Sexually Dimorphic Effects of GHRH on Sleep-Endocrine Activity in Patients with Depression and Normal Controls-Part I: The Sleep EEG

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In patients with depression, enhanced secretion of ACTH and cortisol, a reduction in slow wave sleep (SWS) and a blunted nocturnal growth hormone (GH) surge have been described and attributed, at least partly, to an elevation of corticotropin-releasing hormone (CRH), hence a shift in the ratio between growth hormone-releasing hormone (GHRH) and CRH. We investigated the effects of pulsatile administration of GHRH (4x50 µg, at hourly intervals between 2200 and 0100 h) on the sleep EEG (2300-0700 h) in patients with depression (16 females, 19 males, age range 19-76 years) and matched controls (20 females, 20 males). In patients compared with controls, NREM sleep and in particular stage 2 sleep was greatly decreased at baseline. GHRH treatment enhanced NREM sleep, and in particular stage 2 sleep in men, regardless of diagnosis, while decreasing it in women (F=6.0 and 7.1, p<0.05). In controls, aging was associated with a decrease in NREM sleep, including both SWS and stage 2 sleep (r = -0.45, r=-0.39, p<0.05), while in patients only SWS declined with age (r=-0.49, p<0.05). The significant decrease in NREM sleep including stage 2 sleep in patients with depression and elderly control subjects is compatible with the suggested role of sleep continuity and stage 2 sleep in cognitive functioning. GHRH promoted NREM sleep, stage 2 sleep and sleep continuity and might prove beneficial for improvement of cognitive function, at least in men. These data support the hypothesis that female gender, aging and depression are associated with a shift in the GHRH/CRH ratio towards CRH.

CURRENT CLAIM: Effects of GHRH on sleep regulation are sexually dimorphic.

A major depressive episode is characterized by sleep-endocrine changes, including an increase in time awake and REM density, as well as a reduction in slow wave sleep (SWS) and REM latency (Jarrett et al., 1985; Reynolds and Kupfer, 1987; Steiger et al., 1989, 1994). Hyperactivity of the hypothalamo-pituitary-adrenocortical (HPA) system, including an elevation in central CRH release (Nemeroff, 1988) and elevated plasma levels of cortisol and ACTH (Linkowski et al., 1987; Rubin et al., 1987; Holsboer and Barden, 1996), are observed in depression. Since the ratio between growth-hormone-releasing hormone (GHRH) and CRH is thought to play a critical role for sleep-endocrine regulation (Ehlers and Kupfer, 1987), a shift in that ratio in favor of CRH might contribute to sleep-endocrine changes in depression (Ehlers and Kupfer, 1987; Steiger and Holsboer, 1997). Thus, CRH reduces delta EEG activity in rats (Ehlers et al., 1986; Opp, 1995) and SWS and the nocturnal GH surge in humans (Holsboer et al., 1988), while GHRH has opposite effects, namely a promotion of SWS and the sleep-associated GH surge in animals (Ehlers, 1986; Obá Jr., et al., 1988, 1992) and humans (Steiger et al., 1992; Kerkhofs et al., 1993).

Aging is associated with changes of NREM sleep similar to those seen in depression, including a disruption of sleep continuity and a decrease in SWS, while REM sleep is not markedly affected (Van Coevorden et al., 1991; Lauer et al., 1991; Bliwise, 1993; Hoch et al., 1994). Reduced efficacy of GHRH, possibly involving enhanced somatostatin release and subsequently a shift in the GHRH/CRH ratio in favor of CRH, has been proposed to account for these alterations (Shibasaki, 1984; Ghigo et al., 1990; Van Coevorden et al., 1991).

Since the SWS-promoting effect of GHRH was attenuated in a small group of patients with depression (Steiger et al., 1994) and elderly controls (Guldner et al., 1997), these observations suggest that hyperactivity of the HPA system in depression and the age-related decline in somatotropic activity might curtail stimulatory effects of GHRH on SWS (Steiger, 1995).

Recently, we observed following GHRH administration a sexually dimorphic effect in young normal controls, namely in females unlike in males, a greater increase in sigma than delta EEG activity (Antonijevic et al., 1998). These findings are in agreement with previous studies in rats and humans showing higher sigma EEG activity in females (Gaillard and Blois, 1981; Dijk, 1989; Ehlers et al., 1993). Since animal studies have demonstrated a sexually dimorphic pattern of hypothalamic CRH release (Hiroshige et al., 1973), we have proposed that in women, compared to men, CRH release is greater at the beginning of the night and could contribute to the observed gender differences. Support for this hypothesis is derived from human (Antonijevic et al., 1998a) and animal studies (Ehlers et al., 1986) demonstrating that CRH can promote sigma EEG activity.

