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RAPID COMMUNICATION

Rates of Cerebral Protein Synthesis Are Linked to Slow Wave Sleep in the Rat

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RAMM, P. AND C. T. SMITH. *Rates of cerebral protein synthesis are linked to slow wave sleep in the rat.* PHYSIOL BEHAV **48**(5) 749–753, 1990.—Using L-[1-^{14}C]leucine autoradiography, rates of cerebral and local cerebral protein synthesis were studied during wakefulness, slow wave sleep (SWS) and REM sleep in the rat. In the cerebrum as a whole, the rate at which labelled leucine was incorporated into tissues was positively correlated with the occurrence of slow wave sleep. We failed to observe a significant correlation of protein synthesis rate with either wakefulness or REM sleep. As in the cerebrum as a whole, most discrete brain regions showed moderate positive correlations between the occurrence of SWS and rates of protein synthesis. There were no brain regions in which rates of protein synthesis showed striking correlations with sleep-wake states. Thus, the occurrence of SWS is associated with higher rates of protein synthesis throughout the brain. These data suggest that SWS sleep favors the restoration of cerebral proteins.

Sleep Metabolism Autoradiography Functional mapping Protein synthesis Rat

ENERGY for life processes is ultimately obtained from food. When conditions do not favor efficient food gathering, many organisms sleep. Therefore, a primary function of sleep may be to conserve energy expenditure while the organism is inactive and minimize the usage of food as metabolic fuel. This *conservation hypothesis* of sleep function is supported by reports that, during slow wave sleep (SWS), levels of metabolism are low in the organism as a whole (2) and in many regions of the central nervous system (6,9).

Energy conservation may be, itself, the major function of sleep. However, metabolism fuels all life processes, including production of heat, maintenance of neuronal ionic homeostasis, and biosynthesis. Lower levels of metabolism during sleep could reflect alterations in any or all life processes. Does the energy conservation of sleep reflect a decrease in all forms of cellular work, or is decreased metabolism more strongly linked to some processes than others? It has been suggested that the low levels of metabolism associated with SWS are specifically linked to a decrease in catabolism and that, therefore, competition between metabolic needs for catabolic processes and for biosynthesis is lower during sleep (2). This logic is at the core of numerous suggestions [see (2) for review] that energy conservation during sleep favors the anabolic restoration of tissue components, depleted during periods of high metabolic demand (wakefulness). Various forms of this *restorative hypothesis* have been proposed. Most prevalent are suggestions that SWS and/or rapid eye movement (REM) sleep favor the synthesis of cerebral proteins.

The restorative hypothesis remains controversial [e.g., (4)], as direct demonstrations of rates of cerebral and local cerebral protein synthesis during sleep have not been available. We have used an autoradiographic technique to measure leucine incorporation into brain protein during sleep and wakefulness. L-[1-^{14}C]leucine autoradiography (5, 11, 12), like the similar [^{14}C]-2-deoxyglucose autoradiography, involves the use of isotopically labelled tracer to measure rates of reaction. A single injection of L-[1-^{14}C]-leucine is given intravenously. Arterial blood samples are taken for the next 45 min (while the animal is awake or sleeps) for measurement of precursor specific activity, and the animal is then processed for autoradiography. Regional rates of amino acid incorporation into protein (reflecting rates of protein synthesis) are read from the autoradiographs, using a kinetic model of the exchange of labelled leucine between plasma and tissue, its metabolism in tissue, and its movement through the metabolic pathway for leucine. Autoradiographic mapping of regional rates of cerebral protein synthesis during sleep allows a direct test of the restorative hypothesis.

