plupart des cas que l'élévation de la sécrétion en hexosamines gastriques se produit après injection i.v. des zones 7 et à un degré moindre des zones 8 (Figure 1). Ces zones correspondent à un poids moléculaire de l'ordre de 4 à 5000 (Figure 2). Dans la plupart des cas la zone 9 est inactive. De même, les zones 1, 4, 5 et 6 n'ont pas d'influence sur la sécrétion basale. Nous avons pu observer une diminution de la sécrétion basale en hexosamines après administration des zones 3. La zone 2 provoque aussi une diminution partielle. Le facteur présent dans ces zones est responsable de cette diminution, présente un poids moléculaire de l'ordre de 35000. D'après la courbe d'absorption U.V. qui présente un maximum à 280 nm et la réaction positive avec le réactif du Biuret, ce facteur est vraisemblablement de nature protéique.

La reproductibilité de l'activité mucicrinique dans les zones 7 ou éventuellement 8 nous permet de supposer que nous avons concentré la mucicrine dans cette zone. Les décalages observés dans les essais No. 3 et 4, peuvent s'expliquer par les erreurs de comptage des tubes dues à un fractionnement défectueux du fractionneur utilisé et par les variations de débit dues au tassement du gel dans la colonne après une certaine période d'utilisation.

Après administration des zones 2 et 3 nous avons observé la diminution de la sécrétion basale d'héxosamines gastriques est essentiellement plus faible que la sécrétion basale. Il ne s'agit pas d'une simple inactivation de la mucicrine, parce que la mucicrine n'est pas présente dans cette zone. Il est possible d'expecter que ce phénomène soit produit par un facteur spécifique. Nous avons choisi le terme de la mucimitigine pour désigner le facteur de la muqueuse du tube digestif capable de diminuer la sécrétion basale des héxosamines gastriques et n'influencant pas la pression artérielle. La présence de la mucimitigine est observée dans 7 cas après injection dans la zone 3, les trois cas restant présentent un décalage vers les zones voisines. Le poids moléculaire de la mucimitigine est d'environ 7 à 8 fois supérieur à celui de la mucicrine. Les deux méthodes utilisées concordent à montrer la nature protéique de ce facteur.

Il est intéressant de noter qu'on peut trouver deux activités symétriques, mais avec l'influence biologique inverse, après séparation par filtration sur gel de Séphadex d'un extrait de la même muqueuse duodénale, cet extrait ne manifestant avant séparation qu'une seule de ces deux activités.

The Orthodox-Paradoxical Sleep Cycle in the Rat

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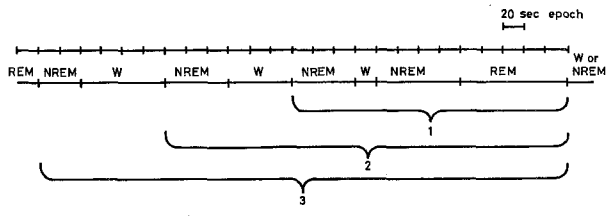
Summary. Under the postulated existence of a mechanism regulating the NREM sleep – REM sleep sequence and a reset of this mechanism by long awakenings, the variability of sleep cycle in the rat was studied. Awakenings of various durations were included in the definition of sleep cycle boundaries. Results show that an intervening awakening of 1 min is close to the limit under which the same cycle seems to be resumed after the awakening and above which the previous cycle is abortive and a new cycle will start after the next sleep onset.

One of the most salient features of mammalian sleep is its organization, that is the regular alternation of non-rapid eye movements (NREM) and rapid eye movements (REM) sleep. The former is mainly characterized by slow waves and spindles on the electroencephalogram (EEG), a low but not abolished muscle tone and the absence of rapid eye movements. The latter is recognized by an EEG of low voltage fast activity, the occurrence of bursts of rapid eye movements, an abolition of basal muscle tone and phasic muscular discharges recorded in the neck muscles. The alternation of these two kinds of sleep allows one to isolate sleep cycles, not without ambiguity however. For some authors, a cycle is comprized between the beginning of a REM phase and the beginning of the next one, whereas for others it extends between the end of two consecutive REM phases. The presence of waking constitutes another difficulty. It may be asked up to what extent an intervening awakening influences the NREM sleep-REM sleep sequence. A response to this question might allow one to determine if waking episodes must be included in the definition of sleep cycles boundaries, and up to what duration.



