

Young Women With Major Depression Live on Higher Homeostatic Sleep Pressure Than Healthy Controls

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There is mounting evidence for the involvement of the sleep-wake cycle and the circadian system in the pathogenesis of major depression. However, only a few studies so far focused on sleep and circadian rhythms under controlled experimental conditions. Thus, it remains unclear whether homeostatic sleep pressure or circadian rhythms, or both, are altered in depression. Here, the authors aimed at quantifying homeostatic and circadian sleep-wake regulatory mechanisms in young women suffering from major depressive disorder and healthy controls during a multiple nap paradigm under constant routine conditions. After an 8-h baseline night, 9 depressed women, 8 healthy young women, and 8 healthy older women underwent a 40-h multiple nap protocol (10 short sleep-wake cycles) followed by an 8-h recovery night. Polysomnographic recordings were done continuously, and subjective sleepiness was assessed. In order to measure circadian output, salivary melatonin samples were collected during scheduled wakefulness, and the circadian modulation of sleep spindles was analyzed with reference to the timing of melatonin secretion. Sleep parameters as well as non-rapid eye movement (NREM) sleep electroencephalographic (EEG) spectra were determined for collapsed left, central, and right frontal, central, parietal, and occipital derivations for the night and nap-sleep episodes in the frequency range .75–25 Hz. Young depressed women showed higher frontal EEG delta activity, as a marker of homeostatic sleep pressure, compared to healthy young and older women across both night sleep episodes together with significantly higher subjective sleepiness. Higher delta sleep EEG activity in the naps during the biological day were observed in young depressed women along with reduced nighttime melatonin secretion as compared to healthy young volunteers. The circadian modulation of sleep spindles between the biological night and day was virtually absent in healthy older women and partially impaired in young depressed women. These data provide strong evidence for higher homeostatic sleep pressure in young moderately depressed women, along with some indications for impairment of the strength of the endogenous circadian output signal involved in sleep-wake regulation. This finding may have important repercussions on the treatment of the illness as such that a selective suppression of EEG slow-wave activity could promote acute mood improvement. (Author correspondence: Christian.cajochen@upkbs.ch)

Keywords: Constant routine, Depressive disorder, EEG slow-wave activity, Nap protocol, Sleep spindles

INTRODUCTION

Major depression today is one of the main diseases causing loss of productive life (WHO, 2003). Vulnerability to depression has been shown to be partly related to genetic and environmental factors, such as stress, emotional trauma, and viral infections, or their interaction with genetic and epigenetic predispositions (Akiskal, 2000; De Kloet et al., 2005; Fava & Kendler, 2000; Rydmark et al., 2006; Schroeder et al., 2010; Tsankova et al., 2007). Compelling evidence has accumulated for an impairment of structural and functional brain plasticity associated with the pathophysiology of major depression (Duman et al., 2000; Krishnan & Nestler,

2008; Manji et al., 2001; Pittenger & Duman, 2008). However, despite considerable research effort for several decades, knowledge of the etiology and pathophysiology of major depression remains fragmented (Krishnan & Nestler, 2008; Maletic et al., 2007; Nestler et al., 2002).

More than 80% of patients with major depression also suffer from insomnia, whereas 15–35% show signs of hypersomnia (Armitage, 2007; Armitage & Hoffmann, 2001; Hawkins et al., 1985). Electroencephalographic (EEG) studies in major depression report abnormalities in sleep architecture, including prolonged sleep latency, shortened rapid eye movement (REM) sleep latency

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and increased REM sleep, decreased slow-wave sleep and EEG slow-wave activity, and a higher rate of awakenings, particularly towards the end of the night sleep episode (Armitage, 2007; Armitage & Hoffmann, 1997; Berger & Riemann, 1993; Borbély et al., 1984; Germain et al., 2004; Knowles et al., 1982; Kupfer et al., 1984; Reynolds & Kupfer, 1987). Interestingly, most of these changes in sleep architecture are also typically observed in healthy aging without depressive symptomatology, and it has been argued that sleep in depression bears similarities to precocious aging (Gillin et al., 1981). However, in contrast to the converging literature on age-related sleep alterations (Ancoli-Israel et al., 2008; Cajochen et al., 2006; Crowley, 2011), the findings on depression-related sleep disturbances are rather inconsistent (Antonijevic, 2006; Armitage et al., 2000a, 2000b; Armitage & Hoffmann, 2001). Major limits to finding common sleep-wake disruptions and circadian rhythm abnormalities in depression arise from the existence of different depression endophenotypes (Antonijevic, 2006).

The sleep-wake cycle, as most other behavioral and physiological processes, such as alertness, cognitive functions, and melatonin secretion, follow a circadian rhythmicity generated by a central pacemaker in the suprachiasmatic nuclei (SCN) located in the anterior hypothalamus (Dijk & Czeisler, 1995; Klein et al., 1991). According to the two-process model of sleep regulation, sleep and wakefulness are coordinated by the interaction of a circadian process, *C*, and a sleep-wake-dependent homeostatic process, *S* (Borbély, 1982). Thereby, the duration and consolidation of sleep depend on the interaction of both processes, whereas the timing of sleep is mainly controlled by process *C* and process *S* reflects the increasing need for sleep during wakefulness and dissipates during non-REM (NREM) sleep (Dijk et al., 1987). The two processes, *C* and *S*, interact in an opposite manner, such that the circadian process promotes wakefulness during the biological day and thereby counteracting the progressively build-up of sleep pressure (process *S*) with increasing time awake (Daan & Beersma, 1984; Dijk & Czeisler, 1995). Interestingly, subjective mood levels also depend, to a substantial degree, on the contribution of the homeostatic and circadian processes in healthy volunteers (Birchler-Pedross et al., 2009; Boivin et al., 1997) and women suffering from winter depression (Koorengevel et al., 2003). Hence, circadian rhythm alterations may not only be involved in the development of sleep disturbances in major depression but also in its pathogenesis (Wirz-Justice, 1995, 2006). In fact, several circadian disturbances have been reported in major depression, such as elevated nocturnal core body temperature, increased cortisol secretion, decreased plasma melatonin levels, and phase advances (earlier timing) of the circadian rhythm of all of these variables (Wehr & Wirz-Justice, 1982; Wirz-Justice, 1995, 2006). Despite the fact that circadian alterations in depression have not been confirmed by all studies (Boivin, 2000; Germain & Kupfer, 2008), several hypotheses have

been developed with reference to circadian, homeostatic, or ultradian abnormalities in depression, such as the phase-advance hypothesis (Wehr & Wirz-Justice, 1981), the low-melatonin hypothesis (Beck-Friis et al., 1985), the so-called *S*-deficiency hypothesis (Borbély & Wirz-Justice, 1982), and the acetylcholine-monoamine imbalance hypothesis (Gillin et al., 1982).

The relative contribution of circadian and homeostatic processes to sleep regulation can only be assessed by applying different sleep-wake manipulation schemes under controlled laboratory conditions in order to dissect the two processes and to unmask endogenous circadian rhythms from external “zeitgebers,” such as light, temperature, food intake, and social interactions (Duffy & Dijk, 2002). Thus, here we aimed at investigating homeostatic and circadian sleep regulation in depressed women under low sleep pressure conditions by applying short sleep-wake cycles (naps) evenly distributed over a 40-h period, allowing for sleep episodes to occur at different circadian phases. Age-matched healthy young and healthy older control women were included in the study. The rationale behind including healthy older volunteers was to determine whether reported similarities in sleep disturbances in major depression and in aging underlie common changes in circadian and homeostatic sleep-wake regulation. To our knowledge, a nap protocol under stringently controlled laboratory conditions (constant routine) has never before been applied in major depressed patients. Our main prediction was that young depressed women, in contrast to young healthy women, would show a reduced circadian wake-promoting signal in the early evening as indexed by more sleep during nap episodes scheduled during this time of day, as we have shown in healthy aging (Knoblauch et al., 2005). Furthermore, we expected a disturbance of the circadian rhythm in salivary melatonin in young depressed women expressed either by lower melatonin concentrations or phase shift of the hormone rhythm, or both, compared to the two healthy control groups.

MATERIALS AND METHODS

Study Participants

Female participants were recruited via advertisements at different universities in the region of Basel, Switzerland, and via selected online portals. Eight healthy young (HY: 20–31 yrs; mean \pm SD = 25.4 \pm 3.8 yrs), eight healthy older (HO: 57–74 yrs; mean \pm SD = 64.4 \pm 5.4 yrs), and nine young women with major depressive disorder (MDD: 19–32 yrs, mean \pm SD = 22.8 \pm 3.9 yrs) were studied. A two-sided *t* test disclosed no significant age differences between the two young groups ($p > .05$). Although the data from the two healthy groups originated from previous studies carried out in the same laboratory (Knoblauch et al., 2005; Münch et al., 2005; 2007), the laboratory setup, equipment, and recording devices as well as the constant routine conditions and data processing remained the same for all three groups.

All participants underwent a defined screening procedure, including questionnaires to screen physical health, drug consumption, and sleep quality, plus a medical examination to assess their somatic state. Additionally, young depressed volunteers filled in a self-report depression-rating questionnaire (Beck Depression Inventory [BDI]; Beck et al., 1961); only participants with a score >12 were considered for a subsequent clinical interview. The BDI (mean \pm SD) of the study participants was 20.3 ± 8.5 . To assess the presence of an episode of major depression disorder, a structured clinical interview for DSM-IV Axis I (SCID-I) according to DSM-IV-TR (*Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision*, of the American Psychiatric Association, 1994) was carried out with the MDD volunteers. This diagnostic tool is symptom based, whereby all diagnostic symptoms are coded as present, subthreshold, or absent. According to the SCID-I, a depressive episode is present if a person is experiencing at least 5 of 9 symptoms the previous ≥ 2 wks for most of the time, and 1 of these 5 symptoms must be either depressed mood or loss of interest. When participating in the study, all MDD women were experiencing either the first or second onset episode of MDD according to DSM-IV criteria (SCID-I value [mean \pm SD] = 5.9 ± 1.4); they had no atypical symptoms, such as hypersomnia, for example, and did not suffer from seasonal depression (SAD) or exhibit any comorbid psychiatric DSM-IV-R disorder. Although the study took place throughout the year, effects of season on the measured output variables were not expected, since only women with MDD, and not SAD, were considered. However, it has to be noted that it was not possible to discern seasonal influences on the measured variables to a satisfactory statistical degree due to the small sample size of the depressed group. Based on the higher prevalence rate of MDD (without comorbidities) in women compared to men and the resulting higher likelihood to find women with MDD, only women were included in the study. None of the MDD participants had any psychiatric medical treatment before and during the study.