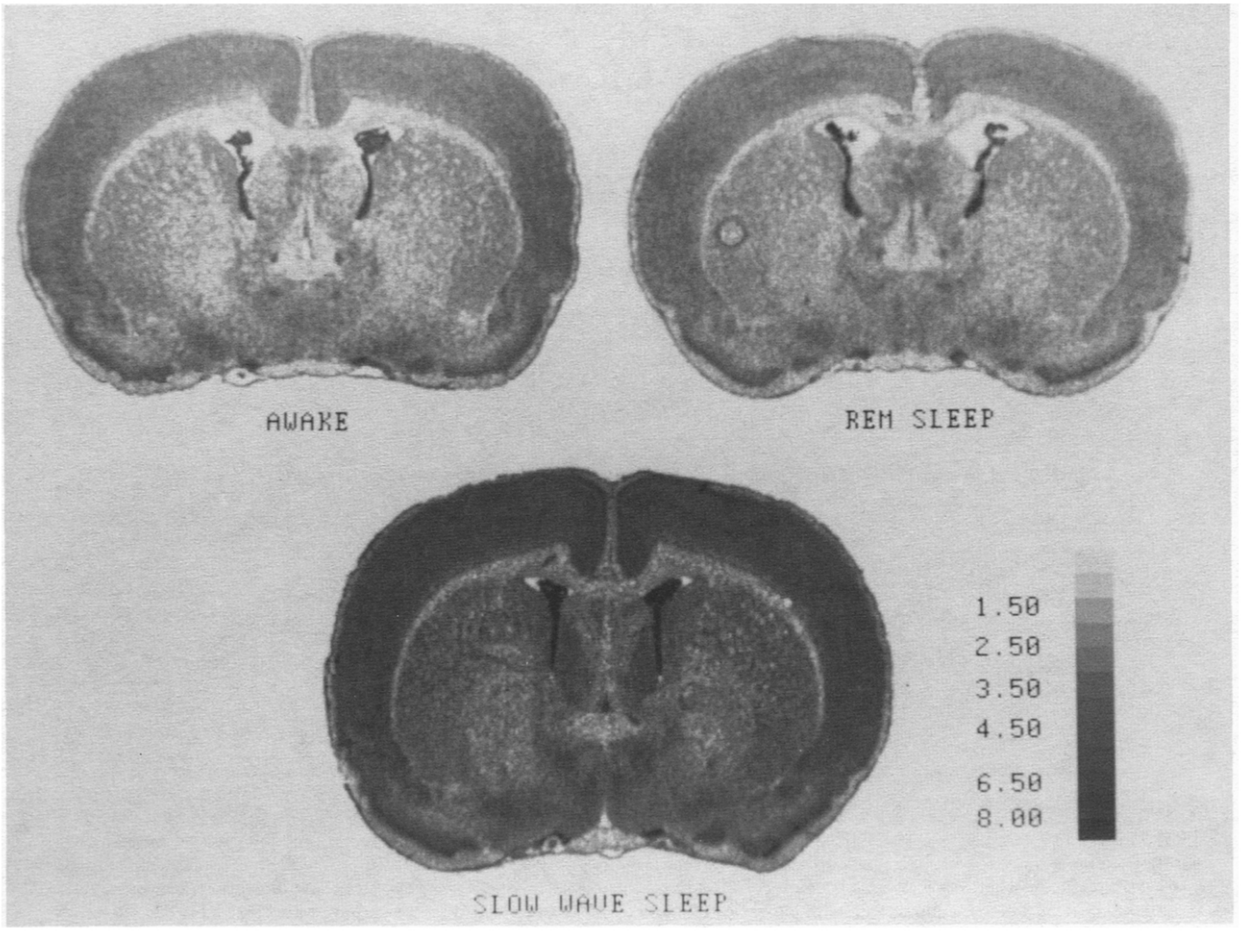

FIG. 1. Autoradiographs of coronal sections of rat brain in representative animals with high state scores for wakefulness, REM and SWS. Note that rates of cerebral protein synthesis are highest in the SWS animal, and are similar in the awake and REM animals.

METHOD

Under barbiturate anesthesia, male Long-Evans hooded rats (275–350 g) received recording electrodes in the hippocampus, over the frontal and parietal cortices, and in the neck muscles. Cannulae were inserted into the femoral vein and artery of one leg, to allow the intravenous administration of L-[1-^{14}C]leucine and arterial sampling of plasma. Four days after surgery, the freely moving animals were REM deprived by a low-stress modification of the platform method. In a warm room (24°C), rats were REM deprived in tanks containing two pedestals (each 6.5 cm diameter) for from 28–72 hours. The pedestals were somewhat wider than those used in many platform REM-deprivation procedures (5 cm is typical), to minimize stress. The rats could move from pedestal to pedestal, and would suffer only a moderate decrease in non-REM sleep (3). However, they could experience very little REM (8).

