Changes in direct current (DC) potentials and infra-slow EEG oscillations at the onset of the luteinizing hormone (LH) pulse

Lisa Marshall,¹ Matthias Mölle,¹ Horst L. Fehm² and Jan Born¹
¹Department of Clinical Neuroendocrinology and
²Department of Internal Medicine, Medical University of Lübeck, H. 23a, Ratzeburger Allee 160, D-23538 Lübeck, Germany

Keywords: human, hypothalamus, neocortex, pulse generator, sleep, slow potentials

Abstract

An essential function of the neuroendocrine system lies in the coordination of hypothalamo-pituitary secretory activity with neocortical neuronal activity. Cortical direct current (DC) potential shifts and EEG were monitored in conjunction with the circulating concentration of luteinizing hormone (LH) in humans while asleep to assess a hypothalamic–neocortical interaction. The onset of an LH pulse was accompanied (i) at frontocortical locations by a transient positive DC potential shift of \( \sim 3 \) min duration and peak amplitude 50 \( \mu \)V; (ii) at frontal and central locations by an increase in power of infra-slow EEG oscillations for periodicities between 64 and 320 s. Results uniquely demonstrate a coupling of hypothalamo-pituitary activity with regulation of neocortical excitability.

Introduction

A coupling of endocrine events to neocortical processing is a basic requirement for integrating needs and behaviour. This coupling may reside in a hypothalamic–neocortical dialogue. Many neuroanatomic pathways relay a direct or indirect interaction between hypothalamus and neocortex (Morecraft et al., 1992; Risold et al., 1997). Stimulation of the hypothalamus has been found to affect, for instance, cortical slow potential activity and sleep-related thalamocortical spindle activity (Aladjalova, 1964; Rowland, 1968; Sutsova & Burikov, 1997), and electrical stimulation of the frontal neocortex can exert an effect, albeit inhibitory, on luteinizing hormone (LH) secretion (Caceres & Taleisnik, 1980; Brutus et al., 1984). The objective of the present study was to demonstrate a temporal link between hypothalamus-dependent endocrine activity and neocortical activity in humans. For this purpose scalp-recorded direct current (DC) potentials and electroencephalogram (EEG) activity were recorded in conjunction with monitoring of LH secretory activity. LH secretion was chosen because it displays a distinct pulsatile pattern known to be closely linked to the hypothalamic release of luteinizing hormone-releasing hormone (LHRH) into the pituitary portal circulation. The neuronal system underlying the rhythmic activation of hypothalamic LHRH cells and the release of the neuropeptide from their terminals has been described as the ‘pulse generator’, and has been localized to the arcuate nucleus within the mediobasal hypothalamus in the monkey (Knobil, 1989). Changes in multiunit activity within the mediobasal hypothalamus parallel to pulsatile LH secretion have been reported in males and females of different mammalian species (Kawakami et al., 1982; Wilson et al., 1984; Mori et al., 1991; Cardenas et al., 1993). In adult humans, LH levels and incremental LH pulse amplitude are on average larger during the night-time sleep than during wakefulness (Fehm et al., 1991; Pietrowsky et al., 1994). Also, during sleep pulsatile activity of this hypothalamo-pituitary axis is more regular, with initiation of LH pulses predominantly occurring during NREM (nonrapid eye movement) sleep (Fehm et al., 1991; Rossmanith & Lauritzen, 1991). In addition, during the night hypothalamic multiunit activity associated with the LHRH/LH pulse generator is increased (O’Byrne et al., 1993). These characteristics led us to focus the investigation on a coupling between endocrine and cortical activity on LHRH/LH activity generated during sleep. DC potentials and EEG activity were measured continuously to determine changes in cortical activity during LHRH/LH secretory pulses for an interval from 15 min prior until 15 min after estimated onset of the LH pulse.

