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REGIONAL CEREBRAL BLOOD FLOW CHANGES DURING SLOW WAVE SLEEP IN HUMANS AS ASSESSED BY POSITRON EMISSION TOMOGRAPHY

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List of Tables and Figures

List of Abbreviations

EEG Electroencephalogram

EOG Electrooculogram

EMG Electromyogram

PET Positron Emission Tomography

SPECT Single Photon Emission Computerized Tomography

MRI Magnetic Resonance Imaging

GABA Gamma-aminobutyric-acid

^{133}Xe Marked Xenon-133

$H_2{}^{15}O$ Water marked with Oxygen-15

FDG Fluorodeoxyglucose

CMR Cerebral Metabolic Rate

$CMRO_2$ Cerebral Metabolic Rate of Oxygen

CMRGlu Cerebral Metabolic Rate of Glucose

rCBF Regional Cerebral Blood Flow

SWS Slow Wave Sleep

REM Rapid-eye-movement

ANCOVA Analysis of Covariance

VOI Volume of Interest

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Preface

The following text is included as a requirement in order to inform the external examiner of McGill Graduate Faculty regulations:

> Candidates have the option of including, as part of the thesis, the text of a paper(s) submitted or to be submitted for publication, or the clearly-duplicated text of a published paper(s). These texts must be bound as an integral part of the thesis.
>
> If this option is chosen, **connecting texts that provide logical bridges between the different papers are mandatory**. The thesis must be written in such a way that it is more than a mere collection of manuscripts; in other words, results of a series of papers must be integrated.
>
> The thesis must still conform to all other requirements of the "Guidelines for Thesis Preparation". **The thesis must include:** A Table of Contents, and abstract in English and French, an introduction which clearly states the rationale and objectives of the study, a comprehensive review of the literature, a final conclusion and summary, and a thorough bibliography or reference list.
>
> Additional material must be provided where appropriate (e.g. in appendices) and in sufficient detail to allow a clear and precise judgement to be made of the importance and originality of the research reported in the thesis.
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The experimental part of this thesis (chapter 2) consists of an original article submitted for publication. For this study, I did all the experimental work, i.e. recruitment and selection of subjects, preliminary studies, EEG recording and analysis, analysis of PET data and drawing of the figures. Under the supervision and guidance of Barbara E. Jones, Tomáš Paus, Jean Gotman and Alan Evans, I also designed the protocol, interpreted the results and wrote the manuscript. The analysis used for the PET data was previously developed by Tomáš Paus and colleagues, hence we worked closely together for its adaptation to the current study. In accordance with safety regulations, David Reutens and Pierre Fiset performed the injections and designed parts of the protocol, to minimize disturbances to the subjects during sleep.

Abstract

Earlier lesion and imaging studies in humans, as well as electrophysiological recordings in other animals, have suggested an important role for the thalamus during slow wave sleep (SWS). This structure has been shown to undergo disfacilitation and active inhibition during SWS, changing its firing pattern and thus altering sensory afference to the cortex during this state.

Regional cerebral blood flow (rCBF) decreases are reported in the human thalamus in association with delta (1-4 Hz) and, even further, with spindle (12-15 Hz) wave activity during SWS. These results support the physiological changes purported to occur in the thalamus during this state. We also found rCBF decreases in association with delta in the brainstem reticular formation, cerebellum, anterior cingulate and orbito-frontal cortex. These changes, together with the thalamic decreases, might provide the physiological substrate for the progressive attenuation of sensory awareness and motor activity that occur during SWS. Regional CBF increases as a function of delta activity were found mainly in the visual and auditory cortices, possibly supporting dream-like imagery during SWS.

Résumé

Des études antérieures portant sur les lesions et des études d'imagerie chez l'humain, ainsi que des enregistrements éléctrophysiologiques chez d'autres animaux, ont suggeré un rôle important du thalamus pendant le sommeil lent (SL). Il a été montré que cette structure subissait une défacilitation et une inhibition active pendant le SOL, changeant son mode de décharge et ainsi altérant l'afference sensorielle au cortex durant cet état.

Dans cet étude nous montrons une diminution du débit sanguin cérébral régional (DSCr) au niveau du thalamus associée à l'activité delta (1-4 Hz) et, une diminution encore plus marquée, avec les fuseaux de sommeil (12-15 Hz) durant le SL. Ces résultats sont en accord avec les changements physiologiques qui ont probablement lieu au niveau du thalamus dans cet état. Nous avons également trouvé des diminutions du DSCr dans la formation réticulaire du tronc cérébral, le cervelet, le cortex cingulé antérieur et le cortex orbito-frontal. Celles-ci, combinées avec les diminutions du thalamus, pourraient donner le substrat physiologique de l'atténuation progressive de la perception sensorielle et de l'activité motrice qui ont lieu pendant le SL. Des augmentations du DSCr en fonction de delta ont été trouvées surtout au niveau du cortex visuel et auditif, favorisant peut-être l'existance d'imagerie de type rêve pendant le SL.

Chapter 1.- General Introduction

Literature Review

The sleep-wake cycle can be defined as a cyclical sequence of three distinct states, defined on the basis of electrophysiological parameters: a) Wake, which is characterized by prominent alpha electroencephalographic (EEG) activity (8-12 Hz) when the subject is relaxed, and low voltage, fast EEG activity (including beta, 20-30 Hz, and gamma, 31-60 Hz) when the subject is alert; b) Rapid-eye-movement (REM) sleep that, besides rapid eye movements, is associated with 'sawtooth' waves (4-7 Hz) and fast, low voltage EEG activity (beta and gamma); and c) slow wave sleep (SWS) that is characterized by the presence of slow waves (1-4 Hz) and sleep spindles (12-15 Hz) in the EEG (Dement and Kleitman, 1957; Rechtschaffen and Kales, 1968). SWS can be defined as a reversible state of disengagement from the external environment that is composed of a cyclical sequence of stages. It is thus further divided into 4 stages: Stage 1, characterized by the disappearance of alpha activity and appearance of slow, rolling eye movements; stage 2 characterized by K-complexes, sleep spindles and some slow or delta waves (less than 25%); stage 3 with some spindles and prominent delta waves (less than 50%) and stage 4 with a predominance of high amplitude delta waves (more than 50%)(see Table 1 and Figure 1; Rechtschaffen and Kales, 1968).

State-stage in sleep-wake cycle	Predominant wave types and frequencies
Alert wakefulness	Gamma, beta
Relaxed wakefulness	Alpha
Stage 1 SWS	Beta, theta, sharp vertex waves
Stage 2 SWS	Sigma, K-complex, delta <25%
Stage 3 SWS	Delta 25-50%
Stage 4 SWS	Delta >50%
REM sleep	Theta, beta, gamma

Table 1: Predominant waves (types and frequencies) present in each state and stage of the sleep-wake cycle.

Neurochemical substrate of sleep-wake states

The three states of the sleep-wake cycle (i.e. Wake, SWS and REM sleep) are the product of distinct neurophysiological processes occurring prior to and during the behavioural manifestation of that state. Different groups of neurons located in the brainstem reticular activating system, including cholinergic, noradrenergic and glutamatergic cells, participate in the neurochemical interplay that generates waking, SWS and REM sleep.

The largest group of cholinergic neurons is located in the pontomesencephalic tegmentum and projects to the intralaminar and ventromedial nuclei of the thalamus, which are part of the non-specific thalamo-cortical projection system, as well as to the thalamic reticular nucleus and to certain thalamic relay nuclei. During waking, these cholinergic neurons fire

tonically at moderate rates, and during REM sleep increase their firing rate. Tonic firing of these cholinergic cells is temporally correlated with cortical arousal: acetylcholine may thus mediate the depolarization of thalamo-cortical relay neurons and the hyperpolarization of thalamic reticular neurons. In addition to cholinergic neurons, noradrenergic neurons located in the brainstem also fire actively during waking, increasing cortical responsiveness during this state (see for review Steriade and McCarley, 1990; Jones, 1993).

In the transition from waking to SWS, cholinergic and noradrenergic cells from the brainstem reticular activating system decrease their firing rate, resulting in a disfacilitation and hyperpolarization of the thalamo-cortical cells onto which they project (see for review Steriade and McCarley, 1990). This hyperpolarization is enough to change the firing pattern of these cells from a tonic to a phasic or burst-like mode (see for review Steriade and Deschênes, 1984; Steriade and Llinás, 1988; Steriade and McCarley, 1990; McCormick, 1992). This change in firing pattern is evident in the EEG, first as sleep spindles and later as slow waves. In association with these changes, external stimuli arriving in the thalamus are no longer conveyed on a one-to-one basis to the cerebral cortex.

A decrease in monoaminergic activity occurring during SWS, followed by a selective increase in cholinergic activity, as mentioned above, are the requisite for the development of REM sleep (McCarley and Hobson, 1975). During REM sleep, the thalamus returns to a tonic firing mode but, unlike during waking, the subject is unaware of external stimuli, due to inhibition of sensory input at lower

levels and to partial blocking of sensory input at the thalamic level (see Pompeiano, 1976).