The HY and HO participants had no sleep disturbances as assessed by the Pittsburgh Sleep Quality Index (PSQI; Buysse et al., 1989), i.e., PSQI value <5 , although for MDD participants a score <8 was allowed, i.e., only mild sleep disturbances. The PSQI score (mean \pm SD) was 6.9 ± 1.4 for MDD, 2 ± 1.7 for HY, and 3.9 ± 1.6 for HO. The PSQI for HY women was significantly lower than it was for the other two groups ($p < .05$), and the MDD women had significant higher values compared to HO women ($p < .001$). All participants spent an adaptation night in the chronobiology laboratory to evaluate sleep quality by polysomnographic recordings and to exclude volunteers with sleep efficiency $<80\%$, periodic leg movements $>10/h$, and apnea-hypopnea index >10 . All study participants were drug-free (verified by urinary toxicologic analysis), nonsmokers, and had no shiftwork or transmeridian flights over >3 time zones for 3 mo prior to the

commencement of the study. Only intermediate chronotypes, as assessed by the diurnal type scale (Torsvall & Åkerstedt, 1980), were considered (mean \pm SD of the diurnal type scale = 16.8 ± 1.7 for MDD, 15.6 ± 3.8 for HY, 17.5 ± 4 for HO; a t test yielded no significant differences between the groups). Medications, other than oral contraceptives, were not allowed; 4 HY and 4 MDD study participants used oral contraceptives. All young volunteers participated in the laboratory part of the study during the follicular phase of their menstrual cycle (days 1–5 after menses onset). All HO women were postmenopausal, and none was on hormone replacement therapy. All study participants gave their signed informed consent. The study procedures, questionnaires, and consent form all were approved by the local Ethics Committee of Basel (EKBB), Switzerland. All procedures conformed to the Declaration of Helsinki, and the study conduct conformed to international ethical and investigative standards (Portaluppi et al., 2010).

Study Protocol

The study comprised a 7-d ambulatory part at home followed by a laboratory part (3.5 d). During the ambulatory part, volunteers were asked to restrict their caffeine intake to only 1 beverage/d, to consume not >5 alcoholic drinks during the entire week, and to abstain from heavy physical exercise. Furthermore, they were asked to keep a regular sleep-wake schedule during the ambulatory study prior to admission to the laboratory. Compliance was verified by sleep logs and ambulatory activity measurements (wrist activity monitor; Cambridge Neuro-Technology Ltd., Cambridge, UK). The timing of the sleep-wake schedule during the protocol was adjusted to individual habitual bedtimes calculated by centering the approximate 8-h sleep episodes during the baseline week at the individual midpoint of sleep of each participant. Habitual bedtimes (mean \pm SD) did not significantly differ between the three groups (MDD = $23:53 \pm 58$ min, HY = $23:37 \pm 81$ min, and HO = $23:24 \pm 52$ min). The protocol comprised a habituation night followed by a baseline night in the chronobiology laboratory. After the baseline night, the participants followed a 40-h multiple nap protocol (10 sleep episodes lasting 75 min each alternating with 11 wake episodes of 150-min duration), which was followed by an 8-h recovery night at habitual bedtimes (Figure 1).

While in the laboratory, participants received no external time cues. Polysomnographic recordings started in the afternoon after the habituation night. During the entire protocol participants remained under constant conditions, such as dim-light <8 lux during wakefulness and 0 lux during sleep episodes, semirecumbent posture position in bed during scheduled wakefulness, regular isocaloric meals, and constant room temperature. A daily heparin injection (Fragmin, .2 mL, 2500 IE/IU; Pfizer AG, Zurich, Switzerland) was given to older volunteers to prevent venous thrombosis. The severity of the depressive episode of the volunteers with major

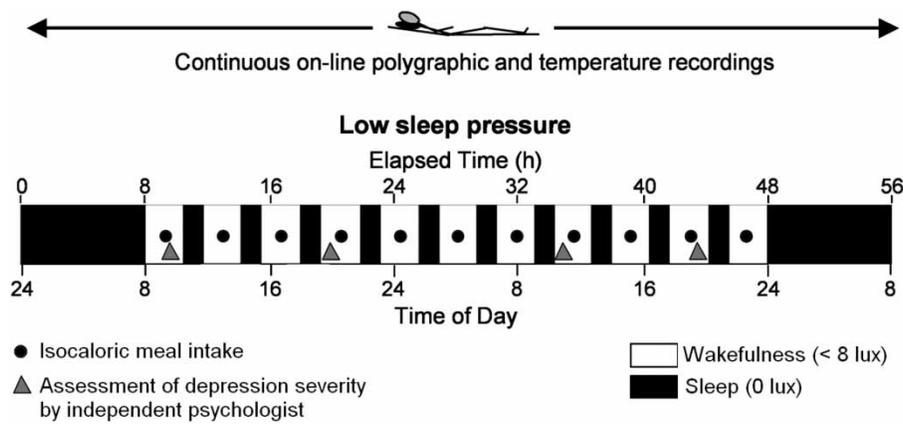


FIGURE 1. Schematic illustration of the short sleep-wake cycle (nap) protocol. The illustration shows the timing of sleep and wakefulness, food intake, and psychological assessments of depression severity in young women with major depression (MDD) in the course of the nap protocol. As a consequence of the alternating sleep/wake cycles of 75 and 150 min, respectively, across a time span of 40 h, sleep pressure levels remain constantly low. The protocol required bedrest conditions and comprised both a baseline night and a recovery night. The timing of the sleep and wake episodes, body posture, light levels, and room temperature were stringently controlled (i.e., constant-routine conditions).

depression was assessed regularly (see Figure 1) by an independent psychologist on the basis of the Montgomery-Asperg Depression Rating Scale (MADRS; Montgomery & Asberg, 1979) and Hamilton Rating Scale for Depression (HAM-D-17; Williams & Terman, 2003).

Polysomnographic Recordings and Analysis

Polysomnographic recordings (Vitaport-3 digital recorder TEMEC Instruments BV, Kerkrade, The Netherlands) during sleep comprised 12 EEG derivations (F3, F4, Fz, C3, C4, Cz, P3, P4, Pz, O1, O2, and Oz referenced against linked mastoids), two electrooculograms, one submental electromyogram, and one electrocardiogram. All EEG signals were filtered at 30 Hz (fourth-order Bessel-type antialiasing low-pass filter, total 24 dB/Oct), and a time constant of 1.0 s was used prior to online digitization (range 610 V, 12 bit AD converter, .15 V/bit; storage sampling rate at 128 Hz). Sleep episodes of all study participants were visually scored on a 20-s epoch basis according to the standard criteria of Rechtschaffen and Kales (1968). EEGs were subjected to spectral analysis using a fast Fourier transform (FFT) with 10% cosine 4-s windows, resulting in a .25-Hz resolution. Sleep stages (1-4), REM sleep, movement time (MT), and wakefulness after sleep onset (WASO) were expressed as percentage of total sleep time (TST) during the respective night for all participants. TST and sleep latencies to stage 1 (SL1), stage 2 (SL2), and REM (RL) were indicated in minutes. Sleep efficiency (SE) was expressed as percentage of TST of the total time between lights-off and lights-on (i.e., time in bed, TIB). NREM sleep was defined as stages 2 to 4 (% of TST). Sleep parameters during the 10 naps were similarly calculated on the basis of the TST during all 10 naps. The time course of different sleep stages during the 10 nap episodes was based on visual sleep scoring of selected sleep stages and is illustrated in Figure 2.

EEG spectra during the baseline and recovery nights were calculated for the first 7 h of NREM sleep in the frequency range from .75 to 25 Hz for all 12 EEG derivations. A mixed-model repeated analysis of variance (rANOVA) with the factors *group* (MDD vs. HY vs. HO women), *night* (baseline vs. recovery), *derivation site* (frontal [F] vs. central [C] vs. parietal [P] vs. occipital [O]), and *hemisphere* (left vs. right) disclosed significant lower left hemisphere EEG values for some of the frequency bins of the delta, theta, and beta ranges (.75, 4.25-4.5, 4.75-6.5, 17.75-18.75, 20.75-21.25, 22-22.25, and 23-23.5 Hz), plus significant ($p < .05$) interaction of the factors *group* \times *hemisphere* \times *derivation site* in three frequency bins of the sigma range (13.75, 14.5-14.75 Hz). Post hoc analysis yielded no significant differences between *groups* and *hemisphere* per *derivation site* (p at least $> .05$; post hoc analysis based on least mean (LSMEANS) statements; Tukey-Kramer adjusted). No other significances were observed (p at least $> .05$). Hence, spectral values for the left and right derivations as well as midline derivations were collapsed for further analyses along the anteroposterior axis resulting in one value for each of the F, C, P, and O sites. Baseline and recovery night values for MDD and HO were graphically illustrated as percentage of the respective values of the HY (100%), whereby the statistical analysis was based on absolute EEG power density values.

NREM-REM sleep cycles were defined according to the criteria of Feinberg and Floyd (1979) with the exception that, for the last sleep cycle, no minimum REM sleep duration was required. Thereafter, each sleep cycle was divided into 10 equal time intervals during NREM sleep and 4 equal time intervals during REM sleep.