To ensure adequate nutrition, a bottle of chocolate milk was continuously accessible. To allow grooming, exercise, and access to solid food during the deprivation period, the rats were removed from the tanks and kept awake for two hours each day in their home cages. REM deprivation ceased when the animals showed an inclination to enter REM within 5 minutes of being placed into their home cages. Most rats received 48 hours of REM deprivation.

On the morning following REM deprivation, the rats were placed in a cage oriented so that the experimenters were not visible. White noise was present (70 dB SPL). The arterial and venous cannulae were connected to tubes that permitted access from outside the cage. Polygraphic recording of EEG and EMG was initiated, and the animals were REM deprived for a further 5–8 hours, using visual inspection of the recordings and manual awakening. Food was not available during this period. Thus, all animals were fasted for about the same period of time before being run and were run about the same time of day.

Physiological condition of the animals was assessed by monitoring rectal temperature, hematocrit and the polygraphic recordings. Animals showing abnormal characteristics on any of these measures, or arterial plasma leucine concentrations below 100 nmol/ml (about 180 nmol/ml is normal), were excluded from the experiment. Further, any animals that did not show at least 20% of SWS during the experimental procedure were excluded. The goal of this final exclusion was to obtain animals that were closely matched for general sleep need and activity level. All animals rested quietly and cycled in and out of sleep. However, some animals spent most of the time asleep, and others did not. Twenty-seven animals (out of 34 run) met the inclusion criteria.

Approximately five hours into the light cycle (12-hour light, 12-hour darkness), 100 μCi/kg of L-[1-^{14}C]leucine was injected as an intravenous pulse. The behavioral state of the animal during injection varied. About equal numbers of animals were injected during wakefulness, SWS and REM. The injection and blood sampling procedure did not wake those animals injected during SWS or REM.

During the next 45 minutes (the leucine incubation period),

TABLE 1

PRODUCT MOMENT CORRELATIONS (r_{xy}) BETWEEN PROTEIN SYNTHESIS IN RAT BRAIN REGIONS, AND WEIGHTED PROPORTIONS OF WAKEFULNESS (W), SLOW WAVE SLEEP (SWS) AND RAPID EYE MOVEMENT SLEEP (REM)

Region	Correlations W	SWS	REM
Cerebral mean	**−.14**	**.54**	**−.31**
Spinal cord			
dorsal horn	−.05	.66	−.50
ventral horn	−.04	.63	−.48
Brain stem			
inferior olive	−.06	.64	−.47
spinal vestibular n.	−.13	.62	−.37
n. seventh nerve	−.06	.55	−.39
raphe dorsalis	−.12	.48	−.27
raphe magnus	−.10	.56	−.36
raphe obscurus	−.10	.52	−.34
raphe pontis	−.14	.58	−.41
mesencephalic raphe n.	−.18	.58	−.32
dorsal cochlear n.	−.12	.62	−.40
superior olive	−.12	.63	−.40
spinal n. nerve V	−.07	.58	−.40
principal n. nerve V	−.08	.53	−.35
motor n. nerve V	−.09	.45	−.28
mesencephalic n. nerve V	−.17	.41	−.18
n. pontis centralis	−.07	.52	−.36
n. pontis oralis	−.06	.46	−.32
locus coeruleus	−.04	.37	−.26
Cerebellum			
granular matter	−.15	.48	−.26
lateral n.	−.13	.52	−.31
internal n.	−.08	.52	−.36
medial n.	−.22	.58	−.31
Mesencephalon			
reticular formation	−.11	.52	−.36
inferior colliculus	−.11	.52	−.33
superior colliculus	−.23	.56	−.25
central gray	−.10	.48	−.29
interpeduncular n.	−.17	.68	−.42
subst. nigra pars reticulata	−.14	.55	−.32
subst. nigra pars compacta	−.11	.53	−.34
zona incerta	−.12	.58	−.37
Thalamus			
posterior	−.08	.53	−.35
ventral posterior	−.12	.55	−.34
centromedian	−.08	.54	−.36
ventral lateral	−.15	.51	−.28
lateral geniculate	−.12	.51	−.30
medial geniculate	−.14	.57	−.33
Hypothalamus			
arcuate	−.17	.57	−.32
lateral	−.21	.50	−.21
ventral medial	−.06	.43	−.29
anterior	−.06	.44	−.30
periventricular	−.24	.46	−.15
Hippocampus			
pyramidal cell layer	−.22	.64	−.33
dentate gyrus, granule cells	−.09	.57	−.38
subiculum	−.02	.53	−.38
Cortex			
entorhinal	−.12	.54	−.33
occipital	−.21	.56	−.28
parietal	−.12	.55	−.35
frontal	−.17	.53	−.29
piriform	−.04	.51	−.37