Materials and methods

Subjects and procedures

Subjects were 11 healthy men (\( n = 7 \)) and women (unmedicated, nonsmokers) between 20 and 27 years, who judged themselves to be good sleepers, had not been on shift work, and who sufficiently fulfilled criteria of a preliminary screening test. For this, subjects were initially asked how well they judge themselves to sleep at home, in a new environment, and if they can sleep lying on their backs. Because restrained head movement is a necessary prerequisite for proper DC potential recording, subjects were then asked to practice at home sleeping throughout the night on their back wearing a cervical collar for 2–3 nights. Subjects documented how long they slept, if they remembered rolling over, and in what position they awoke. Volunteers able to sleep at least 5 h with the cervical collar, and without knowingly changing their position more than about three times during the night, were subsequently recruited for an adaptation night. Normally cycling women were to participate on a night between days 4 and 11 of their menstrual cycle (follicular stage). During the complete recording session subjects remained lying on their backs with their heads fixed. Blood was collected in an adjacent room through a long thin tube.

Correspondence: Dr Lisa Marshall, as above.
E-mail: marshall@kfg.nu-luebeck.de,

Received 8 May 2000, revised 10 August 2000, accepted 31 August 2000
connected to a forearm catheter. The studies took place in a sleep laboratory at the Medical University of Lübeck, and the experimental protocol had been approved by the local ethics committee.

Recording

For determination of LH pulses, blood was sampled every 7.5 min between lights-off and lights-on from \( \approx 23:00 \) to 06:30 h. For standard polysomnographic sleep recordings EEG (0.15–30 Hz), electrooculogram (EOG; 0.045–30 Hz), and submental electromyogram (EMG; 1.5 Hz–1.5 kHz, with two electrodes placed horizontally over the M. submentalis) were amplified by a Nicolet electroencephalograph (Nicolet Biomedical Instruments, WI, USA). The amplified EMG signal was sent further to analogue-to-digital conversion (sampling rate 100 Hz).

DC potential and EEG signals were obtained from electrodes located at F3, Fz, F4, C3, Cz, and C4 of the international 10–20 system. All electrodes were referenced to linked electrodes at the mastoids. DC potential, polysomnographic and temperature measurement were as described previously (Marshall et al., 1998). In brief, at each electrode site a ‘clip-on’ electrode socket was attached with collodion and the scalp was punctured with a sterile hypodermic needle until minor bleeding occurred. Electrode gel (Electrode Electrolyte, TECA Corp., NY, USA) was applied to the socket, and the electrode, also filled with gel, was clipped on. Non-polarizable Ag/AgCl electrodes (8 mm diameter, ZAK GmbH, Simbach/Inn, Germany) were used. Air bubbles had been previously eliminated from the electrode gel by centrifugation (13000 r.p.m. for 3 h at 10°C) and care was taken not to produce any air bubbles when clipping electrodes onto their sockets. Electrode impedance was always \(<5\, \text{k}\Omega\). A direct-current amplifier (Toennies DC/AC amplifier, Jaeger GmbH and Co., KG, Germany; input resistance \( \approx 100\, \text{M}\Omega\)) was used for recording and amplification of DC potentials. Amplification, 2000-fold; low pass filter, 30 Hz; threshold for automatic DC offset correction, \( \pm 4\, \text{mV}\). With short-circuited input, the amplifier drift, if present, was \(<3\, \mu\text{V/h}\). Analog DC/EEG signals were digitized at 100 Hz/channel (CED 1401, Cambridge Electronics, UK) and stored on a PC together with a time marker (every 30 s) for off-line analysis.

Skin temperature was measured near each mastoid reference and on the scalp halfway between each frontalateral (F3, F4) and its corresponding centralateral (C3, C4) electrode (Mini Mitter, OR, USA; \( \pm 0.1^\circ\text{C}\) level of accuracy), and stored every 30 s.

