STUDY OF WAKING-SLEEPING BEHAVIOUR USING AUTOMATIC ANALYSIS AND QUANTIFICATION

Cl. Gottesmann, G. Lacoste, L. Rodrigues, P. Kirkham, J.L. Railo and Ch. Arnaud

Laboratoire de Psychophysiologie. Faculté des Sciences et des Techniques
06034 NICE Cedex

The activation of nerve and muscle cells is characterised by a low voltage activity. The study of these fluctuations of potential difference is the purpose of the electrophysiological approach widely used in clinical medecine on one hand, psychophysiological and neurophysiological research on the other.

This electrical activity has essentially two forms :

- the first is rapid, of digital type, since it is either on or off; this is what one observes in the axons, the nervous conducting paths, and single cell recordings : it is a matter of calculating a response frequency.

- the second consists of variations of potential difference with variable polarity, certain of them can last more than a second. They generally come from complex structures usually studied through several different layers of tissue. The typical example is that of the electroencephalogram recorded on the scalp or the electrocorticogram recorded directly on the cortex, the frequency of which varies from about 0.5-40 c/s. The study of this kind of activity is essential in Man for clinical medecine, in animals for fundamental and applied research.

This approach meets two major difficulties :

- the mass of information from which the critical characteristics are to be drawn.
- visual data analysis, which is slow and open to inconsistency.

This is why many research workers have set out to find automatic analysis methods, which are both rapid and accurate.

For our part, we have undertaken the automatic analysis of the different phases of waking and sleeping in the rat, fundamental behaviour on which all else is based. This approach has been complemented by automatic analysis of brain excitability and psychomotor activity. This research firstly carried out off-line, subsequently on-line, will very soon result in a system allowing the automatic analysis of the same data on animals completly free to move.

Seven phases of the waking-sleeping cycle have been selected, their physiological significance has been specified in our previous works (1, 2) : 1/ active or attentive waking, with theta activity; 2/ normal waking; 3/ slow wave sleep; 4/ deeper sleep characterised by neocortical spindles; 5/ intermediate stage which precedes and follows paradoxical sleep and is characterised by neocortical spindles and theta activity; 6/ paradoxical sleep, where dreams occur in Man; 7/ the periods of rapid eye movements during paradoxical sleep (figure 1).

In order to avoid any loss of information, we have set the unit duration of analysis at one second. It is well known that the nervous system is in permanent unstable equilibrium, even more so in a small animal with higher metabolic rate, having a very polyphasic waking-sleeping rhythm with brief activities such as spindles and the bursts of eye-movements.

Off-line approach

A/ State determination (for references, see 3, 4, 5, 6, 7).

A study of the two cerebral derivations used, has shown that signal analysis by autocorrelation or cross correlation would require computing power far beyond the means available to us and for the processing it would be difficult to reconcile signal length with the one second' "quantum" we had decided on.

Spectral analysis of the two brain derived channels has indicated that in a particular frequency band, the amount of energy in channel 1, makes it possible to distinguish between the principal sleeping and waking phases, with some overlapping. For channel 2, use is made of the ratio of energies in two frequency bands one of which is centred on the theta type activity. For the electrooculogram (channel 3) which is only taken into consideration during paradoxical sleep, the deviations from the base line are detected, then integrated. Finally, for the electromyogram (channel 4), the energy emitted in a particular frequency band is integrated; this too differs during the different behavioural phases, with overlaps. Thus each of the 3 essential derivations (1, 2, 4) is tested a certain number of times per unit of time; the energy collected is integrated over the one second base. This results in an energy value, for each derivation , which is specific to each phase of waking-sleeping behaviour with overlaps at the edges and which constitutes a partial criterion.

Each behavioural phase is also defined by a global criterion resulting from the combination of the three partial criteria. However, in certain cases one of the partial criteria is below the experimentally fixed norm, for the corresponding state. This results in a penalisation of the global criterion, which is a function of the unsatisfactory criterion's deviation from the norm. Above a certain degree of penalisation, the state is retained but qualified as "doubtful". The doubtful states are counted with their probable state and also, separately. They are thus easely deductible.