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Here, we extended our previous studies and further investigated the effects of GHRH on sleep-endocrine regulation in patients with depression, with a focus on the influence of age and gender in patients with depression and matched controls.

**METHODS**

**Subjects**

The study was approved by the Ethics Committee for Human Experiments of the Max Planck Institute of Psychiatry. Written informed consent was obtained from 35 patients with depression (16 females, 19 males) and 40 age- and sex-matched controls (20 females and 20 males). All patients scored above 16 points on the Hamilton Depression Scale (HAMD, 21-item version [American Psychiatric Association, 1987]) in the morning of the first study night. All subjects were of normal height and weight and underwent a rigid medical examination including extensive psychiatric, physical and laboratory investigations (hematology, virology, clinical chemistry, endocrinology, EEG and electrocardiography).

**Control Subjects**

In all subjects any personal or family history of psychiatric disorders, as well as any medical treatment during the past three months, were ruled out in a lengthy interview by a senior psychiatrist. Subjects who reported sleep disturbances or showed signs of sleep apnea or periodic limb movements were excluded.

**Patients**

All patients have been hospitalized for evaluation and treatment of depression. At initial evaluation, all met criteria for major depression according to DSM-III-R (American Psychiatric Association, 1987) with a HAMD score ≥16. The diagnosis was established and previous secondary and comorbid diagnosis were ruled out in an examination conducted by a senior psychiatrist. The patients have not been treated with depot neuroleptics, fluoxetine and irreversible monoamineoxidase-inhibitors for at least eight weeks prior to admission and all patients were drug-free for a minimum of one week prior to the study.

For both patients and controls, subjects who were shift workers and persons who had made a transmeridian flight within the last three months were excluded. Also, abuse of drugs, nicotine, alcohol and caffeine was ruled out.

Of female patients and controls, none were taking oral contraceptives. Nine of the 20 female controls and seven of 16 female patients were peri- or postmenopausal, and none of the female patients and controls were on hormone replacement therapy. Premenopausal patients and controls were not matched with regard to the menstrual cycle, but most recordings were performed during the follicular phase and no recordings were performed during menstruation.

**Study Design**

Each subject spent three successive nights in the sleep laboratory. The first night served as adaptation to the laboratory setting. During the second and third nights (first and second recording nights) the sleep EEG was recorded. During the recording nights, GHRH or placebo was administered at hourly intervals between 2200 h and 0100 h (50 µg GHRH [Clinalfa, Läufelfingen, Switzerland] or 5 ml saline) through an indwelling intravenous catheter connected to plastic tubing that ran through a soundproof lock into the adjacent room. This allowed drug administration and repeated blood sampling in the adjacent laboratory without disturbing the subject’s sleep. The administration of GHRH or saline was randomized. Sleep was allowed between 2300–0700 h, when lights were turned off.

Blood samples were collected every 30 min between 2000 h and 2200 h and every 20 min between 2200 h and 0700 h. Specimens collected before 2200 h served as controls for stress effects after cannulation (1930 h); specimen collected between 2200 h and 0700 h were included in the time-course analysis. No food was permitted during the study until subjects were awakened at 0700 h the next morning.

Some of the hormone data were published separately (Antonijevic et al., 1998b). The majority of the hormone analysis is presented as Part II in this journal.

**Sleep EEG Recording**

Polysomnographic sleep recordings were obtained from 2300–0700 h and consisted of two EEGs (C3-A2, C4-A1; time constant 0.3 sec, low-pass filtering 70 Hz), vertical and horizontal electrooculograms (EOG), an electromyogram (EMG) and an electrocardiogram. The EOG, EEG and EMG signals were filtered (EEG: high pass 0.53 Hz, -3 dB; low pass 70 Hz, -3 dB; -12 dB octave, band-stop between 42 and 62 Hz, -3 dB) and transmitted by an optical fiber system to the polygraph (Schwartzer, ED 24), recording the sleep EEG for visual scoring. By means of a personal computer, EEG signals were additionally sampled by an eight-bit analogue-to-digital converter and stored on disk for further spectral analysis as described elsewhere (Fries et al., 1995). Briefly, spectral EEG analysis was performed for NREM sleep only (Stage 2, 3 and 4 sleep combined). With regard to artifacts, 30 sec epochs were inspected and artifacts removed if necessary. On average 3-5 artifacts for each 8-hour recording had to be removed, with no more than one artifact per hour in any EEG analysis.

