HUMAn SLEEP AND WAKEFULNESS: FUNCTIONAL RELATIONSHIP AND REGULATORY ASPECTS

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HUMAN SLEEP AND WAKEFULNESS: FUNCTIONAL RELATIONSHIP AND REGULATORY ASPECTS

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Meiner lieben Tochter, Olivia Hofer et
à la mémoire de mon très cher papa, Joseph Tinguely
et de ma très chère grand-maman, Jeanne M. Bapst

« L'égalité des chances, c'est pour ceux qui ont de la chance »

LE THÉORÈME DU PERROQUET, Denis Guedj, Editions du Seuil, 1998
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SUMMARY

At the level of the electroencephalogram (EEG), sleep and wakefulness are characterised by different signals reflecting distinct brain activities. To gain more insight into the functional relationships and regulatory aspects of human sleep and wakefulness, the different vigilance states were compared and the analyses also focussed on the commonalities instead of the more common search for distinctions. The thesis is devoted to rapid eye movement sleep (REMS) regulation, the EEG topography in sleep and wakefulness and the effect of napping on sleep inertia and auditory learning.

REMS is characterised by low muscle tone, low amplitude EEG and rapid eye movements. To investigate an atypical feature of non-REMS (NREMS), NREMS with low muscle tone (NLMT), episodes of restricted night-time sleep (23:00 – 03.00 h) and subsequent morning sleep (10:00-13:00 h) were compared. Epochs of NLMT were more frequent in morning sleep than in night sleep. At night, the latency to the first occurrence of NLMT showed a bimodal distribution with modes at sleep onset and close to REMS onset. In morning sleep, the distribution was unimodal with the mode at sleep onset. An episode of NLMT at sleep onset occurred in 35.5% of sleep episodes without sleep onset REMS (SOREMS) in the night and in 60.9% of those in the morning. SOREMS occurred almost exclusively in the morning sleep episodes. These results provide further evidence that NLMT is determined by REMS pressure and suggest that NLMT at sleep onset represents a window for REMS.

To ascertain the extent to which EEG topography is determined by state (NREMS, REMS and waking) and sleep pressure, 27-channel EEG recordings (n=8) were obtained during a baseline night and a recovery night, and intermittently during a 40-h waking period. Sleep deprivation enhanced EEG power in the low-frequency range (1-8 Hz) in all three vigilance states. The largest increase occurred in NREMS in the delta band, in waking in the theta band, while in REMS, there was a peak in both the delta and the theta bands. The response to prolonged waking and the patterns of EEG
topography indicate that REMS is an intermediate state between NREMS and waking. Although state and sleep pressure modulate EEG power, basic topographic features appear to be state-independent. They show characteristic patterns for individuals.

To test whether sleep specifically promotes learning, or whether restful waking yields similar benefits, auditory tone sequence learning was investigated in a nap protocol. Additionally, sleep inertia was studied after this afternoon nap. Subjects (n=50) were assigned to sleep, active-wake or rest groups for a 2-hour experimental phase with polysomnography. Sleep inertia was assessed with an addition task, an auditory reaction time task and the Stanford Sleepiness Scale before (baseline tests) and in five test sessions during the hour after the experimental phase. In the first test session, addition speed in the sleep group was reduced compared with baseline and with the active-wake group. Addition speed in the sleep and rest groups increased substantially from session one to session two and reached a level similar to that of the active-wake group by session five. Auditory reaction speed of the sleep group was reduced compared with speed in baseline and of rest controls but did not differ from that of the active-wake group. The slowest reaction speeds (10th percentile) showed significant recovery after 20 min. The groups reported similar increases in subjective sleepiness after the experimental period. Auditory learning was tested after the baseline tests and after the test sessions of sleep inertia. Compared to active wakefulness, sleep between sessions of auditory learning enhanced performance improvement. Rest provided similar benefits, as could have been predicted on the basis of the perseveration-consolidation hypothesis of memory, which assumes that new memories are initially frail and need time to become resistant to interference from disintegrating factors.

Overall, my thesis revealed a remarkable resemblance between sleep and rest at a behavioural level, and among the states REMS, NREMS and waking and between low and high sleep pressure, both at the level of individual and mean EEG data.
ZUSAMMENFASSUNG

Im Elektroenzephalogramm (EEG) werden Schlaf und Wachsein durch unterschiedliche Signale charakterisiert, die unterschiedliche Hirnaktivitäten widerspiegeln. Um mehr über die funktionelle Beziehung zwischen dem menschlichen Schlaf und dem Wachsein, sowie deren regulierenden Aspekte zu erfahren, wurden die verschiedenen Vigilanzzustände verglichen und die Analysen auch auf Gemeinsamkeiten gerichtet, im Gegensatz zur verbreiteten Suche nach Unterschieden. Meine Arbeit galt folgenden Untersuchungen: der Regulation des Schlafs mit raschen Augenbewegungen (REMS), den EEG Topographien von Schlaf und Wachsein sowie der Auswirkung eines Nachmittagsschlafes auf die Schlafrträgheit und das auditorische Lernen.

REMS wird durch tiefen Muskeltonus, tiefe Amplitude im EEG und durch schnelle Augenbewegungen gekennzeichnet. Um eine atypische Erscheinung im non-REMS (NREMS), d.h. einen tiefen Muskeltonus (NLMT) zu untersuchen, wurde verkürzter Nachtschlaf (23:00 – 03.00 Uhr) mit darauf folgendem Morgenschlaf (10:00-13:00 Uhr) verglichen. NLMT-Epochen kamen im Morgenschlaf häufiger vor als im Nachtschlaf. Nachts zeigte die Latenz bis zum ersten Erscheinen von NLMT eine bimodale Verteilung mit einem ersten Maximum beim Einschlafen und einem zweiten nahe bei REMS-Auftritt. Im Morgenschlaf war die Verteilung unimodal mit dem Maximum beim Einschlafen. Eine NLMT-Episode kurz nach dem Einschlafen kam in 35.5% der Nacht- und in 60.9% der Morgenschlafepisoden vor, die kein SOREMS (REMS kurz nach dem Einschlafen) hatten. SOREMS kam fast ausschliesslich in Morgenschlafepisoden vor. Diese Ergebnisse weisen darauf hin, dass NLMT durch REMS-Druck bedingt ist, und legen nahe, dass NLMT beim Einschlafen ein Fenster für REMS darstellt.

Um das Ausmass zu bestimmen, in welchem die EEG Topographie durch Vigilanzzustand (NREMS; REMS und Wachsein) und Schlafdruck bedingt ist, wurden 27-Kanal EEG-Registrierungen (n=8) einer Kontroll-


Insgesamt offenbarte meine Arbeit bemerkenswerte Ähnlichkeiten zwischen Schlaf- und Ruhezustand auf der Ebene des Verhaltens und zwischen REMS, NREMS und Wachzustand und zwischen tiefem und hohem Schlafdruck sowohl auf der Ebene der individuellen wie auf der Ebene der Durchschnitts-EEG-Werte.
List of abbreviations

Ach: acetylcholine
ANOVA: analysis of variance
BOLD: blood oxygenation level dependent
BL: baseline
BW: busy waking
DEP: deprivation
ECG: electrocardiogram
EEG: electroencephalogram
EMG: electromyogram
EOG: electrooculogram
FIR: finite impulse response
FFT: fast Fourier transform
fMRI: functional magnetic resonance imaging
GABA: gamma-aminobutyric acid
GAD: glutamic acid decarboxylase
GAL: galalin
Hz: Hertz (1/s)
ISI: interstimulus interval
KSS: Karolinska sleepiness scale
LC: locus coeruleus
LDT: laterodorsal tegmental nuclei
MAOI: monoamine oxidase inhibitor
µV: microvolt
NA: noradrenalin
NB: no-break
NLMT: NREMS with low muscle tone
NREMS: non rapid eye movement sleep
PET: Positron Emission Tomography
PGO: ponto-geniculo-occipital
Process S: one of the two fundamental processes of the model of sleep regulation; it keeps track of the sleep and waking time history
PPT: pedunculo-pontine tegmental nuclei
rANOVA: repeated measures ANOVA
rCBF: regional cerebral blood flow
RE / REC: recovery
REMS: rapid eye movement sleep
R-wave: the initial positive deflection of the principal deflection in the electrocardiogram called QRS complex and representing ventricular depolarization
RW: restful waking
SEM: standard error of the mean
SOREMS: sleep onset REMS (REMS latency < 20 min)
SSS: Stanford sleepiness scale
SWA: slow-wave activity (EEG power in the 0.75 – 4.5 Hz range)
SWE: slow wave energy (cumulative SWA in NREMS)
SWS: slow wave sleep (NREMS stages 3 + 4)
TMN: tuberomamillary nucleus
TST: total sleep time
VLPO: ventrolateral preoptic area
W: waking
WASO: waking after sleep onset
5-HT: 5-hydroxytryptamin (serotonin)
Sleep belongs to our daily life since birth or even earlier but unlike other happenings, sleep can not be experienced consciously. Sleep must have an important physiological function. This can be inferred from three facts: first, sleep takes about one third of our lifetime. Second, sleep deprivation has detrimental effects on cognitive behavioural performance (Rajaratnam and Arendt, 2001; Van Dongen et al., 2003) and possibly on health (Rajaratnam and Arendt, 2001; Spiegel et al., 2003). Third, it is widespread in the animal kingdom, although a state of reduced responsiveness may be life threatening. Therefore this recurrent state of diminished awareness must provide an outstanding adaptive advantage. In order to be able to appraise this advantage, it is necessary to find out what the function of sleep is. Several functions have been proposed, but none can satisfactorily explain all the aspects of sleep.
Figure 1.1. The six panels show each a 20-s epoch of wakefulness, NREMS (stages 1-4) and REMS, respectively, with EEG (upper trace), EOG (middle trace) and EMG (lower trace). Scale bars on the left = 75μV
It appears almost certain that sleep subserves not one but many functions. It has been proposed that sleep serves bodily regeneration or energy conservation (Siegel, 2005). Other functions suggested are replenishment of glycogen storage (Benington and Heller, 1995), to provide a pattern of stimulation to maintain the synaptic structure not used during wakefulness (Krueger and Obál, 1993), synaptic downscaling (Tononi and Cirelli, 2003) and memory consolidation (Hasselmo, 1999; Maquet, 2001; Walker and Stickgold, 2004). Glycogenesis, which occurs in astrocytes during slow wave sleep (SWS), has been postulated to provide energy for rapid eye movement sleep (REMS; Jouvet, 1998). To study the function of sleep, many different ways are possible: from simply observing the behaviour of sleeping humans or animals to using sophisticated techniques in various settings.

Sleep architecture and sleep physiology

The most commonly used tool in sleep research is the electroencephalogram (EEG), which was discovered 1929 by Berger (Berger, 1929). The EEG represents brain electrical activity recorded by means of electrodes, which are applied on the scalp in humans. For sleep stage scoring (Fig. 1.1) not only an EEG recording is needed but also the electrooculogram (EOG) measuring eye movements and the electromyogram measuring sub-mental muscle tone (Rechtschaffen and Kales, 1968). Wakefulness is characterised by alpha activity (activity in the frequency range of 8 to 10 Hz) and/or a high frequency, low amplitude EEG. The EMG is usually high and often eye movements and blinks appear in the EOG (Fig. 1.1, upper panel). Human sleep is divided into two substates: non-REMS (NREMS) and REMS, with NREMS comprising four stages (1 to 4, Fig. 1.1) reflecting increasing sleep depth (Rechtschaffen
NREMS and REMS alternate across the night in cycles of approximately 90 minutes. One cycle consists of a NREMS episode followed by a REMS episode. The length of a cycle only slightly varies in length within a subject. Variations across subjects may be quite high, though. The number of cycles correlates with total habitual sleep time (Le Bon et al., 2005; Fig. 1.2). Mean cycle length does not vary between a nocturnal and a morning sleep episode, whereas NREMS and REMS episodes do (Fig. 1.3). NREMS episodes are longer in the first half of the night and shorter in a morning sleep episode, for REMS episodes, it is the opposite. NREMS, particularly the stages 3 and 4, which are often taken together and referred to as slow wave sleep (SWS, Fig. 1.4, upper panels), predominates in the first half of the night. In parallel with

**Figure 1.2** NREM/REMS cycle length as a function of the number of cycles (means and SEM). The shorter the habitual sleep time (indicated in min), the longer the cycles. n=84 subjects (47 women), age 15-45 years. Means and 95 % confidence interval. Data from Table 1 in Le Bon et al., (2001)
SWS, slow wave activity (SWA, EEG power in the frequency range of 0.75 Hz to 4.5 Hz, computed by a fast-Fourier transform of EEG recording) is highest at the beginning and declines roughly exponentially in the course of a night (Fig. 1.4, lower panels). REMS increases in length in the course of a night and prevails in the second half of the night together with stage 2 (Fig. 1.4, upper panels).

After sleep onset, sleep deepens continuously as indicated by the increasing number of slow waves in the EEG, albeit with short interruptions, from stage 1 to stage 4. Stage 1 is characterised by a dominance of theta (4.75 to 8 Hz) activity, sharp waves and slow pendular eye movements. In stage 2, the EEG activity becomes slower and typical phasic events arise: spindles,
waxing and waning short synchronised oscillations in the frequency range of 11 to 15 Hz and K-complexes, conspicuous sharp negative waves immediately followed by a positive component and facultatively by a spindle. In stages 3 and 4, the EEG is dominated by high amplitude slow waves in the frequency range of 0.75 to 4.5 Hz (delta).

At regular intervals, EEG oscillation suddenly changes. It becomes faster and the amplitude smaller, and resembles stage 1. This is the appearance of REMS. Rapid eye movements, which gave this stage the name, take place in episodic bursts. Head and neck muscle tone is very low, decreasing very often before the onset of REMS (Bliwise et al., 1974; Brunner et al., 1990) and being elevated only during short twitches. The rapid eye movements may also be associated with phasic endogenous waves appearing consecutively in the pons (P), lateral geniculate bodies of the thalamus (G) and the occipital cortex (O). These PGO waves have first been recorded in the cat (Mouret et al., 1963).

**Figure 1.4.** Stages (upper panels) and slow wave activity (SWA, 0.75-4.5 Hz; lower panels) for a baseline (left) and a recovery night (right) after prolonged wakefulness of 40 hours. SWA and REMS are mutually exclusive. SWA dominates the first half of the night and REMS the second half. REMS episodes increase in length during the course of a night and SWA declines exponentially. After sleep deprivation, SWA reaches higher levels at the beginning of a night.

*M: movement time; W: waking; R: REMS; 1-4: NREMS stages 1 to 4*
NREMS and REMS are not only characterised by different brain electrical activity, but also by distinct patterns of functional anatomy. During SWS, regional cerebral blood flow (rCBF) is decreased relative to waking in centrencephalic structures, heteromodal association cortices of the orbital, dorsolateral, prefrontal and inferior parietal lobes, but not in unimodal occipitotemporal sensory cortices (Braun et al., 1997; Finelli et al., 1998). During REMS, rCBF is elevated relative to both waking and NREMS in centrencephalic and neocortical regions (Maquet et al., 1996; Braun et al., 1997; Braun et al., 1998; Finelli et al., 1998) and decreased in the dorsolateral prefrontal and lateral orbital cortices, operculum, angular and supramarginal gyri, posterior insula and posterior cingulate cortex (Maquet et al., 1996; Braun et al., 1997; Braun et al., 1998; Finelli et al., 2000b).

Differences between the brain states arise from differential neuronal activity in specific brain regions, which can be seen at the level of electrophysiology (Steriade, 2005) and neurochemistry ((Jones, 2005; Table 1.1). The main neuronal clusters regulating wakefulness (Fig. 1.5) and sleep (Fig 1.6) are shown with their projections. Wakefulness is produced by activity in the ascending arousal system (Fig 1.5). This system consists of cholinergic pedunculopontine (PPT) and laterodorsal tegmental nuclei (LDT). Their neurons project to forebrain structures and the thalamus, which regulate cortical activity. The other component of this system consists of the aminergic nuclei: the noradrenergic locus coeruleus (LC), the serotonergic Raphé nucleus and the histaminergic tuberomammillary nucleus (TMN) projecting diffusely to the cortex. The orexin/hypocretin neurons of the lateral hypothalamic area project to all components of the arousal system, including the cholinergic basal forebrain, and cortex. These neurons increase the activity of the arousal system and help so maintaining wakefulness (Saper et al., 2001; McGinty and Szymbusiak, 2005; Saper et al., 2005). It is hypothesised that sleep is induced by
**Table 1.1. Neurochemicals implicated in controlling the different brain states (Jones, 2005)**

<table>
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<tr>
<th></th>
<th>Waking</th>
<th>NREMS</th>
<th>REMS</th>
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<tr>
<td><strong>Chemicals:</strong></td>
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<tr>
<td>Norepinephrine</td>
<td>Serotonin (at sleep onset, but then lower during SWS and REMS than during waking)</td>
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<td>Acetylcholine</td>
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<td>Epinephrine</td>
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<td>Dopamine</td>
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<tr>
<td>Acetylcholine</td>
<td>Adenosine</td>
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<tr>
<td>Histamine</td>
<td>GABA (release in posterior hypothalamus higher during SWS than during REMS or waking)</td>
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<tr>
<td>Glutamate</td>
<td></td>
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<td></td>
<td><strong>CSF – borne factors and peptides:</strong></td>
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<tr>
<td>Substance P</td>
<td>Opiates (enkephalin, endorphin, dynorphin)</td>
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<td>Vasoactive intestinal peptide (VIP)</td>
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<td>Neurotensin</td>
<td>Corticotropin-releasing factor</td>
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<td>Corticotropin-releasing factor</td>
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<td>Thyrotropin-releasing factor</td>
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<td>Orexin/Hypocretin</td>
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<td><strong>Blood borne factors:</strong></td>
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<tr>
<td>Epinephrine</td>
<td>Serotonin</td>
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<tr>
<td>Norepinephrine</td>
<td>Insuline</td>
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<td>Histamine</td>
<td>Cholecystokinin</td>
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<tr>
<td>Corticotropin</td>
<td>Muramyl peptides (stimulate synthesis and release of cytokines)</td>
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<tr>
<td>Thyrotropin</td>
<td>Delta sleep-inducing peptide</td>
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<tr>
<td>Cortisol</td>
<td>Growth hormone</td>
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<td></td>
<td>Prolactin</td>
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neurons of the ventrolateral preoptic area (VLPO, Saper et al., 2001; McGinty and Szymusiak, 2005; Saper et al., 2005), Fig. 1.6) and of the median preoptic nucleus (McGinty and Szymusiak, 2005). The neurons of the VLPO innervate all components of the ascending arousal system, which project back to the VLPO. They contain the gamma-aminobutyric acid (GABA)-synthesising enzyme glutamic acid decarboxylase (GAD) and the peptide galanin (Saper et al., 2001; Saper et al., 2005). By firing, they inhibit the arousal system to promote sleep (Saper et al., 2001; Saper et al., 2005). During wakefulness, they are silent.

