Topography of EEG Dynamics After Sleep Deprivation in Mice

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Received 3 April 2000; accepted in final form 6 June 2000

INTRODUCTION

The homeostatic regulation of sleep is one of its most prevalent features among mammalian species (Borbély and Achermann 1999; Tobler 2000). Thus electroencephalographic (EEG) slow-wave activity (SWA, mean EEG power density in the 0.75–4.0 Hz band) in non–rapid eye movement (NREM) sleep is a function of the prior history of sleep and wakefulness. SWA is thought to reflect the homeostatic component of the two-process model of sleep regulation. According to this model, originally formulated for the rat and later extended to human sleep, the timing and structure of sleep are determined by the interaction of a homeostatic Process S and a circadian process. Our aim was to investigate the dynamics of SWA in the EEG of two brain regions (frontal and occipital cortex) after sleep deprivation (SD) in two of the mice strains most often used in gene targeting. C57BL/6J (n = 9) and 129/Ola (n = 8) were recorded during a 24-h baseline day, 6-h SD, and 18-h recovery. Both derivations showed a significant increase in SWA in NREM sleep after SD in both strains. In the first hour of recovery, SWA was enhanced more in the frontal derivation than in the occipital derivation and showed a faster decline. This difference resulted in a lower value for the time constant for the decrease of SWA in the occipital cortex) after sleep deprivation (SD) in two of the mice strains most often used in gene targeting. C57BL/6J (n = 9) and 129/Ola (n = 8) were recorded during a 24-h baseline day, 6-h SD, and 18-h recovery. Both derivations showed a significant increase in SWA in NREM sleep after SD in both strains. In the first hour of recovery, SWA was enhanced more in the frontal derivation than in the occipital derivation and showed a faster decline. This difference resulted in a lower value for the time constant for the decrease of SWA in the occipital derivation (frontal: 10.9 ± 2.1 and 6.8 ± 0.9 h in Ola and C57, respectively; occipital: 16.6 ± 2.1 and 14.1 ± 1.5 h; P < 0.02; for each of the strains; paired t-test). Neither time constant differed significantly between the strains. The subdivision of SWA into a slower and faster band (0.75–2.5 Hz and 2.75–4.0 Hz) further highlighted regional differences in the effect of SD. The lower frequency band had a higher initial value in the frontal derivation than in the occipital derivation in both strains. Moreover, in the higher frequency band a prominent reversal took place so that power in the frontal derivation fell below the occipital values in both strains. Thus our results indicate that there may be differences in the brain in the effects of SD on SWA in mice, suggesting regional differences in the dynamics of the homeostatic component of sleep regulation. The data support the hypothesis that sleep has local, use- or waking-dependent features that are reflected in the EEG, as has been shown for humans and the laboratory rat.

This feature of sleep has been consistently shown in a broad range of species, such as humans, cat, mouse, rat, and squirrel (Huber et al. 2000; Larkin and Heller 1998; Strijkstra and Daan 1998; reviewed in Tobler 1995). Furthermore, in humans a night following a nap exhibits reduced SWA compared with normal nights (Dijk et al. 1987; Werth et al. 1996b). The neuronal mechanisms leading to slow waves in the EEG have been elucidated (Amzica and Steriade 1998); however, their function remains unknown.

There is little doubt that the function(s) of sleep must entail restoration, and most probably mainly for the brain (Horne 1985). Moreover, it has been proposed that sleep may not be a global phenomenon encompassing the entire brain, but that slow waves may reflect local recovery processes (Krueger and Obal 1995; Moruzzi 1972). Findings in the bottlenose dolphin contributed to this hypothesis. These animals have the capacity to exhibit “deep” slow-wave sleep only in one brain hemisphere while the EEG in the other hemisphere exhibits a waking pattern (Mukhametov et al. 1977). Furthermore, after unihemispheric sleep deprivation, the deprived brain hemisphere showed a larger increase of deep slow-wave sleep (Oleksenko et al. 1992). Also birds seem to have the capacity to exhibit minor hemispheric asymmetries in the EEG (Amblaner and Ball 1994), but they last only a few seconds and are related to unilateral eye-opening (Rattenborg et al. 1999).

