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Influences of hormone replacement therapy on olfactory and cognitive function in postmenopausal women

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ABSTRACT

Olfactory dysfunction can be an early sign of Alzheimer's disease. Since hormone replacement therapy (HRT) may protect against Alzheimer's disease in postmenopausal women, the question arises as to whether it also protects against olfactory dysfunction in such women. A total of three olfactory and 12 neurocognitive tests were administered to 432 healthy postmenopausal women with varied HRT histories. Serum levels of reproductive hormones were obtained for all subjects; APOE-ε4 haplotype was determined for 77 women. National Adult Reading Test and Odor Memory/Discrimination Test scores were positively influenced by HRT. Odor Identification and Odor Memory/Discrimination Test scores were lower for women who scored poorly on a delayed recall test, a surrogate for mild cognitive impairment. The Wechsler Adult Intelligence Scale, Revised, as a Neuropsychological Instrument Spatial Span Backwards Test scores were higher in women receiving estrogen and progestin HRT and directly correlated with serum testosterone levels, the latter implying a positive effect of testosterone on spatial memory. APOE-ε4 was associated with poorer odor threshold test scores. These data suggest that HRT positively influences a limited number of olfactory and cognitive measures during menopause.

1. Introduction

A number of observational studies and meta-analyses support the view that gonadal hormone replacement therapy (HRT), especially estrogen replacement therapy (ERT), protects postmenopausal women from cognitive decline (LeBlanc et al., 2001), particularly if administered soon after menopause (Fischer et al., 2014). ERT has been reported to reduce the risk of Alzheimer's disease (AD) by greater than 30% (LeBlanc et al., 2001) and to improve attention (Smith et al., 2001), working memory (Duff and Hampson, 2000), and verbal short-term memory (LeBlanc et al., 2001; Sherwin and Sherwin, 2003). Murine studies suggest that estrogens have neurotrophic and neuroprotective effects (Brann et al., 2007), alter spine morphology and synaptic excitability in the hippocampus (Li et al., 2004), promote survival of forebrain cholinergic neurons (Kompoliti et al., 2004), and facilitate cholinergic, dopaminergic, and serotonergic neural transmission (Heikkinen et al., 2002).

That being said, not all studies have found positive effects of HRT on cognition, particularly in older women, and its benefits are widely debated. For example, one study found that 20 weeks of ERT had no influence on the cognitive performance of 58 women 70 years of age and older (Almeida et al., 2006). Similarly, 9 months of opposed ERT did not improve cognition in 52 women ranging in age from 75 to 91 years (Binder et al., 2001). Indeed, negative effects of HRT have been reported. In 532 women older than 65 and those with the highest estrone (E_1) levels had 15% *lower* scores on the Digit Symbol subtest of the Wechsler Adult Intelligence Scale-Revised (WAIS-R) and a longitudinal reduction in the performance on the Trails B test, a test that taps attention, mental flexibility, sequential tasking, and visual scanning (Yaffe et al., 1998). In the Women's Health Initiative Memory Study (Shumaker et al., 2004), women receiving conjugated equine

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estrogen and medroxyprogesterone acetate were more likely to develop dementia relative to controls (hazard ratio = 2.05, 95% confidence interval = 1.21–3.48). This risk was also found to be elevated in women receiving conjugated equine estrogen alone, but the hazard ratio (1.5 [0.8–2.7]) was not statistically significant.

Given that decreased smell function can be an early sign of AD and may predict subsequent cognitive decline in older persons (Devanand et al., 2000), the question arises as to whether HRT influences olfactory test scores in postmenopausal women and, if so, whether such scores are correlated with measures of cognitive function and circulating levels of reproductive hormones. Unfortunately, studies on this point are sparse and inconclusive. Earlier studies reporting improved olfactory function after estrogen treatment had sample sizes ranging from only 1 to 5 and had methodological issues (Doty and Cameron, 2009). Nonetheless, some later studies using larger samples supported these early observations. For example, Sundermann et al. (2006) reported that ERT improved performance in 24 postmenopausal women with AD on an odor recognition memory task. Such improvement was noted in a later study by the same authors for a threshold test in 16 older non-demented women, but this effect was evident only in women positive for the APOE-ε4 allele, a risk factor for AD (Sundermann et al., 2008). More recently, Caruso et al. (2008) noted increased olfactory threshold sensitivity in 46 postmenopausal women who had received opposed ERT for 8 months, although their test procedure confounds olfactory sensitivity with stimulus air pressure (Jones, 1953) and, like most early studies, no controls for sequential order effects were used.

