PERGAMON

Progress in Neurobiology Vol. 59, pp. 1 to 54, 1999

Printed in Great Britain
0301-0082/99/$ - see front matter

PII: S0301-0082(98)00094-X

THE NEUROPHYSIOLOGY OF SLEEP AND WAKING: INTRACEREBRAL CONNECTIONS, FUNCTIONING AND ASCENDING INFLUENCES OF THE MEDULLA OBLONGATA

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(*Received* 16 *October* 1998)

Abstract—This paper focuses on the successive historical papers related to medulla oblongata (M.O.) intracerebral connections, its activities and ascending influences regulating sleep–waking behavior.

The M.O. certainly influences the quantitative and qualitative processes of waking. However, its neurophysiological properties are often concealed by those of the upper-situated brain stem structures.

The M.O., particularly the solitary tract nucleus, is involved in sleep-inducing processes. This nucleus seem to act as a deactivating system of the above situated reticular formation, but it also impacts directly on the thalamocortical slow wave and spindle-inducing processes.

The M.O. is significantly involved in paradoxical sleep mechanisms. Indeed, the mesopontine executive centers are unable to induce paradoxical sleep without the M.O. Moreover, stimulation of the solitary tract nucleus afferents can induce paradoxical sleep, and the M.O. metabolic functioning is specifically disturbed by paradoxical sleep deprivation. Finally, there seems to be a paradoxical sleep Zeitgeber.

Our current knowledge shows that this lowest brain stem level is crucial for sleep–waking mechanisms. It will undoubtedly be further highlighted by future electrophysiologial and neurochemical studies.

CONTENTS

ABBREVIATIONS

AP7	2-Amino-7-phosphonoheptanoic acid
DGG	γ-D-Glutamylglycine
DOPA	Dihydroxyphenylalanine
DSIP	Delta sleep inducing peptide
EEA	Excitatory aminoacid
EEG	Electrocortical
EMG	Electromyogram
EPSP	Excitatory postsynaptic potential
FTL	Lateral tegmental field
GABA	Gamma-amino-butyric acid
GDD	Glutamate diethyl ester
IPSP	Inhibitory postsynaptic potential
MAO	Monoamine oxydase
M.O.	Medulla oblongata
NMDA	*N*-Methyl-D-aspartate
PCPA	*p*-Chlorophenylalanine
PNMT	Phenylethanolamine-*N*-methyltransferase
PGO	Ponto-geniculo-occipital waves
PS-on	Paradoxical sleep on neurons
PS-off	Paradoxical sleep off neurons
REMs	Rapid eye movements of paradoxical sleep
SP	Substance P
SPOL	Sommeil phasique à ondes lentes (slow wave sleep with PGO waves)
5-HT	Serotonin

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1. INTRODUCTION

It is interesting to follow the evolving physiological data and concepts of a neurobiological process. Indeed, historical findings are seldom taken into account in current empirical papers. In this review, we will try to analyze the role of the medulla oblongata (M.O.) on sleep–waking mechanisms. The data, most often, will be presented in their historical sequence, since even the interval between successive publications on the same subject, even by the same team, is of interest.

It would be artificial to study the impact of this brain level on sleep–waking behavior without a preliminary introduction to the knowledge of the whole brain stem (midbrain, pons and M.O.) processes which initially attracted the attention to the M.O.

Since we have already studied the influence of the M.O. on skeletal activities [91], this paper will focus on its intracerebral connections, its own functioning and its influence on the above situated structures.

2. RESULTS

2.1. State of the Art Previous to the Study of the M.O.

The first author to show anatomopathological connections between the brain stem and sleep mechanisms was M. Gayet [84] in 1875. He was a tenured surgeon at the Hotel-Dieu hospital in Lyon. On 23 November 1874, he examined Eugène Perrot, a 28-year-old male. Two months previously, the patient had heard a violent explosion in the factory where he worked. In the following weeks overpowering sleepiness appeared so that he slept almost continuously. However, the patient had no pain, and was totally conscious. Gayet observed that the eyelids covered three-quarters of the eyeballs and could not be raised. It was a case of true paralysis. Moreover, the third motor nerve nuclei were also involved in the paralysis, as the eyes were in divergent strabismus, and the pupils had a tendency to turn upwards. As a result, Perrot was unable to read or write. Gayet [84] immediately concluded that there was a lesion of an inflammatory nature at the level of the third nerve nuclei. The patient died on February 17. Since the weather was cold, the brain was in good shape when a neuroanatomical examination was performed 24 h after the death. The lower part of the cerebral peduncles was healthy but all tissues situated above this location showed signs of a red inflammatory process resembling sclerosis, with some softening. At the highest level, the colliculi were unaffected. However, changes were observed as high as the anterior commissura. Gayet concluded that the salient point was the association of incomplete paralysis of the eyes (as the lesion was limited to the third nerve nuclei) and the almost continuous sleepiness which became more and more marked without evolving into coma.

In 1890, Mauthner [155, 156] published two papers. As already observed by Moruzzi [165] these papers were often mentioned because they were not read. Indeed, they shed no new light on the syndrome described by Gayet [84]. Perhaps the only original idea is that Mauthner [155] suggested that

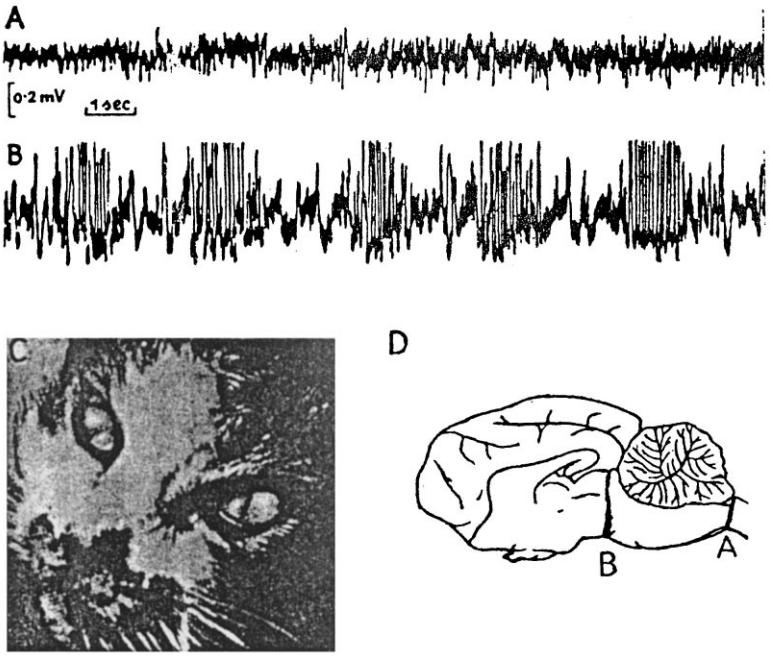

Fig. 1. The intercollicular brain stem transection (cerveau isolé preparation), performed by Bremer in 1935 (B in D), induced cortical slow wave sleep patterns with high amplitude spindles (B) and myosis (C). In contrast, the transection at the lowest level of the M.O. (A in D), which was performed in 1936 and was called encéphale isolé preparation, induced low voltage cortical activity (A). Since the major sensory afferents of cranial nerves enter the brain stem between the two transection levels, Bremer hypothetized that sleep is the consequence of cortical sensory deprivation. From Ref. [37a].

sleep results from tiredness of the central grey which interrupts centripetal and centrifugal influences.

In 1935, the Belgian Frédéric Bremer [35] undertook the first experiment on the brain stem in connection with sleep mechanisms (Fig. 1). The theoretical basis of the research was the (then) common theory of sensory deafferentation of the telencephalon as the basic cause of sleep. After administering ether anesthesia, he made a brainstem transection just behind the third nerve nucleus, at the limit of the mesencephalon and the pons (cerveau isolé preparation). Immediately, the pupils began to contract; after half an hour, they became filiform. The eyeballs progressively turned downwards, and the nictitating membranes relaxed. All these peripheral features resembled those of natural or babiturate-induced sleep. Nevertheless, the myosis could change to mydriasis by stimulation of different parts of the diencephalon. Visual and olfactive stimulations had no effect on pupil and cortex activity. Waning and waxing of 10–15 sec^{-1} waves could be observed in the cortex, similar to effects induced by barbiturates or observed during natural sleep. No electrocortical (EEG) records were shown. Bremer [35] concluded that sleep results from cortical sensory deafferentation (the historical development of the deafferentation hypothesis of sleep can be found in Moruzzi [160]).