The VLPO consists of a cluster specialised for the control of NREMS and an extended region controlling REMS (Saper et al., 2001; McGinty and Szymusiak, 2005; Saper et al., 2005). Regular-spiking neurons represent the majority of neocortical neurons (Steriade, 2005). Their firing rate is characterised by cyclic prolonged hyperpolarisations during NREMS and tonic discharge during REMS (Steriade, 2005). During NREMS, the firing rates of Raphé, LC and TMN neurons are lower than during waking, and the PPT and LDT nuclei are silent (Saper et al., 2001; Steriade, 2005). While Raphé, LC and TMN neurons are virtually silent during REMS, the cholinergic activation of cerebral cortex by basal forebrain neurons, which are driven by brainstem reticular neurons, is even higher than during waking (Saper et al., 2001; Steriade, 2005).
**Figure 1.5.** The ascending arousal system sends projections from the brainstem and posterior hypothalamus throughout the forebrain. Neurons of the laterodorsal tegmental nuclei and pedunculopontine tegmental nuclei (LDT and PPT) (blue circles) send cholinergic fibers (Ach) to many forebrain targets, including the thalamus, which then regulate cortical activity. Aminergic nuclei (green circles) diffusely project throughout much of the forebrain, regulating the activity of cortical and hypothalamic targets directly. Neurons of the tuberomammillary nucleus (TMN) contain histamine (HIST), neurons of the raphé nuclei contain 5-HT and neurons of the locus coeruleus (LC) contain noradrenaline (NA). Sleep-promoting neurons of the ventrolateral preoptic nucleus (VLPO, red circle) contain GABA and galanin (Gal).

Reproduced from (Saper et al., 2001)

**Figure 1.6.** The projections from the ventrolateral preoptic nucleus (VLPO) to the main components of the ascending arousal system. Axons from the VLPO directly innervate the cell bodies and proximal dendrites of neurons in the major monoamine arousal groups. Within the major cholinergic groups, axons from the VLPO mainly innervate interneurons, rather than the principal cholinergic cells.

Abbreviations: LC, locus coeruleus; LDT, laterodorsal tegmental nuclei; PPT, pedunculopontine tegmental nuclei; TMN, tuberomammillary nucleus; VLPO, ventrolateral preoptic nucleus. The blue circle indicates neurons of the LDT and PPT; green circles indicate aminergic nuclei; and the red circle indicates the VLPO.

Reproduced from (Saper et al., 2001)
REM sleep regulation

While it is difficult to directly study the function of sleep, it is possible to make a detour through the investigation of its regulation to try to encircle the problem. For NREMS, especially slow wave activity, the regulation is well described by the Two Process Model of sleep regulation (Borbély, 1982; Daan et al., 1984). The Two Process Model can predict levels of SWA in different experimental situations like in sleep after sleep deprivation (Achermann et al., 1993) or after a daytime nap (Werth et al., 1996). It can also predict changes in SWA in short and long sleepers (Aeschbach et al., 1996). Nothing similar is available for REMS although more than fifty years passed since the discovery of REMS by Aserinsky and Kleitman (1953). Homeostatic and circadian factors can be distinguished, and there are indications of a sleep dependent disinhibition of REMS (see Borbély, 1982; Dijk and Czeisler, 1995; Endo et al., 1998; Werth et al., 2002 for overviews and discussion). Selective REMS deprivation augments the homeostatic ‘REMS pressure’, which is manifested by an increasing number of interventions required to prevent REMS both within a night and across consecutive nights (Dement, 1960; Endo et al., 1998; Werth et al., 2002; for older literature see also Borbély, 1982). The REMS rebound during recovery sleep, however, is smaller than the accumulated deficit (Endo et al., 1998; Werth et al., 2002). So either REMS is intensified in some way or compensated by other features during NREMS or waking, or the process is protracted, as suggested by Dement (1960). Alternatively, this REMS time could also be lost. If this is the case then not all REMS time is needed (Horne, 2000) or it is not possible to make good for it. At least, this produces no obvious detrimental effects. In the treatment of depression with monoamine oxidase inhibitors, REMS is completely suppressed, at least in the beginning of the treatment (Landolt et al., 2003). The only major effect seen is
an improvement of mood. This in turn does not mean that the suppression of REMS is responsible for the amelioration of mood, as some effective antidepressants do not affect REMS (Wilson and Argyropoulos, 2005) and selective REMS deprivation does not necessarily improve the mood (Grözinger et al., 2002). Moreover, REMS re-emerges with chronic MAOI (monoamine oxidase inhibitor) treatment (Landolt and de Boer, 2001).

Circadian aspects of REMS regulation were investigated under free-running conditions (Czeisler et al., 1980a; Weitzman et al., 1980), in forced desynchrony protocols (Dijk and Czeisler, 1995), altered sleep-wake schedules (Carskadon and Dement, 1980; Dantz et al., 1994) and in naps taken at different times of day (Endo et al., 1981). Czeisler et al. (1980a) reported a correlation between the body temperature rhythm as a circadian marker and the rate of REMS accumulation and REMS latency in an environment free of time cues. Sleep onset REMS (SOREMS; REMS latency < 20 min) episodes reliably occurred when subjects went to bed just after the trough of core body temperature (Czeisler et al., 1980a; Czeisler et al., 1980b; Weitzman et al., 1980).

In normal nocturnal sleep, SOREMS episodes are rare (Fig. 1.7). But under certain experimental conditions, e.g. in the case of high REMS pressure, they are not uncommon (Fig. 1.7). SOREMS episodes can be frequent in depressed patients (Kupfer, 1976; Schulz and Tetzlaff, 1982; Schulz and Lund, 1985) and are common in narcoleptic patients (Guilleminault, 2000). In infants, SOREMS episodes are the rule rather than the exception (Coons and Guilleminault, 1984).
Figure 1.7. Vigilance states and slow wave activity (SWA) in the four night sleep (left pairs of panels) and subsequent morning sleep episodes (right pairs of panels) of one subject of the study presented in chapter 2.1. One night and all four morning sleep episodes started with a sleep onset REMS (SOREMS, highlighted in green) episode. Due to the four hours of sleep at night, NREMS pressure is at a lower level at the beginning of the morning sleep episode than at the beginning of night sleep. This results in reduced SWA and increased REMS, which is moreover favoured by the circadian time of the sleep episode. 

Napping and sleep inertia

How long we sleep and whether we sleep during one single night time period or during several episodes distributed over the day is not only determined by biology, but is as much influenced by culture and climate. For instance in Mediterranean regions, the siesta at the hottest period of the day is usual. This is not easily imaginable in northern countries, where noon is for a long period of the year the only time with sunshine. In the western industrious countries, napping is rather the exception and still has the smack of laziness. But exactly this industriousness led to work scheduled around the clock, which may promote naps to further enhance productivity. Productivity is not only a goal in industry, students equally dream of learning more efficiently in shorter time. What could then be more appealing than the conclusion that for learning “a nap is as good as a night” (Mednick et al., 2003)?

The potential benefits of a nap may not be realised immediately, because awakening is typically accompanied by a period of impaired performance known as sleep inertia (Lubin et al., 1976). Thus, sleep inertia represents a serious contraindication to napping if emergency operations at the time of awakening require immediate high levels of performance. Sleep inertia has also to be dissipated before the retest of a learning study because it could mask performance improvement induced by the nap.

This state of confusion at awakening with intrusions of dream like speech and subsequent amnesia for it in severe cases has no correlate in the EEG trace, as has been shown in a study comparing EEG before and after sleep (Dinges, 1990). To measure sleep inertia, sleepiness scales and various performance tasks are used. It is generally assumed that changes in subjective alertness and performance that occur in the time period after awakening reflect recovery from sleep inertia. However, other factors that change with
time, such as learning or loss of motivation, also alter performance. In addition, differences among tasks and different aspects of performance improvement may complicate the outcome of sleep inertia measures. This results in inconsistencies among the findings of the different studies.

Sleepiness, falling asleep and waking up refreshed

Although falling asleep and waking up are assumed to be controlled by a hypothalamic switching mechanism (Saper et al., 2001; McGinty and Szymusiak, 2005; Saper et al., 2005), the transition from wake to sleep and back does usually not occur between full alertness and deep sleep. Drowsiness around the transitions between wakefulness and sleep does not appear nor dissipate synchronously in all brain regions. For instance in drowsy monkeys, single cell recordings from extrastriate area V4 may show a spiking pattern resembling sleep while the animal is performing a visual search task indicating that the primary visual cortex was involved in visual processing and was therefore alert (Pigarev et al., 1997). How much time is needed to fall asleep depends on the time of day, on the sleep-wake history, on the circumstances and on the health of the sleeper. And although sleep and waking are global brain processes, they also have a local aspect. During sleep different brain areas have different amounts of slow wave activity depending on prior use (Kattler et al., 1994; Huber et al., 2004). After awakening different brain regions become fully activated at different rates (Balkin et al., 2002). This can be deduced from an imaging study, in which cerebral blood flow did not increase at the same rate in all brain parts after awakening (Balkin et al., 2002). The local aspect of the awakening process is also evident from the different time courses of different cognitive or reaction time tasks used to assess sleep.
inertia (e.g. Ferrara et al., 2000; Tassi and Muzet, 2000). Although we sleep to feel refreshed, after the awakening in the morning, we feel sleepy for a while before being able to enjoy the restorative effects of the night. Sleepiness immediately after a period of sleep may be greater than before going to sleep if we sleep for longer than about twenty minutes (Horne and Reyner, 1996).

But what is sleepiness actually? In studies, it is defined by some sleepiness scale, visual analogue scale or the Multiple Sleep Latency Test. It is not a clearly defined state, but together with the question of how much sleep we need, a controversial subject (Dinges, 2004; Horne, 2004). Feeling tired can be described in different ways because sleepiness is not a single entity (Horne, 1991). In the EEG, alpha and theta activity are considered physiological markers of sleepiness, with alpha activity decreasing and theta activity increasing with sleepiness (Akerstedt and Folkard, 1997; Finelli et al., 2000a; Strijkstra et al., 2003). In a study, the authors summed both, alpha and theta activity (4-11 Hz), to get more consistent findings, i.e. to get a correlation with the Karolinska Sleepiness Scale (KSS; Horne and Reyner, 1996). In another study, when alertness was medium to high (8-14 on the KSS), the authors found no increase of alpha activity (Akerstedt and Folkard, 1995).

Sleep and learning

Since a few years, the question of whether sleep is beneficial to memory and if indeed it is, how long we must sleep to improve the learned material or skill, has become a topic of high interest and controversy, but also a very prolific area of research. It is investigated in many species from the fly to the human and involves research at the behavioural, neuronal, molecular and genetic level. The topic has recently been reviewed in comprehensive manner (Vertes
and Eastman, 2000; Siegel, 2001; Stickgold et al., 2001; Gais and Born, 2004; Vertes, 2004; Walker and Stickgold, 2004; Walker, 2005). The relationship between sleep and memory remains difficult to characterise because both processes are complex and much is still unknown.

When learning is investigated, plenty of different tasks, performance measures and training schedules are possible not to forget the different amounts of sleep and all the different manners to spend the time between the training sessions and between training and final testing. All these differences in experimental protocols contribute to the great variety of outcomes and interpretations concerning the effect of sleep on learning and memory. There are declarative and procedural tasks, and both categories may be learned implicitly or explicitly. Procedural tasks may be motor or sensory tasks or a combination of both, and may even involve a declarative part. Learning tasks may be simple or complex, novel or familiar, have a long or short training duration and may involve neutral or highly emotional content. To complicate matters further, different aspects of memory processing can be investigated. After the memory acquisition phase there is consolidation, which may include stabilisation and enhancement of memory (Walker, 2005). Afterwards memory association, integration of new information with existing knowledge and past experiences, reorganisation, translocation, reconsolidation and even erasure may take place (Walker and Stickgold, 2004; Walker, 2005). All of these latter stages have not yet been investigated (Walker and Stickgold, 2004; Walker, 2005). Moreover, the memory recall test may be at any time between these stages adding further variety to the testing protocols and with it to the results.
Objectives of my thesis

The aim of my dissertation was to gain more insight into processes, which regulate and characterise sleep and wakefulness. The goal of the study presented in chapter 2.1 was to investigate the relationship of periods of NREMS with low muscle tone to the regulation of REMS. The intention of the following study was to examine the spatial distribution of EEG power over the skull in order to characterise distinct and similar features among the vigilance states (waking, NREMS and REMS) and under conditions of different sleep pressure (chapters 2.2 and 2.3). A further purpose of the thesis was to assess sleep inertia and its time course following an afternoon nap. This analysis is the content of chapter 3.1. The next chapter (3.2) is dedicated to the question of whether sleep specifically benefits learning, or whether a corresponding time spent resting is sufficient to improve cognitive performance.
REM sleep regulation and EEG topography in sleep and waking
Non-rapid eye movement sleep with low muscle tone as a marker of rapid eye movement sleep regulation

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Abstract

Background
It was recently reported that epochs of non-REM sleep (NREMS) with low muscle tone represent a partial correlate of REM sleep (REMS). To further investigate this phenomenon, episodes of restricted night-time sleep (23:00 – 03:00 h) and subsequent morning sleep (10:00 - 13:00 h) were analysed.

Results
Epochs of NREMS with low muscle tone (NLMT) were identified. Their frequency was higher in morning sleep than in night sleep. At night, the latency to the first occurrence of NLMT showed a bimodal distribution with modes at sleep onset and close to REMS onset. In morning sleep, the distribution was unimodal with the mode at sleep onset. An episode of NLMT at sleep onset occurred in 35.5% of the night sleep episodes and in 60.9% of the morning sleep episodes without sleep onset REMS (SOREMS). Also SOREMS occurred predominantly in morning sleep. REMS episodes were longer and NREMS episodes shorter in morning sleep than in night sleep, whereas cycle duration did not differ. Simulating the time course of slow-wave activity revealed a close correspondence between empirical and computed values for night sleep, and some discrepancies for morning sleep.

Conclusions
The results provide further evidence that NREMS with low muscle tone is a marker of REMS regulation. NLMT at sleep onset may represent an early manifestation of REMS.
Background

REM sleep (REMS) is characterised by rapid eye movements, a mixed frequency, low amplitude EEG, and a low submental muscle tone with phasic twitches. At the transition from non-REM sleep (NREMS) to REMS these three features do not appear synchronously. The EMG level may decrease prior to the occurrence of the two other markers, and may also persist for some time after the end of REMS (Bliwise et al., 1974; Brunner et al., 1990a). NREMS with low muscle tone (NLMT) was observed not only before and after REMS but also at sleep onset in a selective REMS deprivation study. NLMT was enhanced by total sleep deprivation and selective REMS deprivation (Werth et al., 2002a). It was proposed that epochs of NLMT could be correlates of REMS and therefore serve as markers of REMS regulation.

The aim of the present study was to further investigate the relationship between NLMT and REMS by analysing the first 3 h of a 4-h nocturnal sleep episode and a subsequent 3-h daytime sleep episode.

Results

REMS latency and SOREMS episodes
REMS latency showed a unimodal distribution with a maximum at 65 min during night sleep and a bimodal distribution with modes at 5 and 55 min and a trough at 25 min during morning sleep (Fig. 2.1.1 A, B). One single sleep onset REMS (SOREMS; REMS latency < 20 min) episode occurred at night, while a total of 40 SOREMS episodes were observed during morning sleep.
**Table 2.1.1: SOREMS episodes, episodes of NLMT, slow wave activity**

<table>
<thead>
<tr>
<th></th>
<th>Night sleep</th>
<th>Morning sleep</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SOREMS episodes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number / total number of sleep episodes</td>
<td>1/63</td>
<td>40/63</td>
</tr>
<tr>
<td>Duration [min], mean±SEM, n=14</td>
<td>12.8±2.8</td>
<td></td>
</tr>
<tr>
<td>Latency [min], mean±SEM, n=14</td>
<td>7.6±1.3</td>
<td></td>
</tr>
<tr>
<td><strong>Frequency of epochs of NLMT</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=16)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number / number of epochs of NREMS [%]</td>
<td>19.7±3.1</td>
<td>36.2±5.0*</td>
</tr>
<tr>
<td><strong>NLMT episodes at sleep onset</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number / total number of sleep episodes without SOREMS</td>
<td>22/62</td>
<td>14/23</td>
</tr>
<tr>
<td>Duration [min], mean±SEM</td>
<td>19.2±6.6 (n=12)</td>
<td>19.9±6.3 (n=7)</td>
</tr>
<tr>
<td>Latency [min], mean±SEM</td>
<td>6.4±1.2 (n=12)</td>
<td>1.5±0.5 (n=7)</td>
</tr>
<tr>
<td><strong>Slow-wave activity</strong> (n=16)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SWA [µV²] mean±SEM</td>
<td>552.6±42.3</td>
<td>361.3±33.7**</td>
</tr>
<tr>
<td>SWE [µV²s] mean±SEM</td>
<td>39772.3±4220.0</td>
<td>20293.7±1853.0**</td>
</tr>
</tbody>
</table>

SOREMS: Sleep Onset REMS episodes (REMS episodes with latency < 20 min).
NLMT: NREMS with low muscle tone. For NLMT at sleep onset, only sleep episodes without SOREMS were considered. SWA: mean slow wave activity (EEG power in the 0.75 – 4.5 Hz range) in the first NREMS episode. SWE: slow wave energy (cumulative SWA in NREMS) in the first NREMS episode.

* p < 0.01, ** p < 0.001; comparison of night and morning sleep episodes by paired t-tests
NREMS with low muscle tone is a marker of REMS regulation.

**Figure 2.1.1:** Distribution of REMS latencies (black bars) in 63 night (A) and 63 morning (B) sleep episodes. Grey bars: latency to the second REMS episode (after a SOREMS episode) measured from sleep onset. C: Distribution of intervals (i.e. length of first NREMS episode) between SOREMS episodes and subsequent REMS episodes. Distribution of the latency to the first occurrence of low muscle tone in NREMS (NLMT) that occurred prior to the first REMS episode in 60 night (D) and 61 morning (E) sleep episodes. In 3 night and 19 morning sleep episodes, no NLMT was observed prior to the first REMS episode. Zero on the abscissa represents sleep onset defined as the first epoch of stage 2 or REMS. Negative values correspond to stage 1. Data were plotted in 10-min bins.
Chapter 2.140

Table 2.1.1). At night, REMS latencies ranged from 0 to 164 minutes. The four longest latencies were from a single individual with a ‘skipped’ first REMS episode in all four nights (i.e. an episode with reduced SWA occurring at the expected time of REMS). In the morning, REMS latencies ranged from 0 to 99 minutes. The duration of NREMS episodes following a SOREMS episode ranged from 40 to 85 min (Fig 2.1.1C); this range is comparable to that of REMS latencies at night (Fig. 2.1.1A).

Seven of 16 subjects showed SOREMS episodes in all four morning sleep episodes, one subject in three, three in two and three in one episode. Only two subjects had no SOREMS episode.

Figure 2.1.2: Time course of (A) EMG variance (i.e. total power), (B) sleep profile (MT, movement time; 1 to 4, NREMS stages 1 to 4), and (C) slow-wave activity (SWA) of an individual for a night and a morning sleep episode. The horizontal red lines in A indicate the threshold that was predetermined to identify episodes of low muscle tone (see Methods). Note the episodes with low level of EMG variance during NREMS at sleep onset (red arrow) and later in the night. In the morning, the episodes of NLMT are longer than in night sleep and often, the muscle tone remained low during the entire recording.
Episodes of NLMT

Epochs of NREMS with low muscle tone (NLMT) are illustrated for one subject (Fig. 2.1.2). The latency to the first appearance of NLMT showed a bimodal distribution with modes at 5 and 55 min in night sleep (Fig. 2.1.1D). The first mode was centred at sleep onset and the second mode was situated in the proximity of REM sleep onset. The distribution closely resembled the distribution of REMS latencies in morning sleep. A unimodal distribution with a mode at 5 min was observed in morning sleep (Fig. 2.1.1E). Epochs of NLMT were more frequent in morning sleep than in the first 3 hours of night sleep (Table 2.1.1; note that in Fig 2.1.1E the sleep episodes with SOREMS are also included).