Data processing and statistical analysis

Serum concentrations of LH were measured by a commercial immunoradiometric assay (Biermann, Bad Nauheim, Germany). Sensitivity of the assay was 0.15 mIU/mL; intra-assay coefficient of variation <1.2% for concentrations <16.4 mIU/mL, interassay coefficient of variation <2.2%, for concentrations <16.8 mIU/mL. Onsets of LH pulses in individual data were detected using the cluster analysis as described (Veldhuis & Johnson, 1988). This method marks all significant increases from nadir to peak clusters, all significant decreases in the data series of known variance, and then designates a peak as a region comprising an increase followed by a decrease. Selected sample size of the nadir and peak clusters (two points each) and the r-statistics for significant increases and decreases resulted in a false positive rate <1%. LH pulse onset was defined as the last nadir sample point preceding a significant peak. Sleep stages (1, 2, 3, 4 and REM sleep), awake time and movement artifacts were scored off-line for 30-sec intervals (Rechtschaffen & Kales, 1968). Stage 3 and 4 correspond to slow-wave sleep (SWS).

To adapt electrophysiological data to a common time scale, average values of DC potentials and integrated EMG activity were calculated for 30-sec epochs with the averaged values assigned to the centre time of the 30-sec epoch. The DC potential, integrated EMG activity and the polysomnographically determined sleep stages were averaged time-locked to the estimated onset of the LH pulse. These averaged data segments covered an interval from 15 min before to 15 min after the LH pulse onset. Data segments containing transitions to REM sleep, frequent transitions between stage 2 sleep and SWS, and periods of wakefulness within \( \pm 15\, \text{min}\) of estimated LH pulse onset were excluded. Exclusion of epochs containing transitions to REM sleep was based on the finding by Fehm et al. (1991) that LH onset occurred predominantly during the mid portion of the NREM sleep phase, and to exclude superimposition with the REM-transition positive shift as described previously (Marshall et al., 1998). Also, data segments with scores of ‘movement time’ (but not of ‘movement arousal’, according to Rechtschaffen & Kales, 1968) occurring within 5 min of estimated LH pulse onset, and segments with changes in scalp temperature (\(>0.5^\circ\text{C}\)) within \( \pm 10\, \text{min}\) of the estimated LH pulse onset were eliminated, because artifacts in DC potential data could not be ruled out. Thus, of the original 30 data segments, 15 segments remained for analyses. The first 5 min of the 30-min data segment served as baseline. For a supplementary analysis the DC potential of the same selected 15 epochs was averaged within \( \pm 60\, \text{min}\) around LH pulse onset. Prior to averaging DC potential data, linear trends occurring across the 30-min and 120-min intervals, respectively, were eliminated.

Differences in DC potential level as well as differences in DC potential slope were assessed by analysis of covariance (ANCOVA) with repeated measures. Factors were Electrode site and Time. Covariates, introduced to control for possible covariations of the DC potential, were either sleep stage, movements (movement arousals/movement time), or integrated EMG activity. For the ANCOVA, sleep stages were given the following values, as in a previous study (Marshall et al., 1998): sleep stage 1, 1; stage 2, 2; stage 3, 3; stage 4, 4; awake, –1; REM sleep, 0. Degrees of freedom were corrected according to Greenhouse–Geisser (Greenhouse & Geisser, 1959). Post hoc contrasts were assessed only when respective main effects or interactions reached statistical significance at the \( P<0.05\) level.

To characterize activity in the EEG frequency bands between 15 min before and after estimated LH pulse onset, a moving fast Fourier transform (FFT) was calculated using 1024-sec windows (1024 data points) shifted every 5 s (frequency resolution 0.0977 Hz). Data of each window had been multiplied by a raised cosine function prior to FFT to taper the signal towards zero at the extremes of the data window, thus reducing errors induced by edge-effects. The moving FFT resulted in a new time series representing the change over time in power within five selected frequency bands: delta (0.49–4 Hz), theta (4–8 Hz), alpha (8–12 Hz), beta (15–25 Hz) and sigma, representing spindle activity (12–15 Hz). For each window of FFT analyses results were assigned to the corresponding centre time. The time series of EEG power were subsequently smoothed by applying a 7-point (i.e. 30-s) moving average.