The 10 nap episodes were categorized either as a biological day or biological night nap in order to compare diurnal and nocturnal EEG sleep spectra. A nap was considered as a day nap if its start time was between the

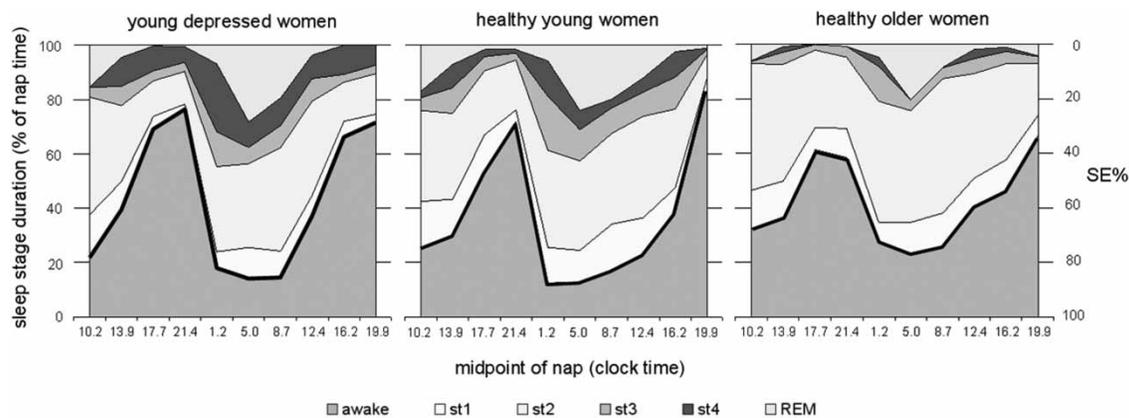


FIGURE 2. Time course of sleep stages across the 40-h nap protocol. The figure displays the mean duration of different sleep stages and wakefulness expressed as % of total nap duration (75 min) for each nap and group. Stage durations are derived from visually scored sleep stages. st1–st4 = sleep stages 1–4. The black bold line indicates the time course of sleep efficiency (SE) (= total sleep time expressed as % of the total nap time) on an inverted scale as indicated by the right ordinate.

melatonin downward and upward crossing times (see “Salivary Melatonin Sampling and Subjective Sleepiness Ratings” below). Subsequently, a nap qualified for the biological night if it occurred during the main melatonin secretion episode between the upward and downward mean crossing times. The mean \pm SD number of biological day and night naps per group, respectively, was $7.2 \pm .67$ and $2.8 \pm .67$ for MDD, $7.6 \pm .52$ and $2.4 \pm .52$ for HY, and $7.8 \pm .46$ and $2.1 \pm .64$ for HO women. These numbers did not differ significantly between groups (p at least $>.05$). Stage 2 sleep duration did not differ significantly between groups and biological day and night according to the interaction effect of the factors *group* and *biological timing* (day vs. night naps) of a two-way rANOVA ($F_{2,22} = .22$; $p > .8$). Moreover, one-way rANOVA performed for each group separately showed no significant difference of stage 2 sleep duration between the biological day and night naps (p at least $>.4$). Hence, the biological day and night EEG sleep spectra analysis was based on EEG spectra derived from NREM stage 2 sleep episodes.

Salivary Melatonin Sampling and Subjective Sleepiness Ratings

Saliva collections were taken every 30 min during scheduled wakefulness throughout the entire 40-h protocol. A direct double-antibody radioimmunoassay (RIA) was used for the melatonin assay (Weber et al., 1997), which was validated by gas chromatography–mass spectroscopy with an analytical least detectable dose of .65 pg/mL (Bühlmann Laboratories, Schönenbuch, Switzerland). All melatonin values were collapsed into 1.25-h bins per subject before averaging across groups, whereby missing values were linearly interpolated. Melatonin, as one of the most reliable endogenous circadian phase measures (Klerman et al., 2002; Shanahan et al., 1997), was used to estimate circadian timing and its phase relationship with sleep-phase preference. Thereby, upward and downward mean crossing times of the 24-h

mean, the average level of melatonin, and the respective secretion duration in between these mean crossing times were calculated for each subject before averaging across groups according to Knoblauch et al. (2005) and Münch et al. (2005) (see Table 2).

Study participants rated their sleepiness every 30 min during scheduled wakefulness across the 40-h study protocol by means of questionnaires. Sleepiness was assessed by the Karolinska Sleepiness Scale (KSS), with a rating range from 1 (very alert) to 9 (very sleepy) (Gillberg et al., 1994). KSS values were collapsed into 1.25-h bins per subject before averaging across groups.

Statistical Analysis

The statistical packages SAS (Version 9.1.3; SAS Institute, Inc.; Cary, NC, USA) and Statistica (STATISTICA for windows, Version 8.0; StatSoft Inc., Tulsa, OK, USA) were used. Sleep stages were analyzed by repeated-measures ANOVA (PROC GLM) and p values were based on Huynh-Feldt corrected degrees of freedom. Post hoc comparisons on sleep stages were based on Duncan’s multiple range test, and the levels of significance of these post hoc comparisons were adjusted according to the false discovery rate procedure (Curran-Everett, 2000). Comparisons of EEG power density, melatonin, and subjective sleepiness were made by mixed-model rANOVA (PROC MIXED), and p values were based on Kenward-Roger’s (1997) corrected degrees of freedom. Contrasts were assessed with the LSMEANS statement, and the respective level of significance was adjusted according to the Tukey-Kramer method (Hayter, 1984). EEG power density was then averaged during NREM sleep per group for the baseline and recovery nights and expressed as percentage of the respective values of the HY group (100%). One-, two-, three-, and four-way mixed-model rANOVAs were used with the categorical factor *group* (MDD vs. HY vs. HO women) and the repetitive factors *derivation*, *night*, and *time interval* (e.g., nap, sleep cycle etc.).

RESULTS

Sleep Stages During Baseline and Recovery Nights

Sleep parameters of the baseline and recovery nights for the MDD, HY, and HO women are summarized in Table 1. A two-way rANOVA disclosed significant differences between groups for TST, SE, WASO, stage 1, stage 4, and SWS. Post hoc comparisons for the significant group effects showed less TST, lower SE, higher WASO values, and shorter stage 4 duration for the HO women compared to both the HY and MDD women (p at least $<.05$). SWS in MDD, but not in HY, women was significantly longer compared to HO women ($p < .01$). Furthermore, MDD had significantly less stage 1 sleep than HO and HY women ($p < .01$).

The nap protocol resulted in significant differences between the baseline and recovery nights for TST, SE, and NREM sleep ($p < .01$), SL1 and SL2 ($p < .001$), MT and stage 1 sleep across groups ($p < .01$), and WASO ($p < .05$). TST, SE, and NREM sleep proportions decreased and wake periods after lights-off and the latency to sleep onset increased in the recovery night compared to baseline night.

Nap-Sleep Episodes

The time course of sleep stages 1–4, REM, and wakefulness across the 10 nap-sleep episodes is displayed in an area diagram where for each nap and group the averaged relative time proportion of selected sleep stages and wakefulness are expressed as a percentage of the total nap duration (Figure 2). A two-way rANOVA on relative sleep

stage values disclosed significant differences between groups for stage 4 sleep ($p < .01$; $F_{2,22} = 8.216$), with a higher sleep stage 4 proportion in MDD than HY and HO women ($p < .05$ and $p < .001$, respectively). Furthermore, HO women showed significantly more stage 2 sleep than the other two groups across the 40-h nap protocol ($p < .01$; $F_{2,22} = 8.902$). The time course of all selected sleep stages, wakefulness, and SE varied significantly ($p < .001$; $F_{9,197} > 3.7$). No group differences for the relative time proportions of sleep stages 1 and 3, REM, wakefulness, and SE, nor any significant interaction of the factors *group* \times *time of day* were observed. Furthermore, a two-way rANOVA disclosed a significant effect of the factor *time of day* for TST and for REM, SL1, and SL2 ($p < .001$). Group differences, however, were only significant for REM sleep latency ($p < .01$; $F_{2,22} = 6.28$), with longer values in HO women compared to the two young groups (p at least $<.05$), and no significant interactions of the factors *group* \times *time of day* were observed ($p > .1$). Total sleep duration across the 10 naps and the baseline night did not differ significantly within the groups ($p > .05$), and SE during the naps was significantly lower for all groups when compared to baseline sleep values (p at least $<.001$; t test for dependent samples; data not shown).

NREM EEG Power Activity During Baseline and Recovery Nights

Figure 3 illustrates the relative EEG spectra during baseline and recovery nights for the MDD and HO women expressed as percentage of the HY women (100%)

TABLE 1. Sleep parameters of the baseline and recovery nights based on visual scoring for all groups

Parameter	Young depressed women		Healthy young women		Healthy older women		2-Way rANOVA					
	Baseline	Recovery	Baseline	Recovery	Baseline	Recovery	Group		Night		Group \times night	
							p	$F_{2,22}$	p	$F_{1,22}$	p	$F_{2,22}$
TST, min	449.9 \pm 21.0	413.2 \pm 26.2	449.5 \pm 17.1	412.2 \pm 53.9	403.2 \pm 47.9	361.6 \pm 54.5	**	6.05	***	19.35	—	.03
SE, %	94.8 \pm 1.5	86.8 \pm 4.8	93.6 \pm 3.5	85.9 \pm 11.3	84 \pm 10	75.4 \pm 11.4	**	6.81	***	23.14	—	.02
WASO, %	2.0 \pm 1.8	5.5 \pm 6.7	2.0 \pm 2.1	8.0 \pm 12.4	16.1 \pm 12.3	19.7 \pm 12.8	**	8.57	*	4.82	—	.17
SL1, min	6.4 \pm 5.2	25.9 \pm 15.8	8.9 \pm 4.0	19.8 \pm 11.8	8.3 \pm 3.9	27.8 \pm 17.5	—	.31	***	44.04	—	1.28
SL2, min	9.3 \pm 6.1	31.6 \pm 20.2	15.7 \pm 8.1	30.5 \pm 15.9	12.2 \pm 5.6	43.1 \pm 30.7	—	.63	***	29.1	—	1.18
RL, min	85.4 \pm 36.6	85.0 \pm 23.3	71.9 \pm 10.8	90.9 \pm 45.4	104 \pm 37.5	101.4 \pm 36.9	—	1.57	—	.38	—	.62
MT, %	.7 \pm .2	.9 \pm .4	.9 \pm .6	1.4 \pm 1.2	.6 \pm .3	1.3 \pm 1.1	—	1.13	**	12.04	—	1.17
St1, %	6.9 \pm 2.7	8.5 \pm 3.2	11.1 \pm 4.1	14.5 \pm 4.8	11.9 \pm 3.4	14.1 \pm 5.3	**	6.13	**	10.15	—	.53
St2, %	51.1 \pm 3.7	51.4 \pm 6.2	49.5 \pm 4.7	49.7 \pm 5.5	60.8 \pm 11.4	55.0 \pm 12.3	—	2.98	—	1.9	—	2.32
St3, %	11.1 \pm 3.6	9.9 \pm 3.8	11.2 \pm 5.1	10.0 \pm 3.1	9.2 \pm 5.0	8.2 \pm 4.9	—	.56	—	3	—	.01
St4, %	10.9 \pm 7.3	11.0 \pm 5.8	8.6 \pm 4.5	6.7 \pm 4.3	2.3 \pm 2.5	2.5 \pm 3.1	**	7.86	—	.37	—	.65
SWS, %	21.9 \pm 8.1	21.0 \pm 6.7	19.8 \pm 7.3	16.7 \pm 6.7	11.4 \pm 7.1	10.7 \pm 7.6	*	5.09	—	2.27	—	.49
NREM, %	73.1 \pm 5.3	72.4 \pm 4.4	69.3 \pm 5.1	66.3 \pm 4.0	72.2 \pm 8.0	65.8 \pm 8.1	—	2	**	9.04	—	2.22
REM, %	20.0 \pm 3.3	19.1 \pm 2.5	19.7 \pm 3.6	19.2 \pm 3.4	15.9 \pm 5.7	20.2 \pm 5.4	—	.52	—	.97	—	2.79

Sleep parameters are averaged separately across the baseline night and the recovery night (mean \pm SD) for young women with major depression (MDD; $n = 9$), young healthy women (HY; $n = 8$), and older healthy women (HO; $n = 8$).