In night sleep, 22 episodes of NLMT at sleep onset were observed (Table 2.1.1). The one subject with four 'skipped' first REMS episodes at night showed neither SOREMS episodes nor episodes of NLMT at sleep onset. The other subject without SOREMS episodes had no episode of NLMT at sleep onset at night but three in the morning.

In 14 of the 23 morning sleep episodes without SOREMS, episodes of NLMT at sleep onset were observed. Their length was similar in night and morning sleep.

Slow wave activity

The mean time course of SWA during night and morning sleep is plotted in Fig. 2.1.3. Mean SWA in the first NREMS episode of morning sleep was lower than in the first NREMS episode of night sleep (Table 2.1.1). Slow-wave energy (cumulative SWA in NREMS) of the first NREMS episode was also lower in the morning than at night.

Simulations of Process S were performed (Fig. 2.1.3D) to test whether the changes in SWA were in accordance with the two-process model of sleep.
The level of $S$ at sleep onset of the 4-hour night-time episode was set to 100%. Based on the average timing of the first NREMS episode and the previously defined time constants (Daan, Beersma et al. 1984), the level of Process $S$ at episode midpoint of the first NREMS episode was calculated as 82.7%. This value equals average SWA of first NREMS episode and served as the reference value for the calculation of relative SWA in the second NREMS episode of the night (61.2%) and the first NREMS episode of the morning (54.5%). * $S$ outside the 95% confidence interval of SWA data. Black bars denote sleep episodes.

**Figure 2.1.3:** Time course of slow-wave activity (SWA) in night (A) and morning sleep episodes (B: mornings without SOREMS episodes; C: mornings with SOREMS episodes). NREMS episodes were subdivided into 20 equal intervals and REMS episodes into 5 equal intervals. Mean values per interval were calculated within subjects prior to averaging across subjects (mean ± SEM). For night sleep, only data of recordings with 2 complete cycles were included. Black bars at the top delineate REMS episodes. D: Simulation of homeostatic Process $S$ (solid line) and time course of empirical slow-wave activity (SWA) in consecutive NREMS episodes (filled circles; ± 95% confidence interval).
NREMS with low muscle tone is a marker of REMS regulation (Daan et al., 1984). Process S represents the homeostatic (i.e., sleep-waking dependent) process of sleep regulation. Its time course was derived from EEG SWA (Daan et al., 1984). The simulations were based on a regular sleep-wake schedule (sleep: 23:00 to 7:00) followed by a 4 h sleep episode at night (23:00 to 3:00) and a 3 h daytime sleep episode (10:00 to 13:00). The time constants (increase of S: 18.2 h; decrease of S: 4.2 h) corresponded to those used in Daan et al. (1984). The simulations revealed a reduced level of S (sleep propensity) at sleep onset in the morning (77.5 %) compared with the level at sleep onset at night (100 %). For night sleep, a close correspondence between empirical SWA and the simulated level of S was observed (i.e., S was within the 95 % confidence interval of empirical SWA of the second NREMS episode). In the first NREMS episode of morning sleep, however, the predicted level of S was above the empirical values (S outside 95 % confidence interval).

Sleep variables derived from visual scoring (Table 2.1.2)
Waking after sleep onset, the duration and percentage (% of total sleep time) of REMS and stage 1 were higher in the morning than at night, whereas the amount of stages 2, 3 and 4 was lower. Sleep latency was shorter in the morning than at night whereas REMS latency did not differ if SOREMS was excluded. Total sleep time, sleep efficiency, and movement time did not differ between night and morning sleep episodes.

Cycle and episode duration (Table 2.1.2)
Sleep cycle duration did not differ between night and morning sleep. REMS episodes, however, were longer and NREMS episodes shorter in morning sleep than in night sleep.
<table>
<thead>
<tr>
<th>Variable</th>
<th>Night [min]</th>
<th>Night [%]</th>
<th>Morning [min]</th>
<th>Morning [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total sleep time</td>
<td>162.92±1.76</td>
<td></td>
<td>165.76±0.97</td>
<td></td>
</tr>
<tr>
<td>Sleep efficiency</td>
<td>90.51±0.98</td>
<td></td>
<td>92.37±0.53</td>
<td></td>
</tr>
<tr>
<td>WASO</td>
<td>2.39±0.61</td>
<td></td>
<td>5.30±0.83**</td>
<td></td>
</tr>
<tr>
<td>Sleep latency</td>
<td>11.47±1.38</td>
<td></td>
<td>5.28±0.58**</td>
<td></td>
</tr>
<tr>
<td>REMS latency</td>
<td>72.26±5.54</td>
<td></td>
<td>58.18±4.00°</td>
<td></td>
</tr>
<tr>
<td>Movement time</td>
<td>3.28±0.36</td>
<td></td>
<td>2.94±0.30</td>
<td></td>
</tr>
<tr>
<td>Stage 1</td>
<td>7.22±0.91</td>
<td>4.56±0.62</td>
<td>12.99±1.80*</td>
<td>7.93±1.10*</td>
</tr>
<tr>
<td>Stage 2</td>
<td>93.01±4.29</td>
<td>57.34±2.90</td>
<td>85.50±4.97*</td>
<td>51.55±3.00*</td>
</tr>
<tr>
<td>Stage 3</td>
<td>29.43±2.79</td>
<td>17.88±1.60</td>
<td>16.29±2.41**</td>
<td>9.86±1.45**</td>
</tr>
<tr>
<td>Stage 4</td>
<td>9.55±2.97</td>
<td>5.76±1.78</td>
<td>3.77±1.57*</td>
<td>2.30±0.95*</td>
</tr>
<tr>
<td>SWS</td>
<td>38.98±4.37</td>
<td>23.64±2.52</td>
<td>20.06±3.53**</td>
<td>12.16±2.14**</td>
</tr>
<tr>
<td>NREMS</td>
<td>131.99±2.51</td>
<td>80.99±1.22</td>
<td>105.56±3.73**</td>
<td>63.71±2.25**</td>
</tr>
<tr>
<td>REMS</td>
<td>23.71±2.17</td>
<td>14.46±1.28</td>
<td>47.21±2.70**</td>
<td>28.36±1.61**</td>
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<tr>
<td>Sleep cycle</td>
<td>89.7±2.4</td>
<td></td>
<td>88.4±4.1</td>
<td></td>
</tr>
<tr>
<td>NREMS episode</td>
<td>70.0±2.1</td>
<td></td>
<td>55.62.0**</td>
<td></td>
</tr>
<tr>
<td>REMS episode</td>
<td>19.7±1.5</td>
<td></td>
<td>32.7±3.7*</td>
<td></td>
</tr>
</tbody>
</table>

*Mean values and standard errors (SEM) are reported (n=16, 63 night and morning recordings of 16 subjects, except for sleep cycle duration: n=15, one subject was excluded because of a ‘skipped’ first REMS episodes in all 4 nights). Mean values were first calculated within subjects prior to averaging across subjects. Stages are in min and in % of total sleep time. For night sleep, only the three first hours after lights out were used for the analyses. Statistics: comparison of night and morning sleep episodes by paired t-test.*

* p < 0.01, ** p < 0.001, °n=9, only subjects with at least one morning sleep episode without SOREMS (Sleep Onset REMS episodes: REMS episodes with latency < 20 min) were included; WASO: waking after sleep onset; SWS: stages 3 and 4, NREMS: stages 2,3 and 4; REMS latency: time from sleep onset (first occurrence of stage 2) to the first occurrence of REMS.*
Discussion

Episodes of NLMT
The present study aimed at clarifying the relationship between a particular feature of NREMS, NLMT, and REMS regulation by analysing restricted nocturnal sleep and subsequent daytime sleep. The results provide evidence that NLMT is determined by REMS propensity.

NLMT occurred more frequently in morning sleep than in night sleep. In morning sleep, the latency to the first epoch of NLMT showed a unimodal distribution. In night sleep, a bimodal pattern prevailed with modes at sleep onset and in the proximity of REMS onset (Fig. 2.1.1D). This distribution corresponds to that of REMS latency in morning sleep (Fig. 2.1.1B). It is also similar to the pattern that had been observed in a selective REMS deprivation protocol (Werth et al., 2002a). The duration of the episodes of NLMT at sleep onset was slightly longer than the duration of SOREMS episodes and their latency was shorter. Low muscle tone usually precedes and outlasts REMS (Bliwise et al., 1974; Brunner et al., 1990b; Werth et al., 2002a). From these results, we conclude that NLMT may represent a window for SOREMS. If the episodes of NLMT at sleep onset would just represent the normal wake-sleep transition then muscle tone would be expected to gradually decrease (Brunner et al., 1990a; Chase and Morales, 2005). As illustrated in Figure 2.1.2 (left) both decrease and increase of muscle activity at sleep onset were typically rather sudden. No specific EEG markers were found to accompany episodes of NLMT as revealed by gross visual inspection.

When NREMS and REMS were considered together, 86% of the morning sleep episodes, but only 37% of the night sleep episodes started with low muscle tone. In morning sleep both the circadian drive (Weitzman et al., 1974; Czeisler et al., 1980; Dijk and Czeisler, 1995) and the reduced NREMS pressure contributed to a high REMS propensity. Low muscle tone may be
considered a REMS marker irrespective of the manifestation of this sleep state and epochs of low muscle tone at sleep onset may reflect the early appearance of REMS. If the drive for REMS is low, NLMT may be the only manifestation of this sleep state. NLMT may therefore be regarded as a facet of REMS regulation as previously proposed (Werth et al., 2002a). In that study, selective REMS deprivation increased NLMT in recovery sleep and NLMT was higher in daytime sleep than in the baseline night (Werth et al., 2002a). In our study, the number of episodes of NLMT increased, in parallel to SOREMS and REMS, in morning sleep compared to night sleep. The present findings challenge the concept of discrete states and favour the concept of interleaved states (Benington and Heller, 1994; Nielsen, 2000; Werth et al., 2002a). Our hypothesis could, for instance, further be tested in a forced desynchrony protocol that allows to separate homeostatic and circadian components (Dijk and Czeisler, 1995).

Low muscle tone is required to score REMS (Rechtschaffen and Kales, 1968). When speaking of muscle tone or EMG, sleep researchers usually refer to recordings of submental or mental muscle activity. One has to keep in mind that the muscle tone of trunk and limb muscles show a different pattern than head and neck muscles: the reduced tonic level during sleep remains rather stable throughout the night without further decrease in REMS (Jacobson et al., 1964). Cells in the medial brainstem reticular formation are thought to control motor movement (Siegel, 2005). They are active during waking and REMS while during NREMS their activity is reduced (Siegel, 2005). During REMS motoneurons in the brainstem are tonically inhibited although central motor systems are highly active (Siegel, 2005). Motoneuron hyperpolarisation and ensuing loss of muscle tone are due to a combination of disfacilitation and inhibition by the co-ordinated action of GABA and glycine release onto the motoneurons and concomitant decrease of norepinephrine and serotonin release onto them (Siegel, 2005). If our assumption is correct,
then the activity of the motoneurons during episodes of NLMT should show a similar pattern as seen during REMS.

**SOREMS**

The high number of SOREMS episodes in morning sleep (Fig. 2.1.1B) showed that this phenomenon is common in experimental protocols and illustrates that there may indeed be a REMS window at sleep onset. SOREMS episodes and increased amounts of REMS were reported for subjects sleeping in the morning after a night with or without sleep (Webb et al., 1966; Endo et al., 1981). In a time-free environment (Czeisler et al., 1980) and in a forced desynchrony protocol (Dijk and Czeisler, 1995), SOREMS occurred at a circadian phase corresponding to morning sleep. Their number decreased in naps scheduled throughout daytime hours (from morning to evening; Bes et al., 1996). After spontaneous wakefulness in a long scotoperiod protocol, SOREMS episodes were more frequent in the early morning hours (Barbato et al., 2002). Not only circadian factors but also NREMS pressure influences SOREMS propensity. Thus sleep initiated at 7:00 h after total sleep deprivation did not result in increased REMS (Dijk et al., 1991; Werth et al., 2002b). In the present study, SOREMS and REMS were enhanced in morning sleep by the high circadian drive and the reduced NREMS propensity. The preceding restricted nocturnal sleep episode not only reduced NREMS propensity (Fig. 2.1.3D) but also induced a partial REMS deprivation, as a large portion of REMS occurs in the second half of the night. The shorter sleep latency in the morning than at night may be due to the increased REMS propensity.
Slow wave activity
Simulations with the two-process model of sleep regulation (Daan et al., 1984) revealed a close correspondence between empirical SWA and the level of S for night sleep. However, the empirical values of SWA were below the predicted level of S in the first NREMS episode of morning sleep. This discrepancy between the data and the model is in accordance with findings of Beersma and co-workers (Beersma et al., 1990) who reported reduced intensity of NREMS under conditions of increased REMS pressure. As already discussed, the 4-h sleep episode in the previous night decreased NREMS pressure, which did not increase again up to the level of night sleep because the time between the night and the morning sleep episode was too short. Concomitantly, the sleep restriction and the circadian phase increased REMS pressure. Thus, increased REMS propensity appears to inhibit the full manifestation of SWA in morning sleep. Therefore not only a high NREMS pressure impedes REMS, but also high REMS drive lowers NREMS intensity (Brunner et al., 1990a). Thus, SWA represented in the model by Process S is not just dependent on sleep-wake history, but additionally on the balance between NREMS and REMS pressure.

Conclusion
We conclude that NLMT is a marker of homeostatic and circadian REMS regulation. At sleep onset, the epochs of low muscle tone represent an early manifestation of REMS. Reduced NREMS pressure associated with enhanced homeostatic and circadian REMS drive in the morning can account for the higher frequency of SOREMS episodes and epochs of NLMT in morning sleep than in night sleep.
Methods

Data of 16 healthy young right-handed men (mean age 22.3 years, range 20 - 25 years) who slept in the laboratory four times (sessions) at weekly intervals for 4 hours at night (beginning at either 22:45 or 23:15) and 3 hours in the subsequent morning (beginning at either 9:45 or 10:15) were analysed. Between the two sleep episodes the subjects remained in the laboratory and were under constant supervision. Subjects were requested to maintain the habitual sleep-wake schedule (23:00 – 07:00) on the night preceding the experimental night. Compliance was verified by means of wrist-worn activity monitors. The subjects gave informed written consent, and the study protocol was approved by the local ethics committee for research on human subjects. Three of the four sessions are from an experiment investigating the effect of electromagnetic fields (EMF) of mobile phones on sleep and the sleep EEG (Huber et al., 2000). In the fourth week, the right hand of the subjects was vibrated intermittently (20 min on; 10 min off) during three hours prior to the morning sleep episode (unpublished data); ten minutes after the end of vibration, lights were switched off. All manipulations were performed only before the morning sessions.

We first tested whether experimental treatment (sessions) affected the variables of interest. Morning sleep episodes were subjected to one-way ANOVAs for repeated measures with the factor ’session’ (1 to 4). All variables analysed except sleep latency (mean ± SEM of the four sessions: 5.7±0.9, 5.3±1.1, 3.0±0.6, 6.9±0.8 min) did not differ between the sessions. Furthermore, the distribution of the values of the variables in the four sessions did not give any indication of an effect of treatment. Therefore, we pooled the data of the four sessions and focused our analysis on the comparison of night and morning sleep episodes. Mean values were calculated
within subjects prior to averaging across subjects. Data of the first 3 hours of
night sleep were compared with the 3-hour morning sleep episodes using two-
tailed paired t-tests.

Polysomnographic recordings were performed during all sleep
episodes. EEG, submental EMG and EOG signals were conditioned by the
following analog filters: a high-pass filter (-3 dB at 0.16 Hz), a low-pass filter
(-3 dB at 102 Hz, <-40 dB at 256 Hz), and a notch filter (50 Hz). Data were
sampled with a frequency of 512 Hz, digitally filtered (EEG and EOG: low-
pass FIR filter, -3 dB at 49 Hz; EMG: band-pass FIR filter, -3 dB points at
15.6 and 54 Hz), and stored with a resolution of 128 Hz. Sleep stages were
visually scored according to standard criteria (Rechtschaffen and Kales, 1968).
Due to technical problems one night recording of a subject and one morning
recording of another subject were incomplete and excluded from data
analysis. The sleep cycles, NREMS and REMS episodes were defined
according to the criteria of Feinberg and Floyd (1979). REMS episodes with
latency shorter than 20 min were defined as sleep onset REMS (SOREMS)
episodes.

EMG variance (i.e. total power) was calculated for consecutive 20-s
epochs after elimination of ECG artefacts, i.e. excluding 30 data points
before and after the occurrence of an R-wave. Epochs of low muscle tone
were identified when the variance of the EMG was below the 90th percentile
level of the EMG variance in REMS epochs (Fig. 2.1.2). The threshold was
determined separately for each sleep episode. All thresholds were visually
controlled. In one recording, the signal-to-noise ratio of the EMG changed in
the course of the sleep episode and the threshold had to be adjusted. Fifty-
eight night-morning pairs were compared; 6 pairs had to be excluded because
in one or both recordings the signal-to-noise ratio deteriorated and prevented
a quantitative analysis. From the latter, 2 morning sleep recordings could be
analysed automatically and additional two night and one morning sleep
NREMS with low muscle tone is a marker of REMS regulation. Episodes by visual inspection. These data were only used for the analysis of NLMT at sleep onset. Episodes of NLMT with latency not longer than 20 min are referred to as NLMT at sleep onset. NLMT latency was defined as the interval between sleep onset (first epoch of stage 2) and the first appearance of NLMT.

Power spectra of consecutive 20-s epochs (FFT routine, Hanning window, averages of five 4-s epochs) were computed for derivation C3A2. Artefacts were identified by visual inspection and a semi-automatic procedure (see Huber et al., 2000 for details). Analysis was restricted to slow-wave activity (SWA; EEG power in the 0.75 – 4.5 Hz range).

List of abbreviations

REMS: rapid eye movement sleep
NREMS: non rapid eye movement sleep
SOREMS: sleep onset REMS (REMS latency < 20 min)
NLMT: NREMS with low muscle tone
EMG: electromyogram
EEG: electroencephalogram
ECG: electrocardiogram
SWA: slow-wave activity (EEG power in the 0.75 – 4.5 Hz range)
SWE: cumulative SWA in NREMS
FIR: finite impulse response
Author’s contributions

GT analysed the data; RH, AAB and PA designed and executed the original experiment; GT, AAB and PA interpreted the data; all authors contributed to the draft and approved the manuscript.
NREMS with low muscle tone is a marker of REMS regulation.
Chapter 2.2

Functional EEG topography in sleep and waking: State-dependent and state-independent features

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Abstract

Power spectra in the non-rapid eye movement sleep (NREMS) electroencephalogram (EEG) have been shown to exhibit frequency-specific topographic features that may point to functional differences in brain regions. Here, we extend the analysis to rapid eye movement sleep (REMS) and waking (W) to determine the extent to which EEG topography is determined by state under two different levels of sleep pressure. Multi-channel EEG recordings were obtained from young men during a baseline night, a 40-h waking period, and a recovery night. Sleep deprivation enhanced EEG power in the low-frequency range (1-8 Hz) in all three vigilance states. In NREMS, the effect was largest in the delta band, in W, in the theta band, while in REMS, there was a peak in both the delta and the theta band. The response of REMS to prolonged waking and its pattern of EEG topography was intermediate between NREMS and W. Cluster analysis revealed a major topographic segregation into three frequency bands (1-8 Hz, 9-15 Hz, 16-24 Hz), which was largely independent of state and sleep pressure. To assess individual topographic traits within each state, the differences between pairs of power maps were compared within (i.e. for baseline and recovery) and between individuals (i.e. separately for baseline and recovery). A high degree of intraindividual correspondence of the power maps was observed. The frequency-specific clustering of power maps suggests that distinct generators underlie EEG frequency bands. Although EEG power is modulated by state and sleep pressure, basic topographic features appear to be state-independent.
Introduction

Typical changes in the electroencephalogram (EEG) occur at the transition between waking and sleep, as well as at the transition between the major sleep states non-rapid eye movement sleep (NREMS) and rapid eye movementsleep (REMS). In fact, the scoring of human sleep is based to a large extent on differences in the amplitude and frequency of the EEG (Rechtschaffen and Kales, 1968). An increase in homeostatic sleep pressure induced by extended waking also gives rise to state-specific changes in the EEG. In NREMS, slow-wave activity (SWA; power in the 0.75-4.5 Hz band) is increased, whereas activity in the spindle frequency range (12-15 Hz) is reduced (Borbély et al., 1981; Dijk et al., 1990; Dijk et al., 1993; Finelli et al., 2001b; Knoblauch et al., 2002; Curcio et al., 2003; reviewed in De Gennaro and Ferrara, 2003; Borbély and Achermann, 2005). In waking, power in the theta range (5-8 Hz) is enhanced with progression of extended wakefulness (Cajochen et al., 1995; Aeschbach et al., 1997; Cajochen et al., 1999; Dumont et al., 1999; Finelli et al., 2000a; Strijksra et al., 2003). The close association between SWA in NREMS and the duration of previous waking has led to the establishment of the two-process model of sleep regulation (Borbély, 1982; Daan et al., 1984; Borbély and Achermann, 2005).

