi: 10.1089/neu.2017.5371
50. Albrecht JS, Hirshon JM, McCunn M, Bechtold KT, Rao V, Simoni-Wastila L, et al. Increased rates of mild traumatic brain injury among older adults in US emergency departments, 2009-2010. *J Head Trauma Rehabil.* (2016) 31:E1–7. doi: 10.1097/HTR.0000000000000190
51. Roos KG, Wasserman EB, Dalton SL, Gray A, Djoko A, Dompier TP, et al. Epidemiology of 3825 injuries sustained in six seasons of National Collegiate Athletic Association men's and women's soccer (2009/2010-2014/2015). *Br J Sports Med.* (2017) 51:1029–34. doi: 10.1136/bjsports-2015-095718
52. Rosene JM, Raksnis B, Silva B, Woefel T, Visich PS, Dompier TP, et al. Comparison of concussion rates between NCAA division i and division iii men's and women's ice hockey players. *Am J Sports Med.* (2017) 45:2622–9. doi: 10.1177/0363546517710005
53. Sutton M, Chan V, Escobar M, Mollayeva T, Hu Z, Colantonio A. Neck injury comorbidity in concussion-related emergency department visits: a population-based study of sex differences across the life span. *J Womens Health*. (2018) 28:473–82. doi: 10.1089/jwh.2018.7282
54. Zuckerman SL, Wegner AM, Roos KG, Djoko A, Dompier TP, Kerr ZY. Injuries sustained in National Collegiate Athletic Association Men's and Women's Basketball, 2009/2010-2014/2015. *Br J Sports Med*. (2018) 52:261–8. doi: 10.1136/bjsports-2016-096005
55. Colvin AC, Mullen J, Lovell MR, West RV, Collins MW, Groh M. The role of concussion history and gender in recovery from soccer-related concussion. *Am J Sports Med.* (2009) 37:1699–704. doi: 10.1177/0363546509332497
56. Preiss-Farzanegan SJ, Chapman B, Wong TM, Wu J, Bazarian JJ. The relationship between gender and postconcussion symptoms after sport-related mild traumatic brain injury. *PMR*. (2009) 1:245–53. doi: 10.1016/j.pmrj.2009.01.011
57. Bazarian JJ, Blyth B, Mookerjee S, He H, McDermott MP. Sex differences in outcome after mild traumatic brain injury. *J Neurotr*. (2010) 27:527–39. doi: 10.1089/neu.2009.1068
58. Covassin T, Elbin RJ, Harris W, Parker T. Kontos A. The role of age and sex in symptoms, neurocognitive performance, and postural stability in athletes after concussion. *Am J Sports Med.* (2012) 40:1303–12. doi: 10.1177/0363546512444554
59. Styrke J, Sojka P, Björnstig U, Bylund PO, Stålnacke BM. Sex-differences in symptoms, disability, and life satisfaction three years after mild traumatic brain injury: a population-based cohort study. *J Rehabil Med.* (2013) 45:749–57. doi: 10.2340/16501977-1215
60. McMahon P, Hricik A, Yue JK, Puccio AM, Inoue T, Lingsma HF, et al. Symptomatology and functional outcome in mild traumatic brain injury: results from the prospective TRACK-TBI study. *J Neurotrauma*. (2014) 31:26–33. doi: 10.1089/neu.2013.2984
61. Blumenfeld RS, Winsell JC, Hicks JW, Small SL. The epidemiology of sports-related head injury and concussion in water polo. *Front Neurol.* (2016) 7:98. doi: 10.3389/fneur.2016.00098
62. Ma R, Lopez VJr., Weinstein, M,G, Chenm JL, Black CM, Gupta AT, et al. Injury profile of American Women's Rugby-7s. *Med Sci Sports Exerc*. (2016) 48:1957–66. doi: 10.1249/MSS.0000000000000997
63. Albanese BJ, Boffa JW, Macatee RJ, Schmidt NB. Anxiety sensitivity mediates gender differences in postconcussive symptoms in a clinical sample. *Psychiatry Res.* (2017) 252:242–6. doi: 10.1016/j.psychres.2017.01.099
64. Brickell TA, Lippa SM, French LM, Kennedy JE, Bailie JM, Lange RT. Female service members and symptom reporting after combat and

non-combat-related mild traumatic brain injury. *J Neurotrauma.* (2017) 34:300–12. doi: 10.1089/neu.2016.4403
65. Chandran A, Barron MJ, Westerman BJ, DiPietro L. Multifactorial examination of sex-differences in head injuries and concussions among collegiate soccer players: NCAA ISS. 2004-2009. *Inj Epidemiol.* (2017) 4:28. doi: 10.1186/s40621-017-0127-6
66. Harrold KG, Hasanaj L, Moehringer N, Zhang I, Nolan R, Serrano L, et al. Rapid sideline performance meets outpatient clinic: results from a multidisciplinary concussion center registry. *J Neurol Sci.* (2017) 379:312–7. doi: 10.1016/j.jns.2017.06.038
67. MacDonald CL, Barber J, Jordan M, Johnson AM, Dikmen S, Fann JR, et al. Early clinical predictors of 5-year outcome after concussive blast traumatic brain injury. *JAMA Neurol.* (2017) 74:821–9. doi: 10.1001/jamaneurol.2017.0143
68. Mollayeva T, Cassidy JD, Shapiro CM, Mollayeva S, Colantonio A. Concussion/mild traumatic brain injury-related chronic pain in males and females: a diagnostic modelling study. *Medicine.* (2017) 96:e5917. doi: 10.1097/MD.0000000000005917
69. Bahraini NH, Hostetter TA, Forster JE, Schneider AL, Brenner LA. A rasch analysis of the neurobehavioral symptom inventory in a national cohort of operation enduring and iraqi freedom veterans with mild traumatic brain injury. *Psychol Assess.* (2018) 30:1013–27. doi: 10.1037/pas0000555
70. Lippa SM, Brickell TA, Bailie JM, French LM, Kennedy JE, Lange RT. Postconcussion symptom reporting after mild traumatic brain injury in female service members: impact of gender, posttraumatic stress disorder, severity of injury, and associated bodily injuries. *J Head Trauma Rehabil.* (2018) 33:101–12. doi: 10.1097/HTR.0000000000000353
71. Terry DP, Huebschmann NA, Maxwell BA, Cook NE, Mannix R, Zafonte R, et al. Preinjury migraine history as a risk factor for prolonged return to school and sports following concussion. *J Neurotrauma.* (2018). doi: 10.1089/neu.2017.5443
72. Varriano B, Tomlinson G, Tarazi A, Wennberg R, Tator C, Tartaglia MC. Age, gender and mechanism of injury interactions in post-concussion syndrome. *Can J Neurol Sci.* (2018) 45:636–42. doi: 10.1017/cjn.2018.322
73. Nelson LD, Temkin NR, Dikmen S, Barber J, Giacino JT, Yuh E, et al. Recovery after mild traumatic brain injury in patients presenting to US level I trauma centers: a transforming research and clinical knowledge in traumatic brain injury (TRACK-TBI) study. *JAMA Neurol.* (2019) 76:1049–59. doi: 10.1001/jamaneurol.2019.1313
74. Yue JK, Levin HS, Suen CG, Morrissey MR, Runyon SJ, Winkler EA, et al. Age and sex-mediated differences in six-month outcomes after mild traumatic brain injury in young adults: a TRACK-TBI study. *Neurol Res.* (2019) 41:609–23. doi: 10.1080/01616412.2019.1602312
75. Combs PR, Ford CB, Campbell KR, Carneiro KA, Mihalik JP. Influence of self-reported fatigue and sex on baseline concussion assessment scores. *Orthop J Sports Med.* (2019) 7:2325967118817515. doi: 10.1177/2325967118817515
76. Kennedy JE, Lu LH, Reid MW, Leal FO, Cooper DB. Correlates of depression in U.S. Military service members with a history of mild traumatic brain injury. *Mil Med.* (2019) 184:148–54. doi: 10.1093/milmed/usy321
77. Putukian M, D'Alonzo BA, Campbell-McGovern CS, Wiebe DJ. The ivy league-big ten epidemiology of concussion study: a report on methods and first findings. *Am J Sports Med.* (2019) 47:1236–47. doi: 10.1177/0363546519830100
78. Spano M, Risucci DA, Etienne M, Petersen KH. Epidemiology of sports related concussion in brazilian jiu-jitsu: a cross-sectional study. *Sports.* (2019) 7:E53. doi: 10.3390/sports7020053
79. Monahan K, O'Leary KD. Head injury and battered women: an initial inquiry. *Health Soc Work.* (1999) 24:269–78. doi: 10.1093/hsw/24.4.269
80. Banks ME. Overlooked but critical: traumatic brain injury as a consequence of interpersonal violence. *Trauma Violence Abuse.* (2007) 8:290–8. doi: 10.1177/1524838007303503
81. Black MC, Breiding MJ. Adverse and health risk behaviors associated health conditions with intimate partner violence - United States, 2005. *Morb Mortal Wkly Rep.* (2008) 57:113–7.
82. Black MC, Basile KC, Breiding MJ, Smith SG, Walters ML, Merrick MT, et al. *The National Intimate Partner and Sexual Violence Survey (NISVS): 2010 Summary Report*. Atlanta, GA: National Center for Injury Prevention and Control, Centers for Disease Control and Prevention. (2011). Available online at: www.cdc.gov/ViolencePrevention/pdf/NISVSExecutiveSummary703a.pdf
83. Wu V, Huff H, Bhandari M. Pattern of physical injury associated with intimate partner violence in women presenting to the emergency department: a systematic review and meta-analysis. *Trauma Violence Abuse.* (2010) 11:71–82. doi: 10.1177/1524838010367503
84. Matteoli M, Piacentino D, Kotzalidis G, Serata D, Rapinesi C, Angeletti G, et al. The clinical and radiological examination of acute intimate partner violence injuries: a retrospective analysis of an italian cohort of women. *Violence Vict.* (2016) 31:85–102. doi: 10.1891/0886-6708.VV-D-14-00107
85. St Ivany A, Schminkey D. Intimate partner violence and traumatic brain injury: state of the science and next steps. *Fam Community Health.* (2016) 39:129–37. doi: 10.1097/FCH.0000000000000094
86. Barnett OW. Why battered women do not leave, Part 2. *Trauma Violence Abuse.* (2001) 2:3–35. doi: 10.1177/1524838001002001001
87. Stark E. *Coercive Control: How Men Entrap Women in Personal Life*. (2007). New York, NY: Oxford University Press.
88. Kwako LE, Glass N, Campbell J, Melvin KC, Barr T, Gill JM. Traumatic brain injury in intimate partner violence: a critical review of outcomes and mechanisms. *Trauma Violence Abuse.* (2011) 12:115–26. doi: 10.1177/1524838011404251
89. Policastro C, Finn MA. Coercive control and physical violence in older adults: analysis using data from the national elder mistreatment study. *J Interpers. Violence.* (2017) 32:311–30. doi: 10.1177/0886260515585545
90. Valera EM, Campbell JC, Gill J, Iverson KM. Correlates of brain injuries in women subjected to intimate partner violence: identifying the dangers and raising awareness. *J Aggress Maltreat Traum.* (2019) 80:695–713. doi: 10.1080/10926771.2019.1581864
91. McCrea M, Guskiewicz KM, Marshall SW, Barr W, Randolph C, Cantu RC, et al. Acute effects and recovery time following concussion in collegiate football players. *JAMA.* (2003) 290:2556–63. doi: 10.1001/jama.290.19.2556
92. Marshall S, Bayley M, McCullagh S, Velikonja D, Berrigan L, Ouchterlony D, et al. Updated clinical practice guidelines for concussion/mild traumatic brain injury and persistent symptoms. *Brain Inj.* (2015) 29:688–700. doi: 10.3109/02699052.2015.1004755
93. Potter S, Leigh E, Wade D, Fleminger S. The Rivermead post concussion symptoms questionnaire. *J Neurol.* (2006) 253:1603–14. doi: 10.1007/s00415-006-0275-z
94. Teasdale G, Skene A, Parker L, Jennett B. Age and outcome of severe head injury. *Acta Neurochir Suppl.* (1979) 28:140–3.
95. Klauber MR, Barrett-Connor E, Marshall LF, Bowers SA. The epidemiology of head injury: a prospective study of an entire community– San Diego County, California, 1978. *Am J Epidemiol.* (1981) 113:500–9. doi: 10.1093/oxfordjournals.aje.a113125
96. Farace E, Alves WM. WM: Do women fare worse: a metaanalysis of gender differences in traumatic brain injury outcome. *J Neuro- Surg.* (2000) 93:539–45. doi: 10.3171/jns.2000.93.4.0539
97. Groswasser Z, Cohen M, Keren O. Female TBI patients recover better than males. *Brain Inj.* (1998) 12:805–8. doi: 10.1080/026990598122197
98. Sperry JL, Nathens AB, Frankel HL, Vanek SL, Moore EE, Maier RV, et al. Inflammation and the Host Response to Injury Investigators. Characterization of the gender dimorphism after injury and hemorrhagic shock: are hormonal differences responsible? *Crit Care Med.* (2008) 36:1838–45. doi: 10.1097/CCM.0b013e3181760c14
99. Magnotti LJ, Fischer PE, Zarzaur BL, Fabian TC, Croce MA. Impact of gender on outcomes after blunt injury: a definitive analysis of more than 36,000 trauma patients. *J Am Coll Surg.* (2008) 206:984–7. doi: 10.1016/j.jamcollsurg.2007.12.038
100. Hoogmartens O, Heselmans A, Van de Velde S, Castrén M, Sjölin H, Sabbe M, et al. Evidence-based prehospital management of severe traumatic brain injury: a comparative analysis of current clinical practice guidelines. *Prehosp Emerg Care.* (2014) 8:265–73. doi: 10.3109/10903127.2013.856506
101. Volovici V, Steyerberg EW, Cnossen MC, Haitsma IK, Dirven CMF, Maas AIR, et al. Evolution of evidence and guideline recommendations for the medical management of severe traumatic brain injury. *J Neurotrauma.* (2019) 36:22. doi: 10.1089/neu.2019.6474