Sleep stages (wakefulness, stage 1 to 4 sleep, stage 2, 3 and 4=NREM sleep, stage 3 and 4 sleep=SWS, REM sleep) were scored visually in all subjects off-line according to conventional criteria (Rechtschaffen and Kales, 1968) by a rater who was unaware of the treatment. All sleep parameters were related to sleep period time (SPT=lasting from sleep onset [first 30 sec epoch containing stage 2, 3 or 4 sleep after "lights off"] to final awakening). Sleep parameters analyzed included SPT, time awake (time spent awake during SPT), the number of awakenings, sleep onset latency (time between
"lights off" and the first occurrence of stage 2 sleep), sleep efficiency index (time asleep/SPT), REM latency (time span between the first occurrence of stage 2 sleep and the first epoch of REM sleep), REM density and the amount of time spent in REM sleep, stage 2 and NREM sleep and SWS during the total night as well as the first and second half of the night. For statistical analysis the time spent in REM sleep, stage 1, 2, 3 and 4 sleep was calculated as percent from SPT.

With regard to EEG spectral analysis, we restricted statistical analysis to delta and sigma EEG activity, and included the ratio between delta and sigma activity (DS ratio) as well as log-transformed DS ratios. The DS ratio was computed to eliminate confounding effects due to differences in input resistance and hence improve between-subjects comparison (Antonijevic et al., 1998). Thus, women had generally higher EEG values than men (Tables 2 & 3) and gender differences in EEG activity have been attributed to a thinner skull thickness and hence lower input resistance in women (Dijk et al., 1989; Armitage, 1995). The DS ratio was chosen, since delta and sigma activity are physiologically related and show an inverse relationship for most of each NREM sleep period: delta-EEG activity is observed when thalamocortical neurones are maximally hyperpolarized, involving GABA-B receptors, while sigma-EEG activity is seen when cells are less hyperpolarized and involve mediation by GABA-A receptors (Steriade et al., 1991; Juhasz et al., 1991, 1994; Nunez et al., 1992).

Due to technical problems with the EEG-recording system, not all sleep-EEG recordings could be analyzed, hence the number of sleep-EEGs included in the statistical analysis is smaller than the number of hormone profiles [see Part II].

Statistical Analysis

Statistical analysis was performed using MANOVA with repeated measures design to examine effects of GHRH treatment (within-subject factor). Diagnosis (patients with depression vs. controls), gender and night of active treatment (first vs. second recording night) were included as between-subject factors. The night of active treatment was included to ensure detection of a possible carry-over effect of GHRH treatment. By significant main or interaction effects of the factors, univariate F-tests followed (D.F. 1, 65) in order to

Table 1A
Conventional Sleep-EEG Analysis-Entire Night During the Placebo Condition

<table>
<thead>
<tr>
<th>Sleep parameter (min or %SPT)</th>
<th>male controls</th>
<th>female controls</th>
<th>male patients</th>
<th>female patients</th>
<th>Gender F value</th>
<th>Diagnosis F value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sleep period time</td>
<td>432.9±11.8</td>
<td>443.3±7.0</td>
<td>413.7±13.6</td>
<td>415.4±20.9</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>Sleep latency</td>
<td>30.6±5.5</td>
<td>19.9±3.0</td>
<td>38.6±10.4</td>
<td>45.9±16.6</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>Sleep efficiency</td>
<td>92.3±2.1</td>
<td>92.8±1.3</td>
<td>76.1±3.9</td>
<td>89.4±3.8</td>
<td>n.s.</td>
<td>F=14.1</td>
</tr>
<tr>
<td>No. awakenings</td>
<td>22±2.4</td>
<td>16.4±2.0</td>
<td>27±3.5</td>
<td>28.1±8.9</td>
<td>n.s.</td>
<td>F=9.1</td>
</tr>
<tr>
<td>Time awake</td>
<td>35.1±7.2</td>
<td>32.6±5.2</td>
<td>98.7±14.1</td>
<td>50.0±17.0</td>
<td>F=4.8</td>
<td>F=12.9</td>
</tr>
<tr>
<td>REM latency</td>
<td>78.3±6.6</td>
<td>65.8±5.9</td>
<td>52.8±12.4</td>
<td>60.7±18.4</td>
<td>n.s.</td>
<td>F=10.1</td>
</tr>
<tr>
<td>REM density</td>
<td>2.1±0.2</td>
<td>2.1±0.1</td>
<td>2.7±0.2</td>
<td>2.9±0.3</td>
<td>n.s.</td>
<td>F=10.6</td>
</tr>
<tr>
<td>REM time</td>
<td>79.6±5.8</td>
<td>84.3±3.5</td>
<td>73.4±9.9</td>
<td>77.8±7.3</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>Stage 1 sleep</td>
<td>38.0±4.4</td>
<td>29.4±3.2</td>
<td>42.4±4.7</td>
<td>33.2±7.2</td>
<td>F=7.5</td>
<td>n.s.</td>
</tr>
<tr>
<td>Stage 2 sleep</td>
<td>246.4±11.6</td>
<td>258.0±6.4</td>
<td>178.1±12.2</td>
<td>208.5±14.3</td>
<td>n.s.</td>
<td>F=16.3</td>
</tr>
<tr>
<td>SWS</td>
<td>31.5±7.3</td>
<td>33.1±4.8</td>
<td>16.9±4.9</td>
<td>41.4±9.1</td>
<td>F=9.9</td>
<td>n.s.</td>
</tr>
<tr>
<td>NREM sleep</td>
<td>277.8±12.5</td>
<td>291.1±7.3</td>
<td>195.0±13.1</td>
<td>249.8±20.1</td>
<td>F=7.9</td>
<td>F=24.3</td>
</tr>
<tr>
<td>SWS (%)</td>
<td>7.1±1.6</td>
<td>7.4±1.1</td>
<td>4.1±1.5</td>
<td>9.7±2.1</td>
<td>F=9.8</td>
<td>n.s.</td>
</tr>
<tr>
<td>NREM (%)</td>
<td>63.9±2.1</td>
<td>65.8±1.5</td>
<td>47.1±2.8</td>
<td>60.5±3.9</td>
<td>F=7.8</td>
<td>F=22.4</td>
</tr>
</tbody>
</table>