Recording the EEG simultaneously from multiple sites made it possible to compute topographic power distributions and thereby to gain new insight into the dynamics of sleep. Specifically, the NREMS-REMS cycles were shown to be reflected by shifts in the power gradients along the antero-posterior axis (Werth et al., 1996). Cluster analysis of the NREMS EEG power maps revealed a segregation into different frequency bands corresponding closely to the traditional frequency bands (i.e. delta, theta, alpha, sigma and beta; Finelli et al., 2001b). This segregation appeared to be impervious to increased sleep pressure on the basis of both mean data (Finelli
et al., 2001b) and individual maps (Finelli et al., 2001a). Therefore, different generators might underlie the frequency bands in the NREMS EEG. In addition, the increase in power in the low-frequency range in NREMS induced by prolonged waking was largest over frontal regions (Cajochen et al., 1999; Finelli et al., 2001b), whereas the corresponding decrease in power in the sigma band was most pronounced over the head vertex (Finelli et al., 2001b; Knoblauch et al., 2003). The power ratio recovery/baseline exhibited a topographic pattern similar to the power ratio between the first and second half of the baseline night (Finelli et al., 2001b). Thus, changes in sleep propensity are reflected in specific regional effects on EEG power. Nevertheless, these changes do not affect the topographical power distribution.

Functional neuroimaging studies with positron emission tomography (PET) complement these findings by showing that the prefrontal cortex is among the brain regions exhibiting the largest reduction of regional cerebral blood flow (rCBF) during NREMS (Maquet et al., 1997; Finelli et al., 2000b; Dang-Vu et al., 2005). Interestingly, a frontal deactivation could also be observed during REMS (Maquet et al., 1996; Finelli et al., 2000b), as well as in wakefulness after 24 hours of sleep deprivation (Thomas et al., 2000). Thus, in addition to state-specific patterns of brain activation and deactivation, those and other imaging studies reviewed in (Maquet, 2000; Nofzinger, 2005) highlight the existence of state-independent functional features of brain dynamics.

The main objective of this study was to explore and compare the functional topography of the EEG in all three vigilance states, NREMS, REMS and waking, and to investigate the response to sleep deprivation. Complementary to sleep data, waking data provide additional information to better interpret the causes of regional changes in the sleep EEG. The
identification of state-dependent and state-independent features provides new insights into EEG dynamics.

Methods

Design of experiment
Eight right-handed, healthy male volunteers (mean age 23 years, range 21–25 years) participated in the study. After a baseline night (23:00 to 07:00 h), preceded by an adaptation night, subjects were kept awake for 40 hours. Subsequent recovery sleep started at 23:00 h and subjects were allowed to sleep 12 hours. During the sleep deprivation period, subjects remained in the sleep laboratory and the surrounding area under constant supervision. The wake EEG was recorded at 3-h intervals starting at 07:00 h after the baseline night. Each session consisted of a 5 min eyes-open period, followed by a 4-5 min eyes-closed period, and a final 5 min eyes-open period. For details of the study see Finelli et al. (2000a; 2001b).

Data acquisition and spectral analysis
The EEG, electrooculogram and submental electromyogram were recorded by a polygraphic amplifier, digitized and transmitted to a personal computer. Twenty-seven EEG leads were placed according to an extended version of the International 10-20 System including FC1, FC2, CP1, CP2, PO1 and PO2. The EEG signals were sampled at 128 Hz during sleep and at 256 Hz during waking. For details see Finelli et al. (2000a; 2001b). Prior to analysis, the data from the 27 referential EEG derivations were transformed to average reference.
Power spectra were calculated for 4-s epochs (linear detrending, Hanning window) resulting in a frequency resolution of 0.25 Hz. The two lowest frequency bins (0.25 and 0.5 Hz) were excluded from the analysis because of their sensitivity to low frequency artifacts. Spectral data were analyzed up to 25 Hz.

**Sleep EEG data and scoring**

The sleep stages were visually scored for 20-s epochs (C3A2 derivation) according to the criteria of Rechtschaffen and Kales (1968).

Power spectra of five consecutive 4-s epochs were matched with the corresponding sleep stage. Artifacts were excluded on a 4-s basis first by visual inspection and then semi-automatically whenever power in the 0.75-4.5 Hz or 20-30 Hz band in any of the derivations exceeded an adaptive threshold (see Finelli et al., 2001b).

Analysis of REMS was restricted to tonic REMS, i.e. 4-s epochs without rapid eye movements (REMs). A REM was defined visually as an EOG deflection unrelated to an EEG event with a peak-to-peak amplitude of at least 75 µV and a slope of at least 150 µV/s. Furthermore, 4-s epochs within a 20-s epoch of REMS with signs of NREMS (low frequency / high amplitude EEG, or containing K-complexes or spindles (Rechtschaffen and Kales, 1968) were excluded.

The length of the recovery sleep analysed was individually matched with the length of baseline sleep (interval between the first occurrence of stage 2 and final awakening). In one subject, only the first 4 h 07 min of baseline sleep was recorded due to technical problems. A mean of 446.5 ± 3.6 (SEM) min of sleep was analysed in baseline (BL) and of 460.7 ± 3.3 min in recovery (RE) nights, respectively. Average power spectra were calculated for NREMS
(stages 2, 3, and 4; BL: 300.5 ± 7.8 min; RE: 337.5 ± 8.3 min) and tonic REMS (BL: 64.2 ± 6.6 min; RE: 65.5 ± 7.2 min).

**Waking EEG data**

Artifacts were identified by visual inspection. Power spectra were calculated for 4-s epochs or multiples thereof starting at the beginning of an artifact-free segment. In addition, an automatic outlier detection algorithm was applied (see Finelli et al., 2000a). We report average spectra calculated for the artifact-free EEG epochs of the eyes open periods recorded at 10:00, 13:00, 16:00 and 19:00 h following baseline sleep (denoted as baseline) and at the corresponding times of the next day of prolonged wakefulness (denoted as deprivation). The first waking recording immediately following the sleep episode was excluded to avoid possible confounding effects of sleep inertia (Hofer-Tinguely et al., 2005; reviewed in Tassi and Muzet, 2000). Due to technical problems, one recording of one subject (at 16.00 h) and data of two derivations (Fp1 and Fp2) of another subject (at 10.00 h) were excluded. In total, a mean of 27.5 ± 3.7 min waking data during BL and 24.5 ± 3.1 min during deprivation contributed to the analysis. It was restricted to the data recorded with eyes open because only a limited amount of artifact free data was available in the eyes closed condition, and to avoid possible confounding effects due to drowsiness associated with increased sleep pressure. Exploratory analyses showed that the waking EEG topographies for the eyes open and eyes closed conditions were similar.

**Data processing and statistics**

*Power maps (Figs. 2.2.1 and 2.2.4).* Topographic power maps were calculated for consecutive 1-Hz bands in the range of 1.0 to 24.75 Hz, and for selected broader bands. The 1-Hz bands are indicated by their lower limits (i.e. 2 Hz denotes the four 0.25-Hz bins centred at 2.0, 2.25, 2.5 and 2.75 Hz).
The mapping software used was adapted from the ICA Toolbox for MATLAB (Version 3.52, Computational Neurobiology Laboratory, The Salk Institute for Biological Studies, La Jolla, CA).

The maxima and minima of the 1-Hz power maps are shown in Fig. 2.2.2. Their location was determined on average maps and the individual power values observed at these locations were used for the statistics.

Comparison of power spectra (Fig. 2.2.3). EEG power spectra of baseline and recovery sleep (NREMS and REMS) and baseline and deprivation (waking) were compared by 2-tailed paired t-tests performed with log-transformed absolute values for each 0.25 Hz bin.

Cluster analysis (Fig. 2.2.5). Hierarchical cluster analysis was performed with the 24 average power maps of 1-Hz bandwidth. Each map was considered as a vector with 27 components and was normalised by z-transformation across all derivations prior to the cluster analysis. All pairs of maps were compared by calculating the Manhattan distance (sum of absolute differences at all derivations) between them and were grouped into a binary, hierarchical cluster tree (dendrogram) according to their proximity (see Finelli et al., 2001b).

Similarity within and between subjects (Fig. 2.2.6). Each 1-Hz map in the frequency range of 1 to 24 Hz was treated as a vector with 27 components and was normalised by z-transformation across all derivations. To assess mutual similarity, pairs of maps within (baseline – recovery for NREMS and REMS, baseline – deprivation for W) and between subjects (in baseline, recovery and deprivation) were compared by calculating the Manhattan distance between them. Finally, distance data of the 24 1-Hz bands of each state were averaged within subjects.
Table 2.2.1. Sleep variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline night [min]</th>
<th>Baseline night [%]</th>
<th>Recovery night [min]</th>
<th>Recovery night [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>TST</td>
<td>446.5±3.6</td>
<td></td>
<td>460.7±3.3*</td>
<td></td>
</tr>
<tr>
<td>NREMS</td>
<td>300.5±7.8</td>
<td>67.3</td>
<td>337.5±8.3*</td>
<td>73.3</td>
</tr>
<tr>
<td>REMS</td>
<td>103.1±6.9</td>
<td>23.1</td>
<td>98.5±8.0</td>
<td>21.4</td>
</tr>
<tr>
<td>Tonic REMS</td>
<td>64.2±6.6</td>
<td>62.3</td>
<td>65.5±7.2</td>
<td>66.5</td>
</tr>
</tbody>
</table>

Values represent means±SEM for 8 subjects in minutes or percent. The duration of recovery sleep analyzed was individually matched with the duration of baseline sleep (interval between the first occurrence of stage 2 and final awakening). TST: total sleep time; NREMS: nonREM sleep stages 2 to 4; REMS: REM sleep. In the second and fourth column, NREMS and REMS are expressed as percentages of TST, and tonic REMS as a percentage of REMS. * p<0.01, paired 2-tailed t-test.

Results

Sleep deprivation induced the typical changes in the sleep variables including a shortening of sleep latency, a reduction of waking after sleep onset and stage 1, and an increase of slow wave sleep, total sleep time and NREMS (Table 1, see also Table 1 in Finelli et al. (2000a). REMS and tonic REMS were not affected by sleep deprivation.
Figure 2.2.1. Topographic distribution of EEG power in NREMS, REMS and waking for baseline recordings. Average absolute power maps (n=8) of 1-Hz bands. The numbers indicate the lower limits of the frequency bands (i.e. 1 denotes the four 0.25 Hz bins centred at 1.0, 1.25, 1.5 and 1.75 Hz). The maps are based on 27 derivations (electrode positions indicated by dots). Values are colour-coded and plotted at the corresponding position on the planar projection of the hemispheric scalp model. Values between electrodes were interpolated (biharmonic spline interpolation). To optimise contrast each map was scaled separately between minimal (min) and maximal (max) power values. Absolute power of maxima and minima are shown in Fig. 2.2.2.
Spectral power maps in NREMS, REMS and waking

Fig. 2.2.1 shows side by side the mean 1-Hz power maps for NREMS, REMS and waking. The maps represent relative power distributions. The corresponding maps of the three vigilance states exhibited mainly similar, but also distinct features.

**Delta and theta band.** In the low-frequency range (1-7 Hz) a predominance of, or a tendency for high values in the frontal area was observed. This power distribution is manifested as a wedge-shaped (NREMS: 1-4 Hz), oval (REMS: 2-7 Hz; W: 4-7 Hz) or rim-shaped (W: 1-3 Hz) frontal maximum, or a circular secondary maximum (NREMS: 5-7 Hz). Note the occipital primary maximum in NREMS (4-7 Hz) and secondary maximum in REMS (1-2; 5-7 Hz).

**Alpha band.** In this frequency range, NREMS differed from both REMS and waking. A very prominent, frontal circular maximum was characteristic for NREMS in the 9-12 Hz range (while the occipital peak of the 4-7 Hz range extended up to 9 Hz). This contrasted with the marked occipital maximum of both REMS and waking.

**Sigma band.** NREMS showed a prominent circular vertex peak in the 13-15 Hz range, corresponding to sleep spindles. Strikingly, in waking the trough in the same area represents almost a mirror image of NREMS. In REMS the occipital maximum (at 13 Hz) was gradually substituted for by a frontal maximum (at 15 Hz and beyond).

**Beta band.** In this frequency band, the maps of NREMS and REMS exhibited both a broad frontal maximum, which in the highest frequencies splitted into two symmetrical circular areas. An occipital rim was evident in the lower beta range, particularly in NREMS.
Figure 2.2.2 Maximum (triangle up) and minimum (triangle down) value for each of the power maps displayed in Fig. 2.2.1. Diamonds above the abscissa indicate significant differences (p<0.05; ANOVA for repeated measures with factor state) between states (NREMS, REMS, waking) of maxima (max) and minima (min). Maxima and minima differed significantly in all frequency bands and states. Inset: Mean power spectra (averaged over all 27 derivations). The line above the abscissa indicates significant differences (p<0.05) between states.

Absolute power spectra in NREMS, REMS and waking

Fig. 2.2.2 shows the maximum and minimum power of the 1-Hz maps illustrated in Fig. 2.2.1. The data of maxima and minima exhibited the typical features of the EEG power spectra, as shown in the inset. Significant differences between the vigilance states are indicated separately for the maximum and minimum values by diamonds above the abscissa. In the 1-7 Hz range both the maximal and minimal values differed between states. NREMS showed the highest and waking the lowest power, while REMS values were intermediate. The alpha peak in waking was conspicuous.

The minima varied significantly in the range of 8-10 Hz and the maxima in the range of 10-12 Hz. The ANOVA reached the level of significance in the 13-
Functional EEG topography in sleep and waking.

15 Hz range for both the maximum and minimum curves. This frequency corresponds to sleep spindles in NREMS. Significance was attained for the minimum curves in the 16-24 Hz range and for the maximum curve in the 18-22 Hz range where waking was highest. Maxima and minima differed significantly in all 1-Hz bands of the three vigilance states. Average power

**Figure 2.2.3.** Effect of sleep deprivation on EEG power. **Top panels:** maxima (max) and minima (min) of 1-Hz ratio maps (not shown) of recovery/baseline in NREMS and REMS and of deprivation/baseline in waking. Power is expressed as percent of baseline. Symbols above the abscissa indicate significant deviations from 100% for the maxima (top), the minima (middle; orientation of the triangles indicates the direction of deviation) and significant differences between minima and maxima (bottom, diamonds; p<0.05; paired 2-tailed t-tests). Ratio maps of selected bands are illustrated in Fig. 4. **Middle panels:** corresponding relative mean EEG power spectra averaged over all 27 derivations. Mean values ± SEM are plotted for 0.25-Hz bins. **Bottom panels:** bars indicate t-values for the comparison recovery sleep - baseline sleep and deprivation - baseline (waking), respectively (p<0.05; 2-tailed paired t-test performed with log transformed absolute values: significant t-values in black, deviations below 100 % are indicated by negative t-values).
spectra differed between the vigilance states over a large frequency range (Fig. 2.2.2, inset).

**Effect of sleep deprivation on power spectra**

Fig. 2.2.3 depicts the ratio of power in recovery sleep over power in baseline sleep for NREMS and REMS, and the ratio of power after prolonged waking over short waking. The maximum and minimum values of ratio maps (top panels) as well as power spectra computed over all derivations (middle panels) are shown.

Sleep deprivation enhanced low-frequency power in all three vigilance states. In NREMS the largest increase occurred in the delta band, in waking in the theta band. REMS showed a bimodal pattern with peaks in the delta and theta bands. In NREMS power in the sigma and beta bands was reduced by sleep deprivation, while in waking power in the 13-16 Hz range was enhanced. With the exception of the highest bins, no effect in higher frequencies was present in REMS.

The effects of sleep deprivation showed a distinct regional specificity that is reflected in the maximum and minimum values. Ratio maps for selected frequency bands are illustrated in Fig. 2.2.4. A global effect encompassing all derivations was present in NREMS in the delta and theta bands (increase; largest over frontal areas), in the sigma band (decrease; largest at pre-central derivations). The increase in the alpha band was limited to the maximum located in frontal areas. In REMS, the enhancement of the delta and theta band, and in waking, the enhancement of the delta, alpha and 13-15 Hz bands were limited to the maximum curves. A more global increase in waking was observed in the theta band, yet it was most pronounced in frontal areas.
Segregation of frequency bands by topography

The similarity of 1-Hz maps (Fig. 2.2.1) was quantified by cluster analysis that yielded a separation into frequency bands (Fig. 2.2.5, upper part). The ordinate represents the 'distance' between different maps or clusters. The dendrograms (upside down U-shaped lines) indicate the partitioning of maps with similar topographies. In NREMS the largest distance (i.e. highest degree of dissimilarity) was present between the 13-15 Hz cluster and all other maps.

Figure 2.2.4. Topographic distribution of the change in power from baseline to recovery sleep (NREMS and REMS) and from baseline to deprivation (waking). Ratio maps (mean power in recovery/deprivation as percentage of mean power in baseline; n = 8) are illustrated for the delta, theta, alpha and sigma range. To optimise contrast each map was scaled separately. At the top right of the ratio maps, maxima (max) and minima (min) of the relative maps are indicated. For the representation of the maps see Fig. 2.2.1.

2 - 4 Hz 5 - 8 Hz 9 - 12 Hz 13 - 15 Hz

NREMS

REMS

Waking
Figure 2.2.5. Top panels: cluster analysis of the 1-Hz power maps of NREMS, REMS and waking for baseline condition represented as dendrograms. Increasing distance represents increasing dissimilarity. Maps were z-transformed prior to analysis (see Methods). Bottom panel: segregation of distinct frequency bands (clusters) at a distance (dist) of 8 and 5 (indicated at right) for baseline and recovery/deprivation. Segregated bands are indicated by arbitrary colours. Colouring of single 1-Hz bands at distance 5 was adapted to that at distance 8.
Such a large distance was not present in REMS. In waking the largest distance was seen between the 1-7 Hz cluster and the maps in the 8-24 Hz range.

