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ORIGINAL INVESTIGATION

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No changes in event-related potentials with estrogen or estrogen plus progesterone treatment in healthy older hysterectomized women: results from a double-blind, placebo-controlled study

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Abstract *Rationale:* The potential to improve cognition in older women with estrogen or estrogen/progesterone therapy is currently a matter of intense debate. Only a few studies conducted so far have used electrophysiological indicators of cognitive information processing as outcome measures in randomised placebo controlled studies. *Objectives:* This study was undertaken to measure changes in event-related potentials (ERPs) after short (4 weeks) or prolonged (24 weeks) hormone treatment in older women. *Methods:* A randomised, double-blind, placebo-controlled study in hysterectomized older women (aged 58–75 years) was performed (n=51). The participants received orally estradiol (2 mg estradiol valerate), estradiol plus progesterone (100 mg micronized progesterone) or placebo for 24 weeks. Using four different paradigms, early and late ERPs were assessed at baseline and after 4 and 24 weeks of treatment. *Results:* Strong hormone increases were observed in the two active treatment groups. However, no significant effects on any of the assessed ERPs were observed in either of the two treatment groups. Similar non-significant findings were obtained for reaction time and error rate. *Conclusions:* Estradiol or estradiol/progesterone treatment appears to have no strong effects on several ERP markers of information processing in older hysterectomized women. The current negative findings might suggest a reduced sensitivity of the aged brain to gonadal steroids.

Keywords Event-related potentials (ERPs) · Hormone replacement therapy (HRT) · Estradiol · Progesterone · Postmenopausal women · Information processing

Abbreviations ADS-K: Short version of the depression scale · ARAS: Ascending reticular activating system · AUC: Area under the curve · BMI: Body mass index (kg/m^2) · CTR: Conditioning-testing ratio · Cz: Centro-central Electrode position · E2: Estradiol · EEG: Electroencephalogram · ERP: Event-related potential · Fz: Fronto-central Electrode position · HEOG: Horizontal electro-occulogram · MMN: Mismatch negativity · MMSE: Mini-mental status examination · Msec: Millisecond(s) · Nd: Negativity difference · P: Progesterone · Pl: Placebo · Pz: Parietal-central Electrode position · RIA: Radio immunoassay · Sec: Second · SEM: Standard error of means · SW: Slow wave · VEOG: Vertical electro-occulogram

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Introduction

Basic neuroscience research has demonstrated that the gonadal steroids estradiol and progesterone can influence multiple brain regions involved in cognitive processes, such as attention and memory (McEwen and Alves 1999). Based on these findings, it has been postulated that estrogen or progesterone treatment after the menopause might enhance cognitive performance of postmenopausal women. However unequivocal findings of effects of hormone treatment on brain functions in postmenopausal women have been published during recent years. Epidemiological studies repeatedly observed superior performance in women taking estrogens or estrogens together with progestins (see for review, Hogervorst et al. 2000; LeBlanc et al. 2001; Rice and Morse 2003). Several mostly small experimental placebo controlled studies resulted in a less clear picture. Sherwin and co-workers found bene-

ficial effects on verbal declarative memory in response to estrogen treatment in relatively young women after surgical menopause (Sherwin 1988; Phillips and Sherwin 1992). Studies in older women reported effects on attention (Fedor-Freybergh 1977) or mental rotation (Duka et al. 2000). A previous study from our group reported no differences between subjects treated with estradiol or placebo, but the patch induced estradiol levels were relatively low. Additional analysis observed a positive association between verbal memory and the treatment induced estradiol levels within the treatment group (Wolf et al. 1999). However, several well conducted studies failed to find any beneficial effects using extensive cognitive test batteries (e.g. Binder et al. 2001; Polo-Kantola et al. 1999). Possible reasons for these discrepancies are discussed in several recent reviews (Hogervorst et al. 2000; LeBlanc et al. 2001; Rice and Morse 2003; Sherwin 2003).

In the WHIMS (Women's Health Initiative Memory Study), a small increased risk of cognitive decline was observed in women treated with equine estrogens and the progestin medroxyprogesterone acetate (MPA; Rapp et al. 2003). Moreover, in this study hormone treatment was associated with an increased risk of dementia (Schumaker et al. 2003), which was in clear contrast to previous epidemiological studies suggesting that hormone treatment can reduce the risk of dementia, especially Alzheimer's disease (Hogervorst et al. 2000; LeBlanc et al. 2001).

Few studies have investigated the cognitive effects of hormone treatment in postmenopausal women with electrophysiological measures. Assessing the overall brain activity with EEG, positive effects on vigilance (increased alpha power) were reported in women with menopausal depression (age range 45–60 years) (Saletu et al. 1995) and in menopausal syndrome patients with a mean age of 58 years (Saletu et al. 2002). Most recently, Krug et al. (2003) observed a reduced dimensional EEG complexity in postmenopausal women (mean age 58 years) in response to short term (3 days) transdermal estradiol treatment, which was accompanied by enhanced convergent and reduced divergent thinking capacity.

Auditory brainstem potentials reflect the conduction time in the cranial nerve and the brain stem. Auditory brainstem potentials can be regarded as an indirect measure of the ascending reticular activating system (ARAS, Swickert and Gilliland 1998). Generally, a shortening of the peak latencies of the auditory brainstem potentials is correlated with a better performance in such tasks, which benefit from an improvement in ARAS-regulated arousal, and vice versa. Using these measures in observational studies, no consistent effects of estrogens or progestins could be observed. Elkind-Hirsch and colleagues (1992) reported enhanced latencies in young women (29–42 years) with premature ovarian failure during the estradiol replacement phase and shortened latencies which were similar to the baseline (non-replaced) values during intake of estradiol plus progestin. Caruso and colleagues, in contrast, observed shortened latencies of brainstem potentials in postmenopausal women (mean age 52 years) taking estradiol (Caruso et al. 2000, 2003) and women taking a combination of estradiol and progesterone (Caruso et al. 2000). Latencies of brainstem responses were shorter in women taking estradiol only, compared with women taking a combination of estradiol plus progestin (Caruso et al. 2000).

Measuring event-related potentials as indices of information processing in the brain, Anderer and colleagues (2003) reported that estrogen induced a P3 latency reduction in women suffering from menopausal insomnia (mean age 58 years). These changes were enhanced in women taking a combination of estradiol and the progestin dienogest. In addition, the authors reported reduced N1 latency and enhanced N1, P2, and P3 amplitude increases with the combined hormone treatment.