One year later, Bremer [36], in an attempt to confirm the deafferentation theory, made transections at different levels of the M.O. but emphasized only the results obtained at its lowest level, or what he called the "high spinal" level (Fig. 1). He observed spontaneous alternation of sleep and waking states. During waking, the animal eyes could explore the environment, generally with a mydriasis characteristic of attention. Ear movements were induced by voices. Sleep occurred spontaneously. When this happened, the eyelids closed, the eyeballs turned downwards with the pupils in myosis and the cortical EEG activity resembled the cerveau isolé preparation. It should be noted that Fig. 1 shows the level of the midbrain and M.O. transections. If the latter is well-situated at the lowest level of the M.O., the cerveau isolé transection passes between the anterior and posterior colliculi at the dorsal level, thus not exactly at the limit of the midbrain and the pons but at a slightly higher level. The EEG records are less convincing than the wonderful ones shown in the following paper by the same author [37] which is a synthesis of previous experiments. On account of their frequency, the characteristic spindles in the cerveau isolé were called "alpha waves". For the first time, he used the term encéphale isolé preparation for the low M.O. transection. In the discussion, Bremer [37] explained again the results of the cerveau isolé preparation by the theory of the sensory deafferentation of the cortex. He claimed that the cranial nerve sensory afferents of the pons and M.O. in the encéphale isolé preparation were crucial for maintaining the 'cortical tonus' characteristic of waking. An interesting point of discussion is the explanation of the myosis of the cerveau isolé. Indeed, it was already known prior to his experiments that a transection in front of the third nerve nucleus induces a permanent myosis because of the disappearance of anterior descending pupillary dilatory influences, which was confirmed by forebrain stimulations. For Bremer, the same result occurs in the midbrain transected animal because the anterior structures are 'asleep'.

In 1944, Magoun [141] began his major contribution on reticular formation. This early research studied the M.O. inhibition and facilitation of motor activity in cats. As observed by Magoun, "since Sherrington's discovery of decerebrate rigidity in 1898, it has been known that the bulbar portion of the brain stem exerts an excitatory influence on neural motor systems, particularly those activating the extensor muscles of the body. That this bulbar region, in addition, contains a mechanism capable of exerting a general inhibitory influence on motor activity does not appear to have been recognized. It was with some astonishment, then, that stimulation of the bulbar reticular formation in the cat was found to bring completely to halt motor activity whether induced reflexly, by brain stem mechanisms or from the motor cortex" (pp. 549–550). Four figures clearly show, on one hand, inhibition of induced knee jerks and blink reflex with a rebound of facilitation of knee jerks after the end of the inhibitory level stimulation and, on the other hand, the facilitation of knee jerks by M.O. activating levels. Magoun [141] did not mention the localization of inhibiting stimulation and only remarked that, up till then, the same results had not been obtained by midbrain stimulation.

Two years later (1946), there appeared the better-known paper by Magoun and Rhines [145] on "an inhibitory mechanism in the bulbar reticular formation" (p. 165). As already shown in the previous paper, M.O. stimulation in cats induced inhibition of decerebrate rigidity and of motor cortex induced responses. The only important new contribution was that the inhibitory reticular formation was localized in the ventromedial part of the M.O. "by exciting a rather long antero-posterior extent". This inhibition "has been obtained after decerebellation and transection through the front end of the medulla" (p. 167). Thus, it originated only from the M.O. level.

The same year, the second most frequently mentioned paper on "brain stem facilitation of cortical motor response" was published by Rhines and Magoun [203]. In cats and monkeys, stimulation from the thalamus to the M.O. facilitated the motor cortex induced responses. In the bulbar reticular formation, the active points were situated "around the periphery of the inhibitory field" (p. 222). The important point was that the authors showed that these facilitatory influences acted at the spinal level. Indeed, Murphy and Gellhorn [165] had previously shown, also in cats and monkeys, a facilitation of cortical induced motor responses by hypothalamic stimulation. But they believed that the hypothalamic facilitatory influences act directly on the motor cortex. Obviously, Murphy and Gellhorn [170] were misled by the fact that hypothalamic stimulation also modified cortical EEG activity in the dial anesthetized animals. Rhines and Magoun [203] clearly demonstrated that there were descending influences since the same hypothalamic facilitation was observed by bulbar pyramid stimulation in ani-

mals in which the entire cortex had been excised on both sides.

In 1948, Magoun [142], working alone, continued to perform (seldom mentioned) research followed by papers published with coworkers. Thus, a 19 line note is fundamental for the knowledge of reticular formation function. This was first based:

1. on the anatomopathological observations of Von Economo [260] showing that lesions at the end of the third ventricle impairs consciousness;
2. on Ranson's [200] researches on monkeys in which the injury of the hypothalamus induced somnolence, finally;
3. on Bailey clinical observations (no references found) that lesions of the periaqueductal grey induces akinetic mutism.

Second, it was obviously linked to his previous work on spinal facilitatory influences induced by midbrain stimulation [203]. This short paper describes coma in monkey induced by midbrain lesions "parallel and a little ventral to the aqueduct but situated in the medial tegmentum above the red nuclei" (p. 752). The animal recovered after 10 days.

A year later, Moruzzi and Magoun [169] published their famous paper. However, it was in a way a partial counter-proof of the previous papers [141, 142, 203], but with EEG control. Cats under chloralose anesthesia, or animals in encéphale isolé preparation, were stimulated in the reticular formation from the M.O. up to the midbrain level. The stimulation induced activation of the EEG when it was "synchronized" (Fig. 2). The EEG responses induced by bulbo-reticular stimulation "were unimpaired following sections of the cerebral peduncles or tectum but were blocked by injury to the mesencephalic tegmentum" (p. 459). The cortical response was shown to be mediated, at least in part, by the "diffuse thalamic projection system", and the thalamic recruiting response described by Morison's group [61, 161] was reduced or suppressed by reticular formation stimulation. Consequently, as shown in their Fig. 3, Moruzzi and Magoun [169] identified the "ascending reticular activating system" extending from the M.O. to the upper part of the midbrain. This paper was followed by several papers on cats [133, 134] and monkeys [79], a synthesis being made by Magoun in 1952 [143] and 1954 [144]. The fundamental finding of Magoun's group was that the sensory afferents are not directly responsible for waking processes, as thought by Bremer and previous authors of the deafferentation theory, although these afferents activate the reticular formation [237] via their collaterals.

In 1954, at the Sainte Marguerite (Quebec) symposium, Moruzzi [163] presented a synthesis of studies made of excellent unicellular recordings of the medio-ventral neurons in the M.O. reticular formation. This review amalgamated the research of Baumgarten and Mollica [19] and Baumgarten *et al.* [20], Mollica *et al.* [158] and Whitlock *et al.* [264]. In fact, although this paper mainly concerned the M.O. descending inhibitory influences, it is worth mentioning that stimulation of the cerebellum principally activated or more seldom inhibited the reticular neurons, which were activated by motor cortex

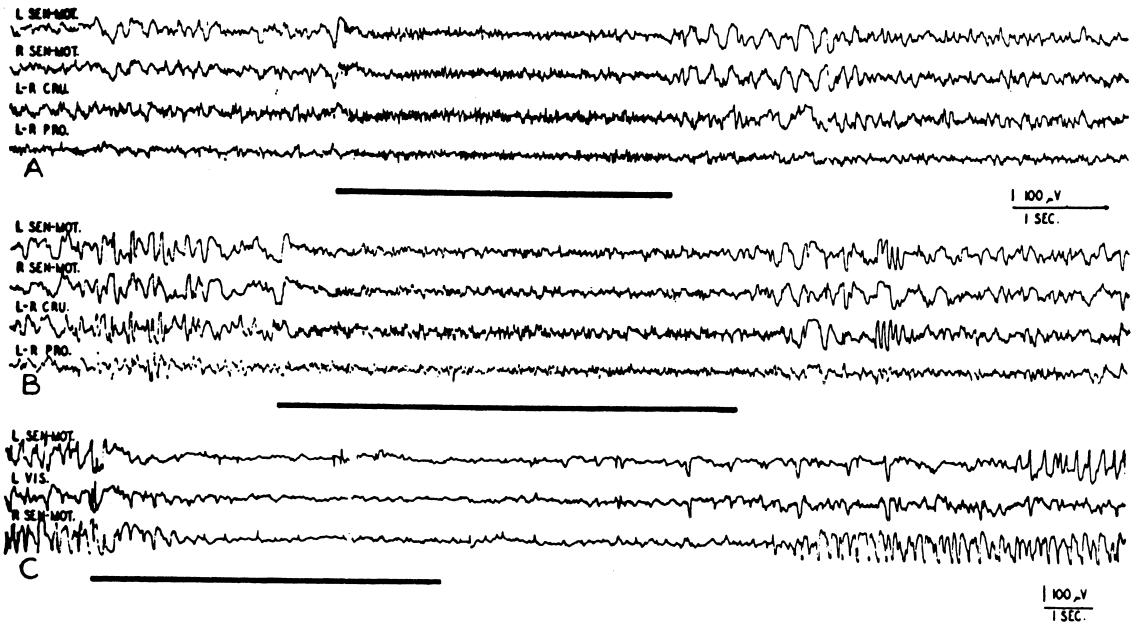

Fig. 2. In 1948, Magoun showed that lesion of the lateral part of the midbrain induces coma. One year later, Moruzzi and Magoun demonstrated in three different cats (A–C) under light chloralose anesthesia, that high frequency electrical stimulation of the bulbo-reticular formation (300 Hz) induces cortical activation. Consequently, the reticular formation, and not the sensory afferents, is responsible for waking processes. L and R Som-Mot, left and right sensory-motor cortex; L and R Cru, left and right cruciate cortex; L–R Pro, left to right proreus gyrus; L Vis, left visual area. Modified from Ref. [169], with Elsevier's permission.