To obtain a general picture of the frequency segregation of power maps in the three states and for the two experimental conditions (i.e. baseline and recovery / deprivation), a composite representation of the map clusters was compiled (Fig. 2.2.5, lower part). The clustering is illustrated by selecting two distance thresholds (8 and 5) limiting a different number of clusters. The higher threshold was derived from the REMS data showing the shortest distances between the maps, and its level was selected to obtain a segregation of REMS maps into 3 to 4 clusters. The lower threshold was selected to achieve further segregation (14 to 17 clusters). Its level was set sufficiently high to avoid a complete lack of clustering (i.e. 24 1-Hz bands).

A common pattern of clustering emerged. A low-frequency cluster (yellow, green) encompassing the 1-7 Hz or 2-8 Hz frequency range, a subsequent cluster extending from 7-8 Hz to 14-15 Hz (blue, black, white), and a high-frequency cluster from 15-16 Hz to 24 Hz (red). They can also be recognised by inspecting the power maps in Fig. 2.2.1.

**Intraindividual and interindindividual distances**

To quantify the degree of correspondence within and between individual spectral maps, the Manhattan distance was calculated for each state. Fig. 2.2.6 shows the distribution of the intraindividual and interindvidual distance measure. Low values corresponding to short distances or high similarity were observed when corresponding maps of the same individual were compared between baseline and recovery / deprivation. This was the case for all three vigilance states. The values showed no (NREMS and REMS) or little (waking) overlap with the interindidual distances, which were computed separately for baseline and recovery / deprivation.
**Discussion**

**EEG power maps: State-independence and state-specificity.**  
This is the first combined analysis of EEG topography of sleep (NREMS and REMS) and waking under conditions of normal and increased sleep pressure. The present study demonstrated that distinct frequency-dependent topographies of EEG power are present not only in NREMS (Buchsbaum et al., 1982; Zeitlhofer et al., 1993; Werth et al., 1997a; Finelli et al., 2001b), but

*Figure 2.2.6. Distributions of mean differences between individual power maps*  
(Manhattan distances between z-transformed maps; see Methods) in the frequency range of 1 to 24 Hz represented as boxplots. Intra-individual distances between baseline and recovery (NREMS and REMS) and baseline and deprivation (waking), respectively (BL-REC and BL-DEP; n=8) and inter-individual distances within (n=28) baseline (BL), recovery (REC) and deprivation (DEP) were determined for 1-Hz maps and averaged. The boxes represent the lower quartile, median, and upper quartile. The whiskers indicate the 5th and 95th percentile. Outliers are indicated by black dots. Intra-individual distances were significantly below inter-individual distances (p<0.05; Wilcoxon rank sum test).
also in waking and REMS. The question therefore arose to what extent the regional distribution of power is state-specific.

The comparison of the topographic patterns in the three vigilance states revealed common features as well as differences. A feature common to all three states was the high level of power in frontal regions in the 1-7 Hz range. The frontal predominance of EEG power is well documented for NREMS (Werth et al., 1996; Werth et al., 1997a; Cajochen et al., 1999; Finelli et al., 2001b; Knoblauch et al., 2002). This feature gradually diminishes in the course of a sleep episode and could reflect a high vulnerability of prefrontal areas to sleep loss (Horne, 1993; Drummond et al., 2000; Harrison and Horne, 2000; Gosselin et al., 2005). The present results support and extend this interpretation by demonstrating that the antero-posterior power gradient is to some extent state-independent and therefore reflects a general regional difference in brain function.

However, also state-specific features were evident in the power maps. We looked therefore more closely for correspondences and differences between the states within the low-frequency range. The power maps of tonic REMS (i.e. REMS epochs with no rapid eye movements) shared features of both NREMS and waking. In particular, in the 2-4 Hz range, the distinct frontal power maximum of REMS had its counterpart in NREMS, while in the 5-7 Hz range, it corresponded more closely to the pattern of waking. Since in the same range, an area of high REMS power was also present in the occipital region, the resemblance to the NREMS pattern was maintained throughout most of the low-frequency range. Finally, in the 1-7 Hz range REMS maxima and minima across the scalp were always interposed between NREMS and waking values.

In the alpha band (9-12 Hz), REMS and waking exhibited a strikingly similar power distribution. The occipital maximum of these states contrasted with the frontal maximum of NREMS. It is necessary to understand each
rhythm in the context of a specific vigilance/sleep state. The fact for example that alpha oscillations can be detected in relaxed wakefulness, REMS, and NREMS does not necessarily imply common underlying mechanisms. Similar EEG signals may be markers of different processes. The topographic analysis may be helpful in discriminating these processes.

Frontal alpha in NREMS and occipital alpha in waking is better documented than occipital alpha in REMS (Dement and Kleitman, 1957; Roth et al., 1999; De Gennaro et al., 2002). Simultaneous acquisition of EEG and imaging data may provide new possibilities to study the neural correlates of oscillatory brain activity. In a PET study, alpha amplitudes in waking were negatively correlated with cerebral blood flow in the occipital cortex (Sadato et al., 1998). In a functional magnetic resonance imaging (fMRI) study alpha power was negatively correlated with the BOLD signal in frontal (Laufs et al., 2003), and mostly parietal cortical areas (Goldman et al., 2002; Moosmann et al., 2003; Feige et al., 2005). Taken together these studies do not yet yield a consistent picture and further correlational analyses are required also for sleep. The available data support a link between the frontal predominance of alpha activity in NREMS and the lower metabolism in frontal cortical areas (see below). Interestingly, in REMS, occipital alpha showed specific functional features: A reduction of alpha activity in REMS was reported after selective REMS deprivation (Endo et al., 1998; Roth et al., 1999). Its topographical distribution and time course, however, were not affected by selective REMS deprivation (Roth et al., 1999). The fact that occipital alpha with eyes closed decreases during prolonged wakefulness (Strijkstra et al., 2003), while in REMS it is higher in the second half of both baseline and recovery night (unpublished data), further suggests that occipital alpha, as putative marker of a waking-like state, may also be influenced by sleep pressure. Further studies are necessary to investigate this hypothesis.
Patterns common to all three states were not present at higher frequencies. The well-known sigma vertex peak of NREMS had no counterpart in REMS or waking; however, the vertex trough in waking looks like a mirror image of the peak in NREMS. Since in the latter, the vertex power corresponds to the predominance of sleep spindles in this area (Zeitlhofer et al., 1993; Werth et al., 1997b; Finelli et al., 2001b; Knoblauch et al., 2002; De Gennaro et al., 2005), the low power in waking may be due to the complete silencing of the spindle generating mechanisms.

**EEG spectra and power maps: Response to sleep pressure**

Not only the baseline distribution of power maps but also their changes in response to sleep deprivation exhibited regional specificity and showed features that are common to all three vigilance states. Consistent with previous findings (Borbély et al., 1981; Dijk et al., 1990; Cajochen et al., 1999; Finelli et al., 2000a; 2001b; Cajochen et al., 2002), prolonged waking enhanced low-frequency power in NREMS, REMS and waking (Fig. 2.2.3). The largest increase in low-frequency power occurred in frontal areas, as we have previously reported for NREMS (Finelli et al., 2001b). The present analysis shows this to be the case also for REMS and waking for the frequency range up to 8 Hz (Fig. 2.2.4). Therefore, the sleep deprivation induced, preferential enhancement of EEG power in frontal regions is another state-independent phenomenon.

In addition to this general, state-independent change, state-specific features can be recognised. In NREMS, the maximum enhancement of power occurs in the lower part of the delta band (Borbély et al., 1981), whereas in waking, it is situated in the theta band (Torsvall and Åkerstedt, 1987; Cajochen et al., 1995; Aeschbach et al., 1997; Finelli et al., 2000a). The bimodal pattern in REMS with the first peak in the delta band and the second peak in the theta band suggests again that this state shares features of both,
waking and NREMS. Also in the higher frequency range, the changes of REMS appear to be intermediate between those of the two other states. While NREMS power was decreased after sleep deprivation at 12 Hz and beyond, and power in waking was increased in the same frequency range, REMS power remained at the baseline level. After its discovery, REMS was referred to as 'emergent stage 1 sleep' based on its EEG pattern (Kleitman, 1963). Stage 1 is a transitional state between waking and sleep. A similar transitional nature of REM sleep emerges from EEG topography and from the response of EEG power to prolonged waking.

The observation, in all three states, of high levels of power in frontal regions in the low-frequency range, together with a preferential enhancement of EEG power in the same frontal regions and frequency range after sleep deprivation, are the most striking state-independent results of this study. Considering that in NREMS, slow-wave activity is a marker of sleep intensity, the regional differences uncovered by the EEG may be a sign of different levels of cortical activation. A higher (lower) level of brain activity is generally paralleled by increased (decreased) cerebral blood flow. Deactivation of frontal cortical areas is indeed the primary result of functional neuroimaging studies of sleep with PET. During NREMS, rCBF is lower in frontal cortical association areas than during waking (Braun et al., 1997; Hofle et al., 1997; Maquet et al., 1997; Andersson et al., 1998; Kajimura et al., 1999; Finelli et al., 2000b; Kjaer et al., 2002; for a review see Maquet, 2000). A recent meta-analysis characterised rCBF correlates of delta activity during baseline NREMS, and showed, among other results, negative correlations between rCBF and delta power in the medial frontal cortex, the orbito-frontal cortex, the anterior cingulate gyrus, and the basal forebrain (Dang-Vu et al., 2005). Similarly, deactivations in dorsolateral prefrontal cortex were observed during REMS (Maquet et al., 1996), together with lower rCBF in parietal cortex. The latter finding could relate to the high occipital low-frequency power observed
in REM sleep in this study. Finally in wakefulness, after sleep deprivation of incremental extent, metabolic parameters revealed by PET during waking were generally reduced in several subcortical and cortical areas, especially heteromodal association cortices (prefrontal and posterior parietal; Thomas et al., 2000). Taken together, these PET findings complement the results from the EEG studies and support the hypothesis that higher-order (frontal) cortical areas may be more susceptible to sleep deprivation, and have a greater need for the restorative processes occurring during sleep.

**Quantification of EEG topography by cluster analysis**

Although inspection of the 1-Hz power maps revealed frequency ranges in which the pattern exhibited little variation, visual analysis alone was insufficient to determine the degree of similarity between the adjacent bins. Cluster analysis, providing a quantitative measure of similarity between maps, was applied to segregate frequency bands. This technique had been successfully applied to NREMS (Finelli et al., 2001b). We now show that a clear segregation of frequency bands occurs also in REMS and waking. In fact, roughly three main clusters were present in all states (i.e. (a) 2 Hz to 7 or 8 Hz; (b) 7 or 8 Hz to 14 or 15 Hz; (c) 14 or 15 Hz to 24 Hz). Clustering was particularly evident in the lowest and highest frequency range, whereas the middle range appeared more heterogeneous.

Interestingly, a similar segregation of frequency bands in the waking EEG was found on the basis of a temporal trend analysis of a single derivation (Dumont et al., 1999).

**Cluster analysis and individual power maps: Response to sleep pressure**

Sleep deprivation did not have a major effect on the clustering of frequency bands based on topographic patterns. Approximately the same frequencies were segregated as for the baseline condition. This independence of relative
maps from sleep pressure, which had been reported for NREMS both at the group level (Finelli et al., 2001b) and also at the topographic individual level (Finelli et al., 2001a), was shown here to extend to REMS and waking.

Power maps in NREMS showed considerable variations between individuals (Finelli et al., 2001a), while individual features were maintained after increasing sleep pressure. In fact, the similarity of the power maps before and after sleep deprivation was so large that they were referred to as individual fingerprints (Finelli et al., 2001a), a feature shared by single-channel spectral profiles across multiple nights (De Gennaro et al., 2005). Also in the present study, the similarity between all pairs of maps within and between individuals was computed. The high degree of intraindividual invariance that was present also in REMS and waking represents a further state-independent feature. We have previously argued that the well documented interindividual variability in anatomical features of the cerebral cortex could account for the variability of power maps (Finelli et al., 2001a). This argument is strengthened by the new observations in REMS and waking.

Concluding remarks

To investigate sleep regulation, EEG analysis has been focused mainly on NREMS, the state with the highest power in the low-frequency range and with sleep spindles as a specific feature. Moreover, slow-wave activity in NREMS is a reliable marker of sleep homeostasis and has served to delineate Process S, one of the two fundamental processes in the sleep regulation model (Borbély, 1982; Daan et al., 1984). In more recent studies, it was demonstrated that also the waking EEG is affected by sleep pressure. Power in the theta band increases as a function of prior waking, while also undergoing a circadian modulation (Aeschbach et al., 1997; Aeschbach et al., 1999; Finelli et al., 2000a; Cajochen et al., 2002). The picture emerging from this study is that basic processes formerly considered to be specific to sleep are present also in
waking. For example, slow-wave activity in NREMS and theta activity in waking seem to have common regulating mechanisms. Thus, on the basis of individual data, the changes induced by sleep deprivation are correlated and most prominent in frontal areas (Finelli et al., 2000a). The present topographic analysis further supports and expands this earlier finding. Low-frequency power exhibits its maximum invariably at frontal regions, and this is also for the response to sleep deprivation. Frontal alpha and vertex sigma are hallmarks of NREMS. Both are correlated with sleep pressure, alpha positively and sigma negatively. Alpha and delta in NREMS, both frontal phenomena, are also closely associated. The close association of frontal alpha with slow-wave activity was evident also from coherence analysis (Achermann and Borbély, 1998). This contrasts with alpha in waking and REMS with its occipital predominance.

The low part of the EEG spectrum seems to be a marker of sleep pressure in all vigilance states. The middle part of the frequency spectrum shows state-specific features. The state-specificity could therefore consist in the characteristic oscillating frequencies in which the major response to sleep pressure is manifested, while the cerebral systems involved may not differ. The latter is underscored by the unchanged pattern of individual power maps before and after sleep deprivation. The low intraindividual variability contrasts with a considerable interindividual variability that encompasses both EEG topography (Finelli et al., 2001a; present study), and the responsiveness to sleep deprivation in NREMS and waking (Finelli et al., 2000a; Finelli et al., 2001b). To fully exploit the potential of EEG power mapping in the functional analysis of sleep and waking, further studies on an individual basis are required.
Supplement

To complement the previous chapter, some additional analyses were performed. The results are presented in this supplement.

Figure 2.3.1 shows the power distribution after prolonged wakefulness in the states NREMS, REMS and wakefulness. The 1-Hz maps are strikingly similar to those of the baseline nights and days (Figure 2.2.1).

Figure 2.3.2 depicts the maxima and minima of power of the 1-Hz maps of figure 2.3.1. Compared to the maxima and minima of baseline condition (Fig. 2.2.2), the distribution over the frequencies and the relationship between the states are virtually unchanged.

Figure 2.3.3 is an overview of the effect of sleep pressure on EEG power spectra in REMS, NREMS and waking. REMS is compared between baseline and recovery and between the first and second half of the baseline night (upper panels). The ratio between REMS and NREMS is displayed for
baseine and recovery nights (middle panels). The two waking conditions (eyes open and eyes closed) are compared between baseline and deprivation days (bottom panels).

Figure 2.3.4 shows the similarity of the power maps within and between subjects. The boxplots represent the distribution of mean differences between individual power maps for each frequency bin separately. Each 1-Hz map was treated as a vector with 27 components and normalised by z-transformation across all derivations. To assess mutual similarity, pairs of maps within (baseline - recovery for NREMS and REMS, baseline – deprivation for waking) and between subjects (in baseline, recovery and deprivation) were compared by calculating the Manhattan distance.
Figure 2.3.1. After extension of wakefulness, the topographic distribution of EEG power in NREMS, REMS and waking recordings is almost identical to the distribution in baseline recordings (see legend of Fig. 2.2.1 for details)
Chapter 2.3

Figure 2.3.2. The maxima and minima of the mean maps in recovery sleep show almost the same distribution over frequencies than those of mean baseline maps. In some frequencies the absolute values differ from the baseline values. (See Fig. 2.2.3)

Figure 2.3.3. (next page) Effect of sleep pressure on EEG power. Relative mean EEG power spectra averaged over all 27 derivations. Mean values are plotted for 0.25-Hz bins (upper panels). Corresponding t-values are indicated by bars (lower panels, p<0.05, 2-tailed paired t-test performed with log transformed absolute values: significant t-values in black, deviations below 100 % are indicated by negative t-values). In tonic REMS, the power difference between the first and the second half of the baseline night encompasses most frequency bins (more bins than between recovery and baseline nights). This might be due to circadian influences on REMS. The ratio between REMS and NREMS is virtually the same in baseline and recovery nights. During prolonged waking, power in the alpha frequency range decreases in the eyes closed condition but remained unchanged in the eyes open condition, while the beta range increased in the eyes open condition but not in the eyes closed condition.
Figure 2.3.4. Lower intra-individual distances than inter-individual distances in EEG power maps in REMS, NREMS and waking. Distributions of mean differences between individual power maps (Manhattan distances between z-transformed maps) for each 1-Hz bin separately represented as boxplots. Intra-individual distances were calculated between baseline and recovery nights (NREMS and REMS) and between baseline and deprivation days (waking), (n=8). Inter-individual distances were computed in baseline and recovery nights and baseline and deprivation days. The boxes represent the lower quartiles, medians, and upper quartiles. The whiskers indicate the 5th and 95th percentiles. Outliers are indicated by black dots. For more details see Fig. 2.2.6
Napping, sleep inertia and learning
Sleep inertia: Performance changes after sleep, rest and active waking

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Abstract

Napping benefits and sustains subsequent performance. Prophylactic naps have been recommended as a means to maintain performance during extended wakefulness, as required during shiftwork. However, napping may cause short-term performance impairments, because awakening from sleep is followed by sleep inertia, a period of hypovigilance and impaired cognitive and behavioral performance. We investigated sleep inertia after an afternoon nap. Healthy 18-28 year-olds (n=50, not sleep deprived) were assigned to sleep, active wake or rest groups for a 2-hour experimental phase with polysomnography starting either at 14:00 or 16:00 for half of each group. Before (baseline, 12:30 or 14:30) and in five sessions during the hour after the experimental phase (16:00-17:00 or 18:00-19:00), subjects completed an addition task, an auditory reaction time task, and the Stanford Sleepiness Scale. In session one, addition speed in the sleep group was reduced compared with baseline and with active wake controls, whereas calculation accuracy did not change. Addition speed in the sleep and rest groups increased substantially from session one to session two and reached a level similar to that of the active wake group by the fifth session. In the first session, auditory reaction speed of the sleep group was reduced compared with baseline and with rest controls but did not differ from the active wake group. The slowest reaction times showed significant recovery after 20 minutes. The groups reported similar increases in subjective sleepiness after the experimental period. These findings provide evidence for performance slowing and recovery during the hour following a two-hour nap opportunity. They highlight the importance of employing multiple control groups and various objective and subjective measures to assess sleep inertia.
Theme: Neural basis of behavior

Topic: Biological rhythms and sleep

Key words: sleep inertia, drowsiness, performance, nap, rest, Stanford Sleepiness Scale
Introduction

Napping may facilitate work that requires extended wakefulness, such as night and shiftwork, emergency operations, or space flights. Naps can improve subsequent performance or prevent decrements in performance (e.g., Dinges et al., 1987; Bonnet, 1991; Horne and Reyner, 1996; Reyner and Horne, 1997). In a study of extended wakefulness subjects were allowed to take a 2-h nap at one of five times during a 56-h period in which they were otherwise required to remain awake (Dinges et al., 1987). Subjects who took a nap at an earlier time point showed longer-lasting performance benefits than did subjects who took a nap later. This result suggests that it would be better for workers to nap before or at the beginning of an extended shift, rather than later during the shift, when they are sleep deprived. Taking a nap before being sleep deprived has the additional advantage that less pronounced sleep inertia would be expected. Sleep inertia, a period of drowsiness and impaired performance after the transition from sleep to wake, is exacerbated by prior sleep deprivation (Balkin and Badia, 1988; Dinges, 1990). However, sleep inertia can also be observed in individuals who are not sleep deprived (Achermann et al., 1995; Jewett et al., 1999; Hayashi et al., 2003a; Hayashi et al., 2003b). Few studies have investigated sleep inertia after daytime napping in individuals who were not previously sleep deprived.