Thalamo-cortical interplay during SWS

The thalamus has been shown to play a key role during SWS, since it is the center of a neuronal interplay that involves the thalamic reticular nucleus and the cortex. This neuronal interplay generates the sleep spindles and slower waves typical of SWS (see for review Steriade et al., 1993). Upon entering stage 2 SWS, the thalamic reticular neurons, which surround the thalamus like a shell, start firing in a phasic, burst-like manner, acting as a pacemaker on the thalamo-cortical neurons which they innervate (Steriade and Llinás, 1988, Steriade et al., 1993). Since the reticular cells are almost exclusively GABAergic (Houser et al., 1980), they hyperpolarize the thalamo-cortical neurons, driving them to fire in a burst-like manner as well, but in opposite phase with the reticular cells. These thalamic bursts of action potentials are seen on the EEG as sleep spindles (12-14 Hz) (Steriade et al., 1993). As sleep deepens and the subject continues into stages 3 and 4 SWS, hyperpolarization of thalamo-cortical cells increases, rendering its cells less responsive to external stimuli, and decreasing the bursting frequency. This decrease is seen on the EEG as the emergence of delta waves (1-4 Hz).

Lesions in patients

Histopathological studies of brain specimen from previously comatose deceased patients have corroborated the important role of the brainstem reticular

activating system in the maintenance of wakefulness and cortical activation (see for review Plum and Posner, 1980; Jones, 1994). The integrity of thalamo-cortical circuits is also known to be necessary for the generation of sleep spindles and delta EEG activity that normally characterize mammalian SWS (Lugaresi et al., 1986; Tinuper et al., 1989; Guilleminault et al., 1993; Weisz et al., 1995; see for review Jones, 1994). However, although physiological changes observed as a result of damaged tissue may provide information about the structure's importance in the functional integrity of the system, normal physiological processes must be studied in order to elucidate the role of those structures in normal sleep.

Neuroimaging techniques

More recently, the development of imaging techniques has provided a new window on the physiological substrate of sleep in humans.

Neural activity requires energy for several processes: maintaining the membrane potential, restoring the resting membrane potential after a depolarization, taking up used transmitter molecules and producing neurotransmitters (Cooper et al., 1991). This energy is thought to come from the oxidative metabolism of glucose, which is not locally stored, but comes from the blood stream. Thus, neural activation, by increasing the energetic demand, would increase the demand for glucose and oxygen and increase in turn the local blood flow. For this reason, cerebral blood flow (CBF) is thought to be coupled to the cerebral metabolic rate (CMR) of oxygen and glucose (Raichle et al., 1976,

Sokoloff, 1981) and all three are used as indicators of neuronal activity for neuroimaging purposes.

CBF, CMRGlu and $CMRO_2$ during different sleep-wake states

Different states and stages of the sleep-wake cycle have been shown to be associated with changing levels of CBF and CMR using measures of glucose or oxygen.

SWS state:

Mainly due to temporal resolution difficulties, SWS has often been studied non-selectively across some or all sleep stages and compared to wakefulness. One of such studies, using positron emission tomography (PET) and fluorodeoxyglucose (FDG) as a tracer, found a uniform global decrease in glucose metabolic rates during SWS (stages 1-4) in male volunteers (Heiss et al., 1985). Buchsbaum et al. (1989), using the same method, also reported a significant reduction of global glucose metabolic rates (23%) in subjects that were in SWS (mainly stages 2-3) as compared to waking controls. This decrease was much greater for the thalamus and basal ganglia than for the cortex.

When SWS stages were discriminated and SWS divided into 'light sleep' (mainly stage 2) and 'deep or delta sleep' (stages 3-4), some differences were apparent between the changes associated to each group.

Stage 2 SWS:

When comparing stage 2 SWS with wakefulness in normal subjects, no significant difference in global CMRGlu was found between the two states, yet a significant decrease in glucose utilization was found in the thalamus (20%) (Maquet et al., 1992). Stage 2 SWS has also been associated with a small but significant (5%) decrease in global $CMRO_2$, as measured in normal volunteers with the Kety-Schmidt technique and ^{133}Xe as the inert gas (Madsen et al., 1991b). Using the same tracer, a significant decline in global CBF (14-20%) was found during stages 1-2 SWS, especially in the brainstem-cerebellar region of normal volunteers (Sakai et al., 1980). Another study reported no global changes in CBF during stages 1-2 SWS as compared to wakefulness, but regional increases were found in the cerebellum and decreases in the secondary sensory system (Andersson et al., 1995).

Stages 3-4 SWS:

As sleep deepens from stage 2 to stages 3-4 SWS, cerebral metabolism appears to further decrease. Global glucose metabolism, as measured with PET and FDG, has been shown to decrease significantly (44%) during stages 3-4 SWS as compared to wakefulness in normal subjects (Maquet et al., 1990). In this study, thalamic nuclei had a significantly lower glucose utilization than the average cortical values. Stages 3-4 SWS have also been associated with a decrease in global $CMRO_2$ (25%) and global CBF (18%) in normal subjects when using the Kety-Schmidt technique (Madsen et al., 1991c). With the same

technique, global CBF decreases were 28% during stages 3-4 SWS compared to waking (Sakai et al., 1980). However, when measuring CBF with $H_2{}^{15}O$ as a tracer, no global CBF changes during stages 3-4 SWS were reported in normal subjects, although significant regional CBF decreases were found in areas of the cortical secondary sensory system and increases were found in the cerebellum (Andersson et al., 1995). Hajak et al. (1994) found that the CBF in the left middle cerebral artery of volunteers decreased during deepening SWS, reaching the lowest level in stage 4. However, spontaneous or provoked changes in sleep stage patterns as well as awakenings from SWS, were not regularly followed by corresponding changes in CBF (Hajak et al., 1994).

REM sleep:

Although behaviourally, REM sleep is very different from wakefulness, these two states are generally considered to have similar metabolic rates. Buchsbaum et al. (1989) and Maquet et al. (1990) using PET and FDG, found no significant global metabolic difference between REM sleep and waking. Nevertheless, regional increases were reported in the left anterior and middle temporal cortex, left lateral occipital cortex and the brainstem during REM sleep compared to wakefulness (Maquet et al., 1990). Global CBF has also been shown to increase (41-47%) during REM sleep as compared to waking (Sakai et al., 1980). Regional CBF, measured with single photon emission computerized tomography (SPECT) and ^{99m}Tc-*dl*-hexamethylpropyleneamine as a tracer, was significantly increased in the associative visual area (4%) during REM sleep associated with

visual dreaming, but decreased in the inferior frontal cortex (9%) of healthy volunteers (Madsen et al., 1991a). The authors speculate it might be the result of the strong visual imagery and lack of organization typical of bizarre dreaming patterns during REM sleep (Madsen et al., 1991a). A recent study, using $H_2{}^{15}O$ to assess rCBF, showed an rCBF increase in several structures in association with REM sleep (Maquet et al., 1996). These structures included the pontine tegmentum, left thalamus, anterior cingulate cortex, right parietal operculum, bilateral amygdaloid complexes and entorhinal cortex. Regional CBF decreases in association with REM sleep were found bilaterally in the dorsolateral prefrontal cortex, the supramarginal gyrus and posterior cingulate cortex and precuneus (Maquet et al., 1996). The authors claim that the pattern of activation 'provides a biological basis for the processing of some types of memory during REM sleep' (Maquet et al., 1996).

In summary, small decreases in global $CMRO_2$ and CBF have been reported during stage 2 SWS, but no changes in global CMRGlu were observed. As the sleeper progresses into stages 3-4 SWS, greater decreases in global CBF, CMRGlu and $CMRO_2$ were seen. During these stages (stage 2 and 3-4 SWS), regional decreases superimposed on any global changes were also observed in the thalamus, basal ganglia and brainstem-cerebellar region. It is to note, however, that the majority of studies required prolonged acquisition times (30-45 min), and in the one where acquisition time was short (1 min), no global CBF changes were found.

During REM sleep, no differences in global CMRGlu were reported as compared to wakefulness. Regional increases were found in the left temporal and occipital areas, and in the brainstem. Global CBF, on the other hand, appears to increase during REM sleep, with regional increases evident in the associative visual cortex and in inferomedial temporal regions involved in memory processing.

Proposed Study

Objectives

The scope of the present study was to investigate regional changes in brain activity that occur in association with SWS and, moreover, with specific EEG activity patterns such as delta waves and sleep spindles. This was done with the expectation of further understanding the physiological substrate underlying the generation and maintenance of SWS and its associated EEG activity in humans.

To accomplish this, we assessed rCBF by means of PET with the $H_2{}^{15}O$-bolus technique. The high spatial resolution of PET combined with the short acquisition time (60 seconds) was expected to allow the detection of spatially and temporally distinct changes that have not previously emerged with other techniques. Older techniques that allow the measurement of global brain perfusion and metabolism, but not the regional changes, obscure possible significant changes in opposite directions in different structures. Also, other techniques that have adequate spatial resolution, such as PET with FDG as the

tracer, require prolonged acquisition times, necessitating the inclusion of data from different sleep stages, thus obscuring changes unique to a particular stage.

Furthermore, we used the recently developed across-subject covariance analysis (Paus et al., 1995) in order to find an association between rCBF and progressive changes in specific EEG frequency band activities. With this analysis, and in accordance with the thalamo-cortical system's interplay mentioned earlier, rCBF decreases, reflecting neuronal activity decreases, would be expected in the thalamic area in association with delta activity and also with sleep spindles.

Since global CBF changes have been shown to occur during SWS by some authors, it would have been beneficial to measure absolute CBF in our study. However, arterial blood samples were not drawn so that disturbances to natural sleep were minimized. Indeed, this study might not have been viable, had arterial sampling been attempted. In any event, since the aim of this study was to assess *regional* changes in brain activity that would be superimposed upon any possible global changes, normalized rCBF values had to be calculated. Normalized measures of brain activity are standardly used in neuroimaging studies to assess regional changes even when absolute values are known.