To prove statistical significance of very slow periodic fluctuations observed at visual inspection in the above-mentioned time series of power, autocorrelations were calculated for the intervals of all 15 data segments with SPSS statistical software (SPSS Inc., Chicago, USA). The cumulative distribution of the time-lags for the statistically significant autocorrelations determined across the 15 data segments indicated considerable jitter in the period length of these oscillations, with most of the ‘infra-slow’ EEG oscillations occurring in a band corresponding to period lengths between 64 and 320 s (Fig. 4). For this band, average power was calculated by FFT for evaluating the time course of infra-slow oscillations in relation to the LH pulse.
onset. The average power for the 64–320 s band was calculated separately for the time series (temporal resolution 5 s) of delta, theta, alpha, beta and sigma activity as well as for the time series of DC potential data, using again a moving FFT. Window length of these FFTs was 10.67 min (128 data points, resulting in a frequency resolution of 0.00156 Hz), and windows were shifted every minute. Resulting values were assigned to the centre time of the FFT window; thus the first window began 5 min before and the last window ended 5 min after the 30-min interval centred around the estimated LH pulse onset.

Changes in EEG power and in infra-slow oscillatory power (corresponding to the 64–320 s range of period length) occurring before and after LH pulse onset were statistically evaluated using an ANCOVA model as described for DC potential data.

Results
In general, sleep parameters were close to typical sleep under laboratory conditions with the somewhat increased time spent awake, and decreased time spent in stage 4 probably due to the restrained position of head and body during sleep. Mean ± SEM total sleep time was 433.1 ± 8.1 min, and percentages of time spent awake, in sleep stages 1–4 and in REM sleep were 9.7 ± 0.02, 9.8 ± 0.03, 48.7 ± 0.03, 9.8 ± 0.01, 6.2 ± 0.01 and 15.6 ± 0.01%, respectively. Mean LH concentration increased by 3.88 ± 0.55 mU/mL within 15 min after estimated pulse onset and reached a peak value of 7.19 ± 0.84 mU/mL. Thereafter LH concentration gradually declined with baseline levels not fully recovered by 60 min after estimated LH pulse onset (Fig. 2A).

DC potential changes at the onset of the LH pulse
Close to the time of LH pulse onset (0 min) a prominent large positive shift occurred being most obvious in recordings over the frontal cortex (F3, Fz, F4) where peak amplitudes between 40 and 50 μV as compared to baseline were measured (Fig. 1B). ANCOVA confirmed that the DC potential level between −1 and +2 min (referenced to estimated LH pulse onset) was significantly more positive than baseline over the frontal cortex (Fig. 1C). At central electrodes (C3, Cz, C4) a parallel increase in positivity of the DC potential remained nonsignificant compared with baseline (*P > 0.2). Also, the covariate sleep stage remained nonsignificant (*P > 0.4), indicating that the DC potential changes were not contaminated by sleep stage-related influences (Fig. 1D). Likewise neither movement arousal (Fig. 1E) nor integrated EMG activity when used as covariate obtained any significance (*P > 0.2).

The transient character of the large positive DC potential shift around the time of LH pulse onset is indicated by the clear change in direction of slope between the −3 to +1 min interval and the +2 to +6 min interval (*F(1,13) = 10.50, P < 0.007; covariates sleep stage, movement arousal, integrated EMG: all *P > 0.4; *F(5,70) = 5.46, P < 0.003 for the Electrode–Time interaction). Mean slope values for the rising and falling intervals of the large positive DC potential shift were, respectively, for F3 10.2 vs. −13.2 μV/min (*F(1,13) = 10.57, P < 0.01), for Fz 10.0 vs. −9.5 μV/min (*F(1,13) = 5.45, P < 0.05), and for F4 13.0 vs. −13.4 μV/min (*F(1,13) = 15.73, P < 0.01). At ≈8 min after the LH pulse onset the DC potential at frontal locations

