European Journal of Neuroscience, Vol. 9, pp. 271–279, 1997

Positive Correlations Between Cerebral Protein Synthesis Rates and Deep Sleep in *Macaca mulatta*

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Keywords: autoradiography, deep sleep, leucine, NREM sleep, rhesus monkey

Abstract

Local rates of cerebral protein synthesis ($lCPS_{leu}$) were determined with the autoradiographic L-[1-^{14}C]leucine method in seven awake and seven asleep, adult rhesus monkeys conditioned to sleep in a restraining chair in a darkened, ventilated chamber while EEG, EOG, and EMG were monitored. Prior to the period of measurement all animals slept for 1–4 h. Controls were awakened after at least one period of rapid-eye-movement (REM) sleep. Experimental animals were allowed to remain asleep, and they exhibited non-REM sleep for 71–99% of the experimental period. Statistically significant differences in $lCPS_{leu}$ between control and experimental animals were found in four of the 57 regions of brain examined, but these effects may have occurred by chance. In the sleeping animals, however, correlations between $lCPS_{leu}$ and percent time in deep sleep were positive in all regions and were statistically significant ($P \leq 0.05$) in 35 of the regions. When time in deep sleep was weighted for the integrated specific activity of leucine in grey matter, positive correlations were statistically significant ($P \leq 0.05$) in 18 regions in the experimental animals. These results suggest that rates of protein synthesis are increased in many regions of the brain during deep sleep compared with light sleep.

Introduction

A popular hypothesis regarding the function of sleep is that during wakefulness cerebral energy metabolism is used primarily for the maintenance of cellular ionic gradients whereas during sleep, when energy requirements are reduced, the brain's energy resources are diverted to protein synthesis for the restoration of cell structure and function (Adam and Oswald, 1983). Whole brain energy metabolism is, indeed, reduced by 25–30% in non rapid-eye-movement (NREM) sleep compared to the awake state in both monkeys (Kennedy *et al.*, 1982; Nakamura *et al.*, 1983) and human subjects (Sakai *et al.*, 1980; Heiss *et al.*, 1985; Buchsbaum *et al.*, 1989; Maquet *et al.*, 1990, 1992; Madsen *et al.*, 1991; Boyle *et al.*, 1994). Non-REM sleep includes both the light (stages 1 and 2) and deep (stages 3 and 4) phases of sleep. Although the energy requirements for biosynthesis of proteins are relatively minor, the biosynthetic process is sensitive to the cellular energy charge (Chapman *et al.*, 1971; Ayuso-Parrilla and Parrilla, 1975), which may be increased in brain during deep or slow wave sleep, a state in which the brain is relatively unresponsive to outside stimulation.

The purpose of this study was to determine the effects of natural sleep, i.e. sleep with no previous sleep deprivation, on local rates of cerebral protein synthesis ($lCPS_{leu}$). The study was conducted in the adult rhesus monkey because in the monkey, unlike several other species, no sleep deprivation is necessary for spontaneous NREM sleep periods to be of sufficient duration for the [^{14}C]leucine method (Smith *et al.*, 1988) to be applied.

Materials and methods

Chemicals

Chemicals were obtained from the following sources: L-[1-^{14}C]Leucine (specific activity, 59 mCi/mmol), Amersham, Arlington Heights, IL; L-norleucine, Cyclochemical, Los Angeles, CA; 5-sulphosalicylic acid, Fluka Chemie AG, Buchs, Switzerland.

Animals

All procedures were carried out in accordance with the National Institutes of Health Guidelines on the Care and Use of Animals and an animal study protocol approved by the NIMH Animal Care and Use Committee. Eighteen adult rhesus monkeys *(Macaca mulatta)*,

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Received 17 June 1996, revised 16 September 1996, accepted 18 September 1996

of both sexes (11 female and seven male), weighing 4.2–6.6 kg were prepared several weeks before the experiment by surgical implantation under halothane anaesthesia of electrodes for monitoring electroencephalogram (EEG), electromyogram (EMG), and electro-oculogram (EOG). The animals spent daytime hours in individual cages in the animal facility under controlled conditions of normal humidity and temperature, with standard alternating 12 h periods of light and darkness (light, 0700–1900 h). They were fed Purina Monkey Chow once daily and given water *ad libitum*. At the same time each night, animals were gradually conditioned to sleep in a restraining chair in a darkened, ventilated chamber in which white noise (70 dB) was continuously generated. On the day of the experiment, between 3:00 and 5:00 p.m., catheters were inserted into a femoral artery and vein under light halothane anaesthesia to permit the administration of [^{14}C]leucine and arterial sampling. Following a 3-h recovery period, animals were placed in the chamber and allowed to sleep soundly for 1-4 h prior to initiation of the measurement of protein synthesis. Sleep was monitored by polygraph recordings of EEG, EOG, and EMG, and the animal's behaviour was continuously observed by a video camera with infrared light. Epochs of 20 or 30 s duration were scored as awake, light sleep (stage 1–2), deep sleep (stage 3–4), or REM sleep (Crowley *et al.*, 1972). To ensure that animals were actually in a prolonged sleep state, the study was not initiated until the end of the first extended REM period that followed at least 1 h of sleep. At this time experimental animals were allowed to remain in NREM sleep and the 1 h study was initiated by the i.v. injection of [^{14}C]leucine. Control animals were awakened, administered the [^{14}C]leucine, and kept awake, if necessary, by occasional, gentle jostling of the chamber. In one of the control animals there was no REM period during the 3.5 h of sleep prior to administration of [^{14}C]leucine.