Definition of sleep cycle length. REM: REM sleep; NREM: NREM sleep; W: waking. Notice that, in this example, the 1st waking phase (left) is 4 epochs long, the 2nd (middle) 3 epochs long and the last one (right) 1 epoch long. Under definition 1, sleep cycles may contain waking phases and no more than 1 epoch; under definition 2, they may contain waking phases of no more than 3 consecutive epochs, and under definition 3, they contain all sleep epochs between the end of 2 consecutive REM phases. Waking phases are not included in sleep cycles duration. Thus, this sleep cylcle lasts 12 epochs (4 min), 15 epochs (5 min) and 17 epochs (5 min 40 sec) under definition 1, 2 and 3 respectively.

It seems reasonable to hypothetize the existence of some kind of biological and probably sleep-dependent clock regulating sleep cycles, and more specifically determining the amount of NREM sleep necessary before the appearance of REM sleep. A very short awakening is not likely to reset this mechanism; on the contrary, it is hard to believe that a sleep cycle can resume after a very long awakening. It follows that between these two extremes, there must be some critical value of awakening duration below which the sleep cycle clock is not reset and above which the counting is reset to 0. It has been shown in man that, when a cycle contains waking, its NREM part is lengthened (BREZINOVA[1]).

[1] V. BREZINOVA, Electroenceph. clin. Neurophysiol. *36*, 275 (1974).

Statistics of sleep cycle duration in 5 rats according to various definitions of the cycle (see text, under Methods)

Definition of sleep cycle	$\bar{X} \pm S_x$ (min)	CV%	Range of individual means	Range of individual CV%
Day 0	1.39 ± 0.23	17	1.10– 1.71	57– 75
1	4.27 ± 0.71	17	3.49– 5.40	64– 83
2	6.76 ± 0.58	9	5.78– 7.22	61– 81
3	8.57 ± 1.25	15	7.2 –10.6	71– 87
Night 0	1.30 ± 0.33	25	0.83– 1.72	40– 69
1	4.12 ± 1.00	24	3.27– 5.49	63– 83
2	6.91 ± 1.81	26	4.97– 9.82	57– 67
3	12.84 ± 5.97	46	8.97–23.23	62–101

0. REM phase only; 1. NREM-REM cycle containing no waking phases longer than 1 epoch (20 sec); 2. NREM-REM cylce containing no waking phases longer than 3 epochs (1 min); 3. NREM-REM cycle counted from the end of the preceding REM phase. Values indicated in the 1st column are interindividual means and standard deviation, calculated using the 5 individual means. $N = 231$ and 194 for day and night respectively. $CV\% = S_x/\bar{X} \cdot 100$.

In order to approach this problem, it seemed appropriate to study the variability of sleep cycle in the rat, by including or not in its definition awakening episodes of various durations.

Methods. 5 male albino wistar rats, weighting about 300 g, were used. They were chronically implanted with 4 cortical electrodes (2 bipolar leads), 2 periocular electrodes and 2 electrodes in neck muscles. For surgery they were pretreated with atropine and anaesthetized by pentobarbital (55 mg/kg). After surgery, they were placed in their recording cages and allowed 5 days for recovery. They were then connected to the EEG machine by means of wires and rotating connector, and left 7 further days for habituation under a 12/12 light-dark schedule, with indirect and attenuated light turned on at 07.00 h. The animals had free access to food and water. They were then continuously recorded for 24 h at slow paper speed (2 mm/sec). The carbon writing system of the EEG machine (Schwartzer) allows an easy recognition of sleep stages inspite of this slow speed.

Recordings were visually scored by 20 sec epochs, with the usual criteria for recognition of waking, NREM sleep, intermediate sleep (counted thereafter with NREM sleep) and REM sleep.