In addition to the duration of waking prior to the sleep episode, other factors may influence sleep inertia, including sleep stage on awakening (Koulack and Schultz, 1974; Dinges et al., 1985; Jewett et al., 1999), eye movement density (Koulack and Schultz, 1974), slow-wave activity (a putative marker of sleep intensity; defined as EEG power within the range of 0.75 – 4.5 Hz (Achermann et al., 1995)), duration of the nap (Tietzel and Lack, 2001), and circadian time (Dinges et al., 1985; Naitoh et al., 1993). Sleep regulation and neurobehavioral functioning are commonly modelled by an
interaction of a homeostatic process (reflecting sleep-wake history) and a circadian process (reflecting the endogenous rhythm of approximately 24 hours) (Daan et al., 1984; Achermann and Borbély, 2003). However, these two factors were insufficient to mathematically model changes in sleepiness and performance related to sleep inertia. To account for sleepiness and performance changes after awakening, a third factor was implemented: a short-lived exponential deviation that represents sleep inertia (Åkerstedt and Folkard, 1990; Folkard and Åkerstedt, 1992; Achermann and Borbély, 1994; Jewett and Kronauer, 1999). Several prior studies investigated the time course of sleep inertia (Achermann et al., 1995; Jewett et al., 1999; Ferrara et al., 2000). It lasted minutes to hours, depending partly on the task and dependent variables used to assess it (reviewed in Muzet et al., 1995; Ferrara and De Gennaro, 2000; Tassi and Muzet, 2000).

The present study investigated sleep inertia using both objective measures of performance and subjective assessment of sleepiness. These objective and subjective measures were administered at a high frequency during the hour after the sleep opportunity period. It is generally assumed that changes in subjective alertness and performance that occur in the time period after awakening reflect recovery from sleep inertia. In many experimental paradigms, waking control conditions are not feasible without introducing sleep deprivation. The present study investigated sleep inertia after an afternoon nap with both active wake and rest control groups to control for other factors that might cause changes in performance after awakening, such as learning, boredom, lying down and being in the dark. The subjects in all groups were recorded polysomnographically during the experimental period, and they all adhered to an 8-h sleep schedule during the three nights before the study.
Methods

Subjects and Design
Participants were 50 healthy subjects aged 18-28 years. They were assigned to sleep (n=18, mean age 23 years), active wake (n=16, mean age 23 years), or rest groups (n=16, mean age 23 years). Half of the subjects in each group were women. The experimental phase was scheduled at two different times (early: 13:55-15:55, or late: 15:55-17:55) to increase the number of subjects we could test in one day. Half of the subjects in each group were assigned to the early time and half to the late time, except that in the sleep group, eight subjects were scheduled at the early and ten subjects at the late test time.

Participants were instructed to keep a regular sleep schedule from 23:00-07:00 and to abstain from caffeine and alcohol for 3 days before the study. Compliance with the request of maintaining a regular sleep schedule before the experiment was verified by continuous recording of wrist-activity (monitor worn on non-dominant arm) and sleep logs before the start of the experiment. Two subjects (not included in the numbers given) did not adhere to the sleep schedule and were excluded from the study. Subjects met the following criteria: right handed (Oldfield, 1971), non-smoker, moderate alcohol (≤ five alcoholic beverages per week) and caffeine (equivalent of ≤ 3 cups coffee per day) consumption, no drug intake, no history of head injury or neurologic or psychiatric disease, no shift work or atypical sleep schedule, no flights over more than two time zones in the two months before the study, and no medications (with the exception of oral contraceptives). All subjects had hearing thresholds ≤ 35 dB hearing level in the range 250-3000 Hz. Study procedures were approved by the local ethics committee and written informed consent was obtained from all subjects.
Sleep inertia assessments

Addition task: Pairs of 2-digit numbers appeared on the computer screen, one below the other. Subjects summed these numbers and entered the answer on the numeric keypad. The next pair of numbers appeared immediately. We instructed subjects to work as quickly and accurately as possible. The duration of the task was 3 min. A similar task was previously used to assess sleep inertia (Jewett et al., 1999).

Auditory reaction time task: The auditory reaction time task consisted of 48 presentations of a tone of 1000 Hz and 50 ms duration that was presented through earphones at a loudness of 70 dB sound pressure level. The interstimulus interval (ISI) varied randomly between 2 and 7 s (in increments of 1s) with 4 occurrences of each ISI in each half of the task. The first presentation of each session was excluded; therefore 47 tone presentations were analysed. The task lasted approximately 4 min. Subjects were instructed to press a button on a response box using their right index finger as soon as they heard a tone. This auditory reaction time task is similar to one previously used to assess sleep inertia (Ferrara et al., 2000).

Stanford Sleepiness Scale: The Stanford Sleepiness Scale is a 7-point anchored scale with descriptors of varying sleepiness levels. We used a German translation of the Stanford Sleepiness Scale (Sturm and Clarenbach, 1997).

Procedure
The experiments were conducted in temperature-controlled and soundproof rooms of the sleep laboratory at the Institute of Pharmacology and Toxicology, University of Zurich. To ensure that subjects were well acquainted with the tests and to reduce practice effects, subjects practised
each performance test twice on a day before the study. On the day of the study, electrodes were applied and then subjects completed a baseline session with all three sleep inertia measures. The baseline session occurred at 12:30 or 14:30. A demanding auditory learning task of 45 min followed (Gottselig et al., 2004). After a 25-min break, during which subjects had a small snack, used the restroom, and the electrode impedances were checked, we informed subjects of their group assignment for the two-hour experimental phase. All subjects were recorded polysomnographically during this two-hour phase. Subjects in the sleep and rest groups lay in bed in a darkened single bedroom for the full two hours.

**Sleep group.** We instructed subjects to remain in bed and to try to sleep.

**Rest group.** Subjects were instructed to lie in the dark for two hours and to try to relax without falling asleep. They were informed that we would monitor their polysomnographic signals and that we would alert them by sounding a tone over the intercom if they showed signs of falling asleep (rolling eye movements and reduced alpha activity or EEG slowing).

**Active wake group.** Subjects watched an educational film (1 hour and 50 min) about the universe in a lighted room (approximately 130 lux). The volume was set at a constant level for the entire experiment, with a mean loudness of approximately 60 dB sound pressure level. They sat relaxed in comfortable chairs and were allowed to stand up to stretch if they felt sleepy. They were supervised at all times to ensure that they did not fall asleep. We instructed them to attend the film carefully, because afterwards they would have to answer a set of questions about it. The 44 true-false questions required approximately 10 min to complete.
At the end of the experimental phase, the lights were turned on to awaken the sleep group and to signify the end of the experimental period in the rest group. At this time, all subjects were required to get up immediately and walk to a computer in the hallway outside their room. They began the sleep inertia tests five minutes after lights were switched on. The tests were repeated five times at 12-min intervals over the course of the hour following the experimental phase. We will refer to these sessions as sessions 1 to 5. There were breaks of 4-5 min between sessions.

Polysomnographic recordings
During the experimental phase the electroencephalogram (EEG), submental electromyogram, the electrooculogram, and the electrocardiogram were continuously recorded with a polygraphic amplifier (for signal conditioning and sampling see Endo et al., 1998). Scorers blind to the experimental conditions visually scored the recordings based on the C3A2 derivation using standard criteria (Rechtschaffen and Kales, 1968). We set an a priori criterion that subjects had to sleep at least 30 min to be included in the sleep group; any subject who slept less would be replaced with another subject.

Data analysis
Dependent variables. For the addition task, percentage correct was calculated as a measure of accuracy and the number of sums attempted was taken as a measure of speed. We lost the accuracy data for one subject because the NumLock key was mistakenly pressed, resulting in failure to record accuracy, while we obtained data for speed. To normalise the data from the auditory reaction time task, we took the inverse of reaction time, which we refer to as speed (Belenky et al., 2003). Because of a technical problem, data for the auditory reaction time test were lost in one subject in session 1.
Statistics. For all sleep inertia assessments, data in the sessions after the experimental phase were calculated as a deviation from the individual baseline (referred to as relative speed or accuracy). The data were submitted to repeated measures ANOVA with the Huynh-Feldt correction for sphericity violation. The ANOVAs employed the factors experiment time (early or late), group (sleep, rest or active wakefulness) and session (1-5). Because initial analyses showed no significant main effect or interactions involving the factor experiment time, we performed the analyses with the factors group and session. In the case of significant interactions, ANOVAs with the factor session were performed separately for each group. Planned comparisons consisting of paired or unpaired t-tests were used to test for differences between sessions or groups when the ANOVAs revealed significant effects. Values reported are means and standard errors. In the sleep group, six of the subjects were already awake for longer than two minutes at the end of the experimental period. Analysis of the performances did not show any significant differences between the two subgroups (awake, asleep); therefore, the two subgroups were combined. Excluding the subjects who were awake for longer than 2 minutes (n = 6, range: 7 – 58 min) at the end of the experimental period in the sleep group did not change the statistical results. We analysed the relationship between relative performance in the first session after the experimental phase and the duration of total sleep time, slow wave sleep (stages 3 and 4), stage 2, REM sleep and time awake since final awakening by computing Spearman rank correlation coefficients.
Sleep inertia: performance changes after sleep, rest and active waking

Results

Sleep variables
The sleep variables for the sleep group are shown in Table 3.1.1. Sleep duration ranged from 40.7 to 110.7 min. Thus, all subjects were able to sleep and exceeded the criterion duration of 30 min. Thirteen of the eighteen subjects exhibited REM sleep. The rest group spent a mean of 2.6 ± 1.5 min in stage 1 and one subject showed 0.3 min and another 5.0 min of stage 2. A median of 15 interventions via intercom was needed to alert the subjects (range 0 - 50). In the active wake group one subject showed 1.3 min of stage 1 and 4 min of stage 2 and another one 0.3 min of stage 1.

With one exception in the 10th percentile of reaction speed, excluding the subjects of the rest and active groups with stage 2 sleep did not change the statistical results.

Addition task
Percent correct. Accuracy on the addition task did not differ among groups in the baseline session (F(2,47) = 1.54, p = 0.23). Relative accuracy did not change over sessions (F(4, 184) = 1.80, p = 0.15) and there were no differences in relative accuracy among groups (group effect: F(2, 46) = 0.41, p = 0.67; group * session interaction: F(8, 184) = 0.65, p = 0.70). The overall accuracy averaged across sessions and groups was 92.6 ± 0.8 %.

Number of sums attempted (Fig. 3.1.1). The baseline performance on the addition task did not differ among groups (F(2,47) = 1.18, p = 0.32; sleep: 46.89 ± 2.52; rest: 41.25 ± 2.68; active wakefulness: 44.25 ± 2.68). Analysis of the relative speed revealed a significant group * session interaction (group * session: F(8, 188) = 2.35, p = 0.02; session: F(4,188) = 11.00, p < 0.01; group:
Table 3.1.1. Sleep variables$^1$.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Duration (min)</th>
<th>SEM$^2$</th>
<th>% of TST</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Sleep Time (TST)</td>
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<td>5.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sleep Latency</td>
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<td>1.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Waking After Sleep Onset</td>
<td>14.7</td>
<td>3.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sleep Efficiency$^3$</td>
<td></td>
<td></td>
<td>71.4</td>
<td>4.2</td>
</tr>
<tr>
<td>Stage 1</td>
<td>6.0</td>
<td>0.8</td>
<td>7.8</td>
<td>1.2</td>
</tr>
<tr>
<td>Stage 2</td>
<td>39.1</td>
<td>3.7</td>
<td>47.3</td>
<td>4.3</td>
</tr>
<tr>
<td>Stage 3$^5$</td>
<td>10.3</td>
<td>2.0</td>
<td>11.9</td>
<td>2.3</td>
</tr>
<tr>
<td>Stage 4$^5$</td>
<td>19.1</td>
<td>3.7</td>
<td>21.3</td>
<td>4.4</td>
</tr>
<tr>
<td>SWS (stages 3 and 4)$^5$</td>
<td>29.5</td>
<td>3.9</td>
<td>33.1</td>
<td>4.6</td>
</tr>
<tr>
<td>Non-REM Sleep$^4$</td>
<td>68.6</td>
<td>4.6</td>
<td>80.4</td>
<td>2.3</td>
</tr>
<tr>
<td>REM Sleep$^5$</td>
<td>10.7</td>
<td>2.5</td>
<td>11.8</td>
<td>2.5</td>
</tr>
<tr>
<td>REM Sleep Latency</td>
<td>62.4</td>
<td>3.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^1$ Data in the table are for the sleep group, n=18, except REM Sleep Latency, n = 13.

$^2$ Standard error of the mean.

$^3$ TST expressed as percentage of time in bed.

$^4$ Defined as stages 2, 3, and 4.

$^5$ Data of all subjects were included in the calculations, if a state was missing a value of zero was used (stage 3: 1 subject; stage 4 and SWS: 2 subjects; REMS: 5 subjects).

F(2,47) = 2.02, p = 0.14) and a significant session effect for the sleep (F(4, 68) = 12.13, p < 0.01) and the rest (F(4, 60) = 4.11, p < 0.01) groups. No changes across sessions were observed in the active wake group (F(4,60) = 1.86, p = 0.13). Relative speed increased from the first to the last session (sleep: t(17) = 7.51, p < 0.01; rest: t(15) = 2.73, p = 0.02), from session 1 to session 2 (sleep:
Sleep inertia: performance changes after sleep, rest and active waking

t(17) = 3.50, p < 0.01; rest: t(15) = 2.74, p = 0.02), and from session 4 to 5 (sleep: t(17) = 4.00, p < 0.01). In the last session, all groups reached a similar level that exceeded baseline level (t(49) = 3.90, p < 0.01). The strongest effects of sleep inertia would be expected in session 1, immediately after the experimental phase. In session 1, the sleep group performed worse than in the baseline session (t(17) = 2.58, p = 0.02) and worse than the active wake group (t(32) = 2.81, p < 0.01) and tended to perform worse than the rest group (t(32) = 1.75, p = 0.09).

Auditory reaction time task

10th percentile of reaction speed (slowest reaction times) (Fig. 3.1.2, upper panel). The 10th percentile of speed did not differ among groups during baseline (F(2, 47) = 0.40, p = 0.67; sleep: 4.27 ± 0.15 s⁻¹; rest: 4.09 ± 0.16 s⁻¹; active wakefulness: 4.25 ± 0.16 s⁻¹). Analysis of relative speed of the slowest reaction times revealed a session * group interaction (session * group: F(8, 184) = 2.02, p < 0.05; session: F(4,184) = 1.31, p = 0.27; group: F(2,46) = 3.93, p = 0.03). There was a significant session effect in the sleep group (F(4, 68) = 3.94, p = 0.01) but not in the control groups (rest: F(4, 60) = 0.81, p = 0.52; active wake: F(4, 56) = 0.70, p = 0.56). The sleep group showed significant recovery (session 5 > session1, t(17) = 2.63, p = 0.02), yet relative speed in session 5 was still below baseline (t(17) = 4.51, p < 0.01). Tests of performance averaged over the five sessions revealed that the active wake group tended to perform slower than in baseline (t(15) = 2.00, p = 0.06, without the subject showing stage 2 sleep: t(14) = 2.16, p = 0.05), whereas performance in the rest group did not differ from baseline (t(15) = 0.03, p = 0.97).
Mean speed (Fig. 3.1.2, middle panel). Baseline performance did not differ among groups ($F(2, 47) = 0.57, p = 0.57$; sleep: $5.13 \pm 0.17 \text{ s}^{-1}$; rest: $4.92 \pm 0.18 \text{ s}^{-1}$; active wakefulness: $5.17 \pm 0.18 \text{ s}^{-1}$). Analysis of mean relative speed revealed no session effect or group * session interaction (session: $F(4, 184) = 1.51, p = 0.21$; group * session: $F(8, 184) = 0.97, p = 0.45$). The groups differed in mean relative speed (main effect of group: $F(2, 46) = 3.56, p = 0.04$; means over sessions: sleep group: $-0.33 \pm 0.09$; rest group: $0.02 \pm 0.1$; active wake group: $-0.20 \pm 0.10$). The sleep group performed slower than in baseline ($t(17) = 3.90, p < 0.01$) and slower than the rest group ($t(32) = 2.67, p = 0.01$), but did not differ from the active wake group ($t(32) = 0.99, p = 0.33$). The active wake group also performed slower than in baseline ($t(15) = \ldots$).
2.22, p = 0.04), while performance in the rest group did not differ from baseline (t(15) = 0.16, p = 0.87).

**90th percentile of reaction speed (fastest reaction times)** (Fig. 3.1.2, lower panel). The 90th percentile of speed did not differ among groups during baseline (F(2, 47) = 0.55, p = 0.58; sleep: 6.04 ± 0.20 s⁻¹; rest: 5.78 ± 0.21 s⁻¹; active wakefulness: 6.04 ± 0.21 s⁻¹). There was no session effect or group * session interaction (session: F(4, 184) = 0.66, p = 0.61; group * session: F(8, 184) = 0.67, p = 0.70). The relative speed of the fastest reaction times differed among groups (group main effect: F(2, 46) = 3.46, p = 0.04; means over the five sessions were: sleep group: −0.38 ± 0.12; rest group: 0.003 ± 0.10; active wake group: −0.19 ± 0.09). The sleep group had larger decrements of relative speed than the rest group (t(32) = 2.42, p = 0.02) and did not differ from the active wake group (t(32) = 1.27, p = 0.22). The sleep group performed slower than in baseline (t(17) = 3.22, p < 0.01), the active wake group tended to be slower than in baseline (t(15) = 2.06, p = 0.06) and the rest group did not differ from baseline (t(15) = 0.03, p = 0.98).

**Stanford Sleepiness Scale**
Sleepiness ratings are shown in Fig. 3.1.3. Baseline ratings of subjective sleepiness did not differ among groups (F(2, 47) = 1.08, p = 0.35; sleep: 2.06 ± 0.18; rest: 2.19 ± 0.19; active wakefulness: 2.44 ± 0.19). Analysis of relative changes revealed a main effect of session (F(4, 188) = 8.13, p < 0.01) but no effect of group and no group * session interaction (group: F(2,47) = 0.23, p = 0.79; group * session: F(8,188) = 0.41, p = 0.86). In session 1, subjects rated themselves as sleepier than in baseline (t(49) = 5.66, p < 0.01) and than in session 5 (t(49) = 3.44, p < 0.01). In session 5, subjects still felt sleepier than in baseline (t(49) = 2.62, p < 0.01).
Figure 3.1.2. Auditory reaction time task. The dependent variable was speed, defined as \(1/\text{reaction time}\). Performance in the five sessions following the experimental phase (rest, sleep and active wakefulness) was expressed as the difference from the individual baseline. Error bars represent standard errors. Values are staggered along the x-axis to clearly show the error bars. Top: 10\(^{th}\) percentile of speed (slowest reaction times). Middle: Mean speed. Bottom: 90\(^{th}\) percentile of speed (fastest reaction times).
Relationship between performance and sleep variables

Eight subjects of the sleep group were woken from stage 2 and one from stage 4. The other nine subjects had already been awake for 1-58 min (time since final awakening). The subjects who were asleep or awake for less than 2 min at the end of the experimental period had longer total sleep time than the six subjects who were already awake (t(16) = 3.39, p = 0.02).