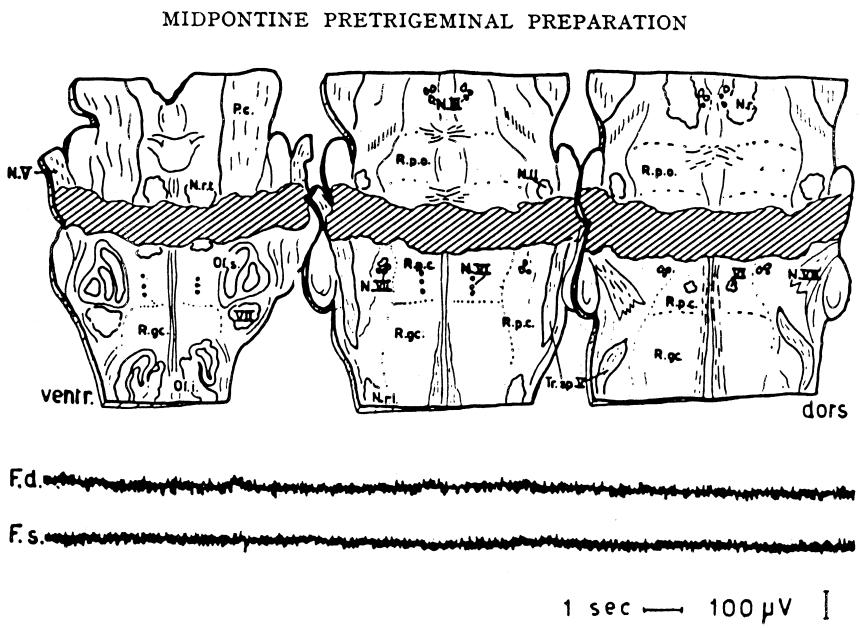

Fig. 3. The acute midpontine pretrigeminal preparation performed in cats by Batini *et al.* (1959) shows a dramatic increase of cortical waking activities in the right and left frontal cortex (Fd and Fs, respectively), in spite of the suppression of the majority of sensory afferents (particularly the V nerve nucleus). This experiment displayed that waking processes are sustained by the tonus of the anterior part of the reticular formation. It also confirmed that sensory afferents are of secondary importance. Moreover, it showed that structures situated behind the transection promote sleep processes. Modified from Ref. [17], with permission.

stimulation. Peripheral stimulations either increased or inhibited cell discharges. The specific reticular projections of cerebellar vermal influences were shown afterwards [83]. Moreover, pyramidal nerve spikes were recorded from the decussatio in cats with cerveau isolé transections sparing the pes pedunculi. In this case, spikes were observed during the cortical spindles, while olfactory or forebrain subcortical stimulation suppressed them. In the last part of the review, Moruzzi [163] discussed the function of the M.O. ascending influences induced by the facilitating levels. He concluded: "the lower reticular formation is essential for the maintenance of wakefulness but not for eliciting an EEG arousal" (p. 45).

Several cytoarchitectonic studies of the reticular formation were undertaken over a period of 4 years. They will be analyzed together. In 1954, Olszewski [181] first studied the reticular formation in humans. Except for the medio-ventral part of the M.O. with its descending inhibitory influences, previous research showed that ascending influences function as a single entity (in French the facilitatory reticular formation was informally called the "blood sausage": V. Bloch dixit). Olszewski's histological study clearly demonstrated that "the reticular formation is not a morphological unit, but is composed of many nuclei of very different structure" (p. 54). At M.O. level, he distinguished many nuclei, emphasizing the centralis and gigantocellularis nuclei where three kinds of nerve cells could be distinguished.

The second study was undertaken a year later by Brodal and Rossi [39] on kittens. Lesions of the upper brain stem showed that numerous reticular neurons, from the M.O. up to the midbrain, send out ascending axons. This was the case for giant, large, medium and small body cells situated in two-thirds of the reticular formation along its entire extent. Large cells were relatively abundant in the reticular formation of the pons and M.O. Some ascending fibers had a partial crossing. Finally, "the findings support the assumption that at least the majority of the long ascending fibers from the reticular formation of the brain stem reach the posterior hypothalamus" (p. 78).

In another study (which in fact should have featured at the beginning of the next paragraph), Scheibel and Scheibel [223] studied more than 4000 cats, dogs, mice and rats and a few young macaques and human infants over 2.5 years. Using the Golgi method, they confirmed that all along the reticular core the majority of neurons have long-distance axons: "No short-axoned Golgi type II cells exist in the brain stem reticular core" (p. 37). Many of them bifurcate into rostral projections up to the thalamus, and into caudal projections to the spinal cord. "The great majority of reticular axons...course...through the cell-fiber maze of the reticular formation itself" (p. 39). The axons emit collaterals along their entire trajectory. This paper also recalls the major convergence and interaction of afferents on neurons, particularly as seen in the M.O. medio-ventral reticular formation [224].

The last, almost contemporary, study was performed by Nauta and Kuypers [174]. On cats, the authors made small electrolytic lesions along the brain stem. The animals were allowed to survive for

7–11 days and the axon degeneration was identified by silver impregnation. The histology showed that the ascending reticular "system originates throughout the longitudinal extent of the M.O. and pons... The longest medullary contributions to the main ascending reticular system appear to arise in the medial, magnocellular region of the tegmentum" (p. 23). "The parvocellular region of the medullary tegmentum appeared to emit short projections which spread medially into the magnocellular reticular formation" (p. 24). Their figure 2 clearly shows that the majority of medullary ascending reticular influences ascend ipsilaterally up to the medial thalamic level. Consequently, the overall results stress the potentially important contribution of the medullary level in modulating the functioning of higher structures.

2.2. Study of the M.O.

2.2.1. *1958–1967*

In 1958, there was published the first of a series of papers by Moruzzi's group in Pisa, on pontine brain stem transections. These major studies were based first on Rossi and Zirondoli's [206] data which showed that transections of the anterior pons, in front of the pretrigeminal nucleus, induce slow wave sleep patterns while transections caudal to this nucleus induce EEG patterns as in the encéphale isolé preparations. Moreover, Roger *et al.* [204] showed in the encéphale isolé preparation that the destruction of the trigeminal nucleus induces a tendency to sleep patterns. Consequently, in 1958, Batini *et al.* [16] made, in cats under ether anesthesia, a transection in front of the pretrigeminal nucleus (Fig. 3). All animals showed spontaneous respiration and decerebration rigidity. The animals were recorded up to 9 days. "When the transection was performed through the middle part of the pons (pretrigeminal midpontine preparation), the EEG patterns consisted of low voltage, fast rhythms, similar to those characteristic of alert behavior in the normal cat. A peculiar feature was their persistence throughout the survival time, with infrequent and short lasting interruptions by sleep patterns" (p. 31). While in normal cats the waking patterns last from 20 to 50% of recording time, it was 70–90% of time after transection. The eyes of the animal showed vertical movements to follow objects in the visual field and pupillary dilatations were observed after emotional stimulus. The waking patterns were replaced by cortical sleep activities by low doses of pentobarbital. However, rostropontine transections induced sleep patterns with myosis. The authors advanced the hypothesis that caudal structures could influence sleep-induction.

A year later (1959), Batini *et al.* [17] published the corresponding full paper about the midpontine pretrigeminal preparation. The research was performed on 85 cats. The same results were described. The authors explained that only vertical eye movements in response to stimuli could be observed since the sixth nerve nucleus (rectus lateralis) was eliminated. Carefully, they explain that the eye movements by themselves "are not a proof of a conscious perception". However, the pupillary dilatations observed only in a small number of preparations suggested this to be the case. During the infrequent and short-lasting periods of synchronized EEG patterns, there was an absence of ocular responses. Later on, results clearly showed that this preparation can be conditioned [1]. The neuroanatomy showed that the transections were situated at the caudal part of the nucleus reticularis pontis oralis. The authors claimed that, contrary to the opinion of Roger *et al.* [204] opinion, the trigeminal nucleus afferents are not responsible for waking processes. Again, they conclude that "synchronizing and possibly sleep inducing mechanisms lying mainly, though not exclusively, in the lower brain stem would provide a substantial contribution to precipitating EEG and behavioral sleep patterns in the normal cat and in the encephale isolé preparation" (p. 8). Consequently, the postulated influences are most likely situated in the caudal pons or M.O.

In the second paper of the set published the same year, Batini *et al.* [15] studied the neural mechanisms underlying the enduring EEG and behavioral activation of the midpontine pretrigeminal preparation. They first showed that the slow wave sleep patterns in the encéphale isolé preparation with trigeminal nucleus lesion do not stem from variations of arterial pressure or ventilation (hypocapnia). They confirmed that pontine rostral transections reverse the EEG activation to slow wave sleep patterns. They showed by transections at different levels that the synchronizing influences seem to originate caudal to the rostral part of the M.O., since transections at this level induce EEG activation, and rostral to the spinal level, since the encéphale isolé shows alternation of waking and slow wave sleep patterns.

In the third paper published in 1959, Batini *et al.* [18] performed olfactory and visual deafferentations in the midpontine pretrigeminal preparation. Olfactory deafferentation alone did not produce a modification of the EEG. Dual deafferentation induced slow wave patterns. However, 1 or 2 days later, the EEG activation reappeared. Consequently, they concluded that there existed "an autochthonous reticular tonus responsible for waking EEG activity" (p. 30).