This analysis is undertaken on an IBM 1800 with 16 K words of 16 bits which processes the data at thirty-two times the speed of acquisition, which is done in analogue form on magnetic tape. The signals undergo a pre-processing by means of pass-band filters centred

on zones determined by spectral analysis. Each channel is sampled at 25-50 Hz depending on the derivation. The data are then digitised.

Different rejection logics were used : for example, to eliminate heart pulses, high energy signals which are surimposed on the electromyogram; also transient states like the short periods of clonus activities during paradoxical sleep that could be taken for attentive waking.

Output :

Cards are punched with the successive states and their duration, in binary code. The doubtful states are given in decreasing order of their probability of appearance. The processing condenses 24 hours of recording into a maximum of 300 cards.

These data are then grouped by quarter of an hour, hour and total length of the recording. The values produced in a listing (figure 2A)show the percentage of time passed in each of the seven states, in waking as a whole, in sleeping as a whole, and the global percentage of doubtful states grouping together the individual percentages for each of the seven states.

Finally, an output in the form of histograms (figure 2B)indicates the evolution by quarter of an hour, of the percentage of time passed in each state and the average percentage for the whole recording.

Reliability :

The contingency studied by the C test (12) indicates the following values :

Corrector A - Corrector A : C = .89
Corrector B - Corrector B : C = .83
Corrector A - Corrector B : C = .87
Corrector A - Computer : C = .81
Corrector B - Computer ; C = .81

The perfect contingency in our experimental conditions (global contingency between 6 states) is .91. The mean percentage of correspondence, state by state (13), between the two correctors and the computer is 82%. For the different stages it is :

attentive or active waking	: 86%
normal waking	: 79,5%
slow wave sleep	: 83%
spindles	: 78,5%
intermediate state	: 81%
paradoxical sleep	: 85,5%

These results are satisfactory. In fact, since the publication of our technique KOHN et al. (14) have described a method with which they dissociate only three phases, with a 12 second period of integration and they obtain a 91% correspondence (15).

B/ Determination of brain excitability

1. Study of evoked potentials (for references, see 6, 7, 8, 9).

A central or peripheral stimulation induces in the nervous pathways and centres with which it comes into contact, an activity said to be "evoked", which gives indications about the level of excitability of the central zone studied.

The "evoked potential" is recorded in analogue form on a magnetic tape. During off-line treatment, at 16 times the acquisition speed, a synchronising signal preceding the evoked potential opens an analysis window of variable duration with a high definition (2 samples per millisecond), during which the response is registered by the computer, then stored with the responses collected in the same behavioural phase. The responses which occur during "doubtful" states are eliminated.

The mean amplitude and dispersion are automatically calculated for each sample point making up the evoked potential. Only a predetermined number of responses is retained, by hour and by state.

The output of results takes place every twelve hours in graphical analogue form. The standard deviation is also marked on the same figure ($\frac{\sigma}{2}$) -figure 3A-.

2. Analysis of induced states (for references, see 6,7,9).

Equally, a central or peripheral stimulation can also induce a change in behaviour and in the spontaneous global electrophysiological activities . A stimulus can momentarily wake the animal etc ... This possible influence is tested by the automatic comparison of the animal's state in the second which precedes and the second which follows the stimulation .

In order to avoid any interference with the state determination, the stimulus intended to induce an evoked potential always occurs at the limit of a period of state analysis .

The results of this analysis are produced as a listing showing the percentage of induced states in each phase of the cycle and the number of samples counted (figure 3B).

C/ Psychomotor activity (for references, see 7, 8, 9) .

It is important to know the rate and the distribution of psychomotor activity during the circadian period in order to characterise the behaviour of a "normal" animal and that of an experimental animal subjected to various disturbances : selective lesions of the nervous system or pharmacological influences . In order to detect movements of the preparation the electromyogram channel undergoes a second processing which allows the fluctuations in energy in successive periods of analysis to be tested . Above an experimentally determined threshold, this fluctuation is counted as a movement, which is then classified with the state during which it occurred .

This approach has, in addition, the advantage of distinguishing the drugs which create a dissociation between the behaviour and the electrocorticogram, such as morphine and atropine which tend to induce a cortical activity like slow wave sleep although the animal has a waking behaviour and moves about in its cage . This diagnostic is established where an animal present a significant increase in its movements during slow wave sleep, a phase which is usually associated with resting behaviour .