Addition task. In the first post-sleep session, relative addition speed was negatively correlated with total sleep time (r = -0.68, p < 0.01, n = 18) and stage 2 (r = -0.59, p = 0.01), but not with slow wave sleep (i.e., stages 3 and 4; r = -0.11, p = 0.67) or REM sleep (r = -0.24, p = 0.35). Relative addition speed correlated marginally with time since final awakening (r = 0.45, p = 0.06, n = 18); if subjects who were asleep at the end of the experimental
period were excluded from the analysis, there was no correlation (r = 0.33, p = 0.39, n = 9).

**Auditory reaction time task.** In the first post-sleep session, mean relative speed was negatively correlated with total sleep time (r = −0.50, p = 0.03, n = 18) but not with stage 2 (r = −0.27, p = 0.27), slow wave sleep (r = −0.41, p = 0.09) or REM sleep (r = 0.12, p = 0.63). Relative speed correlated significantly with time since final awakening (r = 0.49, p = 0.04, n = 18), but this was no longer the case if subjects who were asleep at the end of the experimental period were not included in the analysis (r = 0.38, p = 0.31, n = 9).

**Sleepiness ratings.** In the first post-sleep session, relative sleepiness changes were not correlated with any sleep variable (total sleep time: r = 0.20, p = 0.44; stage 2: r = 0.02, p = 0.93; slow wave sleep: r = 0.17, p = 0.49; REM sleep: r = 0.23, p = 0.37; n = 18). Relative sleepiness changes did not correlate with time since final awakening (r = −0.41, p = 0.10, n = 18; only subjects already awake: r = −0.28, p = 0.47, n = 9).

**Discussion**

The present study provides evidence for performance slowing after an afternoon nap in subjects who were not previously sleep deprived. The absence of sleep deprivation was supported by the time it took volunteers of the sleep group to fall asleep. The mean sleep latency of approximately 14 minutes (Table 3.1.1) was in the expected range for healthy subjects at this time of day (Mitler et al., 2000). Subjects in the sleep group slept for a mean of
85.3 \pm 5.1\text{ min}. The main finding was that immediately after the experimental period, the sleep group showed performance slowing on addition and auditory reaction time tasks, and there was evidence of recovery over the course of the hour after the experimental period. The dynamics of recovery appeared to differ depending on the task used to assess inertia. In contrast to previous studies with control groups (e.g. Naitoh et al., 1993; Salamé et al., 1995), our design included two distinctly different control groups (a rest and an active wake group), which underwent polysomnographic recording as did the sleep group.

**Addition task**

Immediately after the nap, the sleep group attempted fewer sums than in baseline. Performance recovered and reached the level of the control groups over the course of one hour. This duration of recovery from sleep inertia is similar to that reported by Jewett et al. (Jewett et al., 1999), who found a rapid increase in performance during the first hour after sleep, which levelled off after 2 hours. We found that after a period of sleep, only performance speed (i.e., the number of sums attempted) was diminished, whereas accuracy was not affected. This finding is consistent with the results of Salamé et al. (Salamé et al., 1995), who found a reduction in speed but no change in accuracy of performance on spatial memory and logical reasoning tasks following a night time nap. In contrast, Ferrara et al. suggested that sleep inertia influences accuracy more than it affects speed (Ferrara et al., 2000). Balkin and Badia (Balkin and Badia, 1988) found that both speed and accuracy increased over time during the 20 min after awakening and decreased across consecutive nights of sleep restriction. In the latter two studies subjects were sleep deprived (total or slow wave sleep deprived), whereas our subjects were not. Thus, sleep deprivation may help explain the discrepant results. Instructions regarding the relative importance of speed and accuracy can also influence
performance. However, in the previously discussed studies as well as in our study, accuracy and speed were given equal emphasis. Our study suggests that sleep inertia causes subjects to perform slower but not less accurately than before sleep. Tassi and Muzet (Tassi and Muzet, 2000) likewise concluded that in subjects who are not sleep deprived, sleep inertia affects speed more than accuracy.

Relative addition speed in our study increased from the first to the last session and reached a level significantly above baseline. The increase over baseline in all groups suggested that subjects learned to calculate sums more quickly with practice. Thus, in tasks that involve learning, part of the performance recovery after sleep may reflect the ability to manifest learning in performance. At the final time point assessed, the extent of performance improvement did not differ significantly among groups, suggesting no beneficial effects of the nap at this time.

After the experimental period there were no significant performance changes in the active wake group, whereas the rest group, like the sleep group, showed improvement over time, including a significant increase between session 1 and session 2. The performance of the rest group was between that of the sleep and active wake groups. Assuming that learning has occurred, performance in the first session would have been improved by learning, thereby masking a decrement due to inertia. The similarity in the time course of performance changes in the rest and sleep groups may indicate the presence of a sleep inertia-like phenomenon or “rest inertia” in the rest group. In a recent study, a similar finding was reported and the authors proposed to use the term “relaxation inertia” instead of “sleep inertia” to underline the independence from sleep *per se* (Kräuchi et al., 2004). Resting and sleep are accompanied by similar changes in physiology. For example, Kräuchi et al. reported that both lying down (Kräuchi et al., 1997) and darkness after turning-off the light (Kräuchi et al., 2001) were associated with warming of
the extremities. Upon awakening, cooling of the extremities and dissipation of sleep inertia showed a similar time course (Kräuchi et al., 2004). A study of Dinges et al. (Dinges et al., 1981) also suggested that lying down in a dark quiet room affects subsequent performance. In that study immediate post-nap performance on a descending subtraction task was worse after a nap taken in a recumbent position in a dark, quiet room than after a nap taken in a sitting position in a lounge chair with the lights on. Differences in results among sleep inertia studies may thus arise not only from differences in the tasks employed and in the timing of their administration, but also from the circumstances in which they are performed. Some researchers ask subjects to perform sleep inertia tasks while lying in bed in the dark, whereas others require subjects to get up and perform tasks in a lighted room, as we did.

**Auditory reaction time task**

As expected, the sleep group showed the largest performance decrements on the auditory reaction time test. The slowest reaction times recovered significantly from the first to the second session, but remained below baseline up to the last session. Therefore, decrements in auditory reaction speed may last longer than one hour, even when subjects are not sleep deprived. In another study with a very similar task, performance was still below baseline 75 min after awakening from a night of sleep with slow wave sleep deprivation (Ferrara et al., 2000). However, these decrements in auditory reaction speed may not be a specific effect of sleep, because the auditory reaction speed of the active wake group was also consistently impaired after the experimental phase when compared with baseline. On the other hand performance of the rest group was not impaired. The active wake group watched a documentary film that was preceded, as in the other groups, by a challenging 45-minute auditory learning test (Gottselig et al., 2004). As subjects knew that afterwards they would have to answer questions about the content of the film, they also
had to listen carefully to the information provided. Thus, prolonged auditory stimulation may have caused auditory information overload in the active wake group, resulting in reduced reaction speed to auditory stimuli. Similarly, performance on visual discrimination tasks was reduced after repeated within-day testing (Ludwig and Skrandies, 2002; Mednick et al., 2002). Alternatively, it is possible that subjects have recovered from sleep inertia after the second session, and did not return to baseline level, because of boredom, for instance. Nevertheless, there are no reasons to assume that the sleep and wake groups were bored whereas the rest group, which performed at baseline level, was not.

Performance of the rest group did not change over time, nor was it different from baseline or from the performance of the active wake group. The rest group, however, performed better than the sleep group. Therefore, lying in bed in a dark quiet room does not seem to affect subsequent performance on an auditory reaction time task. This may seem at odds with the results from the addition task, but most studies on sleep inertia show that performance on different tasks is differently affected by sleep inertia. Likewise, performance on some tasks may be affected by “rest inertia” whereas performance of other tasks is not.

**Stanford Sleepiness Scale**

Subjects in all groups rated themselves sleepier in the first session after the experimental period compared with baseline. In a study without controls, one would conclude that the nap caused increased sleepiness. The fact that increased sleepiness was also observed in the active wake and rest groups suggests that factors that were not specific to sleep must have contributed to the sleepiness changes. If there were differences in sleepiness among the groups, the Stanford Sleepiness Scale was not sensitive enough to detect them, or the subjects may have experienced different dimensions of sleepiness that
are intermingled in the descriptors of the Stanford Sleepiness Scale (reviewed in Horne, 1991).

Increased sleepiness is related to increased vasodilatation, which is not only induced by lying down or being in darkness but also by relaxation (Baker et al., 1976; Kräuchi et al., 1997; Kräuchi et al., 2001; Kräuchi et al., 2004). Thus, the extent of relaxation, and resulting changes in body temperature distribution through vasodilatation may not have differed sufficiently among the groups to cause differing levels of sleepiness.

**Experimental considerations**

The use of a between-subjects design allowed us to avoid carryover effects inherent to performance tasks. The groups were well matched in terms of their age, gender and ability to perform the task. Although our experiment took place in the afternoon with subjects who were not sleep deprived, microsleep episodes occurred in resting and active wake subjects. These microsleep episodes highlight the importance of doing polysomnographic recordings in control groups. Recordings in controls would be even more important in studies of subjects who are sleep deprived and during the night time studies, when inadvertent sleep is more likely.

It was not possible to assess whether sleep inertia was dependent on the sleep stage from which subjects were awakened, because there was not sufficient variability in our sample. More specifically, eight subjects were woken from stage 2, one was woken from stage 4, and the remaining participants were already awake at the end of the sleep opportunity period.

Dissipation of sleep inertia and body posture changes are associated with changes in body temperature distribution (Kräuchi et al., 1997; Kräuchi et al., 2004). Improvement on various performance measures, such as slowest reaction times and addition performance, is positively correlated with elevated
core body temperature (Wright JR. et al., 2002). Therefore, in future sleep inertia studies it would be useful to include measures of core and peripheral body temperature (Kräuchi et al., 1997; Kräuchi et al., 2004).

Conclusions
Sleep inertia is a major factor that accounts for changes in human alertness and performance after a period of sleep, but surprisingly little is known about its mechanisms. For example, the physiological underpinnings of sleep inertia (Balkin et al., 2002) and the pharmacological treatment of sleep inertia are areas that are only beginning to be explored (e.g. Van Dongen et al., 2001). Few previous studies have investigated sleep inertia after daytime sleep. Sleep inertia after daytime naps is of practical and clinical relevance. For example, daytime napping may help to counteract sleepiness in patients with sleep disorders (reviewed in Takahashi, 2003). It would be interesting to investigate sleep inertia after daytime naps in the elderly, who nap more frequently than younger people do (Huang et al., 2002). The extent and time course of sleep inertia may be age dependent.

The present study demonstrated consistent performance slowing on an addition task and on an auditory reaction time task after awakening from an afternoon nap. Results from the control groups revealed that performance slowing observed after a period of sleep is not always specifically attributable to the brain state of sleep. For instance, after a 2-hour period of rest in a dark quiet environment, calculation speed was reduced compared with the following sessions. In addition, reaction speed in the active wake group showed a persistent reduction compared with baseline. These results indicate that the careful choice of appropriate control conditions is essential in assessing the specificity and underlying mechanisms of sleep inertia.

In accordance with previous research, the results suggested that after awakening from sleep, performance on different tasks recovers at different
rates (e.g. Ferrara et al., 2000). These tasks may involve distinct neuroanatomical areas. Previous studies have documented regional differences in sleep processes as a result of prior waking activities (e.g. Kattler et al., 1994; Huber et al., 2004). Differences in the rate of recovery from sleep inertia on different tasks could reflect differences in the time course of awakening of different parts of the brain. Consistent with this idea, a recent PET study demonstrated that after a period of sleep, waking patterns of regional cerebral blood flow were re-established at different rates in different brain areas (Balkin et al., 2002).
Sleep and rest facilitate auditory learning


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Abstract

Sleep is superior to waking for promoting performance improvements between sessions of visual perceptual (Stickgold et al., 2001) and motor (Smith and MacNeill, 1994; Fischer et al., 2002; Walker et al., 2002) learning tasks. Few studies have investigated possible effects of sleep on auditory learning (Fenn et al., 2003; Atienza et al., 2004). A key issue is whether sleep specifically promotes learning, or whether restful waking yields similar benefits (Tononi and Cirelli, 2001). According to the “interference hypothesis,” sleep facilitates learning because it prevents interference from ongoing sensory input, learning (e.g., Walker et al., 2003a) and other cognitive activities that normally occur during waking. We tested this hypothesis by comparing effects of sleep, busy waking (watching a film) and restful waking (lying in the dark) on auditory tone sequence learning. Consistent with recent findings for human language learning (Fenn et al., 2003), we found that compared with busy waking, sleep between sessions of auditory tone sequence learning enhanced performance improvements. Restful waking provided similar benefits, as predicted based on the interference hypothesis. These findings indicate that physiological, behavioural and environmental conditions that accompany restful waking are sufficient to facilitate learning and may contribute to the facilitation of learning that occurs during sleep.

Key words: sleep, napping, rest, auditory perceptual learning, retroactive interference, plasticity
Introduction

Sleep may promote learning and neural plasticity (Buzsáki, 1989; Sejnowski and Destexhe, 2000; Tononi and Cirelli, 2001). Sleep stages (Horne and Walmsley, 1976; Cantero et al., 2002), as well as EEG slow-wave activity (Kattler et al., 1994; Vyazovskiy et al., 2000; Miyamoto et al., 2003), regional cerebral blood flow (e.g., Peigneux et al., 2003) and neuronal activity (e.g., Wilson and McNaughton, 1994; Nádasdy et al., 1999; Dave and Margoliash, 2000; Hirase et al., 2001) during sleep are related to the activity of the brain during prior wakefulness. These changes in sleep as a result of waking activity may reflect processes involved in synaptic reorganization (Buzsáki, 1989; Buzsaki, 1998; Tononi and Cirelli, 2003). Consistent with a functional role of sleep in neural plasticity, sleep enhanced visual cortical plasticity induced by monocular deprivation during a critical period of development in cats (Frank et al., 2001). Numerous human and animal studies suggested that REM or nonREM sleep or both improve memory retrieval (reviewed in Sejnowski and Destexhe, 2000; Stickgold et al., 2001; Tononi and Cirelli, 2001). Increasing evidence suggests that sleep promotes visual perceptual (reviewed in Stickgold et al., 2001) and motor (Smith and MacNeill, 1994; Fischer et al., 2002; Walker et al., 2002) learning in humans. However, the proposed involvement of sleep in learning and memory remains controversial (e.g., Vertes and Eastman, 2000; Siegel, 2001).

The present study addressed the question of whether sleep specifically facilitates learning, or whether resting can provide similar benefits (Tononi and Cirelli, 2001). We investigated the effect of an afternoon nap on learning of a challenging auditory tone perception task. The nap paradigm had the advantage that it permitted waking control groups without necessitating sleep
deprivation. Napping was previously found to have large effects on visual perceptual learning (Mednick et al., 2003).

Experimental procedures

Subjects
Participants (n=64) were 18-28 year-olds with hearing thresholds ≤35 dB hearing level (250-3000 Hz). They were right-handed nonsmokers with moderate alcohol (≤5 drinks/week) and caffeine (≤3 cups coffee/day) consumption, no flights involving time shifts for two months before the study, and no history of neurologic or psychiatric disease. For three days before the study subjects maintained a regular sleep schedule (23:00-07:00, verified by wrist actimetry), refrained from napping, and abstained from alcohol and caffeine. Procedures were approved by the local ethics committee and subjects gave written informed consent.

Design
Subjects were randomly assigned to sleep, restful waking (RW), busy waking (BW) or no-break (NB) groups (n=16 per group), with the constraints that groups were matched in age and gender. In each group, mean age was 23 years and half of the subjects were female. The experimental period started at 13:55 or 15:55 (half of each group at each time). Experiment times and gender were completely counterbalanced. In the NB group, subjects completed immediately consecutive learning sessions that began or ended at times matched to those of other groups (one quarter of the subjects at each time). The level achieved, learning score, and reaction times did not differ among groups in session 1, indicating no differences in their ability to perform the
Table 3.2.1. Timing of experimental procedures

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Time 1</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Session 1:</strong> Auditory learning task, preceded and followed by a subjective sleepiness rating</td>
<td>12:45-13:30</td>
</tr>
<tr>
<td>Restroom break, snack, impedance tests, subjects informed of group assignments</td>
<td>13:30-13:55</td>
</tr>
<tr>
<td><strong>Experimental manipulation,</strong> concurrent polysomnographic recording</td>
<td>13:55-15:55</td>
</tr>
<tr>
<td>Sleep group: sleep opportunity period</td>
<td></td>
</tr>
<tr>
<td>Restful waking (RW) group: lay awake in bed in dark room</td>
<td></td>
</tr>
<tr>
<td>Busy waking (BW) group: watched film</td>
<td></td>
</tr>
<tr>
<td>Time to permit recovery from sleep inertia with reaction time and addition tests, subjective sleepiness ratings, and rest breaks supervised in the laboratory</td>
<td>15:55-17:00</td>
</tr>
<tr>
<td><strong>Session 2:</strong> Auditory learning task, preceded and followed by a subjective sleepiness rating</td>
<td>17:00-17:45</td>
</tr>
</tbody>
</table>

¹Half of the subjects in the sleep, RW, and BW completed the procedures at the times given and half completed the procedures two hours later. In the no-break group, subjects completed immediately consecutive learning sessions, such that Session 1 or Session 2 was performed at times matched to those of other groups (one quarter of the subjects at each time).

²A German translation of the Stanford Sleepiness Scale was used.

Procedure

Procedures are summarised in Table 3.2.1. Electrodes were applied to sleep, rest, and wake groups for polysomnographic recordings during the task.
After the first session, subjects had a 25-min. break when electrode impedances were checked and subjects were informed of group assignments. During the immediately following 2-hour experimental period, subjects in sleep and RW groups lay in bed in a darkened single bedroom. The RW group was instructed to relax without falling asleep. If rolling eye movements or reduced alpha activity appeared, we alerted subjects by sounding a tone over the intercom. BW subjects sat in a lighted room under experimenter supervision and watched an educational film. They were instructed to attend carefully because they would answer questions about the film afterwards. To allow time for recovery from sleep inertia, the second learning session began 65 min. after the experimental period. Subjects spent intervening time in the same controlled manner (with short tests and rest breaks) in the laboratory. The NB group completed the two learning sessions with no intervening time between sessions.

Data analysis
The alpha criterion for statistical analyses was 0.05. The level achieved was analysed with ordinal logistic regression. The learning score assessed performance accuracy and weighted correct answers for each level based on theoretical considerations of difficulty (Fig. 3.2.1b). Zero points were given for incorrect answers. The number of points a subject receives for a correct answer should be inversely related to the probability of getting a correct answer by chance, because an improbable correct answer is more likely to reflect knowledge. In level 1, the probability of getting a correct answer by chance is 1/4, so 4 points were given for a correct answer. Upon reaching level 2, subjects had already learned to discriminate single patterns. Thus, it is assumed that one of the two patterns (e.g., the square) is identified correctly,
leading to a 1/3 probability of getting the second pattern correct by chance. Seven points are given for correctly identifying the sequence, because the first pattern is worth 4 points (as in level 1), and identifying the second pattern is worth 3 points (reciprocal of the chance probability). Following analogous considerations, correct answers received 9 points in level 3 and 10 points in level 4. Learning scores were averaged over all trials in each session and subjected to repeated measures ANOVA (rANOVA) with group and session factors. Paired t-tests (two-tailed) assessed changes in performance between sessions. We used one-tailed unpaired t-tests to test predictions that performance changes in (1) the sleep and the RW groups would be greater than the BW group (2) the sleep group would be greater than the RW group, (3) the NB group would be greater than the BW group (4) the sleep and RW groups would be greater than the NB group. Predictions were based on the interference hypothesis and on previous findings (Mednick et al., 2002; Walker et al., 2002; Fenn et al., 2003).