Values are means±SEM. F value according to MANOVA (D.F. 1, 65) is indicated for p<0.05; n.s.=not significant.

Table 1B
Conventional Sleep-EEG Analysis-First and Second Half of the Night During the Placebo Condition

<table>
<thead>
<tr>
<th>Sleep parameter (min or %SPT)</th>
<th>male controls</th>
<th>female controls</th>
<th>male patients</th>
<th>female patients</th>
<th>Gender F value</th>
<th>Diagnosis F value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SWS - 1st half</td>
<td>24.3±5.4</td>
<td>31.3±4.4</td>
<td>14.3±4.3</td>
<td>31.4±6.5</td>
<td>F=15.0</td>
<td>n.s.</td>
</tr>
<tr>
<td>SWS - 2nd half</td>
<td>7.2±2.3</td>
<td>1.8±0.8</td>
<td>2.6±1.1</td>
<td>10.0±4.4</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>Stage 2 sleep-1st half</td>
<td>129.7±7.2</td>
<td>133.8±5.2</td>
<td>97.8±8.5</td>
<td>101.0±9.7</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>Stage 2 sleep-2nd half</td>
<td>116.4±6.6</td>
<td>124.0±5.1</td>
<td>80.1±8.9</td>
<td>107.4±8.4</td>
<td>F=5.2</td>
<td>F=20.1</td>
</tr>
<tr>
<td>NREM (%) -1st half</td>
<td>158.9±7.4</td>
<td>167.1±4.6</td>
<td>121.6±7.4</td>
<td>140.5±12.7</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>NREM (%) -2nd half</td>
<td>119.0±6.5</td>
<td>124.0±4.1</td>
<td>73.4±7.9</td>
<td>109.3±9.2</td>
<td>F=6.5</td>
<td>F=22.4</td>
</tr>
</tbody>
</table>

Values are means±SEM. F value according to MANOVA (D.F. 1, 65) is indicated for p<0.05; n.s.=not significant.
identify the parameters which contributed significantly to the effect. Furthermore, age was included as covariate. Upon significant influence of age, correlation analysis with the SPSS for Windows system, using Pearson’s product-moment correlation two-tailed, was performed for patients and controls separately to estimate linear relationships between age the respective variable.

A value for \( p \leq 0.05 \) was considered significant; for \( p > 0.1 \) data were considered to reflect a trend for a significant difference. In order to keep the Type I error less than 0.05, posteriori tests (\( F \)-tests) were carried out at a reduced level of significance (adjusted alpha according to the Bonferroni procedure). All data are expressed as means±SEM.

**RESULTS**

**Demographic Data**

Patients and controls did not differ in age (male controls: 37.7±3.1 years [range 22-63], female controls: 42.5±4.2 years [range 19-73], male patients: 42.6±3.7 years [range 19-72] and female patients: 46.9±4.6 years [range 25-76]). Male and female patients were very similar with regard to the HAMD Score (male patients: 25.5±1.2 [range 17-40], female patients: 25.5±1.4 [range 18-41]). In twelve of 19 male and nine of 16 female patients the current depressive episode was the first episode; the number of previous episodes in the other patients ranged between one and four. Two male patients were diagnosed with bipolar disorder, while all other patients were classified as unipolar depressed patients.

**Visual Sleep-EEG Analysis**

No significant effect of the night of active treatment (pulsatile administration of GHRH during either the first or second recording night) was observed for conventional sleep-EEG parameters. Therefore, this factor is not indicated in the subsequent results (though it was included in the statistical analysis).