In contrast to the aforementioned studies are ones that failed to observe an effect of estrogens or HRT on olfactory function. A cross-sectional study of 62 postmenopausal women found no influences of opposed or unopposed ERT on a range of olfactory tests (Hughes et al., 2002). In a second component of this study, no effects of HRT were found for the 24 women who were tested longitudinally. More recently, olfactory thresholds to phenyl ethanol, mercaptan, glacial acetic acid, and eucalyptol were not influenced by in vitro fertilization procedures that enhanced ovarian production of estradiol (E_2) (Robinson et al., 2007). Thresholds for 6 women under the low and high 17β-E_2 conditions did not differ, nor did those from 7 subjects before and after the steepest rise in 17β-E_2 levels.

The present study tested odor identification, odor discrimination/memory, odor threshold sensitivity, and a range of neuropsychological measures in a large number of postmenopausal women who had never taken, had previously taken, or were currently taking opposed or unopposed ERT. Associations between the test measures and serum levels of follicle stimulating hormone (FSH), E_1, E_2, progesterone, testosterone (T), dehydroepiandrosterone sulfate (DHEA-S), and cortisol (C) were also obtained. The time of HRT initiation relative to menopause was examined, as were associations among the olfactory, cognitive, and hormonal measures. The APOE genotype was obtained from a subgroup of subjects to determine whether having the ε4 haplotype influenced the test scores.

2. Methods

Each subject received a series of olfactory and cognitive tests during a 4–5 hour test session. Adequate breaks were interspersed between the tests. Peripheral venous blood was then collected for the hormone assays. The test administrators had no access to the results of these analyses.

2.1. Subjects

The study population comprised 432 healthy postmenopausal women with varying histories of HRT treatment (Table 1). Each had ≥12 months of amenorrhea or surgical menopause with bilateral oophorectomy and serum FSH levels >40 IU. Thirty-two percent had undergone hysterectomy. Among the HRT groups, 158 were taking oral preparations, 11 were taking transdermal preparations, and 11 were taking both oral and transdermal preparations. The form of hormone administration was unknown in the remainder of the women. All subjects had received complete medical and gynecological examinations before testing. Exclusion criteria included the presence or history of severe nasal or respiratory disorders, stroke,

Table 1
Demographics of study group

Group	Subgroup	N	Age Mean ± SD Median Range 95% CI	Education Mean ± SD Median Range 95% CI	Age at menopause Mean ± SD Median Range 95% CI	Cumulative HRT Mean ± SD Median Range 95% CI
Current HRT	Current unopposed ERT	33	66.0 ± 9.5 62.0 55–83 62.6–69.4	15.1 ± 2.9 16.0 11–20 14.1–16.2	44.6 ± 7.9 43.0 32–58 48.6–50.0	15.3 ± 10.2 14.0 2–38 11.7–18.9
	Current opposed ERT	24	60.7 ± 5.8 58.5 55–76 58.2–63.1	15.9 ± 3.1 16.0 12–22 14.6–17.2	49.9 ± 4.1 50.0 40–56 48.0–51.8	9.5 ± 5.8 10.0 0.13–23 7.0–12.0
Past HRT	Past unopposed ERT	62	69.3 ± 8.2 68.0 54–85 67.2–71.4	14.5 ± 2.4 14.0 11–20 13.8–15.1	44.5 ± 6.4 44.0 28–55 42.2–46.8	9.8 ± 7.9 9.5 0.01–30 7.7–11.9
	Past opposed ERT	99	65.4 ± 7.3 65.0 55–84 63.9–66.8	15.6 ± 2.8 16.0 11–22 15.0–16.1	51.3 ± 4.1 52.0 35–62 50.4–52.2	7.1 ± 6.2 5.0 0.03–30 5.8–8.4
Never HRT	Never ERT	214	67.5 ± 9.7 67.0 52–89 66.2–68.8	14.5 ± 3.1 14.0 8–28 14.1–14.9	50.0 ± 6.2 51.0 20–60 48.8–50.7	—

All values in years
Key: CI, confidence interval; ERT, estrogen replacement therapy; HRT, hormone replacement therapy; SD, standard deviation.