Stimulated by the lack of ERP studies in older healthy women not suffering from menopausal symptoms or depression, the present experiment was undertaken in order to test the effects of estradiol or estradiol/progesterone treatment in older women. In addition emphasis was placed on comparing short-term (occurring within weeks) and long-term (occurring within months) effects of the hormone treatment.

Materials and methods

Subjects

Older hysterectomized postmenopausal women (age: 58–75 years) were recruited via different local media. The following inclusion and exclusion criteria were screened by phone: Previous hysterectomy; no estrogen treatment within the past 12 months; absence of cancers, tumors, deep vein thrombosis, metabolic diseases cardiovascular diseases, neurological or psychiatric disorders. In addition, subjects had to be non-smokers with a body mass index (kg/m^2) between 20 and 34. Interested potential participants fulfilling the above mentioned criteria were invited for a visit at the university. In the first screening part presence of dementia was tested using the Mini-Mental Status Examination (MMSE; Folstein et al. 1975). In addition, presence of depression was assessed with the German short version of the center for epidemiological studies depression scale (ADS-K; Hautzinger and Bailer 1993). Finally, verbal intelligence was assessed using the German version of the Wechsler Adult Intelligence Scale (WAIS-R), test of verbal comprehension. The latter test was used to balance the three treatment Groups on verbal intelligence (see below). The second screening part consisted of a thorough medical examination consisting of a mammography, an ultrasonic examination of the breast and the vaginal tract and a pap smear. In addition, a medical history was obtained. Lastly, a blood sample was taken for the assessment of several thrombosis risk factors. A total of 51 women was initially included into the study after the screening procedure. All subjects provided written informed consent and the study was approved by a national and a local ethic committee. Subjects received 250 Euro as reimbursement.

Hormone treatment

Subjects were allocated to one of three treatment groups (see below), which were balanced with respect to age, verbal intelligence, and BMI using the minimization procedure (related to the biased coin procedure) as proposed by Pocock and Simon (1975). This procedure guarantees appropriate randomization of subjects sequentially entering a study. Advantages of this randomization procedure are, that Groups similar in size are obtained and that balancing of potential confounders (in the current study age, intelligence and BMI) is possible.

The estradiol group (E2): subjects received orally 2 mg estradiol (estradiol valerate, Gynokadin; Dr. Kade, Berlin, Germany), to be taken in the mornings. In the evenings they received a placebo pill. The estradiol/progesterone group (E2/P): subjects received estradiol in the morning and 100 mg micronized progesterone in the evenings (Utrogest; Dr. Kade, Berlin, Germany). The placebo group (Pl): subjects received placebo tablets (Dr. Kade, Berlin, Germany) in the morning and in the evening.

Subjects included into the analysis

Fifty-one healthy elderly postmenopausal women were initially included. Nine dropped out, three immediately after the medical check-up because of second thoughts about the study and six during the study because of minor health problems in general unrelated to the treatment (except for one subject with strong breast pain in response to hormone treatment). In addition, seven subjects were excluded after the completion of data collection (five due to non-compliance with treatment and two due to psychological problems occurring during the study period in response to a critical life events (loss of a partner and traffic accident). In addition, one subject was excluded due to hearing problems (tinnitus). Since there were too many artefacts in one of the postmenopausal women in the placebo group in the ERPs, this person was also excluded from further analyses (see below). Thus 33 women were included in the final statistical analysis reported in this paper. Data from 11 women in the estradiol group [age (mean±SEM): 63.91±1.28 years; BMI: 27.09±1.38 kg/m^2), ten in the estradiol/progesterone group (age: 64.80±1.28 years; BMI: 26.00±.47 kg/m^2) and 12 in the placebo group (age: 63.83±.92 years; BMI: 26.75±1.09 kg/m^2) were included into the data analysis.

Experimental design

Subjects were given three university appointments, firstly, a baseline test (baseline) before start of the treatment, secondly, a test session after 4 weeks of treatment (4 weeks) to test for short-term effects of the treatment and thirdly, after 24 weeks treatment (24 weeks) to test for long-term effects of the treatment. Each session consisted of two parts: in the first part ERP measures were obtained by four auditory paradigms and in the second part the subjects had to perform several cognitive tests. These results will be published elsewhere (Wolf et al. 2005). Each subject was tested at all three appointments at the same time of day.

The study was based on a 3 (Group)×3 (Session) plan. In addition, for ERP parameters the factor Electrode position was used. Physiological as well as behavioural measures were taken. The study was undertaken in a laboratory of the university of Duesseldorf, which contained an acoustically and electrically shielded chamber (Industrial Acoustics Company) where the experiment took place.

Paradigms for ERP recordings

Four different paradigms were applied to measure electrophysiological correlates of the cortical arousal response and orienting and controlled processing of auditory stimulation.

In the conditioning-testing paradigm, stimuli were presented binaurally as a series pairs of conditioning and testing clicks (880 Hz, 75 dB SPL intensity, 100-ms duration). The interval between the subsequent pairs (testing stimulus of pair 1 to conditioning stimulus of pair 2, etc.) was 10 s. Averaged responses to 48 pairs of stimulus presentations were obtained from subjects at a conditioning-testing interval of 500 ms. This paradigm was applied to measure the P50 component and the function of the sensory gating via measuring the conditioning-testing ratio (CTR).

The habituation paradigm consisted of 200 identical stimuli. Tones of 1 kHz and 75 dB SPL intensity and a duration of 60 ms were presented binaurally and the subjects had to mentally count the number of tones heard. N1 and P2 components and the resulting measure of the vertex potential as indicators of the cortical arousal and orienting response were obtained in this task. To measure the course of habituation the whole trial was divided into four blocks consisting of 50 stimuli each.

In the oddball paradigm, subjects were presented with two types of stimuli. Standards (background stimuli) consisted of 1 kHz tones with an intensity of 75 dB SPL and a duration of 60 ms, whereas targets had a frequency of 1200 Hz with an intensity of 75 dB SPL and a duration of 60 ms. In each of the two blocks, there were 200 tones with 80% standards and 20% targets. Subjects had to react to targets as fast as possible by pressing a button. This paradigm was taken to measure the N2 and the P3 components and the slow wave (SW) as indicators of controlled processing.