102. Gessel LM, Fields SK, Collins CL, Dick RW, Comstock RD. Concussions among United States high school and collegiate athletes. *J Athl Train.* (2007) 42:495–503.
103. Cameron KL, Marshall SW, Sturdivant RX, Lincoln AE. Trends in the incidence of physician-diagnosed mild traumatic brain injury among active duty U.S. military personnel between 1997 and 2007. *J Neurotrauma.* (2012) 29:1313–21. doi: 10.1089/neu.2011.2168
104. McAllister TW, Ford JC, Flashman LA, Maerlender A, Green-wald RM, Beckwith JG, et al. Effect of head impacts on diffusivity measures in a cohort of collegiate contact sport athletes. *Neurology.* (2014) 82:63–9. doi: 10.1212/01.wnl.0000438220.16190.42
105. McKee AC, Stein TD, Kiernan PT, Alvarez VE. The neuropathology of chronic traumatic encephalopathy. *Brain Pathol.* (2015) 25:350–64. doi: 10.1111/bpa.12248
106. Johannes CB, Crawford SL. Menstrual bleeding, hormones, and the menopausal transition. *Semin Reprod Endocrinol.* (1999) 17:299–309. doi: 10.1055/s-2007-1016240
107. O'Connor KA, Holman DJ, Wood JW. Menstrual cycle variability and the perimenopause. *Am J Human Biol.* (2001) 13:465–78. doi: 10.1002/ajhb.1078
108. Maas D, Jochen A, Lalande B. Age-related changes in male gonadal function. Implications for therapy. *Drugs Aging.* (1997) 11:45–60. doi: 10.2165/00002512-199711010-00005
109. Gilthorpe MS, Wilson RC, Moles DR, Bedi R. Variations in admissions to hospital for head injury and assault to the head. Part 1: Age and gender. *Br J Oral Maxillofac Surg.* (1999) 37:294–300. doi: 10.1054/bjom.1998.0039
110. Dick RW. Is there a gender difference in concussion incidence and outcomes? *Br J Sports Med.* (2009) 43:i46–i50. doi: 10.1136/bjsm.2009.058172
111. Brook EM, Luo X, Curry EJ, Matzkin EG. A heads up on concussions: are there sex-related differences? *Phys Sportsmed.* (2016) 44:20–8. doi: 10.1080/00913847.2016.1142834
112. Edwards 3rd G, Moreno-Gonzalez I, Soto C. Amyloid-beta and tau pathology following repetitive mild traumatic brain injury. *Biochem Biophys Res Commun.* (2017) 483:1137–42. doi: 10.1016/j.bbrc.2016.07.123
113. Ramos-Cejudo J, Wisniewski T, Marmar C, Zetterberg H, Blennow K, Leon M,J., et al. Traumatic brain injury and Alzheimer's disease: the cerebrovascular link. *EBioMedicine.* (2018) 28:21–30. doi: 10.1016/j.ebiom.2018.01.021
114. Farlow MR, Lahiri DK, Poirier J, Davignon J, Schneider L, Hui SL. Treatment outcome of tacrine therapy depends on apolipoprotein genotype and gender of the subjects with Alzheimer's disease. *Neurology.* (1998) 50:669–77. doi: 10.1212/wnl.50.3.669
115. Sundermann EE, Biegon A, Rubin LH, Lipton RB, Mowrey W, Landau S, et al. Better verbal memory in women than men in MCI despite similar levels of hippocampal atrophy. *Neurology.* (2016) 86:1368–76. doi: 10.1212/WNL.0000000000002570
116. Sundermann EE, Maki PM, Rubin LH, Lipton RB, Landau S, Biegon A, et al. Female advantage in verbal memory: Evidence of sex-specific cognitive reserve. *Neurology.* (2016) 87:1916–24. doi: 10.1212/WNL.0000000000003288
117. Omalu BI, DeKosky ST, Minster RL, Kamboh M, I., Hamilton RL, Wecht CH. Chronic traumatic encephalopathy in a National Football League player. *Neurosurgery.* (2005) 57:128-34; discussion 128–134. doi: 10.1227/01.NEU.0000163407.92769.ED
118. Goldstein LE, Fisher AM, Tagge CA, Zhang X, Velisek L, Sullivan JA, et al. Chronic traumatic encephalopathy in blast-exposed military veterans and a blast neurotrauma mouse model. *Sci Transl Med.* (2012) 4:134ra60. doi: 10.1126/scitranslmed.3003716
119. McKee AC, Robinson ME. Military-related traumatic brain injury and neurodegeneration. *Alzheimers Dement.* (2014) 10:S242–53. doi: 10.1016/j.jalz.2014.04.003
120. Alosco ML, Stein TD, Tripodis Y, Chua AS, Kowall NW, Huber BR, et al. Association of white matter rarefaction, arteriolosclerosis, and tau with dementia in chronic traumatic encephalopathy. *JAMA Neurol.* (2019) 76:1298–308. doi: 10.1001/jamaneurol.2019.2244
121. Roberts GW, Whitwell H, Acland PR, Bruton CJ. Dementia in a punch-drunk wife. *Lancet.* (1990) 335:918–9. doi: 10.1016/0140-6736(90)90520-f
122. Kroshus E, Baugh CM, Stein CJ, Austin SB, Calzo JP. Concussion reporting, sex, and conformity to traditional gender norms in young adults. *J Adolesc.* (2017) 54:110–9. doi: 10.1016/j.adolescence.2016.11.002
123. Sullivan L, Molcho M. Gender differences in concussion-related knowledge, attitudes and reporting-behaviors among high school student-athletes. *Int J Adolesc Med Health*. (2018). doi: 10.1515/ijamh-2018-0031
124. Alsalaheen B, McCloskey K, Bean R, Almeida A, Eckner J, Lorincz M. Women and men use different strategies to stabilize the head in response to impulsive loads: implications for concussion injury risk. *J Orthop Sports Phys Ther.* (2019) 49:779–86. doi: 10.2519/jospt.2019.8760
125. Grafton ST, Ralston AB, Ralston JD. Monitoring of postural sway with a head-mounted wearable device: effects of gender, participant state, and concussion. *Med Devices (Auckl).* (2019) 12:151–64. doi: 10.2147/MDER.S205357
126. Mihalik JP, Amalfe SA, Roby PR, Ford CB, Lynall RC, Riegler KE, et al. Sex and sport differences in college lacrosse and soccer head impact biomechanics. *Med Sci Sports Exerc.* (2020) 52:2349–56. doi: 10.1249/mss.0000000000002382
127. Wei J, Xiao GM. The neuroprotective effects of progesterone on traumatic brain injury: current status and future prospects. *Acta Pharmacol Sin.* (2013) 34:1485–90. doi: 10.1038/aps.2013.160
128. Wunderle K, Hoeger, KM, Wasserman E. Menstrual phase as predictor of outcome after mild traumatic brain injury in women. *J Head Trauma Rehab.* (2014) 29:E1–E8. doi: 10.1097/HTR.0000000000000006
129. Wagner AK, McCullough EH, Niyonkuru C, Ozawa H, Loucks TL, Dobos JA, et al. Acute serum hormone levels: characterization and prognosis after severe traumatic brain injury. *J Neurotrauma.* (2011) 28:871–88. doi: 10.1089/neu.2010.1586
130. Kesler SR, Adams HF, Blasey CM, Bigler ED. Premorbid intellectual functioning, education, and brain size in traumatic brain injury: an investigation of the cognitive reserve hypothesis. *Appl Neuropsychol.* (2003) 10:153–62. doi: 10.1207/S15324826AN1003_04
131. Ystad MA, Lundervold AJ, Wehling E, Espeseth T, Rootwelt H, Westlye LT, et al. Hippocampal volumes are important predictors for memory function in elderly women. *BMC Med Imaging.* (2009) 9:17. doi: 10.1186/1471-2342-9-17
132. Umile ET, Sandel ME, Alavi A, Terry CM, Plotkin RC. Dynamic imaging in mild traumatic brain injury: support for the theory of medial temporal vulnerability. *Arch Phys Med Rehab.* (2002) 83:1506–13. doi: 10.1053/apmr.2002.35092
133. Livny A, Biegon A, Kushnir T, Harnof S, Hoffmann C, Fruchter E, et al. Cognitive deficits post-traumatic brain injury and their association with injury severity and gray matter volumes. *J Neurotrauma.* (2017) 34:1466–72. doi: 10.1089/neu.2016.4598
134. Ruigrok AN, Salimi-Khorshidi G, Lai MC, Baron-Cohen S, Lombardo MV, Tait RJ, et al. A meta-analysis of sex differences in human brain structure. *Neurosci Biobehav Rev.* (2014) 39:34–50. doi: 10.1016/j.neubiorev.2013.12.004
135. Escorial S, Roman FJ, Martinez K, Burgaleta M, Karama S, Colom R. Sex differences in neocortical structure and cognitive performance: a surface-based morphometry study. *Neuroimage.* (2015) 104:355–65. doi: 10.1016/j.neuroimage.2014.09.035
136. Luders E, Gaser C, Narr KL, Toga AW. Why sex matters: brain size independent differences in gray matter distributions between men and women. *J Neurosci.* (2009) 29:14265–70. doi: 10.1523/JNEUROSCI.2261-09.2009
137. Pakkenberg B, Gundersen HJ. Neocortical neuron number in humans: effect of sex and age. *J Comp Neurol.* (1997) 384:312–20.
138. Martínez K, Janssen J, Pineda-Pardo JÁ, Carmona S, Román FJ, Alemán-Gómez Y, et al. Individual differences in the dominance of interhemispheric connections predict cognitive ability beyond sex and brain size. *Neuroimage.* (2017) 155:234–44. doi: 10.1016/j.neuroimage.2017.04.029
139. Biegon A, Eberling JL, Richardson BC, Roos MS, Wong ST, Reed BR, et al. Human corpus callosum in aging and Alzheimer's disease: a magnetic resonance imaging study. *Neurobiol Aging.* (1994) 15:393–7. doi: 10.1016/0197-4580(94)90070-1
140. Gur RC, Mozley PD, Resnick SM, Gottlieb GL, Kohn M, Zimmerman R, et al. Gender differences in age effect on brain atrophy measured by