Fig. 1. Time courses of LH concentration, DC potential, sleep stage and movements for the interval from 15 min before to 15 min after the estimated onset of the LH pulse. (A) Mean ± SEM LH concentration; (B) DC potential collapsed across frontal electrode sites (F3, Fz, F4; heavy line) and central electrode sites (C3, Cz, C4; thin line). (The mean potential value of the first 60 s was set to 0 μV). The hatched horizontal bar indicates the 5-min interval used as baseline (−15 to −10 min); (C) P-values for the effect of Time (compared to baseline) in ANCOVA with sleep stage as covariate. P-values refer to changes in the DC potential at frontal sites. Changes at central sites remained statistically nonsignificant; (D) Averaged sleep stage (± SEM); (E) Number of movements (movement time given the value 2; movement arousal given the value 1) summed across the 15 data segments.
recovered to baseline levels (Fig. 1). The small yet distinct negative dip in the DC potential ≈ 3 min before estimated LH pulse onset was not statistically significant, although clearly visible in several single data segments.

**DC potential changes within ±60 min of LH pulse onset**

A supplementary analysis of the DC potential ±60 min around LH pulse onset examined whether the DC potential shift associated with the LH pulse onset bore any relationship to the NREM-REM sleep cycle and related DC potential changes (Hoffmann *et al.*, 1996; Marshall *et al.*, 1998). Figure 2 reveals that within a ±60 min interval the immediate change in DC potential at LH onset was preceded by a pronounced DC potential shift toward negative values being steepest ≈ 30 min prior to LH pulse onset. Apparently, this negative shift coincides with a shift in sleep stage from preceding REM sleep to NREM sleep. Thereafter a more gradual DC potential shift in positive direction developed, onto which the immediate changes in DC potential at LH pulse onset were superimposed. This gradual shift was reflected in a significantly more positive mean DC potential level between 25 and 30 min after LH pulse onset as compared to the potential level between 20 and 15 min before pulse onset ($F_{(1,13)}= 6.76, P<0.05$; $P>0.1$ for covariate Sleep stage). Comparing the potential level within intervals even more distant from LH pulse onset yielded no more significant changes. Both the strong negative shift in the DC potential seen in Fig. 2 and the subsequent gradual positive slope have been documented in pervious experiments (Marshall *et al.*, 1998) where they were termed ‘NREM-transition negative shift’ and ‘NREM-positive slope’. They indicate a close link between DC-potential changes and the NREM-REM sleep cycle, upon which potential changes related to LHRH/LH secretory activity appear to be superimposed during the NREM phase.

**Delta, theta, alpha, beta and sigma power**

Consistent with the criteria of sleep stage scoring, ANCOVA revealed that power within all EEG frequency bands correlated with the sleep stage covariate ($P<0.05$). However, analyses with reference to the time course of the LH pulse did not reveal any significant change in EEG power in the 0.5–25 Hz frequency range. Figure 3 reveals, for two individuals, LH concentration and the associated time series of EEG power, together with infra-slow EEG activity.

**Infra-slow periodic EEG activity**

Inspection of individual records indicated that slow periodic fluctuations were superimposed on the time series of EEG power (Fig. 3). Autocorrelation functions for the intervals confirmed the occurrence of significant infra-slow oscillations in the time series of EEG power which were distributed in a rather broad range between 60 and 320 s (Fig. 4). A main periodicity appears to occur with a time lag of ≈90 s, being most apparent in the time series of alpha and sigma power. Given the wide range of significant periodicities, average power was determined by FFT for a broad band covering the periodicities between 64 and 320 s to further analyse the changes in time of this infra-slow periodic activity time-locked to the LH peak onset. (In fact, when upper or lower subdivisions of this band were separately evaluated, basically the same changes in time were revealed as those reported below for the broad 64–320 s band.) DC potential data were not included in this analysis, because respective autocorrelation functions did not confirm a sufficient number of significant correlation coefficients (<45) for this range of infra-slow oscillations (Fig. 4).
Compared to baseline, significant enhancements in infra-slow oscillatory power were observed between 0 and 5 min after the estimated LH pulse onset for delta, theta, alpha and beta bands, but not in the sigma band \((P<0.05\), for all ANOVA main effects of Time, Fig. 5). In addition, significant \((P<0.05)\) Time–Electrode site interactions indicated differential topographical distributions in all EEG bands except for theta. Accordingly, increases in infra-slow oscillatory activity around the time of LH pulse onset were most distinct for delta at central sites (C3, Cz, C4), for alpha at frontal sites (F3, Fz, F4) and for beta activity at lateral sites (F3, F4, C3, C4). For the theta band, the increase in infra-slow oscillatory activity around the estimated LH pulse onset was preceded by a transient but statistically significant suppression of infra-slow activity.