TIB = total time in bed (from lights-off to lights-on); TST = total sleep time; SE = sleep efficiency (TST/TIB \times 100); WASO = wake after sleep onset (in % of TST); SL1 = sleep latency to stage 1; SL2 = sleep latency to stage 2; RL = REM latency; MT = movement time after sleep onset (in % of TST); St1–St4 = sleep stages 1–4 (in % of TST); SWS = slow-wave sleep (sum of stages 3 and 4 in % of TST); NREM = non-REM sleep (sum of sleep stages 2–4 in % of TST); REM = REM sleep in % of TST.

* $p < .05$; ** $p < .01$; *** $p < .001$.

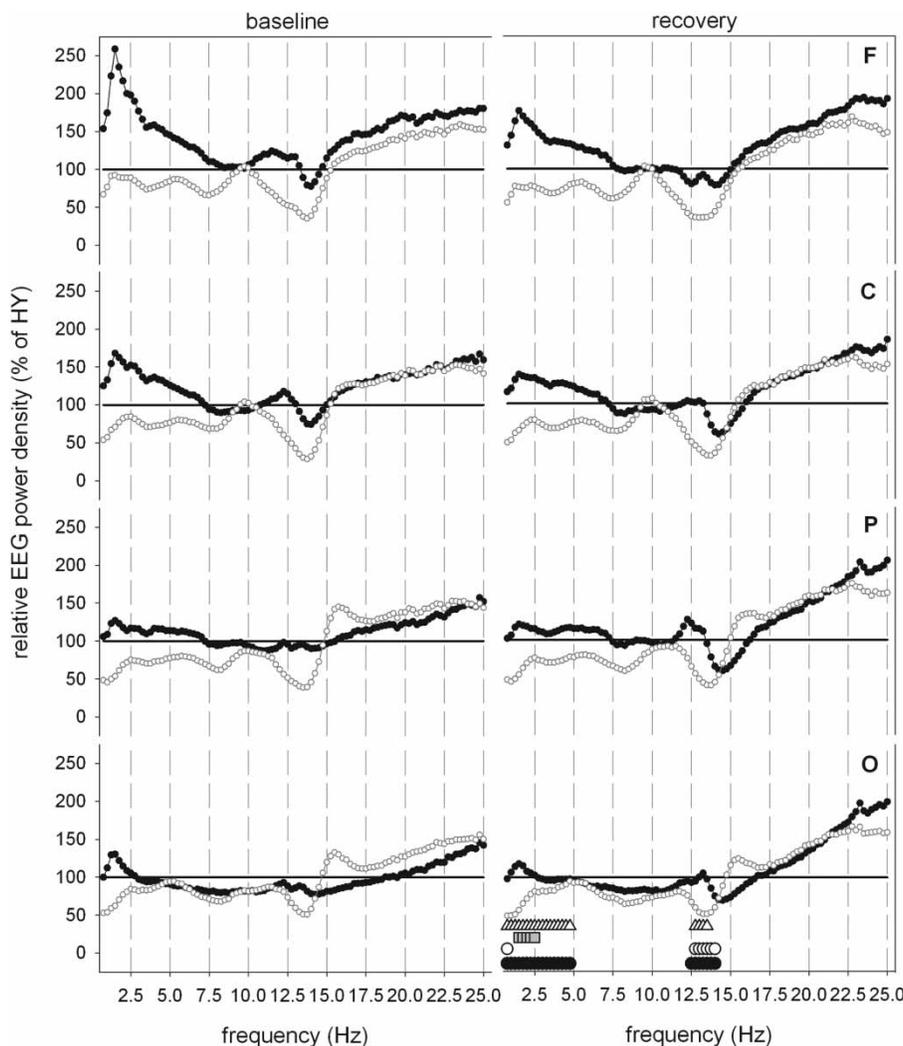


FIGURE 3. Relative NREM EEG spectra during baseline and recovery nights. Relative EEG spectral values are shown for .75–25 Hz for F, C, P, and O derivations (collapsed left, central, and right values for Fz, F3, F4, Cz, C3, C4, Pz, P3, P4, and Oz, O1, O2, respectively) for healthy young (HY; reference line at 100%; $n = 8$), healthy older (HO; in % of HY; gray open circles; $n = 8$), and major depressed (MDD; in % of HY; black filled circles; $n = 8$) women. Significant differences for the factor group (filled black circles) are indicated as well as respective post hoc comparisons: open circles = HY > HO; shaded square = MDD > HY; open triangle up = MDD > HO (statistics are based on the absolute EEG spectra values, mixed-model three-way rANOVA, post hoc analysis with LSMEANS procedure, Tukey-Kramer adjusted p values).

during NREM sleep in the frequency range of .75 and 25 Hz for F, C, P, and O derivations. Mixed-model three-way rANOVA (on absolute EEG spectral values) disclosed a significant difference for the main factor *group* ($p < .05$; $F_{2,22} > 3.53$) in the delta, one frequency bin in the theta range (.75–4.5 and 4.75 Hz), and in the sigma range (12.5–14 Hz). Post hoc inspection of the data showed significantly ($p < .05$) higher EEG power of MDD than HY women for some of the frequency bins in the delta range (1.5–2.5 Hz) and than HO women in the delta range and in some of the frequency bins of the theta and sigma ranges (.75–4.5, 4.75, and 12.75–13.5 Hz). Furthermore, HY women showed significantly ($p < .05$) higher EEG power than HO women for one of the frequency bins in the theta range and in the sigma range (.75 and 12.75–14 Hz). Subsequent analysis (e.g., time course of delta power/sleep cycle; see Figure 5) with all three groups were based on a collapsed delta band in

the range of 1.5–2.5 Hz, because these frequency bins showed significant differences between MDD and the two other groups across the baseline and recovery nights.

Mixed-model three-way rANOVA showed significant ($p < .05$; $F_{3,66} > 2.51$) differences for the main factor *derivation* for all frequency bins, except for the theta frequencies 6.25 and 6.5 Hz. A post hoc analysis yielded a significant ($p < .01$) frontal predominance compared to the central, parietal, and occipital derivations in the delta frequency range of .75–2.25 Hz. In the higher frequencies, differences between frontal and central derivations were not significant, but they were between the parietal and occipital derivations. The interaction of the main factors *group* \times *derivation* yielded significance for the frequency bins in the delta range (.75–4.5 Hz), some of the frequency bins in the theta range (4.75–7 Hz; 1), in the alpha and sigma ranges (11.5–12.25, 13.25–14.25, and 15.25–16 Hz), and in the beta range (16.25–21.5 and 22–22.5 Hz)

($p < .05$; $F_{6,66} > 2.8$). Post hoc analysis yielded higher frontal values in MDD than in HY and HO women in frequency bins of the delta range (1–3 Hz) as well as higher values in MDD than in HY women in the beta frequency bins (18.25–20 Hz) ($p < .05$, LSMEANS procedure with Tukey-Kramer adjusted p values).

There was no significant interaction of the factors *group* × *night*, but the interaction *derivation* × *night* yielded significance in the theta frequency bin of 5 Hz, for frequency bins in the alpha range (11.25–12 Hz), for frequency bins in the sigma spindle range (13.5–15.5 Hz), and in the beta frequency range (22.75–23, 23.75, and 24.75 Hz) ($p < .05$; $F_{3,60} > 3.02$). Significantly higher values in frontocentral derivations were found in the theta 5-Hz bin during the recovery night compared to the baseline night, higher frontal values during the recovery compared to the baseline night for the alpha frequencies 11.25–12 Hz, higher central and parietal values for the sigma spindle range (13.5–15.5 Hz), and higher values in all derivations during the recovery night

compared to the baseline night for the beta frequency bins 22.75–23, 23.75, and 24.75 Hz ($p < .05$). Finally, three-way mixed model rANOVA yielded significance for the interaction *group* × *night* × *derivation* in the sigma spindle range (14.75–15.75 Hz; $p < .05$; $F_{6,56} > 2.77$), with no significant differences between groups per night and derivation after Tukey-Kramer adjustment of the p values of the post hoc analysis.