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Chapter 2.- Regional Cerebral Blood Flow Changes as a Function of Delta and Spindle Activity during Slow Wave Sleep in Humans

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Abstract

In the present study, we investigated changes in regional cerebral blood flow (rCBF) in humans during the progression from relaxed wakefulness through slow wave sleep (SWS). These changes were examined as a function of spindle (12-15 Hz) and delta (1.5-4 Hz) electroencephalographic (EEG) activity of SWS. Regional CBF was studied with positron emission tomography (PET) using the $H_2{}^{15}O$-bolus method. A maximum of six 60-s scans was performed per subject during periods of wakefulness and stages 1 to 4 of SWS, as determined by on-line EEG monitoring. Spectral analysis was performed off-line on the EEG epochs corresponding to the scans for computation of activity in specific frequency bands. The relationship between EEG frequency band activity and normalized rCBF was determined by means of a voxel-by-voxel analysis of covariance. Delta activity covaried negatively with rCBF most markedly in the thalamus, and also in the brainstem reticular formation, cerebellum, anterior cingulate and orbito-frontal cortex. After removing the effect of delta, a significant negative covariation between spindle activity and the residual rCBF was evident in the medial thalamus. These negative covariations may reflect the disfacilitation and active inhibition of thalamo-cortical relay neurons in association with delta and spindles, as well as the neural substrates underlying the progressive attenuation of sensory awareness, motor responsiveness and arousal that occur during SWS. Delta activity covaried positively with rCBF in the visual and auditory cortex possibly reflecting processes of dream-like mentation purported to occur during SWS.

Key words : reticular formation, thalamus, visual cortex, EEG, PET, consciousness

Introduction

Across the sleep-wake cycle, the brain undergoes fundamental changes in activity which are associated with different levels and states of consciousness. These changes have been extensively studied in non-human mammals. During the passage from wakefulness into slow-wave sleep (SWS), neurons in the brainstem reticular activating system, including noradrenergic and cholinergic cells, have been shown to decrease their firing rate (see for review Steriade and McCarley, 1990). As a result, thalamic neurons undergo disfacilitation and become slightly hyperpolarized, tending to change their firing mode from single spikes to rhythmic bursts (see for review Steriade and Deschênes, 1984; Steriade and Llinás, 1988; Steriade and McCarley, 1990; McCormick, 1992). In addition, GABAergic neurons of the thalamic reticular nucleus fire in prolonged bursts, directly hyperpolarizing the thalamo-cortical cells onto which they project, and entraining them into a spindle rhythmicity (12-14 Hz) during stage 2, and delta rhythmicity (1-4 Hz) during subsequent stages 3-4 SWS (Steriade et al., 1994).

In humans, the importance of the brainstem reticular activating system in the maintenance of wakefulness and cortical activation has been documented in cases of lesions in comatose patients (see for review Plum and Posner, 1980; Jones, 1994). It is also known that the integrity of thalamo-cortical circuits is important for the elaboration of spindle and delta electroencephalographic activity, which normally characterizes mammalian SWS (Lugaresi et al., 1986;

Guilleminault et al. 1993; Weisz et al., 1995; see for review Jones, 1994). However, the changes that occur in these systems with natural sleep progression, remain to be explored.

Neuroimaging techniques now allow direct assessment of cerebral hemodynamics and metabolism, providing a non-invasive approach to the study of sleep physiology in humans. Global decreases of cerebral blood flow (CBF) (Sakai et al, 1980; Meyer et al., 1987; Madsen, 1991c), oxygen metabolism (Madsen, 1991b,c) and glucose metabolism (Buchsbaum et al., 1989; Maquet et al., 1990, 1992) have been reported during different stages of SWS as compared to waking. Using positron emission tomography (PET), a regional decrease in glucose metabolism in the thalamus was found to be significantly greater than the global decrease (Maquet et al., 1990, 1992). During rapid-eye-movement (REM) sleep, compared to waking, no significant global changes in CBF and oxygen metabolism (Madsen 1991c) or glucose metabolism (Buchsbaum et al., 1989; Maquet et al., 1990) have been documented. However, significant regional increases were reported in several limbic and cortical areas, including the visual cortex (Buchsbaum et al., 1989; Maquet et al., 1990; Madsen et al., 1991a; Maquet et al., 1996).

In this study, we sought further understanding of the neural substrates that underlie the generation and maintenance of SWS and associated electroencephalographic (EEG) activity in normal humans. We thus examined regional CBF (rCBF) changes using PET with the $H_2{}^{15}O$-bolus technique in the

progression from wake through stages of SWS. Normalized rCBF was correlated with delta and spindle EEG activity using a voxel-by-voxel analysis of covariance. Preliminary results from this work have been reported in abstract form (Hofle et al., 1995).

Materials and Methods

Subjects and experimental design.

Eighteen normal volunteers (11 males and 7 females, mean age 25.1 years) participated in this study. They were asked to limit their sleep to four hours (from 2 to 6 am) the night before the study, and could thus be considered slightly sleep-deprived, as has been the case in previous PET studies (Maquet et al., 1990, 1996). Subjects were also instructed not to take any alcohol, coffee or tea for at least 24 hours before the experiment. The study was approved by the local ethics committee, and subjects gave written informed consent.

Subjects were asked to report to the lab at 19:30 h, when they were fitted with electrodes for EEG, electrooculogram (EOG) and electromyogram (EMG) (see below). In an initial sham study, subjects (n=18) lay supine in the scanner and an i.v. catheter was taped to their wrist (not inserted in the vein) for simulation of the PET experiment, including mock bolus injections during sleep. In this way, each subject was allowed to become acquainted with the recording situation prior to the real PET study and to withdraw from the study if unable to sleep under the experimental conditions. In the real PET studies, the subjects lay

on the bed, with the head restrained in a customized head-holder, and an i.v. line in the left antecubital vein.

For data acquisition, ambient lighting was dimmed and subjects were asked to close their eyes and relax. The EEG/PET studies extended from approximately 22:00 to 1:00 h (see Fig. 1B).

Eleven volunteers performed satisfactorily in the sham study, and proceeded to the real PET study. A maximum of six rCBF scans was obtained for each subject. Scans were acquired throughout the sleep-wake cycle, with the aim to obtain at least one scan in each of the major sleep-wake state-stages. By visual assessment of the EEG on the computer monitor, the following state-stages were identified (according to Rechtschaffen and Kales, 1968): quiet wakefulness, determined by the presence of alpha (~8-12 Hz) in the EEG; stage 2 SWS, characterized by the presence of sleep spindles (~12-14 Hz); and stages 3-4 SWS, distinguished by a large percentage of delta waves (~1-4 Hz) (see Fig. 1A). This classification was performed on-line by the investigators and, once a wake state or sleep stage was established, the $H_2{}^{15}O$-bolus injection was performed within 2 minutes.

The analysis presented here was based on data obtained in the six subjects who were able to reach at least stage-2 sleep during the PET study. They were three men and three women, all right-handed with a mean age of 24.2 years.

EEG recording and analysis.

Scalp electrodes were placed over the left hemisphere using the international 10-20 system. EEG was recorded from F_3, C_Z, P_3 and O_1 referential to A_2. Bipolar recordings of EOG and EMG (from the chin) were obtained. The resistance was less than 10 KOhms for all electrodes.

GRASS amplifiers were used for polygraphic recording and the output sent to a computer equipped with software for on-line digitalization, recording and display of signals (*Rhythm* software, *Stellate Systems*, Montreal). Data were stored at a sampling rate of 256 Hz for subsequent off-line spectral analysis (*Rhythm* software, *Stellate Systems*). EEG was filtered below 0.3 and above 100 Hz, EOG was filtered below 0.3 and above 30 Hz and EMG below 10 and above 100 Hz.

EEG records from PET studies were scored according to standardized criteria (Rechtschaffen and Kales, 1968). Spectra were obtained for each 60-second scan. Most 60-s EEG-epochs were artifact-free (32 cases). However, EEG and CBF data were eliminated in the two cases where movement artifacts occurred. Activity (expressed in arbitrary amplitude units) was determined in the 60-s epochs in the following bands: a) delta (1.5-4.0 Hz); b) theta (4.5-8.0 Hz); c) alpha (8.5-11.5 Hz); d) sigma (12.0-15.0 Hz); e) beta (18.0-30.0 Hz) and f) gamma (30.5-57.0 Hz). With the spectral analysis employed here, EEG activity could not be measured in the recently characterized 'slow oscillation' range of <1 Hz (see Steriade et al., 1994). For the present analysis, data from P_3-A_2 leads

were employed for statistical analysis. A Spearman ranked correlation test was performed between activity in alpha, sigma and delta bands and sleep-wake stages, to characterize the relationship of each band with sleep progression.

Measurement and localization of rCBF.

PET scans were obtained using a Scanditronix PC-2048B 8-ring 15-slice tomograph, with the 9-cm axial field-of-view covering the brain approximately from z= 48 to z= -44 mm. Employing the $H_2{}^{15}O$-bolus technique (Raichle et al., 1983), counts were measured during a 60-s scan following a 40 mCi $H_2{}^{15}O$-bolus injection. Arterial blood samples were not drawn in order to minimize disturbances to natural sleep, hence absolute values of CBF were not calculated in this study. Since a linear relationship exists between PET counts and CBF, the counts can be used as direct indices of CBF for each scan in the absence of arterial sampling (Herscovitch et al., 1983).