**Discussion**

Results indicate (i) that the onset of LH pulses is accompanied by a transient positive DC potential shift commencing shortly before the estimated LH pulse onset and ceasing =8 min later; (ii) The positive DC potential shift concentrated over frontal cortical areas; (iii) LH pulse onset was associated with an increase in infra-slow oscillations in EEG activity occurring over a wide range of EEG frequencies.
Fig. 4. (A) Autocorrelation functions for the time series of the DC potential and of delta, theta, alpha, beta and sigma power shown for two individual data segments (07, 09). Autocorrelation functions were calculated over intervals revealing slow periodic fluctuations. Vertical lines indicate the 5% level of significance. Resolution, 5 s. (B) Cumulative distribution of statistically significant positive time lags for the autocorrelation functions of all 15 data segments, for the time series of the DC potential as well as of delta, theta, alpha, beta and sigma power. Y-axis reflects the number of statistically significant autocorrelations across the 15 data segments for a given time lag. For simplicity diagrams are only shown for positive time lags; correlation coefficients with lag <10 s were omitted. Note, while infra-slow periodic activity was poorly expressed in the time series of the DC potential, significant infra-slow oscillations overlaying EEG activity considerably accumulated in a broad range of time lags (periodicities) between ≈64 and 320 s.

**Indications of a hypothalamic-neocortical interaction**

Whilst the data provide clear evidence for concurrent changes in neocortical activity and endocrine activity of the LHRH/LH system, the question arises what type of hypothalamic-neocortical interaction can be inferred. It can be excluded that the electrocortical changes in the DC potential and infra-slow EEG oscillations were a consequence of the LH changes in peripheral blood. Significant changes in cortical activity were well established before the LH plasma concentration reached maximum levels. In addition, when the DC potential shift was ceasing 8 min after estimated LH pulse onset mean LH concentration in blood was still enhanced and even rising (Figs 1 and 2).

LH in the present study was used as an indicator of activation by the LHRH pulse generator because LHRH in portal blood in humans cannot be measured directly. In studies where LHRH and LH have been measured simultaneously large-amplitude LH pulses have been reported to accompany or be directly preceded by LHRH pulses, whereby the accompanying LH pulse, measured with 10-min collection intervals, always occurred abruptly and peaked within 1–2 collection intervals, similarly to the present study (Moenter et al., 1992). The durations of the positive DC shift and of the increase in infra-slow periodic EEG activity are similar to several temporal measures obtained for neuronal and neurosecretory activity associated with the LHRH pulse generating mechanism in mammals.
Enhanced multiunit activity of 1–5 min duration has been reported in conjunction with LHRH/LH neurosecretory activity (Kawakami et al., 1982; Williams et al., 1990; Goubillon et al., 1995), and release of LHRH into portal blood persisted for 2–8 min, subsequent to and preceding a rapid onset and cessation (Moenter et al., 1992). These temporal characteristics suggest a close linkage of the LHRH pulse generation to the positive DC potential shift starting shortly before the estimated LH pulse onset and lasting >8 min, as well as to the increase in infra-slow oscillatory power commencing around the same time. A higher sampling rate of venous blood in the present study may have reduced some temporal jitter between LH concentration and electrophysiological measures. However, the inert release and distribution of LH into peripheral vessels contribute to the fact that plasma LH represents only an estimate of the actual timing of the LHRH pulse generator. Studies in rabbits and cats have shown that shifts in the cortical DC potential as well as infra-slow oscillatory activity in the neocortex can be readily induced by electrical stimulation of various medial hypothalamic sites (Aladjalova, 1964; Rowland, 1968). Moreover, Aladjalova measured infra-slow oscillations simultaneously but with different period lengths in the hypothalamus and cerebral cortex, after hypothalamic stimulation. The latter studies imply that changes in neocortical electrical activity in association with pituitary LH secretion result from a direct or indirect collateral hypothalamic output to cortical structures. Although the temporal resolution of the present data do not allow exclusion of an inverse relationship, evidence that stimulation of LHRH/LH secretory activity could be a consequence of changes in activity at the cortical level is scarce (Caceres & Taleisnik, 1980; Braitus et al., 1984).