Sleep could be regarded as a use-dependent local phenomenon serving to stimulate synapses insufficiently used during wakefulness to maintain neuronal connections (Krueger and Obal 1995). According to this hypothesis, synaptic connectivity is strengthened locally and modulates EEG synchronization during sleep. An alternative hypothesis proposed a restoration of brain glycogen levels during sleep, which are thought to be depleted during the brain activity related to wakefulness (Benington and Heller 1995). Recent evidence supports the notion that a previous experience can affect the cell firing of specific neurons during sleep (Poe et al. 2000). Thus in rats an experience-dependent reversal of the phase of multiunit firing of hippocampal cells was shown in REM sleep following running in specific tracks.

It is well known from animal studies that the EEG pattern differs according to the position of the cortical electrodes, but most reports have remained at the descriptive level. Spectral analysis of the EEG recorded from a frontal and occipital derivation in the rat, or from six bipolar derivations in humans revealed state- and frequency-specific differences between the...
derivations (Schwierin et al. 1999; Werth et al. 1997). After 24-h sleep deprivation (SD) in the rat, a larger increase in the 1.75- to 4.5-Hz band was observed in frontal versus occipital power (Schwierin et al. 1999). This differential response to SD may reflect regional, use-dependent aspects of sleep regulation. A frontal predominance of SWA has been described in humans (Werth et al. 1996a), which supports the notion that the prefrontal cortex is the site of greatest cortical brain work during wakefulness (Horne 1993). In addition, local activation of the left somatosensory cortex by vibration of the right hand in humans resulted in a shift of power in the sleep EEG of the somatosensory cortex toward the left hemisphere (Kattler et al. 1993), and visual stimulation in macaque monkeys suggested that sleep does not occur simultaneously in all cortical areas of the brain (Pigarev et al. 1997). Such studies are supported by results from regional distribution of cerebral blood flow (Gur et al. 1995; Swanson et al. 1992) and positron emission tomography in humans, which reflect changes in the functional organization of the brain by the different patterns of regional blood flow (Braun et al. 1998; Finelli et al. 2000; Gillin et al. 1996; Maquet et al. 1996, 1997).

Due to the large use of gene targeting in mice, these rodents have become important models to investigate the mechanisms and function(s) of sleep (Boutrel et al. 1999; Chemelli et al. 1999; Franken et al. 2000; Huber et al. 1999; Tobler et al. 1996). Our aim was to investigate the dynamics of EEG activity of two cortical derivations after SD in two strains of inbred mice that are most often used in gene targeting experiments.

**METHODS**

**Mice**

Adult male mice, of the inbred strain 129/Ola (Ola; n = 8) and C57BL/6J (C57; n = 9) were used. They were maintained in a 12-h light–12-h dark cycle (lights from 08:00 to 20:00 h; OSRAM Dulux EL energy saving lamp, 7 W, 10–40 lux at the level of the mice), kept individually in Macrolon cages (36 × 20 × 35 cm) and in sound-attenuated chambers. Food and water were available ad libitum. Adaptation to these conditions was at least 3 wk. Ambient temperature in the boxes during the recording days was 22.5 ± 0.4°C.