Measurement of physiological variables

Several variables were measured immediately before the pulse of [^{14}C]leucine and again 20 min later to evaluate the normalcy of each animal's physiological state. Mean arterial blood pressure was measured with an air-damped mercury manometer. Arterial blood pCO_2, pO_2, and pH were determined with a Corning 158 pH/Blood Gas Analyzer (Corning Ltd, Halstead, Essex, UK). Arterial blood haematocrit was determined in blood samples collected in capillary tubes that were subsequently sealed and centrifuged in a Beckman Microfuge B (Beckman Instruments, Fullerton, CA).

Measurement of local rates of leucine incorporation into protein

The experimental period was initiated by an intravenous pulse of [1-^{14}C]leucine (100 μCi/kg body weight contained in 0.5–1.0 ml of physiological saline). Timed arterial blood samples were collected during the following 60 min for determination of the time courses of plasma [^{14}C]leucine and leucine concentrations. The blood samples were immediately centrifuged, and the plasma deproteinized at 4°C by the addition of a solution of sulphosalicylic acid to make the final concentration 4% (wt/vol) sulphosalicylic acid. [^{14}C]Leucine and leucine concentrations in the acid-soluble fractions were assayed by liquid scintillation counting with external standardization and by amino acid analysis with norleucine as an internal standard. At the end of the 60 min experimental period, the animals were killed by an i.v. dose of thiopental immediately followed by a saturated solution of KCl in sufficient amount to stop the heart, and the brain was removed rapidly and frozen in isopentane cooled to –40°C with dry ice. Coronal brain sections, 20 μm thick, were cut in a cryostat maintained at –20°C and prepared for autoradiography as previously described (Sun *et al.*, 1992). In each animal local ^{14}C concentrations in individual brain regions were determined by analysis of the autoradiograms with a computerized image processing system (M1, Imaging Research Inc., St Catharines, Ontario, Canada). The concentration of ^{14}C in each region of interest was determined from the regional optical density in the autoradiogram and a ^{14}C concentration–optical density calibration curve determined from the autoradiographic representations of calibrated [^{14}C]plastic standards. Local rates of leucine incorporation into protein were calculated by means of the operational equation of the [1-^{14}C]leucine method (Smith *et al.*, 1988) with a value of 0.58 for λ_i:

$$R_i = \frac{P_i^*(T)}{\lambda_i \int_0^T [C_p^*(t)/C_p]dt} \quad [1]$$

where R_i is the rate of leucine incorporation into protein in region i, $P^*_i(T)$ is the ^{14}C-labelled protein concentration in region i at time T, C^*_p and C_p are the concentrations of labelled and unlabelled leucine respectively in arterial plasma, T is the time of killing, t is variable time, and λ_i is the fraction of leucine in the precursor pool for protein synthesis in the tissue that is derived from the plasma. The factor λ_i is needed in the equation to derive the integrated specific activity of leucine in the precursor pool in the tissue from the integrated specific activity measured in the arterial plasma; λ_i corrects for the dilution of the labelled leucine entering the precursor pool by unlabelled leucine recycled from protein degradation in the tissue. The value of λ_i for the brain as a whole has been determined in the rat (Smith *et al.*, 1988) and in the sheep (Abrams *et al.*, 1995) and found to be 0.58 ± 0.03 (mean ± SD) and 0.57 ± 0.07 (mean ± SD) respectively. We have assumed that the value of λ_i in the monkey is similar to that determined in rat and sheep and that it is constant from region to region and in asleep and awake states. These are reasonable assumptions inasmuch as previous studies in normal, conscious, adult rats have shown that the value of λ_i in most grey matter regions falls within ±5% of the value of 0.58, the average measured in the brain as a whole (Sun *et al.*, 1992), and remains so even in conditions in which $lCPS_{leu}$ has been shown to change, including the regenerating hypoglossal nucleus of the adult rat in which $lCPS_{leu}$ is increased by 20–30% (Sun *et al.*, 1993). If slow wave sleep were to affect rates of protein degradation, the value of λ_i could be affected, and rates of protein synthesis calculated with control values of λ_i would be in error. Significant changes in the value of λ_i have, however, thus far not been observed, except during postnatal development in the rat (Sun *et al.*, 1995).

Measurement of protein synthesis rates was carried out in local regions selected so as to survey the major systems of brain and to include most of the regions implicated by others as important for sleep on the basis of the effects of lesions or of local electrical or pharmacological stimulation (Sterman and Clemente 1962; Jouvet, 1967; Moruzzi, 1972; McGinty *et al.*, 1974; Steriade and Hobson, 1976; Bremer, 1977; Morrison, 1979; Steriade *et al.*, 1994). These regions could be identified in the autoradiograms by comparison with the Nissl-stained tissue sections from which the autoradiograms were prepared.

Statistics

Two control and two experimental animals were not included in the analyses because their endogenous plasma leucine concentrations fell during the experimental period. Values of $lCPS_{leu}$ in seven control