**Effect of Diagnosis**

Depression was associated with significantly more awakenings \( (F=9.1, p<0.05, \text{Table 1A}) \), a reduced sleep efficiency index \( (F=14.1, p<0.05) \), a shorter REM latency \( (F=10.1, p<0.05) \) and a higher REM density \( (F=10.6, p<0.05) \). In contrast, sleep period time (SPT), sleep latency and SWS duration were not significantly affected by diagnosis.

All further statistical analysis of the sleep EEG was based on sleep stages expressed as percent from SPT. Depression was associated with a significantly longer time spent awake \( (F=12.7, p<0.05) \), a highly significant reduction in stage 2 sleep for the entire and the second half of the night \( (F=16.3 \text{ and } 20.1, \text{ respectively, each } p<0.01, \text{ Tables 1A & B}) \) and a reduction in stage 2, 3 and 4 sleep combined (NREM sleep) during the entire and second half of the night \( (F=22.4 \text{ and } 22.4, \text{ respectively, each } p<0.05) \). With regard to SWS duration, there was a significant gender x diagnosis interaction with female controls spending less, while female patients spent more time in SWS during the second half of the night than the corresponding male groups \( (F=6.7, \text{ respectively, each } p<0.05) \). No correlation between the HAMD score and any of the above sleep-EEG parameters was noted.

**Effect of Gender**

During the entire night, females compared to males spent, regardless of diagnosis, significantly less time awake \( (F=4.8, p<0.05) \), corresponding to less intermittent awakenings, \( F=6.0, p<0.05, \text{ Table 1A}) \) and in stage 1 sleep \( (F=7.5, p<0.05, \text{ Table 1A}) \), but more time in SWS \( (F=9.8, p<0.05) \). For stage 1 sleep and SWS the gender differences was even more pronounced during the first half of the night \( (F=19.1 \text{ and } 15.0, \text{ respectively, each } p<0.01, \text{ Table 1B}) \). Correspondingly, females spent, regardless of diagnosis, more time in NREM sleep compared with males during the entire and the second half of the night \( (F=7.8 \text{ and } 6.5, \text{ each } p<0.05, \text{ Tables 1A & B}) \) and more time in stage 2 sleep during the second half of the night \( (F=5.2, p<0.05, \text{ Table 1B}) \).

**Figure 1: Effects of GHRH on the sleep EEG - significant gender x treatment interaction.** Following administration of GHRH a significant gender x treatment interaction was observed for sleep efficiency, time awake, stage 2 sleep and NREM sleep; thus, in males sleep efficiency, stage 2 and NREM sleep increased following GHRH; but decreased in women, regardless of diagnosis \( (F=6.5, 7.1 \text{ and } 6.0, \text{ respectively, each } p<0.05) \). Also, GHRH led to a decrease in time awake in men, but an increase in women, regardless of diagnosis \( (F=3.8, p<0.05) \). Bars indicate the percent change (means±SEM) during GHRH treatment compared to placebo for females (black bars) and males (hatched bars).
Effect of Treatment

Treatment, per se, had no significant effect on the variables assessed, while a significant treatment x gender interaction for time awake ($F=3.8$, $p<0.05$, Figure 1), the sleep efficiency index ($F=6.5$, $p<0.05$), stage 2 sleep ($F=7.1$, $p<0.05$) and NREM sleep ($F=6.0$, $p<0.05$) was noted: in men, regardless of diagnosis, GHRH treatment reduced time awake and enhanced sleep efficiency, stage 2 and NREM sleep. In contrast, in women, time awake was increased, while sleep efficiency, stage 2 and NREM sleep were decreased by GHRH (Figure 1).

Effect of Aging

Age was positively correlated with time awake ($F=9.6$, $t=3.1$) and number of awakenings ($F=12.5$, $t=3.5$) and negatively with sleep efficiency ($F=17.3$, $t=-4.2$), SWS ($F=27.5$, $t=-5.2$), particularly during the first half of the night ($F=33.6$, $t=-2.1$), stage 2 sleep during the second half of the night ($F=8.3$, $t=-2.9$) and NREM sleep during the entire night, as well as the first and second half ($F=21.1$, $23.0$ and $8.7$, respectively, $t=-4.6$, $-4.8$ and $-3.0$).