In the dichotic-listening paradigm, subjects were presented with a set of standards and targets in one ear (standard: 1 kHz, target: 1,2 kHz; 75 dB SPL intensity, duration of 60 ms) and an independent set of standards and targets in the other ear (standard: 600 Hz, target: 800 Hz; 75 dB SPL intensity, duration of 60 ms). The participants were instructed to only focus their attention on the set of stimuli in one ear and to react to the targets in this set. Each

possible combination of set and attended ear was presented to the subjects resulting in four different trials. This paradigm was used to obtain the mismatch negativity (MMN) and the negativity difference (Nd) waves as measures of conscious and unconscious analyses.

In the habituation, the oddball, and the dichotic-listening paradigm, there were randomised interstimulus intervals of 1, 2 or 3 s. The order of the four paradigms was permuted and randomised between the subjects.

Behavioural measures

Reaction times (RT) to target stimuli in the oddball and dichotic-listening paradigm as well as incorrect responses (IR) were measured. Reaction times were only analysed if they occurred within a 200- to 1000-ms interval after a target.

EEG recordings and data analysis

For ERP determination, the eletroencephalogram (EEG) was recorded from Ag/AgCl electrodes (diameter 8 mm; Falk Minow Services, Germany) attached at midline positions (Fz, Cz, Pz) according to the 10–20 system. The reference electrodes were placed at each mastoid and the ground electrode on the forehead. Additionally, a vertical electro-oculogram (VEOG) and a horizontal electro-occulogram (HEOG) were obtained by placing two electrodes sub- and supraorbitally and at the temples, respectively. The subjects were instructed to refrain from blinking as much as possible and to keep their eyes on a fixation mark during the main trials. The Electrode positions were cleaned with the abrasive paste "Grasspaste" and the electrodes filled with the electrode paste "Elefix" (Nihon Khoden Europe). The skin impedance was kept below 5 kΩ for each electrode.

A SynAmps-amplifier (Neuroscan, USA) was used to amplify EEG and ocular potentials. The recordings were digitalised with a sampling rate of 250 Hz and were continuously recorded. The low-pass filter was set to 30 Hz and as high-pass filter a DC correction was used. The recordings were stored for later analysis.

Hormone analyses

Serum estradiol and progesterone levels were determined using commercially available RIAs (radio immunoassays; ESTR-CTK-4 and PROG-CTK-4 from DiaSorin, Saluggia, Italy) with a sensitivity of 3 pg/ml (estradiol) and 30 pg/ml (progesterone) respectively. Inter- and intra assay variations were below 15% for both assays. Expected hormone levels for postmenopausal women with those assays as provided by the manufacturer are below 55 pg/ml for estradiol and below 1.6 ng/ml for progesterone.

ERP analysis

ERP data analysis was undertaken with the "Brainvision" software (Brain Products, Germany). The off-line analysis consisted of a segmentation for each tone (100 ms pre-stimulus until 900 ms post-stimulus), an ocular correction, a baseline correction (post-stimulus potentials referred to the level of 100-ms pre-stimulus interval) and an artefact rejection (±50 μV). Afterwards, each trial and each electrode position were averaged separately. The peaks of the different components were defined as the maximal negativity or positivity in an a priori defined time interval.

In the conditioning-testing paradigm, the P50 peak amplitude was measured relative to the preceding negative peak (Nb), which was defined as the negative peak within 0–80 ms post-stimulus. The P50 peak amplitude to the conditioning stimulus was defined as the positive peak within 30–120 ms post-stimulus, whereas the P50 peak amplitude to the testing stimulus was defined as the positive peak at Cz within ±20 ms of the P50 peak amplitude to the conditioning stimulus at Cz. The CTR was expressed as a percentage of the P50 amplitude to the test stimulus divided by the P50 amplitude to the conditioning stimulus For data analysis, conditioning-testing paradigm ratios above 200% were truncated to 200% to prevent outliners from having a disproportionate effect on group means (see also Nagamoto et al. 1991).

In the habituation paradigm, the vertex potential was determined as peak-to-peak amplitude between the N1 (N1 peak amplitude: negative peak within 90–190 ms post stimulus) and P2 (P2 peak amplitude: positive peak within 190–290 ms post stimulus) peak amplitudes.

In the oddball paradigm, the N2 peak amplitude was defined as the negative peak within 230–370 ms post stimulus and the P3 peak amplitude as the positive peak within 270–600 ms post stimulus. Since the area under the curve (AUC) of the SW was calculated for each electrode position separately, it was determined between 540–840 ms post stimulus at Fz, between 515 and 900 ms post stimulus at Cz, and between 465 and 900 ms post-stimulus at Pz.

In the dichotic-listening paradigm, the MMN was determined as AUC of the difference curve between the ERP to the unattended targets and the ERP to unattended standards (at Fz and Cz between 150- and 590-ms post stimulus, and at Pz between 150- and 390-ms post stimulus). The Nd (difference curve between the ERP to attended standards and the ERP to unattended standards) was divided into two component. The early component was determined as the area under the curve between 215 and 340 ms post stimulus at Fz, and between 140- and 490-ms post-stimulus at Cz and between 140- and 390-ms post-stimulus at Pz. The late component was calculated of the area between the Nd curve and the baseline between 490 and 900 ms post-stimulus at Fz and Cz, and between 680 and 900 ms post-stimulus at Pz. Additionally the latencies for the P50 (in the conditioning-testing paradigm), the N1 and P2 (in the habituation paradigm) and the

Table 1 Hormone concentration of the three experimental groups [mean±(SEM)]

Group	Estradiol (pg/ml)			Progesterone (ng/ml)		
	Baseline	4 weeks	24 weeks	Baseline	4 weeks	24 weeks
Estradiol	20.52 (3.99)	133.77* (9.33)	139.67* (13.40)	0.14 (0.02)	0.13 (0.03)	0.12 (0.02)
Estradiol/progesterone	22.91 (3.46)	135.26* (14.47)	135.08* (16.90)	0.15 (0.03)	3.95* (0.54)	4.45* (0.68)
Placebo	23.13 (5.35)	31.82 (6.05)	27.75 (3.74)	0.20 (0.05)	0.20 (0.05)	0.18 (0.04)

* $P<0.05$ compared to baseline and compared to the respective placebo condition

N2 and P3 peak amplitudes (in the oddball paradigm) were determined.