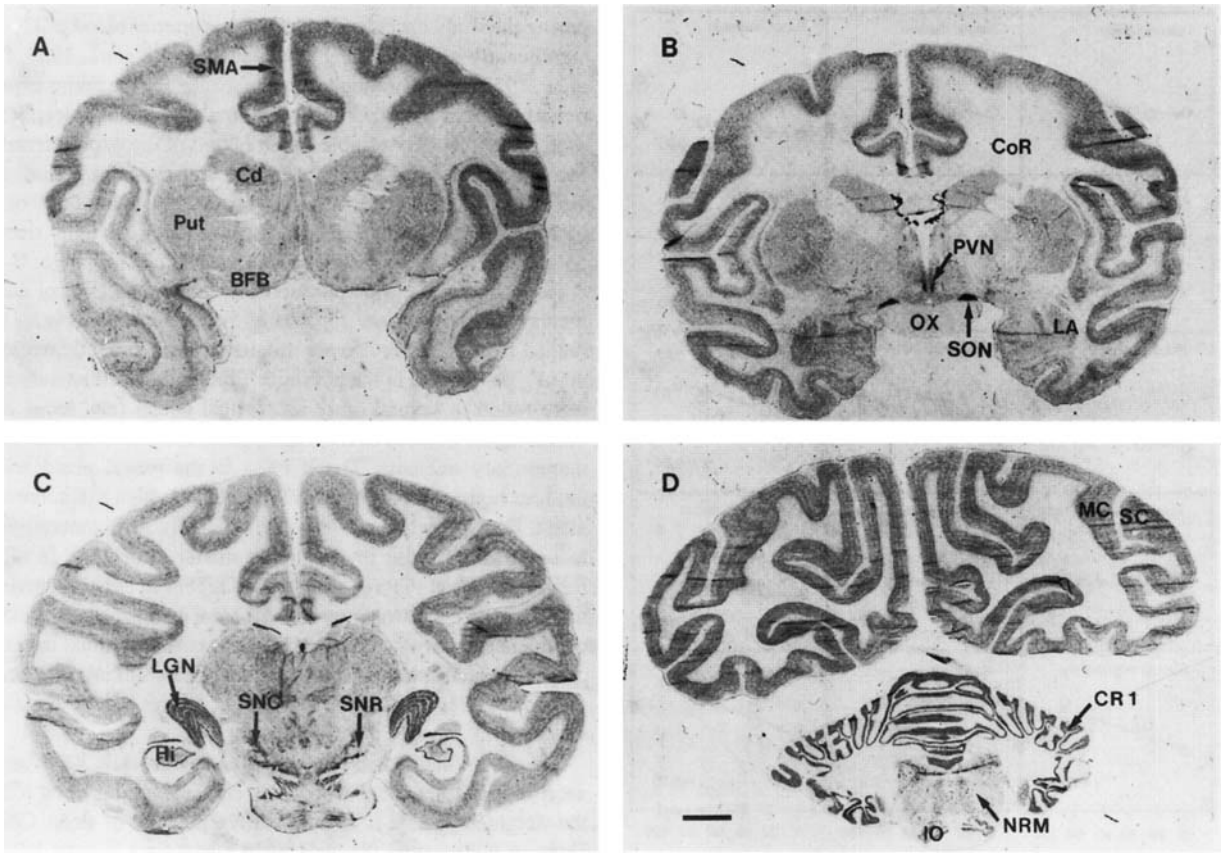

FIG. 1. Autoradiograms of coronal sections of monkey brain labelled with [1-^{14}C]leucine. Sections were fixed and washed with phosphate-buffered formalin to wash out free, unincorporated [^{14}C]leucine and possible water-soluble labelled metabolic products in the tissue (Smith *et al.*, 1988). Four different levels of the brain (A–D) are illustrated. Dorsal is up and the right side of the brain is on the right. The scale bar in the lower right hand corner represents 1 cm. Abbreviations: SMA, supplementary motor area; Cd, caudate nucleus; Put, putamen; BFB, basal forebrain; CoR, corona radiata; PVN, paraventricular nucleus; SON, supraoptic nucleus; OX, optic chiasma; LA, lateral basal amygdala; LGN, lateral geniculate nucleus; SNC, substantia nigra compacta; SNR, substantia nigra reticulata; Hi, hippocampus; MC, motor cortex; SC, sensory cortex; CR1, cerebellar hemisphere, crus 1; NRM, magnocellular reticular nucleus; IO, inferior olive.

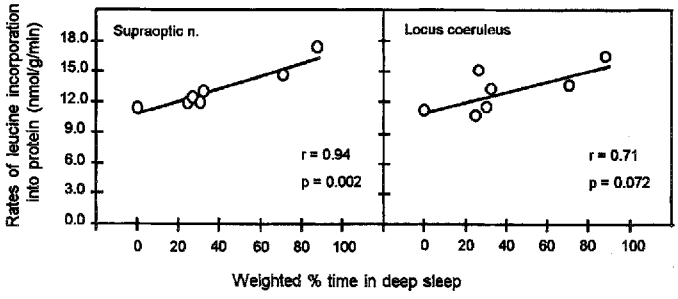

FIG. 2. Linear relationship between weighted percent time in deep sleep and rate of leucine incorporation into protein in supraoptic nuclei (left) and locus coeruleus (right) of sleeping monkeys. Each point represents the result obtained in a single monkey. The least squares best-fitting linear regression line is shown for illustration.

