

ELECTROCORTICOGRAPHIC ACTIVITY OF THE PREPYRIFORM CORTEX DURING THE SLEEP-WAKEFULNESS CYCLE OF THE FREELY-BEHAVING RAT

J. A. RICARDO¹, N. NEGRAO AND J. S. C. PEREIRA

*Department of Physiology and Pharmacology, Institute of Biomedical Sciences,
University of Sao Paulo;
Functional Neurosurgery Research Center, University of Sao Paulo
Medical School;
Department of Statistics, Institute of Mathematics and Statistics,
University of Sao Paulo, Brazil*

INTRODUCTION

There is already a voluminous body of both descriptive and analytical published work on the sleep-wakefulness cycle (SWC) and the electrocorticographic (ECoG) activity of the neocortex (NEO) (see 1, 25, 26). This is also true, to a lesser extent, of the ECoG activity of the hippocampus (part of the archicortex) (see 25, 33). It seems fair to say that, comparatively, the study of the prepyriform cortex (PPC) (part of the paleocortex) in this context is not as advanced. In fact, there are several papers on the *in vitro* electrophysiological activity of the PPC (*e.g.*, 11), and also some investigations of the intrinsic PPC neuronal circuitry (3, 9). However, only the Freeman group (2, 7, 8) has reported data on the ECoG patterns exhibited by the PPC during different phases of the SWC in freely-behaving animals (cats), and the general description given by these authors could certainly be refined nowadays by attempting to make a more detailed parceling of the SWC and by paying

¹Address for reprints: Departamento de Fisiologia e Farmacologia Instituto de Ciências Biomédicas da USP, Cidade Universitária, 05508 Sao Paulo, SP, Brazil.

more attention to transition points that demarcate the phase alternations along the SWC.

The analysis of the relationships between the ECoG activities of the great subdivisions of the mammalian cerebral cortex gained a considerable impetus after the classic Green and Arduini (14) comparative study of the neo- and archicortical activities in rabbits, cats and monkeys. As far as the PPC is concerned, mention can be made here of a group of investigations (19, 21, 27) in which the neo-, archi-, and paleocortical ECoG activities were simultaneously recorded. It should be emphasized, however, that all of the studies of this last group were done on acute, curare-immobilized preparations, and that very often the activity of unspecified regions of the amygdaloid complex was taken as reflecting the paleocortical one.

The purpose of the present investigation was to provide a detailed descriptive characterization of the PPC ECoG during the spontaneous SWC of freely-behaving rats. Considering that the PPC, which receives massive projections from the olfactory bulb (OB) (30), seems to be involved in the organization of sexual, emotional and alimentary behavioral patterns (13, 23), we hope that such an investigation may prove to be useful in future inquiries into some electrophysiological-behavioral correlations.

METHODS

1. *Experimental procedures.* The experiments were performed on 9 male adult Wistar albino rats weighing 220-320 g. Stereotaxic surgery for electrode implantation was carried out when the animals were under sodium pentobarbital anesthesia (45 mg/kg, i.p.). Pairs of parallel, side by side, electrodes for short bipolar recording of the ECoG of some brain structures were made of nichrome wire thoroughly varnish-insulated, except for a 0.5 mm tip. The two tips of each electrode pair were fixed 1 mm apart. In all 9 rats pairs of electrodes were implanted in Krieg's (22) anterior neocortical area 2 (1 mm caudal to the bregmatic suture, 5 mm lateral to the longitudinal suture, and 1.5 mm below the cortical surface) (32), in the PPC (A, 8.2-6.6; L, 5.5-6; H, 0.5 mm above the bony floor) (15), and in the entorhinal area (A, 3.4-0.6; L, 7-5.5; H, 0.5 mm above the bony floor) (15). In 5 rats, additional electrode pairs were implanted in the OB (2.5 mm rostral to the frontal pole, 0.6 mm lateral to the midline, and 2 mm below the dorsal surface of the OB) and in 1 animal a pair of electrodes was also implanted in the ventral hippocampus (A, 2.6; L, 5.2; H, -2.5) (15). Two stainless steel electrodes were implanted in the dorsal neck muscles of all 9 animals for electromyogram (EMG) recording. All the electrodes were connected to the pins of a miniature socket, which was fixed to the skull by acrylic dental cement. Four to ten days were allowed for recovery after surgery. An 8-channel Kaiser electroencephalograph was used for

recording. Recording sessions took place between 9:00 a.m. and 4:00 p.m. During recording the unanesthetized animals were left undisturbed in a small Farady cage. After completion of the experiments the correctness of electrode placements was microscopically checked in Nissl-stained frontal sections of the brain.

2. *Data processing.* The entire ECoG and EMG records were examined by visual inspection. Since this is a critical point in the present work, it is essential to describe at length the partition and the nomenclature of the SWC phases and of the PPC ECoG patterns that were adopted.

Initially, considering only the NEO, EMG and (in the 5 rats in which this was possible) OB activities, the SWC was parceled into phases of alert wakefulness (AW), relaxed wakefulness (RW), synchronized sleep (SS), drowsiness (D), paradoxical sleep (PS), preparadoxical phase (PRE), and intermediary phase (INT), according to the following criteria: *i*) AW: very pronounced NEO desynchronization, gross movement artifacts superimposed on a high muscle tone background in the EMG, and (when this information was available) bursts of rhythmic activity in the OB of the kind described during alert states by Hernández-Péon *et al.* (17); *ii*) RW: NEO desynchronized but less so than in AW, EMG showing an absence of gross movement artifacts and often also a smaller degree of basal muscle tone when compared with AW, and absence of the electrical bursts in the OB; *iii*) SS: continuous and pronounced synchronization in the NEO in the presence of muscle tone; *iv*) D: mixing of a synchronized (less regular and pronounced than that of SS) and a relatively desynchronized activity in the NEO in the presence of muscle tone; *v*) PS: desynchronization in the NEO together with neck muscle atony; *vi*) PRE (see 32): synchronized NEO activity in the absence of muscle tone. This phase can either precede or follow a PS episode, or it can even be interposed between two typical PS phases; *vii*) INT: mixing of desynchronized and synchronized activities in the NEO in the presence of muscle tone, with the synchronization manifesting itself basically as sleep spindles, whose number and internal frequency generally increase in this phase. This phase seems to correspond to a similar one already described in the literature (5, 12, 18, 28, 31). In order to be individualized in the present analysis a SWC phase had to have a minimal duration of about 2 sec.