The results are given as a listing in the same way as for the states . The motor activity arising in the different states is indicated in the form of a percentage of the duration of the state .

On line approach

The off line approach, rapid and rich in information, is interesting but it nervertheless presents the following difficulties :

- it is not always possible to detect experimental problems and artefacts during the data acquisition ; the difficulties only appear after analysis.
- the determination of central excitability requires the number of stimulations to be increased, with the computer undertaking a sorting process afterwards .
- on a practical level, the analysis done outside the laboratory is very expensive, which has caused this technique to be abandoned .

The purchase of a small computer (TEXAS 980A) has allowed us to undertake the study of a program for analysis and quantification in real time . Eliminating the difficulties outlined above, it makes it possible in addition to envisage a real-time investigation of metabolic changes . At the moment, we are studying the coupling of a push-pull cannula technique, for the perfusion of a central structure by means of 2 concentric or parallel tubes, with the time basis of the computer . In this way, the output could be studied taking into account the precise state during which it was obtained, and it be easier to look for possible assimilation or elimination by the central nervous system, in terms of the different behavioural phases .

It is difficult to indicate the precise method used as this investigation is the subject of a contract . The real-time program has a better performance than the off-line one and allows the feed-back necessary for the determination of central excitability with a minimal number of stimulations .

The output data are the same as for the off-line program . Figure 4 gives an example of recording analysed in real time without the inter-

vention of an organisational logic for the stages which would enable the rare aberrant states to be eliminated.

Real time processing of telemetry data

Ideally one wishes to work on animal which are free to move. Up to now, we have only been able to use rats equipped with recording cables, which, though light, cause the animal discomfort and favour the dislodging of the acrylic cement cap fixed to the skull.

Since a short while ago the laboratory has had at its disposal the prototype of a polygraphic microtelemetric system : 4 channels, 4 gr., 3 weeks autonomy (11). At the moment, we are in a position to demonstrate the processing in real-time of biological signals received by this method (figure 5). Although adjustments may be necessary, the results are very encouraging. A miniature telemetry technique for electrical and pharmacological stimulations will soon be added, offering a bright future for research in psychophysiology and ethology.

Conclusion :

We would like to perfect rapid and viable techniques of analysis for electrophysiological and metabolic activities in an animal living as normally as possible, a necessary condition for the advancement of knowledge of the physiological basis of behaviour.

* We wish to thank M. Rodi for his valuable assistance.

* This work was supported by D.R.M.E. (74/138), D.G.R.S.T. (75 7 0092) and C.N.R.S. grants.