(four female and three male) and seven experimental (five female and two male) monkeys were compared by means of Student's *t*-tests. Effects of total NREM sleep, light sleep, and deep sleep on $lCPS_{leu}$ were evaluated by correlating $lCPS_{leu}$ in the experimental monkeys with percent time in NREM sleep, light sleep, or deep sleep. The controls were excluded from the correlations because these animals were awake throughout the leucine study, i.e. in a totally different behavioural state from the experimental animals; in the awake state protein synthesis could be influenced by factors not present during sleep, and such factors could confound the correlational analyses. Percent time in NREM sleep, light sleep, and deep sleep was weighted for the integrated specific activity of leucine present in grey matter during the NREM sleep, light sleep, or deep sleep periods (as described by Abrams *et al.*, 1988). Grey matter leucine specific activity curves for each monkey were generated from the time course of the leucine specific activity measured in arterial plasma and the rate constant for the turnover of the leucine pool in grey matter:

$$SA(T) = ke^{-kT}\int_0^T \frac{C_p^*(t)}{C_p} e^{kt}dt \qquad [2]$$

where $SA(T)$ represents the leucine specific activity in grey matter at time T, k is the rate constant for the turnover of the leucine pool in grey matter, C^*_p and C_p are the concentrations of labelled and unlabelled leucine, respectively, in arterial plasma, and t is variable time. We have used a value of 0.2 min^{-1} for k, the average value for grey matter regions determined in the conscious, adult rat (Smith, 1991). This value is equivalent to a 3.5 min half-life ($T_{1/2} = 0.693/k$). Regression lines and correlation coefficients and their P-values relating $lCPS_{leu}$ to the weighted percent time in NREM sleep, light sleep, or deep sleep were calculated by means of a least squares best fit weighted for the measured value for $lCPS_{leu}$.

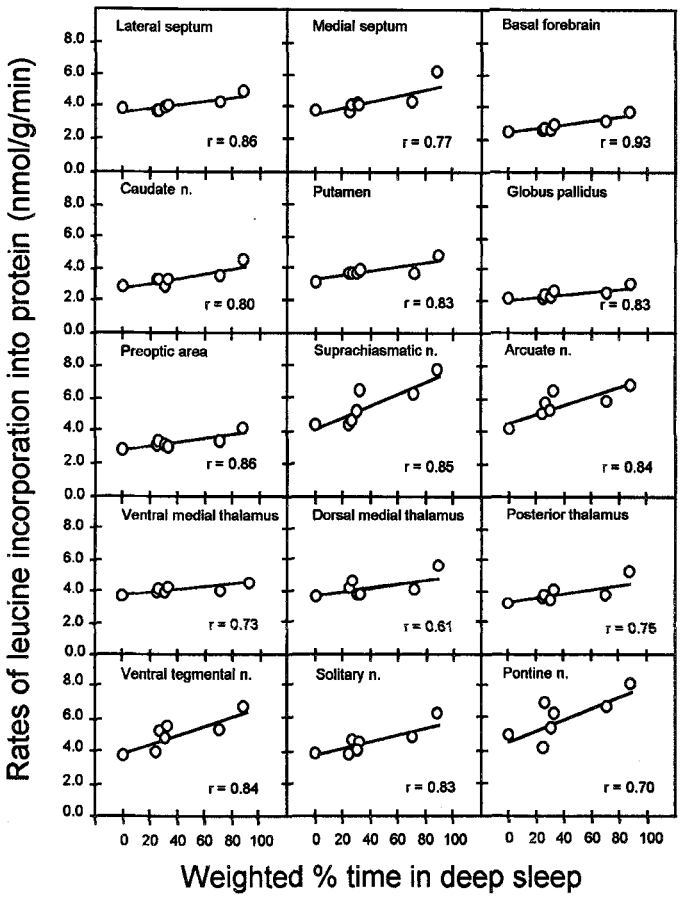

FIG. 3. Linear relationship between weighted percent time in deep sleep and rate of leucine incorporation into protein in (from top to bottom rows) limbic system, basal ganglia, hypothalamus, thalamus, and brain stem of sleeping monkeys. Each point represents the result obtained in a single monkey. The best-fitting linear regression lines are shown for illustration. *P*-values are given in Tables 3–5.

TABLE 1. Physiological variables in awake and sleeping monkeys

	Awake (7)	Asleep (7)
Body weight (kg)	4.97 ± 0.33	5.25 ± 0.22
Mean arterial blood pressure (mm Hg)	101 ± 3	103 ± 4
Haematocrit (%)	41 ± 0.9	39 ± 0.5
Arterial plasma leucine concentration (μM)	173 ± 9	235 ± 41
Arterial blood		
pH	7.44 ± 0.01	7.42 ± 0.01
pO_2 (mmHg)	88.0 ± 1.3	90.7 ± 3.0
pCO_2 (mmHg)	37.1 ± 1.2	42.4 ± 1.2[a]

Values are means ± SEM for the number of monkeys indicated in parentheses. Measurements for arterial blood pH were made in only six awake monkeys. Measurements for mean arterial blood pressure and plasma leucine concentration were made before administration of radiolabelled leucine. Measurements for haematocrit, arterial blood pH, pO_2 , and pCO_2 were made 10–45 min after administration of radiolabelled leucine.
[a]Statistically significantly different from value in awake monkeys, $P < 0.01$, Student's *t*-test.

Results

Behavioural observations and physiological measurement (Table 1) indicated that the monkeys were in a normal physiological state during the study except, as expected, the arterial blood pCO_2 increased significantly in the sleeping animals (Mangold *et al.*, 1955; Birchfield *et al.*, 1958). Control monkeys were awake for the entire experimental period whereas the experimental animals remained asleep 93% of the time (range, 84–99%; Table 2). Only two of the experimental animals had epochs of REM sleep during the experimental period, and these were relatively short (1–8 min). Hence, they spent 90% of the time in NREM sleep (range, 71–99%), consisting of light sleep (mean, 53%; range, 9–89%) and deep sleep (mean, 37%; range, 0–84%).