In the last paper of the series published in the Archives Italiennes de Biologie by Moruzzi's group in 1959, Magni *et al.* [140] studied in cats the influence of injections of thiopental in different parts of the brain stem of the encéphale isolé preparation (Fig. 4). They made careful ligatures to isolate the lower brain stem structures from the anterior ones. Intracarotid injections of low doses of barbiturate induced homolateral synchronized patterns while high doses induced bilateral effects. The intracarotid injection induced sleep patterns by inactivating the anterior pons and midbrain reticular formation. In contrast, the intravertebral injection of low doses (which irrigate the posterior pons and the M.O.) induced a short latency and constant EEG activation. Moreover, apart from the effect of the barbiturate on the EEG, the intravertebral low dose injection suppressed reflexes commanded by the lower brain stem, which are normally very resistant to anesthesia. The authors concluded that the "exist-

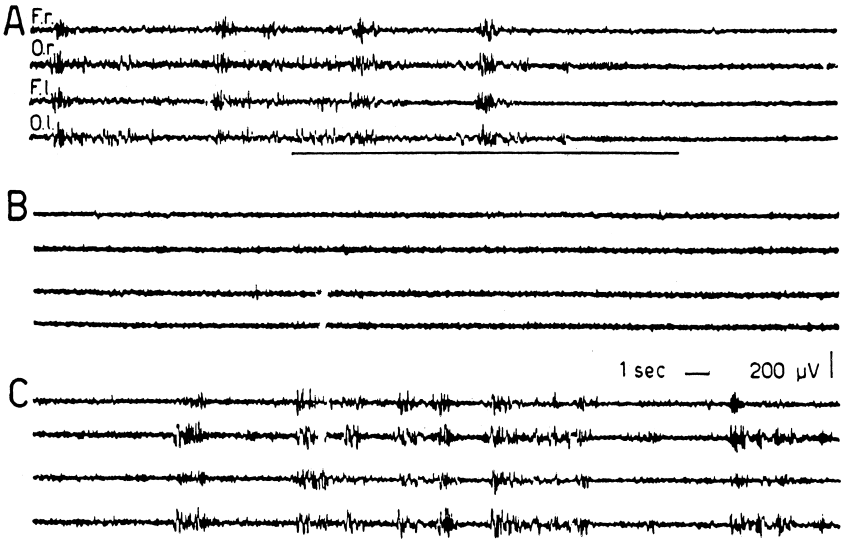

Fig. 4. Magni *et al.* (1959) performed clamping of brain stem vessels in order to control the functioning of caudal pons structures. Infusion of thiopental in the vertebral artery (black signal) induced a cortical arousal reaction in the sleeping cat. This experiment confirmed the importance of the posterior part of the hindbrain for slow wave sleep inducing mechanisms. There is a 5 sec interval between A and B and between B and C. Fr and Fl, right and left frontal cortex; Or and Ol, right and left occipital cortex. From Ref. [140], with permission.

ence of a EEG synchronizing mechanism in the medulla appears as the simplest explanation'' (p. 44).

Finally, Cordeau and Mancia [54], of the same Pisa group, published the same year a paper the title of which is clear ''Evidence for the existence of an electroencephalographic synchronization mechanism originating in the lower brain stem''. On chronic midpontine pretrigeminal hemisections undertaken in cats, the authors showed that although the ipsilateral cortex remained at low voltage, the contralateral hemisphere showed a greater tendency to synchronize. The same asymmetry was found with more caudal hemisections down to the middle level of the M.O. The asymmetry disappeared with more caudal transections. The authors hypothesized ''bulbar synchronizing structures'' which could act directly on cortical neurons, or on the ascending brain stem activating reticular system or on the thalamic pacemaker of spindles. Finally, based on their knowledge at that time, the authors raised the question, though providing no answer, regarding the functional relationship between the bulbar synchronizing center and the inhibitory reticular formation described by Magoun [141].

The following year (1960), Moruzzi [164] wrote a detailed review on the interpretation of previous results regarding the synchronizing influences of the brain stem. In the last part of the paper, he discussed the possible functioning processes of falling asleep, and concluded on two hypotheses:

1. besides the reticular activating system, there is an antagonist group of synchronizing, and possibly sleep inducing, structures mainly (though not exclusively) localized in the lower brain stem;
2. the reticular activating system is excited by any sudden change in the environment (orienting reflex, Pavlov's external inhibition) while the synchronizing structures of the brain stem are endowed with the opposite property of responding with an avalanching increase of their activity whenever a prolonged sequence of monotonous sensory stimulation is applied to a large number of receptors.

These two hypotheses account satisfactorily for the habituation of the EEG arousal (as described by Sharpless and Jasper [228]) and for the ''sleep elicited during habituation or by conditioned stimuli'' (p. 252) (as shown in Pavlov's group).

Researching along similar lines, Bonvallet and Bloch [26], in 1960, published a paper in French on the bulbar control of cortical activations. A year later, a second paper in English by the same authors [27] was published in *Science*. This paper was essentially a translation of the previous one. The authors used cats transected at T2 level, under Flaxedil and slightly nembutalized (3–7 mg kg^{-1}). ''Short stimulation of the mesencephalic reticular formation induces a brief and intense burst of cortical activation but an identical stimulus applied a few seconds later results in a second cortical response which is less intense and much shorter than the first one. However, in the same preparation, after a prebulbar brainstem transection, the two cortical bursts of activation produced by two consecutive reticular stimuli are of the same intensity and duration'' (p. 1134). The reduced duration of activity of the second stimulation in the intact cat does not result from reticular fatigue but from an active inhibitory mechanism lying below the transection. Moreover, when a reticular or somesthetic nerve stimulation induces a cortical activation, slow wave sleep patterns reappear in < 40 sec, despite continuous stimulation. Following medial bulbar transec-

tion, the activation does not disappear 3 or 4 min after stimulation. Finally, when reticular stimulations of progressive intensity are delivered to reach the usually cortical activation threshold within 1 or 2 min, the cortical activity remains synchronized. After medial prebulbar transection or novocainization of the caudal M.O., a progressive increase of cortical activation occurs. Similar results were obtained in unanesthetized encéphale isolé preparations. The authors concluded that the bulbar deactivation structures are the same as those described by Moruzzi's group. At last, they hypothesized "that it is the reticular activating system itself which triggers the reticular bulbar antagonistic mechanism" (p. 1134).

At the same time, Ho *et al.* [100] demonstrated the predominance of cortical slow wave sleep patterns in the encéphale isolé preparation. The deafferentation theory postulated by Bremer [35] was based on the results obtained with the cerveau isolé preparation. The authors wanted to verify this theory on the low brain stem transected animal. Indeed, Bremer [36] was the first to claim that the animal showed alternating waking and slow wave sleep activities. However, Ho *et al.* [100] noticed that several authors produced figures with recordings of encéphale isolé preparations with cortical synchronized activities. As a result, they undertook spinal transections at C_1 level in animals placed in "a large electrically shielded, sound-proof, light-tight box in which the air was kept moist and warm" (p. 86). The results clearly showed that, soon after transection, cortical slow wave sleep patterns were predominant which could last several hours. The same synchronized patterns could be observed soon after spinal injection of novocaine. The authors concluded that "when electrical activation and attentive wakefulness occur in this (encéphale isolé) preparation, they should be considered the result of circumstances which alter the fundamental sleepy state in which it usually exists" (p. 89). This result was confirmed a year later by Magnes *et al.* [138] (p. 36 see below).

Indeed, in 1961, Moruzzi's group [138] published the results of research showing cortical synchronization produced in encéphale isolé cats by low-frequency electrical stimulation in the region of the M.O. solitary tract nucleus. Magnes *et al.* [138] first explained that they chose to stimulate this structure since "this area receives afferent fibers from baroreceptors of the carotid sinus and the arch of the aorta. (Moreover), Koch [123] in 1932 showed that distension of the carotid sinus may cause a sleep-like state in the unanesthetized dog or monkey and Bonvallet *et al.* [32], in 1953, were able to produce EEG synchronization in the encéphale isolé preparation, hence in the complete absence of changes of blood pressure and respiration, by stimulating the baroreceptors of the carotid sinus in the same fashion" (p. 34). The authors also recalled that several researchers were already able to induce EEG synchronization by midbrain low rate stimulation. Magnes *et al.* [138] showed that the EEG waves induced by 1 to 10 c sec^{-1} stimulation of the nucleus of the solitary tract were similar to spontaneous slow waves (Fig. 5). "In fact it was usually impossible to distinguish the pattern elicited by electrical stimulation of these medullary structures from the spontaneous spindle bursts that occasionally appeared in the same cat" (p. 37). However, these waves differed from those induced by the midline nuclei of the thalamus. Indeed:

1. there was no evidence of recruitment of cortical neurons even with threshold stimuli;
2. the repetition rate of the elicited slow waves was unrelated to that of the stimulus over a fairly wide range;
3. in many experiments intermittent synchronization of the EEG persisted for some time after interrupting the electrical stimulus, though prior to stimulation the background of activity had been desynchronized (p. 37).