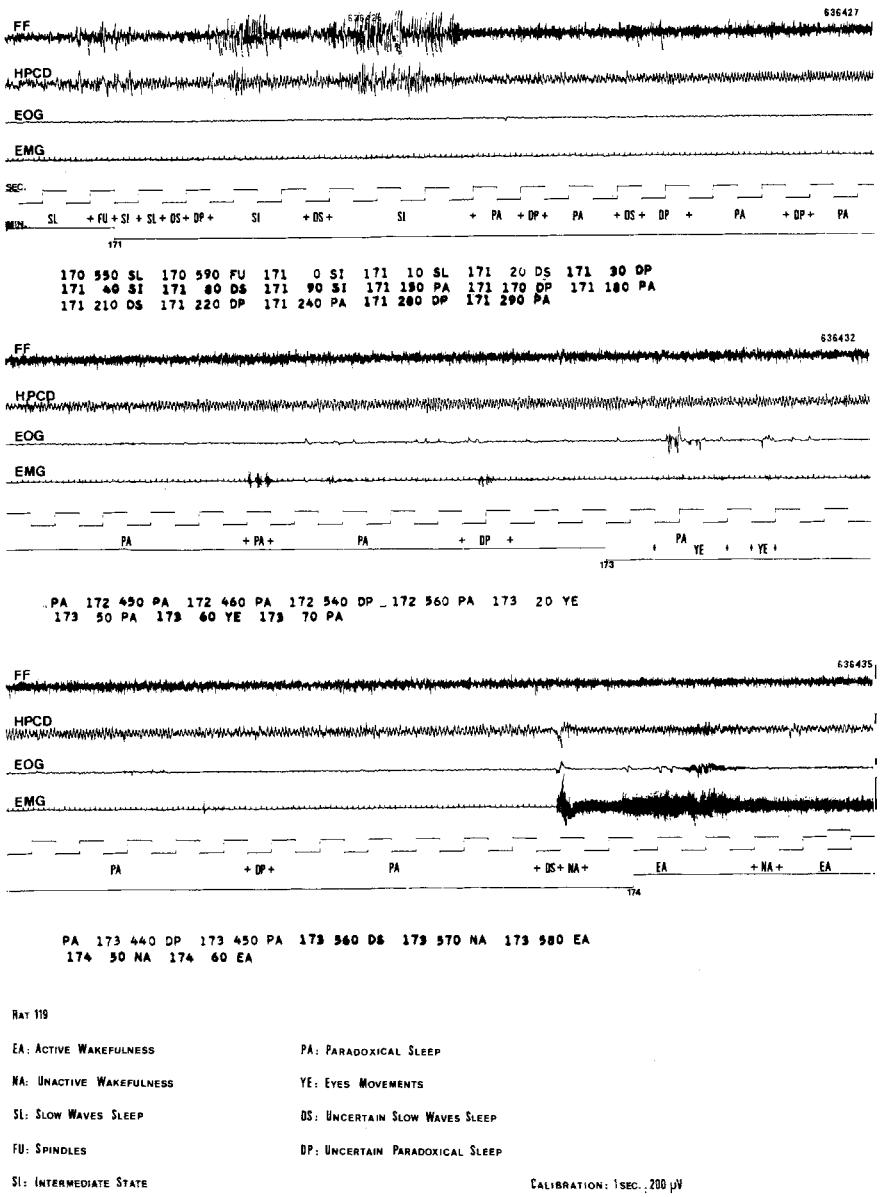


Figure 1 : DIFFERENT PHASES OF THE WAKING-SLEEPING CYCLE DETECTED BY THE OFF-LINE PROGRAM . Under each of the three parts of this recording is the corresponding listing . The first three numbers indicate the minute of recording (min.) the following three give the seconds (55.0).
On the central trace, note the intervention of the prohibitory logic (min.172, sec. 45) which indicates the appearance of paradoxical sleep despite the electromyogram which could have resulted in active waking .

Abreviations : FF : frontal cortex - HPCD : dorsal hippocampus - EOG : electrooculogram - EMG : electromyogram .

From GOTTESMANN et al., 1973 .

A

*** ETATS *** RAT 214 S JOURNEE DU 21 2 72

*** POURCENTAGES PAR TRANCHES DE 0 H 15 MN 0 S ***

TRANCHE	EVEIL ACTIF		EVEIL NON ACTIF		SOMMEIL LENT		STADE DE FUSEAUX		STADE INTERMEDIAIRE		SOMMEIL PARADOXAL			EVEIL	SOMMEIL DOUTEUX	
0	17.4	0.7	1.3	0.0	44.1	5.8	9.6	0.0	19.8	0.7	7.2	0.0	0.4	18.7	80.7	7.6
1	55.5	0.0	37.6	0.0	3.8	0.2	1.3	0.0	1.5	0.0	0.0	0.0	0.0	93.1	6.6	0.2
2	68.7	0.0	31.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	99.9	0.0	0.0
3	49.1	0.0	19.2	0.0	21.5	2.3	3.3	0.1	6.1	0.3	0.5	0.0	0.1	68.3	31.4	2.8
*****	47.6	0.1	22.3	0.0	17.3	2.0	3.5	0.0	6.6	0.2	1.9	0.0	0.1	70.0	29.6	2.6