After having done this parceling of the SWC, we turned our attention exclusively to the PPC ECoG, which was subdivided into the following categories of ECoG patterns: *i*) \overline{AW} : marked desynchronization constituting the background over which appear, intermittently, bursts of electrical deflections whose frequency varied between approximately 42 and 48 c/sec and which often grouped themselves according to a spindle configuration. This is the only PPC ECoG pattern in which these characteristic electrical bursts appear; *ii*) \overline{RW} : PPC desynchronized, but less so than in episodes classified as \overline{AW} ; *iii*) \overline{SS} : pronounced synchronized activity constantly present in the PPC; *iv*) \overline{D} : mixing of a synchronized (less regular and pronounced than that of \overline{SS}) and a relatively desynchronized activity in the PPC; *v*) \overline{DES} : generic term denoting the presence in the PPC of a desynchronized activity without superimposed electrical bursts and applied when such a pattern occurred during SWC phases that were neither AW nor RW.

Once completed the partition of SWC phases and PPC ECoG patterns according to the criteria outlined above, the following two general questions were posed:

i) Given a certain SWC phase, how was the degree of synchronization of the PPC ECoG record in relation to that of the NEO one? To answer this question the average percentage of occurrence of given associations between SWC phases and PPC ECoG patterns was determined.

ii) Which were the relationships between the alternations of different SWC phases and PPC ECoG patterns? In order to simplify the analysis of this second problem, we have been concerned only with the characteri-

zation of the PPC pattern alternations that took place during any one of the following types of temporal sequences ("transitions") of SWC phases: Wakefulness of any kind, *i.e.*, either AW or RW (W)-D-SS, W-SS, SS-W, SS-D-W, SS-INT-W, SS-INT-PS, SS-PS. "Subtransition" was the term applied to the alternation of 2 immediately sequential phases in the case of transitions composed of 3 phases (*e.g.*, the RW-D-SS transition comprises the RW-D and D-SS subtransitions). A "transition" in the PPC ECoG was defined as corresponding to any one of the following types of temporal sequences of ECoG patterns in the PPC (\bar{W} = either $\bar{A}W$ or $\bar{R}W$): \bar{W} (or $\bar{D}ES$)- \bar{D} - $\bar{S}S$, \bar{W} (or $\bar{D}ES$)- $\bar{S}S$, $\bar{S}S$ - \bar{D} - \bar{W} (or $\bar{D}ES$), $\bar{S}S$ - \bar{W} (or $\bar{D}ES$); any other kind of temporal sequence of PPC patterns (*e.g.*, $\bar{S}S$ - \bar{D} - $\bar{S}S$, \bar{W} - \bar{D} - \bar{W}) was defined as a "fluctuation". Only PPC transitions were taken into account in our analysis.

After having established that the SWC and the PPC transitions proceeded always in the same general direction as far as the degree of cortical synchronization (or desynchronization) was concerned, we started out the analysis of the transitions by determining the time relationships between the attainment of an equivalent degree of synchronization (or desynchronization) in PPC and NEO. Thus, taking the SWC transitions or subtransitions as reference points, there were 3 possibilities for the change in PPC: it could precede, be simultaneous with or succeed the alteration in NEO. We have determined, for each type of SWC transition or subtransition, the average percentage of times in which each of these 3 possible events was verified. In the cases in which the PPC transitions preceded the correspondent SWC ones two parameters have been determined: *i*) The specific PPC pattern(s) whose appearance was most commonly responsible for the time discrepancies; *ii*) The mean magnitude of the time discrepancies associated with these specific PPC patterns, in every case in which the available number of observations allowed it.

The examination of the ECoG of OB, entorhinal area and hippocampus was very limited in scope. In the OB we have observed only the presence or absence of the bursts of rhythmic activity previously shown (17) to be correlated with alert states. In the entorhinal area and the hippocampus we have paid attention only to the occurrence of typical theta rhythm.

RESULTS

1. Associations between SWC phases and PPC ECoG patterns.

— The ECoG behavior of the PPC differs considerably when the 2 types of wakefulness phases discerned in the present analysis are compared. In AW phases an $\bar{A}W$ pattern was invariably seen in the PPC, which means that the degree of background desynchronization shown by the PPC was always equivalent to that of the NEO desynchronization. In RW phases (Fig. 1), on the other hand, the PPC was more synchronized than the NEO in the great majority ($97 \pm 1\%$) (mean value \pm S.E.M.) of cases. It should be noticed, also, that in AW episodes there were, superimposed on the marked desynchronization of the PPC record, bursts of potential deflections concomitant with the OB similar bursts (Fig. 2). These PPC bursts, whose frequency varied between approximately 42 and 48

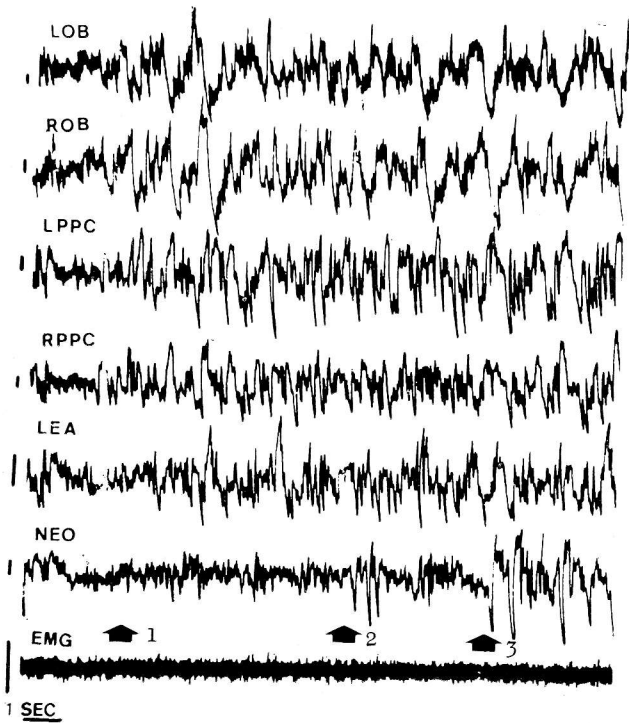


Fig. 1. — ECoG and EMG patterns during a RW-D-SS transition of the SWC.