Rates of protein synthesis were assayed in 112 brain regions, using L-[1-^{14}C]leucine autoradiography. Regions selected for presentation in the table exhibit highly significant correlations ($p<0.01$), or have been previously implicated in sleep mechanism or functions. The correlations reflect the extent of linear relation between incorporation of leucine into protein and the sleep/wake state of the animal. Positive correlations show that as a particular state increases, protein synthesis rate also increases. Significance lelvels: $r_{xy}=.40$, $p<0.05$; $r_{xy}=.50$, $p<0.01$; $r_{xy}=.60$, $p<0.001$. As many correlations are presented, the possibility of type I error suggests caution in evaluating the significance of individual regional correlation values.

EEG and EMG recordings were made and timed 125 μl arterial blood samples (0, 0.25, 0.5, 1, 2, 3, 4, 5, 10, 15, 20, 30, 45 minutes) were taken, deproteinized with 10% perchloric acid and centrifuged for the measurement of plasma levels of [^{14}C]leucine and of free leucine.

Following the leucine incubation period, the animals were given an overdose of barbiturate. The brain was rapidly extracted and cryostat sections of brain tissue were cut at 20 μ. The sections were mounted on gelatinized hot slides (60°C) and repeatedly washed in buffered formalin. Autoradiographs were then prepared using standard procedures. Deproteinized arterial plasma samples were assayed for levels of L-[1-^{14}C]leucine by liquid scintillation counting, and additional time 0 samples were assayed for free leucine by amino acid analysis (Beckman Amino Acid Analyzer, Model 121 MB).

Rates of regional cerebral protein synthesis were calculated using the time courses of the concentrations of L-[1-^{14}C]leucine and leucine in the plasma, and the local tissue concentrations of [^{14}C] in the autoradiographs as measured by computer image analysis (MCID Image Analyzer, Imaging Research Inc., St. Catharines, Ontario). Behavioral states of the animals were scored by analyzing the polygraphic records for each 30-second epoch occurring during the leucine incubation period.

There is a potential problem in analyzing tracer autoradiographic data from a sleep experiment. In sensory or pharmacological studies, animals can be exposed to a single stimulus condition which is maintained throughout the isotope incubation period. In contrast, our animals did not spend the entire incubation period in one state. A given animal might show 10 min in REM, 30 min in SWS and 5 min in wakefulness. As most of the labelled leucine is incorporated into tissue during the first minutes, groups could be created from animals showing mainly SWS (e.g., more than 10 min of SWS in the first 15 min), or mainly wakefulness. Unfortunately, this procedure would preclude the analysis of REM, which does not last for many consecutive minutes in the rat.

Analysis of REM required that states be scored on a continuum for wakefulness, SWS and REM [Abrams *et al.* (1); Ramm and Frost (9,10)]. Thus, each 30-sec epoch occurring during the leucine incubation period was weighted by the tissue level of free