For each subject, high-resolution T1-weighted magnetic resonance images (MRI; 160 contiguous sagittal slices, 1-mm thick) were obtained from a Philips Gyroscan ACS (1.5T). PET count images were reconstructed with an 18-mm Hanning filter and normalized for differences in global CBF by means of ratio normalization, i.e. the counts at each voxel (3-D image-element) were divided by the mean counts calculated across all brain voxels (Fox et al., 1988). All results reported in this paper refer to these normalized, or relative, rCBF values. Finally, the images were co-registered with individual MRI's (Woods et al., 1993), and

transformed into a standardized stereotaxic space (Talairach and Tournoux, 1988) by means of an automated feature-matching algorithm (Collins et al., 1994).

Statistical analysis of rCBF and EEG changes.

The data set consisted of normalized rCBF obtained in 6 subjects, scanned 5 or 6 times each, yielding a total of 32 rCBF volumes and corresponding EEG data. The relationship between normalized rCBF and absolute EEG activity was assessed by means of analysis of covariance (ANCOVA) (Sokal and Rohlf, 1981), with subjects as a main effect and EEG activity obtained for each scan as a covariate (for complete analysis description see Paus et al., 1996). Subject effects were removed, and the parameter of interest was the slope of the rCBF, EEG activity linear regression. A *t*-statistic map was calculated that assessed whether the slope of the regression at a given 3-D image-element (voxel) was significantly different from zero. The size of a voxel was 1.34 x 1.72 x 1.5 mm in X, Y and Z dimensions, respectively. The degrees of freedom of the estimate of the standard deviation (s) were increased by pooling s across all brain voxels, so that the *t*-statistic distribution was normal and the *t*-values were equivalent to *z*-scores. The presence of significant focal changes was tested by a method based on three-dimensional Gaussian random field theory, which corrects for the multiple comparisons involved in searching across a volume (Worsley et al., 1992). Values equal to or exceeding a criterion of t=4.5 were deemed statistically significant ($p<0.00001$, two-tailed, uncorrected). Correcting for multiple comparisons, a t-value of 4.5 yields a false positive rate of 0.06 in 600 resolution elements (each of

which has dimensions of 18 x 18 x 7.7 mm), constituting the scanned volume of the whole brain.

Results

On-line EEG recording allowed determination of sleep stage according to the predominant patterns of alpha for relaxed wake, spindles for stage 2, and delta for stage 3-4 (Fig. 1A). In six subjects, the bolus injections delivered during these EEG patterns and stages (Fig. 1B) were successfully performed without arousing them. Six scans were obtained in wake, seven in drowsy, two in stage 1, nine in stage 2, four in stage 3 and four in stage 4 SWS (for a total of 32 scans across 6 state-stages). Spectral analysis revealed typical peaks of alpha, sigma (spindles) and delta activity in the scanned epochs for relaxed wake, stage 2 and stage 3-4 respectively (Fig. 1C). Across scans and subjects, the average frequency band activity varied systematically for alpha, sigma and delta in the progression from relaxed wake through drowsy and stages 1-4 SWS (across 6 state-stages, Fig. 1D). As an indicator of the wake state, alpha activity (8.5-11.5 Hz) was found to decrease non-significantly with sleep as reflected in a negative correlation of alpha with 1 (wake) through 6 (stage 4 SWS) state-stages [$r=-0.29$, n.s.] (Fig. 1D). Sigma band activity (12-15 Hz) increased in the early stages of sleep to be maximal in stage 2 and decrease slightly in stages 3-4 SWS, having an overall positive correlation with state-stage [$r=0.43$, $p<0.01$]. Delta activity (1.5-4 Hz) increased progressively in the passage from wake through the stages of SWS, reaching its maximum in stage 4 SWS, and having a significant positive

correlation with state-stage [$r=0.77$, $p<0.01$]. To examine regional blood flow changes as a function of SWS EEG activity, normalized rCBF was examined first as a function of delta activity and subsequently as a function of sigma activity after removing the effect of delta.

In an analysis of covariance, the largest significant decrease in normalized rCBF as a function of delta was found in the thalamus, centered over the midline-medial thalamus (Table 1, Fig. 2). In addition, a significant negative peak was present in the ponto-mesencephalic tegmentum on the right side (Table 1). Significant negative covariations were also found in the cerebellar hemispheres, the left temporal muscle, and in two regions of the cerebral cortex, namely the anterior cingulate and orbito-frontal cortices (Fig. 2 and Table 1).

A highly significant positive covariation between normalized rCBF and delta activity was found in several cortical regions, most particularly the visual cortex in both hemispheres (Fig. 3, Table 1). Other maxima of positive covariation were found in the cortex, including the posterior superior temporal gyrus on the left (Fig. 3, Table 1). This locus was centered over the temporal plane (BA 22) and did not appear to include the primary auditory cortex in Heschl's transverse gyri. Another focus was located near the latter one, in the inferior parietal lobule (supramarginal gyrus, BA 40, Table 1). Other loci of positive covariation were centered over the left central sulcus (BA 3/4) and bilaterally in the anterior middle temporal gyri (BA 21).

In order to assess contributions to changes in rCBF by sigma (spindles), further regressions were performed removing (in addition to the subject effect) the activity in the delta frequency band. In this case, the only significant covariation between such residuals of rCBF and sigma was localized in the thalamus and restricted to the medial thalamus (Fig. 2B).

The relationship between mean number of non-normalized counts in the entire scanned volume of the brain and delta EEG activity was examined in an attempt to assess possible global changes in brain activity during sleep. This correlation was found to be non-significant [r=0.267, p=0.14].

Discussion

This study demonstrates a significant decrease in normalized rCBF in several subcortical and cortical regions as a function of EEG delta activity in the progression from waking through SWS. The negative covariation between rCBF and delta was largest in the thalamus, where a significant negative correlation was also found for spindling (sigma) after removing the effect of delta activity, suggesting a marked disfacilitation and inhibition of the thalamus in association with delta activity and spindles of SWS. In contrast to a decrease in some cortical areas, a significant increase in normalized rCBF was found in visual and other cortical regions as a function of delta activity, possibly revealing the cortical substrate of dream-like imagery during SWS.

Methodological considerations

The aim of the present study was to examine regional changes in brain activity as a function of EEG SWS patterns. For this purpose, we analyzed normalized rCBF values based upon radioactivity counts, since these values are directly proportional to regional differences that are superimposed upon any generalized change in global blood flow. Such values are standardly used in studies of regional activation and have been applied in other sleep-wake studies (Maquet et al., 1996). Moreover, by employing normalized values of glucose cerebral metabolic rate (CMRGlu), regional changes were found to be superimposed upon global CMRGlu decreases during SWS (Maquet et al., 1990). Previous studies measuring absolute CBF also reported global decreases in blood flow during SWS (Madsen and Vorstrup, 1991) that were parallel to those reported for glucose metabolism (Maquet et al., 1990, 1992). On the other hand, earlier studies had shown slight increases in global CBF during SWS (Mangold et al., 1955). A recent PET study in humans found no significant change in global CBF (Andersson et al., 1995). In the present study, no significant correlation was found between mean brain counts and delta activity, also suggesting a lack of significant change in global CBF. A lack of change may be the result of the relatively brief duration of SWS episodes that occurred during the PET experiment, and also the sampling in the early part of the night when global blood flow changes appear to be minimal (Hajak et al., 1994). Nevertheless, our results indicate that, superimposed upon any possible global CBF changes,

differential regional changes in CBF occur during SWS that reflect differential regional changes in brain activity during that state.

The present results using analysis of covariance confirmed our preliminary results of significant changes in normalized rCBF between waking, stage 2 and stages 3-4 sleep, obtained in the same group of subjects, with the more common subtraction method (Hofle et al., 1995). However, since sleep stages are defined by EEG activity, and it is this activity which reflects the electrophysiological changes that occur during sleep, rCBF was examined here as a function of delta and spindle wave activity. Delta activity varied in a continuous manner across sleep stages and was thus used as the main covariate. Sigma, reflecting spindling, also varied continuously, but in a different manner from delta, reflecting the maximal occurrence of spindles in the early stages of sleep, and was thus examined after removing the effect of delta.

Negative covariation of rCBF with delta or sigma

Our data show a highly significant decrease in normalized rCBF in the thalamus as a function of delta activity, and, in a more restricted region of the thalamus, as a function of spindling. These results concur with previous PET studies in which significant decreases in normalized rCMRGlu had been found in the thalamus, in association with stages 2 and 3-4 of SWS (Maquet et al., 1990, 1992).

There is considerable evidence to suggest that excitatory neurotransmission is associated with increased rCBF, in part through mediation

by local nitric oxide release, and thus that increases or decreases in excitatory neurotransmission would be reflected by increases or decreases in rCBF (Knowles et al., 1989; Northington et al., 1992; see Iadecola, 1993; Paus et al., 1995; Gjedde, 1997). Decreases in CBF have also been shown as a result of pharmacological stimulation of $GABA_A$ receptors, suggesting that inhibitory postsynaptic neurotransmission may be associated with decreases in rCBF (Roland and Friberg, 1988; see Roland, 1993; Gjedde, 1997). Hence, the rCBF decrease observed here in the thalamus could reflect the disfacilitation of thalamo-cortical relay neurons by decreased excitatory input from the brainstem reticular activating system but also the active inhibition from GABAergic thalamic reticularis cells (Steriade et al., 1994).