Upon closer analysis of the baseline (placebo) condition, the positive correlation with age for time awake and the number of awakenings was only noted in controls ($r=0.44$ and $r=0.31$, each $p<0.05$), but not in patients ($r=0.10$ and $r=0.33$, respectively). Also, sleep efficiency, stage 2 sleep in the second half of the night and NREM sleep during the entire night were inversely related to age only in controls ($r=-0.45$, $r=-0.39$ and $r=-0.41$, respectively, each $p<0.01$), but not in patients ($r=-0.04$, $r=-0.05$ and $r=-0.24$, respectively, Figure 2). SWS was inversely related to age in both patients ($r=-0.49$, $p<0.01$) and controls ($r=-0.45$, $p<0.01$, Figure 2).
Spectral EEG Analysis

Effect of Diagnosis
For the DS ratio during the entire night, we observed a significant diagnosis x gender interaction ($F=4.4$, $p<0.05$, Table 2) with female gender being associated with a smaller DS ratio in controls, but a greater DS ratio in patients. The DS ratio was significantly reduced in the first half of the night (but not in the second half of the night) in patients compared to controls ($F=4.5$, $p<0.05$, Table 3). Using log-transformation of the DS ratio, the diagnosis x gender interaction was marginally significant for the entire night and the first half of the night ($F=2.4$ and $F=2.7$, each $p<0.1$, Tables 2 and 3). Similarly, a marginally significant diagnosis x gender interaction was observed for the log-transformed DS$_{14-16}$ Hz ratio ($F=3.6$, $p<0.1$, Table 2). No correlation between the HAMD score and sleep-EEG spectra was noted.

Effect of Gender
As expected, female gender was associated with significantly higher EEG values for the delta and sigma range (Table 2).

Effect of Treatment
No effect of GHRH treatment on sleep-EEG spectra was observed.

Effect of Aging
In the group of all subjects, age was inversely correlated with delta EEG activity ($F=12.4$, $p<0.01$) and sigma activity ($F=10.6$, $p<0.01$) during the entire night. Closer analysis revealed that during the baseline condition, only in controls but not in patients, there was an inverse correlation between age and delta activity during the entire night ($r=-0.42$, $p<0.05$, respectively, Figure 3). In contrast, in patients but not in controls, sigma activity during the entire night was inversely related to age ($r=-0.44$, $p<0.05$, respectively, Figure 3).

Finally, age was inversely related to the DS$_{10-12}$ Hz ratio ($F=8.8$, $p<0.05$) and the DS$_{14-16}$ Hz ratio during the entire night ($F=5.2$, $p<0.05$). Again, closer analysis showed that this inverse relation between age and the DS ratio was apparent in controls only ($r=-0.45$ and $r=-0.33$, respectively, each $p<0.05$), but not in patients ($r=-0.05$ and $r=-0.14$, respectively).

### Table 2

<table>
<thead>
<tr>
<th>Sleep parameter</th>
<th>male controls</th>
<th>female controls</th>
<th>male patients</th>
<th>female patients</th>
<th>Gender $F$ value</th>
<th>Diagnosis $F$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delta $\mu$V $^2$/s</td>
<td>235.5±30.1</td>
<td>274.5±28.4</td>
<td>177.0±22.1</td>
<td>303.9±37.4</td>
<td>$F=12.2$</td>
<td>n.s.</td>
</tr>
<tr>
<td>Sigma $\mu$V$^2$/s</td>
<td>8.3±0.8</td>
<td>11.0±1.2</td>
<td>9.0±1.2</td>
<td>12.9±1.9</td>
<td>$F=9.8$</td>
<td>n.s.</td>
</tr>
<tr>
<td>DS</td>
<td>39.2±4.5</td>
<td>35.6±4.2</td>
<td>28.5±4.0</td>
<td>34.7±4.1</td>
<td>n.s.</td>
<td>$F=4.4$</td>
</tr>
<tr>
<td>Log DS</td>
<td>1.5±0.05</td>
<td>1.49±0.05</td>
<td>1.39±0.06</td>
<td>1.48±0.07</td>
<td>n.s.</td>
<td>(F=2.4)</td>
</tr>
<tr>
<td>DS 10-12 Hz</td>
<td>61.0±8.5</td>
<td>60.8±8.1</td>
<td>47.3±4.1</td>
<td>52.7±4.7</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>Log DS 10-12 Hz</td>
<td>1.67±0.07</td>
<td>1.70±0.08</td>
<td>1.61±0.06</td>
<td>1.67±0.12</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>DS 12-14 Hz</td>
<td>69.6±7.8</td>
<td>62.8±7.1</td>
<td>51.1±7.9</td>
<td>61.5±7.5</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>Log DS 12-14 Hz</td>
<td>1.71±0.08</td>
<td>1.74±0.07</td>
<td>1.63±0.10</td>
<td>1.79±0.12</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>DS 14-16 Hz</td>
<td>169.1±17.3</td>
<td>135.3±18.2</td>
<td>110.1±13.1</td>
<td>144.6±17.1</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>Log DS 14-16 Hz</td>
<td>2.19±0.05</td>
<td>2.00±0.05</td>
<td>1.98±0.04</td>
<td>2.10±0.07</td>
<td>n.s.</td>
<td>(F=3.6)</td>
</tr>
</tbody>
</table>

Values are means±SEM. $F$ value according to MANOVA (D.F. 1, 65) is indicated for $p<0.05$; n.s.=not significant.