B

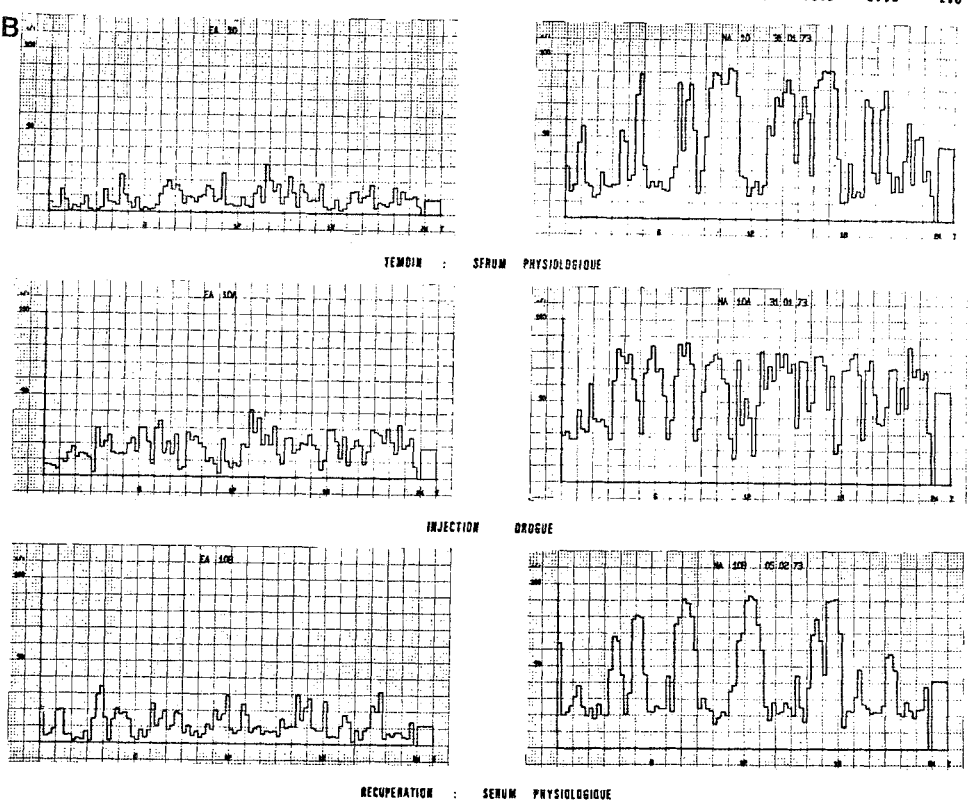


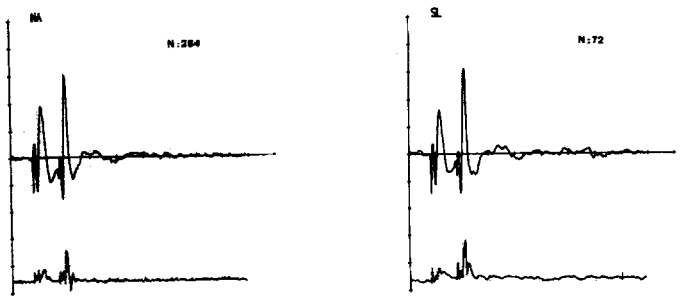
Figure 2 : A/ STATES OUTPUT LISTING . Each column, indicates for every quarter of an hour and every hour, the percentage of the total time passed in each state with the percentage of "doubtfuls" at the side . The last three columns collect together the values for waking as a whole, sleeping as a whole, and all the "doubtfuls".

B/ STATES OUTPUT ON A HISTOGRAM . An example taken from the study of a psychotropic drug . Evolution of active waking (left hand column) and normal waking (right hand column) ; during the control period (top histograms), the administering of the drug (middle), the recuperation period (bottom) . Note, on the right, the average for each state for the duration of recording.

For abreviations, see figure 1

From TASSET and GOTTESMANN (*unpublished*)

A



B

RAT 214 S JOURNEE DU 21 2 72

NOMBRE D'OBSERVATIONS			ETATS INDUITS EN °/°						
			EA	NA	SL	FU	SI	PA	YE
EA	592	287	64	29	5	0	1	2	0
NA	430	221	40	54	5	0	0	1	0
SL	450	226	4	3	60	12	20	0	0
FU	203	72	2	1	29	35	32	0	0
SI	337	167	1	0	29	20	50	0	0
PA	71	49	7	3	20	0	1	69	0
YE	0	0	0	0	0	0	0	0	0

Figure 3 : AUTOMATIC ANALYSIS OF CEREBRAL REACTIVITY IN THE RAT .