Table 2
Age- and NART-IQ-adjusted mean and SEM values for olfactory and neuropsychological test scores of HRT groups

Group	Sub-group	UPSIT	OMT	PEA	CVLT LD	CVLT TOT	ACT	BVMT DR	BVMT TR
Current HRT	Current unopposed ERT ($n = 33$)	30.55 (0.84)	6.55 (0.42)	−4.54 (0.30)	10.29 (0.55)	46.80 (1.64)	30.61 (1.17)	9.16 (0.51)	21.61 (1.24)
	Current opposed ERT ($n = 24$)	31.68 (0.98)	6.00 (0.49)	−4.91 (0.35)	10.42 (0.64)	49.38 (1.92)	30.02 (1.33)	8.54 (0.61)	21.93 (1.48)
Past HRT	Past unopposed ERT ($n = 62$)	32.61 (0.62)	6.20 (0.31)	−4.78 (0.22)	11.02 (0.40)	50.28 (1.20)	29.58 (0.83)	8.52 (0.37)	21.52 (0.91)
	Past opposed ERT ($n = 99$)	32.79 (0.48)	5.38 (0.24)	−4.65 (0.17)	10.57 (0.31)	49.74 (0.94)	30.13 (0.65)	8.81 (0.29)	22.09 (0.48)
Never HRT	Never ERT ($n = 214$)	32.63 (0.33)	5.56 (0.16)	−4.80 (0.12)	10.32 (0.21)	48.48 (0.64)	29.70 (0.44)	8.33 (0.20)	20.41 (0.48)
	p-Values	0.16	**0.05**	0.87	0.62	0.37	0.94	0.49	0.34

Group	Sub-group	DS TOT	DS TOT-B	SS TOT	SS TOT-B	Stroop CWT	CPT V	CPT RT	NART
Current HRT	Current unopposed ERT ($n = 33$)	18.00 (0.63)	7.30 (0.36)	13.05 (0.49)	5.72 (0.76)	−0.75 (1.27)	26.80 (0.79)	476.98 (20.05)	113.26 (2.05)
	Current opposed ERT ($n = 24$)	16.47 (0.74)	6.43 (0.43)	14.35 (0.58)	6.55 (0.89)	−0.54 (1.45)	27.85 (0.93)	505.32 (23.54)	115.39 (2.40)
Past HRT	Past unopposed ERT ($n = 62$)	17.50 (0.46)	7.15 (0.27)	13.29 (0.36)	6.27 (0.55)	0.93 (0.92)	28.23 (0.58)	488.79 (14.73)	108.62 (1.51)
	Past opposed ERT ($n = 99$)	16.72 (0.36)	6.86 (0.21)	13.75 (0.28)	6.40 (0.43)	0.76 (0.71)	27.88 (0.46)	501.86 (11.52)	112.90 (1.17)
Never HRT	Never ERT ($n = 214$)	16.83 (0.24)	6.78 (0.14)	13.13 (0.19)	6.52 (0.29)	0.32 (0.48)	27.64 (0.31)	488.99 (7.85)	108.87 (0.80)
	p-Values	0.26	0.43	0.16	0.90	0.76	0.68	0.78	**0.004**

The age-adjusted NART IQ measure is presented. *p* values for the individual ANCOVAs are indicated at the bottom of each column. Significant *p*-values indicated in bold type. See text for details.
Key: ACT, Auditory Consonant Trigrams-total score; BVMTDR, Brief Visuospatial Memory Test-delayed recall score; BVMTTR, Brief Visuospatial Memory Tests-total score; CPT V, Continuous Performance Task-vigilance score adjusted for false positives; CPT RT, Continuous Performance Task-response time in milliseconds; CVLT LD, California Verbal Learning Test-long delay score; CVLT TOT, California Verbal Learning Test-total score; DS TOT, Digit Span-total score; DS TOTB, Digit Span-backwards score; OMT, 12-item Odor Memory Test; NART, National Adult Reading Test; PEA, phenyl ethanol single staircase detection threshold test (log vol/vol); SS TOT, Spatial Span-total score; SS TOT-B, Spatial Span-backwards score; Stroop CWT, Stroop Color Word Test interference score; UPSIT, University of Pennsylvania Smell Identification Test.