As a further, though indirect, indication of a hypothalamic–neocortical interaction, the temporal association between LH pulse onset and previously reported phases of the DC potential change during the NREM-REM sleep cycle (i.e. the NREM-transition negative shift and the NREM-positive slope) could also be taken (Marshall et al., 1998). This data confirms the strong link of LHRH/LH secretory activity to the NREM phase of the NREM-REM sleep cycle observed in numerous foregoing studies (e.g. Fehm et al., 1991; Driver et al., 1996).

Cortical mechanisms of DC potentials and slow periodic EEG activity

At present the exact sources of the large transient positive DC potential shift at frontal locations around the time of LH pulse onset are unclear. Slow cortical DC potential changes during wakefulness have been described as a measure reflecting a tonic tuning of excitability of cortical neuronal networks (Birbaumer et al., 1990; Elbert, 1993). It has become established to associate the occurrence of slow negative DC potential shifts during tasks with widespread synchronized membrane depolarizations of pyramidal apical dendrites and, accordingly, to associate DC potential shifts of positive polarity with reduced depolarization and hyperpolarization of apical dendrites. However, widespread hyperpolarizations in deeper cortical layers underlying slow oscillatory activity may also produce a superficial potential of negative polarity (Steriade et al., 1993). Further, it is of note that if activity is concentrated in the orbito-frontal region it cannot be excluded that the direction of polarity at the frontal cortex is reversed due to the convexity of the orbito-frontal region.

Regardless of the issue of exactly where the potential-generating dipole is located in the neocortex, which would require more fine-grained topographical analyses, our results indicate that modulations in the DC potential and in infra-slow EEG oscillations are temporally
linked, with enhanced infra-slow periodic EEG activity and the positive DC potential shift obtaining significance around the same time. Yet, although the positive DC potential shift and the increase in infra-slow periodic EEG activity may share some of the generator mechanisms in cortical and subcortical tissues these phenomena do not reflect identical processes because there are discrete distinctions in topography and time course. First, changes in infra-slow periodic EEG activity in conjunction with LH release were observed at frontal, but also at central, electrode sites whereas the large transient positive DC potential shift was clearly limited to frontal locations (Figs 1 and 5). Second, the DC potential level at the end of the 30-min interval did not differ from baseline level in contrast to the infra-slow oscillations in delta, theta and alpha activity which at that time were still enhanced. This suggests the shifts in the DC potential to be more closely linked to a temporally restricted hypothalamic–frontocortical interaction. Also, infra-slow oscillatory activity in the DC potential was much weaker than that in the EEG bands. Interestingly, neuronal membrane potential level has been reported to influence amplitude and frequency of a very slow oscillatory activity in thalamic neurons (Leresche et al., 1991; Albrecht et al., 1998), and very slow periodic excitability changes were also reported in neurons of the hippocampus (Penttonen et al., 1999).