### Table 3

<table>
<thead>
<tr>
<th>Sleep parameter</th>
<th>placebo control male patients</th>
<th>GHRH male patients</th>
<th>placebo control female patients</th>
<th>GHRH female patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delta - H1 $\mu$V$^2$/s</td>
<td>286.6±38.5</td>
<td>334.0±32.1</td>
<td>309.2±33.2</td>
<td>363.5±47.8</td>
</tr>
<tr>
<td>Delta - H2 $\mu$V$^2$/s</td>
<td>192.5±26.3</td>
<td>198.2±28.4</td>
<td>187.7±26.1</td>
<td>239.9±32.9</td>
</tr>
<tr>
<td>Sigma – H1 $\mu$V$^2$/s</td>
<td>8.4±0.8</td>
<td>10.7±1.0</td>
<td>10.6±4.1</td>
<td>12.4±1.7</td>
</tr>
<tr>
<td>Sigma – H2 $\mu$V$^2$/s</td>
<td>8.1±0.8</td>
<td>11.2±1.2</td>
<td>11.2±1.1</td>
<td>13.5±2.3</td>
</tr>
<tr>
<td>DS - H1</td>
<td>46.2±5.6</td>
<td>42.1±4.8</td>
<td>39.4±4.2</td>
<td>40.0±4.3D</td>
</tr>
<tr>
<td>Log DS – H1</td>
<td>1.60±0.06</td>
<td>1.56±0.06</td>
<td>1.54±0.06</td>
<td>1.54±0.07</td>
</tr>
<tr>
<td>DS - H2</td>
<td>32.1±4.3</td>
<td>25.0±2.8</td>
<td>22.3±2.7</td>
<td>26.3±4.3</td>
</tr>
<tr>
<td>Log DS - H2</td>
<td>1.42±0.07</td>
<td>1.34±0.05</td>
<td>1.28±0.07</td>
<td>1.31±0.08</td>
</tr>
</tbody>
</table>

Values are means±SEM. D=significant effect of diagnosis. Statistical details see text.
DISCUSSION

The major findings of the present study include: 1) a pronounced reduction in NREM, and particularly stage 2 sleep in patients with depression; 2) sexually dimorphic effects of GHRH on sleep-EEG regulation, including promotion of NREM, stage 2 sleep and sleep continuity in men, regardless of diagnosis, but a reduction in stage 2 sleep and sleep continuity in women; and 3) greatly diminished age-associated alterations of the sleep EEG in patients with depression. We observed a striking reduction in NREM sleep that was accounted for by a predominant reduction in stage 2 sleep but not SWS, in patients with depression compared to controls. A reduction in stage 2 sleep in depression has been noted before (Lauer et al., 1991; Holsboer-Trachsler et al., 1994; Stefos et al., 1998) and has been associated with the severity of depression (T. Coe, 1994; Stefos et al., 1998). We described in controls, but not in patients, an inverse correlation between NREM sleep and stage 2 sleep and age, particularly during the second half of the night when stage 2 sleep predominates. Interestingly, frequent awakenings reduce stage 2 sleep more than would be expected from the resulting time awake (Salzarulo et al., 1997), and a reduction in stage 2 sleep has been described to impair memory consolidation (Salzarulo et al., 1997; Schulz and Salzarulo, 1997). In addition, stage 2 sleep is decreased in elderly subjects with cognitive impairment (Spiegel et al., 1999) and sleep-spindles and K-complexes, which characterize stage 2 sleep, are significantly reduced in patients with dementia (Reynolds et al., 1988). Taken together, our data open up the hypothesis that a deterioration in cognitive functions in elderly subjects and patients with depression might involve a reduction in stage 2 sleep.

With regard to the promotion of sleep continuity and SWS by GHRH in animals (Obsdal et al., 1988, 1996) and humans (Steiger et al., 1992; Kerkhofs et al., 1993; Guldner et al., 1997), we observed a sexual dimorphism: in men, regardless of diagnosis, GHRH treatment decreased time awake and increased sleep efficiency and stage 2 sleep, while opposite effects were observed in women (for corresponding endocrine findings see also the subsequent paper). Thus, the present study confirms our previous data on the promotion of sleep continuity by GHRH in men (Guldner et al., 1997) and explicates the previously described lack of effect of GHRH in a mixed group of patients with depression (Steiger et al., 1994), due to opposite effects of GHRH in men and women. The NREM and stage 2 sleep promoting effect of GHRH opens up the possibility that GHRH could contribute to improve cognitive functioning, at least in men.