For reasons given in the discussion, we chose to define the end of a sleep cycle by the end of a REM phase. Thus, a sleep cycle begins with NREM sleep and ends with REM sleep. Waking episodes are not counted in the sleep cycle duration but are used to define the onset of the cycle (Figure). The first epoch of the cycle is the epoch of NREM sleep following: 1. 2 or more consecutive epochs of waking; 2. 4 or more consecutive epochs of waking; 3. the last REM phase. It follows that in definition 1, the cycle may contain 1 or several waking phases of a maximum duration of 1 epoch (20 sec); in definition 2, it may contain 1 or several waking phases of a maximum duration of 3 epochs; in definition 3, it may contain waking phases of any number of epochs and therefore contains all NREM sleep epochs between the end of 2 REM phases.

Results and discussion. The range of individual $S_x/\bar{X} \cdot 100$ (CV%) of cycle length, calculated according to the different definitions of sleep cycle, is given in the Table. In almost all animals, CV% are lower when cycles are calculated under definition 2 than under definition 1 and 3. Furthermore, the interindividual CV% (1st column) during the day is lower for definition 2, whereas during the night it is not different from the one calculated under definition 1. Thus, it is clear that counting all sleep epochs between the end of 2 REM phases irrespective of the length of waking episodes with which they are interspersed increases the variability of cycle length. The fact that the calculation of cycle length, according to definition 2, yields the smallest variability seems to indicate that a waking episode slightly longer than 1 min is close to the limit under which the same cycle seems to resume after the awakening and above which the previous cycle is abortive and a new cycle will start after the next sleep onset. Thus, definition 2 can be accepted as a reasonable limit for setting sleep cycle boundaries.

The values of the rat sleep cycle length given in literature differ from one another. They are most commonly given for the interval between 2 REM sleep phases. It is, however, not always stated if waking is included in the count or not: 11 min (Weiss and Roldan[2]), 11.9 min (Pellet and Béraud[3]), 5–10 min (Michel et al.[4]), 8.5 min for periods uninterrupted by waking (Twyver[5]), 16 min (Timo-Iaria et al.[6]). The values we have found under definition 3, 8.6 and 12.8 min, for day and night respectively, are consistent with these values, perhaps somewhat low for the day values.

For a number of reasons, it is more apropriate to begin the count of a cycle with NREM sleep and to end it with REM sleep, in rat as well as in man. This is especially obvious in rat, since most of the REM sleep episodes are followed by waking (80.9% according to Timo-Iaria[6]), whereas waking is very rare between NREM sleep and REM sleep. Thus, the switch of REM sleep to NREM sleep seems to be fragile as regards sleep continuity. Moreover, if the count of a cycle is started at the first epoch of REM sleep, the initial episode of NREM sleep must be discarded, which seems illogical, especially in man. Finally, there are biochemical arguments indicating that the sequence NREM sleep-REM sleep is more likely to have a physiological base than the reverse (Jouvet[7]; Jouvet and Pujol[8]).

An accurate cycle length measurement is important from the point of view of interspecific comparisons. Dallaire et al.[9], who mentioned the frequent ambiguity in the definition of sleep cycles, concluded that differences observed in mammalians sleep cycle durations are better accounted for by encephalization index than by body weight or metabolic rate.

[2] T. Weiss and E. Roldán, Experientia *20*, 281 (1964).
[3] J. Pellet and G. Béraud, Physiol. Behav. *2*, 131 (1967).
[4] F. Michel, M. Klein, M. Jouvet and J.-L. Valatx, C. r. Soc. Biol., Paris *155*, 2384 (1968).
[5] H. van Twyver, Physiol. Behav. *4*, 901 (1969).
[6] C. Timo-Iaria, N. Negro, W. R. Schmidek, K. Hoshino, C. E. Lobato de Menezes and T. Leme da Rocha, Physiol. Behav. *5*, 1057 (1970).
[7] M. Jouvet, Science *163*, 32 (1969).
[8] M. Jouvet and J.-F Pujol, Adv. biochem. Psychopharmac. *11*, 199 (1974).
[9] A. Dallaire, P.-L. Toutain and Y. Ruckebusch, Pyhsiol. Behav. *13*, 395 (1974).