After automatic peak detection, the individual averaged curves were manually inspected and rejected if the peaks were not clearly visible. Since there were too many artefacts in one of the postmenopausal women in the placebo group the data of only 33 participants were further analysed. Additionally, the measures of some other subjects were not included if the maxima or minima could not be identified correctly. In this case all the data in the particular paradigm of the subject was taken out of further analyses, so that there were some differences in the number of participants between the four paradigms. Additionally, some subjects had excessive artefacts in various paradigms and could not be used in analyses involving this paradigm. Thus, data from 11 (E2 group), nine (E2/P group), and ten (Pl group) postmenopausal women were compared in the conditioning-testing paradigm. In the oddball paradigm data from 11 (E2 group), ten (E2/P group), and ten (Pl group) postmenopausal women could be obtained. The data from all participants could be analysed in the habituation paradigm and dichotic-listening paradigm (11 (E2 group), ten (E2/P group), and 12 (Pl group) postmenopausal women).

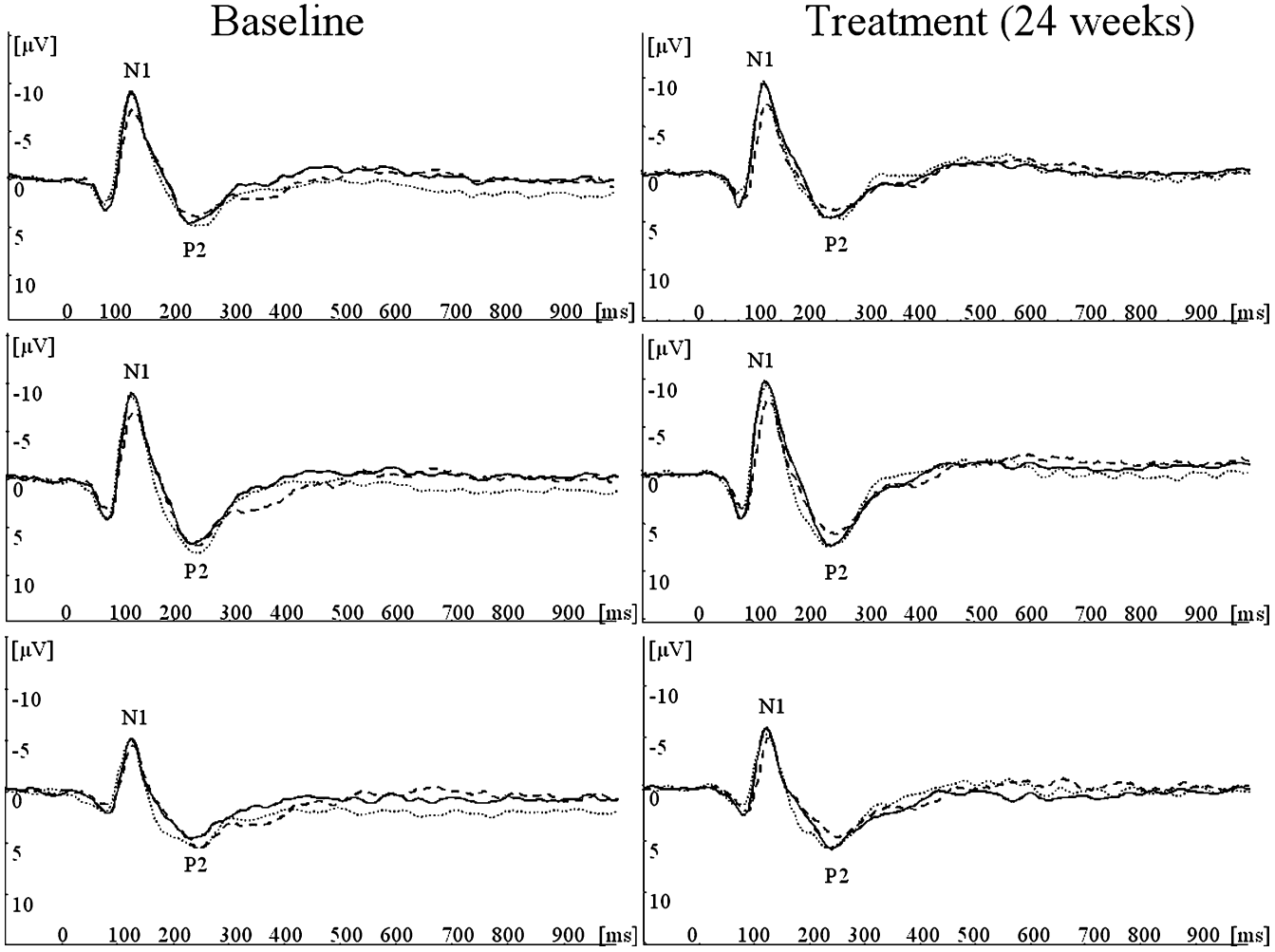

Fig. 1 Grand averages of ERPs in the estradiol group (*solid line*), in the estradiol/progesterone group (*dashed line*) and the placebo group (*dotted line*) during the habituation paradigm at baseline (*left row*) and after 24 weeks of treatment (*right row*). The *first line* shows the grand averages at the electrode position Fz, the *middle line* at Cz and the *last line* at Pz

Statistical analysis

An ANOVA with the factors Group (estradiol, estradiol/progesterone, placebo) and Session (baseline, 4 and 24 weeks of treatment) was performed to test for the presence of a significant Group by Session interaction for hormonal and behavioural data. For the ERP data analysis a third factor, Electrode position (Fz, Cz, Pz), was introduced. Greenhouse–Geisser corrected *F*- and *P*-values are reported. Follow up analysis of significant interactions were done using Bonferroni corrected ANOVAs and *t*-tests.

Results

Hormones

Estradiol and progesterone concentrations during the course of the study are summarized in Table 1. An ANOVA revealed a significant interaction Session by Group for estradiol [$F(4,60)=22.85$, $P<0.001$] and for progesterone ($F(4,60)=39.03$, $P<0.001$]. Further analyses for each session separately revealed that estradiol levels were significantly elevated in both treatment groups at both sessions (4 and 24 weeks). Progesterone levels were significantly increased in the combined treatment group at both sessions (4 and 24 weeks). No hormonal changes occurred in the placebo group.

ERPs

Figures 1 and 2 show grand-averaged ERPs to the stimuli in the habituation paradigm and to the targets in the oddball paradigm, respectively, in the three groups at baseline and after 24 weeks of treatment and at each Electrode position.