An intriguing candidate mediating hypothalamic influences on cortical excitability, DC potentials and infra-slow EEG activity is neuropeptide Y (NPY). Abundant NPY binding sites have been found in human frontal cortex (Statnick et al., 1997; Caberlotto et al., 1998), and NPY release precedes LHRRH by ~5 min (Woller et al., 1992). NPY has been shown to produce shifts in EEG frequencies and amplitude, particularly over the frontal cortex, and to influence cortical excitability in vitro as well as after intracerebroventricular administration in rats (Zini et al., 1984; Ehlers et al., 1997; Klapstein & Colmers, 1997; Woldbye et al., 1997). NPY closely interacts with the noradrenergic system in LHRRH pulse generation (Wuttke et al., 1996; Terasawa, 1998), which points to a possible contribution of brainstem noradrenergic afferents in the joint modulation of neocortical DC potential and infra-slow oscillatory activity (Terzano et al., 1985; Scheuler et al., 1990; Novak et al., 1992). However, LHRRH itself could also be involved in the transfer of action to the neocortex. Receptor sites for LHRRH have been identified in the rat frontal cortex, limited to the rhinal sulcus (Jennes & Conn, 1994), which would correspond with the distinct frontal focus of the changes in the DC potential observed in the present study.

These neuropeptides and transmitters within the neocortex may also affect glial cells which, due to their ability to spatially buffer K⁺, are supposed to participate in the generation of slow potentials (Lux et al., 1986; Birbaumer et al., 1990). Glial cells have been shown to respond to electrical and various neurochemical stimuli, including NPY, with an increase in intracellular Ca²⁺ concentration (Laming, 1989; Gimpi et al., 1993; Pasti et al., 1997; Verkhatsky et al., 1998). In cultured astrocytes, oscillatory activity has been described with periodicities up to the minute range (Pasti et al., 1997). Thus neuronal–glial interactions may contribute to the enhancement of slow periodic EEG activity or DC potential positivity.

The changes in the DC potential and infra-slow periodic EEG activity associated with LH pulse onset suggest a coordinated control of neocortical activity in conjunction with hypothalamo-pituitary activation of LH release. The concordant increase in infra-slow EEG oscillations at LH pulse onset might point, in addition, to an entrainment of neuroendocrine activity with longer lasting oscillations of autonomic nervous system functions (Delamont et al., 1999). Possibly, the linkage between neuroendocrine secretory activity and neocortical excitability as expressed in the DC potential changes pertains also to other hypothalamo-pituitary endocrine systems which would argue against a specific relevance within the reproductive axis. Rather, such a link could serve a more general function, tuning activity between the endocrine and central nervous systems. On the basis of the present data it may be speculated that neocortical excitability is down-regulated in temporal proximity to LH pulse onset, thus coinciding with the inhibitory effect of neocortical stimulation on LH secretion (Caceres & Taleisnik, 1980; Bruttus et al., 1984). Also, long-lasting effects on hippocampal excitability after in vitro LHRRH administration have been observed (Chen et al., 1993). Assuming that the association between LHRRH/LH secretory activity and DC potential change disclosed here during night-time sleep can be likewise demonstrated during the awake phase, the modulation in neocortical excitability in conjonction with LH pulses may also acutely affect various aspects of ongoing behavioural regulation. However, such actions remain to be specified.

**Acknowledgements**

This work was supported by a DFG grant to J.B. and H.L.F. We gratefully thank Ms J. Ries, Ms C. Beyer, Mr J. Czyborra and Mr J. Hoffmann for collecting data and for preliminary analyses, as well as Ms C. Zinke and Ms A-K. Jürs for further technical assistance.

**Abbreviations**

DC, direct current; EEG, electroencephalogram; EMG, electromyogram; FFT, fast Fourier transform; LH, luteinizing hormone; LHRRH, luteinizing hormone-releasing hormone; NPY, neuropeptide Y; NREM, nonrapid eye movement; REM, rapid eye movement.

**References**


Fehm, H.L., Claussing, J., Kern, W., Pietrowsky, R. & Born, J. (1991) Sleep-

DC shifts, infra-slow oscillations and LH