Subsequently, relative NREM spectra per sleep cycle (recovery night EEG spectra per cycle as a percentage of respective baseline values per subject averaged across groups) and group were calculated (Figure 4). Mixed-model three-way rANOVA with the factors *group*, *cycle*, and *derivation* showed significant interaction of the factors *group* × *cycle* ($p < .05$; $F_{4,56} > 3.06$), with higher values of HY than MDD women in NREM sleep cycle 3 in some of the delta and theta frequency bins (1–2, 2.75, 3.25, 4, and 5–5.25 Hz; $p < .05$). Furthermore, the relative EEG power density during NREM sleep cycles 1 and 2 were significantly lower than during

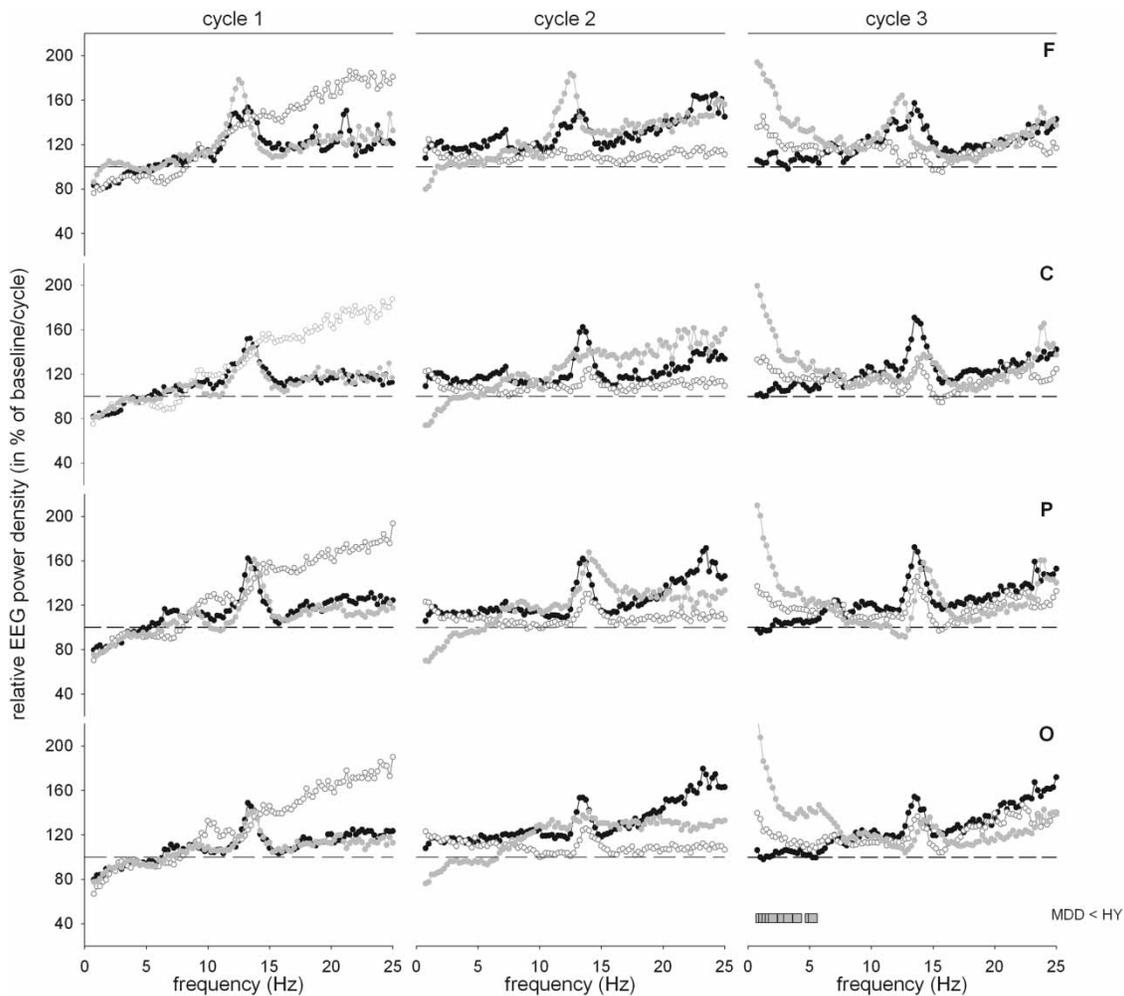


FIGURE 4 Relative EEG power density during the recovery night for NREM sleep cycles 1–3 between .75 and 25 Hz. The figure illustrates relative EEG spectra values (recovery night in % of baseline night/sleep cycle) for NREM sleep cycles 1–3 for F, C, P, and O derivations between .75 and 25 Hz for healthy young (HY; gray filled circles; $n = 8$), healthy older (HO; gray open circles; $n = 7$), and young depressed (MDD; black filled circles; $n = 7$) women. Significant post hoc comparisons for the interaction of the factors *group* × *cycle* are indicated near the abscissae (gray shaded squares = MDD < HY; $p < .05$).

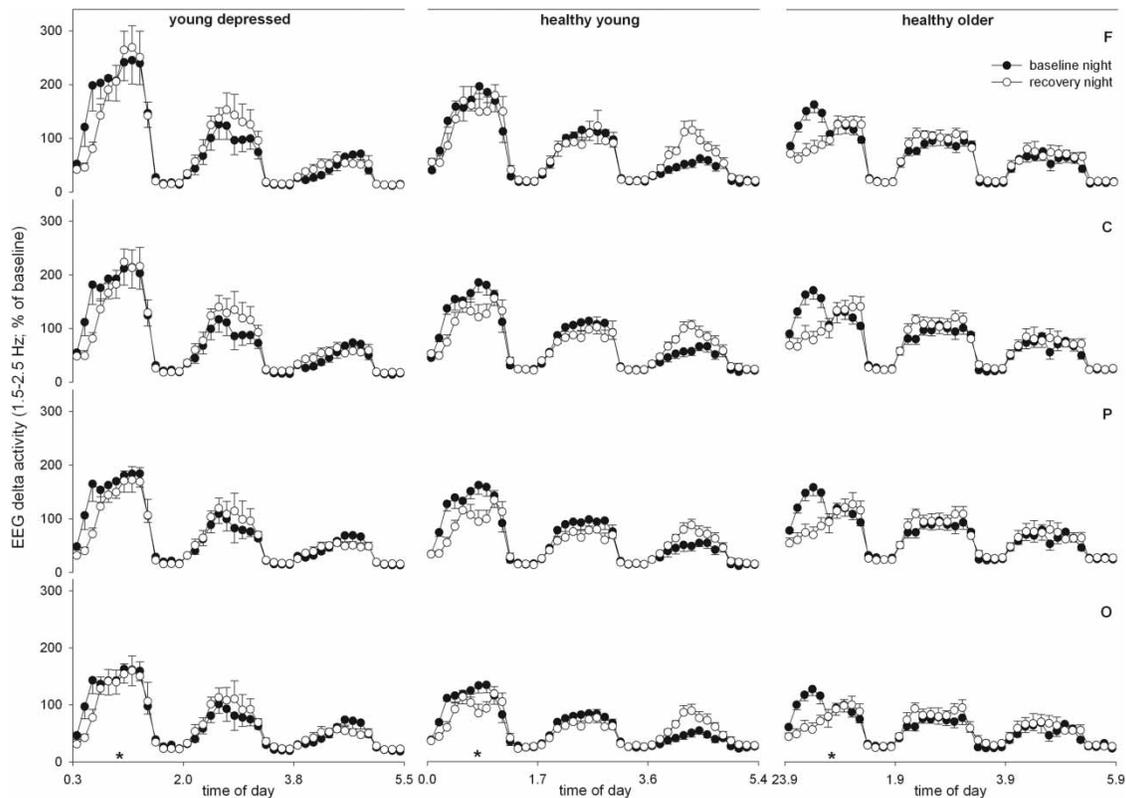


FIGURE 5. EEG delta power activity for NREM-REM sleep cycles 1-3. EEG delta activity (1.5-2.5 Hz)/sleep cycle 1-3 for collapsed derivations (F, C, P, O) is expressed for each group separately as a percentage of the respective baseline value (young depressed, $n = 7$; healthy elderly, $n = 7$; healthy young, $n = 8$; mean \pm SEM). Black filled circles = baseline night; black open circles = recovery night. Asterisks indicate significant post hoc comparisons of mean NREM EEG values/cycle between baseline and recovery night within each group ($p < .05$; LSMEANS procedure, Tukey-Kramer adjusted).

sleep cycle 3 in HY women in the delta and theta frequency bins 1-5.75 Hz ($p < .01$).

There were no significant differences of EEG power density between NREM sleep cycles 1-3 in MDD and HO women (post hoc comparisons within each group, $p > .05$) as well as between these two groups per cycle (post hoc comparison between groups, $p > .05$). Furthermore, mixed-model three-way rANOVA showed significant interaction of the factors *group* \times *cycle* \times *derivation* for some frequency bins of the delta, theta, alpha, sigma, and beta ranges (4.25-5, 5.5-7, 10-10.25, and 15.25-16.25 Hz; $p < .05$).

In order to analyze the time course of the EEG delta power in detail, percentiles (see Materials and Methods) in the frequency range between 1.5 and 2.5 Hz (= significant EEG band between MDD and the other two groups across baseline and recovery nights as illustrated in Figure 3) were calculated as a percentage of the mean delta activity during the baseline night for NREM-REM sleep cycles 1-3 for F, C, P, and O derivations (Figure 5). A mixed-model four-way rANOVA disclosed no significant effect of the main factors *group* and *night* ($p > .2$), but of the factors *derivation* and *cycle* ($p < .001$; $F_{3,48} = 41.86$ and $F_{2,42} = 107.29$, respectively). Furthermore, significant interactions of the factors *group* \times *night* \times *cycle* ($p < .001$; $F_{4,325} = 9.5$) and *group* \times *night* \times

cycle \times *derivation* ($p < .001$; $F_{42,322} = 2.09$) were observed. Significant post hoc comparisons for the three-way interaction are displayed in Figure 5 and show significantly lower EEG delta power during the first NREM-REM sleep cycle in the recovery night compared to the baseline night within each of the three groups ($p < .05$; LSMEANS procedure). There was no difference of the duration of the NREM-REM cycles 1-3 between the three groups during the baseline or recovery night ($p > .6$, mixed-model two-way rANOVA; data not shown).