The best conditions to obtain synchronized activity was "a faint suggestion of spontaneous synchronization" (p. 40). Thus, the synchronization could outlast the stimulation. When the degree of activation was too high, or the synchronization already high, it was difficult to induce synchronized patterns. However, higher frequency stimulation (i.e. 26 c sec^{-1}) induced arousal. In neighbouring M.O. levels, stimulation at low frequency even induced low voltage cortical activity. The anatomophysiological relationship showed that the synchronization processes were only induced by stimulation of the solitary tract, the nucleus of the solitary tract and also the nucleus reticularis ventralis. Finally, the synchronized response may cross in the brain stem. In the discussion, the authors stated that synchronization could be related "either to a reciprocal inhibition of the brain stem activating system leading to the release of the diencephalic pacemaker or to direct excitation of EEG synchronizing structures of the diencephalon, mainly the thalamus" (p. 63). In favor of the first hypothesis is the fact that EEG synchronization may significantly outlast termination of bulbar stimulation. On the other hand, the fact that a single pulse was able to induce a slow wave burst and that the frequency of the wave may slightly increase as the frequency of stimulation increases "speaks in favour of a more direct effect on the thalamic centers. Obviously, these two hypotheses are not mutually exclusive. In fact, the best way to explain the results would be if the two mechanisms were working hand in hand" (p. 64).

The same year, Favale *et al.* [76] stimulated the reticular formation from the midbrain to the medulla in 40 cats. This paper was published in the same issue of the *Archives Italiennes de Biologie* as the previous paper by Magnes *et al.* [138]. Favale *et al.* [76] observed synchronization of the EEG at the most suitable rate of stimulation situated between 4 and 14 c sec^{-1}. At the M.O. level, they obtained effects when stimulating the nucleus gigantocellularis, the nucleus parvocellularis and the nucleus ventralis. They did not stimulate the lateral and paramedian reticular nuclei projecting to the cerebellum. At all positive points, synchronization most often was not obtained by the first pulses but by the following ones. EEG synchronization was obtained in relaxed animals when the previous EEG activity was slightly synchronized. No effect was observed during fully activated EEG activity.

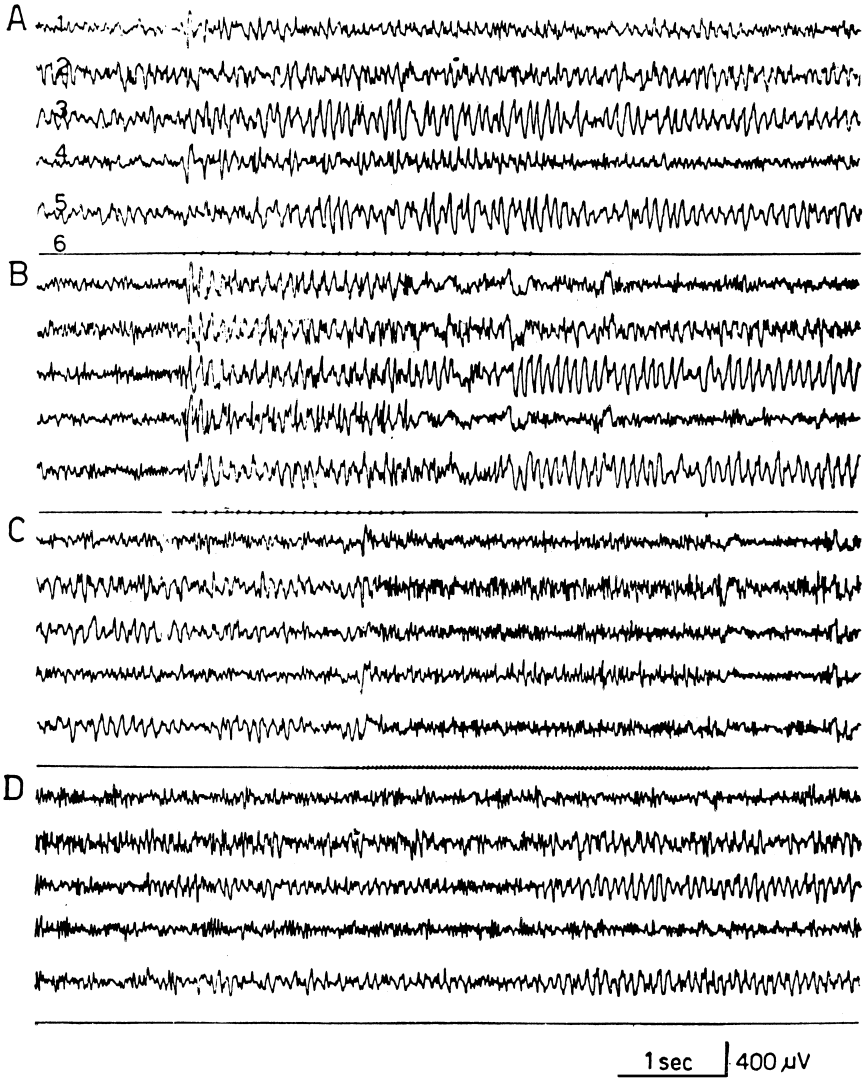

Fig. 5. Magnes *et al.* (1961) observed in cats that stimulation of the solitary tract nucleus, at low frequency (6 sec^{-1} in A, 10 sec^{-1} in B), induces slow high amplitude patterns in the cortex, whereas higher frequencies (26 c sec^{-1} in C) induce activation with an after-effect in the (D) continuous recording. 1, Right parietal cortex; 2, right temporal; 3, right occipital; 4, left parietal; 5, left occipital; 6, stimulus marker. From Ref. [138], with permission.

Behavioral sleep inducing effects could be observed when the synchronization outlasted the duration of stimulation. In the discussion, the authors first analyzed the hypothesis that the effect was caused by reticular synaptic fatigue or by "reticular units endowed with an antagonist (EEG synchronizing or sleep-inducing) influence on the cerebrum" (p. 18). They concluded that the strict temporal link between stimulation and EEG response of 'phasic'* type and the quick and complete reversibility of the 'tonic' effect did not argue in favor of reticular fatigue. They thought, on the contrary, that the "EEG and behavioral effects are probably due to the excitation of...neurons having an EEG-synchronizing and sleep-inducing influence" (p. 20). Are the corresponding influences acting on the reticular activating core or directly on thalamic EEG synchronizing structures? The authors think that the 'phasic' effect seems to be related to direct thalamic influences while the 'tonic' effects could result from influences on the activating reticular core. Favale *et al.* [76] refer to the previous paper by Magnes *et al.* [138] to suggest the role of the nucleus of the solitary tract as the source of the synchronizing influences.

Several investigations into the control of ascending sensory influences in the M.O. relay nuclei were undertaken within the space of a few years. They will be analyzed together. It is worth mentioning that Hernandez-Peon *et al.* [99] in 1956, and Scherrer and Hernandez-Peon [225] in 1958, had published two papers containing the same figures showing that stimulation of the midbrain and pontine reticular formation reduced the postsynaptic

*The authors refer to Moruzzi and Magoun's [169], Hagbarth and Kerr [96] and Sharpless and Jasper's [228] dissociation of phasic and tonic central processes.

evoked potential induced in the M.O. gracilis nucleus by dorsal column stimulation. The presynaptic component was unchanged. Moreover, Hagbarth and Kerr [96] in 1954, had already shown that stimulation of the midbrain and M.O. reticular formation decreases the amplitude of sensory evoked potentials, the primary response remaining unchanged. Direct pyramidal postsynaptic inhibitory influences on the gracilis and cuneate nuclei, without reticular mediation, were also observed by Magni *et al.* [139]. However, Towe and Jabbur [248] in 1961 studied extensively cortical influences on the unit activity of the cuneate nucleus in pentobarbital anesthetized cats immobilized by flaxedil or decamethonium. The M.O. response was induced by cutaneous electrical and natural stimulation at various times following cortical conditioning stimulation. Only the sensorimotor cortex was able to modulate cuneate nucleus activity. The peripherally induced responses were either facilitated or inhibited, but rarely both. The depressive influence resulted from active inhibition and not from any occlusive mechanism. Stimulation of the contralateral cortex was more effective than stimulation of the ipsilateral cortex. The cortical depressive influence persisted after midbrain reticular lesion but disappeared after bilateral section of the bulbar pyramidal tract. "It is thus evident that the cerebral cortex is capable of modulating its input from the dorsal column nuclei" (p. 497). The cortical facilitating activity of the same M.O. nucleus studied by Jabbur and Towe [111], also in 1961, concerned 78 out of 189 neurons. The latency of contralateral stimulation was shorter than that of ipsilateral stimulation. The effect also disappeared after pyramidal tract transection while slight inhibition persisted. Consequently, "the excitatory pathway from the cortex to the dorsal column nuclei appears to be exclusively the pyramidal system (while) the inhibitory pathway...appears to involve the pyramidal system and an extrapyramidal system at least as far as the bulb" (p. 508).