A/ STUDY OF EVOKED POTENTIALS .

The upper curve shows the mean amplitude of two successive potentials . The lower one represents the corresponding dispersion for the different components of the response .

B/ ANALYSIS OF INDUCED STATES .

In the left column, the numbers indicate the number of stimulations taken into account for each state for this analysis . On the right, written in percentages, are the states observed in the second following stimulation . Thus, 430 stimulations in normal waking were followed by normal waking in 54% of the cases, by active waking in 40 % of the cases, etc...

Note that this animal did not have an electrooculogram.

The second column of numbers (287, 221, etc...) shows the number of samples counted for the evoked potential analysis, the reduction being the result of logical prohibition .

For abreviations, see figure 1

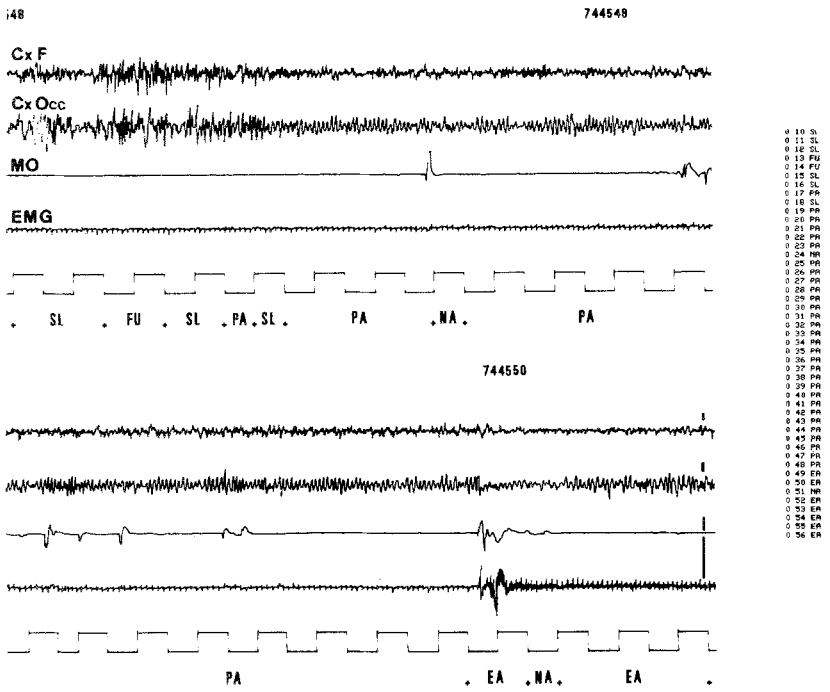


Figure 4 : DIFFERENT PHASES OF WAKING AND SLEEPING ANALYSED BY THE REAL-TIME PROGRAM .

Continuous recording with corresponding listing on the right .
The analysis has been undertaken without the organisational logic which allows the elimination of the rare aberrant states . Eye movements (MO) of paradoxical sleep are not analysed . Channel 1 is filtered .