magnetic rensonace imaging. *Proc Natl Acad Sci USA*. (1991) 88:2845–9. doi: 10.1073/pnas.88.7.2845
141. Blatter DD, Bigler ED, Gale SD, Johnson SC, Anderson CV, Burnett BM, et al. Quantitative volumetric analysis of brain MR: normative database spanning 5 decades of life. *Am J Neuroradiol.* (1995) 16:241–51.
142. Coffey CE, Lucke JF, Saxton JA, Ratcliff G, Unitas LJ, Billig B, et al. Sex differences in brain aging: a quantitative magnetic resonance imaging study. *Arch Neurol.* (1998) 55:169–79. doi: 10.1001/archneur.55.2.169
143. Pruessner JC, Collins DL, Pruessner M, Evans AC. Age and gender predict volume decline in the anterior and posterior hippocampus in early adulthood. *J Neurosci.* (2001) 21:194–200. doi: 10.1523/JNEUROSCI.21-01-00194.2001
144. Bigler ED, Blatter. D. D., Anderson CV, Johnson SV, Gale SD, Hopkins RO, et al. Hippocampal volume in normal aging and traumatic brain injury. *AJNR Am J Neuroradiol.* (1997) 18:11–23.
145. Yount R, Raschke KA, Biru M, Tate DF, Miller MJ, Abildskov T, et al. Traumatic brain injury and atrophy of the cingulate gyrus. *J Neuropsychiatry Clin Neurosci.* (2002) 14:416–23. doi: 10.1176/jnp.14.4.416
146. Levine B, Kovacevic N, Nica EI, Cheung G, Gao F, Schwartz ML, et al. The Toronto traumatic brain injury study: injury severity and quantified MRI. *Neurology*. (2008) 70:771–8. doi: 10.1212/01.wnl.0000304108.32283.aa
147. Schönberger M, Ponsford J, Reutens D, Beare R, O'Sullivan R. The Relationship between age, injury severity, and MRI findings after traumatic brain injury. *J Neurotrauma*. (2009) 26:2157–67. doi: 10.1089/neu.2009.0939
148. Zhou Y, Kierans A, Kenul D, Ge Y, Rath J, Reaume J, et al. Mild traumatic brain injury: longitudinal regional brain volume changes. *Radiology*. (2013) 267:880–90. doi: 10.1148/radiol.13122542
149. Silberstein SD, Merriam GR. Physiology of the menstrual cycle. *Cephalalgia*. (2000) 20:148–54. doi: 10.1046/j.1468-2982.2000.00034.x
150. Stachenfeld NS, Silva C, Keefe DL, Kokoszka CA, Nadel ER. Effects of oral contraceptives on body fluid regulation. *J Appl Physiol.* (1999) 87:1016–25. doi: 10.1152/jappl.1999.87.3.1016
151. Watanabe H, Lau DC, Guyn HL, Wong NL. Effect of progesterone therapy on arginine vasopressin and atrial natriuretic factor in premenstrual syndrome. *Clin Invest Med.* (1997) 20:211–23.
152. Jones JS, Nevai J, Freeman MP, McNinch DE. Emergency department presentation of idiopathic intracranial hypertension. *Am J Emerg Med*. (1999) 17:517–21. doi: 10.1016/s0735-6757(99)90188-2
153. Wall M. Idiopathic intracranial hypertension: mechanisms of visual loss and disease management. *Semin Neurol.* (2000) 20:89–95.
154. Unterberg AW, Stover J, Kress B, Kiening KL. Edema and brain trauma. *Neuroscience.* (2004) 129:1021–9. doi: 10.1016/j.neuroscience
155. Alberico AM, Ward JD, Choi SC, Marmarou A, Young HF. Outcome after severe head injury: relationship to mass lesions, diffuse injury, and ICP course in pediatric and adult patients. *J Neurosurg.* (1987) 67:648–56. doi: 10.3171/jns.1987.67.5.0648
156. Sheth KN, Stein DM, Aarabi B, Hu P, Kufera JA, Scalea TM, et al. Intracranial pressure dose and outcome in traumatic brain injury. *Neurocrit Care.* (2013) 18:26–32. doi: 10.1007/s12028-012-9780-3
157. Kahraman S, Dutton RP, Hu P, Xiao Y, Aarabi B, Stein DM, et al. Automated measurement of "pressure times time dose" of intracranial hypertension best predicts outcome after severe traumatic brain injury. *J Trauma.* (2010) 69:110–8. doi: 10.1097/TA.0b013e3181c99853
158. Yao X, Uchida K, Papadopoulos MC, Zador Z, Manley GT, Verkman AS. Mildly reduced brain swelling and improved neurological outcome in aquaporin-4 knockout mice following controlled cortical impact brain injury. *J Neurotrauma.* (2015) 32:1458–64. doi: 10.1089/neu.2014.3675
159. Liang F, Luo C, Xu G, Su F, He X, Long S, et al. Deletion of aquaporin-4 is neuroprotective during the acute stage of micro traumatic brain injury in mice. *Neurosci Lett.* (2015) 598:29–35. doi: 10.1016/j.neulet.2015.05.006

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Prediabetes Is Associated With Brain Hypometabolism and Cognitive Decline in a Sex-Dependent Manner: A Longitudinal Study of Nondemented Older Adults

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Although type 2 diabetes is a well-known risk factor for Alzheimer's disease (AD), little is known about how its precursor—prediabetes—impacts neuropsychological function and brain health. Thus, we examined the relationship between prediabetes and AD-related biological and cognitive/clinical markers in a well-characterized sample drawn from the Alzheimer's Disease Neuroimaging Initiative. Additionally, because women show higher rates of AD and generally more atherogenic lipid profiles than men, particularly in the context of diabetes, we examined whether sex moderates any observed associations. The total sample of 911 nondemented and non-diabetic participants [normal control = 540; mild cognitive impairment (MCI) = 371] included 391 prediabetic (fasting blood glucose: 100–125 mg/dL) and 520 normoglycemic individuals (age range: 55–91). Linear mixed effects models, adjusted for demographics and vascular and AD risk factors, examined the independent and interactive effects of prediabetes and sex on 2–6 year trajectories of FDG-PET measured cerebral metabolic glucose rate (CMRglu), hippocampal/intracranial volume ratio (HV/IV), cerebrospinal fluid phosphorylated tau-$_{181}$/amyloid-β_{1-42} ratio (p-tau$_{181}$/Aβ_{1-42}), cognitive function (executive function, language, and episodic memory) and the development of dementia. Analyses were repeated in the MCI subsample. In the total sample, prediabetic status had an adverse effect on CMRglu across time regardless of sex, whereas prediabetes had an adverse effect on executive function across time in women only. Within the MCI subsample, prediabetic status was associated with lower CMRglu and poorer executive function and language performance across time within women, whereas these associations were not seen within men. In the total sample and MCI subsample,

prediabetes did not relate to HV/IV, p-tau$_{181}$/Aβ_{1-42}, memory function or dementia risk regardless of sex; however, among incident dementia cases, prediabetic status related to earlier age of dementia onset in women but not in men. Results suggest that prediabetes may affect cognition through altered brain metabolism, and that women may be more vulnerable to the negative effects of glucose intolerance.

Keywords: prediabetes, sex, brain metabolism, Alzheimer's disease, hippocampal volume, amyloid-beta, phosphorylated tau, cognitive function

INTRODUCTION

Type 2 diabetes (T2D) is a well-established risk factor for accelerated cognitive decline, mild cognitive impairment (MCI) and Alzheimer's disease (AD) (1–6). Additionally, there are consistent reports of associations between T2D and AD-related brain changes in non-demented older adults (7–18). For example, T2D is associated with smaller brain volumes, accelerated rates of brain atrophy, reduced cerebral blood flow in predilection sites for AD pathology (e.g., medial temporal lobe, inferior parietal regions), reductions in cerebral glucose metabolic rate (CMRglu) (7–15, 19), and higher cerebrospinal fluid (CSF) levels of the AD pathological marker hyperphosphorylated tau (p-tau) (16–18). Even among nondiabetics, elevated blood glucose has been associated with more severe AD pathology (i.e., higher medial temporal lobe neurofibrillary tangle pathology) (20). Associations between T2D and the other AD pathological hallmark characteristic, amyloid-β (Aβ), are less clear (8, 17, 21, 22).

Despite these well-established associations with T2D, far less is known about the effects of its precursor condition, prediabetes, on brain health and cognitive function. The impact of prediabetes on the brain is of great public health significance considering that half of adults aged 60 and older are estimated to have prediabetes (23), yet most are unaware that they have this condition. Although enough insulin is produced to thwart a T2D diagnosis, prediabetes is characterized by exposure to abnormally high levels of insulin for years, which results in insulin resistance and, in turn, impaired fasting glucose (24, 25). Because insulin and insulin-like growth factors regulate many biological processes such as axonal growth, protein synthesis, and gene expression (26, 27), insulin resistance and the resultant oxidative stress adversely affect all of these processes, which often occur before onset of overt T2D (28–30). In fact, older adults (age $\geq$ 65) with prediabetes have shown a two times greater risk of incident AD over 3,691 person-years (24); however, the specific effect of prediabetes on cognitive function and brain health in older adults without dementia is unclear. In a small study of participants with prediabetes ($n = 23$), there was an association between greater insulin resistance and a reduction in CMRglu in frontal, parietotemporal, and cingulate regions (14). A recent meta-analysis demonstrated that CSF levels of Aβ, p-tau and total tau (t-tau) were not associated with prediabetes status overall; however, prediabetes was associated with lower Aβ levels (indicative of greater Aβ plaque deposition in the brain) and higher t-tau levels among participants with memory impairment, suggesting that prediabetes may accelerate AD progression (18). Larger, longitudinal studies, such as the current study, are needed to understand the links between prediabetes, AD brain changes, and cognitive decline that may contribute to AD risk.

There are important, well-documented sex differences in both AD and in the manifestation of T2D. Compared to men, women have a higher prevalence of AD (31–33) and show a two times faster rate of decline in MCI (34) and greater severity in clinical AD dementia (35–37). In terms of T2D, women typically have a more favorable cardiovascular risk profile than men but lose that advantage with menopause (38–40) and/or T2D onset (41). In fact, the risk of cardiovascular events among individuals with T2D is estimated to be 50% greater in women than in men (42, 43), although this estimate varies across studies (44, 45). In a study of sex differences in risk factors for myocardial infarction ($N = 15{,}152$ cases and 14,820 controls), diabetic women were at almost double the risk of myocardial infarction compared to diabetic men (43). The more adverse cardiovascular profile in diabetic women vs. diabetic men is thought to be due to women showing greater lipid imbalances and more severe insulin sensitivity and inflammation compared to men (42, 44, 46). These differences in women vs. men present many years before T2D diagnosis, suggesting that these differences exist at the prediabetes stage (42, 44, 46).

Despite these sex differences in AD and in the T2D/prediabetes profile, studies have not yet examined sex differences in the relationship between these conditions and AD outcomes. Herein, we used longitudinal data drawn from the Alzheimer's Disease Neuroimaging Initiative (ADNI) to examine the relationship between prediabetes and changes in AD biological (hippocampal volume, brain glucose metabolism, AD CSF biomarkers) and cognitive/clinical markers (memory, executive function, and language performance, progression to dementia) in nondemented, middle-aged and older adults, and whether these relationships are moderated by sex. This research will help provide insight into (1) the impact of prediabetes on cognitive function in older adults without dementia, (2) the neural mechanisms underlying the link between prediabetes/T2D and AD, and (3) whether these associations differ in men vs. women. We hypothesized that prediabetic status will be associated with more advanced AD biomarkers and faster cognitive decline over time, particularly with frontal-mediated cognition (e.g., executive function) that is commonly influenced by vascular mechanisms (47–50) compared to normoglycemic individuals, and that these associations would be stronger in women vs. men.

TABLE 1 | Baseline sample size for each AD marker in the overall and MCI subsample as a function of sex and prediabetes status.

Diagnostic Group/AD marker	Total Sample, *N*	Maximum follow-up period examined (months)	Women		Men	
			Prediabetic, *N*	Normo-glycemic, *N*	Prediabetic, *N*	Normo-glycemic, *N*
Overall						
HV/IV	803	36	154	222	194	233
CMRglu	911	72	167	252	222	270
p-tau$_{181}$/Aβ_{1-42} ratio	718	48	134	206	172	206
AVLT Z-scores	910	60	167	251	222	270
TMT Part B Z-scores	902	60	165	249	219	269
BNT Z-scores	910	60	167	251	222	270
Incident dementia analysis*	850	All available	154	239	203	254
MCI						
HV/IV	330	24	50	89	98	93
CMRglu	354	36	58	104	81	111
p-tau$_{181}$/Aβ_{1-42} ratio	290	48	46	88	76	80
AVLT Z-scores	370	48	58	103	98	111
TMT Par B Z-scores	368	48	58	103	96	111
BNT Z-scores	370	48	58	103	98	111
Incident dementia analysis*	341	all available	55	96	87	103

**Cox proportional hazards models estimating incident dementia rates were conducted in participants with at-least one follow-up visit. MCI, Mild Cognitive Impairment; HV/IV, hippocampal/intracranial volume x 10^3; CMRGlu, cerebral metabolic glucose rate; p-tau$_{181}$/Aβ_{1-42}, phosphorylated tau/amyloid-β; AVLT, Rey Auditory Verbal Learning Test; TMT, Trail Making Test; BNT, Boston Naming Test.*

MATERIALS AND METHODS

Participants and Data Source

Data were extracted from the ADNI database. ADNI data is publically available at adni.loni.usc.edu. ADNI is a longitudinal, multi-site, cohort study that began in 2003 as a public-private partnership. Information about ADNI can be found at www.adni-info.org. The primary goal of ADNI has been to test whether serial neuroimaging measures and other biological and clinical markers can be combined to measure the progression of MCI and early AD. ADNI study visits involve neuroimaging, neuropsychological and clinical assessments. This research was approved by the Institutional Review Boards of all participating sites, and written informed consent was obtained for all participants. The general enrollment inclusion/exclusion criteria for ADNI have been described elsewhere (51). This specific study included participants who had the following data points at their baseline ADNI visit: (1) fasting glucose data as part of the FDG-PET imaging, (2) at-least one of the AD markers of interest and (3) relevant covariate data ($n = 1{,}294$). We excluded participants with a dementia diagnosis at baseline ($n = 240$). Given the limited number of participants with diabetes in ADNI and our interest in the relationship between prediabetes and AD, we also excluded those who were diabetic according to self-reported medical history and criteria from the World Health Organization (WHO), including fasting blood glucose levels $\geq$ 126 mg/dL and/or self-reported diabetic medication ($n = 145$) (52). Sample size varied by AD marker with the largest sample size including 911 participants who had brain glucose metabolism data since measuring fasting blood glucose was conducted as part of the [18F]fludeoxyglucose (FDG)-PET scan. See **Table 1** for sample size for each AD marker by sex and prediabetes status. Among the largest sample ($N = 911$), 46% were female and 41% were MCI with 391 prediabetic and 520 normoglycemic individuals (144 participants from ADNI1 and 765 from ADNI2/GO).