EEG Spectra During Nap Episodes

Nap-sleep episodes were grouped according to their occurrence either during the biological day or biological night (see Materials and Methods and Figure S1) and the EEG values during the biological night stage 2 sleep were expressed as a percentage of the respective values during the biological day (Figure 6). A mixed-model two-way rANOVA disclosed a significant effect of the main factor *group* ($p < .05$; $F_{2,22} > 6.15$), with significantly ($p < .05$) lower relative EEG values of MDD compared to HY women in some of the frequency bins of the alpha and sigma ranges (10.5-11.5 and 13.5-13.75 Hz). Furthermore, HO women exhibited significantly ($p < .05$) lower relative EEG values during the biological night in some frequency bins of the theta, alpha, and sigma ranges

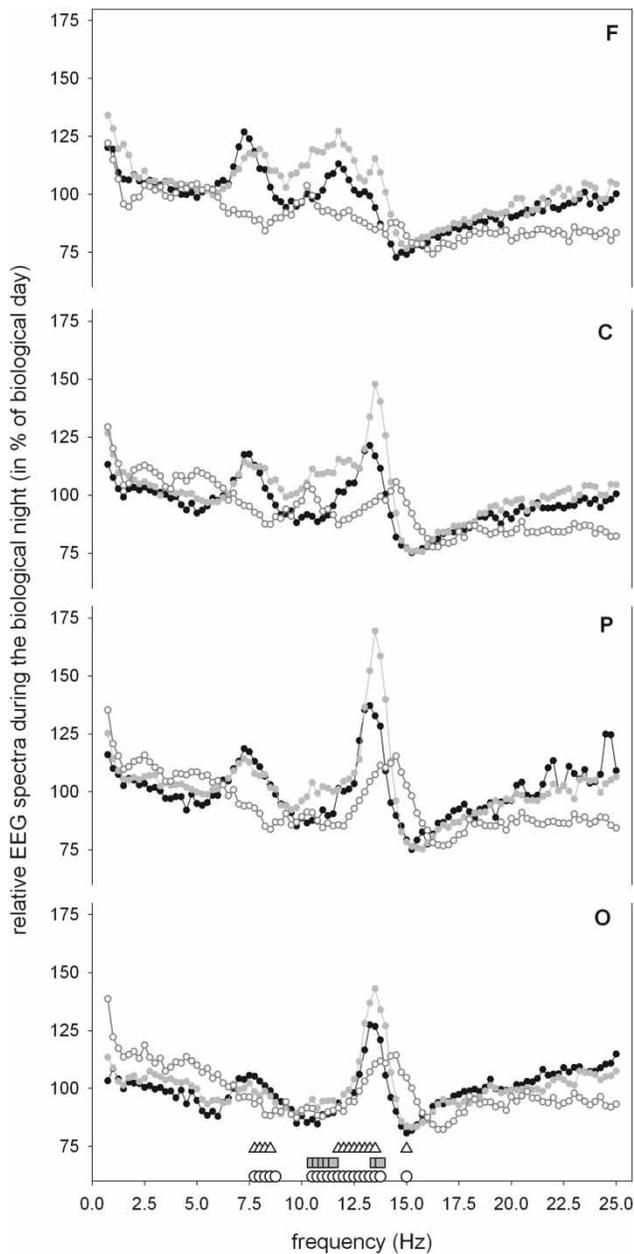


FIGURE 6 Relative EEG spectra during the biological night. The figure illustrates relative EEG spectra during the biological night as a percentage of the respective biological day values (all EEG spectra were analyzed during NREM sleep stage 2). Post hoc analysis on significant differences between the three groups are indicated near the abscissa (open black circles = HY vs. HO; shaded square = MDD vs. HY; open triangle up = MDD vs. HO; p at least $<.05$; LSMEANS statement, Tukey-Kramer adjusted). Young depressed women (MDD, $n=9$) = black filled circles; healthy young women (HY, $n=8$) = gray filled circles; healthy older women (HO, $n=8$) = gray open circles. F = frontal derivations, C = central derivations, P = parietal derivations, O = occipital derivations.

compared to HY (7.75–8.75 and 10.5–13.75 Hz) and MDD women (7.75–8.5 and 11.75–13.5 Hz), whereas in the 15-Hz frequency bin HO showed higher EEG values than the two young groups. Importantly, at 13.5 Hz there was a

significant difference between all three groups ($p < .05$). Furthermore, the interaction of the factors *group* \times *derivation* was significant in the frequency bins 1–3, 6–6.25, 8–9, 9.5, 10, 10.5–12.25, 13.5–13.75, and 15.25 Hz ($p < .05$; $F_{6,66} > 2.27$). Post hoc analysis on this interaction showed no significance; hence, group differences were not dependent on derivation, although they seem to be most pronounced in parietal derivations according to visual inspection of Figure 6.

Circadian Variables

Salivary melatonin, as a robust circadian rhythm marker, sampled every 30 min over the entire 40-h protocol was collapsed into 1.25-h bins (Figure 7, top panel). Mixed-model two-way rANOVA disclosed significant effects of the main factors *group* ($p < .05$; $F_{2,22} = 4.59$) and *session* ($p < .001$; $F_{21,462} = 29.61$), as well as of their interaction ($p < .001$; $F_{42,462} = 2.06$). Post hoc analysis showed significantly ($p < .05$) higher melatonin values of HY than HO women during two night sessions as indicated in Figure 7. No significant differences were observed between the MDD and the other two groups.

The time course of mean subjective sleepiness per group is illustrated in Figure 7 (lower panel). The ratings were derived from the Karolinska Sleepiness Scale (KSS), and the values were collapsed into 1.25-h bins as done with melatonin. Mixed-model two-way rANOVA disclosed significant effects of the main factors *group* ($p < .001$; $F_{2,22} = 14.07$), with significantly higher subjective sleepiness of MDD than the other two groups ($p < .01$). Furthermore, the analysis showed significance for the factor *session* ($p < .001$; $F_{21,462} = 20.28$), and a tendency to significance for the interaction *group* \times *session* ($p = .054$; $F_{42,462} = 1.40$).

Different phase markers of melatonin secretion over the 40-h protocol were calculated for each subject and subsequently averaged across groups (Table 2). The three groups differed significantly neither in the upward nor downward mean crossing time of melatonin ($p > .1$, t test for independent samples; $t = -.06$ and $.76$, respectively). Similarly, no significant group differences occurred with reference to the temporal midpoint of the melatonin peak, mean bedtime clock hours, and the circadian phase angles (expressed as differences between habitual bedtime and the upward or downward mean crossing time, respectively) ($p > .1$; $t = 1.18$, $.48$, and $.61$, respectively). The HY participants exhibited a higher mean amount of melatonin secreted between the upward and downward mean crossing times compared to HO women ($p < .05$; $t = 2.68$), and MDD women had a significantly longer duration of melatonin secretion between the upward and downward crossing times than HO women ($p < .05$; $t = 2.25$).

DISCUSSION

Our data indicate clear alterations of homeostatic sleep pressure in MDD compared to HY and HO

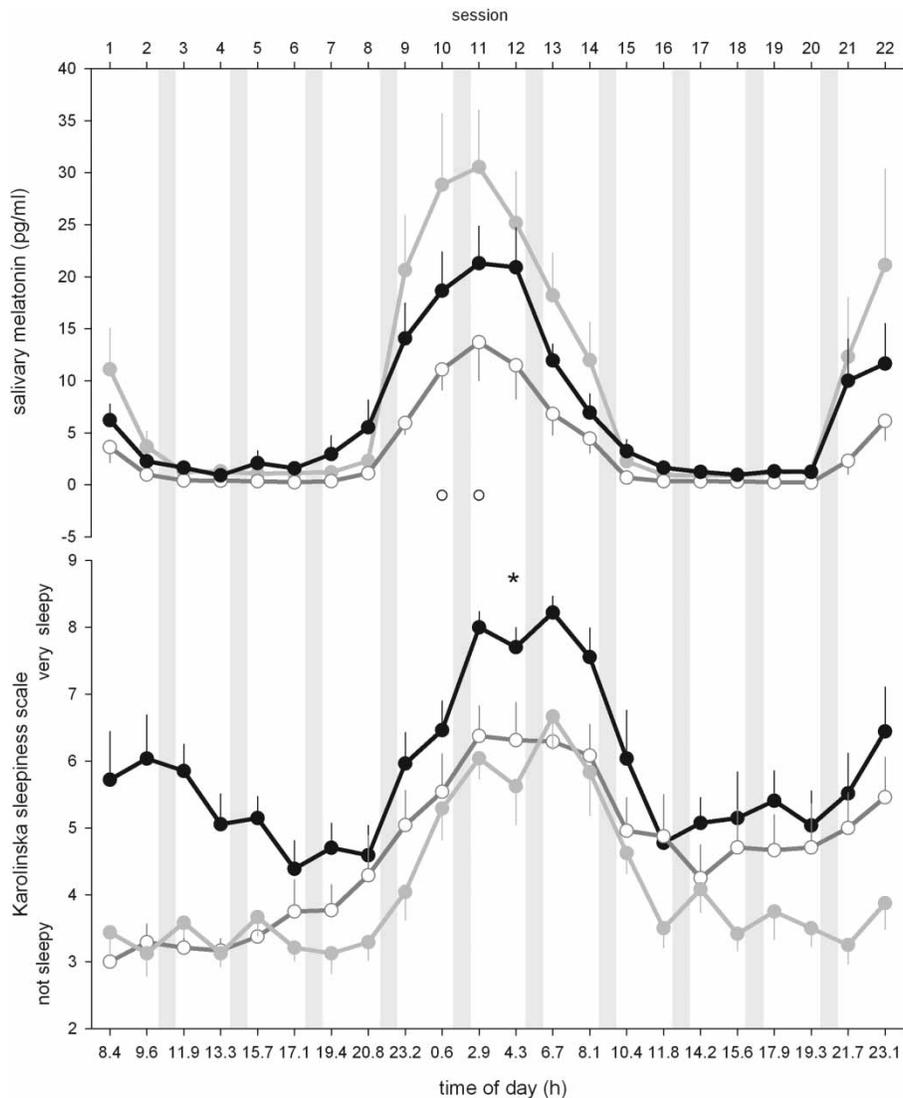


FIGURE 7 Mean salivary melatonin levels and subjective sleepiness ratings during the 40-h nap protocol. The top panel displays mean (\pm SEM) salivary melatonin values and subjective sleepiness ratings are displayed in the lower panel for healthy young (HY, gray filled circles; $n=8$), young depressed (MDD, black filled circles; $n=9$), and healthy older (HO, gray open circles; $n=8$) volunteers. Half-hourly values for both variables were binned in 1.25-h segments across the 40-h protocol. Significant post hoc comparisons of the interaction of the factors *group* \times *session* are indicated by small open circles at the bottom line of the top panel (open circle = HY > HO; p at least $<.05$; post hoc analysis based on LSMEANS statement with Tukey-Kramer adjustment of p values). MDD women were significantly sleepier during the entire protocol compared to HY and HO women (indicated with asterisks in the lower panel, $p < .01$, mixed-model two-way rANOVA). Gray bars indicate schematically the scheduled nap episodes.

women, as indexed by significantly higher frontal EEG delta activity across the baseline, recovery nights, and the 10 nap episodes along with more stage 4 sleep and significantly higher subjective sleepiness levels. These changes occurred with minimal changes in sleep architecture in the young depressed women. These results were rather unexpected, since a reduction in EEG delta activity and SWS along with more REM sleep, particularly at the beginning of the night, was previously reported in many studies (Armitage, 2007; Armitage & Hoffmann, 1997; Berger & Riemann, 1993). Furthermore, the MDD and HO women showed reduced nighttime melatonin levels and increased sleepiness levels compared to the HY women.