We have suggested before that in young normal controls the sexually dimorphic effects of GHRH on sleep regulation might reflect greater hypothalamic CRH release at the beginning of the night in women than men (Antonijevic et al., 1998). Also, nocturnal GH secretion is characterized predominantly by one large GH surge shortly after sleep onset in men, but smaller and more frequent pulses in women (Ho et al., 1987; Antonijevic et al., 1998), suggesting that in females, the predominance of GHRH relative to CRH release at the beginning of the night is less pronounced than in men. Thus, pulsatile GHRH administration during the first half of the night might better mimic the normal secretory pattern of hypothalamic releasing hormones in men than women. In addition, a more easily disturbed sleep in elderly women compared to men has been noted before (Vitiello et al., 1996) and could further contribute to the sexually dimorphic effects of GHRH.

Besides the decrease in NREM and stage 2 sleep, we observed a number of additional age-associated changes in the sleep EEG of normal controls, which have been reported before, including a disrupted sleep continuity with an increase in time awake and intermittent awakenings and a reduction in sleep efficiency and SWS (Reynolds et al., 1990; Van Coe, 1991; Lauer et al., 1991; Bilwise, 1993; Hoch et al., 1994). In contrast, in patients with depression only a reduction in SWS with increasing age was noted, while time awake and intermittent awakenings were greatly increased and sleep efficiency greatly decreased compared with controls, but were not further affected by age. Though our data seem in contrast to previous reports (Kupfer et al., 1982; Reynolds et al., 1990; Lauer et al., 1991), we have included only inpatients rather than in- and outpatients, and our patients were more severely depressed according to the Hamilton Depression Score than in some other studies (Kupfer et al., 1982; Reynolds et al., 1990). Furthermore, in previous reports not all age-related changes of the sleep EEG in controls were observed in patients (Kupfer et al., 1982; Lauer et al., 1991), supporting the hypothesis that an acute depressive episode is characterized by typical sleep-endocrine alterations which can mask the effects of aging.

In addition, we computed the ratio between delta and sigma activity (DS ratio) to eliminate confounding factors, such as input resistance, that can affect spectral EEG activity. The DS ratio was reduced in patients with depression particularly during the first half of the night, in agreement with previous studies reporting a significant decrease in delta EEG activity in depression (Kupfer et al., 1984, 1985). However, the decrease in delta relative to sigma activity was primarily observed in male patients, resulting in a significant interaction between diagnosis and gender. Also, the lack of effect of diagnosis on SWS was due to the slightly higher amount of SWS in female patients compared with controls, while in male patients SWS was markedly reduced. Thus, the hypothesis that oversecrecion of CRH attenuates maximal hyperpolarization and hence delta activity and SWS (Holsboer et al., 1988; Steiger, 1995), while promoting sigma activity (Ehlers et al., 1986; Antonijevic et al., 1998a) is compatible with the observation in male patients. In addition, most sleep parameters that have been associated with depression were more pronounced in male patients, while in females, depression was primarily associated with longer sleep latency and a greater number of awakenings.

With regard to aging, we observed in controls an age-associated decline in delta activity that has been proposed to reflect attenuated hyperpolarization of thalamic neurones via activation of GABA-B receptors (Tourigne and Albin, 1994; Billard et al., 1995; Murck et al., 1997), while with increasing age GABA-A receptor mediated functions are reinforced (Lippa et al., 1981; Abdulla et al., 1995). In support of this
hypothesis, we observed an inverse correlation between the DS10-12 Hz and DS14-16 Hz ratios with age in controls, indicating a greater decline in delta relative to sigma activity (which involves GABA-A receptor mediation [Borbély et al., 1985]). The lack of such correlation in patients with depression, together with the observed decline in sigma rather than delta activity with age, supports the hypothesis that in depression the level of hyperpolarization within thalamocortical neurones is reduced.

In summary, we described a pronounced reduction in NREM and stage 2 sleep in depression and an age-associated decline in NREM and stage 2 sleep in controls. GHRH promoted NREM, stage 2 sleep and sleep continuity in men, regardless of diagnosis, while in women opposite effects were noted. Thus, we put forward the hypotheses that: 1) In women compared to men activity of hypothalamic CRH neurones is greater at the beginning of the night and curtails effects of exogenous GHRH; and 2) GHRH might, via promotion of NREM, stage 2 sleep and sleep continuity, contribute to cognitive functioning. Finally, the sleep EEG of patients with depression was, unlike in controls, not significantly affected by age, suggesting that an acute depressive episode can mask sleep-endocrine changes normally seen during aging.

REFERENCES


43. Reynolds CF3rd, Kupfer DJ, Houck PR, Hoch CC, Stack JA, Berman SR, Zimmer B. Reliable discrimination of elderly depressed and demented patients by electroencephalographic sleep data. *Arch Gen Psychiatry* 1988; 45: 258-64.