Conditioning-testing paradigm

No significant Group by Session or Group by Session by Electrode position interaction occurred for the P50 am-

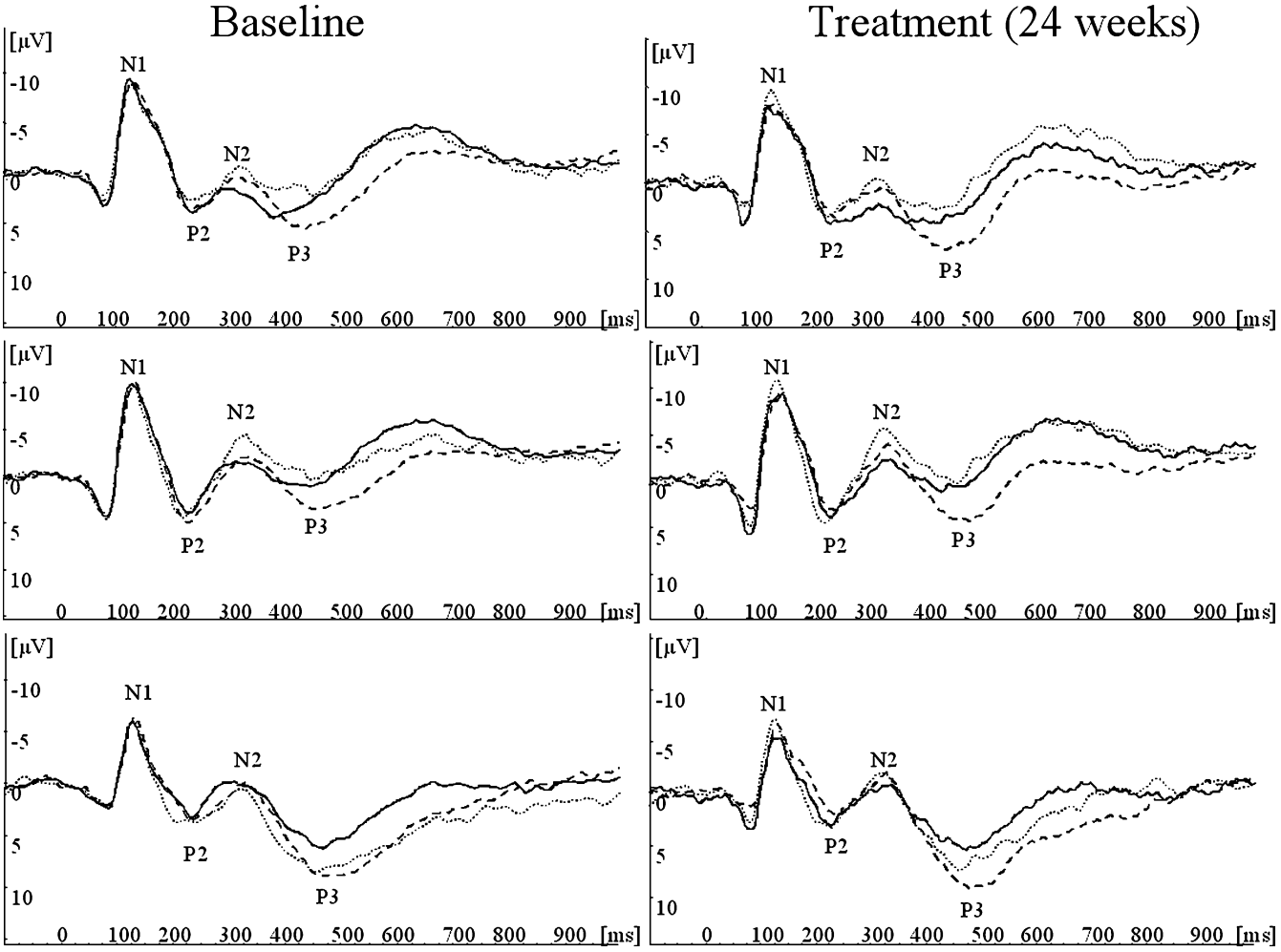

Fig. 2 Grand averages of ERPs in the estradiol group (*solid line*), in the estradiol/progesterone group (*dashed line*) and the placebo group (*dotted line*) to the target stimuli during the oddball paradigm at baseline (*left row*) and after 24 weeks of treatment (*right row*). The *first line* shows the grand averages at the electrode position Fz, the *middle line* at Cz and the *last line* at Pz

Table 2 Amplitudes (μV) and latencies (ms) of four ERP components [mean±(SEM)] in the three hormone groups across the sessions