Arrow 1 indicates the beginning of RW phase. RW-D and D-SS subtransitions are signaled by arrows 2 and 3, respectively.

Abbreviations: LOB, ROB: left and right olfactory bulb; LPPC, RPPC: left and right prepyriform cortex; LEA: left entorhinal area; NEO: neo-cortex; EMG: electromyogram of dorsal neck muscles.

Calibration: 100 μ V and 1 sec.

c/sec, were absent during all other phases. In AW phases a theta activity in the hippocampus and the entorhinal area was often observed.

An equivalent degree of synchronization of PPC and NEO records was noticed in the vast majority ($86 \pm 1\%$) of SS episodes (Fig. 3) and almost always ($99 \pm 0\%$) in the case of PS phases (Fig. 4). Typical sleep spindles were never seen in the PPC ECoG. During PRE episodes the PPC record was desynchronized in most ($78 \pm 4\%$) instances. As far as the INT phase is concerned, it was

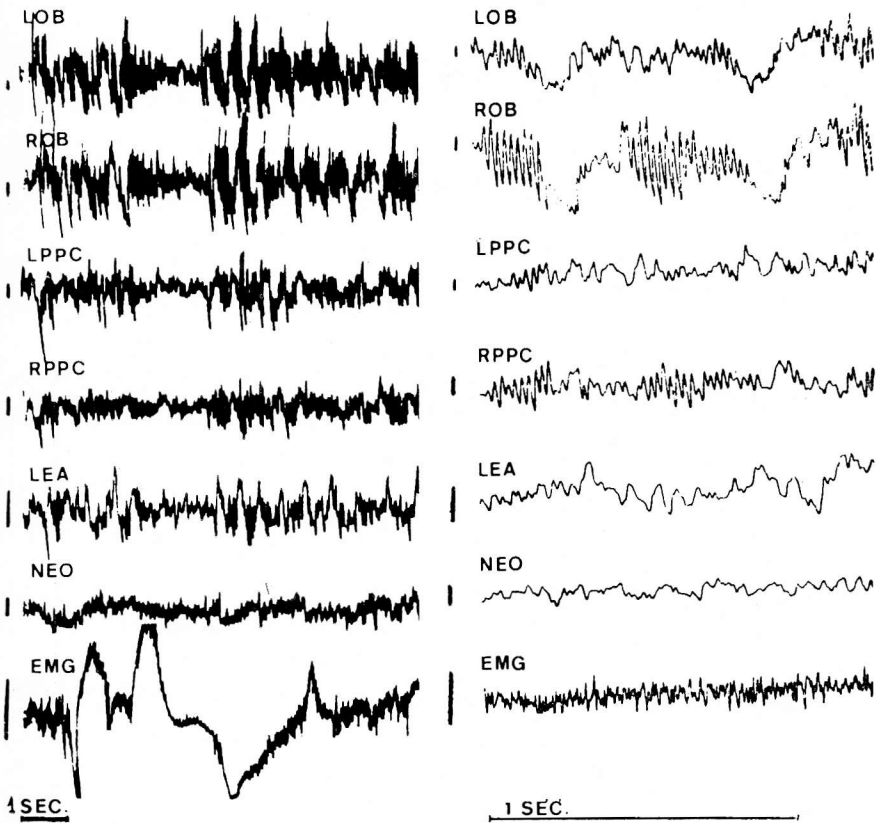


Fig. 2. - ECoG and EMG patterns during an AW phase of the SWC.

The record shown at right, obtained at a higher speed, clearly illustrates the concomitance of olfactory bulb and prepyriform cortex electrical bursts (compare ROB with RPPC). Abbreviations and calibration as in Fig. 1.

observed that almost always it followed a phase of SS and heralded an episode of either PS ($51 \pm 4\%$ of times) or wakefulness of any kind ($45 \pm 3\%$ of times). During INT phases the PPC was most ($81 \pm 3\%$) of the times more desynchronized than the NEO (Figs. 5 and 6). Very often, during INT, PS and PRE phases the presence of a typical theta activity (either in isolation or mixed with other rhythms) was noticed in the entorhinal cortex and the hippocampus (Figs. 4 and 5).

Finally, considering the D phases of the SWC, it was seen that

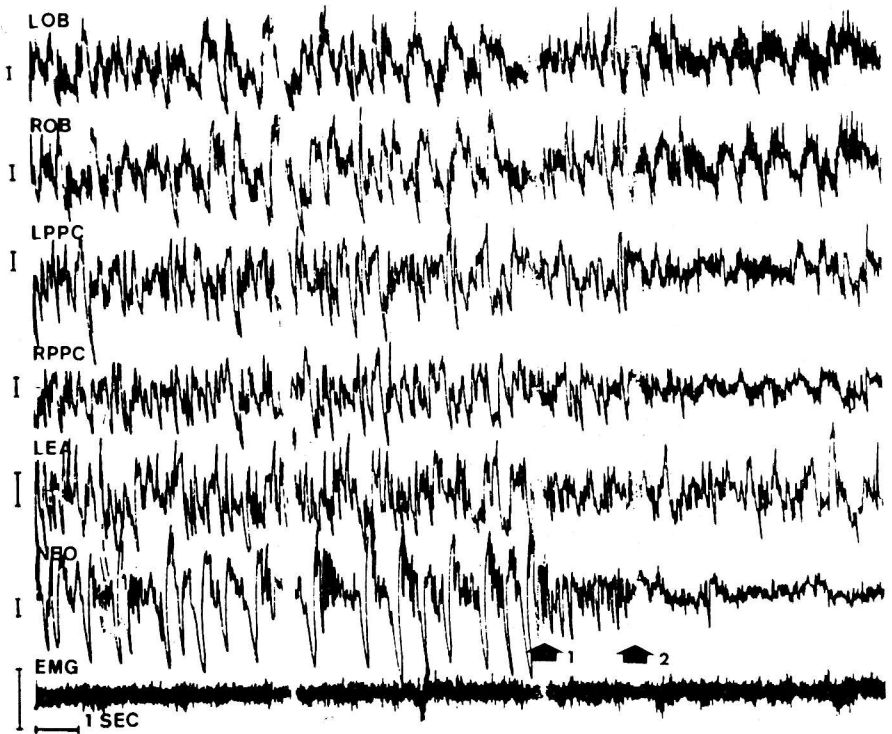


Fig. 3. - *ECoG and EMG patterns during an SS-D-AW transition of the SWC.*

The SS-D and D-AW subtransitions are indicated by arrows 1 and 2, respectively. Abbreviations and calibration as in Fig. 1.