carcinoma, hypercoagulable states, gallbladder disease, porphyria, parkinsonism, mental retardation, chronic alcohol or other substance abuse, liver disease, seizure disorder, bipolar disorder, schizophrenia, psychosis, multiple sclerosis, Korsakoff psychosis, head trauma leading to loss of consciousness, or chronic or major depression, as indicated by past treatment with antidepressants.

2.2. Olfactory tests

Three well-validated standardized olfactory tests were administered: the *University of Pennsylvania Smell Identification Test* [UPSIT; a 40-odor forced-choice test of the ability to identify odors (Doty et al., 1984)], the *Odor Memory/Discrimination Test* [OMT; a 12-item 4-alternative test in which a target odorant must be discerned from a set of foils at 10-, 30-, or 60-seconds delay intervals (Choudhury et al., 2003)], and a single staircase *Detection Threshold Test* using the odorant phenyl ethyl alcohol (PEA) (Deems and Doty, 1987). The OMT was chosen because of reports that short-term memory may be particularly sensitive to HRT (LeBlanc et al., 2001; Sherwin and Sherwin, 2003). The UPSIT and the PEA threshold tests were chosen on the basis of their sensitivity to age (Doty and Kamath, 2014) and a range of neurological disorders (Doty, 2012).

2.3. Cognitive tests

Six neuropsychological tests were administered: the *Digit Span and Spatial Span* tests of The Wechsler Adult Intelligence Scale, Revised, as a Neuropsychological Instrument (WAIS-R NI; attention, cognitive flexibility, and multiple forms of memory) (Kaplan et al., 1991), the Gordon Diagnostic Systems *Continuous Performance Test* (sustained attention) (Gordon and Mettelman, 1988), the *California Verbal Learning Test, Second Edition* (CVLT-II; verbal learning and memory) (Delis et al., 1987), the *Brief Visuospatial Memory Test-Revised* (BVMT-R; visuospatial learning and memory) (Benedict, 1997), the *Auditory Consonant Trigrams* Test (short-term auditory verbal memory and divided working memory) (Stuff et al., 1988), and the *Stroop Color-Word Test* (cognitive flexibility and resistance to interference) (Golden, 1978). The *National Adult Reading Test* (NART-IQ) was selected to be a surrogate measure for premorbid verbal IQ and full-scale IQ of the WAIS-R NI (Crawford et al., 2001) and has been shown to be sensitive to AD-related language deterioration (Schlosser and Ivison, 1989). The NART-IQ also served as a covariate along with age in a number of analyses.

2.4. Hormone analyses

RIA kits from Diagnostic Products Corporation, Los Angeles, CA, USA, were used to measure E_2 (Coat-A-Count, #TKE21), P (Coat-A-Count, #TKPG1), FSH (Count-A-Count, IRMA #IKFS1), C (Coat-A-Count, #TKC01); DHEA-S (Coat-A-Count, #TKDS1), and T (Count-A-Count, #TKTT1). The RIA kit from Diagnostic Systems Laboratories, Inc, Webster, TX, USA, was used to measure E_1 (DSL-8700). Hormonal measurements were made in batch assays using log-logit transformations to determine serum concentrations by interpolation from a standard curve. RIA trac Plus (Bio Rad, Anaheim, CA, USA) tri-level assay controls were used in all cases. The mean of 2 duplicate assay measurements for each sample was used.