The group of Eccles then published a series of papers on the same subject. In the first, published in 1962, Andersen *et al.* [5] described in cats a presynaptic inhibition regulating transmission processes in the cuneate nucleus. This inhibition was induced by conditioning stimuli applied either to afferents or to the contralateral sensorimotor cortex. They induced an increase in excitability in the primary afferent terminals which decreased progressively when going downwards from the synaptic terminals to the lower brain stem level. The authors hypothesized that the neurons which had been activated by cortical stimulation in Jabbur and Towe's [111] study were interneurons responsible for the presynaptic depolarisation of the synaptic terminals. This depolarization induces an inhibition by reducing or suppressing transmitter release, as shown by Wall [262].

In the first corresponding full paper published in 1964, Andersen *et al.* [6] detailed the results of antidromic stimulation of the cuneate nucleus afferents. They showed that "when the test pulse was preceded by conditioning volley in the ulnar or the superficial radial nerve or a brief cortical tetanus the first part of the antidromic response increased indicating increased excitability of the (recorded) median nerve terminals" (p. 93). They gave detailed results showing that excitability dropped rapidly at more caudal levels.

In the second contemporaneous full paper, Andersen *et al.* [7] distinguished relay cells from interneurons in the cuneate nucleus. The first ones (78 out of 147 recorded cells) were invaded antidromically from the lemniscus medialis and orthodromically activated by afferent nerves. The second

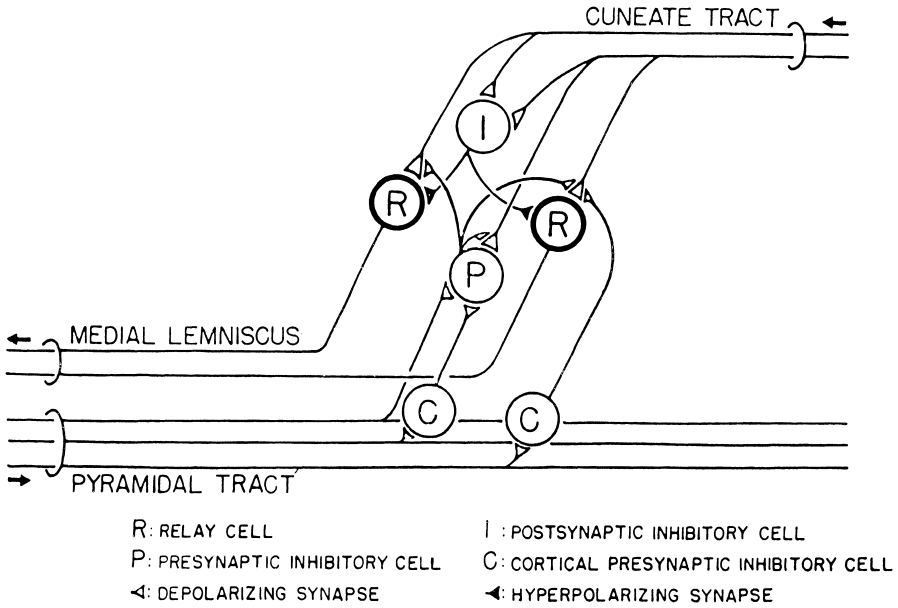

Fig. 6. Transmission processes in the cuneate nucleus. Andersen *et al.* (1964) identified in cats both presynaptic and postsynaptic inhibitory influences on the relay cells which transmit sensory and proprioceptive information to the lemniscus medialis. From Ref. [7], with permission.

group (69 cells) "were not antidromically invaded by volleys from the lemniscus medialis. They were further subdivided according to whether they were excited from the cortex or from peripheral nerves" (p. 1094). The authors hypothesized that there are presynaptic inhibitory acting interneurons activated either by the pyramidal tract or by the cuneate tract and postsynaptic acting interneurons activated by the cuneate tract.

The third full paper also published in 1964 by Andersen *et al.* [4] demonstrated this postsynaptic inhibition by inhibitory postsynaptic potentials (IPSP) recorded by intracellular recordings with glass microelectrodes (Fig. 6). This complementary inhibition could be induced by afferent and cortical stimulation. The authors also mentioned that their results cannot be identical to those of Scherrer and Hernandez-Peon [225] who found an inhibition by cortical and reticular stimulation with a latency of about three seconds, a maximal effect after about 30 sec, the duration ranging between 90 and 120 sec. Here, the duration is only about 200 msec. From the general standpoint of sleep regulation, these results show that structures situated above the M.O. level influence sensory afferent information at the first relay. In particular, "the cerebral cortex participates in the negative feedback controls that are exerted on the transmission through the cuneate nucleus of impulses destined for the cerebral cortex" [4] (p. 1113).

Already in 1962, there appeared the full paper on neurophysiological sleep–waking processes by Jouvet [113] in the *Archives Italiennes de Biologie*. In relation to our topic, the significant data concern the results obtained by retropontine transections made in chronic cat preparations. Indeed, Jouvet and Michel [115] had previously shown pontine and peripheral criteria of paradoxical sleep which appear in the animals with transections in front of the pons. These retropontine animals never showed disappearance of dorsal neck muscle activity. There was a clear-cut predominance of cortical activated activities (which is in agreement with previous results from Moruzzi's group). However, Jouvet [113] stated that some periods resembled cephalic sleep. Nevertheless, without myographic criteria it was not possible to assert that it corresponded to paradoxical sleep.

Bonvallet and Allen [25] in 1963, continued their study on the M.O. ascending antagonistic influences of waking processes. This paper demonstrated that the nucleus of the solitary tract is the origin of these influences, confirming the Moruzzi group hypothesis. First, the authors made complete low M.O. transections* in encéphale isolé cats. Following stimulation of the activating reticular formation, they induced long-lasting inhibition of the ciliary nerve and substantial lengthening of correlative activation of the EEG. The same effects were observed after limited lateral transections. These tonic modifications were characterized by fluctuations of the cortical activation and pupillary constrictory tone. The authors further showed by lesions that the ventromedial inhibitory reticular formation [145] is not responsible for these effects. The active zone was the rostral part of the solitary tract nucleus. Since this structure is innervated by cardiovascular afferents, they verified that the transection of the IXth and Xth nerves did not suppress these EEG and pupillary effects which, nevertheless, were spontaneously prolonged after this deafferentation. It was already known that the reticular activating system controls EEG activation [169] and pupillary function (by increasing the sympathetic pupillary-dilating tonus and inhibiting the parasympathetic constrictory influences of the Edinger–Westphal nucleus [30]). Since these two physiological parameters change simultaneously after lesion of the nucleus of the solitary tract, the authors concluded that this structure directly antagonizes the influences of the reticular activating system (Fig. 7). Indeed, the effects on pupils persist in mesencephalic animals. Consequently, it was thought possible that the reticular activating system could be controlled by antagonistic M.O. ascending influences, as well as by cortical diffuse descending influences previously identified by Hugelin and Bonvallet [108, 109]. At the congress hold in Lyon in September 1963, Bonvallet and Dell [28] emphasized that there is a double component in the reticular formation activating response, a phasic stereotyped one and a tonic one, which are often indissociable. However, the M.O. inactivating influences act only on the latter which is markedly prolonged with fluctuations after lesion of the nucleus of the solitary tract. The first response could result from the activation of paucisynaptic circuits and perhaps corresponds to the startle reaction. The late response would imply plurisynaptic circuits which are probably looped. In this paper the M.O. inactivating ascending influences were confirmed by coagulation of the cephalic pole of the solitary tract nucleus which induced "considerably prolonged responses of all recorded activities to brief sensory or mesencephalic stimulation" (p. 133).

Also in 1963, Cordeau *et al.* [55] in Montreal studied the effects of a direct brain stem injection of adrenaline and acetylcholine in chronic cats. Up till then, only circulating influences of adrenaline [31, 208] and acetylcholine acting compounds [34] had been studied. The authors injected slowly, over a period of 100 sec, 20 mg of adrenaline and acetylcholine in the M.O., pons and midbrain reticular formation. At M.O. level, adrenaline induced behavioral arousal and EEG activation. However, it was often accompanied by retching and vomiting or profuse salivation and frequent swallowing. "In these cases arousal may have been secondary to the autonomic effects of the adrenaline injection" (p. 35). But in some cases arousal occurred "without any overt evidence of nausea". Acetylcholine injections "were usually followed by behavioral sleep and electrocortical synchronization. In most instances, the slowing of the EEG trace began some 60–120 sec following the end of injection, but it could occur earlier" (p. 35). It is worth

*Although the authors called the transection 'low', it had to be situated in front of the solitary tract nucleus. Indeed, their figure 1(A) shows that the transection was not situated at the lowest M.O. level.