Neurons of the brainstem reticular activating system have been shown to decrease their firing rate prior to the onset of SWS in animals (see Steriade and McCarley, 1990), changes which might be reflected in the rCBF decreases seen here in the ponto-mesencephalic tegmentum of humans during SWS. This decrease in excitatory neurotransmission would result in a disfacilitation of the thalamic neurons, particularly the nuclei of the diffuse thalamo-cortical projection system, onto which neurons of the brainstem reticular formation, including glutamatergic, cholinergic and noradrenergic neurons, project (Jones and Yang, 1985; see Jones, 1995). As a result of this disfacilitation, thalamic neurons would become sufficiently hyperpolarized to change their firing mode from tonic to phasic as occurs in the transition to SWS (Steriade et al., 1994).

Moreover, the thalamic reticularis neurons, which contain GABA (Houser et al., 1980) and project onto thalamic relay neurons, including midline, medial and intralaminar nuclei of the diffuse thalamo-cortical projection system (Steriade et al., 1984; Jones, 1985; Velayos et al., 1988), would begin to burst, hyperpolarizing the thalamo-cortical neurons and entraining them first in a spindle and then delta rhythmicity (von Krosigk et al., 1993; Steriade et al., 1994). Thalamic neurons oscillate in a slower rhythm with long-lasting hyperpolarizations in association with the 'slow oscillation' on the cortex (<1 Hz) (Contreras et al., 1996), a recently described sleep rhythm (see Steriade et al., 1994) which was not measured in the present study. In our results, the rCBF correlation with delta (accounting for ~65% of the variation in thalamic rCBF) could reflect the progressive hyperpolarization of thalamic neurons that occurs across SWS (Hirsch et al., 1983; Contreras and Steriade, 1995). The additional covariation with spindling (~35% of the variation in the residual thalamic rCBF), which was localized in the midline-medial thalamus, may reflect the active inhibition of thalamo-cortical neurons in association with spindling in the early stages of sleep and a focused dampening of the diffuse thalamo-cortical projection system during sleep initiation. The early inhibition of this system may underlie the loss of consciousness that occurs with SWS.

Changes in normalized rCBF in the cerebellum also correlated negatively with delta activity. This might be a reflection of the decreased muscle tone and proprioception characteristic of sleep and is consistent with the decreased

mobility of the sleeping subject. In addition, the present results may suggest dampening during sleep of certain processes involving neocerebellar-neocortical circuits that are important in higher order cognitive processes (see Leiner et al., 1993).

Negative covariation of normalized rCBF and delta was seen in frontal regions of the cortex (anterior cingulate and orbito-frontal cortex), which receive the most dense projections from the diffuse thalamo-cortical projection system together with afferents from the dorsomedial nucleus. The rCBF decrease in the anterior cingulate cortex, whose activity has been previously linked to changes in arousal during waking (Paus et al., 1997), most likely reflects an attenuation of this cortical arousal system during sleep.

Positive covariation of rCBF with delta

Our results show that during SWS normalized rCBF changes were heterogeneous across different regions of the cerebral cortex. Whereas delta covaried negatively with rCBF in anterior cingulate and orbito-frontal cortex, it covaried positively with rCBF in another set of areas. Thus, delta activity and rCBF covaried positively in primary and adjacent secondary visual cortex, as well as in secondary auditory cortex, suggesting increased regional activity in these areas during SWS as compared to relaxed wakefulness with eyes closed. Regional CBF increases have been reported when awake subjects imagine different objects with their eyes closed (Kosslyn et al., 1995) or imagine sounds in absence of external auditory stimuli (Zatorre et al., 1996a). In addition to the

positive covariations in the left secondary auditory cortex over planum temporale (BA 22), rCBF versus delta positive covariations were also found in the left inferior parietal lobule (BA 40). Regional CBF increases in these areas have been linked with auditory-phonological processing (Howard et al., 1992; Paulesu et al., 1993; Zatorre et al., 1992, 1996b). Regional activation in this complex of areas may therefore reflect the occurrence of visual, auditory and, perhaps, verbal imagery during SWS.

Although in early studies, 'dreaming' was reported almost exclusively from REM sleep awakenings (Dement and Kleitman, 1957), 'dream' reports have been subsequently obtained from all stages of sleep at rates of ~50-75% for stages 2-4 SWS, as compared to ~80-90% for REM sleep (Foulkes, 1962; Tracy and Tracy, 1974; Cavallero et al., 1992). Reports from SWS were slightly less vivid, shorter and more thought-like than those from REM sleep, yet the majority of non-REM reports involved both 'primary visual experience' in addition to 'secondary cognitive elaboration' (Foulkes, 1962; Molinari and Foulkes, 1969; Foulkes and Pope, 1973). Since our subjects were not awakened after each scan, the subjective experience of 'dreaming' could not be confirmed; however, our PET results appear to suggest the presence of visual and auditory imagery or processing during SWS.

Conclusions

Thalamic rCBF decreases dramatically as a function of delta and spindle activity, reflecting the disfacilitation and active inhibition of thalamo-cortical

neurons that occur during SWS and possibly underlie the loss of consciousness and sensory awareness characteristic of that state. Despite this closing of the afferent gateway to the cerebral cortex, certain areas, including the visual and secondary auditory cortex, appear relatively more active thus revealing a possible substrate for dream-like mentation during SWS.

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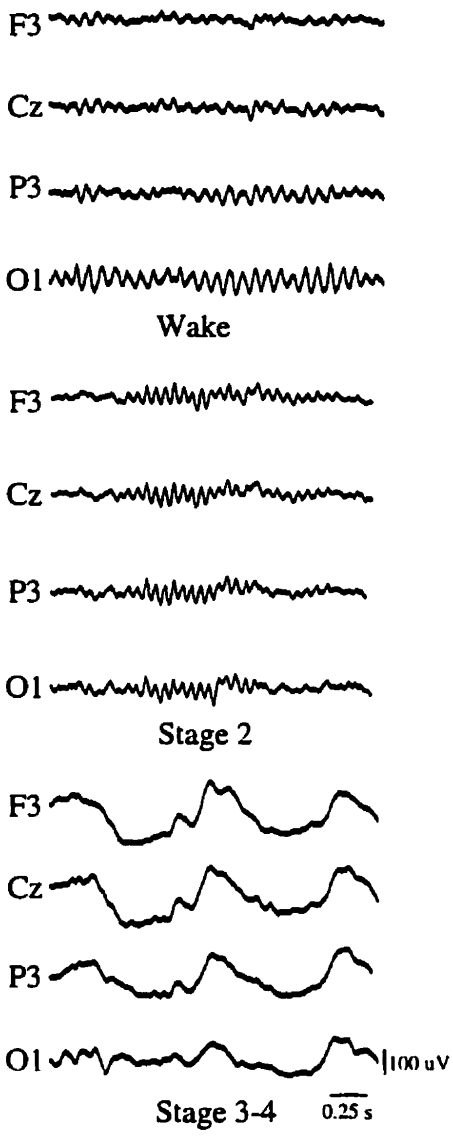
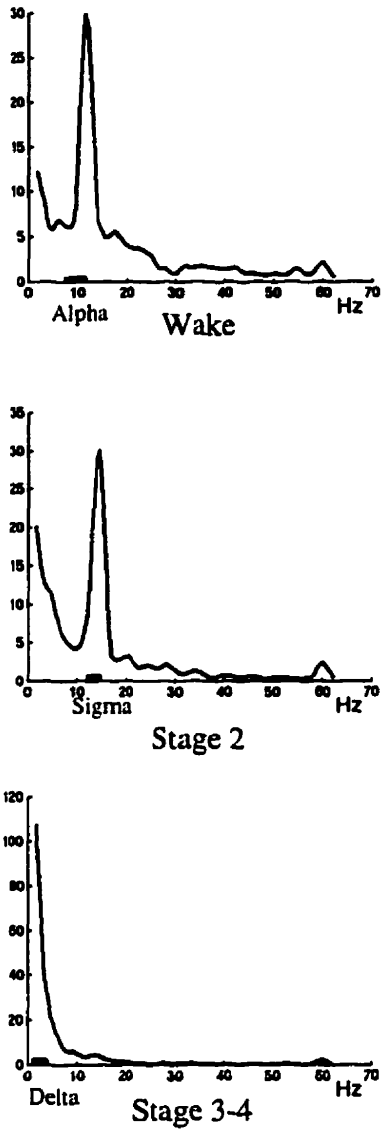
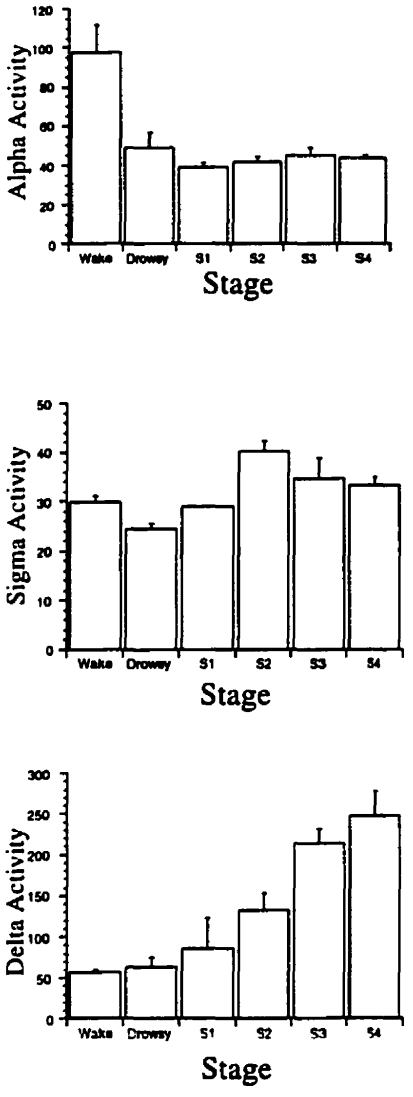
A. EEG