Table 3
Mean, SEM, and 95% CI serum hormone levels of HRT groups

Group	FSH mil/mL	Estrone pg/mL	Estradiol pg/mL	Progesterone ng/mL	Cortisol μg/dL	DHEA-S μg/dL	Testosterone ng/dL
Current unopposed ERT ($n = 33$)	49.39 (5.39)	119.61 (9.81)	77.56 (6.25)	0.17 (0.08)	9.95 (0.79)	46.97 (10.47)	17.24 (3.37)
Current opposed ERT ($n = 24$)	48.84 (5.84)	149.56 (10.67)	84.97 (6.77)	0.82 (0.08)	9.72 (0.85)	82.50 (11.34)	21.33 (3.55)
Past unopposed ERT ($n = 62$)	67.70 (3.75)	33.93 (6.57)	11.66 (4.79)	0.22 (0.05)	9.88 (0.54)	55.29 (7.28)	18.91 (2.15)
Past opposed ERT ($n = 99$)	75.67 (2.79)	28.91 (5.06)	9.47 (3.37)	0.22 (0.04)	9.92 (0.41)	55.06 (5.43)	22.06 (1.63)
Never ERT ($n = 211$)	64.17 (1.95)	35.02 (3.50)	14.82 (2.48)	0.22 (0.03)	9.61 (0.29)	66.10 (3.8)	24.18 (1.14)
p-Values	**0.000**	**0.000**	**0.000**	**0.000**	0.969	0.061	0.111

p-Values from ANCOVAs computed across hormone groups with age and NART IQ as covariates, with significant *p*-values indicated in bold type.

Table 4
Partial correlation coefficients (p values) between olfactory and cognitive test scores

Group	CVLT LD	CVLT TOT	ACT	BVMT DR	BVMT TR	DS TOT	DS TOT-B	SS TOT	SS TOT-B	Stroop CWT	CPT V	CPT RT
UPSIT	0.19	0.19	0.11	0.21	0.21	0.06	0.08	0.06	0.01	0.10	0.09	−0.03
	0.000	**0.000**	**0.026**	**0.000**	**0.000**	0.247	0.095	0.226	0.808	**0.038**	0.064	0.519
OMT	0.09	0.10	0.07	0.15	0.12	0.04	0.08	0.05	0.10	0.07	0.08	−0.12
	0.052	**0.032**	0.147	**0.001**	**0.013**	0.413	0.088	0.333	**0.047**	0.156	0.089	**0.011**
PEA	−0.11	−0.14	−0.09	−0.07	−0.05	−0.07	−0.10	−0.04	0.00	−0.03	−0.17	+0.04
	0.020	**0.004**	0.152	0.161	0.320	0.156	**0.036**	0.370	0.926	0.588	**0.001**	0.403

Age and NART IQ effects are parceled out. p-Values are uncorrected for inflated alpha from multiple assessments. Nominally significant p-values are bolded. Only p-values $\leq$ 0.001 are statistically significant following the Bonferroni correction for inflated α. The negative correlations with PEA indicate positive associations with threshold *sensitivity* because negative PEA threshold scores reflect better performance.
Key: ACT, Auditory Consonant Trigrams-total score; BVMT DR, Brief Visuospatial Memory Test-delayed recall score; BVMT TR, Brief Visuospatial Memory Tests-total score; CPT V, Continuous Performance Task-vigilance score adjusted for false positives; CPT RT, Continuous Performance Task-response time in milliseconds; CVLT LD, California Verbal Learning Test-long delay score; CVLT TOT, California Verbal Learning Test-total score; DS TOT, Digit Span-total score; DS TOTB, Digit Span-backwards score; OMT, 12-item Odor Memory/Discrimination Test; NART, National Adult Reading Test; PEA, phenyl ethanol single staircase detection threshold test (log vol/vol); SS TOT, Spatial Span-total score; SS TOT-B, Spatial Span-backwards score; UPSIT, University of Pennsylvania Smell Identification.

2.5. APOE genotyping

Blood samples from 77 subjects were tested for the APOE-ε4 allele using a polymerase chain reaction procedure described in detail elsewhere (Addya et al., 1997).

2.6. Statistical analyses

In the case of E_1, E_2, T, DHEA-S, and C, log transformations were performed to better normalize the data. We initially compared scores for each test measure among the HRT groups listed in Table 2 using analysis of covariance (ANCOVA) with age and NART-IQ scores as covariates. When NART-IQ was used as a covariate, it eliminated the education effect between the groups of Table 1. When NART-IQ itself was assessed, age served as the covariate. The hormone levels detailed in Table 3 were similarly compared across groups. Additionally, olfactory test measures were compared between subjects who scored above and below 40 on the delayed recall measure of the CVLT-II, a surrogate measure for mild cognitive impairment (MCI). This ANCOVA included the between-subject factors of HRT group and CVLT-II category and the covariates of age and NART-IQ scores.