**Surgery**

Implantation of EEG and electromyographic (EMG) electrodes occurred under deep anesthesia (pentobarbital sodium, Nembutal, 80 mg/kg ip; volume, approximately 0.5 ml). Three gold-plated miniature screws (diameter 0.9 mm) placed over the right occipital cortex (2–3 mm lateral to the midline, 2 mm posterior to bregma), the right frontal cortex (1 mm anterior to bregma, 1 mm lateral to midline) and the cerebellum (at midline, 1 mm posterior to lambda) served as epidural

| Table 1. Mean EEG power over all vigilance states for the 24-h baseline |
|---------------------------|---------------------------|---------------------------|
|                           | Total (0.25–25 Hz)        | SWA (0.75–4.0 Hz)         |
|                           | Frontal                   | Occipital                 | P           | Frontal | Occipital | P           |
| 129/Ola                   | 8                         | 2.397 ± 224.0             | 3.010 ± 369.7 | 0.10    | 837.1 ± 88.2 | 655.3 ± 76.6 | 0.10 |
| C57BL/6J                  | 9                         | 1.961 ± 225.8             | 2.433 ± 275.6 | 0.06    | 617.7 ± 74.2 | 544.6 ± 61.1 | 0.19 |

Values are means ± SE in μV²; n is number of mice. SWA is the EEG power density in non–rapid eye movement sleep in the 0.75- to 4.0-Hz range. P is the P value frontal vs. occipital derivation, 2-tailed paired t-test. EEG, electroencephalographic; SWA, slow-wave activity.
and the corresponding interaction, repeated measures with between factor "strain" and within factor "derivation" was not significant). For description of the procedure see METHODS.

**TABLE 2. Estimation of the time constant (hours) for the decrease of slow-wave activity in non–rapid eye movement sleep after sleep deprivation**

<table>
<thead>
<tr>
<th></th>
<th>Occipital</th>
<th>Frontal</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>129/Ola</td>
<td>16.6 (2.1)</td>
<td>10.9 (2.1)</td>
<td>0.02</td>
</tr>
<tr>
<td>C57BL/6J</td>
<td>14.1 (1.5)</td>
<td>6.8 (0.9)</td>
<td>0.004</td>
</tr>
</tbody>
</table>

Mean values in hours (± SE). Difference between occipital and frontal derivation (Wilcoxon signed-rank test for paired samples, 2-way ANOVA for repeated measures with between factor "strain" and within factor "derivation" and the corresponding interaction. P < 0.0001 for the factor “derivation,” the factor “strain” and the interaction “strain×derivation” was not significant). For description of the procedure see METHODS.

EEG electrodes. Two gold wires (diameter 0.2 mm) inserted into the neck muscle served to record the EMG. The electrodes were connected to stainless steel wires that were glued to the skull with dental cement. At least 3 wk were allowed for recovery. Age at recording onset was as follows: Ola, 16.6 ± 0.8 (mean ± SE); C57, 16.6 ± 1.2 wk.

**Experimental protocol and data acquisition**

After a 24-h baseline recording the mice were recorded during a 6-h SD starting at light onset and the remaining 18-h recovery. SD was performed by gentle handling (Huber et al. 2000). The bipolar EEG recordings (occipital-cerebellum; frontal-cerebellum) and EMG signals were amplified (amplification factor ~2,000), conditioned by analog filters (high-pass filter: –3 dB at 0.016 Hz; low-pass filter: –3 dB at 40 Hz; less than –35 dB at 128 Hz), sampled with either 256 or 512 Hz, digitally filtered (EEG: low-pass FIR filter 25 Hz; EMG: band-pass FIR filter 20–50 Hz) and stored with a resolution of 128 Hz. EEG power spectra were computed for consecutive 4-s epochs by a fast Fourier transform (FFT) routine within the frequency range of 0.25–25.0 Hz. Between 0.25 and 5.0 Hz, the values were expressed in 0.5-Hz bins and between 5.25 and 25.0 Hz in 1-Hz bins. EMG signals were integrated over 4 s and ambient temperature inside the chambers was recorded at 4-s intervals. All data were recorded simultaneously and stored on optical disks. The EEG channels were calibrated with a 10-Hz sine wave, 300-μVpp signal that was recorded before each experiment.