C. Spectral Activity



D. Frequency Band Activity



B. Hypnogram

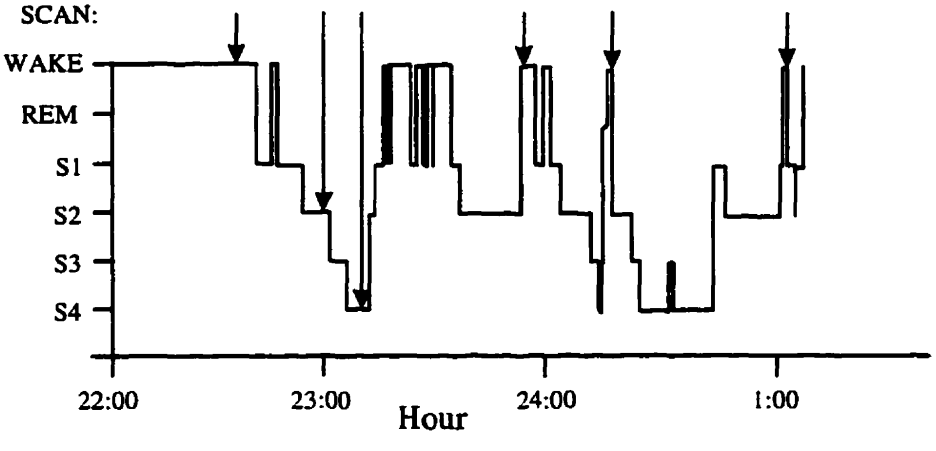


Figure 1

EEG during wake and sleep stages when scans were performed following $H_2{}^{15}O$ injections. A) EEG samples taken from actual scan periods showing typical patterns for each state or stage (wake, stage 2 and stages 3-4 SWS), according to which injections were performed. B) Hypnogram of a typical study, showing scans in one subject during different state-stages of sleep and wake. C) Spectra of EEG epochs (from A) showing typical peaks in alpha, sigma and delta bands (marked by bars) during each stage, respectively. D) Average frequency band activity ($\bar{x}\pm$ s.e.m.) for alpha, sigma and delta, respectively, across wake, drowsy and sleep stages 1 through 4 SWS for all subjects (n=6) and scans (n=32). C) and D) Values taken from P_3 electrode and EEG activity expressed in arbitrary amplitude units; s.e.m.= standard error of the mean.

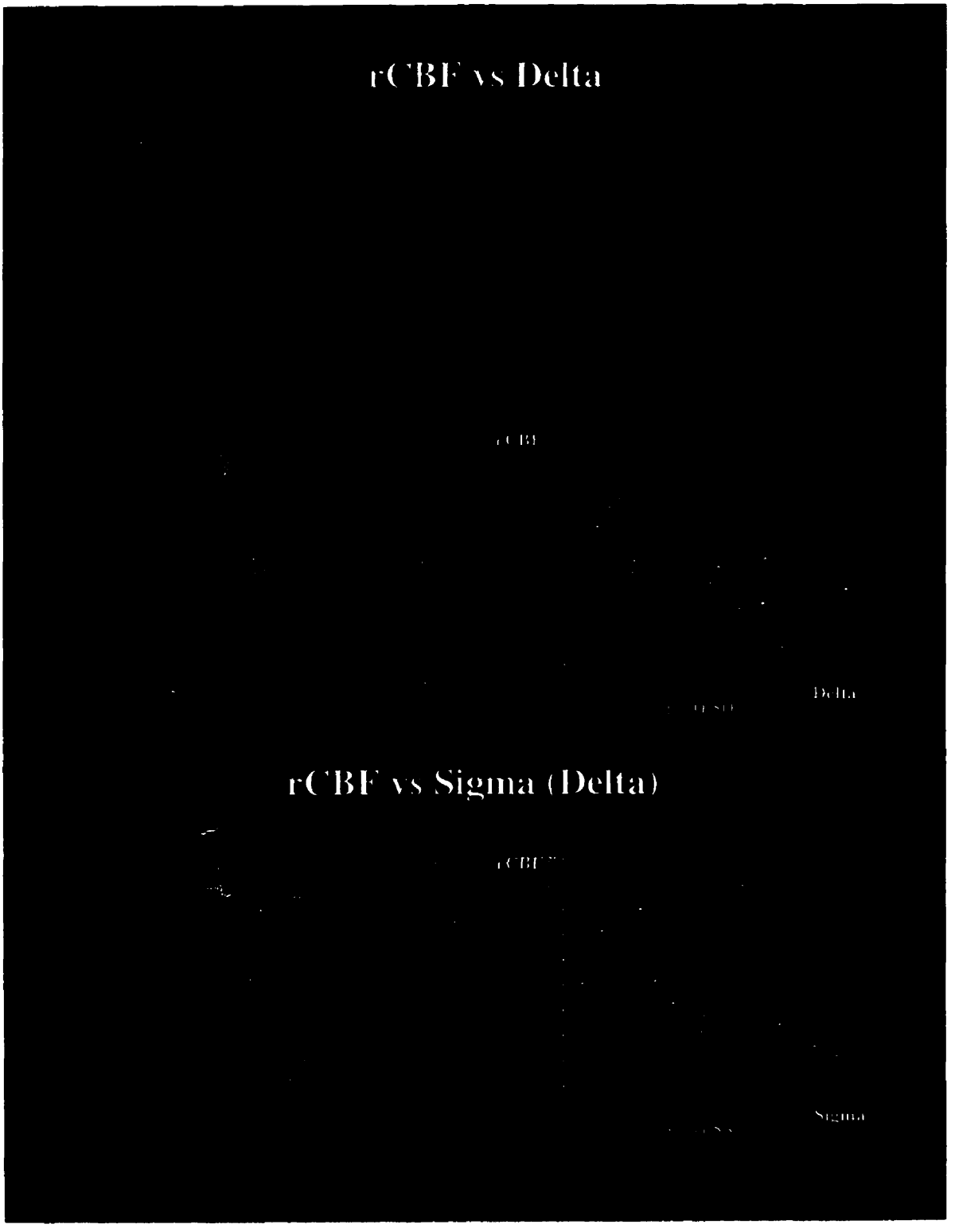

rCBF vs Delta
rCBF
Delta
rCBF vs Sigma (Delta)
rCBF
Sigma

Figure 2

Normalized rCBF decreases as a function of delta and sigma (spindle) EEG activity. The merged rCBF/magnetic resonance images indicate the location of maximal significant negative covariation between normalized rCBF and delta or sigma activity, with the range of *t*-values for the PET data coded by color scale. Top: rCBF vs. Delta. Maximal significant negative covariation of rCBF as a function of delta activity, centered over the thalamus and shown in the sagittal, coronal and horizontal planes. Also evident: anterior cingulate and cerebellum. Image sections are centered at the following coordinates (Talairach and Tournoux, 1988): X= 0 mm, Y= -16 mm, Z= 6 mm. Bottom: rCBF vs. Sigma (-Delta). Maximal significant negative covariation of rCBF as a function of sigma after removing the effect of delta, centered over medial thalamus. Image sections are centered at the following coordinates (Talairach and Tournoux, 1988): X= -1 mm, Y= -16 mm, Z= 9 mm. The scatterplots shown beside the PET/MRI images illustrate the nature of the covariations by plotting the residuals of normalized rCBF, obtained in the thalamus, against the residuals of absolute delta activity after removing the effect of subject (top) or the residuals of absolute sigma activity, after removing the effect of subject and delta (bottom). For this purpose, the rCBF values were extracted from an 8-mm radius spherical volume-of-interest centered over the medial thalamus. Each point represents one scan/subject, and the line is the linear regression. Dots in the plot are colour coded: blue=wake, cyan=drowsy, green=stage 1, yellow=stage 2, red=stage 3,

white=stage 4. rCBF, regional cerebral blood flow; PET, positron emission tomography; MRI, magnetic resonance imaging.

Figure 3

Normalized rCBF increases as a function of delta activity. The merged rCBF/magnetic resonance images indicate the location of maximal positive significance for normalized rCBF and delta covariation, centered in the primary visual cortex bilaterally, showing also the left secondary auditory cortex (see Table 1). Image sections are centered at the following coordinates (Talairach and Tournoux, 1988): X= 11 mm, Y= -80 mm, Z= 14 mm. The scatterplot shown beside the images illustrates the nature of the covariation by plotting the residuals of normalized rCBF, obtained in the visual cortex (volume-of-interest, r= 8 mm), against the residuals of absolute delta activity after removing the effect of subject. See Fig. 2 for details. rCBF, regional cerebral blood flow; PET, positron emission tomography; MRI, magnetic resonance imaging.