Clinical Diagnosis

Diagnosis of NC vs. MCI was based on the Jak/Bondi diagnostic method (53). This method included six neuropsychological tests representing three cognitive domains: Trail-Making Tests A and B (psychomotor speed/executive function), Category Fluency and Boston Naming Test (language) and the Rey Auditory Verbal Learning Test (AVLT) Delayed Recall and Recognition Tests (episodic memory). An impaired score was defined as >1 SD below the age-corrected normative mean. MCI diagnosis required one of three criteria: (1) impaired score on two tests within a cognitive domain; (2) one impaired score in each of the three cognitive domains; and/or (3) a score of 9 on the Functional Assessment Questionnaire indicating dependence in at-least three daily activities. If no criterion was met, participants were considered cognitively normal. Diagnostic criteria for dementia was based on the standard NINCDS/ADRDA criteria (54).

Prediabetes Classification

As part of the FDG-PET protocol, participants had blood drawn following a fast of at-least 4 h (water only) and blood glucose was measured. Prediabetic status was ascribed based on blood draw from the baseline visit and was defined as fasting glucose blood levels of 100–125 mg/dL based on guidelines from the American Diabetes Association (55).

AD Markers

Structural MRI

Structural MRI scans were collected on a 1.5T scanner according to a standardized protocol (56). Hippocampal volume data were analyzed using FreeSurfer version 4.3 (https://surfer.nmr.mgh.harvard.edu) at the University of California–San Francisco (57). To control for individual differences in head size, we created a ratio measure of hippocampal volume to intracranial volume (HV/IV) using the formula, hippocampal volume/intracranial volume $\times 10^3$.

Cerebral Metabolic Glucose Rate

CMRglu was measured by FDG-PET. Images were preprocessed following a standard procedure described at http://adni.loni.usc.edu/methods/pet-analysis/pre-processing/. ADNI investigators at the University of California–Berkeley established a "Meta-Regions of Interest" (MetaROI) of brain regions that commonly demonstrate metabolic changes in MCI/AD and correlate with cognitive performance in a meta-analysis (58, 59). The "MetaROI" was comprised of bilateral posterior cingulate gyrus, bilateral angular gyrus, and middle/inferior temporal gyrus. Standardized uptake value ratios (SUVRs) were calculated by averaging FDG uptake across the MetaROI and dividing by a reference region of pons and cerebellum (58, 59). The protocol for image analysis is described in http://adni.loni.usc.edu/methods/pet-analysis-method/pet-analysis/#pet-pre-processing-container.

Cerebrospinal Fluid Phosphorylated-tau-181 (p-tau$_{181}$)/Aβ_{1-42} Ratio

We examined the ratio of CSF levels of hyperphosphorylated tau-181 (p-tau$_{181}$) to Aβ_{1-42} proteins (p-tau$_{181}$/Aβ_{1-42} ratio) which has been shown to predict cognitive decline in individuals with MCI (60).

Cognitive Performance

Within the ADNI neuropsychological test battery, we examined tests measuring cognitive domains that typically show impairments early in the AD trajectory including language [Boston Naming Test (BNT)], executive function [Trail Making Test (TMT) Part B], and episodic memory (AVLT - Delayed Recall) (61). Individual scores from these tests were converted to z-scores based on age- and education-adjusted regression coefficients derived from a normative control group (n = 328) that remained cognitively normal (as identified via ADNI criteria) throughout follow-up in ADNI. The TMT Part B z-scores were multiplied by −1 so that higher scores indicated better performance.

Statistical Analyses

Differences in sample characteristics and baseline AD markers between sex and prediabetes status were examined using independent t-tests for continuous variables and Chi-square tests for categorical variables. We conducted linear mixed effects models with a random intercept to examine the separate and interactive effects of sex and baseline prediabetes status on (1) AD biological (HV/IV, CMRglu, p-tau$_{181}$/Aβ_{1-42} ratio) and clinical markers (AVLT, BNT and TMT Part B z-scores) across time and (2) the change in these markers over 2–6 years (depending on the follow-up period that varied by AD marker in ADNI; see **Table 1**). The effect of the predictors on change in AD markers was modeled by examining all two- and three-way interactions with time, which was modeled continuously (i.e., sex X time, prediabetes X time and sex X prediabetes X time). Models with three-way interactions (i.e., sex by prediabetes by time) controlled for all two-way interactions and main effects. Separate linear mixed effects models were conducted for each AD-marker outcome and each model included data from time points in ADNI that had at-least 10 participants per group (i.e., female/male diabetic/normoglycemic) resulting in at-least 40 participants total at a time point. Among participants with at-least one follow-up visit (N = 850), Cox proportional hazards models estimated hazard ratios (HR) and 95% confidence intervals (CI) for risk of developing dementia during follow-up as a function of sex, prediabetes, and sex X prediabetes. Among those who developed dementia during follow-up, we conducted a linear regression to examine the effect of sex, prediabetes and sex X prediabetes on the age at dementia-onset. In all analyses, male sex was compared to female sex (reference group) and the prediabetes group was compared to the normoglycemic group (reference group). Significant interactions were probed via sex- and prediabetes-stratified analyses. Interaction terms were removed from the model if $p \geq 0.10$ in order to assess main effects. Covariates in statistical models included demographic variables (age, education, race/ethnicity), apolipoprotein ε4 allele (APOE4), and indices of cardiovascular risk given sex differences in cardiovascular risk profiles beyond prediabetes/T2D (38–40). We selected cardiovascular risk indices as covariates that commonly cluster with diabetes (62) and are available in ADNI including body mass index (BMI), pulse pressure (systolic blood pressure – diastolic blood pressure), and history of any self-reported cardiovascular event (e.g., hypertension, hyperlipidemia, atrial fibrillation, coronary artery disease, and stroke). In analyses examining risk of dementia and age at dementia-onset, we additionally adjusted for baseline AD biomarkers. Any covariate that was not significant in the multivariable regression model at a $p \leq 0.10$ threshold level was removed from the final model. Analyses were repeated in a subsample of participants with MCI at baseline in order to assess the effect of prediabetes in the prodromal stage of AD. The follow-up period for most AD markers was shorter in the MCI subsample given the smaller sample size at follow-up visits. Analyses were performed using SPSS 24 (SPSS Inc., Chicago, Illinois). Significance was defined as $\alpha = 0.05$ (two-sided).

RESULTS

Sample Characteristics

Prediabetes was more prevalent in men (n = 223, 45%) vs. women (n = 168, 40%), although the difference was not significant (χ^2 = 2.5, p = 0.11). See **Table 2** for sample characteristics and baseline AD markers by sex and prediabetic status. At baseline, women were younger with fewer years of

TABLE 2 | Baseline sample characteristics and AD markers by sex and prediabetic status.

	Women			Men			Sex *p*-value	Pre-diabetic Status *p*-value
	Normo-glycemic Mean (SD)	Prediabetic Mean (SD)	*p*-value	Normo-glycemic Mean (SD)	Prediabetic Mean (SD)	*p*-value		
N (%)	251 (59.9%)	168 (40.1%)		269 (54.7%)	223 (45.3%)	0.11	–	–
Follow-up time, years	4.2 (2.4)	3.8 (2.4)	0.10	4.4 (2.9)	4.1 (2.6)	0.19	0.22	0.04
Age	72.2 (7.3)	72.2 (6.7)	0.93	73.9 (6.9)	73.9 (7.0)	0.95	<0.001	0.88
Education, years	15.8 (2.6)	15.7 (2.7)	0.74	16.7 (2.6)	16.5 (2.7)	0.28	<0.001	0.27
White, *N* (%)	229 (91.2%)	158 (94.0%)	0.16	258 (95.9%)	203 (91.0%)	0.03	0.40	0.55
APOE4 carrier, *N* (%)	102 (40.6%)	75 (44.6%)	0.42	117 (43.5%)	87 (39.0%)	0.31	0.85	0.85
MMSE score	28.5 (1.5)	28.3 (1.7)	0.28	28.2 (1.7)	28.1 (1.6)	0.69	0.02	0.31
MCI diagnosis, *N* (%)	104 (41.4%)	58 (34.5%)	0.16	111 (41.3%)	98 (43.9%)	0.55	0.22	0.62
Incident dementia cases, *N* (%)	55 (21.8%)	32 (19.2%)	0.51	67 (24.6%)	44 (19.7%)	0.19	0.50	0.16
Body mass index	26.5 (5.2)	27.4 (5.8)	0.08	26.8 (3.4)	27.5 (4.1)	0.04	0.47	0.006
History of CVD, *N* (%)	146 (58.2%)	117 (69.6%)	0.02	192 (71.3%)	152 (68.2%)	0.44	0.03	0.30
Pulse Pressure* (mm Hg)	59.6 (18.3)	60.7 (14.7)	0.52	58.8 (13.0)	58.5 (14.8)	0.81	0.18	0.82
Baseline fasting blood glucose level (mg/dL)	87.8 (11.1)	107.8 (6.7)	<0.001	90.1 (8.5)	109.0 (6.5)	<0.001	0.002	<0.001
AD biomarkers								
HV/IV ratio	4.89 (0.75)	4.84 (0.78)	0.57	4.56 (0.75)	4.56 (0.72)	0.96	0.001	0.70
CMRglu	1.30 (0.13)	1.27 (0.13)	0.005	1.26 (0.14)	1.25 (0.12)	0.21	0.001	0.004
p-tau/Aβ ratio	0.03 (0.03)	0.03 (0.03)	0.78	0.03 (0.03)	0.03 (0.03)	0.43	0.75	0.76
AD cognitive markers								
AVLT Z score	−0.5 (1.2)	−0.3 (1.1)	0.34	−0.8 (1.0)	−0.9 (1.0)	0.48	<0.001	0.84
TMT Part B Z score	−0.4 (1.4)	−0.6 (1.5)	0.23	−0.6 (1.6)	−0.4 (1.2)	0.04	0.60	0.45
BNT Z score	−0.5 (1.4)	−0.7 (1.7)	0.18	−0.4 (1.5)	−0.5 (1.4)	0.37	0.13	0.12

*Table reflects sample characteristics of the largest sample examined (N = 911). "Sex p-value" and "Prediabetic Status p-value" columns reflect differences by sex across prediabetic status groups and differences by prediabetic status across sex, respectively. P-values within the sex columns reflect differences between prediabetes status within sex and are derived. All p-values are derived from univariate analyses of variance for continuous variables and Chi-square tests for categorical variables. *Pulse pressure, systolic blood pressure (mm Hg) – diastolic blood pressure (mm Hg); MCI, Mild Cognitive Impairment; MMSE, Mini Mental State Exam; HV/IV, hippocampal/intracranial volume × 10^3; CMRGlu, cerebral metabolic glucose rate; AVLT, Rey Auditory Verbal Learning Test; TMT, Trail Making Test; BNT, Boston Naming Test.*

education, a lower prevalence of past cardiovascular events, a lower mean baseline fasting blood glucose level, higher mean HV/IV ratio, and CMRglu. From a cognitive standpoint, women showed lower MMSE and AVLT scores compared to men (*p*s < 0.05) at baseline. Compared to normoglycemics, prediabetics had a significantly higher BMI and lower CMRglu at baseline and fewer years of follow-up (*p*s < 0.05). Among men only, the proportion of White participants was significantly lower in prediabetic vs. normoglycemic ($p = 0.03$). Among women only, the proportion of those with a history of CVD was significantly higher in prediabetics vs. normoglycemics ($p = 0.02$). Although BMI was higher in prediabetics vs. normoglycemics in both sexes, this difference was only significant in men ($p = 0.04$). In unadjusted analyses examining AD markers by sex and prediabetic status, baseline CMRglu was significantly lower in prediabetic vs. normoglycemic women ($p = 0.005$); however, baseline CMRglu did not differ between prediabetic and normoglycemic men ($p = 0.21$). Mean baseline executive function (TMT Part B) z-score was significantly lower in normoglycemic men vs. prediabetic men ($p = 0.04$). Baseline HV/IV, p-tau$_{181}$/Aβ_{1-42} ratio, and episodic memory (AVLT) and language (BNT) z-scores did not differ by prediabetic status within men or women (*p*s ≥ 0.05). One-hundred ninety eight participants developed dementia during follow-up (73% MCI and 27% cognitively normal at baseline).

Results of statistical tests modeling the interactive and separate effects of sex and prediabetes on the outcomes of (1) change in AD markers over time, (2) average AD marker level across time, and (3) risk of dementia and age at dementia onset are described below and separated by results in the overall sample vs. the MCI subsample. Parameter estimates from linear mixed effects regressions modeling AD markers over time are displayed in **Table 3** (overall sample) and **Table 4** (MCI subsample). The trajectories of AD markers by sex and prediabetic status are displayed in **Figure 1** (overall sample) and **Figure 2** (MCI subsample).

Sex x Prediabetes Interactive Effects in the Overall Sample and MCI Subgroup

Outcome: Change in AD Markers Over Time

In the overall sample and MCI subgroup, we found no Sex x Prediabetes interactive effects on change in AD markers (i.e., Sex x Prediabetes x Time interactions *p*s ≥ 0.05).

TABLE 3 | Estimates of linear mixed effects models predicting change in AD markers by sex and prediabetes status in the overall sample.