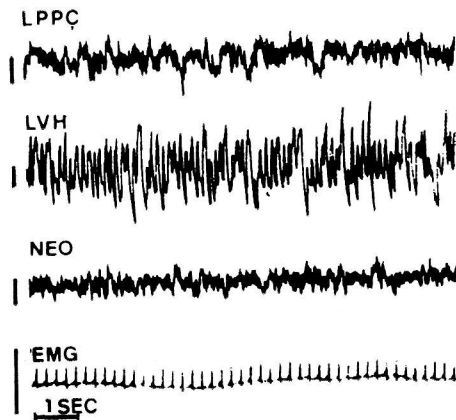


Fig. 4. - *ECoG and EMG patterns during a PS episode.*

LVH: left ventral hippocampus. Other abbreviations and calibration as in Fig. 1.

when the D phases under scrutiny were incorporated within W-D-SS transitions (Fig. 1), in most ($77 \pm 2\%$) cases the PPC was more synchronized than the NEO. This fact reflects the tendency, which will be seen below, for the W-D-SS transitions to be preceded by the correspondent PPC ones. In the case of D phases incorporated within W-D-W and SS-D-SS sequences, the degree of PPC synchronization was, almost always, either equivalent ($51 \pm 4\%$ and $40 \pm 3\%$, respectively) to, or greater ($48 \pm 4\%$ and $58 \pm 3\%$, respectively) than that of the NEO ECoG.

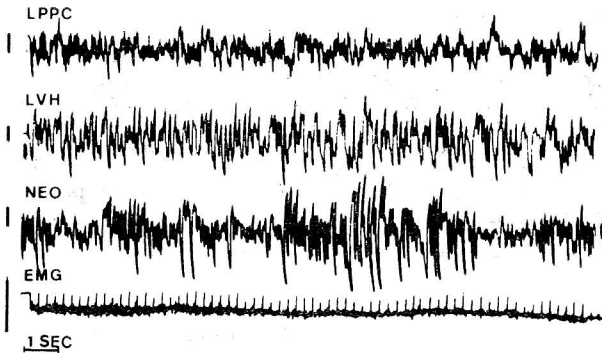


Fig. 5. - ECoG and EMG patterns during an INT phase of the SWC. Abbreviations and calibration as in Figs. 1 and 4.

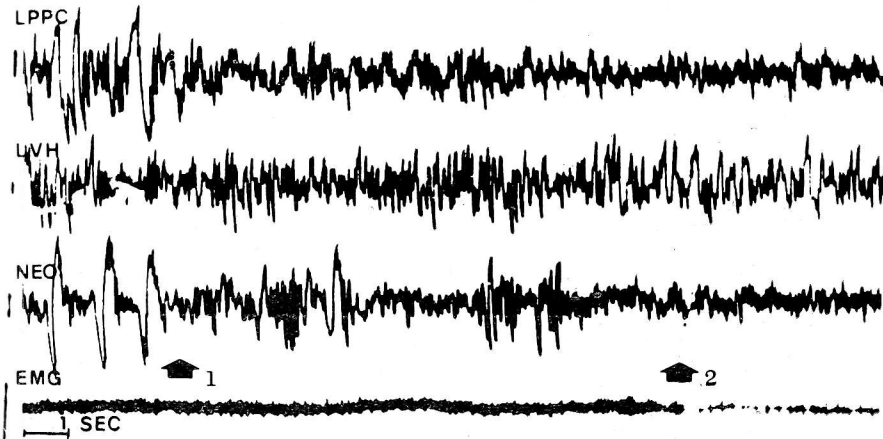


Fig. 6. - ECoG and EMG patterns during an SS-INT-PS transition of the SWC.

SS-INT and INT-PS subtransitions are signaled by arrows 1 and 2, respectively. Abbreviations and calibration as in Figs. 1 and 4.

2. *Relationships between transitions of different SWC phases and PPC ECoG patterns.* — The most general observation made in connection with this problem was that the SWC and the PPC transitions proceeded always in the same general direction as far as the degree of cortical synchronization was concerned, *i.e.*, whenever the NEO ECoG changed from a less synchronized to a more synchronized pattern (or *vice versa*), the PPC ECoG also changed in a similar way.

TABLE I. — *Time behavior of the PPC ECoG pattern transitions in relation to the correspondent SWC ones.*

The first of the two numbers between brackets in the first column represents the number of instances in which the respective transition was observed, whereas the second one indicates the number of rats in which the respective transition was observed. The symbol — denotes inexistence of the phenomenon. Abbreviations: SWC: sleep-wakefulness cycle; PPC: prepyriform cortex; AW, RW: alert and relaxed wakefulness; D: drowsiness; SS, PS: synchronized and paradoxical sleep; INT: intermediary phase; SEM: standard error of the mean.

Reference point	Percentage of occurrence of each kind of time behavior of the PPC transition in relation to the correspondent SWC one (mean \pm SEM) ¹		
	Precedes	Simultaneous	Succeeds
RW-D-SS transition (176; 9)			
RW-D subtransition	99 \pm 1	1 \pm 1	—
D-SS subtransition	75 \pm 4	21 \pm 3	4 \pm 2
AW-D-SS transition (53; 7)			
AW-D subtransition	—	100	—
D-SS subtransition	58 \pm 7	36 \pm 7	6 \pm 3
RW-SS transition (84; 8)	93 \pm 3	6 \pm 3	0 \pm 0 +
SS-INT-PS transition (95; 9)			
SS-INT subtransition	50 \pm 5	49 \pm 5	1 \pm 1
INT-PS subtransition	100	—	—
SS-AW transition (80; 8)	8 \pm 4	92 \pm 4	—
SS-D-AW transition (24; 6)			
SS-D subtransition	19 \pm 2	56 \pm 8	24 \pm 8
D-AW subtransition	8 \pm 6	92 \pm 6	—
SS-INT-AW transition (19; 5)			
SS-INT subtransition	43 \pm 12	47 \pm 9	10 \pm 7
INT-AW subtransition	100	—	—

¹ Mean of the individual percentages determined for each animal which exhibited the respective SWC transition.