For those behavioral measures for which the ANCOVA was significant, pairwise post hoc comparisons among the groups were made using Tukey's honest significant difference test. Additionally, the performance of the women currently taking any type of HRT (24 opposed, 33 unopposed HRT) was compared with that of the women who were not currently taking HRT (99 past opposed, 62 past unopposed, 214 never). We also compared the test scores of the women receiving opposed ERT and the women receiving unopposed ERT with women who were not taking any type of HRT. Partial correlations assessed associations between the olfactory and cognitive test scores, as well as between these scores and the serum hormone levels, while parceling out potential confounds of age and NART-IQ.

To determine whether the time of HRT initiation relative to menopause or oophorectomy influenced the behavioral measures, an ANCOVA was performed on each variable. For all variables except NART-IQ, the between-subjects factor was age (years) of HRT initiation (<49 years, $n = 62$; 49–55 years, $n = 99$; >55 years, $n = 57$); covariates were NART-IQ, cumulative HRT use, and current age. In other analyses, the test scores of the top and bottom age-of-initiation quartiles, as well as of the top and bottom deciles, were compared to further address this issue. Finally, partial correlations controlling for cumulative HRT use were computed between each olfactory and cognitive test score and the age of HRT initiation. Separate analyses were similarly performed on the opposed and unopposed current ERT users and the opposed and unopposed previous ERT users.

3. Results

3.1. Subject characteristics

The women in the Current Opposed ERT group were younger than those in the Past Unopposed ERT group and the Never ERT group (Table 1; $p < 0.003$). In addition, the women in the Past Opposed ERT group were younger than the women in the Past Unopposed ERT group ($p = 0.046$). The women of the Current Unopposed ERT group, most of whom had hysterectomies, had an earlier menopause than the women of the Current Opposed ERT and Past Opposed ERT groups ($p \leq 0.031$). The Past Unopposed ERT group was significantly younger at menopause than both the Current and the Past Opposed ERT groups (respective $p = 0.007$ and 0.001).

Some HRT groups also differed in education and in the cumulative duration of HRT use (i.e., total years of HRT use independent of periods of disuse). The Past Opposed ERT group had more education than the Never ERT group ($p = 0.025$). The Current Unopposed ERT group had a significantly longer cumulative duration of HRT than the women in the 3 other HRT groups ($p < 0.001$), whereas the Past Unopposed ERT group had significantly longer cumulative duration of HRT than the Past Opposed ERT group ($p = 0.022$).

3.2. Olfactory and neuropsychological test scores

The average olfactory and psychological test scores of the 5 hormone groups are presented in Table 2; those for the hormone levels are presented in Table 3. As can be seen from Table 2, the overall ANCOVAs found significant differences among the hormone groups for OMT and NART-IQ scores. As shown in Table 3, the levels of FSH and the ovarian steroids differed among the hormone treatment conditions, as would be expected. Although there was a tendency for DHEA-S also to differ among the HRT groups, the 0.05 level of statistical significance was not reached.

The olfactory scores were variably and weakly correlated with a number of cognitive measures (Table 4), most notably with scores on the CVLT-II and the BVMT-R. A correlation was also present between the olfactory threshold measures and the Continuous Performance Test V.

3.3. Differences in test scores among specific hormone treatment groups

The adjusted mean (SEM) OMT scores were higher in the Current HRT group than in the combined Past and Never HRT groups (6.33 [0.32] vs. 5.54 [0.12]; $p = 0.037$); the delay interval was not statistically significant for any comparison ($p > 0.20$). The Current Unopposed ERT group outperformed the combined Past and Never HRT groups on this test (6.51 [0.41] vs. 5.54 [0.12]; $p = 0.029$]. This was not the case for the Current Opposed ERT group (6.01 [0.49] vs. 5.54 [0.12]; $p = 0.413$). Note that these means differ slightly from those in Table 2 because they are age and NART-IQ adjusted for the *specific* comparisons that were made.