Table 1. Significant changes in rCBF as a function of delta activity

		Coordinates (mm)			
Structure	Side	x	y	z	*t*
A. Negative covariations (normalized rCBF decreases)					
Anterior Cingulate Cortex (BA 24)	Midline	0	36	22	-5.4
Anterior Cingulate Cortex (BA 24/32)	Midline	1	42	-3	-4.6
Orbito-frontal Cortex (BA 11)	Right	13	15	-21	-4.4
Medial Thalamus [1]	Midline	0	-16	6	-13.6
Ponto-mesencephalic Tegmentum	Right	5	-33	-21	-4.8
Cerebellar Hemisphere (Crus I posterior)*	Left	-32	-74	-36	-8.9
Cerebellar Hemisphere (Crus I posterior)	Right	39	-62	-38	-7.6
Base of Pons	Midline	0	-33	-39	-4.5
Temporalis muscle	Left	-51	39	-27	-5.5
B. Positive covariations (normalized rCBF increases)					
Medial (Calcarine/Cuneus) Occipital Cortex (BA 17/18)[2]	Right	12	-80	14	7.5
Medial (Calcarine) Occipital Cortex (BA 17)	Left	-13	-73	6	7.5
Posterior Superior Temporal Gyrus (BA 22)	Left	-54	-26	3	6.1
Inferior Parietal Lobule (BA 40)	Left	-40	-47	22	4.9
Pericentral Cortex (BA 3/4)	Left	-44	-11	26	5.0
Anterior Middle Temporal Gyrus (BA 21)	Left	-40	-9	-22	4.8
Anterior Middle Temporal Gyrus (BA 21)	Right	40	-14	-28	4.5

Table Legend: Standard stereotaxic coordinates (Talairach & Tournoux, 1988) are given for the local maxima, as indicated by the highest *t*-statistic, within an area of significant negative ($t < -4.5$) or positive ($t > 4.5$) covariation between normalized rCBF and absolute activity in the delta frequency band (1.5-4 Hz). x, distance to right (+) or left (-) of the midsagital line; y, distance anterior (+) or posterior (-) to the anterior commissure; z, distance above (+) or below (-) the intercommissural line. For cortical locations, Brodmann's cytoarchitectonic areas are given (BA). *According to Schmahmann et al., 1996. rCBF, regional cerebral blood flow. Peaks [1,2] depicted in Figs. 2 (top) and 3, respectively.

Chapter 3.- General Discussion

The following Discussion is strongly based on the 'Discussion' presented in Chapter 2, with expansions added where necessary, especially in 'Methodological considerations' and 'Positive covariations of rCBF and delta'.

Methodological considerations

In an ideal sleep study, volunteers should be on a regular sleeping schedule (not sleep-deprived), left alone and sleep undisturbed in a quiet, familiar room, with a comfortable bed and freedom of movement. However, these conditions are very difficult to meet in a laboratory setting, especially if the volunteer has to sleep in a PET scanner with the head restrained and an i.v. catheter in his/her arm.

In order to increase the likelihood of sleeping under difficult experimental conditions, volunteers in this study had to be partially sleep-deprived. This procedure was also undertaken to shorten sleep latency. Discomfort caused by head-holder, bed and i.v. increases with time, hence a long sleep latency drastically decreases the chances of falling asleep at all, since discomfort may be too great. For these reasons, partial sleep-deprivation has frequently been used in neuroimaging sleep studies (Heiss et al., 1985; Maquet et al., 1990; Maquet et al., 1996). Other changes reported during recovery sleep in association with previous sleep-deprivation include the enhancement of EEG delta power (Borbely et al., 1981), a change in sleep architecture by increasing the amount of

stage 3-4 SWS at the expense of stages 1 and 2 SWS, and the interference with vigilance as assessed by continuous performance tests (Wu et al., 1991). However, potential changes in CBF or brain metabolism during recovery sleep have not yet been explored. While changes in CBF are possible, we do not expect them to be of great magnitude since subjects were allowed to sleep 4 hours the preceding night. Moreover, we measured rCBF as a function of EEG activity by using ANCOVA, and the relationship of these variables would not be expected to change as a result of sleep deprivation even if delta were increased in amplitude.

Arterial blood samples were not drawn in this study since sleeping conditions for volunteers were already difficult, hence absolute values of CBF were not calculated. However, since a linear relationship exists between PET counts and CBF when using the $H_2{}^{15}O$-bolus technique, the counts can be used as direct indices of CBF for each scan in the absence of arterial sampling (Herscovitch et al., 1983). This procedure is standardly used in blood flow activation studies.

Nevertheless, in our study, it would have been useful to calculate absolute values of CBF to assess any possible global changes in this variable. Global values of CBF have been shown to decrease during stages 3-4 SWS (Madsen et al., 1991b), decreases that were parallel to those reported for CMRGlu throughout SWS (Maquet et al., 1990, 1992). On the other hand, earlier studies had shown slight increases in global CBF during SWS in humans (Mangold et al., 1955) and cats (Reivich, 1968). Also, a recent PET study in humans, using $H_2{}^{15}O$ as a tracer,

found no significant change in global CBF (Andersson et al., 1995). In our study, no significant correlation was found between mean brain counts and delta activity, suggesting a lack of systematic change in global CBF. This possible lack of change may be the result of the sampling in the early part of the night when global blood flow changes appear to be minimal (Hajak et al., 1994; Kuboyama et al., 1997). Also, it should be noted that the majority of imaging studies where significant decreases in CBF or CMRGlu were found required prolonged (30-45 minutes) periods of SWS. On the contrary, in this and in another study where no global CBF changes were found (Andersson et al., 1995), data acquisition was short (60 seconds). Thus, a lack of systematic change in brain counts could be the result of the sampling in the early part of the night and of the relatively brief periods of SWS employed in our study.

Nevertheless, as stated in the Introduction, the aim of the present study was to examine regional changes in brain activity, for which normalized rather than absolute values of CBF or glucose metabolism are standardly used, and have been used in other sleep-wake studies (Maquet et al., 1990, 1996).

Throughout this thesis, decreases and increases in rCBF were assumed to reflect decreases and increases in excitatory neurotransmission (see also Paus et al., 1995). This view is based on the presumed role of nitric oxide, a vasodilator, in coupling rCBF to changes in synaptic activity (Northington et al., 1992). Regional decreases in CBF have also been shown to be associated with inhibitory neurotransmission through pharmacological activation of $GABA_A$ receptors

(Roland and Friberg, 1988). The rCBF decreases discussed in this thesis could then reflect either disfacilitation or active inhibitory neurotransmission.

Implications of reported findings

The present study shows a strong decrease in rCBF in the thalamus as measured in an analysis of covariance with delta EEG activity and furthermore with spindle activity during SWS. Regional CBF decreases as a function of delta activity were also found in the brainstem reticular formation, cerebellum, anterior cingulate, orbito-frontal cortex and temporalis muscle. In the cerebral cortex, rCBF changes were not homogeneous and increases were found mainly in the primary and secondary visual cortex and the secondary auditory cortex.

Negative covariations of rCBF with delta and sigma

Thalamus and brainstem:

The data presented in this thesis show a highly significant decrease in rCBF in the thalamus as a function of delta activity, and, in a more restricted region, as a function of spindling. These results support the presumed role of the thalamus in the production of sleep spindles and delta EEG activity during SWS.

Neurons of the brainstem reticular activating system have been shown to decrease their firing rate just prior to the onset of SWS in non-human mammals (see Steriade and McCarley, 1990). These changes might be reflected in the rCBF decreases seen in our study in humans in the ponto-mesencephalic tegmentum

during SWS. The decrease in excitatory neurotransmission that originates in the brainstem reticular activating system would result in a disfacilitation of the thalamic neurons. The disfacilitation would be largest in the nuclei of the non-specific thalamo-cortical projection system, onto which neurons of the brainstem reticular formation project (Jones and Yang, 1985). As a result of this disfacilitation, thalamic neurons become sufficiently hyperpolarized to change their firing mode from tonic to phasic as occurs in the transition to SWS (Steriade et al., 1994). Moreover, the GABAergic thalamic reticularis neurons (Houser et al., 1980) that project onto thalamic relay neurons, begin to burst, hyperpolarizing the thalamo-cortical neurons and entraining them first in a spindle and then in a delta rhythmicity (von Krosigk et al., 1993; Steriade et al., 1994). In our results, the rCBF correlation with delta could reflect the progressive hyperpolarization of thalamic neurons that occurs across SWS (Hirsch et al., 1983; Contreras and Steriade, 1995). The additional covariation with spindling, localized in the medial thalamus, may reflect the active inhibition of thalamo-cortical neurons in association with spindling in the early stages of sleep. The early inhibition of the non-specific thalamo-cortical system may underlie the loss of consciousness that occurs with SWS.

Our results of rCBF decreases in the thalamus during SWS concur with previous PET studies in which significant regional decreases in glucose metabolism had been found in the thalamus in association with stages 2 and 3-4 of SWS (Buchsbaum et al., 1989; Maquet et al., 1990, 1992). However, in our

study, it is the first time that decreases in rCBF, possibly reflecting decreases in neural activity, are found in the human thalamus in association with specific cortical EEG activity patterns, i.e. delta and sleep spindles.

Other structures:

Normalized rCBF covaried negatively with delta EEG activity bilaterally in the cerebellum. These changes might reflect the decreased muscle tone and proprioception characteristic of sleep and is consistent with the decreased mobility of the sleeping subject. Likewise, rCBF decreases observed in the temporalis muscle could reflect general muscle relaxation and muscle tone decrease during sleep. In addition, the present results may suggest dampening during sleep of certain processes involving neocerebellar-neocortical circuits that are important in higher order cognitive processes (see for review Leiner et al., 1993).