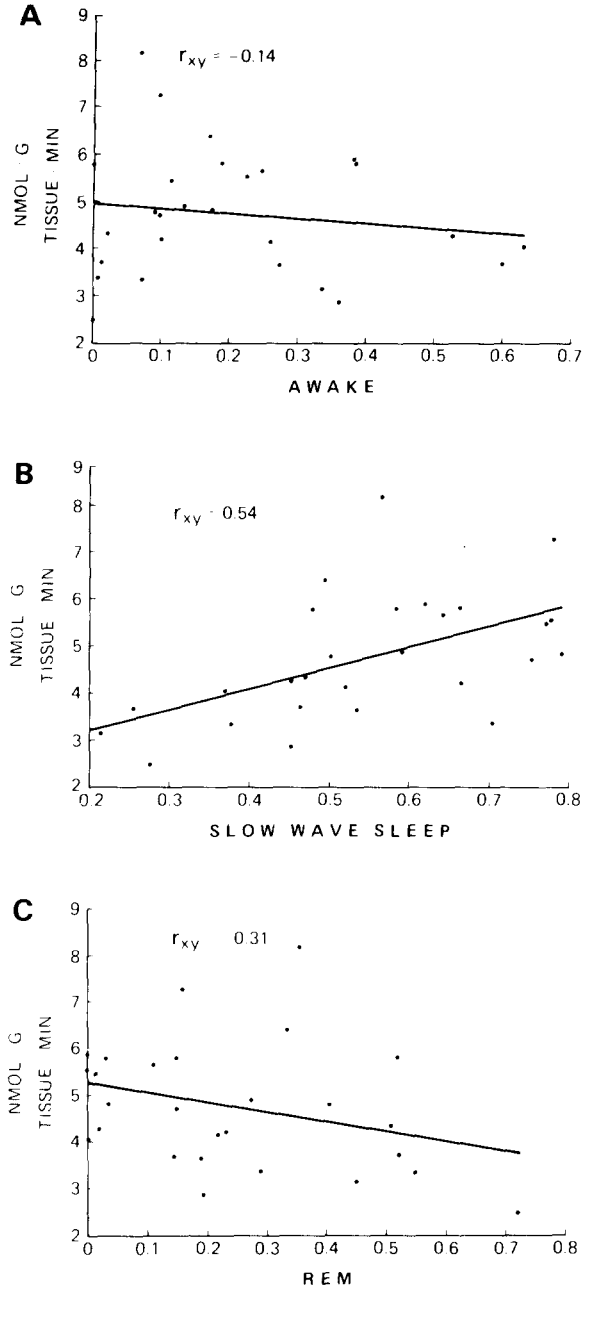

FIG. 2. Product moment correlations (r_{xy}) between cerebral protein synthesis (Y-axis) and (on the X-axis) weighted proportions of wakefulness (W), slow wave sleep (SWS) and rapid eye movement sleep (REM). The correlations reflect the extent of linear relation between rates of protein synthesis and the sleep/wake state of the animal. Positive correlations show that as a particular state increases, brain protein synthesis rate also increases. Two-tailed significance levels: $r_{xy} = .40$, $p<0.05$; $r_{xy} = .50$, $p<0.01$.

L-[1-^{14}C]leucine available during that epoch. The amount of free [^{14}C]leucine in tissue during each epoch was calculated using the following equation:

$$Ce^*(T) = k1^* e^{-(k2+k3+k4)T} \int_0^T Cp^* e^{(k2+k3+k4)t} dt.$$

Ce*(T) is the free L-[1-^{14}C]leucine in tissue. Cp* is the concentration of labelled tracer in tissue, as measured by autoradiography. The constants k1, k2, k3 and k4 represent the rate constants for carrier-mediated transport of leucine from plasma to tissue, for carrier-mediated transport back from tissue to plasma, for metabolic degradation of leucine and for incorporation of leucine into protein [details in (11,12)]. The weighted time value for each state was calculated as follows [equation after form in (1)]:

$$\text{Weighted time in a state} = \frac{\sum_{i=1} \int_{si}^{ti} Ce^*(t)\, dt}{\int_0^T Ce^*(t)\, dt},$$

where Ce*(t) is the tissue concentration of free L-[1-^{14}C]leucine in tissue at time t; i is the epoch of the state, si the beginning time and ti the end time of the ith episode of the state; n is the total number of epochs of the state; and T is total duration of the leucine incubation period.

To summarize, the state scores are not simply time in each state. Because the concentration of precursor (free L-[1-^{14}C] leucine) varies, an epoch is less important if it occurs at the end of the leucine incubation period than at the beginning. Therefore, state time is an inaccurate measure and what is needed is the amount of precursor incorporated into tissue during each state. Our procedure weights each epoch by its relative importance to provide an estimate of the proportion of labelled precursor incorporated into protein during wakefulness, SWS and REM.

RESULTS

The REM deprivation paradigm was successful in producing long REM periods during the leucine incubation period. The longest continuous REM period we observed was 8.5 minutes, as compared to the normal rat REM period of 1–2 minutes. We observed REM which was qualitatively similar to normal REM in that all the electrographic and behavioral criteria for normal REM sleep were present.

The mean rate of cerebral protein synthesis across all animals was 4.32 nmol/g tissue/min (SEM = 0.26). The mean arterial plasma leucine concentration was 170.72 (SEM = 9.52). These values agree well with those previously reported (5) for young adult rats.