Sleep Stages and Homeostatic Sleep Regulation

Young depressed women (MDD) showed no significant differences in sleep architecture during the baseline and recovery nights compared to HY women, except for less stage 1 sleep (most likely, but not statistically significant, at the cost of more stage 4 sleep). Less stage 1 sleep indicates faster transition to higher NREM sleep stages in our depressed cohort. These results contrast commonly reported sleep pattern alterations associated with major depression, such as reduced SWS, shortened REM sleep latencies, and increased REM sleep (Benca et al., 1992; Buysse et al., 1990; Reynolds et al., 1982). However, it has been shown that changes in sleep architecture in depression depend on sex, age, depression subtype, and

TABLE 2. Melatonin timing, mean secretion, and phase angles for each group

Variable	Mean \pm SD			<i>t</i> test
	MDD	HY	HO	<i>p</i>
Melatonin upward mean crossing time (h; clock time)	22.15 \pm 1.44	22.19 \pm 1.39	21.83 \pm 1.51	n.s.
Melatonin downward mean crossing time (h; clock time)	8.47 \pm 1.50	8.01 \pm .99	7.11 \pm 1.67	n.s.
Midpoint of melatonin peak (h)	3.31 \pm 1.33	3.10 \pm 1.09	2.47 \pm 1.57	n.s.
Mean duration of melatonin secretion (h)*	10.32 \pm 1.25	9.82 \pm 1.03	9.29 \pm .55	** MDD > HO
Mean melatonin secretion (pg/mL)	18.43 \pm 10.01	25.83 \pm 14.93	10.69 \pm 6.2	** HY > HO
Mean bedtime (h; clock time)	23.89 \pm .96	23.61 \pm 1.35	23.41 \pm .86	n.s.
Phase angle 1 (h; bedtime upward mean crossing time)	1.74 \pm 1.34	1.43 \pm .70	1.58 \pm 1.28	n.s.
Phase angle 2 (h; bedtime downward mean crossing time)	8.58 \pm 1.54	8.40 \pm 1.35	7.71 \pm 1.33	n.s.

The table shows different characteristics of melatonin secretion for each group (mean \pm SD). Furthermore, the phase angle between external and internal circadian phase, expressed as the difference between habitual bedtime and upward or downward melatonin mean crossing time, respectively, was calculated.

MDD = young depressed (n = 9), HY = healthy young (n = 8), HO = healthy older (n = 8) women.

*With reference to secretion between the upward and downward mean crossing times; ***p* < .05 (*t* test for independent samples).

severity of illness; therefore, these findings cannot be generalized to all individuals with depression. In particular, it should be emphasized that our recruitment criteria for depressed women excluded individuals with major sleep problems, such as decreased SE or prolonged latency to sleep onset, in order to investigate rather the influence of depression per se on sleep-wake regulation and to allow for a more stringent comparison with the non-sleep-disturbed healthy control women. Hence, our results are only representative of a specific group of MDD patients, i. e., young moderately depressed women without sleep disturbances and without pharmacotherapy.

Our cohort of HY and HO women originated from previous studies where we found less TST, less stage 4 sleep, and lower SE in older women (Knoblauch et al., 2005; Münch et al., 2005, 2007). We now extend these findings by showing that young depressed (MDD) women differ similarly as young healthy women differ from healthy older women with reference to the aforementioned sleep parameters. However, unexpectedly, our MDD women had significantly more stage 4 sleep across the 10 nap episodes relative to baseline night values compared to the other two groups.

Commonly reported sleep pattern alterations in depression, such as reduced deep SWS, for example, have also been attributed to increased activity of the neural arousal system as described by the hyperarousal theory of depression (Van den Burg & Van den Hoofdaker, 1975). One often-mentioned mechanism for hyperarousal in depression is sustained activation of the hypothalamic-pituitary-adrenal (HPA) axis, leading to increase in corticotropin-releasing hormone (CRH) and cortisol, which in turn causes excessive wakefulness and sleep disturbances (Nestler et al., 2002). Paradoxically, we observed significantly higher mean subjective tension and, thus, arousal as assessed by visual analogue scale (data not shown), together with significantly higher subjective sleepiness across the 40-h nap protocol and more deep SWS (sleep stage 4) during the nap episodes in our

MDD participants. We concluded that these rather contradictory findings may be indicative for our particular sample of young moderately depressed women who experience either the first or second onset episode of MDD and reflect as such a first sign for elevated risk of developing sleep disturbances thereafter if the major depression disorder persists or aggravates in severity.

Two EEG markers of homeostatic sleep pressure have been described: frontal delta activity (.75–4.5 Hz) during NREM sleep and low-frequency EEG activity in frontal brain regions during wakefulness, which rises during wakefulness and parallels the sleep-wake-dependent increase of delta activity during subsequent sleep (Cajochen et al., 1995). We observed a significant relative decline of EEG delta activity in all three groups during the first NREM-REM sleep cycle of the recovery night. This result not only confirms previous findings on the effect of napping to diminish homeostatic sleep pressure during subsequent night-sleep episodes (Campbell & Feinberg, 2005; Werth et al., 1996), but it also extends them to women with major depression. The young depressed women had significantly higher absolute EEG delta activity compared to the other two groups across both baseline and recovery nights in frontal brain regions, whereas relative EEG spectra values (recovery night spectra expressed as a percentage of respective baseline values; data not shown) did not significantly differ. Moreover, our MDD women exhibited significantly higher delta sleep EEG activity compared to the HY women during naps occurring during the biological day (see Figure S1). Hence, we conclude that the more pronounced increase in EEG delta activity in young women with MDD reflects higher homeostatic sleep pressure, rather than an impairment in the homeostatic response to low sleep pressure conditions. Although this conclusion remains to be confirmed by analysis of the low-frequency EEG activity during wakefulness, the significantly higher subjective sleepiness ratings of our young depressed women compared to both other groups

provides further evidence for our conclusion, as a close correlation between these objective and subjective sleepiness parameters has been reported previously (Cajochen et al., 2001). It has been suggested recently that SWS and EEG slow-wave activity may play a crucial role in depression, as selective EEG slow-wave deprivation, without changing habitual sleep duration, improves mood in depressed volunteers and has been, therefore, associated with the known antidepressant effect of a night sleep deprivation (Landsness et al., 2011). There is also evidence that an enhancement of SWS potentially decreases positive mood in certain depressed patients while having a contrary effect in healthy controls (Cheng et al., 2010).

Different neurological aspects may contribute to higher homeostatic sleep pressure in depression. Recently, the neural correlates of rumination (recursive self-focused thinking) in depression have been identified through a functional magnetic resonance imaging (fMRI) study (Cooney et al., 2010). Depressed individuals showed higher activation in the medial and dorsolateral prefrontal cortex and in limbic structures during rumination than healthy controls. Although it is not clear yet whether the activation of the dorsolateral prefrontal cortex reflects impairment of neural regulatory mechanisms or neural recruitment towards cognitive demand, one may still speculate that during rumination additional synaptic potentiation and, hence, changes in cortical plasticity may occur. This provides a link to the synaptic homeostasis hypothesis of sleep. It is proposed that plastic changes during wakefulness leading to an increase of synaptic strength are strongly correlated to the amount of slow-wave activity during subsequent sleep, particularly in cortical areas (Tononi & Cirelli, 2003). Within the framework of this hypothesis, SWS serves to downscale synaptic strength to an energetically sustainable level. We can combine the cortical correlates of rumination, cortical activation during wakefulness, and its correlation with SWS to understand our results of higher frontal delta activity in depressed young women. Our depressed women may have higher rumination levels, but this was not measured. It remains to be studied whether rumination in depression may induce higher synaptic potentiation and subsequently lead to higher NREM sleep slow-wave activity.

From a molecular perspective, homeostatic sleep regulation has been suggested to be associated with the nucleoside *adenosine* (Landolt, 2008; Porkka-Heiskanen et al., 1997). Brain adenosine levels rise during prolonged wakefulness and decrease during sleep (Porkka-Heiskanen et al., 2000; Strecker et al., 2000). Caffeine, an adenosine antagonist, increases alertness and decreases sleepiness (Landolt et al., 1995). Furthermore, individuals with elevated adenosine levels due to a functional polymorphism in adenosine deaminase show increased levels of SWS and less nocturnal awakenings (Reitey et al., 2005). This argument suggests that the increased level of SWS and EEG slow-wave activity in our cohort

of young depressed women could be a result of increased adenosine levels. So far, studies on adenosine deaminase levels in depression have shown both increased and decreased levels (Elgun et al., 1999; Herken et al., 2007).

The HY women differed from the other two groups of women with respect to the ultradian slow-wave activity regulation across NREM-REM cycles during the recovery night, such that they exhibited a significant intrasleep rebound of EEG slow-wave activity during the NREM sleep episode 3 compared to prior NREM sleep episodes, which was not present in the MDD and HO women. The resurgence of EEG slow-wave activity during the later parts of the sleep episodes have been observed either after selective SWS deprivation or during extended sleep episodes of up to 14.9 h (Dijk & Beersma, 1989; Dijk et al., 1991). Whereas in the former case SWS rebound occurred because homeostatic sleep pressure was still present from prior wakefulness, the intrasleep rebound in connection with prolonged sleep duration has been suggested to represent a circasemidian sleep-dependent rhythm (Broughton, 1988; Broughton & Mullington, 1992). As neither of these explanations serves to explain the observed slow-wave activity intrasleep rebound in HY women in our study, it remains unclear whether it reflects a sleep-wake-dependent (homeostatic) process or if, due to the low sleep pressure conditions, it is a response to a sleep-promoting signal of the circadian pacemaker around the sleep maintenance zone.