Negative covariation of normalized rCBF and delta was also seen in the anterior cingulate (BA 24/32) and orbito-frontal cortex (BA 11). Considering the changes reported in the thalamus, an rCBF decrease in anterior cingulate and orbito-frontal cortex would be expected, since these cortical regions receive the most dense projections from the non-specific thalamo-cortical projection system and afferents from the dorsomedial nucleus of the thalamus. The rCBF decrease in the anterior cingulate cortex, whose activity has been previously linked to changes in arousal during waking (Paus et al., 1997), most likely reflects an attenuation of this cortical arousal system during sleep.

Positive covariations of rCBF and delta

In our study, rCBF changes in association with delta EEG activity were not parallel across cortical regions. Whereas delta covaried negatively with rCBF in anterior cingulate and orbito-frontal cortex, it covaried positively with rCBF in another set of areas. These areas included primary and adjacent secondary visual cortex, secondary auditory cortex, inferior parietal lobule and sensory-motor cortex.

The significant covariation between delta and rCBF in the primary and adjacent secondary visual cortex, as well as in the secondary auditory cortex, suggests an increased activity in these areas during SWS as compared to the quiet waking state. It should be noted that, during the whole procedure, subjects had their eyes closed and the only constant source of auditory stimulation was the humming sound of the scanner. Brain activity in primary sensory cortical regions has previously been observed in the absence of external stimuli that could induce such changes. For instance, rCBF increases have been observed in the primary visual cortex when awake subjects imagine different objects with their eyes closed (Kosslyn et al., 1995). Also, rCBF increases have been found in the auditory cortex when subjects imagine sounds in the absence of external stimuli (Zatorre et al., 1996a).

Positive covariations between rCBF and delta activity in the left secondary auditory cortex were located over the planum temporale (BA 22), and were accompanied by rCBF increases in the left inferior parietal lobule (supramarginal

gyrus, BA 40). Regional CBF increases in these areas have been previously linked with auditory/phonological processing (Howard et al., 1992; Paulesu et al., 1993; Zatorre et al., 1992, 1996b). The increased activity observed in the left sensory-motor cortex (BA 3/4) is consistent with similar activations seen in previous studies in association with silent word repetition (Paulesu et al., 1993) and whispering of syllables (Paus et al., 1996).

Regional activation in the complex of areas described above may then reflect the occurrence of visual, auditory and, perhaps, verbal imagery during SWS. It is of interest to note that many of the brain regions previously shown in the imagery studies and in the present study (Brodman's areas 18, 21, 22 and 4) were also observed during visual and auditory hallucinations in a drug-naïve schizophrenic patient (Silbersweig et al., 1995).

During REM sleep as compared to waking, brain activity increases have been observed in the visual cortex that are similar to those reported here for SWS (Madsen et al., 1991a; Maquet et al., 1990, 1996). Such a similarity between SWS and REM sleep, suggests the presence of visual imagery during both states. Nevertheless, some differences arise when comparing the activation patterns during REM and SWS. During REM sleep, rCBF increases were found mainly in the pontine tegmentum, anterior cingulate cortex, parietal operculum, amygdaloid complexes and entorhinal cortex (Maquet et al., 1996), which were not present in our study. Maquet et al. (1996) speculate that the activation

patterns observed might provide a neurophysiological substrate for the processing of affective laden memories during REM sleep.

Although in early studies 'dreaming' was reported almost exclusively from REM sleep awakenings (Dement and Kleitman, 1957), 'dream' reports have been subsequently obtained from all stages of sleep (Foulkes, 1962; Tracy and Tracy, 1974; Cavallero et al., 1992). Since our subjects were not awakened after each scan, the subjective experience of 'dreaming' could not be confirmed in this study. Reports from SWS appear to be slightly less vivid, shorter and more thought-like than those from REM sleep, yet the majority of non-REM reports involved both visual and auditory imagery (Foulkes, 1962), which is consistent with our results. Hence, differences between REM and SWS 'dreaming' may be the result of differences in the cognitive processes taking place during that state, rather than in the underlying sensory activity. Furthermore, some of these differences might be accounted for by the processing of affective laden memories during REM sleep that would not take place during SWS.

Summary and Conclusions

The work presented in this thesis evidences a very strong thalamic rCBF decrease in association with delta activity during the progression of SWS. After removing changes associated with delta activity, a further rCBF decrease in association with sigma activity (sleep spindles) was found over the medial thalamus. These results may reflect the thalamic disfacilitation during SWS and

furthermore the active inhibition imposed upon thalamo-cortical cells during sleep spindles in stage 2 SWS.

Other areas of decreased rCBF in association with delta activity include the brainstem reticular formation, cerebellum, anterior cingulate and orbito-frontal cortex. These changes, together with the thalamic rCBF decreases described above, may provide the neural substrate underlying the loss of consciousness, and decrease in sensory awareness and responsiveness characteristic of SWS.

Despite this closing of the afferent gateway to the cerebral cortex during SWS, significant rCBF increases in association with delta activity were found in several cortical areas including the visual and auditory cortices. These results could possibly reflect processes of dream-like mentation purported to occur during SWS.

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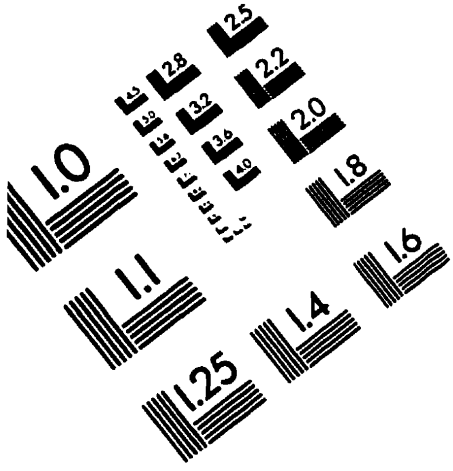
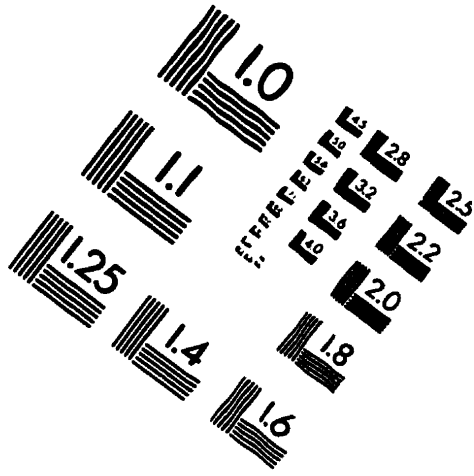
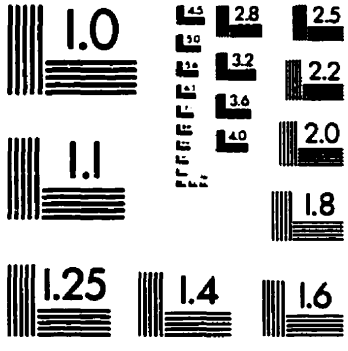
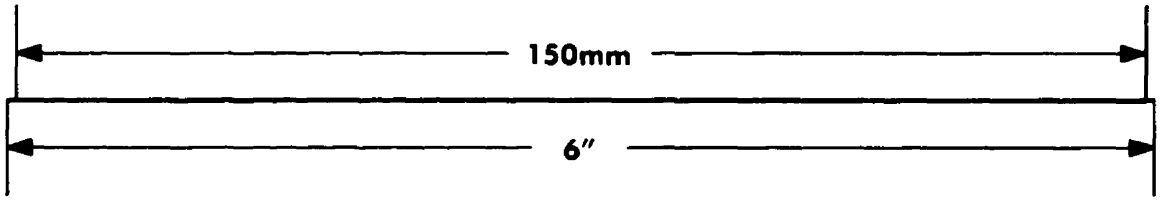
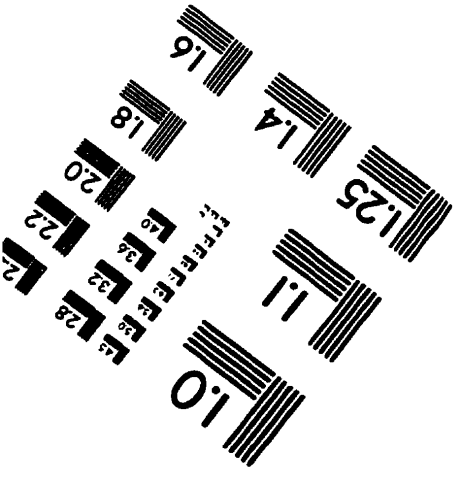
IMAGE EVALUATION
TEST TARGET (QA-3)

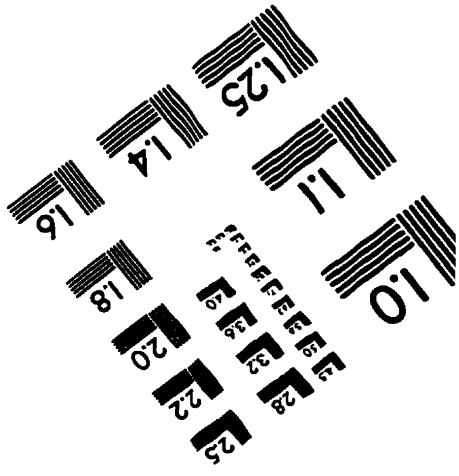












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