	Sex			Prediabetic Status			Time			Sex X prediabetes			Prediabetes X time			Sex X Time			Sex X Prediabetes X time		
	b	**SE**	***p***	**b**	**SE**	***p***	**b**	**SE**	***p***	**b**	**SE**	***p***	**b**	**SE**	***p***	**b**	**SE**	***p***	**b**	**SE**	***p***
AD biomarker																					
HV/IV	−0.27	0.09	**0.003**	−0.14	0.09	0.13	−0.007	0.001	**<0.001**	0.11	0.18	0.53	0.001	0.001	0.51	0.001	0.001	0.33	0.001	0.002	0.71
CMRglu	−0.03	0.01	0.001	−0.04	0.01	**0.001**	−0.002	0.0002	**<0.001**	0.03	0.02	0.10	0.0004	0.0002	0.05	0.0004	0.0002	0.05	-0.3×10^{-4}	0.0004	0.93
p-tau/ Aβ ratio	−0.003	0.003	0.20	−0.002	0.003	0.36	0.0001	0.3×10^{-4}	**0.003**	0.005	0.003	0.17	0.0001	0.6×10^{-4}	0.07	0.5×10^{-4}	0.5×10^{-4}	0.31	−0.0001	0.0001	0.26
AD cognitive markers																					
AVLT z-score	−0.36	0.05	**<0.001**	0.02	0.05	0.72	−0.004	0.0007	**<0.001**	−0.12	0.11	0.27	0.0002	0.001	0.90	0.002	0.001	0.18	−0.003	0.003	0.24
TMT Part B z-score	−0.18	0.12	0.14	−0.23	0.14	0.10	−0.009	0.002	**<0.001**	0.47	0.18	**0.01**	−0.003	0.002	0.27	0.001	0.002	0.63	−0.002	0.005	0.70
BNT z-score	0.20	0.08	**0.03**	−0.19	0.09	**0.03**	−0.006	0.001	**<0.001**	0.29	0.18	0.10	0.002	0.003	0.55	0.002	0.003	0.41	−0.001	0.005	0.84

Bolded values indicate statistical significance. All analyses included the following covariates: age, education, race, APOE4 status, body mass index, pulse pressure and history of cardiovascular events. Covariates were removed from the final model if not a significant predictor of the outcome. SE, standard error; HV/IV, hippocampal/intracranial volume × 10^3; CMRglu, cerebral metabolic glucose rate; AVLT, Rey Auditory Verbal Learning Test; TMT, Trail Making Test; BNT, Boston Naming Test.

TABLE 4 | Estimates of linear mixed effects models predicting change in AD markers by sex and prediabetes status in MCI subsample.

	Sex			Prediabetic status			Time			Sex X prediabetes			Prediabetes X time			Sex X time			Sex X prediabetes X time		
	b	**S.E.**	***p***	**b**	**S.E.**	***p***	**b**	**S.E.**	***p***	**b**	**S.E.**	***p***	**b**	**S.E.**	***p***	**b**	**S.E.**	***p***	**b**	**S.E.**	***p***
AD biomarker																					
HV/IV	−0.39	0.11	**<0.001**	−0.20	0.13	0.13	−0.01	0.0009	**<0.001**	0.19	0.17	0.26	−0.002	0.001	0.07	0.003	0.001	0.003	0.0002	0.002	0.94
CMRglu	−0.04	0.02	**0.04**	−0.06	0.02	**0.004**	−0.002	0.0003	**<0.001**	0.07	0.03	**0.02**	0.0002	0.0004	0.68	0.0001	0.0004	0.78	0.0006	0.0008	0.45
p-tau/ Aβ ratio	−0.002	0.003	0.54	0.0009	0.003	0.80	0.0001	0.00004	**<0.001**	0.01	0.007	0.08	0.0001	0.0001	0.23	−0.00001	0.0001	0.46	−0.0002	0.0002	0.26
AD cognitive markers																					
AVLT Z score	−0.07	0.08	0.41	0.006	0.08	0.94	−0.004	0.001	**<0.001**	−0.02	0.16	0.90	−0.003	0.002	0.14	0.003	0.002	0.11	0.003	0.004	0.40
TMT Part B Z score	−0.57	0.28	**0.04**	−0.69	0.34	**0.04**	−0.02	0.005	**<0.001**	1.07	0.46	**0.02**	−0.009	0.01	0.23	0.002	0.01	0.77	0.002	0.01	0.89
BNT Z score	−0.35	0.43	0.41	−1.58	0.53	**0.004**	−0.03	0.01	**0.006**	1.45	0.71	**0.04**	−0.004	0.01	0.75	0.007	0.01	0.60	0.002	0.03	0.94

Bolded values indicate statistical significance. All analyses included the following covariates: age, education, race, APOE4 status, body mass index, pulse pressure, and history of cardiovascular events. Covariates were removed from the final model if not a significant predictor of the outcome. MCI, Mild Cognitive Impairment; HV/IV, hippocampal/intracranial volume × 10^3. CMRglu, cerebral metabolic glucose rate; AVLT, Rey Auditory Verbal Learning Test; TMT, Trail Making Test; BNT, Boston Naming Test.

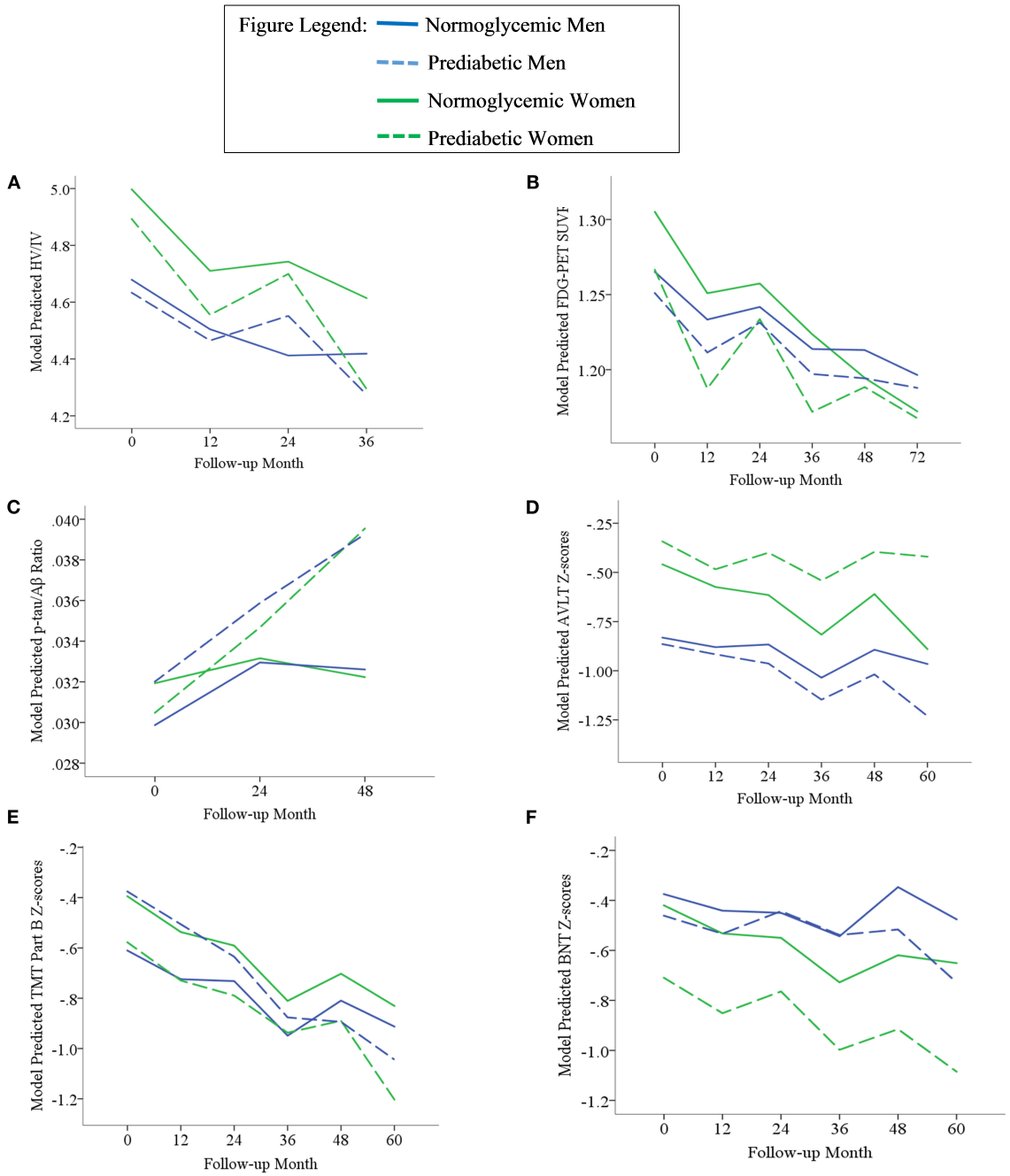

FIGURE 1 | Trajectories of AD biomarkers **(A–C)** and cognitive test z-scores **(D–F)** by prediabetes status and sex in the overall sample. TMT Part B z-scores were multiplied by −1 so that higher scores indicated better performance. Z-scores are age- and education-adjusted based on a normative control group that remained cognitively normal throughout follow-up in ADNI. HV/IV, ratio of hippocampal volume to intracranial volume; PET, positron emission tomography; FDG, fludeoxyglucose; SUVR, standaradized uptake volume ratio; p-tau, phosphorylated tau; Aβ, amyloid beta; BNT, Bostin Naming Test; TMT, Trail Making Test; AVLT, Rey Auditory Verbal Learning Test.

Outcome: Average AD Marker Across Time

In the overall sample, the Sex x Prediabetes interaction was significant only for executive function performance, whereby, on average, prediabetics performed significantly worse than normoglycemics, but only among women (B = −0.31, SE = 0.12, $p = 0.01$). Conversely, prediabetic men actually showed better executive function performance than normoglycemic men (B = 0.24, SE = 0.12, $p = 0.04$).

Within the MCI subgroup, there were significant Sex x Prediabetes interactions for the CMRGlu ($p = 0.02$), executive

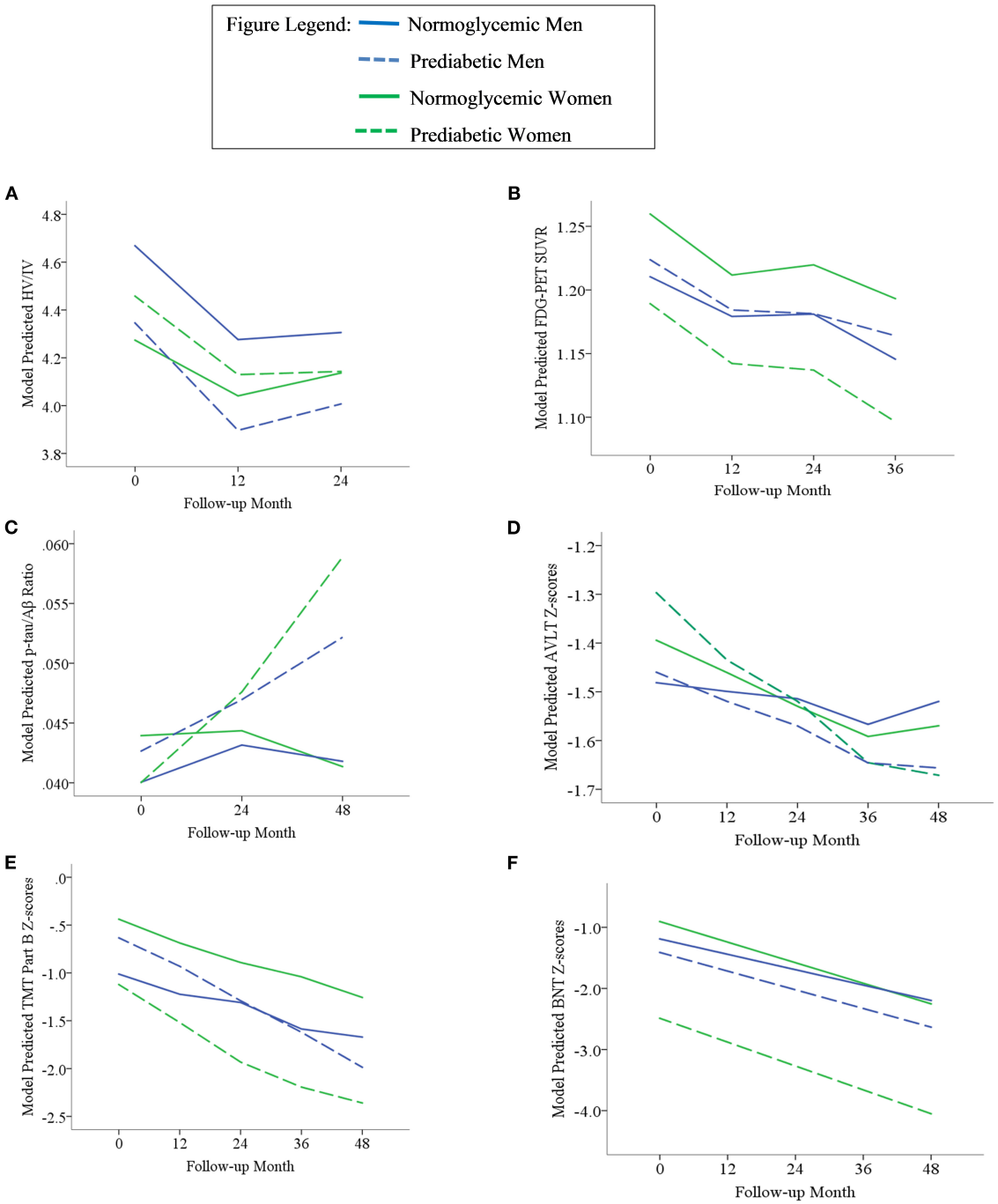

FIGURE 2 | Trajectories of AD biomarkers **(A–C)** and cognitive test z-scores **(D–F)** by prediabetes status and sex in the MCI subsample. TMT Part B z-scores were multiplied by −1 so that higher scores indicated better performance. Z-scores are age- and education-adjusted based on a normative control group that remained cognitively normal throughout follow-up in ADNI. HV/IV, ratio of hippocampal volume to intracranial volume; PET, positron emission tomography; FDG, fludeoxyglucose; SUVR, standaradized uptake volume ratio; p-tau, phosphorylated tau; Aβ, amyloid beta; BNT, Bostin Naming Test; TMT, Trail Making Test; AVLT, Rey Auditory Verbal Learning Test.

function (p = 0.02) and language outcomes (p = 0.04). The interactions revealed that, on average, prediabetic women exhibited lower CMRglu (B = −0.06, SE = 0.02, p = 0.005) and poorer executive function (B = −0.76, SE = 0.32, p = 0.02) and language performance (B = −1.69, SE = 0.54, p = 0.003) relative to normoglycemic women, whereas prediabetes did not relate to CMRglu (B = 0.01, SE = 0.02, p = 0.61), executive function (B = 0.41, SE = 0.31, p = 0.19) or

language performance (B = −0.09, SE = 0.46, p = 0.84) in men.