Table I lists the results of the quantitative analysis of the behavior of the PPC ECoG transitions in relation to the SWC ones. First of all, it can be seen that only in few instances the PPC transitions occurred after the correspondent SWC ones.

In the group of the W-D-SS transitions (Table I), when the transitions are considered in their entirety (*i.e.*, when the attention is focused on the D-SS subtransitions), a tendency can be observed for the SWC transitions to be preceded by the correspondent PPC ones. This tendency is much more pronounced in the cases in which the RW phases were involved (Fig. 1), but is still present when the sequences involving AW are examined. The vast majority of the RW-SS transitions was preceded by the correspondent PPC pattern alternations (Table I). Results on AW-SS transitions are not presented because the scarce occurrence of such sequences in our material rendered the available data unsuitable for quantitative treatment.

When the SS-INT-PS transitions are considered in their entirety, it can be seen in Table I that the appearance of a less synchronized activity in the PPC always preceded the correspondent alteration in the NEO ECoG (Fig. 6). The SS-PS transitions occurred too infrequently to be discussed here.

A conspicuous difference in the behavior of the PPC transitions can be noticed (Table I) when the SS-INT-AW transitions, on the one hand, are compared with the SS-AW and SS-D-AW ones, on the other. In fact, whereas in the former transitions (considered in their entirety) a more desynchronized ECoG pattern always appeared first in PPC and then in NEO, in the latter ones the ECoG pattern alterations appeared simultaneously in PPC and NEO in the vast majority of cases (Fig. 3). Results on the SS-RW, SS-D-RW and SS-INT-RW transitions are not reported because in these cases the PPC ECoG frequently showed only a fluctuation instead of a transition; this resulted in a total number of useful observations that was insufficient for a quantitative analysis.

Table II lists the magnitudes of the time discrepancies observed in cases in which the PPC ECoG pattern transitions preceded the correspondent SWC ones. Two general remarks seem appropriate: *i*) there is a similarity among the mean magnitudes of the time discrepancies in cases of different SWC transitions. In fact, half of the values given in Table II fall within the relatively narrow interval of 8.5-9.6 sec; *ii*) especially when SWC transitions involving the INT phase are considered, the mean magnitudes of the time discre-

TABLE II. - *Time discrepancies in cases in which the PPC ECoG pattern transitions preceded the correspondent SWC ones.*

The values presented are those associated with the specific PPC pattern(s) whose appearance was most commonly responsible for the time discrepancies. The numbers between brackets that follow the mean time discrepancies represent the number of measurements encompassed in the respective mean. Numbers between brackets in the first column and abbreviations as in Table I.

Reference point	Magnitude of time discrepancy (mean \pm SEM in sec) ¹
RW-D-SS transition (176; 9)	
RW-D subtransition	8.5 \pm 0.4 (95)
D-SS subtransition	14.5 \pm 0.8 (126)
AW-D-SS transition (53; 7)	
D-SS subtransition	11.2 \pm 1.4 (30)
RW-SS transition (84; 8)	9.3 \pm 0.8 (34)
SS-INT-PS transition (95; 9)	
SS-INT subtransition	9.1 \pm 1.0 (32)
INT-PS subtransition	30.4 \pm 2.2 (49)
SS-AW transition (80; 8)	9.6 \pm 2.7 (6)
SS-INT-AW transition (19; 5)	
INT-AW subtransition	27.4 \pm 4.0 (15)

¹ Mean of all observed values assumed by the respective variable.

pancies attain quite considerable values, up to a maximum of 30.4 \pm 2.2 sec.

DISCUSSION

The present study represents the first published systematic description of the PPC ECoG during the different phases of the spontaneous SWC of freely-behaving rats.

Associations between SWC phases and PPC ECoG patterns. It has been reported (19, 27) that in animals studied under acute conditions the level of the ECoG activity (in terms of synchronization vs. desynchronization) is, in most instances, equivalent in neo- and paleocortical structures. The present findings show that in the case of freely-behaving rats such a statement would be correct only when certain SWC phases (AW, SS and PS) are considered; in other

phases (RW, D, INT, PRE) the level of the ECoG activity is usually different in NEO and PPC. Moreover, it has also been reported (14) that there is, frequently, during the spontaneous SWC, an inverse relationship between the archicortical (hippocampal) and neocortical ECoG activities. The present results indicate that such an unqualified generalization cannot either be properly extrapolated to the case of the paleocortical (PPC) ECoG behavior.

The bursts of rhythmic activity described in the present work in the rat PPC ECoG during AW episodes had not been previously reported in this species (27), but their occurrence in AW phases has already been pointed out in other species (*e.g.*, 6-8). The concomitance of these bursts in OB and PPC, presently described in rats, has also been seen in dogs and monkeys (6), and the dependence of the PPC bursts on projections from the OB to the PPC has been pointed out (2). In relation to the AW pattern, presently reported to be invariably associated with AW phases, we prefer to emphasize that the degree of background desynchronization observed in the PPC ECoG was equivalent to that of the NEO desynchronization, rather than stressing the intermittent occurrence of the PPC electrical bursts. This is done for 2 reasons: *i*) during AW episodes rather long segments of PPC record that were free from superimposed bursts were observed; *ii*) this procedure discloses the existence of a marked difference in the degree of PPC synchronization relatively to that of NEO when AW and RW phases are compared; such a difference has not been previously reported in the literature. The present description of a greater degree of synchronization in PPC when compared to NEO in the vast majority of RW episodes might be in contradiction with the reported PPC desynchronization during NEO ECoG alerting responses associated with a lack of overt behavioral activity in cats (7). Assuming that these conditions are analogous to those prevailing in the RW phases discerned in the present work, the apparent contradiction with our results might depend on species differences, or on the fact that Freeman (7) did not attempt to do a systematic analysis of the PPC ECoG patterns during the various SWC phases.