In a manner analogous to that of the OMT scores, the mean NART-IQ scores were higher in the Current HRT group than in the combined Past and Never HRT groups (114.09 [1.57] vs. 109.91 [0.61]; $p = 0.014$). However, unlike the situation with the OMT scores, the Current Opposed ERT group, but not the Current Unopposed ERT group, had significantly higher average NART-IQ scores than the combined Past and Never HRT groups (115.01 [2.43] vs. 109.93 [0.61]; $p = 0.044$). The Past Opposed ERT group had a larger test score than the Never HRT group (112.89 [1.17] vs. 108.87 [0.80]; $p = 0.037$). As with the OMT scores, the NART-IQ score means differ slightly from those in Table 2 because they were age-adjusted for the involved specific comparisons.

Interestingly, women currently receiving opposed ERT performed significantly better than women currently receiving unopposed ERT on the Spatial Span Backwards Test of the WAIS-R NI (6.68 [0.16] vs. 5.70 [0.33]; $p = 0.020$). This finding implies that the ability to recognize successively longer spatial sequences and denote them in reverse order was better in women who were taking estrogen and progestin preparations than in those who were taking estrogen alone preparations. No other measures differentiated between the Opposed and Unopposed ERT groups.

3.4. Correlations between behavioral tests and serum hormone levels

Only 1 correlation between behavioral tests and serum hormone levels was found to be significant after adjusting for age, NART-IQ, and multiple statistical comparisons; namely, that between T and the Spatial Span Backwards Test of the WAIS-R NI ($r = 0.16$, uncorrected $p < 0.009$). Although a weak association, it is of interest that this is the same neuropsychological measure that was influenced by the combination HRT preparations noted above.

3.5. Relationship of test scores to the time of HRT initiation

ANCOVAs using age, NART-IQ, and duration of HRT as covariates found that none of the olfactory or neuropsychological test measures were meaningfully associated with the time of HRT initiation, regardless of whether the cut-off points were set at quartiles, deciles, or other cut points that we explored (all $p > 0.12$). This was also the case for NART-IQ, where age and duration of HRT use were used as covariates.

3.6. Relationship of test scores to APOE allele status

Fourteen (18%) of the 77 subjects, for whom genetic data were available possessed 1 or more copies of the APOE-ε4 allele. One was a current HRT user (opposed ERT), 3 were past HRT users (past opposed ERT), and 10 had never taken HRT. Although these sample sizes precluded a determination as to whether an interaction was present between the HRT group and APOE gene status, comparisons between test scores of subjects with and without the APOE-ε4 allele were possible. PEA detection thresholds were higher (i.e., sensitivity was lower) in the 14 subjects who had one or more APOE-ε4 alleles than in the 63 subjects who did not (−3.65 [0.52] vs. −4.83 [0.24]; $p = 0.04$). Two other measures also appeared compromised in the APOE-ε4 group, although the level of significance was 0.06 in both cases: the Auditory Consonant Trigrams Test (25.05 [1.45] vs. 28.10 [0.68]) and the total score on the WAIS-R NI Digit Span Test (14.88 [0.78] vs. 16.55 [0.37]).

3.7. Influence of proxy MCI status on olfactory test scores

We identified 60 women with long delay interval recall scores (≥1.5 standard deviation [SD] below the mean) on the CVLT-II, a surrogate measure for probable MCI (Petersen et al., 2001). Independent of the HRT group, these subjects scored significantly lower than the other subjects on both the OMT (respective means [SEMs] = 5.02 [0.31] and 5.76 [0.12]; $p = 0.03$) and the UPSIT (respective means [SEMs] = 31.03 [0.63] and 32.59 [0.24]; $p = 0.02$). Such an association was not found for the odor threshold test ($p > 0.05$).

4. Discussion

This cross-sectional study evaluated the influences of HRT on tests of odor identification, detection, memory/discrimination, and cognition in 432 healthy postmenopausal women with disparate histories of HRT and for whom serum levels of a range of reproductive hormones were measured. Scores on 2 tests were higher in women taking HRT: the OMT and the NART-IQ.