Outcome: Incidence of Dementia and Age at Onset

Among participants with at-least one follow-up visit in the overall sample, (N = 850), the interactive effect of Sex x Prediabetes on risk of dementia was not significant (HR = 0.75, 95% CI = 0.36–1.54, p = 0.43). Among those who developed dementia, there was a significant Sex x Prediabetes interactive effect on age at dementia onset (B = −1.67, SE = 0.72, p = 0.02) that was unchanged when adjusting for baseline HV/IV and p-tau$_{181}$/Aβ_{1-42} ratio but attenuated when adjusting for baseline CMRglu (B = −1.28, SE = 0.68, p = 0.06). The interaction was driven by a significant association between prediabetic status and age at dementia onset among women (B = −0.91, SE = 0.40, p = 0.03) but not men (B = 0.43, SE = 0.48, p = 0.37). Specifically, female prediabetics (M = 75.14, SD = 0.32) developed dementia at an earlier age than female normoglycemics (M = 76.05, SD = 0.25); however, this female-specific relationship of prediabetes to age at dementia onset was attenuated when adjusting for CMRglu (B = −0.75, SE = 0.41, p = 0.07).

In the MCI subgroup, the interactive effect of Sex x Prediabetes on risk of dementia was not significant (HR = 0.68, 95% CI = 0.29–1.60, p = 0.38). Among those who developed dementia, the Sex x Prediabetes interactive effect on age at dementia onset trended toward significance (B = −1.01, SE = 0.60, p = 0.09). This interaction became significant when adjusting for baseline HV/IV and p-tau$_{181}$/Aβ_{1-42} ratio (B = −1.28, SE = 0.63, p = 0.04), but was attenuated with adjustment for CMRglu (B = −0.97, SE = 0.62, p = 0.012). The interaction was driven by a relationship between prediabetic status and age at dementia onset in women (B = −0.78, SE = 0.37, p = 0.04) but not in men (B = 0.54, SE = 0.48, p = 0.26), and this female-specific relationship was significant with and without adjustment for baseline AD biomarkers (HV/IV, p-tau$_{181}$/Aβ_{1-42} ratio and CMRglu). Specifically, female prediabetics (M = 73.49, SD = 8.32) developed dementia at an earlier age than female normoglycemics (M = 74.31, SD = 6.72).

Main Effects of Prediabetes and Sex in Overall Sample and MCI Subgroup

Outcome: Change in AD Markers Over Time

In the overall sample, a marginally significant Prediabetes x Time interaction (p = 0.05) indicated that CMRglu in normoglycemics declined faster over time from their higher baseline measure (B = −0.0015, SE = 0.0001, p < 0.001) compared to prediabetics (B = −0.0011, SE = 0.0001, p < 0.001). A marginally significant Sex x Time interaction (p = 0.05) indicated that CMRglu declined faster over time in women (B = −0.0016, SE = 0.0002, p < 0.001) compared to men (B = −0.0011, SE = 0.0001, p < 0.001). Although all AD markers showed significant progression over time (i.e., main effect of time, p's< 0.001), this change did not differ by prediabetic status or sex for any marker besides CMRglu.

Within the MCI subgroup, AD markers showed significant progression over time (i.e., main effect of time, ps < 0.01); however, this change did not differ by prediabetic status (i.e., non-significant Prediabetes X Time interactions, ps > 0.05). A significant Sex x Time interaction on HV/IV (p = 0.003) indicated that the initially higher HV/IV in MCI women showed faster decline over time (B = −0.012, SE = 0.001, p < 0.001) compared to MCI men (B = −0.008, SE = 0.001, p < 0.001). There was no effect of sex on change in other AD markers over time (ps > 0.05).

Outcome: Average AD Marker Across Time

In the overall sample, there were significant main effects of prediabetes on CMRglu (p = 0.001) and language performance (p = 0.03), whereby prediabetics demonstrated a lower average CMRglu and language z-score than normoglycemics across time points. There was a main effect of sex on HV/IV, episodic memory and language z-scores (ps < 0.05). Consistent with the broader literature (63–65), women demonstrated a higher average HV/IV and episodic memory z-score than men across time points. Conversely, women demonstrated a lower average language z-score compared to men across time points.

Within the MCI subgroup, there were main effects of prediabetic status on CMRglu, executive function and language outcomes in MCI, whereby, compared to normoglycemics, prediabetics demonstrated a lower average CMRglu, executive function z-score and language z-score across time points. There were also main effects of sex on HV/IV, CMRglu and executive function performance in the MCI subgoup, whereby women demonstrated a higher average HV/IV and CMRglu and a lower average executive function z-score than men across time points.

Outcome: Incidence of Dementia and Age at Onset

In the overall sample, there was no main effect of prediabetic status on risk of dementia (HR = 0.83, 95% CI = 0.61–1.14, p = 0.25). There was a main effect of sex on risk of dementia (HR = 0.72, 95% CI = 0.53–0.99, p = 0.04), whereby the risk was nearly 30% lower in men vs. women. Among those who developed dementia during follow-up, there was no main effect of prediabetic status (B = 0.44, SE = 0.43, p = 0.31) or sex (B = 1.51, SE = 0.95, p = 0.11) on age at dementia onset. Results were unchanged when adjusting for baseline AD biomarkers.

In the MCI subgroup, there were no main effects of prediabetic status (HR = 0.74, 95% CI = 0.48–1.15, p = 0.18) or sex (HR = 0.77, 95% CI = 0.51–1.17, p = 0.22) on risk of dementia. Similarly, among those who developed dementia during follow-up, there were no main effects of prediabetes (B = 0.50, SE = 0.42, p = 0.24) or sex (B = 1.44, SE = 0.94, p = 0.13) on age at dementia onset. Results were unchanged when adjusting for baseline AD biomarkers.

DISCUSSION

Among non-demented older adults, we found that prediabetes at baseline related to brain hypometabolism in both women and men; however, in line with our hypotheses, the adverse effects of prediabetes on cognitive outcomes were female-specific and limited to more frontal-mediated cognitive domains (i.e., executive function and language). Prediabetes was associated with poorer executive function performance in women only in the overall sample, whereas prediabetes curiously related to better executive function performance in men. When limiting analyses to those with MCI, the relationship between prediabetic

status and hypometabolism became female-specific and the female-specific relationship between prediabetes and poorer executive function performance persisted, whereas the opposing relationship in men was no longer observed. Among those with MCI, we also observed a female-specific relationship between prediabetes and poorer language performance. Prediabetes did not relate to the p-tau$_{181}$/Aβ_{1-42} ratio, HV/IV or memory performance in the overall sample or in the MCI subsample. In either the overall or MCI subsample, there was no effect of prediabetes on incident dementia rates regardless of sex; however, among incident dementia cases, prediabetes was associated with an earlier age of dementia onset in women but not in men.

The finding that brain hypometabolism was associated with prediabetes suggests that altered brain metabolism may be an early neural mechanism by which prediabetes/T2D aversely impacts cognition and AD risk. In fact, among incident dementia cases, we found that the significant Sex X Prediabetes interaction on age at dementia onset was attenuated when adjusting for CMRglu suggesting that brain hypometabolism may be a mechanism underlying what appears to be a hastening of the AD trajectory in female prediabetics vs. female normoglycemics. There is biological plausibility for this finding as animal studies have shown that impaired insulin modulation negatively affects brain glucose utilization (66, 67) likely through impeded delivery of glucose to the central nervous system [CNS; (68)]. Hypometabolism is characteristic of both prediabetes and AD and has been implicated in other AD mechanisms including tau protein hyperphosphorylation, vascular dysfunction, and inflammation (14, 15, 69). Thus, hypometabolism may represent a convergent pathway by which prediabetes predisposes one to AD brain changes. Our results in the overall sample showed significantly lower CMRglu in prediabetics throughout follow-up; however, a steeper rate of CMRglu decline over time in normoglycemics vs. prediabetics resulted in similar CMRglu at the final follow-up measure in prediabetics and normoglycemics. This pattern of results suggests that prediabetes may contribute to or compound the effects of AD and/or brain aging on physiological brain changes and, thus, accelerate these changes so that they occur earlier in the trajectory. Unlike results in the overall sample, the lower CMRglu in prediabetics vs. normoglycemics was consistent throughout follow-up in the MCI group. although the follow-up period examined was shorter in the MCI subsample. It is possible that we would have seen the normoglycemics decline faster to meet the already low rates in prediabetics with more follow-up time in the MCI group.

Our findings of an association between prediabetes and brain hypometabolism are consistent with preliminary findings from a previous, smaller study ($n = 23$) that reported an association between greater insulin resistance and hypometabolism in frontal, parietotemporal, and cingulate regions among prediabetics (14). Additionally, a previous ADNI study found an association between T2D and brain hypometabolism in MCI (13); however, prediabetes or sex differences were not examined. We saw no effect of prediabetes on HV/IV. Similarly, Schneider et al. (11) reported that smaller brain volume was associated with more severe diabetes (defined by higher HbA1c and longer disease duration) but not prediabetes or less-severe diabetes suggesting that the effects of insulin resistance on brain structure presumably do not manifest until a later disease stage (i.e., where structural changes are typically observed subsequent to altered brain function). We saw no significant effect of prediabetes on the p-tau$_{181}$/Aβ_{1-42} ratio; however, we observed trends for a steeper increase in the ratio over time in the prediabetes vs. normoglycemic group in the overall sample and for a female-specific relationship between prediabetes and a higher ratio in the MCI subsample. These trends suggest that the effects of prediabetes on AD pathological markers and their sex differences may occur subsequent to effects on brain function and manifest with greater follow-up time. Consistently, a meta-analysis found that prediabetes was not associated with more advanced AD pathological markers in CSF (lower Aβ levels and higher p-tau and total tau levels) (18). However, in a subgroup analysis among studies with samples that were recruited through memory clinics, prediabetes was associated with lower Aβ levels and higher total tau, but not p-tau levels (18). Consistent with the idea that a relationship between prediabetes and AD pathological markers may manifest at a later AD stage, these clinic samples may represent more severe cognitive impairment than the MCI diagnosis in ADNI.

Regarding sex differences, we hypothesized that associations between prediabetic status and brain and cognitive outcomes would be stronger in women than in men. Results were partially consistent with hypotheses in that the associations of prediabetes with poorer executive function (overall sample and MCI subsample) and language performance (MCI subsample only) were not only stronger in women but were female-specific. In the overall sample, the relationship between prediabetes and brain hypometabolism across time was observed regardless of sex; however, prediabetes was associated with hypometabolism at baseline in women and not in men, and, in the MCI subsample, the longitudinal relationship became female-specific. One explanation for our sex disparities in findings may be that insulin resistance is more severe in women vs. men at the prediabetic stage and therefore shows more adverse effects. Arguing against this explanation, the mean fasting glucose level was actually lower in prediabetic women vs. prediabetic men in both the overall and MCI samples although not significantly ($ps > 0.05$). An alternative explanation is that women are more vulnerable than men to the adverse effects of a given level of insulin resistance. These more adverse effects of insulin resistance in women, possibly in combination with other early-stage AD brain changes, may surpass a threshold of brain insult that leads to greater cognitive deficits in prediabetics vs. normoglycemics among women. The reason for the sex disparity is unclear, although sex differences often suggest sex hormone mechanisms. Insulin concentrations have been reported to decline in men from the 5th to 8th decade of life but increase in women purportedly related to shifts in sex hormone status that occur with menopause (70). Another contributing factor that is related to prediabetes could be the higher percentage of body fat, particularly subcutaneous, and the adipose tissue derived leptin hormone in women vs. men (71, 72). Adiposity and its associated hormones are strongly associated with low-grade inflammation and this association is more robust in women vs. men (73–75)

so that both normal and prediabetic women have shown higher levels of inflammatory markers than men (75).