ERP component		Baseline			4 weeks			24 weeks		
		Fz	Cz	Pz	Fz	Cz	Pz	Fz	Cz	Pz
N1 amplitudes (μV)	E2	−9.55	−9.58	−5.46	−10.12	−10.52	−5.90	−10.36	−10.77	−6.27
		(1.34)	(1.35)	(0.87)	(1.11)	(1.43)	(1.11)	(0.84)	(1.11)	(0.84)
	E2/P	−7.57	−7.79	−5.04	−7.00	−6.96	−4.41	−7.77	−7.96	−5.06
		(1.11)	(1.22)	(0.95)	(1.18)	(1.13)	(0.95)	(1.15)	(1.26)	(0.96)
	Pl	−9.47	−9.08	−5.51	−9.21	−8.81	−5.39	−9.92	−9.80	−6.13
		(0.94)	(0.89)	(0.63)	(0.78)	(0.64)	(0.44)	(0.95)	(1.00)	(0.67)
N1 latencies (ms)	E2	124.36	128.36	127.64	125.82	126.91	126.55	124.73	124.36	124.36
		(2.38)	(2.56)	(2.05)	(2.55)	(2.02)	(1.89)	(3.18)	(2.77)	(1.98)
	E2/P	127.20	134.00	128.80	131.60	132.80	132.80	127.60	129.60	131.60
		(2.22)	(4.63)	(2.85)	(2.10)	(1.77)	(1.96)	(3.84)	(3.64)	(3.24)
	Pl	121.33	122.67	120.00	126.00	127.00	129.00	121.67	125.67	128.00
		(2.33)	(2.89)	(2.74)	(3.70)	(3.69)	(3.20)	(1.89)	(2.12)	(3.48)
P2 amplitudes (μV)	E2	5.57	7.68	5.28	4.57	6.98	4.83	5.43	8.19	6.36
		(0.82)	(0.83)	(0.67)	(0.83)	(1.12)	(0.87)	(0.72)	(0.93)	(0.90)
	E2/P	4.64	7.51	5.97	4.92	7.19	5.72	4.50	6.46	4.82
		(0.79)	(1.04)	(0.83)	(1.00)	(1.15)	(0.83)	(1.21)	(1.37)	(0.89)
	Pl	5.99	8.54	6.26	5.26	8.00	6.05	5.84	8.55	7.02
		(0.65)	(0.78)	(0.70)	(0.94)	(0.97)	(0.55)	(0.72)	(1.00)	(0.91)
P2 latencies (ms)	E2	243.64	238.18	229.45	244.36	245.09	244.73	237.45	240.00	236.36
		(5.77)	(6.13)	(7.80)	(9.49)	(8.73)	(11.00)	(5.66)	(5.42)	(5.27)
	E2/P	228.80	233.60	237.60	245.20	244.40	244.00	240.80	244.80	245.20
		(7.18)	(5.47)	(6.09)	(6.61)	(6.18)	(9.69)	(8.90)	(6.90)	(7.23)
	Pl	242.00	244.33	233.00	235.67	237.00	232.67	239.00	237.00	229.33
		(7.36)	(5.62)	(7.50)	(7.71)	(6.57)	(7.01)	(6.50)	(8.05)	(8.01)
N2 amplitudes (μV)	E2	−0.13	−3.71	−1.65	0.10	−3.44	−2.28	0.05	−4.20	−2.06
		(1.14)	(1.48)	(1.31)	(1.77)	(1.75)	(1.22)	(1.53)	(1.51)	(0.57)
	E2/P	−0.77	−3.09	−0.89	−2.27	−5.22	−2.63	−1.18	−4.90	−2.99
		(1.37)	(1.74)	(1.19)	(1.35)	(1.66)	(1.16)	(1.42)	(1.87)	(1.07)
	Pl	−2.10	−5.31	−0.57	−1.75	−5.77	−1.80	−2.04	−6.63	−3.28
		(1.61)	(2.33)	(0.99)	(2.25)	(2.40)	(1.37)	(2.23)	(2.78)	(1.72)
N2 latencies (ms)	E2	305.45	311.64	309.09	304.36	326.55	309.45	314.91	317.82	321.09
		(5.82)	(6.98)	(6.15)	(12.19)	(8.15)	(8.82)	(7.85)	(10.91)	(7.97)
	E2/P	328.00	326.80	323.60	309.60	317.20	318.40	314.80	329.20	318.40
		(8.82)	(8.66)	(8.48)	(8.58)	(8.43)	(7.23)	(8.20)	(5.99)	(7.94)
	Pl	296.40	301.60	300.00	300.80	304.80	303.20	308.80	314.40	301.20
		(11.80)	(10.70)	(9.96)	(9.52)	(9.29)	(8.98)	(14.38)	(9.03)	(11.67)
P3 amplitudes (μV)	E2	5.82	3.61	7.68	7.28	5.27	9.00	5.88	3.32	6.87
		(1.14)	(1.41)	(1.19)	(1.79)	(2.09)	(1.42)	(1.66)	(1.85)	(1.11)
	E2/P	7.02	5.54	10.79	7.07	5.67	10.86	8.62	6.25	10.93
		(1.02)	(1.79)	(1.11)	(.86)	(1.62)	(0.85)	(1.15)	(2.21)	(1.42)
	Pl	4.50	3.13	10.29	6.56	4.07	11.17	5.36	2.45	9.29
		(1.96)	(2.59)	(1.62)	(2.53)	(3.13)	(1.87)	(1.85)	(2.59)	(1.67)
P3 latencies (ms)	E2	401.45	421.45	444.73	402.18	441.82	464.00	416.73	422.91	436.73
		(16.72)	(15.37)	(16.87)	(21.58)	(9.08)	(10.80)	(15.20)	(17.31)	(14.83)
	E2/P	446.80	456.40	479.20	453.20	496.60	480.00	449.20	466.80	474.80
		(15.94)	(17.43)	(13.84)	(14.21)	(11.09)	(10.70)	(11.78)	(11.87)	(10.73)
	Pl	405.20	420.40	445.20	405.60	422.80	472.00	411.60	439.60	449.20
		(18.77)	(28.67)	(18.30)	(9.82)	(15.66)	(15.25)	(20.24)	(16.30)	(15.47)

N1 and P2 values are from the habituation paradigm whereas N2 and P3 values are from the oddball paradigm (target tone)

Table 3 Behavioural data (mean±SEM) in the three hormone groups across the sessions. *IR* Incorrect responses, *RT* reaction time

Behavioural data	Baseline			4 weeks			24 weeks			*F*(4,62) values
	E2	E2/P	Pl	E2	E2/P	Pl	E2	E2/P	Pl	
IR (Oddball)	7.91 (3.38)	3.50 (1.42)	5.54 (1.68)	5.45 (1.90)	2.50 (.56)	4.00 (1.31)	5.55 (2.47)	2.60 (0.91)	3.38 (0.92)	*F*=0.23 *P*=0.91
RT in ms (Oddball)	547.64 (18.28)	543.96 (12.64)	515.06 (10.56)	543.18 (18.49)	552.09 (15.16)	518.26 (13.36)	549.52 (20.94)	538.18 (15.95)	530.18 (10.80)	*F*=0.62 *P*=0.63
IR (Dichotic listening)	32.18 (10.53)	21.60 (5.49)	18.62 (4.72)	21.36 (6.68)	10.70 (2.00)	14.46 (2.63)	17.18 (5.54)	15.10 (3.10)	11.77 (1.89)	*F*=1.64 *P*=0.20
RT in ms (Dichotic listening)	608.02 (17.78)	626.68 (11.92)	586.72 (13.00)	604.27 (20.38)	625.77 (13.98)	599.22 (14.90)	597.65 (19.74)	623.50 (15.73)	578.05 (13.07)	*F*=0.72 *P*=0.57

F- and *P*-values are for the Group by Session interaction

plitudes to the conditioning and testing stimulus and the CTR (data not shown).

Habituation paradigm

No significant Group by Session or Group by Session by Electrode position interaction occurred for the N1, P2, and the vertex-potential amplitudes. N1 and P2 values are presented in Table 2.

Oddball paradigm

No significant Group by Session or Group by Session by Electrode position interaction occurred for the N2 and the P3 amplitudes and the SW. N2 and P3 values are presented in Table 2.