Rates of leucine incorporation into protein in control and experimental monkeys were regionally heterogeneous (Fig. 1). Values ranged from 12.2 nmol/g per minute in the supraoptic nucleus to 0.9 nmol/g per minute in the pyramids (Tables 3–5). Relatively high rates were found in several other subcortical nuclei (e.g. locus coeruleus, dorsal motor nucleus of the vagus, paraventricular nucleus and lateral mammillary nucleus). The $lCPS_{leu}$ in the pineal gland was among the four highest found in the 57 regions in which measurements were made. Rates in white matter were relatively low, generally one-third to one-fifth of those in most grey matter structures. In all but one region, the mean $lCPS_{leu}$ was slightly higher in experimental monkeys than in awake controls, but the differences reached the 0.05 level of confidence in only four regions: inferior parietal cortex, lateral septum, cerebellar hemispheres, and locus coeruleus. Taking into account the number of regions examined, these effects could have occurred by chance.

The data obtained in the experimental animals were analysed to ascertain whether there were any relationships between $lCPS_{leu}$ and the weighted percent time in the different states of sleep. Correlations between $lCPS_{leu}$ and the weighted percent time in total NREM sleep were generally positive but statistically significant ($P \leq 0.05$) in only three of the 57 regions. Correlations with weighted percent time in light sleep were negative in all 57 regions examined; these were statistically significant ($P \leq 0.05$) in nine of the regions and approached statistical significance ($P \leq 0.10$) in six. Finally, the correlations with weighted percent time in deep sleep were positive in all 57 regions examined; 18 were statistically significant ($P \leq 0.05$), and 16 more approached statistical significance ($P \leq 0.10$) (Tables 3–5, Figs 2 and 3).

Discussion

The results of the present study suggest that deep sleep, in comparison with light sleep, is accompanied by widely distributed increases in rates of cerebral protein synthesis. Although no anatomical or functional system was selectively affected, effects were particularly prevalent in the limbic system, basal ganglia, and brain stem. Total NREM sleep tended to be positively correlated with $lCPS_{leu}$, and correlations of $lCPS_{leu}$ with light sleep were generally negative, but in both cases the correlations were not statistically robust. Effects of REM sleep were not analysed because only two of the sleeping monkeys had any periods of REM sleep during the experimental period.

The two animals with the greatest (>70%) weighted time in deep sleep were the only two of the seven sleeping animals that had any REM sleep during the study, but in both cases the duration of REM sleep was short and occurred in the later portion of the [^{14}C]leucine clearance curve. Specifically, in the case of SM6, REM sleep occurred between 41 and 48 min after the pulse of [^{14}C]leucine, and in the case of SM7, REM sleep occurred between 33 and 36 min and again between 37 and 38 min after the pulse. Events later in the clearance curve, i.e. 30 min after the pulse, when arterial plasma [^{14}C]leucine concentrations have fallen to less than 4% of peak values, have

TABLE 2. Behavioural state in sleeping monkeys

Monkey	Time in behavioural state				
	Awake	Asleep			
		Light sleep[a]	Deep sleep[b]	Total NREM	REM
Percentage of total experimental time					
SM1 (F)	1.0	59.0	40.0	99.0	0.0
SM2 (F)	9.0	52.0	39.0	91.0	0.0
SM3 (F)	5.0	65.0	30.0	95.0	0.0
SM4 (F)	11.0	89.0	0.0	89.0	0.0
SM5 (M)	5.0	64.0	31.0	95.0	0.0
SM6 (F)	16.0	33.0	38.0	71.0	13.0
SM7 (M)	2.0	9.0	84.0	93.0	5.0
Weighted for integrated tissue leucine specific activity[c]					
SM1 (F)	0.4	67.2	32.4	99.6	0.0
SM2 (F)	13.7	61.5	24.7	86.3	0.0
SM3 (F)	12.2	61.5	24.7	87.8	0.0
SM4 (F)	30.4	68.8	0.0	69.6	0.0
SM5 (M)	4.3	65.1	30.6	95.7	0.0
SM6 (F)	9.1	15.2	70.8	86.0	4.9
SM7 (M)	2.0	6.6	88.3	94.9	3.1

F and M denote female and male respectively.
[a]Sleep stages 1 and 2.
[b]Sleep stages 3 and 4.
[c]Percent of the total experimental time weighted for the area under the curve of the time course of the tissue leucine specific activity calculated with a tissue leucine half-life of 3.5 min.

TABLE 3. Telencephalon: local rates of cerebral protein synthesis in sleeping monkeys

Region	Local rate of cerebral protein synthesis (nmol leucine/g/min)		Product moment correlations: $lCPS_{leu}$ and deep sleep in sleeping monkeys (7)	
	Awake (7)	Asleep (7)	r_{xy}	*P*-value
Cortex				
Medial orbital	4.2 ± 0.2	4.6 ± 0.3	0.65	0.118
Supplementary motor area	4.4 ± 0.3	4.7 ± 0.3	0.75	0.050
Cingulate	4.3 ± 0.3	4.8 ± 0.3	0.67	0.102
Premotor	4.0 ± 0.3	4.2 ± 0.2	0.72	0.069
Sensory	5.1 ± 0.3	5.6 ± 0.3	0.58	0.168
Motor	5.0 ± 0.3	5.2 ± 0.3	0.66	0.108
Inferior parietal	4.4 ± 0.2	5.1 ± 0.2*	0.56	0.190
Striate	5.9 ± 0.3	6.5 ± 0.4	0.52	0.229
Limbic system				
CA1 area of hippocampus	4.2 ± 0.2	4.5 ± 0.3	0.68	0.094
Parahippocampal gyrus	4.2 ± 0.2	4.4 ± 0.3	0.73	0.062
Medial basal amygdala	3.3 ± 0.2	3.4 ± 0.2	0.63	0.126
Lateral basal amygdala	4.2 ± 0.2	4.4 ± 0.3	0.62	0.137
Lateral septum	3.7 ± 0.2	4.1 ± 0.2[a]	0.86	0.013
Medial septum	3.9 ± 0.2	4.3 ± 0.3	0.77	0.044
Basal forebrain	2.9 ± 0.2	2.9 ± 0.2	0.93	0.003
Basal ganglia				
Caudate nucleus	3.3 ± 0.2	3.4 ± 0.2	0.80	0.031
Putamen	3.6 ± 0.2	3.8 ± 0.2	0.83	0.022
Globus pallidus	2.3 ± 0.1	2.4 ± 0.1	0.83	0.021
White matter				
Corona radiata	1.0 ± 0.1	1.2 ± 0.1	0.70	0.078
Optic chiasm	1.3 ± 0.1	1.5 ± 0.1	0.88	0.009