Circadian Sleep-Wake Modulation

The distribution of the 10 nap episodes over a time period of 40 h allowed sleep to occur at different circadian phases, thus resulting in significantly varying SE and TST during the naps. This circadian control of sleep duration is in accordance with the two-process model of sleep regulation and previous study findings (Borbély, 1982; Czeisler et al., 1980; Zulley et al., 1981).

Whereas it has been demonstrated that EEG slow-wave activity is distinctively dependent on the prior history of sleep and wakefulness, the modulation of sleep spindles (12–15 Hz) is mainly under circadian control, with high values during the early subjective night and low values in the early morning (Dijk & Czeisler, 1995). Furthermore, a melatonin-related shift of spindle frequency peaks has been shown in healthy young subjects, such that during the biological day when melatonin secretion is low spindle peaks occur at higher frequencies than during the biological night (Dijk et al., 1995; Knoblauch et al., 2002, 2003). This spindle modulation between the biological day and night was clearly reflected in the HY women by both a significant low (12.25–13.75 Hz) and a significant high (14.75–15.25 Hz) spindle peak difference. This circadian modulation of spindle frequencies was less pronounced in the young MDD women, as differences between biological day and night were only significant in the high spindle frequencies (14.75–15.25 Hz). In HO women, no significant circadian modulation was

observed. Correspondingly, the relative nocturnal increase in low spindle frequencies was significantly higher in HY than young MDD (13.5–13.75 Hz) and HO (12–13.75 Hz) women. Furthermore, the nocturnal decrease in the higher spindle frequency range (15 Hz) was not only significantly less intense in HO compared to HY women, but it was also significantly less pronounced compared to young MDD women. The lack of a circadian modulation of sleep spindles between the biological night and day together with significant lower values of mean melatonin secretion in HO compared to HY women during the biological night have been reported to add evidence for a weaker circadian signal in sleep-wake regulation (Münch et al., 2005). Our young depressed subjects thereby appear to display some of these aspects observed in healthy aging, but to a lesser degree, as the circadian modulation of spindle peaks seemed to be partially intact and melatonin secretion only shows a tendency to lower values compared to age-matched healthy individuals.

The internal coincidence hypothesis states that sleeping at the wrong circadian phase is depressogenic (Wehr et al., 1979). Although this hypothesis was based on the assumption that the circadian clock is advanced compared to sleep timing, more recent studies rather suggested a delayed circadian phase of dim-light melatonin onset relative to sleep timing to be involved in the modulation of depressive symptomatology (Emens et al., 2009). However, a recent study observed no significant difference between external and internal circadian phase as expressed by the difference between midsleep time and core body temperature minimum and dim-light melatonin onset respectively in depressive patients compared to healthy individuals (Hasler et al., 2010). Similarly, we found no phase advance or delay of the rhythm of melatonin (upward and downward mean crossing times) relative to habitual sleep time in young depressed women. Mean bed- and wake-up times also did not differ between the three groups. The only finding was a significant longer melatonin secretion duration compared to healthy older women, indicating a different circadian coding for the duration of the biological night. Taken together, the observed changes in sleep of young depressed women was most likely not due to a circadian misalignment of sleep-wake timing with respect to the endogenous circadian pacemaker.

Our results provide strong evidence that young moderately depressed women live on a higher homeostatic sleep pressure. Hence, our data on SWS and EEG slow-wave activity do not support a homeostatic deficiency in young depressed women without sleep problems as proposed by the S-deficiency hypothesis for depressed patients with sleep problems (Borbély, 1987; Borbély & Wirz-Justice, 1982). Our data clearly show dissimilar homeostatic sleep-wake regulation in depression than in healthy aging. Furthermore, the observed sleep-wake alterations in depression could not be related to a circadian misalignment of sleep-wake timing and the endogenous pacemaker. Although our data do not fully

highlight alterations of circadian processes involved in sleep-wake regulation, the less pronounced modulation of the spindle frequency peaks between biological day and night and reduced nighttime melatonin secretion suggest changes in the strength of the circadian output signal in depression. Along these lines, higher levels of sleepiness and EEG slow-wave activity during the biological day could reflect an imbalance of the opponent interaction between the circadian and homeostatic processes in depression, such that a weaker circadian output signal leads to an overexpression of sleep-wake homeostatic influences, which may be probably further boosted by increased rumination by the depressed women as discussed above.

Our study provides first insights into homeostatic and circadian sleep-wake regulation in young women with major depression under low sleep pressure conditions to unmask the circadian component. Contrary to our hypothesis, they showed higher sleep pressure in all sleep episodes. A parallel investigation on an independent group of young depressed women studied under high sleep pressure in a constant routine protocol also revealed higher sleep pressure before and after sleep deprivation (unpublished data), providing a confirmation of our findings.

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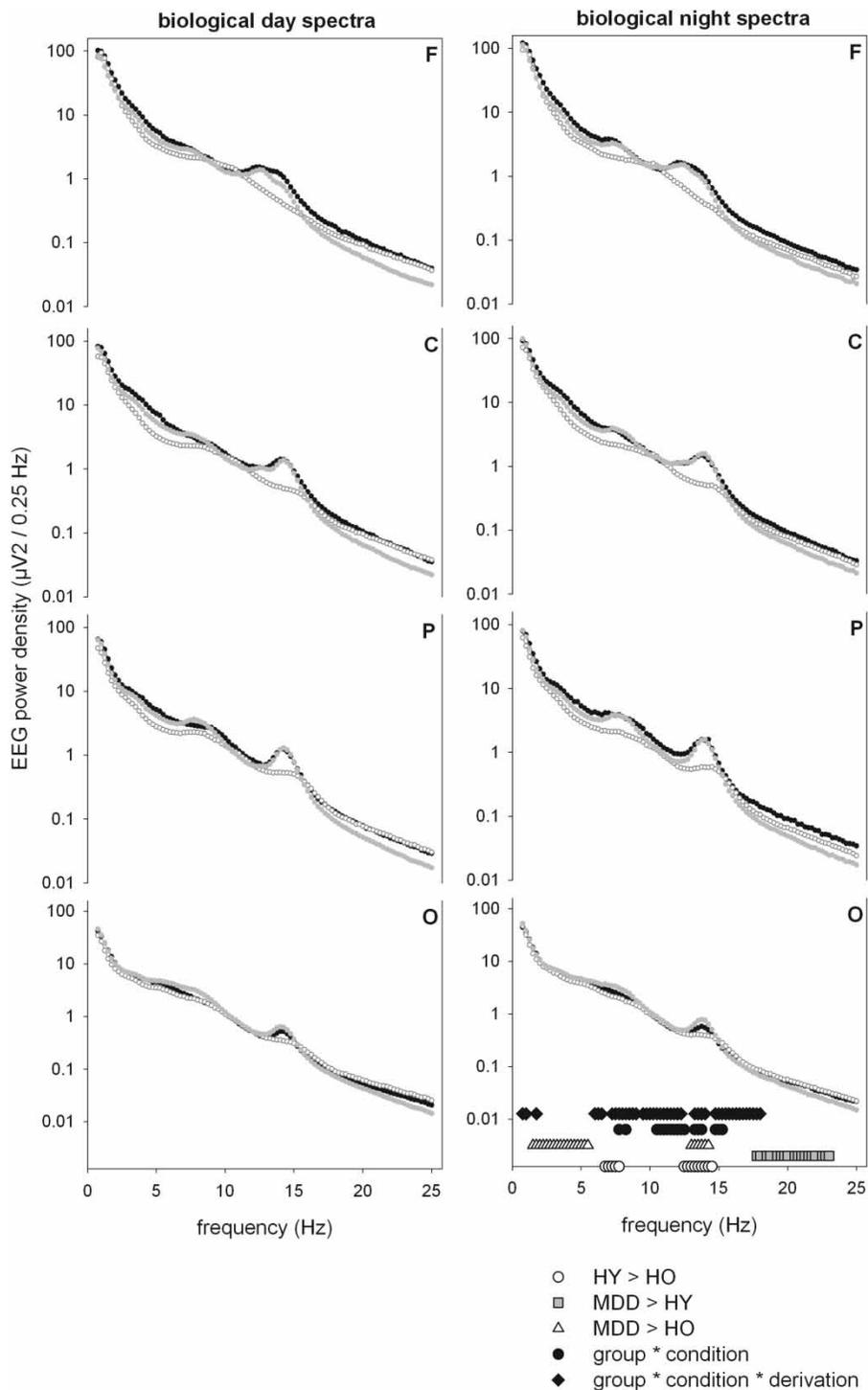


FIGURE S1 EEG power spectra for the nap-sleep episodes during the biological night and day. Absolute biological day and biological night EEG sleep spectra are derived from NREM stage 2 nap-sleep episodes. Spectra are shown in the frequency range .75–25 Hz for lateral and central collapsed F, C, P, and O derivations for young depressed (MDD; black filled circles; $n = 9$), healthy young (HY; gray filled circles; $n = 8$), and healthy older (HO; gray open circles; $n = 8$) women. Post hoc analysis on significant differences between the groups, significant interactions between biological day and night and the groups, and significant interactions of the factors *group* \times *condition* \times *derivation* are indicated near the abscissa (p at least $<.05$). Post-hoc analysis on the 3-way interaction showed significant higher diurnal spectra values in MDD than in HY for the delta bin 1.75 Hz for the frontal derivations ($p < .01$; LSMEANS statement, Tukey-Kramer adjusted). Furthermore, the analysis of the interaction *group* \times *derivation* (data not shown) disclosed significant higher spectra values for the frontal derivations for MDD than HY (frequency bins: 1.75–2.25 Hz; $p < .01$; 17.5–20 Hz and 20.5–20.75 Hz; $p < .05$) and HO (frequency bins: 2–4.25 Hz and 11.75–13.25 Hz; $p < .05$).