The comparable degree of synchronization in PPC and NEO presently observed in most SS episodes had already been reported by several authors, both in cats (7, 8, 19) and rats (27). We have also confirmed that, as verified by Freeman (7), no typical sleep spindles can be seen in PPC with short intracortical bipolar ECoG

exclusive possibilities could be envisaged: *i*) Common, or interrelated, subcortical structures could be involved in the control of both PPC and NEO activities. An anatomical basis for such mechanisms certainly exists; for example, the *locus coeruleus*, thought to be importantly involved in the SWC control (see 25), directly innervates both PPC and NEO areas (29). *ii*) The PPC could participate in the control of NEO activity; this possibility is supported by the observation that electrical stimulation of the PPC can bring about either a desynchronization or a synchronization of the NEO ECoG (4, 20). *iii*) The NEO could be involved in the control of the PPC ECoG activity, conceivably through, for instance, the cortical projections to the reticular formation (*e.g.*, 24) and to unspecific thalamic nuclei (*e.g.*, 10); both the reticular formation (16) and the unspecific thalamus (7) seem to have access to the PPC.

SUMMARY

The electrocorticographic (ECoG) behavior of the prepyriform cortex (PPC) was studied during the spontaneous sleep-wakefulness cycle (SWC) of freely-behaving rats in which recording electrodes had been chronically implanted in PPC, neocortex (NEO), entorhinal area, olfactory bulb (OB), hippocampus, and dorsal neck muscles. The different phases of the SWC were discerned by taking into account the NEO, OB and neck musculature electrical activities, and criteria were set for the identification of various PPC ECoG patterns.

The existence of rather regular associations between the PPC ECoG and the various SWC phases was demonstrated, but it was also seen that these associations defied simplistic generalizations. Thus, whereas the level of ECoG activity (in terms of synchronization vs. desynchronization) was, in the majority of instances, equivalent in PPC and NEO in some SWC phases (alert wakefulness, synchronized sleep and paradoxical sleep), the same was not true in the case of the relaxed wakefulness, drowsiness, intermediary and preparadoxical phases. In the same context, when the time relationships between the alternations of different SWC phases and PPC ECoG patterns were analyzed, it became clear that although only rarely the PPC transition occurred after the correspondent SWC one, the former could either precede, or be simultaneous with the latter, depending on the particular SWC transition being considered.

The neural control of the coupling of PPC and NEO activities.

Acknowledgements.

Paulo Research Foundation. Authors are greatly indebted to S. Lagnado (Universidade Federal do Rio de Janeiro) for the manuscript, to Dr. Y. R. de Mello for the translation to Mr. R. Vieira and M. A. de Mello for the final proof.

1. ANDERSEN, P. and S. H. H. *Pha Rhythm. New York*, 1968.
2. BECKER, C. J. and J. W. *after loss of peripheral input. J. Neurophysiol.*, 32: 597-599, 1968.
3. BIEDENBACH, M. A. *Olfactory cortex as a source of rhythmic activity. Biol. Sci.*, 32: 204-214, 1968.
4. BREMER, F. et TERPSTRA, J. *physiologiques du sommeil et de la formation du réveil. Arch. int. Pharmacodyn.*, 12: 789-796, 1974.
5. DALLAIRE, A. and J. P. *with observations on the EEG. J. Neurophysiol.*, 12: 789-796, 1974.
6. DOMINO, E. F. *an EEG phenomenon in some patients. EEG clin. Neurophysiol.*, 1968.
7. FREEMAN, W. J. *Electrical activity. J. Neurophysiol.*, 1968.
8. FREEMAN, W. J. *Olfactory cortex and behavior. J. Neurophysiol.*, 1968.
9. FREEMAN, W. J. *Electrical potentials in prepyriform cortex. J. Neurophysiol.*, 1968.
10. FRENCH, J. D., H. J. *jections from cortex to monkey. J. Neurophysiol.*, 1968.
11. FUJII, T. *Effects of electrical stimulation in vitro. EEG clin. Neurophysiol.*, 1968.
12. GOTTESMANN, C., B., RODI, M., *Rondeletti, Programme pour l'analyse des phases de l'éveil et du sommeil. EEG clin. Neurophysiol.*, 1965: 373-376, 1971.
13. GREEN, J. D. *The role of the reticular formation and the reticular formation. H. et al. (Eds.), Brain and Cerebellum. Brown and Co., 1968.*
14. GREEN, J. D. and J. W. *arousal. J. Neurophysiol.*, 1968.
15. GROOT, J. de *The role of the reticular formation. Ned. Akad. Wet. Sch.*, 1968.

recording. In this context, it is interesting that Kaada (20) was not able to observe barbiturate spindles in PPC, and that Freeman (7) could not elicit recruiting responses in PPC by electrical stimulation of the midline thalamus.

The ECoG patterns exhibited by the PPC during PS, PRE and INT episodes have not been previously reported in the literature. The INT phase deserves some special comments at this point. In fact, the phase distinguished under this name in the present work seems to correspond, with greater or lesser precision, to a similar one described in the pony (5), the cat (18, 28) and the rat (12, 31). However, only Parmeggiani and Zanocco (28) refer that such a phase can precede either an episode of vigilance or a PS phase, offering no quantitative qualification of this statement; in the other papers cited above the phase under scrutiny is mentioned as preceding only PS episodes. Dallaire and Ruckebusch (5) suggested that during the INT phase the animal would rapidly explore its surroundings before going to a PS phase. According to the present data, given an INT phase, the probabilities of it being followed by an episode of PS or by a phase of wakefulness of any kind are about the same. Considering this fact, it might be helpful, in an attempt to test Dallaire and Ruckebusch's (5) suggestion, to verify if it is possible to establish any correlation between conditions in the animal's surroundings and the occurrence of either a W or a PS episode after an INT phase. The fact that the PPC is more desynchronized than the NEO during most of INT episodes might also be helpful in future attempts to disclose the biological significance of the INT phase.