Values are the means ± SEM for the number of monkeys indicated in parentheses except in the optic chiasm in which $lCPS_{leu}$ could be measured in only six of the awake animals.
[a]Statistically significantly different from awake animals; $P \leq 0.05$, Student's *t*-test.

diminishing effects on the determined $lCPS_{leu}$ because the concentration of [^{14}C]leucine in the tissue is relatively low at this time (see Fig. 4). In both SM6 and 7, the integrated specific activity of [^{14}C]leucine in the tissue during REM was less than 5% of the total tissue integrated [^{14}C]leucine specific activity.

The observed effect of deep sleep on $lCPS_{leu}$ is based on product

TABLE 4. Diencephalon and mesencephalon: local rates of cerebral protein synthesis in sleeping monkeys

Region	Local rate of cerebral protein synthesis (nmol leucine/g/min)		Product moment correlations: $lCPS_{leu}$ and deep sleep in sleeping monkeys (7)	
	Awake (7)	Asleep (7)	r_{xy}	P-value
Diencephalon				
Thalamus				
Dorsomedial nucleus	4.0 ± 0.3	4.2 ± 0.3	0.61	0.150
Centrolateral nucleus	4.5 ± 0.3	4.8 ± 0.3	0.63	0.133
Lateral dorsal	3.5 ± 0.2	3.6 ± 0.3	0.57	0.179
Ventral posterior lateral	4.0 ± 0.3	4.1 ± 0.2	0.78	0.038
Lateral geniculate nucleus	6.7 ± 0.4	7.2 ± 0.5	0.48	0.278
Hypothalamus				
Preoptic area	3.0 ± 0.2	3.3 ± 0.2	0.86	0.013
Suprachiasmatic nucleus	5.5 ± 0.5	5.6 ± 0.5	0.85	0.017
Supraoptic nucleus	12.2 ± 0.9	13.3 ± 0.8	0.94	0.002
Parventricular nucleus	8.4 ± 0.6	9.5 ± 0.7	0.58	0.174
Anterior hypothalamus	3.6 ± 0.2	3.7 ± 0.2	0.55	0.203
Lateral hypothalamus	3.1 ± 0.1	3.3 ± 0.1	0.51	0.243
Ventromedial nucleus	3.8 ± 0.2	4.1 ± 0.2	0.73	0.063
Dorsomedial nucleus	4.5 ± 0.2	4.8 ± 0.3	0.71	0.076
Median eminence	5.3 ± 0.6	5.3 ± 0.3	0.68	0.096
Arcuate nucleus	6.3 ± 0.6	5.7 ± 0.4	0.84	0.018
Ventral anterior nucleus	3.4 ± 0.2	3.6 ± 0.2	0.63	0.132
Posterior nucleus	3.8 ± 0.3	3.9 ± 0.2	0.75	0.050
Medial mammillary nucleus	5.0 ± 0.4	5.2 ± 0.4	0.70	0.081
Lateral mammillary nucleus	7.3 ± 0.5	8.0 ± 0.5	0.61	0.145
Interpeduncular nucleus	3.9 ± 0.3	4.4 ± 0.2	0.52	0.233
Mesencephalon				
Locus coeruleus	11.1 ± 0.7	13.2 ± 0.8[a]	0.71	0.072
Dorsal raphe	5.4 ± 0.4	6.1 ± 0.4	0.53	0.225
Ventral raphe	3.6 ± 0.4	4.0 ± 0.3	0.92	0.004
Pyramids	0.9 ± 0.0	0.9 ± 0.1	0.54	0.210

Values are the means ± SEM for the number of monkeys indicated in parentheses except in the suprachiasmatic nucleus, median eminence and arcuate nucleus, in which $lCPS_{leu}$ was measured in six, four and five awake monkeys respectively.
[a]Statistically significantly different from awake animals, $P \leq 0.05$, Student's t-test.

moment correlations between the weighted time in deep sleep and $lCPS_{leu}$. The weighted time in deep sleep was calculated for each monkey based on the animal's sleep record and the time course of the tissue leucine specific activity which was calculated from the time course of the plasma leucine specific activity and the rate constant for the turnover of the leucine pool in grey matter (equation [2]). The shape of the calculated tissue leucine specific activity curve depends on the value assigned to the half-life of the tissue free leucine pool (Fig. 4), and the weighted percent time in the various phases of sleep depends on the shape of the curve. A value of 3.5 min for the half-life of the tissue free leucine pool determined in conscious, adult, male rats (Smith, 1991) was initially used for these calculations. It is conceivable that in the adult monkey and, in particular, in the sleeping monkey the half-life could be longer than 3.5 min. To test the dependence of these correlations on the value of the half-life we determined the weighted percent time in deep sleep with values of 0, 6.9, 13.9, and 34.7 min for the half-life of the tissue free leucine pool. The results of these analyses show that, with longer half-lives, correlations between $lCPS_{leu}$ and weighted percent time in deep sleep remain positive, and statistical significance ($P \leq 0.05$) is reached in an increasing number of regions when the curves are calculated with longer half-lives; for example, with half-lives of 0 and 34.7 min, correlations in 16 and 33 of the 57 regions respectively were statistically significant. Correlations between $lCPS_{leu}$ and unweighted time in deep sleep (i.e., with an infinite half-life) correlations were significant in 35 regions. Our results (Table 3–5), therefore, are a conservative appraisal of the relationship between deep sleep and $lCPS_{leu}$. It would appear that deep sleep is associated with generalized, as opposed to regionally specific, increases in $lCPS_{leu}$.