Dichotic-listening paradigm

No significant Group by Session or Group by Session by Electrode position interaction occurred for the MMN and the early and late component of the Nd (data not shown).

Behaviour

No significant Group by Session interaction occurred for incorrect responses (sum of missed hits and false alarms) and the reaction times. Results are presented in Table 3.

Additional statistical analysis combining the two hormone groups

Similar non-significant findings were obtained when all analysis described above (ERP as well as behaviour) were re-run with the data of the two hormone groups pooled together (n=21).

Power analysis

We calculated the power of the present study to detect a large or a medium effect as suggested by Cohen (1977). The Group by Session interaction of the repeated measurement ANOVAs was the effect of interest. The software package G*power was used (Erdfelder et al. 1996) and all necessary parameters were estimated from the data for the P3 amplitude, since this was one of the primary outcome measures. Power analysis was done for the three-group design (E2, E2/Prog, Placebo) as well as for the two-group design (hormones (n=21) against placebo). The study was sufficiently powered to detect a large effect (0.80 and 0.90 respectively). The power to detect a medium effect was 0.41 and 0.53, respectively.

Discussion

In the current study, hormonal treatment led to strong estradiol and progesterone increases resulting in plasma levels typically observed in young women. Despite the effective hormonal replacement no changes in several ERP components measured 4 and 24 weeks after initiation of the treatment were detected. Similar non-significant findings were observed in the cognitive tests (see Wolf et al. 2005). All study participants had a previous hysterectomy thus allowing to compare the effects of estradiol mono-therapy with the effects of estradiol/progesterone combination therapy in a truly double blind fashion. Participants in this study were healthy older asymptomatic women (i.e. women without menopausal symptoms), who had not been taking hormone replacement therapy for at least 12 months. In fact most of the participants had not taken any estrogens for more than a decade. Therefore, the negative results of the current study cannot be extrapolated to younger women or women without such a long period of estrogen depletion.

Using the same electrophysiological paradigms we previously investigated young women during the course of the menstrual cycle and observed changes in early as well as late ERP components (Walpurger et al. 2004). Moreover, when data of the above-mentioned group of

young women were compared with baseline data from the older subjects of the current study, the 'typical' age associated alterations were observed (e.g. reduction in latency of the late ERP components; see for review Kügler et al. 1993; Polich 1996). These two observations demonstrate that the ERP paradigms used in the current study are sensitive to hormonal fluctuations as well as to aging.

The relatively small sample size of the present study certainly raises the issue of statistical power. Power analysis revealed sufficient power to detect a large effect, while the power to detect a mediun effect was limited. However, large beneficial effects on memory have been reported in studies with young women tested immediately after surgical menopause (Phillips and Sherwin 1992). Moreover, we observed one medium and one large effect in a menstrual cycle study using the same paradigms (Walpurger et al. 2004). Data in older postmenopausal women in contrast are more mixed, with non-significant findings in cognitive test batteries being reported in about 50% of the studies (Hogervorst et al. 2000; LeBlanc et al. 2001; Rice and Morse 2003; Sherwin 2003).

Another aspect worth considering when discussing the current negative findings is the length of the treatment. Several recent studies observed rather rapid effect of estradiol on some aspects of cognitions, with the earliest changes being reported after 3 days of treatment (e.g. Duka et al. 2000; Krug et al. 2003). Since in the present study the first treatment effects were assessed after 4 weeks, the possibility that some temporary favourable effects had already disappeared again can not be ruled out.

Some previous electrophysiological studies revealed effects of estrogen or estrogen/progesterone treatment on brain activity measured with EEG or ERPs. Subjects in those studies were either symptomatic women, suffering from menopausal insomnia (Anderer et al. 2003) or depression (Saletu et al. 1995, 2002) or younger women (mean age 58 years), when compared to subjects in the present study (Krug et al. 2003). In addition, two observational studies reported that brainstem potentials were modified by gonadal hormones in asymptomatic postmenopausal women aged 47–55 years (Caruso et al. 2000, 2003). Taken together, these studies might suggest that only women suffering from menopausal symptoms and/or women of younger age respond to estradiol or estradiol/progesterone treatment.

Behavioural studies in aged rodents have observed that the brain loses its sensitivity for estradiol after surgically induced estradiol depletion within a period of several months (e.g. Gibbs 2000), even though "estrogen priming" with repeated injections might be able to overcome some of these effects (Markowska and Savonenko 2002). In addition, long-term estrogen deprivation leads to changes in receptor density as well as to structural changes in the brain (Toran-Allerand 2000). However, recent research in rodents has also suggested that an age associated decrease in the number of hippocampal synapses containing estrogen receptors might cause a reduced sensitivity of these synapses to estradiol (Adams and Morrison 2003). Less is known about similar alterations in other brain regions involved in cognition.

These findings in rats as well as some of the previous electrophysiological findings in humans seem to suggest that higher age per se or prolonged estrogen depletion leads to a blunted or absent response of the brain to gonadal steroids. One line of future experimental studies in humans could be to look for the existence of a "critical time window" for beneficial effects of estrogens (Resnick and Henderson 2002). These studies would focus on younger women during or immediately after the menopausal transition.

In sum, the present small study conducted in healthy older postmenopausal women suggests that oral treatment with either estradiol or estradiol/progesterone has no strong effects on several electrophysiological measures of cognitive processing. The absence of beneficial effects was apparent after both a short (4 weeks) and a more prolonged (24 weeks) treatment period. The current study is in contrast to some previous studies in younger or symptomatic women, which might suggest that beneficial effects of gonadal steroid treatment are restricted to these cohorts.