**Vigilance states and analysis**

Vigilance states were determined for 4-s epochs as described previously (Tobler et al. 1997). Epochs containing EEG artifacts were excluded from spectral analysis (% of recording time: Ola, 10.3 ± 2.1; C57, 7.8 ± 1.9; >85% of all EEG artifacts were in waking). Vigilance states could always be determined.

Overall effects within a strain were analyzed by two-way ANOVA for repeated measures with factors “time” (1-h intervals) and “frequency bin” or “derivation” (frontal and occipital). Contrasts were tested by post hoc two-tailed t-tests only if a factor or interaction between factors reached significance.

**Time constant of the decrease of slow-wave activity**

To obtain an approximation of the time constant of the decrease of SWA in NREM sleep, an exponential function was fitted to the first six 1-h intervals after SD (Franken et al. 1991; Huber et al. 2000).

**RESULTS**

**Baseline**

Invariably, independent of strain and derivation, power in the delta range was highest in NREM sleep compared with REM sleep and waking (Fig. 1). Furthermore, power in REM sleep exceeded NREM sleep within the theta range in the occipital derivation only. A peak in waking within the theta band was more prominent in the occipital derivation compared with the frontal one in both strains, but did not exceed the values observed in REM sleep (Fig. 1). Neither the 24-h mean integrated power (0.25–25.0 Hz) over the three vigilance states nor power within the slow-wave range (0.75–4.0 Hz) in NREM sleep differed between the derivations (Table 1).

**Sleep deprivation**

After 6-h SD EEG power density in NREM sleep was massively enhanced in both derivations and strains, especially...
in the delta range (Fig. 2). In the frontal derivation the effect in the first 2 h was most prominent in the frequency bins between 0.75 and 1.5 Hz, and larger than in the occipital derivation. In the course of recovery the occipital and frontal changes affected a progressively narrowing band within the delta range in C57. A similar effect was seen in Ola in a broader frequency range. Moreover, in Ola all frequencies were initially enhanced in both derivations, and power in some of the higher frequencies remained enhanced till the end of the light period. In contrast, in C57 power in the higher frequencies in the frontal derivation was below baseline and also below power of the occipital derivation after the first 2 h of recovery (Fig. 2, right panel).

The estimation of the time constants of the decrease of SWA in NREM sleep for the first 6 h after SD revealed a significant difference between the frontal and occipital derivation in both strains (Table 2). Thus SWA in the frontal derivation showed a faster decrease. No difference was found between the strains (2-way ANOVA was not significant for factor “strain” and interaction “strain” vs. “derivation”).

The subdivision of SWA into a slow and a faster frequency band highlighted regional differences in the effect of SD. The time course of the two bands during recovery is illustrated in Fig. 3. Thus in both strains the slow band (0.75–2.5 Hz) in the frontal derivation was initially enhanced to a higher level compared with the occipital derivation. For the fast band (2.75–4.0 Hz), this result was similar but did not reach significance. However, in contrast to the slow band, a significant reversal took place in the course of recovery. Thus after the initial increase the fast band of the frontal derivation fell below the values of the occipital one in both strains. The strains differed in the time point and duration of this reversal. Moreover, in C57 similar differences between the bands already were apparent during the baseline, with higher frontal than occipital values in the lower band at the beginning of the light period and a more rapid subsequent decline (data not shown).

**DISCUSSION**

Vigilance state–specific differences were found in the EEG spectra of the frontal and occipital derivation in both strains, despite the strain differences in the spectra (Fig. 1). The prominent occipital peak in theta power in REM sleep and waking as well as its reduced appearance in the frontal derivation was similar in both strains and comparable to data obtained from similar cortical regions in the rat (Brigmann 1995; Schwerin et al. 1999). Also in the rabbit, EEG recordings from the motor cortex showed little theta activity in REM sleep or waking while in the sensory cortex a prominent theta rhythm was recorded in both states (Tobler et al. 1990). In mice the occipital electrode was over the somatosensory cortex and the hippocampus while the frontal electrode was over the motor cortex. Therefore it can be assumed that the occipital electrode primarily reflects the hippocampal theta rhythm (Vertes and Kocsis 1997).
Our main finding was the regional difference in time course of SWA in NREM sleep. Especially the initial increase of SWA after SD was larger in the frontal than in the occipital derivation and showed a faster decline (Fig. 3). This topographic difference was similar in the rat, where frequencies between 1.75 and 4.5 Hz showed a larger frontal power increase after SD compared with occipital power (Schwierin et al. 1999).