Rates of protein synthesis determined with the [1-^{14}C]leucine method are calculated from the regional concentration of ^{14}C fixed in the tissue, the time course of the arterial plasma leucine specific activity, and λ_i, a factor in the equation that corrects the integrated leucine specific activity calculated from the plasma specific activity for dilution by unlabelled leucine derived from the steady state breakdown of protein in the tissue (equation [1]). We have not yet determined the value of λ_i in the adult rhesus monkey and have, therefore, assumed that the value is similar to that determined in conscious, adult rats (Smith *et al.*, 1988) and in conscious sheep (Abrams *et al.*, 1995). If the actual value of λ_i in the monkey is higher or lower than the value determined in these two disparate species our calculated values for $lCPS_{leu}$ would be over- or under-estimates respectively. The error, however, would be expected to be similar in all regions and in both control and experimental animals. The only circumstance in which an error in our estimate of the value of λ_i would affect the conclusions drawn from the present study would be if the value of λ_i itself were affected by the state of sleep. This is unlikely because results of studies in rats show that the value of λ_i is stable under several diverse conditions, e.g., normal ageing (Smith *et al.*, 1995b), ketamine anaesthesia (C. B. Smith and Y. Sun, unpublished results), repetitive electrical stimulation (Smith *et al.*, 1992), and neural regeneration (Sun *et al.*, 1993). Only during postnatal development were significant changes in the value of λ_i observed (Sun *et al.*, 1995). During normal ageing (Smith *et al.*,

TABLE 5. Brain stem, cerebellum and pineal gland: local rates of cerebral protein synthesis in sleeping monkeys

Region	Local rate of cerebral protein synthesis (nmol leucine/g/min)		Product moment correlations: $lCPS_{leu}$ and deep sleep in sleeping monkeys (7)	
	Awake (7)	Asleep (7)	r_{xy}	P-value
Brain stem				
Pontine nucleus	5.4 ± 0.3	6.1 ± 0.5	0.70	0.081
Ventral tegmental nucleus	4.7 ± 0.3	5.0 ± 0.4	0.84	0.018
Gigantocellular tegmental nucleus	2.6 ± 0.2	2.9 ± 0.2	0.44	0.327
Vestibular nucleus	5.2 ± 0.4	5.9 ± 0.5	0.67	0.100
Magnocellular reticular nucleus	3.5 ± 0.2	3.9 ± 0.4	0.68	0.091
Solitary nucleus	4.2 ± 0.2	4.6 ± 0.3	0.83	0.022
Dorsal motor nucleus of vagus	9.2 ± 0.6	11.0 ± 0.8	0.76	0.045
Hypoglossal nucleus	5.7 ± 0.3	6.5 ± 0.6	0.55	0.197
Cerebellum				
Fastigial nucleus	3.7 ± 0.2	4.1 ± 0.3	0.67	0.102
Interpositus nucleus	3.9 ± 0.3	4.4 ± 0.3	0.72	0.070
Dentate nucleus	4.3 ± 0.3	4.6 ± 0.3	0.69	0.084
Cerebellar hemisphere, crus 1	6.4 ± 0.3	7.5 ± 0.5[a]	0.63	0.131
Pineal gland	9.1 ± 0.6	10.1 ± 0.9	0.23	0.625

Values are mean ± SEM for the number of monkeys indicated in parentheses, except for the pineal gland, in which $lCPS_{leu}$ was measured in six awake animals. [a]Statistically significantly different from awake animals, $P \leq 0.05$, Student's t-test.