We noted a significant but weak correlation between SWS and plasma leucine level ($r_{xy} = .45$, $p<0.05$). That is, animals with high proportions of SWS tended to also exhibit higher levels of plasma leucine, but considerable variability was present.

The mean rate of cerebral protein synthesis was calculated by taking a mean of synthesis rates in all brain regions measured (N = 112). Similar results were obtained by reading rates of protein synthesis in large volumes of brain tissue (scanning entire sections through all brain levels). Higher rates of cerebral protein synthesis were associated with higher SWS state scores (Figs. 1 and 2). We did not observe a link between rates of cerebral protein synthesis and either wakefulness or REM. The SWS correlation was significantly different from both the wakefulness and REM correlations ($p<0.001$, Fisher Z-transformation). The REM and wakefulness correlations were not significantly different from each other ($p>0.05$). In summary, SWS showed a significant positive correlation with cerebral protein synthesis, a correlation that was different from those associated with wakefulness and REM.

We also conducted a t-test, comparing animals with SWS state scores higher than 50% (n = 17) and those with SWS state scores lower than 50% (n = 10). The high SWS group exhibited a significantly higher mean rate of cerebral protein synthesis than the low SWS group ($p<0.05$).

The global pattern of state-dependent protein synthesis was replicated regionally (Table 1). Protein synthesis in most brain regions exhibited moderate positive correlations with SWS and weakly negative (nonsignificant or weakly significant) correlations with REM.

DISCUSSION

Our data suggest that SWS favors cerebral protein synthesis, but must be evaluated in the light of several cautions. For one thing, we used short periods of platform REM deprivation to achieve adequate levels of REM during the incubation period. The platform technique results in deprivation of REM and, to a lesser extent, SWS (3,8). In consequence, our rats exhibited signs of increased sleep need during the leucine incubation period. They fell asleep more quickly and were more difficult to arouse than would be the case in rested rats. It is possible that the increased sleep need enhanced the relation between sleep and rates of protein synthesis. Fully rested animals might have less need for the synthetic functions of sleep and might not show higher rates of protein synthesis during sleep. We are testing this hypothesis by observing rates of cerebral protein synthesis under various conditions of REM and total sleep deprivation.

Although our deprivation procedure did tire the rats, we believe it unlikely that it produced highly abnormal internal conditions. Extended REM deprivation has been shown to result in higher levels of energy expenditure and accelerated protein catabolism as reflected by increased plasma urea nitrogen (7). However, these effects are not observed until REM deprivation continues for much longer periods (weeks) than were used here. We also made every effort to minimize stress from the platform technique and to maximize the normalcy of our animals by using only those with normal body temperature, plasma leucine, hematocrit, and electrophysiological characteristics. Therefore, we can reasonably assume that sleep in our REM-deprived rats models sleep in tired, nondeprived rats.

A methodological caution is that our observed rates of protein synthesis probably underestimate actual rates of protein synthesis. The L-[1-^{14}C]leucine method assumes that unlabelled leucine derived from protein degradation does not recycle into the precursor pool for protein synthesis. However, such recycling has been shown to occur (12) and, therefore, the rates of protein synthesis obtained with this method are minimal estimates.

A final caution is that our observations are not a certain indication that net levels of cerebral proteins increase during SWS. Higher rates of protein turnover might offset higher rates of synthesis. Although this question of protein turnover during sleep remains to be investigated, net tissue protein content may not be a critical variable for sleep function. Rather, it may be that synthesis of *new* proteins during SWS is restorative, whatever the rate of protein turnover.

The observation that SWS is linked to protein synthesis recalls our previous observation that cerebral metabolism (measured with [^{14}C]-2-deoxyglucose) is linked to SWS (10). Thus, we found that SWS is linked to lower metabolic rates and higher protein synthesis rates, while we failed to observe clear links between REM and either cerebral metabolism or cerebral protein synthesis. The [^{14}C]-2-deoxyglucose and L-[1-^{14}C]leucine findings converge to suggest that a decrease in metabolic demand during SWS does not result from a decrease in biosynthesis. Decreased metabolic demand for other aspects of cellular work may actually favor biosynthetic processes during SWS. The functions of REM remain elusive.

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