In humans EEG power in NREM sleep derived from anterior recordings exceeded power of more posterior derivations in the 2-Hz band in the first two NREM-REM sleep cycles (Wermth et al. 1996a). These topographic differences support the hypotheses that sleep has local, use-dependent features (Krueger and Obal 1995). It is tempting to assume that the differences in EEG power between derivations during sleep reflect differences in neuronal activity occurring during waking in the brain region below the recording site (frontal: motor cortex; occipital: somatosensory cortex). This interpretation is supported by results from Swanson et al. (1992), which showed increased glycogen utilization in the somatosensory cortex after facial vibrissae stimulation indicating a regionally increased metabolism.

The two-process model of sleep regulation describes the timing and structure of sleep on the basis of a circadian and homeostatic process (Borbély 1982; Daan et al. 1984). With this model SWA in NREM sleep was simulated successfully for a considerable number of experimental protocols, including the rat and mouse (e.g., Borbély and Achermann 1999; Franken et al. 1991; Huber et al. 2000). Our results show that regional differences in the dynamics of SWA become more apparent when the slow-wave band is subdivided into two frequency bands. Thus the initial, larger increase of SWA in the frontal derivation is mainly due to the contribution of frequencies belonging to the lower delta range (0.75–2.5 Hz), whereas the faster frontal power decline in the course of recovery can be attributed to the higher delta range (2.75–4.0 Hz). The lower delta frequency band corresponds to the frequency of slow cortical oscillations (<1 Hz) and thalamic clocklike oscillations (1–4 Hz), while the higher delta frequency band corresponds to the intrinsic delta activity of cortical neurons (3–4 Hz) (Amzica and Steriade 1998). Since the former two oscillations are related to hyperpolarization of neurons during sleep (Amzica and Steriade 1998), our data could indicate topographic differences of the cerebral cortex in its response to the activity of the thalamocortical system. The time course differences in regional EEG power in the two bands within the “delta” range may reflect differences in the recovery process of distinct neuronal groups.

The estimation of the time constants of the decrease of SWA resulted in a faster SWA decline in the frontal derivation compared with the occipital derivation, and did not differ between the two strains (Table 2). This result supports our previous lack of strain differences in both the time constant for SWA increase and decrease in the comparison between 129/SvJ and C57 (Huber et al. 2000). The different time constants for the two derivations (Table 2), support a difference in the underlying dynamics and could reflect different rates of recovery. This is therefore a first indication of topographic differences in the dynamics of the homeostatic Process S of the two-process model of sleep regulation in rodents (Borbély 1982; Daan et al. 1984).

In general, the strain differences in SWA were minor. However, the regional changes in the frequencies above the SWA-band were not consistent between the strains (Fig. 2). Interestingly, in Ola, power in these frequencies exceeded baseline in both derivations, and in the frontal derivation they were above occipital power. In contrast, in C57 there was no increase in frequencies above 10 Hz in either derivation, but a decrease below baseline values in frontal power. This finding in C57 is comparable to the larger decrease after SD in similar frequencies in the rat in a frontal derivation compared with an occipital derivation (Schwierin et al. 1999).

It remains to be investigated whether particular brain regions are more activated in the course of sleep deprivation, leading to a different time course of power in specific frequency bands during recovery.

We thank Drs. A. Borbély and P. Achermann for comments on the manuscript. This study was supported by the Swiss National Grant Foundation (3100-05300597).

REFERENCES