Prediabetes related to poorer executive function and language performance only among women with MCI sample suggesting that prediabetes may only affect cognitive function among women on the AD trajectory. Considering that 83% of the MCI sample was biomarker positive for CSF Aβ and/or pTau levels, as determined based on established cutpoints (76), this suggests that the negative effect of prediabetes on cognition may only manifest when compounded by AD pathology. In line with this theory was our finding that prediabetic status did not affect rates of incident dementia in either sex but, among incident dementia cases, prediabetes was associated with an earlier age of dementia onset among women only. This might further suggest that prediabetes plays a role in the acceleration or severity of AD pathological cascades in women rather than the initiation of that cascade.

As hypothesized, prediabetes was associated with poorer executive function and language performance in women with MCI, but not with episodic memory performance, regardless of sex. This disparity could be because frontal-mediated cognitive impairments are commonly reported in relation to vascular mechanisms and vascular dementia (47–50). In support of our results is a meta-analysis of over 100,000 dementia cases that reported that T2D was a stronger risk factor specifically for vascular dementia in women compared to men (77). Cardiovascular risk factors in older adults are associated with microvascular changes in the cerebral white matter (78), which, regardless of brain location, are associated with diminished executive function (48). In addition, Type 2 diabetes and elevated pulse pressure have each been associated with declines in language abilities (2, 79). Disruptions of frontal-subcortical networks may lead to reduced abilities to retrieve semantic information, manifesting as word-finding difficulties. Similarly, a study from the National Alzheimer's Coordinating Center's Uniform Data examining longitudinal cognitive profiles in diabetic patients with MCI ($N = 4{,}114$) found that diabetes was associated with lower cognitive performance, primarily in non-memory domains (80). A more psychometric explanation for the association of prediabetes with language and executive functioning but not memory is also possible. The MCI group showed the poorest performance in the memory domain relative to other domains regardless of sex or prediabetes status, likely because ADNI targets the precursor stage to AD dementia—amnestic MCI—in their recruitment. The lower memory scores among the MCI participants may result in a restricted range that limits the ability to observe an effect of prediabetes.

There are study limitations. For example, the number of follow-up assessments differed by AD marker suggesting that our statistical power to detect significant effects varied across AD markers. We did not apply a statistical correction for our multiple comparisons; however, we felt this was not necessary given that we had apriori hypotheses concerning the specific outcomes that would relate to prediabetes and the specific direction of those relationships, with results mostly supporting hypotheses. In light of the observed effects in frontally-mediated cognitive domains, we would have ideally examined CMRglu specifically in more frontal regions; however, the number of ADNI participants with frontal lobe CMRglu in addition to our other variables of interest did not allow for statistically powered analyses. Lastly, ADNI is a convenience sample of mostly white and well-educated volunteers compared with the general US population, which limits the generalizability of results to the general population and particularly to other race/ethnic groups, which have differing rates of prediabetes and other cardiovascular comorbidities. Strengths of the current study include (1) a large sample that is well-characterized for AD neurocognitive deficits and biomarkers, (2) a longitudinal analyses of change in AD markers over time, (3) determining whether sex plays a moderating role in associations between prediabetes and AD-related outcomes, and (4) examination of prediabetes in relation to multiple cognitive domains and biomarkers of brain structure, function, and pathology.

In conclusion, although the adverse effects of T2D on brain and cognitive health are well documented, the effects of prediabetes on these outcomes are far less understood. Our results suggest that even prediabetes shows adverse effects on brain metabolism in older men and women. In light of previous findings relating T2D to reduced brain volume and AD pathology, our results suggest that functional changes may be seen before brain volume changes in the early trajectory of T2D. In women only, prediabetes was associated with poorer executive function overall and language performance specifically in the context of MCI. This suggests that women may be more susceptible than men to the negative effects of prediabetes on brain and cognition, and that these adverse effects may accelerate the progression of AD. Large-scale prospective studies are needed to further investigate sex disparities in the effect of prediabetes and T2D on other markers of brain health including white matter integrity and dementia progression rates. A large focus should be on modifiable risk factors such as prediabetes/T2D given that we still do not have any established treatments for progressive cognitive impairment. Practitioners should be advised that women with prediabetes may be at higher risk for cognitive decline and should target this population for careful assessment and intervention that could possibly delay the onset or progression of AD.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were conducted by the Alzheimer's Disease Neuroimaging Initiative (ADNI), and were approved by the Institutional Review Boards of all participating sites. Participants provided their written informed consent to participate in the study.

AUTHOR CONTRIBUTIONS

ES and LD-W conceived and designed the research project. ES, KT, and AW aggregated the data. ES conducted statistical analyses, wrote the paper, and has primary responsibility for final content. All authors consulted on study design, statistical analyses, manuscript editing, read, and approved the final manuscript.

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REFERENCES

1. Accardi G, Caruso C, Colonna-Romano G, Camarda C, Monastero R, Candore G. Can Alzheimer disease be a form of type 3 diabetes? *Rejuvenation Res.* (2012) 15:217–21. doi: 10.1089/rej.2011.1289
2. Bangen KJ, Beiser A, Delano-Wood L, Nation DA, Lamar M, Libon DJ, et al. APOE genotype modifies the relationship between midlife vascular risk factors and later cognitive decline. *J Stroke Cerebrovasc Dis.* (2013) 22:1381–69. doi: 10.1016/j.jstrokecerebrovasdis.2013.03.013
3. Cheng G, Huang C, Deng H, Wang H. Diabetes as a risk factor for dementia and mild cognitive impairment: a meta-analysis of longitudinal studies. *Intern Med J.* (2012) 42:484–91. doi: 10.1111/j.1445-5994.2012.02758.x
4. Leibson CL, Rocca WA, Hanson VA, Cha R, Kokmen E, O'Brien PC, et al. The risk of dementia among persons with diabetes mellitus: a population-based cohort study. *Ann N Y Acad Sci.* (1997) 826:422–7. doi: 10.1111/j.1749-6632.1997.tb48496.x
5. Ott A, Stolk RP, van Harskamp F, Pols HA, Hofman A, Breteler MM. Diabetes mellitus and the risk of dementia: the Rotterdam study. *Neurology.* (1999) 53:1937–42. doi: 10.1212/WNL.53.9.1937
6. Peila R, Rodriguez BL, Launer LJ. Honolulu-Asia aging S. Type 2 diabetes, APOE gene, and the risk for dementia and related pathologies: the Honolulu-Asia aging study. *Diabetes.* (2002) 51:1256–62. doi: 10.2337/diabetes.51.4.1256
7. Moran C, Phan TG, Chen J, Blizzard L, Beare R, Venn A, et al. Brain atrophy in type 2 diabetes: regional distribution and influence on cognition. *Diabetes Care.* (2013) 36:4036–42. doi: 10.2337/dc13-0143
8. Moran C, Beare R, Phan TG, Bruce DG, Callisaya ML, Srikanth V. Type 2 diabetes mellitus and biomarkers of neurodegeneration. *Neurology.* (2015) 85:1123–30. doi: 10.1212/WNL.0000000000001982
9. Biessels GJ, Reijmer YD. Brain changes underlying cognitive dysfunction in diabetes: what can we learn from MRI? *Diabetes.* (2014) 63:2244–52. doi: 10.2337/db14-0348
10. van Elderen SG, de Roos A, de Craen AJ, Westendorp RG, Blauw GJ, Jukema JW, et al. Progression of brain atrophy and cognitive decline in diabetes mellitus: a 3-year follow-up. *Neurology.* (2010) 75:997–1002. doi: 10.1212/WNL.0b013e3181f25f06
11. Schneider ALC, Selvin E, Sharrett AR, Griswold M, Coresh J, Jack CR Jr, et al. Diabetes, prediabetes, and brain volumes and subclinical cerebrovascular disease on MRI: the atherosclerosis risk in communities neurocognitive study (ARIC-NCS). *Diabetes Care.* (2017) 40:1514–21. doi: 10.2337/dc17-1185
12. Callisaya ML, Beare R, Moran C, Phan T, Wang W, Srikanth VK. Type 2 diabetes mellitus, brain atrophy and cognitive decline in older people: a longitudinal study. *Diabetologia.* (2019) 62:448–58. doi: 10.1007/s00125-018-4778-9
13. Li W, Risacher SL, Huang E, Saykin AJ. Alzheimer's disease neuroimaging initiative. Type 2 diabetes mellitus is associated with brain atrophy and hypometabolism in the ADNI cohort. *Neurology.* (2016) 87:595–600. doi: 10.1212/WNL.0000000000002950
14. Baker LD, Cross DJ, Minoshima S, Belongia D, Watson GS, Craft S. Insulin resistance and Alzheimer-like reductions in regional cerebral glucose metabolism for cognitively normal adults with prediabetes or early type 2 diabetes. *Arch Neurol.* (2011) 68:51–7. doi: 10.1001/archneurol.2010.225
15. Willette AA, Bendlin BB, Starks EJ, Birdsill AC, Johnson SC, Christian BT, et al. Association of insulin resistance with cerebral glucose uptake in late middle-aged adults at risk for Alzheimer disease. *JAMA Neurol.* (2015) 72:1013–20. doi: 10.1001/jamaneurol.2015.0613
16. Verdile G, Fuller SJ, Martins RN. The role of type 2 diabetes in neurodegeneration. *Neurobiol Dis.* (2015) 84:22–38. doi: 10.1016/j.nbd.2015.04.008
17. Laws SM, Gaskin S, Woodfield A, Srikanth V, Bruce D, Fraser PE, et al. Insulin resistance is associated with reductions in specific cognitive domains and increases in CSF tau in cognitively normal adults. *Sci Rep.* (2017) 7:9766. doi: 10.1038/s41598-017-09577-4
18. Lu Y, Jiang X, Liu S, Li M. Changes in cerebrospinal fluid tau and β-amyloid levels in diabetic and prediabetic patients: a meta-analysis. *Front Aging Neurosci.* (2018) 10:271. doi: 10.3389/fnagi.2018.00271