Reaction times (RT; latency from stimulus onset to final button press) from the first block of level 1 (approximately the first 5 minutes of the task) were transformed (1/RT), analysed with rANOVA and reverse transformed (Fig. 3.2.2b).

Results and discussion

The sleep group slept for 77 min. on average (Table 3.2.2), and all individuals slept for more than 30 minutes. Two subjects in the RW group and one subject in the BW group exhibited very short periods of sleep that constituted
### Table 3.2.2. Sleep variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>Duration (min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sleep Latency(^2)</td>
<td>13.6 ± 2.1</td>
</tr>
<tr>
<td>Stage 1</td>
<td>6.1 ± 0.8</td>
</tr>
<tr>
<td>Stage 2</td>
<td>39.4 ± 4.2</td>
</tr>
<tr>
<td>Stage 3</td>
<td>9.2 ± 1.9</td>
</tr>
<tr>
<td>Stage 4</td>
<td>19.2 ± 4.2</td>
</tr>
<tr>
<td>Slow Wave Sleep (Stages 3 &amp; 4)</td>
<td>28.3 ± 4.2</td>
</tr>
<tr>
<td>REM Sleep(^3)</td>
<td>13.6 ± 2.2</td>
</tr>
<tr>
<td>REM Sleep Latency(^3)</td>
<td>64.0 ± 4.0</td>
</tr>
<tr>
<td>Total Sleep Time (TST)</td>
<td>77.1 ± 5.6</td>
</tr>
<tr>
<td>Waking After Sleep Onset</td>
<td>16.3 ± 4.1</td>
</tr>
<tr>
<td>Sleep Efficiency(^4) (%)</td>
<td>64.4 ± 4.7</td>
</tr>
</tbody>
</table>

\(^1\)Data from the sleep group, n=16.

\(^2\)Latency to stage 2

\(^3\)Eleven of the subjects exhibited REM sleep, and only these n=11 were included in calculating REM sleep duration and latency.

\(^4\)The percentage of time when the subjects were asleep (stages 2, 3, 4, or REM sleep) relative to the total time in bed.

0.3 - 4% of the experimental period. Excluding these subjects did not alter statistical results. Previous studies of human sleep and learning have generally not included polysomnographic recordings of waking groups. Awareness of recordings motivates participants and their supervisors to ensure that wakefulness is maintained; the occurrence of microsleeps despite this
Sleep and rest facilitate auditory learning awareness underscores the advantages of conducting polysomnographic recordings in waking groups.

On the auditory learning task, the maximum level achieved increased from session 1 to session 2 ($\chi^2=3.83$, $p=0.05$), indicating overall improvement. The change in level achieved did not differ among groups ($\chi^2=2.25$, $p=0.52$). Three, 57, and 4 subjects respectively reached levels 1, 2, and 3 in the first session; 55, 8, and 1 subjects respectively reached levels 2, 3, and 4 in the second session.

The learning score (Fig.3.2.1b) provided a more sensitive measure of performance accuracy. Changes in learning scores from session 1 to session 2 differed among groups (Fig 3.2.2a), as indicated by a significant group * session interaction ($F(3, 60)=3.59$, $p=0.02$). Sleep and RW groups showed significant improvement from session 1 to session 2; the BW group did not. Although performance improvement in the sleep group was greater than in the RW group, this difference did not reach statistical significance. Performance increases in both sleep and RW groups were significantly larger than in the BW group.

As predicted based on the interference hypothesis, the sleep and RW groups showed greater improvement than did the BW group. Whereas sensory input was dramatically reduced in sleep and RW groups, the BW group watched a film involving visual and auditory stimulation. Interference due to ongoing stimulation may have prevented performance improvements in the BW group. Previous studies provided only partial control for such interference (Mednick et al., 2002; Walker et al., 2002). To prevent waking activities from interfering with learning, it may be necessary to limit motor activity and sensory input from all modalities, as in the restful waking group of the present study.
Figure 3.2.1. Auditory learning task (Leek and Watson 1988). a. Auditory tone patterns. Subjects identified pattern(s) by pressing corresponding button(s) labelled with the symbols shown. Stimuli were presented in blocks of 48 trials at 70 dB sound pressure level. Initially, single patterns were presented. Visual feedback indicated whether the response was correct or incorrect and the correct answer was shown. After each block, percentage correct for that block was displayed. If subjects obtained ≥ 90% correct, the length of the stimulus sequence was increased by one pattern in the next block. For example, if a subject obtained ≥ 90% correct in identifying single patterns (level 1), subsequently sequences of two patterns were presented (level 2). Sequence length could increase up to four patterns. The interval between patterns within a sequence was 50 ms. The same pattern never occurred more than once within a sequence. Accuracy was emphasised over speed. b. Learning scores. Performance in each level was weighted according to theoretical considerations of difficulty (see methods). The actual accuracies achieved support our weighting system. In the first block of levels 1 and 2 in the first session, subjects achieved means of 80±1% and 43±2% correct. The theoretical weighting of 1.8 closely resembles the relative empirical difficulty of 1.9. Abbreviations: $p_i = $ probability of correct answer in level $i$, $s_i = $ score for correct answer in level $i$. 

\[
\begin{align*}
\pi_1 &= \frac{1}{4} \\
\pi_2 &= \frac{1}{3} \\
\pi_3 &= \frac{1}{2} \\
\pi_4 &= \frac{1}{1} \\
\end{align*}
\]

\[
\begin{align*}
s_1 &= \frac{1}{\pi_1} = 4 \\
s_2 &= \frac{1}{\pi_2} = 7 \\
s_3 &= \frac{1}{\pi_3} = 9 \\
s_4 &= \frac{1}{\pi_4} = 10 \\
\end{align*}
\]
Sleep and rest facilitate auditory learning. Another possible source of waking interference is learning. Learning while watching the film may have interfered with the BW group’s performance in the second auditory learning session. Learning new information can interfere with recall of previously learned information, a phenomenon known as retroactive interference. Based on the finding that subjects re-learned a list of syllables more quickly if they memorised it immediately before going to bed than if they memorised it in the daytime (Heine, 1914), Heine suggested that sleep benefits learning by preventing retroactive interference that normally occurs during waking. Our results are consistent with Heine’s interpretation.

Physiological processes common to sleep and restful waking may actively facilitate learning and serve to prevent waking interference. Neuronal replay that is postulated to facilitate learning (e.g., (Wilson and McNaughton, 1994; Nádasdy et al., 1999; Dave and Margoliash, 2000)) could occur during restful waking as well as during sleep. Hippocampal sharp waves may provide a mechanism for neuronal replay (Nádasdy et al., 1999) and consequent neural plasticity, and they occur during immobile waking as well as during slow wave sleep (Buzsáki, 1989; Buzsáki, 1998).

If sleep and rest actively facilitate learning, one might expect greater between-session improvement if subjects sleep or rest between sessions than if they complete immediately consecutive sessions. However, improvement in the sleep and RW groups did not differ significantly from the NB group. This finding does not preclude the possibility that sleep and rest actively promote learning, because continuous learning processes and between-session performance improvements might involve different mechanisms (Walker et al., 2003b). The performance increase in the NB group was significant and larger than in the BW group (Fig. 3.2.2a), as expected based on the interference hypothesis. A previous study likewise showed that a group with no break between language learning sessions showed greater between-session improvement.

**Figure 3.2.2. Changes in performance from session 1 to session 2.**

- **a.** Changes in learning scores. Increases of 24%, 18%, 6%, and 18% were observed in sleep (s), restful waking (rw), busy waking (bw), and no-break (nb) groups, respectively. Performance in session 1 did not differ among groups (F(3,60)=0.90, p=0.45). Symbols on bars indicate changes between sessions. P-value symbols: ***<0.001, **<0.01, *<0.05, ns: not significant. Statistics for differences between sessions: ts=6.22, ps<0.0001, trw=4.47, prw=0.0004, tbw=1.68, pbw=0.11, tsnb=5.18, pnb=0.0001. Statistics for differences between groups: tsw=3.07, psw=0.002; trw-bw=1.82, prw-bw=0.04; tsnb-bw=1.81, pnb-bw=0.04; tsw-bw=1.60, psw-bw=0.06; tsw-bw=1.31, psw-bw=0.10; tsw-bw=0.18, prw-bw=0.43. 
- **b.** Reaction times. Performance became faster from session 1 to session 2 (F(1, 60)=15.47, p<0.001). This change did not differ among groups (group * session interaction: F(3, 60)=0.54, p=0.66). Speed of performance did not differ among groups in session 1 (F(3,60)=0.58, p=0.63). Error bars indicate s.e.m.
improvement than a group that was awake for 12 hours between sessions, while the no-break group did not differ from a group that had a night of sleep (Fenn et al., 2003).

Improved attention after sleep or rest might reduce retroactive interference or contribute to subsequent performance improvements by enhancing automaticity of brain responses to auditory stimuli (Atienza et al., 2004). Potentially decreased concentration due to sustained attention might have contributed to the lack of improvement in the BW group, yet the NB group sustained attention to the auditory learning task for the longest continuous period and nonetheless showed larger improvements than did the wake group. Moreover, BW, RW and sleep groups rated their subjective sleepiness similarly (Table 3.2.3), and reaction times on the learning task did not differ among groups (Fig. 3.2.2b), suggesting that differences in alertness (e.g., residual sleep inertia) cannot account for the results.

Table 3.2.3. Subjective sleepiness ratings

<table>
<thead>
<tr>
<th></th>
<th>Before session 1</th>
<th>After session 1</th>
<th>Before session 2</th>
<th>After session 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW</td>
<td>2.44 ± 0.20</td>
<td>3.00 ± 0.30</td>
<td>2.81 ± 0.28</td>
<td>3.19 ± 0.29</td>
</tr>
<tr>
<td>RW</td>
<td>2.19 ± 0.16</td>
<td>3.31 ± 0.22</td>
<td>2.63 ± 0.26</td>
<td>3.13 ± 0.27</td>
</tr>
<tr>
<td>Sleep</td>
<td>2.13 ± 0.20</td>
<td>3.06 ± 0.28</td>
<td>2.38 ± 0.20</td>
<td>2.69 ± 0.25</td>
</tr>
</tbody>
</table>

1Values are means ± standard errors (n=16 per group). Ratings on a scale of 1 to 7 were made using the Stanford Sleepiness Scale; higher ratings correspond to greater sleepiness. Repeated measures ANOVA showed no differences between groups (group main effect: F(2, 45)=3.26, p= 0.45, group * time interaction: F(6, 135)=0.63, p=0.68, Huynh-Feldt ε = 0.86). No-break subjects did not rate their sleepiness. Abbreviations: BW: busy waking, RW: restful waking.
Compared with busy waking, sleep facilitated learning of auditory tone patterns. Subjects who rested or performed continuously also showed performance improvements, and these improvements did not differ significantly from those observed in the sleep group. These findings support the idea that sleep and rest promote auditory learning by reducing interference that normally occurs during waking. Other physiological, behavioural and environmental changes common to sleep and rest may also facilitate learning. Our findings do not exclude the possibility that sleep-specific processes provide additional benefits to auditory learning beyond the benefits of restful waking; the sleep group showed greater improvement than the restful waking group, although this difference did not reach significance. Comparing sleep with resting conditions and quiescent states such as meditation or hypnosis promises to enrich future investigations of the influence of sleep on learning.
Concluding remarks

Sleep and wakefulness are two obviously different states. The most typical distinction being that sleep represents a state of mind with diminished awareness for the environment, while wakefulness is characterised by varying degrees of consciousness. Usually, the decision to go to sleep is triggered by increasing sleepiness. After awakening, we need some time to dissipate sleepiness, before being able to enjoy the refreshment of a good night of sleep.

The scoring rules for human sleep suggest that the sleep states are discrete entities. But the first study (chap 2.1) of this thesis supports the view that REMS and NREMS are interleaved states (Benington and Heller, 1994; Nielsen, 2000; Werth et al., 2002). Low muscle tone in NREMS can be considered as a feature of REMS. The quantity of SOREMS episodes in addition to the number of episodes of NLMT at sleep onset not only in
morning sleep without SOREMS, but also at night suggest that there is a REMS window at sleep onset.

The second study (chap. 2.2) shows that, besides having very typical features of topographical distributions of EEG power, the two sub-states also share basic topographical features with each other and with wakefulness. Figures 2.2.1 and 2.3.1 displaying the 1-Hz bin maps show that REMS represents an intermediate state between NREMS and waking. At some frequencies, REMS maps resemble those of NREMS (2-4 Hz), at other frequencies those of waking (9-12 Hz), while they represent a mixture of both patterns at yet other frequencies (5-8 Hz). Increasing sleep pressure, which greatly augments power in lower frequencies during a prolonged period of wakefulness, does not change the distribution of relative power in the maps. In all three states, the resemblance of a map depicting relative power distribution over the skull during a baseline night to the one during a recovery night of a given subject is greater than the resemblance between maps of two subjects during a baseline or a recovery night (Fig. 2.2.6). These individual characteristics suggest that analyses of single subjects might shed more light on certain aspects of the EEG, which are blurred by averaging. Cluster analysis of the topographical power maps revealed segregation into three frequency partitions, which were largely independent of state and sleep pressure (Fig. 2.2.5). Further similarities between the states were seen in the response to sleep deprivation. EEG power was enhanced in the low frequency range in all three vigilance states (Fig 2.3.3).

The other two studies also point at commonalities between sleep and waking. The study presented in chapter 3.1 is exceptional because it investigated sleep inertia using both waking and rest control groups in non-sleep deprived subjects. In many experimental paradigms, waking control conditions are not feasible without introducing sleep deprivation. The frequency of testing employed was higher than in most previous studies, and
two different tasks were used to assess sleep inertia, in addition to sleepiness ratings.

New insights came from the results of the addition task (Fig. 3.1.1). Lying in a dark, quiet room also affects subsequent performance. I proposed the term ‘rest inertia’ for it. Although performance was not below baseline after the rest period, the time course of improvement was strikingly similar to the one of the nap group. Specifically, performance improved from the first to the last session and from session 1 to 2, while in the active wake group, there was no change across sessions. Learning occurred in this task in all groups, as inferred from the significant improvement in the last test session relative to baseline. Therefore, one could argue that performance of the rest group would have been below baseline without the confounding effect of learning. Since both recumbence (Kräuchi et al., 1997) and darkness (Krauchi et al., 2005) have an effect on body temperature distribution, it is possible that one physiological correlate of inertia is temperature distribution (Kräuchi et al., 2004).

The results of the perceptual auditory learning task in the study in chapter 3.2 also showed effects of rest on performance. The restful waking group performed better than the busy waking group. In a previous study using a rest condition, the authors did not find any restorative effect of the resting condition on performance of a visual texture discrimination task (Mednick et al., 2002). But their rest condition was different from ours, and resembles more our active waking condition. Their subjects were blindfolded to prevent visual input while they listened to short stories. Our subjects had no perceptual input at all because they were lying in a dark quiet room. The performance of the sleep and rest groups did not improve relative to the performance of the no-break group, as might have been expected from the results of a visual texture discrimination task (Mednick et al., 2002; Mednick et al., 2003). Reasons for the discrepancy may be that subjects did not spend
enough time awake before the nap or that the interval between learning and retest phase was too short. Nevertheless, the sleep and rest groups improved relative to an active-wake group, showing that consolidation had taken place in the absence of competing or disrupting factors such as sensory input or cognitive activity, which are also referred to as interference (Walker et al., 2003). The perseveration-consolidation hypothesis of memory positing that memories need time to become resistant to interference has already been proposed in 1900 by Müller and Pilzecker (McGaugh, 2000).

As already discussed for sleep inertia, the many different learning tasks and output variables measured in different settings produce such a variety of results that they may appear to be contradictory. In our study, behavioural performance enhancement on an auditory learning task was similar after rest and sleep. These results do not indicate a specific effect of sleep in performance enhancement for this task, but they do not prove either, that sleep could not play a specific role. To investigate the relationship between sleep and memory, simple elements have to be manipulated without touching basic mechanisms of sleep not related to learning so that the restorative function of sleep is intact. Otherwise, indirect effects on learning not related to learning mechanisms would confound the results. A conclusive discussion of this theme is not yet possible but new findings and theories emerge almost every day.

Usually, the distinctions between and the characteristics of the different states are analysed and stressed. In the present studies, we chose the opposite approach and found some striking features shared by the vigilance states.
Chapter 5

References


Dement, W., 1960. The effect of dream deprivation: The need for a certain amount of dreaming each night is suggested by recent experiments. Science 131: 1705-1707.


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Journal papers

J. M. Gottselig, D. Brandeis, G. Hofer-Tinguely, A. A. Borbély, P. Achermann

*Human central auditory plasticity associated with tone sequence learning*
Learning and Memory 2004; 11:162-171


*Sleep and rest facilitate auditory learning*
Neuroscience 2004, 127; 557–561


*Sleep inertia: performance changes after sleep, rest and active waking*
Cognitive Brain Research 2005, 22, 323-331

G. Tinguely, R. Huber, A. A. Borbély and P. Achermann

*Non-rapid eye movement sleep with low muscle tone as a marker of rapid eye movement sleep regulation*
BMC Neuroscience 2006, 7:2

G. Tinguely, L. A. Finelli, H. P. Landolt, A. A. Borbély and P. Achermann

*Functional EEG topography in sleep and waking: State-dependent and state-independent features*
Manuscript accepted by NeuroImage
S. J. Regel, **G. Tinguely**, J. Schuderer, M. Adam, N. Kuster, H.-P. Landolt, P. Achermann

*Dose-response effects of electromagnetic fields on cognitive performance and sleep EEG*

Manuscript in preparation

S. J. Regel, J. M. Gottselig, J. Schuderer, **G. Tinguely**, N. Kuster, H.-P. Landolt, J. V. Rétey, P. Achermann

*Effects of electromagnetic fields on cognitive performance and waking EEG*

Manuscript in preparation
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Joint meeting of the Associated Professional Sleep Societies (APSS) with the World Federation of Sleep Research Societies, Chicago, 6/03

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„Is Sleep-Onset REM Sleep An Exceptional Feature Of Sleep?“

Sleep 26, A60-A60, 2003

Julie M. Gottselig, **Gilberte Hofer-Tinguely**, Sabine Regel, Julia Retey, Anna Gerber, Hans-Peter Landolt, Alexander A. Borbély, Peter Achermann

„Reduced Power After a Power Nap“

Sleep 26, A431-A431, 2003
Joint Meeting of the Swiss Society for Neuroscience and the Swiss Society of Psychiatry and Psychotherapy, Fribourg, 1/03

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J. Sleep Res. 11, Suppl. 1: 100-101, 2002

Neuroscience Centre, Zurich (ZNZ), PhD retreat in Valens, Switzerland, 5/02

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**Gilberte Hofer-Tinguely**, Peter Achermann.

„REM Sleep Regulation: Comparison of Daytime and Nighttime Sleep“
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