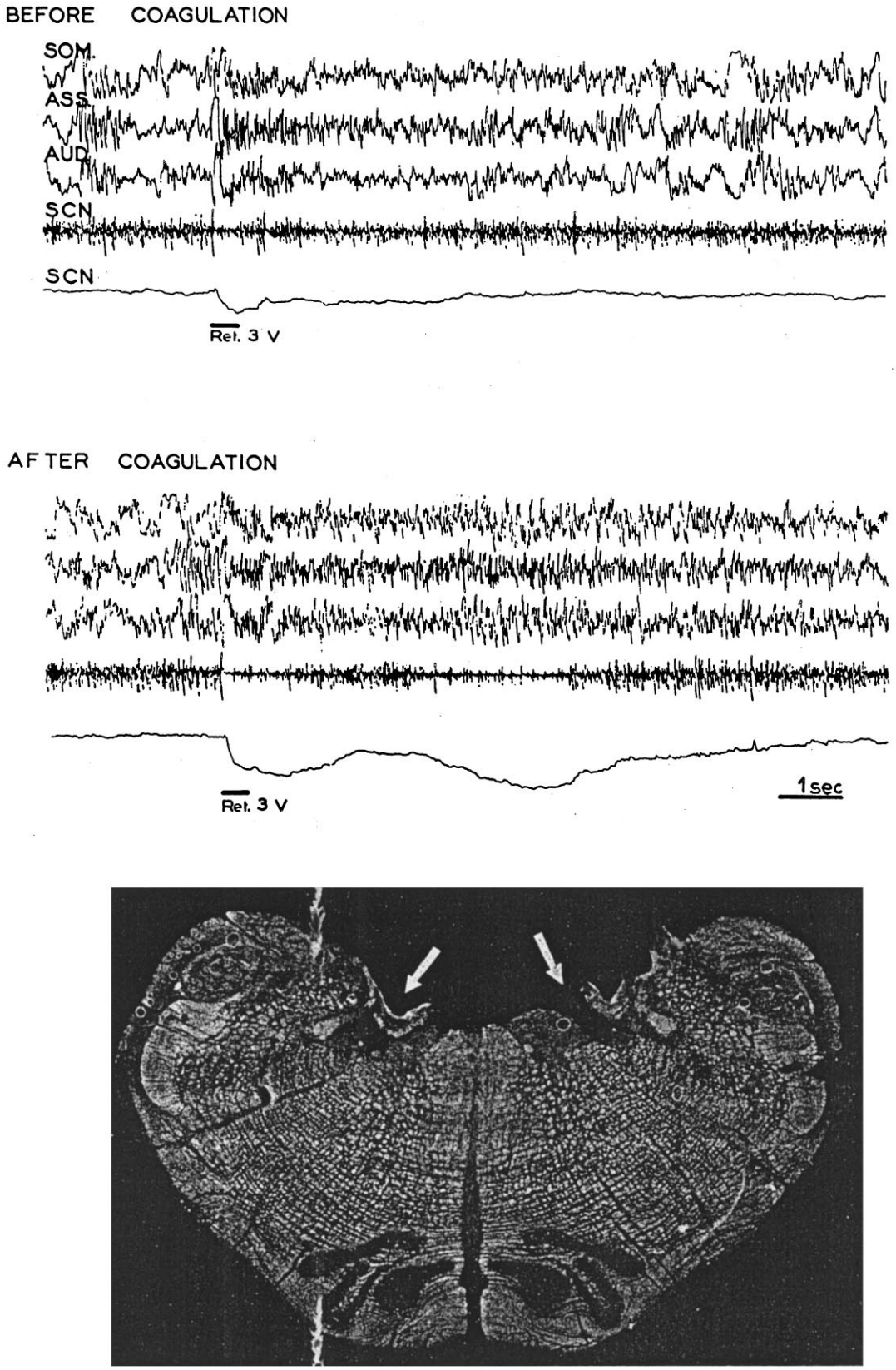

Fig. 7. Bonvallet and Allen (1963) performed in cats small bilateral lesions of the solitary tract nucleus level (bottom). They induced a significant increase of the duration of cortical activation generated by stimulation of the mesencephalic reticular formation. The inhibition of short ciliary nerve responses was also of much longer duration. This experiment confirmed the importance of this structure as origin of influences antagonist of the waking system. Som, ASS, AUD, Somesthetic, associative, auditive cortex; SCN, direct and integrated recordings of ciliary nerve activity. From Ref. [25], with permission. Bottom: from Ref. [28], with Elsevier's permission.

noting that, in one case after M.O. injection and after rostral pontine tegmentum injection of acetylcholine, the authors were able to induce paradoxical sleep. It was probably the first observation, definitively established 1 year later by George *et al.* [85] after pontine cholinomimetic compound injection.

In 1964, Moruzzi's group (Berlucchi *et al.*) [23] further studied in encéphale isolé preparations, the M.O. antagonistic influences of the reticular activating system by cooling the floor of the fourth ventricle in front of the obex, under which the nucleus of the solitary tract is situated (Fig. 8). Curiously, we could not find quantitative details regarding the level of temperature lowering induced by the thermode. The cooling triggered EEG activation and pupillary dilation which lasted as long as the cooling procedure. In some cases, the responses were induced after adding very small doses of Nembutal (0.5–5 mg kg^{-1}). "The synchronization reappeared when the thermode was turned off, but the rate of recovery depended upon the background activity before the cooling inactivation. In the preparation which had a marked tendency to fall asleep spontaneously, the EEG synchronization reappeared within a short period of time, ranging from a few seconds to 1 or 2 min after cooling. However, when the preparation turned out to be remarkably resistant to EEG synchronization, so that minute doses of Nembutal had to be injected in order to produce a proper background of reversible sleep, the EEG arousal had a tendency to outlast the period of cooling and occasionally the EEG synchronization reappeared only after injecting another dose of Nembutal" (pp. 378–379). During the EEG activation, the animal was clearly awake with searching movements of the eye and pupillary dilation. On the contrary, EEG synchronization was observed after upper pons cooling. The authors concluded that these results confirm the M.O. inactivating and synchronizing influences already shown by Magni *et al.* [140] by intravertebral injection of barbiturate. However, its origin (the solitary tract nucleus) was

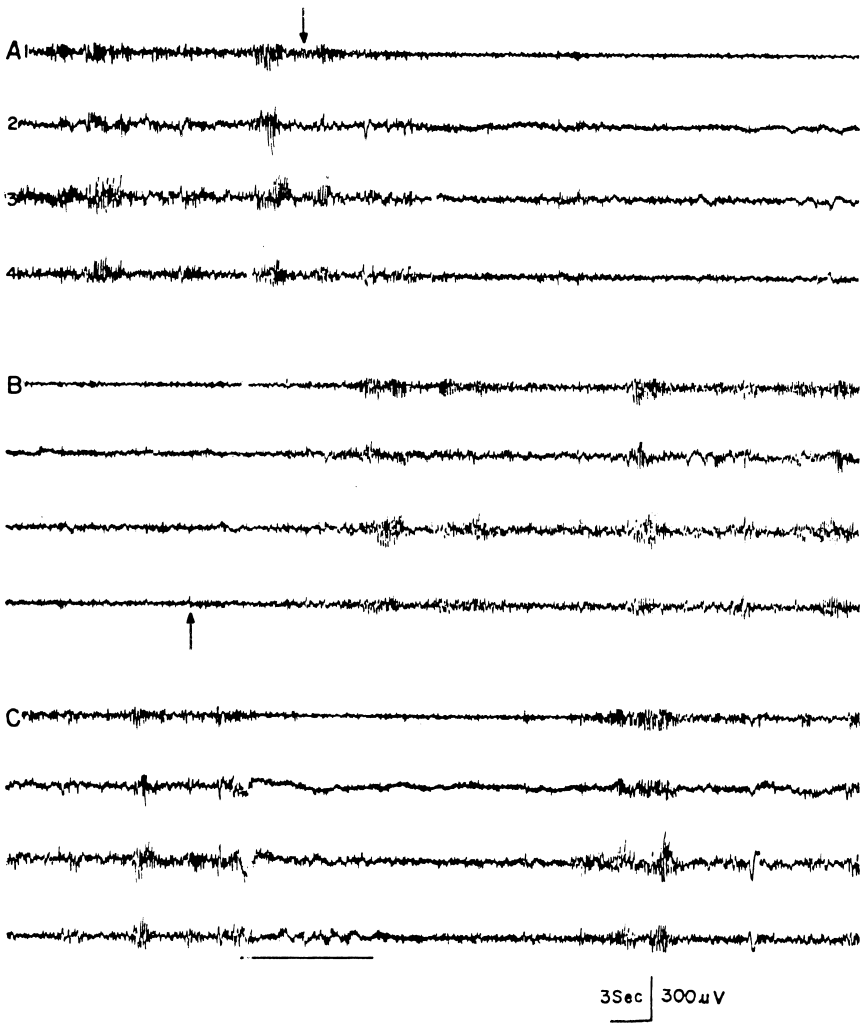

Fig. 8. Berlucchi *et al.* (1964) also confirmed in cats the waking antagonistic influences of the solitary tract nucleus level by thermode local cooling which induced cortical arousal. The arrow shows the beginning and end of cooling. The continuous line indicates an acoustic stimulation (whistle). Uninterrupted recording. 1, Left fronto-parietal cortex; 2, left parieto-occipital; 3, right fronto-parietal; 4, right parieto-occipital. From Ref. [23] with permission.

more clearly shown by Bonvallet and Allen's [25] paper. In 1965, Berlucchi *et al*. [24] presenting the same results, emphasized the fact that "the M.O. tonic activity of synchronizing centers, antagonist to the reticular activating system, prevails over the activating effect" (p. 89).