Finally, confirming previous reports from several laboratories (*e.g.*, 33), we have observed the frequent occurrence of a theta rhythm in the hippocampal ECoG during AW and PS episodes. The present description of a hippocampal theta activity in the INT phases brings to mind the fact that such a rhythm has already been noticed (12) in the hippocampus of rats during SWC phases that probably corresponded to the INT phase discerned in our analysis.

Relationships between transitions of different SWC phases and PPC ECoG patterns. This problem has not been systematically examined in the literature so far. The only pertinent data were published by Imamura and Kawamura (19), according to whom, in most instances, the ECoG transitions take place simultaneously in neo- and paleocortical structures. Such an unqualified statement stands in clear contradiction with the present results, and the discrepancy

might depend on differences in subject species, experimental conditions and/or data processing procedures.

An interesting comparison can be made between our findings and those reported by Green and Arduini (14) in their comparative analysis of the archicortical (hippocampal) and neocortical ECoG activities. In fact, these authors have seen that, quite often, the hippocampal and the neocortical transitions proceeded in the opposite general direction, in the sense that when a more synchronized, or a more desynchronized, pattern appeared in the NEO, the hippocampal record underwent a desynchronization or a synchronization, respectively. This picture stands in sharp contrast to that emerging from the present study, according to which the PPC and the SWC transitions proceeded always in the same general direction. Notwithstanding this basic difference between hippocampal (archicortical) and PPC (paleocortical) records, however, Green and Arduini (14) have noticed that quite often the hippocampal transitions preceded the correspondent NEO ones, a phenomenon also verified with the PPC transitions in the present work.

Finally, the mean magnitudes of the time discrepancies observed in cases in which the PPC transitions preceded the SWC ones were relatively large (several seconds, up to a maximum of 30.4 ± 2.2 sec). This fact tends to discard the possibility of ascribing these cases of no-simultaneity to simple experimental errors inherent to the adopted data processing procedures. The observation that the mean magnitudes of the time discrepancies were similar in several different groups of transitions (Table II) remains an intriguing coincidence, suggesting perhaps the existence of common aspects in the neural control mechanisms responsible for various types of transitions.

Neural control mechanisms. Our results show the existence of rather regular associations between the PPC and the NEO ECoG activities. It remains to consider here the neural control mechanisms possibly responsible for this coupling. Obviously, no purely descriptive study such as the present one could provide concrete answers to this question. The best that can be done at this juncture is to briefly outline general categories of control mechanisms that could be operative in this context, remaining for future investigations to explore these working hypotheses along experimental lines.

Very much in line with Green and Arduini's (14) hypotheses about the control mechanisms possibly responsible for the coupling of hippocampal and NEO ECoG activities, the following nonmutually

exclusive possibilities could be envisaged: *i*) Common, or interrelated, subcortical structures could be involved in the control of both PPC and NEO activities. An anatomical basis for such mechanisms certainly exists; for example, the *locus coeruleus*, thought to be importantly involved in the SWC control (see 25), directly innervates both PPC and NEO areas (29). *ii*) The PPC could participate in the control of NEO activity; this possibility is supported by the observation that electrical stimulation of the PPC can bring about either a desynchronization or a synchronization of the NEO ECoG (4, 20). *iii*) The NEO could be involved in the control of the PPC ECoG activity, conceivably through, for instance, the cortical projections to the reticular formation (*e.g.*, 24) and to unspecific thalamic nuclei (*e.g.*, 10); both the reticular formation (16) and the unspecific thalamus (7) seem to have access to the PPC.

SUMMARY

The electrocorticographic (ECoG) behavior of the prepyriform cortex (PPC) was studied during the spontaneous sleep-wakefulness cycle (SWC) of freely-behaving rats in which recording electrodes had been chronically implanted in PPC, neocortex (NEO), entorhinal area, olfactory bulb (OB), hippocampus, and dorsal neck muscles. The different phases of the SWC were discerned by taking into account the NEO, OB and neck musculature electrical activities, and criteria were set for the identification of various PPC ECoG patterns.

The existence of rather regular associations between the PPC ECoG and the various SWC phases was demonstrated, but it was also seen that these associations defied simplistic generalizations. Thus, whereas the level of ECoG activity (in terms of synchronization vs. desynchronization) was, in the majority of instances, equivalent in PPC and NEO in some SWC phases (alert wakefulness, synchronized sleep and paradoxical sleep), the same was not true in the case of the relaxed wakefulness, drowsiness, intermediary and preparadoxical phases. In the same context, when the time relationships between the alternations of different SWC phases and PPC ECoG patterns were analyzed, it became clear that although only rarely the PPC transition occurred after the correspondent SWC one, the former could either precede, or be simultaneous with the latter, depending on the particular SWC transition being considered.

The neural control mechanisms possibly responsible for the coupling of PPC and NEO ECoG activities are briefly discussed.

Acknowledgements. — This research was supported by the State of Sao Paulo Research Foundation (FAPESP) under Grant Médicas 68/149. The Authors are greatly indebted to Drs. P. J. Morgane (Worcester Foundation for Experimental Biology, U.S.A.), C. Timo-Iaria, W. R. Schmidek and S. J. S. Lagnado (University of Sao Paulo) for their critical reading of the manuscript, to Dr. Y. R. Leite for her help in the statistical procedures, and to Mr. R. Vieira and Mr. N. Teixeira for their skillful technical assistance.