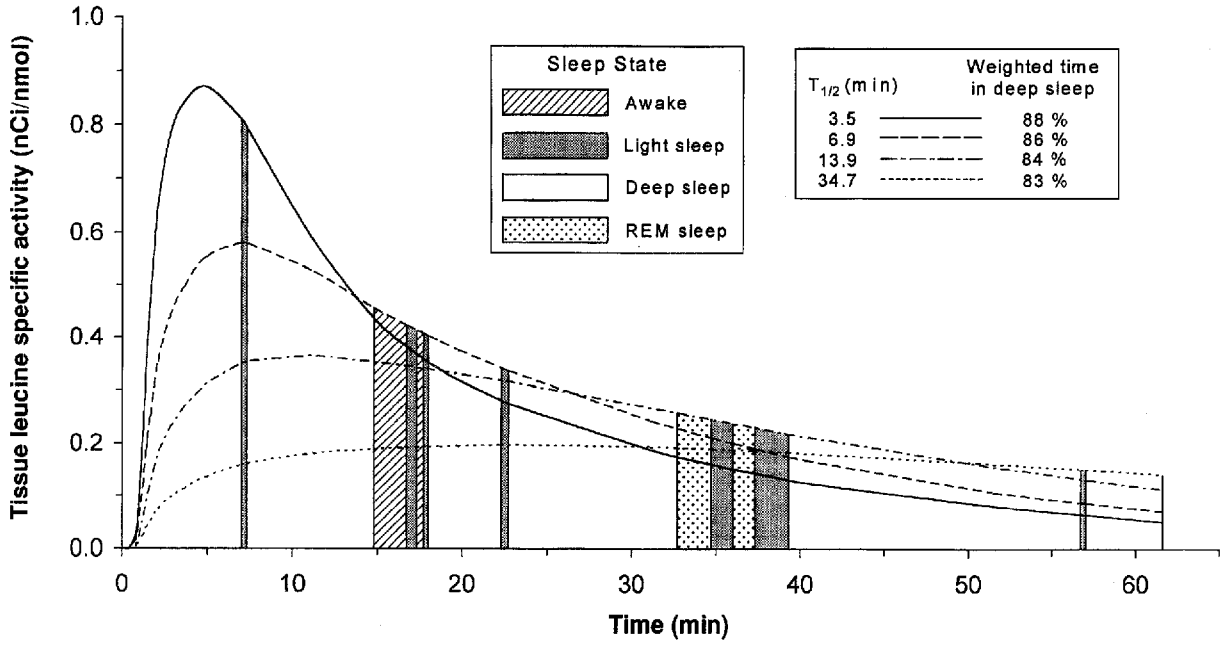

FIG. 4. Time course of the tissue leucine specific activity for monkey #SM7 calculated with half-lives of 3.5 (solid line) and 34.7 min (dashed line) for the tissue free leucine pool. The area under the curve has been hatched vertically, stippled, or left blank to indicate the sleep state of the monkey during the experimental interval.

1995b) and neural regeneration (Sun *et al.*, 1993) the value of λ_i remains constant even when $lCPS_{leu}$ is decreased or increased respectively.

Direct comparison of the absolute rates of protein synthesis in awake and asleep monkeys showed statistically significant effects in only four of the 57 regions examined. Taking into account the fact that 57 regions were analysed, these effects could have occurred by chance. In most regions the means and standard errors were similar for both groups of animals even though the experimental animals were a very heterogeneous group with respect to percent time in deep sleep. Awake controls were also heterogeneous with respect to drowsiness, the amount of time before the [^{14}C]leucine injection spent in deep sleep, the interval between the last time in deep sleep and the [^{14}C]leucine injection, and the amount of time awake immediately before the [^{14}C]leucine injection. Although none of these variables alone appeared to correlate with regional rates of protein synthesis in the controls, it is possible that some of them acted in concert to affect $lCPS_{leu}$. Analysis of these effects, however, would require more than seven animals.

To our knowledge, this is the first report that rates of cerebral protein synthesis are increased in naturally occurring deep sleep. Positive correlations between weighted time in deep or slow wave sleep and $lCPS_{leu}$ were reported in rats that had been previously deprived of REM sleep for 48 h (Ramm and Smith, 1990). In that

study, correlations were statistically significant in 49 of the 112 regions examined, and no statistically significant correlations were found between weighted time in either wakefulenss or REM sleep and $lCPS_{leu}$. Other studies of the effects of sleep on cerebral protein synthesis in rats following REM deprivation (Bobillier *et al.*, 1974; Shapiro and Girdwood, 1981) have yielded variable results, probably due to problems in measurement of precursor pool specific activity. There is some indirect evidence of a relationship between rates of cerebral protein synthesis and sleep. Treatment of rats with inhibitors of protein synthesis completely suppressed REM sleep and influenced the diurnal variation of slow wave sleep (Uezu *et al.*, 1981). Giuditta *et al.* (1980) observed that after a subarachnoidal injection of radiolabelled orotate the concentration of radioactive RNA per nucleus increased with the degree of synchronization of the EEG, a measure of depth of sleep. Others have reported circadian oscillations in DNA synthesis (Zucconi *et al.*, 1988), RNA synthesis (Merritt and Sulkowsi, 1970) and protein synthesis (Richardson and Rose, 1971; ter Haar, 1977) in rat and rabbit brain.

If future studies confirm our finding that regional rates of cerebral protein synthesis are positively correlated with high amplitude, low frequency EEG sleep (deep sleep), these results may be consistent with the hypothesis that NREM sleep is heterogeneous in appearance and function and that deep sleep may be particularly related to the so-called restorative functions of sleep. Based partially on the well known increase of deep sleep during recovery of sleep following sleep deprivation, Feinberg *et al.* (1974), Borbely *et al.* (1982) and others have suggested that deep sleep reverses the effects of prolonged wakefulness. That is, deep sleep and the associated increase in rates of cerebral protein synthesis may play a particularly important role in the homeostatic function of sleep. There has been widespread speculation that memory consolidation requires sleep, and it has been shown that inhibitors of protein synthesis block long term memory (reviewed by Agranoff, 1981). The results of our study may have some relevance for this hypothesis.

Acknowledgements

The authors wish to thank Ms J. Jehle for her expert technical assistance in the preparation of tissue sections for autoradiography, Ms G. Deibler for carrying out the amino acid analyses, Dr K. Pettigrew for advice on statistical analyses and Ms K. Schmidt for the program to calculate weighted product moment correlation coefficients. These results were presented in part at the XIVth International Symposium on Cerebral Blood Flow and Metabolism, Bologna, Italy, 1989 (Nakanishi *et al.*, 1989) and the 25th Annual Meeting of the Society for Neuroscience, San Diego, CA, 1995 (Smith *et al.*, 1995a).

Abbreviations

$lCPS_{leu}$	local rate of incorporation of leucine into protein
NREM	non-rapid eye movement
REM	rapid eye movement

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