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References

Adams MM, Morrison JH (2003) Estrogen and the aging hippocampal synapse. Cereb Cortex 13:1271–1275

Anderer P, Semlitsch HV, Saletu B, Saletu-Zyhlarz G, Gruber D, Metka M, Huber J, Gräser T, Oettel M (2003) Effects of hormone replacement therapy on perceptual and cognitive event-related potentials in menopausal insomnia. Psychoneuroendocrinology 28:419–445

Binder EF, Schechtman KB, Birge SJ, Williams DB, Kohrt WM (2001) Effects of hormone replacement therapy on cognitive performance in elderly women. Maturitas 38:137–146

Caruso S, Cianci A, Grasso D, Agnello C, Galvani F, Maiolino L, Serra A (2000) Auditory brainstem response in postmenopausal women treated with hormone replacement therapy: a pilot study. Menopause 7:178–183

Caruso S, Maiolino L, Agnello C, Garozzo A, di Mari L, Serra A (2003) Effects of patch or gel estrogen therapies on auditory brainstem response in surgically postmenopausal women: a prospective, randomized study. Fertil Steril 79:556–561

Cohen J (1977) Statistical power analysis for the social sciences. Academic, New York

Duka T, Tasker R, McGowan JF (2000) The effects of 3-week estrogen hormone replacement on cognition in elderly healthy females. Psychopharmacology 149:129–139

Elkind-Hirsch KE, Wallace E, Stach BA, Jerger JF (1992) Cyclic steroid replacement alters auditory brainstem responses in young women with premature ovarian failure. Hearing Res 64:93–98

Erdfelder E, Faul F, Buchner A (1996) G*Power: a general power analysis program. Behav Res Meth Instrum Comput 28:1–11

Fedor-Freybergh P (1977) The influence of oestrogens on the well-being and mental performance in climacteric and postmenopausal women. Acta Obstet Gynecol Scand, Suppl 64:1–91

Folstein MF, Folstein SE, McHugh PR (1975) "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 12:189–198

Gibbs RB (2000) Long-term treatment with estrogen and progesterone enhances acquisition of a spatial memory task by ovariectomized aged rats. Neurobiol Aging 21:107–116

Hautzinger M, Bailer M (1993) ADS. Allgemeine depressions skala. Beltz, Weinheim, Germany

Hogervorst E, Williams J, Budge M, Riedel W, Jolles J (2000) The nature of the effect of female gonadal hormone replacement therapy on cognitive function in postmenopausal women: a meta-analysis. Neuroscience 101:485–512

Krug R, Mölle M, Dodt C, Fehm HL, Born J (2003) Acute influences of estrogen and testosterone on divergent and convergent thinking in postmenopausal women. Neuropsychopharmacology 28:1538–1545

Kügler CFA, Taghavy A, Platt D (1993) The event-related P300 potential analyses of cognitive human brain aging: a review. Gerontology 39:280–303

LeBlanc ES, Janowsky J, Chan BKS, Nelson HD (2001) Hormone replacement therapy and cognition: systematic review and meta-analysis. JAMA 285:1489–1499

Markowska AL, Savonenko AV (2002) Effectiveness of estrogen replacement in restoration of cognitive function after long-term estrogen withdrawal in aging rats. J Neurosci 22:10985–10995

McEwen BS, Alves SE (1999) Estrogen actions in the central nervous system. Endocr Rev 20:279–307

Nagamoto HT, Adler LE, Waldo MC, Griffith J, Freedman R (1991) Gating of auditory response in schizophrenics and normal controls. Effects of recording site and stimulation interval of the P50 wave. Schizophr Res 4:31–40

Phillips SM, Sherwin BB (1992) Effects of estrogen on memory function on surgically menopausal women. Psychoneuroendocrinology 17:485–495

Pocock SJ, Simon R (1975) Sequential treatment assignment with balancing for prognostic factors in the controlled clinical trial. Biometrics 31:103–115

Polich J (1996) Meta-analyses of P300 normative aging studies. Psychophysiology 33:334–353

Polo-Kantola P, Erkkola R, Irjala K, Pullinen S, Virtanen I, Polo O (1999) Effect of short-term transdermal estrogen replacement therapy on sleep: a randomized, double-blind crossover trial in postmenopausal women. Fertil Steril 71:873–880

Rapp SR, Espeland MA, Shumaker SA, Henderson VW, Brunner RL, Manson JAE, Gass MLS, Stefanick ML, Lane DS, Hays J, Johnson KC, Coker LH, Dailey M, Bowen D (2003) Effects of estrogen plus progestin on global cognitive function on postmenopausal women. JAMA 289:2663–2672

Resnick SM, Henderson VW (2002) Hormone therapy and risk of Alzheimer disease: a critical time. JAMA 288:2170–2172

Rice K, Morse C (2003) Measuring cognition in menopause research: a review of test use. Climacteric 6:2–22

Saletu B, Brandstätter N, Metka M, Stamenkovic M, Anderer P, Semlitsch HV, Heytmanek G, Huber J, Grünberger J, Linzmayer L, Kurz C, Decker K, Binder G, Knogler W, Koll B (1995) Double-blind, placebo-controlled, hormonal, syndromal and EEG mapping studies with transdermal oestradiol therapy in menopausal depression. Psychopharmacology 122:321–329

Saletu B, Anderer P, Gruber D, Metka M, Huber J, Saletu-Zyhlarz GM (2002) Hormone replacement therapy and vigilance: double-blind, placebo-controlled EEG-mapping studies with an estrogen-progestogen combination (Climodin, Lafamme) versus estrogen alone in menopausal syndrome patients. Maturitas 43:165–181

Sherwin BB (1988) Estrogen and/or androgen replacement therapy and cognitive functioning in surgically menopausal women. Psychoneuroendocrinology 13:345–357

Sherwin BB (2003) Estrogen and cognitive functioning in women. Endocr Rev 24:133–151

Shumaker SA, Legault C, Thal L, Wallace RB, Ockene JK, Hendrix SL, Jones BN III, Assaf AR, Jackson RD, Morley KJ, Wassertheil-Smoller S, Wactawski-Wende J (2003) Estrogen plus progestin and the incidence of dementia and mild cognitive impairment in postmenopausal women: the women's health initiative memory study: a randomized controlled trial. JAMA 289:2651–2662

Swickert R, Gilliland K (1998) Relationship between the brainstem auditory evoked response and extraversion, impulsivity, and sociability. J Res Pers 32:314–330.

Toran-Allerand CD (2000) Estrogen as a treatment for Alzheimer disease. JAMA 284:307–308

Walpurger V, Pietrowsky R, Kirschbaum C, Wolf OT (2004) Effects of the menstrual cycle on auditory event-related potentials. Horm Behav 46:600–606

Wolf OT, Kudielka BM, Hellhammer DH, Törber S, McEwen BS, Kirschbaum C (1999) Two weeks of transdermal estradiol treatment in postmenopausal elderly women and its effect on memory and mood: verbal memory changes are associated with the treatment induced estradiol levels. Psychoneuroendocrinology 24:727–741

Wolf OT, Heinrich AB, Hanstein B, Kirschbaum C (2005) Estradiol or estradiol/progesterone treatment in older women: no strong effects on cognition. Neurobiol Aging (in press)