19. Bangen KJ, Werhane ML, Weigand AJ, Edmonds EC, Delano-Wood L, Thomas KR, et al. Reduced regional cerebral blood flow relates to poorer cognition in older adults with type 2 diabetes. *Front Aging Neurosci.* (2018) 10:270. doi: 10.3389/fnagi.2018.00270
20. Bangen KJ, Himali JJ, Beiser AS, Nation DA, Libon DJ, Fox CS, et al. Interaction between midlife blood glucose and APOE genotype predicts later Alzheimer pathology. *J Alzheimers Dis.* (2016) 53:1553–62. doi: 10.3233/JAD-160163
21. Starks EJ, Patrick O'Grady J, Hoscheidt SM, Racine AM, Carlsson CM, Zetterberg H, et al. Insulin resistance is associated with higher cerebrospinal fluid tau levels in asymptomatic APOEε4 carriers. *J Alzheimers Dis.* (2015) 46:525–33. doi: 10.3233/JAD-150072
22. Li W, Risacher SL, Gao S, Boehm SL 2nd, Elmendorf JS, Saykin AJ. Type 2 diabetes mellitus and cerebrospinal fluid Alzheimer's disease biomarker amyloid β1-42 in Alzheimer's disease neuroimaging initiative participants. *Alzheimers Dement.* (2017) 10:94–8. doi: 10.1016/j.dadm.2017.11.002
23. Luchsinger JA. Diabetes, related conditions, and dementia. *J Neurol Sci.* (2010) 299:35–8. doi: 10.1016/j.jns.2010.08.063
24. Luchsinger JA, Tang MX, Shea S, Mayeux R. Hyperinsulinemia and risk of Alzheimer disease. *Neurology.* (2004) 63:1187–92. doi: 10.1212/01.WNL.0000140292.04932.87
25. Yaffe K, Blackwell T, Kanaya AM, Davidowitz N, Barrett-Connor E, Krueger K. Diabetes, impaired fasting glucose, and development of cognitive impairment in older women. *Neurology.* (2004) 63:658–63. doi: 10.1212/01.WNL.0000134666.64593.BA
26. Wozniak M, Rydzewski B, Baker SP, Raizada MK. The cellular and physiological actions of insulin in the central nervous system. *Neurochem Int.* (1993) 22:1–10. doi: 10.1016/0197-0186(93)90062-A
27. D'Ercole AJ, Ye P, Calikoglu AS, Gutierrez-Ospina G. The role of the insulin-like growth factors in the central nervous system. *Mol Neurobiol.* (1996) 13:227–55. doi: 10.1007/BF02740625
28. Bell GI. Lilly lecture 1990. Molecular defects in diabetes mellitus. *Diabetes.* (1991) 40:413–22. doi: 10.2337/diab.40.4.413
29. Taylor SI. Lilly lecture: molecular mechanisms of insulin resistance. Lessons from patients with mutations in the insulin-receptor gene. *Diabetes.* (1992) 41:1473–90. doi: 10.2337/diabetes.41.11.1473
30. Bitra VR, Rapaka D, Akula A. Prediabetes and Alzheimer's disease. *Indian J Pharm Sci.* (2015) 77:511–4. doi: 10.4103/0250-474X.169026
31. Alzheimer's Association. 2020 Alzheimer's disease facts and figures. *Alzheimers Dement.* (2020) 10:e47–92. doi: 10.1016/j.jalz.2014.02.001
32. Heyman A, Fillenbaum G, Prosnitz B, Raiford K, Burchett B, Clark C. Estimated prevalence of dementia among elderly black and white community residents. *Arch Neurol.* (1991) 48:594–8. doi: 10.1001/archneur.1991.00530180046016
33. Andersen K, Launer LJ, Dewey ME, Letenneur L, Ott A, Copeland JR, et al. Gender differences in the incidence of AD and vascular dementia: the EURODEM studies. EURODEM Incidence Research Group. *Neurology.* (1999) 53:1992–7. doi: 10.1212/WNL.53.9.1992
34. Lin KA, Choudhury KR, Rathakrishnan BG, Marks DM, Petrella JR, Doraiswamy PM, et al. Marked gender differences in progression of mild cognitive impairment over 8 years. *Alzheimers Dement.* (2015) 1:103–10. doi: 10.1016/j.trci.2015.07.001
35. Barnes LL, Wilson RS, Bienias JL, Schneider JA, Evans DA, Bennett DA. Sex differences in the clinical manifestations of Alzheimer disease pathology. *Arch Gen Psychiatry.* (2005) 62:685–91. doi: 10.1001/archpsyc.62.6.685
36. Corder EH, Ghebremedhin E, Taylor MG, Thal DR, Ohm TG, Braak H. The biphasic relationship between regional brain senile plaque and neurofibrillary tangle distributions: modification by age, sex, and APOE polymorphism. *Ann N Y Acad Sci.* (2004) 1019:24–8. doi: 10.1196/annals.1297.005
37. Irvine K, Laws KR, Gale TM, Kondel TK. Greater cognitive deterioration in women than men with Alzheimer's disease: a meta analysis. *J Clin Exp Neuropsychol.* (2012) 34:989–98. doi: 10.1080/13803395.2012.712676
38. Benjamin EJ, Blaha MJ, Chiuve SE, Cushman M, Das SR, Deo R, et al. Heart disease and stroke statistics-−2017 update: a report from the American Heart Association. *Circulation.* (2017) 135:e146–603. doi: 10.1161/CIR.0000000000000485
39. Kannel WB, Hjortland MC, McNamara PM, Gordon T. Menopause and risk of cardiovascular disease: the Framingham study. *Ann Intern Med.* (1976) 85:447. doi: 10.7326/0003-4819-85-4-447
40. Willeit J, Kiechl S. Prevalence and risk factors of asymptomatic extracranial carotid artery atherosclerosis: a population-based study. *Arterioscler Thromb.* (1993) 13:661. doi: 10.1161/01.ATV.13.5.661
41. Barrett-Connor EL, Cohn BA, Wingard DL, Edelstein SL. Why is diabetes mellitus a stronger risk factor for fatal ischemic heart disease in women than in men? The Rancho Bernardo Study. *JAMA.* (1991) 265:627–31. doi: 10.1001/jama.265.5.627
42. Huxley R, Barzi F, Woodfard M. Excess risk of fatal coronary heart disease associated with diabetes in men and women: meta-analysis of 37 prospective cohort studies. *BMJ.* (2006) 332:73–8. doi: 10.1136/bmj.38678.389583.7C
43. Yusuf S, Hawken S, Ôunpuu S, Dans T, Avezum A, Lanas F, et al. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. *Lancet.* (2004) 364:937–52. doi: 10.1016/S0140-6736(04)17018-9
44. Kanaya AM, Grady D, Barret-Connor E. Explaining the sex difference in coronary heart disease mortality among patients with type 2 diabetes mellitus: a metaanalysis. *Arch Intern Med.* (2002) 162:1737–45. doi: 10.1001/archinte.162.15.1737
45. Kannel WB, McGee DL. Diabetes and glucose tolerance as risk factors for cardiovascular disease: the Framingham study. *Diabetes Care.* (1979) 2:120–6. doi: 10.2337/diacare.2.2.120
46. Carnevale Schianca GP, Colli E, Onolfo S, Pedrazzoli R, Fra GP, Bartoli E. Individuation of different metabolic phenotypes in normal glucose tolerance test. *Acta Diabetol.* (2010) 47:167–72. doi: 10.1007/s00592-009-0165-9
47. Traykov L, Baudic S, Thibaudet MC, Rigaud AS, Smagghe A, Boller F. Neuropsychological deficit in early subcortical vascular dementia: comparison to Alzheimer's disease. *Dement Geriatr Cogn Disord.* (2002) 14:26–32. doi: 10.1159/000058330
48. Tullberg M, Fletcher E, DeCarli C, Mungas D, Reed BR, Harvey DJ, et al. White matter lesions impair frontal lobe function regardless of their location. *Neurology.* (2004) 63:246–53. doi: 10.1212/01.WNL.0000130530.55104.B5
49. O'Brien JT, Erkinjuntti T, Reisberg B, Roman G, Sawada T, Pantoni L, et al. Vascular cognitive impairment. *Lancet Neurol.* (2003) 2:89–98. doi: 10.1016/S1474-4422(03)00305-3
50. Graham NL, Emery T, Hodges JR. Distinctive cognitive profiles in Alzheimer's disease and subcortical vascular dementia. *J Neurol Neurosurg Psychiatry.* (2004) 75:61–71.
51. Petersen RC, Aisen PS, Beckett LA, Donohue MC, Gamst AC, Harvey DJ, et al. Alzheimer's disease neuroimaging initiative (ADNI): clinical characterization. *Neurology.* (2010) 74:201–9. doi: 10.1212/WNL.0b013e3181cb3e25
52. World Health Organization. *Definition, Diagnosis and Classification of Diabetes Mellitus and Its Complications. Report of a WHO Consultation. Part 1: Diagnosis and Classification of Diabetes Mellitus. Report No: WHO/NCD/NCS/99.2.* Geneva, Switzerland: World Health Organization (1999). Available online at: http://www.staff.ncl.ac.uk/philip.home/who_dmg.pdf (accessed August, 2019).
53. Bondi MW, Edmonds EC, Jak AJ, Clark L, Delano-Wood LR, McDonald CR, et al. Neuropsychological criteria for mild cognitive impairment improves diagnostic precision, biomarker associations, and progression rates. *J Alzheimers Dis.* (2014) 42:275–89. doi: 10.3233/JAD-140276
54. McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology.* (1984) 34:939–44. doi: 10.1212/WNL.34.7.939
55. American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care.* (2008) 31 (Suppl. 1):S55–60. doi: 10.2337/dc08-S055
56. Jack CR Jr, Bernstein MA, Fox NC, Thompson P, Alexander G, Harvey D, et al. The Alzheimer's disease neuroimaging initiative (ADNI): MRI methods. *J Magn Reson Imaging.* (2008) 27:685–91. doi: 10.1002/jmri.21049
57. Hsu YY, Schuff N, Du AT, Mark K, Zhu X, Hardin D, et al. Comparison of automated and manual MRI volumetry of hippocampus in normal aging and dementia. *J Magn Reson Imaging.* (2002) 16:305–10. doi: 10.1002/jmri.10163
58. Landau SM, Harvey D, Madison CM, Koeppe RA, Reiman EM, Foster NL, et al. Associations between cognitive, functional, and FDG-PET

measures of decline in AD and MCI. *Neurobiol Aging.* (2011) 32:1207–18. doi: 10.1016/j.neurobiolaging.2009.07.002
59. Jagust WJ, Bandy D, Chen K, Foster NL, Landau SM, Mathis CA, et al. The Alzheimer's disease neuroimaging initiative positron emission tomography core. *Alzheimers Dement.* (2010) 6:221–9. doi: 10.1016/j.jalz.2010.03.003
60. Landau SM, Harvey D, Madison CM, Reiman EM, Foster NL, Aisen PS, et al. Comparing predictors of conversion and decline in mild cognitive impairment. *Neurology.* (2010) 75:230–8. doi: 10.1212/WNL.0b013e3181e8e8b8
61. Han DS, Nguyen CP, Stricker NH, Nation DA. Detectable neuropsychological differences in early preclinical Alzheimer's disease: a meta-analysis. *Neuropsychol Rev.* (2017) 27:305–25. doi: 10.1007/s11065-017-9366-0
62. Smith SC Jr. Multiple risk factors for cardiovascular disease and diabetes mellitus. *Am J Med.* (2007) 120 (3 Suppl. 1):S3–11. doi: 10.1016/j.amjmed.2007.01.002
63. Gur RC, Turetsky BI, Matsui M, Yan M, Bilker W, Hughett P, et al. Sex differences in brain gray and white matter in healthy young adults: correlations with cognitive performance. *J Neurosci.* (1999) 19:4065–72. doi: 10.1523/JNEUROSCI.19-10-04065.1999
64. Filipek PA, Richelme C, Kennedy DN, Caviness VS Jr. The young adult human brain: An MRI-based morphometric analysis. *Cereb Cortex.* (1994) 4:344–60. doi: 10.1093/cercor/4.4.344
65. Kramer JH, Delis DC, Daniel MH. Sex differences in verbal learning. *J Clin Psychol.* (1988) 44:907–15. doi: 10.1002/1097-4679(198811)44:6<907::AID-JCLP2270440610>3.0.CO;2-8
66. Grillo CA, Piroli GG, Hendry RM, Reagan LP. Insulin-stimulated translocation of GLUT4 to the plasma membrane in rat hippocampus is PI3-kinase dependent. *Brain Res.* (2009) 1296:35–45. doi: 10.1016/j.brainres.2009.08.005
67. Lucignani G, Namba H, Nehlig A, Porrino LJ, Kennedy C, Sokoloff L. Effects of insulin on local cerebral glucose utilization in the rat. *J Cereb Blood Flow Metab.* (1987) 7:309–14. doi: 10.1038/jcbfm.1987.68
68. Craft S. The role of metabolic disorders in Alzheimer disease and vascular dementia: two roads converged. *Arch Neurol.* (2009) 66:300–5. doi: 10.1001/archneurol.2009.27
69. Langbaum JB, Chen K, Lee W, Reschke C, Bandy D, Fleisher AS, et al. Alzheimer's disease neuroimaging initiative. Categorical and correlational analyses of baseline fluorodeoxyglucose positron emission tomography images from the Alzheimer's Disease Neuroimaging Initiative (ADNI). *Neuroimage.* (2009) 45:1107–16. doi: 10.1016/j.neuroimage.2008.12.072
70. Willeit J, Kiechl S, Egger G, Oberhollenzer M, Oberhollenzer F, Muggeo M, et al. The role of insulin in age-related sex differences of cardiovascular risk profile and morbidity. *Atherosclerosis.* (1997) 130:183–9. doi: 10.1016/S0021-9150(96)06038-8
71. Bredella MA. Sex differences in body composition. *Adv Exp Med Biol.* (2017) 1043:9–27. doi: 10.1007/978-3-319-70178-3_2
72. Jackson AS, Stanforth PR, Gagnon J, Rankinen T, Leon AS, Rao DC, et al. The effect of sex, age and race on estimating percentage body fat from body mass index: the heritage family study. *Int J Obes Relat Metab Disord.* (2002) 26:789–96. doi: 10.1038/sj.ijo.0802006
73. Suboc TM, Dharmashankar K, Wang J, Ying R, Couillard A, Tanner MJ, et al. Moderate obesity and endothelial dysfunction in humans: influence of gender and systemic inflammation. *Physiol Rep.* (2013) 1:e00058. doi: 10.1002/phy2.58
74. Peters SA, Huxley RR, Sattar N, Woodward M. Sex differences in the excess risk of cardiovascular diseases associated with type 2 diabetes: potential explanations and clinical implications. *Curr Cardiovasc Risk Rep.* (2015) 9:36. doi: 10.1007/s12170-015-0462-5
75. Saltevo J, Kautiainen H, Vanhala M. Gender differences in adiponectin and low-grade inflammation among individuals with normal glucose tolerance, prediabetes, and type 2 diabetes. *Gend Med.* (2009) 6:463–70. doi: 10.1016/j.genm.2009.09.006
76. Hansson O, Seibyl J, Stomrud E, Zetterberg H, Trojanowski JQ, Bittner T, et al. CSF biomarkers of Alzheimer's disease concord with amyloid-beta PET and predict clinical progression: a study of fully automated immunoassays in BioFINDER and ADNI cohorts. *Alzheimers Dement.* (2018) 14:1470–81. doi: 10.1016/j.jalz.2018.01.010
77. Chatterjee S, Peters SA, Woodward M, Mejia Arango S, Batty GD, Beckett N, et al. Type 2 diabetes as a risk factor for dementia in women compared with men: a pooled analysis of 2.3 million people comprising more than 100,000 cases of dementia. *Diabetes Care.* (2016) 39:300–7. doi: 10.2337/dc15-1588
78. Pugh KG, Lipsitz LA. The microvascular frontal-subcortical syndrome of aging. *Neurobiol Aging.* (2002) 23:421–31. doi: 10.1016/S0197-4580(01)00319-0
79. Nation DA, Wierenga CE, Delano-Wood L, Jak AJ, Delis DC, Salmon DP, et al. Elevated pulse pressure is associated with age-related decline in language ability. *JINS.* (2010) 16:933–8. doi: 10.1017/S1355617710000548
80. Sano M, Zhu CW, Grossman H, Schimming C. Longitudinal cognitive profiles in diabetes: results from the national Alzheimer's coordinating center's uniform data. *J Am Geriatr Soc.* (2017) 65:2198–204. doi: 10.1111/jgs.15014

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