REFERENCES

1. ANDERSEN, P. and ANDERSSON, S. A. *Physiological Basis of the Alpha Rhythm*. New York, Appleton-Century-Crofts, 235 pp., 1968.
2. BECKER, C. J. and FREEMAN, W. J. Prepyriform electrical activity after loss of peripheral or central input, or both. *Physiol. Behav.*, 3: 597-599, 1968.
3. BIEDENBACH, M. A. and STEVENS, C. F. Synaptic organization of cat olfactory cortex as revealed by intracellular recording. *J. Neurophysiol.*, 32: 204-214, 1969.
4. BREMER, F. et TERZUOLO, C. Contribution à l'étude des mécanismes physiologiques du maintien de l'activité vigile du cerveau. Intéraction de la formation réticulée et de l'écorce cérébrale dans le processus du réveil. *Arch. int. Physiol.*, 62: 157-178, 1954.
5. DALLAIRE, A. and RUCKEBUSCH, Y. Sleep patterns in the pony with observations on partial perceptual deprivation. *Physiol. Behav.*, 12: 789-796, 1974.
6. DOMINO, E. F. and UEKI, S. An analysis of the electrical burst phenomenon in some rhinencephalic structures of the dog and monkey. *EEG clin. Neurophysiol.*, 12: 635-648, 1960.
7. FREEMAN, W. J. Distribution in time and space of prepyriform electrical activity. *J. Neurophysiol.*, 22: 644-665, 1959.
8. FREEMAN, W. J. Correlation of electrical activity of prepyriform cortex and behavior in cat. *J. Neurophysiol.*, 23: 111-131, 1960.
9. FREEMAN, W. J. Relations between unit activity and evoked potentials in prepyriform cortex of cats. *J. Neurophysiol.*, 31: 337-348, 1968.
10. FRENCH, J. D., HERNANDEZ-PEON, R. and LIVINGSTON, R. B. Projections from cortex to cephalic brain stem (reticular formation) in monkey. *J. Neurophysiol.*, 18: 74-95, 1955.
11. FUJII, T. Effects of cooling on guinea pig olfactory cortex maintained *in vitro*. *EEG clin. Neurophysiol.*, 43: 238-247, 1977.
12. GOTTESMANN, C., MENDOZA, J. L. J. de, LACOSTE, G., LALLEMENT, B., RODI, M., RODRIGUES, L. et TASSET, M. Elaboration d'un programme pour l'analyse et la quantification automatiques des différentes phases de l'éveil et du sommeil chez le Rat. *C. R. Soc. Biol. Paris*, 165: 373-376, 1971.
13. GREEN, J. D. The rhinencephalon: aspects of its relation to behavior and the reticular activating system. Pp. 607-619. In JASPER, H. H. et al. (Eds.), *Reticular Formation of the Brain*. Boston, Little, Brown and Co., 1958.
14. GREEN, J. D. and ARDUINI, A. A. Hippocampal electrical activity in arousal. *J. Neurophysiol.*, 17: 533-557, 1954.
15. GROOT, J. de The rat forebrain in stereotaxic coordinates. *Verh. Kon. Ned. Akad. Wet. B. Naturkunde*, 2: 1-40, 1959.

16. GUEVARA-AGUILAR, R., AGUILAR-BATURONI, H. U., ARÈCHIGA, H. and ALCOCER-CUARON, C. Efferent evoked responses in the olfactory pathway of the cat. *EEG clin. Neurophysiol.*, 34: 23-32, 1973.
17. HERNÁNDEZ-PEON, R., LAVIN, A., ALCOCER-CUARON, C. and MARCELIN, J. P. Electrical activity of the olfactory bulb during wakefulness and sleep. *EEG clin. Neurophysiol.*, 12: 41-58, 1960.
18. HOWE, R. C. and STERMAN, M. B. Cortical-subcortical EEG correlates of suppressed motor behavior during sleep and waking in the cat. *EEG clin. Neurophysiol.*, 32: 681-695, 1972.
19. IMAMURA, G. and KAWAMURA, H. Activation pattern in lower level in the neo-, paleo-, and archicortices. *Jap. J. Physiol.*, 12: 494-505, 1962.
20. KÅADA, B. R. Somato-motor, autonomic and electrocorticographic responses to electrical stimulation of "rhinencephalic" and other structures in primates, cat and dog. *Acta physiol. scand.*, 24: Suppl. 83: 1-285, 1951.
21. KAWAMURA, H., NAKAMURA Y. and TOKIZANE, T. Effect of acute brain stem lesions on the electrical activities of the limbic system and neocortex. *Jap. J. Physiol.*, 11: 564-575, 1961.
22. KRIEG, W. J. S. Connections of the cerebral cortex. I. The albino rat: topography of cortical areas. *J. comp. Neurol.*, 84: 221-275, 1946.
23. LEUNG, P. M. and ROGERS, Q. R. Importance of prepyriform cortex in food-intake response of rats to amino acids. *Amer. J. Physiol.*, 221: 929-935, 1971.
24. LINDSLEY, D. F., RANF, S. K., SHERWOOD, M. F. and PRESTON, W. G. Corticifugal influences on the activity of reticular formation neurons in cats. *Exp. Neurol.*, 36: 250-262, 1972.
25. MORGANÉ, P. J. and STERN, W. C. Chemical anatomy of brain circuits in relation to sleep and wakefulness. Pp. 1-131. In WEITZMAN, E. D. (Ed.), *Advances in Sleep Research*, Vol. 1. New York, Spectrum Publ. 1974.
26. MORUZZI, G. The sleep-waking cycle. *Ergebn. physiol.*, 64: 1-165, 1972.
27. OSHIMA, K., MIYAMA, T. and KAWAMURA, H. Studies on EEG of the neo-, paleo-, and archicortices in albino rat. *Jap. J. Physiol.*, 12: 601-610, 1962.
28. PARMEGGIANI, P. L. and ZANOCCO, G. A study on the bioelectrical rhythms of cortical and subcortical structures during activated sleep. *Arch. ital. Biol.*, 101: 385-412, 1963.
29. PICKEL, V. M., SEGAL, M. and BLOOM, F. E. A radioautographic study of the efferent pathways of the nucleus locus coeruleus. *J. comp. Neurol.*, 155: 15-42, 1974.
30. PRICE, J. L. An autoradiographic study of complementary laminar patterns of termination of afferent fibers to the olfactory cortex. *J. comp. Neurol.*, 150: 87-108, 1973.
31. SAYERS, A. and STILLE, G. The caudate spindle during various sleep stages. *Experientia*, 28: 282-283, 1972.
32. TIMO-IARIA, C., NEGRAO, N., SCHMIDEK, W. R., HOSHINO, K., MENEZES, C. E. L. de and ROCHA, T. L. da Phases and states of sleep in the rat. *Physiol. Behav.*, 5: 1057-1062, 1970.
33. WINSON, J. Patterns of hippocampal theta rhythm in the freely moving rat. *EEG clin. Neurophysiol.*, 36: 291-301, 1974.