Our finding of an influence of HRT on the odor memory/discrimination task appears to be in accord with the study of Sundermann et al. (2006) in which HRT enhanced performance on an odor recognition memory test in 24 postmenopausal women. However, their subjects had AD, unlike ours, and we controlled for potential confounding influences of IQ in our analyses. These authors later reported, in non-demented older persons, that HRT enhanced *n*-butanol odor threshold sensitivity only in women who possessed the APOE-ε4 allele, and that overall, this allele had no influence on their threshold measure (Sundermann et al., 2008). This differs from our finding of poorer threshold sensitivity to PEA in the APOE-ε4 group. We were unable to determine whether the ε4 haplotype was associated with our finding of an HRT effect on the OMT because only 1 ε4 person was currently receiving HRT (opposed ERT). Our negative findings of the influences of HRT on the other olfactory measures accord well with the findings of the earlier studies of Robinson et al. (2007) and Hughes et al. (2002), the latter of which also included the UPSIT.

All 3 olfactory tests significantly correlated, albeit weakly, with at least some of the neuropsychological test measures of this study (Table 4). The psychological tests that correlated most strongly with the olfactory measures, such as the CVLT–II and BVMT-R, largely tap verbal or visuospatial memory. The higher NART-IQ scores among the women currently taking HRT suggests that HRT may mitigate decrease in language and/or intellectual function that occurs during the menopause. NART-IQ scores are known to correlate with age-related cognitive decline, as measured by the Mini-Mental State Examination (Cockburn et al., 2000).

Although none of the subjects in our study reported experiencing any significant memory or cognitive problems, we identified a subgroup of women who exhibited memory difficulties consistent with a diagnosis of MCI-Amnestic Type, that is, scores falling 1.5 SD or more below the mean of the long delay recall measure of the CVLT-II. These women scored significantly lower than the other women on both the OMT and the UPSIT, but not on the odor threshold test. These observations are in agreement with a number

of studies in which MCI patients who score lower than non-MCI patients on the UPSIT have a higher likelihood of later converting to AD (Conti et al., 2013; Devanand et al., 2000). To our knowledge, there have been no earlier investigations of odor memory performance in patients with MCI, although the current data suggest that similar relationships between this diagnosis and the ability to remember odors may exist.

It should be noted that the influence of HRT on the OMT may reflect something other than or in addition to an influence of hormones on olfaction or odor memory, per se. For example, this test has operational elements that might confound the findings, such as the use of odors that can be semantically labeled or identified. Thus, the memory of the label, rather than that of the odor per se, could be what is influenced by HRT (Wharton et al., 2011). An argument could be made that if olfactory function in general was influenced by HRT, then more than 1 type of olfactory test would be expected to be affected, given that most olfactory test measures are correlated with one another (Doty et al., 1994).

Our finding of a statistically significant correlation between T levels and scores on the Spatial Span Backwards Test of the WAIS-R NI implies that the ability to recognize successively longer spatial sequences and denote them in reverse order (i.e., non-verbal working memory) is positively associated with plasma T levels in older women. Although we are unaware of any previous studies examining the influences of T on this specific measure in any age group, positive associations have been reported in younger women between other measures of spatial cognition and serum levels of T (Aleman et al., 2004; Burkitt et al., 2007; Hausmann et al., 2000). It is of interest that women taking opposed ERT outperformed women who were taking unopposed ERT on the same task. Most of the women on opposed ERT were taking preparations containing medroxyprogesterone acetate, a pro-androgenic, and anti-estrogenic progestin that also binds to C and GABA receptors (Belelli and Herd, 2003; Hapgood et al., 2014). Although it is unknown whether the positive effect on the Spatial Span Backwards Test reflects the androgenic effects of the involved progestins, it is noteworthy that this measure was the only one that correlated with T levels in the overall study sample.

In conclusion, the present study suggests that, after controlling for age and IQ, odor memory/discrimination is positively influenced by HRT. The APOE-ε4 haplotype was found to have a detrimental effect on olfactory threshold sensitivity, whereas HRT was found to have a positive effect on NART-IQ scores, presumably reflecting influences on language. UPSIT and OMT scores were lower for women with probable MCI. Scores on the Spatial Span Backwards Test were higher in women taking opposed than unopposed ERT, and were positively correlated with levels of serum T. It is not known whether the improved performance in the Opposed ERT group was a reflection of androgenic effects of the involved progestins, although this is certainly possible.

5. Disclosure statement

Richard L. Doty is President and major shareholder of Sensonics International, the manufacturer and distributor of the olfactory tests used in this study.

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