For abreviations, see figure 1

Scale : 1 sec. 100 μV

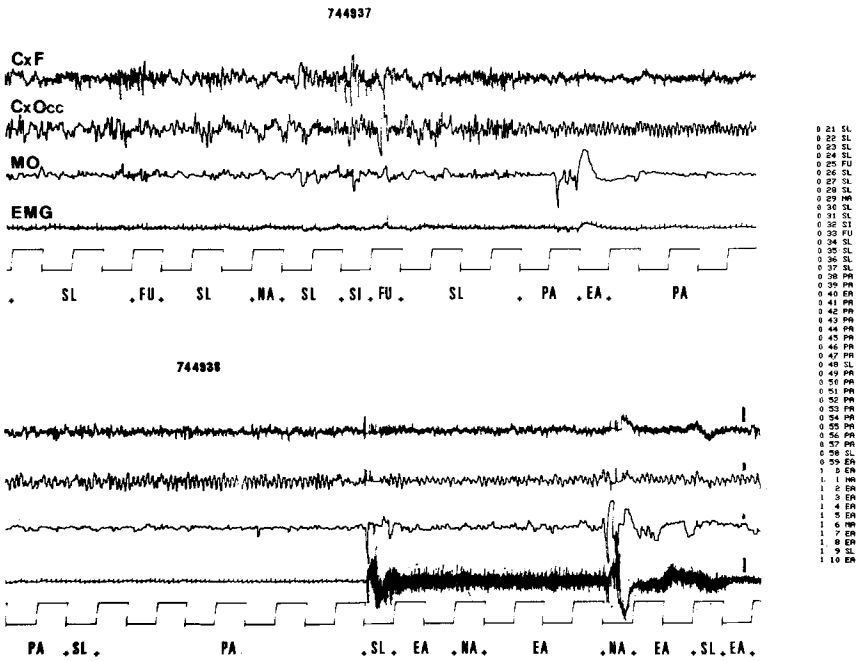


Figure 5 : REAL-TIME ANALYSIS OF A RAT RECORDED BY TELEMETRY .

Continuous recording with corresponding listing on the right .
As in figure 4, the processing was completed without the use of logical organisation of the states . Note the two short field losses in the awake rat . Eye movements (MO) of paradoxical sleep are not analysed .

For abreviations, see figure 1.

Scale : 1 sec. 200 µV

1. GOTTESMANN Cl. : Recherche sur la Psychophysiologie du sommeil chez le Rat. Presses du Palais-Royal, Paris, 1967, 156 pages.

2. GOTTESMANN Cl. : Année Psychologique, 1971, 71, 451-488.

3. GOTTESMANN Cl., JUAN DE MENDOZA J.L., LACOSTE G., LALLEMENT B., RODRIGUES L. et TASSET M. : C.R. Acad. Sciences Paris, 1971, t. 272, 301-302.

4. GOTTESMANN Cl., JUAN DE MENDOZA J.L., LACOSTE G., LALLEMENT B. RODI M., RODRIGUES L. et TASSET M. : C.R. Soc. Biol. Paris, 1971, 165, n° 2, 373.

5. GOTTESMANN Cl., JUAN DE MENDOZA J.L., LACOSTE G., LALLEMENT B., RODI M., RODRIGUES L. et TASSET M. : Psychophysiology, 1972, Vol. 9, n° 1, 142.

6. GOTTESMANN Cl., TASSET M., JUAN DE MENDOZA J.L., LACOSTE G., RODI M., RODRIGUES L. et ROUX R. : Symposium de Wurzburg "La Nature du Sommeil". Gustav Fischer Verlag, 19/3, 98-101.

7. GOTTESMANN Cl., LACOSTE G. JUAN DE MENDOZA J.L., TASSET M., RODRIGUES L.: Informatique Médicale, Colloques IRIA, 1973, t. 1, 295-309.

8. GOTTESMANN Cl., TASSET M., RALLO J.L., JUAN DE MENDOZA J.L., GAUTHIER P. LACOSTE G., RODI M., RODRIGUES L. et ROUX R. : C.R. Acad. Sciences Paris, 1972, 274, 3601-3602.

9. GOTTESMANN Cl., TASSET M., RALLO J.L., JUAN DE MENDOZA J.L., GAUTHIER P., LACOSTE G., RODI M., RODRIGUES L. et ROUX R. : Psychophysiology, 1973.

10. GOTTESMANN Cl., LACOSTE G. et RODRIGUES L. : A.P.S.S. Edinburgh, 19/5, Psychophysiology (in press).

11. GOTTESMANN Cl., RODI M. et RALLO J.L. : BIOCAPT Paris 1975 (in preparation)

12. SIEGEL S. : Non parametric statistics for behavioural sciences. The Graw Hill Co, New-York, 1956.

13. JOHNSON C. : Use of computer in sleep research. A.P.S.S. Bruges, 1971.

14. KOHN M., LITCHFIELD D., BRANCHEY M. and BREBBIA D.R.: Electroenceph. clin. Neurophysiol., 1974, 37, 518-520.

15. BRANCHEY M., BREBBIA D.R., KOHN M. and LITCHFIELD D. : Electroenceph. clin. Neurophysiol., 1974, 37, 501-506.