During this period crucial papers were published on neurochemical anatomy. Although Eränkö [72] had shown in 1955 that noradrenaline and adrenaline neurons of the adrenal medulla become fluorescent when fixed in formalin, it was Falck *et al*. [74] in 1962 who clearly demonstrated that catecholamines become fluorescent when condensed with formaldehyde vapours. "Dopamine, noradrenaline and adrenaline were treated with an excess of formaldehyde either in acid or at pH 5" (p. 348). They became "intensely green to yellow. However, adrenaline gave a much weaker fluorescence which furthermore develops more slowly in the formaldehyde treatment" (p. 349). "The three OH groups seemed to be essential for the reaction" (p. 353).

In 1964, Dahlström and Fuxe [57], in their well-known paper, described the central distribution of mononamine-containing neurons. Four hundred rats were used for the study. The catecholaminergic "nerve cells showed a weak to strong green fluorescence in normal animals and animals treated with monoamine (MAO)-inhibitors. The light emitted may be more yellow-green if the formaldehyde treatment is prolonged to 3 h" (p. 12). Serotoninergic (5-HT) nerve cells showed "a very weak or weak or not at all yellow fluorescence without treatment but a medium or usually strong yellow fluorescence after the administration of potent MAO-inhibitors" (p. 12). At the M.O. level, the authors first distinguished catecholaminergic groups. Group A_1 mostly contained descending axons. Group A_2 was seen with varicose fibers ending in the area of the nucleus of the solitary tract, where the terminals were later shown to be axo-dendritic [80]. The serotoninergic cell bodies were situated in the raphe nuclei. Group B_1 "surrounded the medial and ventral surface of the pyramidal tract from the level of the motor decussation up to the beginning of the nerve facialis. Most of the cells were present within the nucleus raphe pallidus" [57] (p. 15). Group B_2 was within the nucleus raphe obscurus. Group B_3 was mainly localized in the nucleus raphe magnus. "It must be pointed out, however, that no distinct borderline existed between groups B_1 and B_3" (p. 16). Many axons descended to the spinal level. Finally, in the postrema area, there were noradrenergic and serotoninergic nerve cells and in the nucleus of the solitary tract there were 5-HT terminals with no close contact with the cell bodies [80]. These papers opened up the field of the monoamine contemporary history of "wet neurophysiology" [226], as mentioned by Jouvet [114].

Also in 1964, Fuxe and Owman [81] described monoaminergic neurons in the area postrema. The study, performed in rats, guinea-pigs, rabbits, cats, dogs and monkeys, showed green fluorescent-colored cells characteristic of catecholamines and yellow-colored serotoninergic neurons. The number of catecholaminergic cells varied with the species and the terminals partly ended in the solitary tract nucleus. Some axon terminals were hypothesized to end in the lateral reticular formation. Serotoninergic cells were found only in rats. Fine nerve fibers with green and yellow varicosities were observed within the area. This paper improved previous findings suggesting that "the area postrema…is built of non nervous tissue only" (p. 342).

All data relative to the regulation of reticular formation up to 1964 were reviewed by Moruzzi [166].

The variations of cuneate (and gracilis) nucleus transmission level during sleep–waking cycle were studied in 1965 by Favale *et al*. [75] in cats. The peripheral subcutaneous stimulation induced a lemniscal "fast positive deflection (initial latency 3–3.7 msec) followed by a slower and smaller negative deflection" (p. 361). "The afferent transmission at the level of the first station (gracilis and cuneatus nuclei) does not seem to be affected by the depth of sleep, being unchanged during deep sleep (paradoxical sleep) as compared to light sleep (slow wave sleep)" (p. 367). During arousal from paradoxical sleep, there was a reduction of the response. Finally, during strong arousal, the responses were decreased or unchanged. Consequently, unlike the thalamic level, the M.O. did not play a major modulation role of somatic afferents during the sleep–waking cycle. However, it is worth observing that, just previously, Okuma and Fujimori [180] had showed that the response recorded in the bulbar reticular formation after cutaneous stimulation decreased slightly from waking to slow wave sleep and markedly during paradoxical sleep. These responses occurred with a long latency (peak latency of about 30–40 msec for the second negative component). Finally, the response induced in the midbrain reticular formation by M.O. reticular pulses also varied during the sleep–waking cycle. The initial short latency components slightly increased from waking to paradoxical sleep but the following negative component showed no significant changes in slow wave sleep but decreased markedly during paradoxical sleep.

In 1965 again, Padel and Dell [183] recorded in cats several brain stem regions with microelectrodes. At M.O. level, they found an increase of firing in the solitary tract nucleus during spontaneous drowsiness or after stimulation of aortic baroceptive fibers which induced closing of pupils after 3 or 4 sec and slow waves and spindles after 10–15 sec. The authors concluded that these results confirm the active role played by this structure during falling asleep. It should be added that previous and contemporaneous data showed that this kind of vegetative afferent stimulation, although most often inducing EEG activation [29, 32, 52, 93, 267], is capable of inducing slow waves and spindles [21, 29, 32, 52, 93]. These synchronizing effects generally appear after longer duration stimulation [29] and are preferentially obtained by stimulation of rapid conducting fibers while the stimulation frequency "is of little consequence in the manifestation of this response, for low (< 25/sec) and high (100/sec) frequencies were effective" [51] (p. 243) (Fig. 9).

Caspers [45], in the Lyon meeting report published in 1965, showed in rats that the cortical

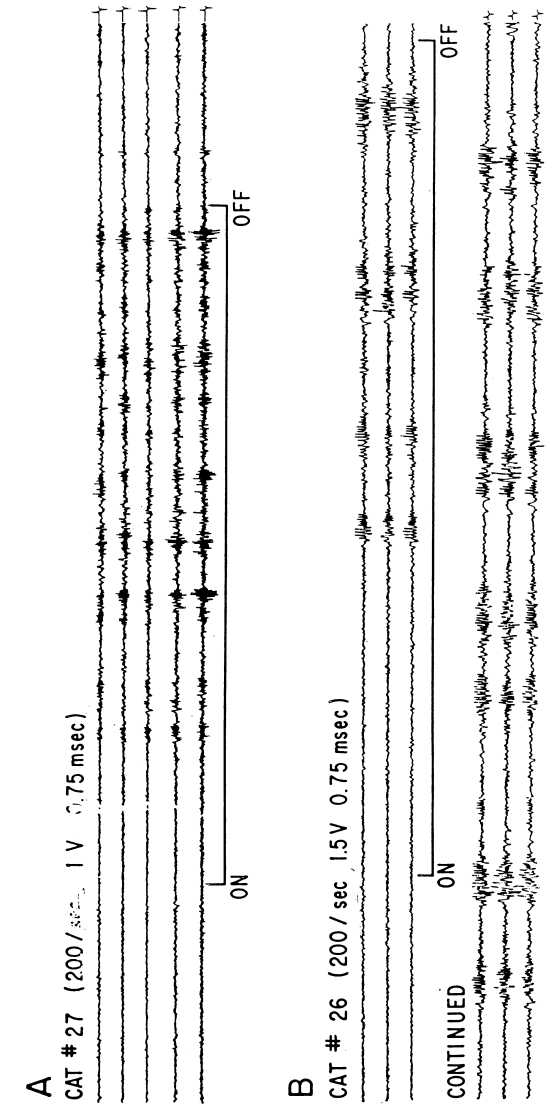

Fig. 9. Chase *et al.* (1966) stimulated unilaterally the cut end of vagus nerve of cats and, with a latency, induced cortical slow wave sleep patterns. This fact was previously mentioned by Bonvallet and Sigg (1958). LFP and RFP, Left and right fronto-parietal cortex; LPO and RPO, left and right parieto-occipital cortex; TR–FR, right temporo-frontal cortex. From Ref. [52], with permission.

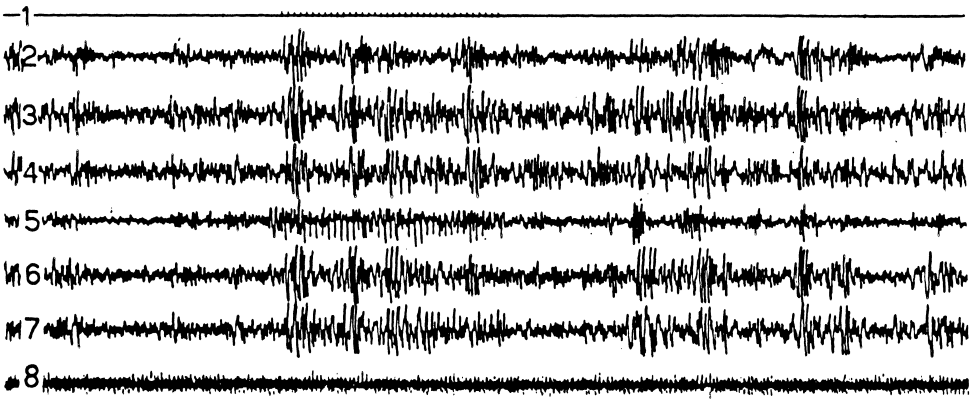

Fig. 10. The team of Pompeiano (1965) showed in cats that low frequency stimulation (5 Hz) of a cutaneous nerve, at low intensity, induces phasic and tonic synchronization of the EEG by primary action on the cuneate nucleus. The fact that the effect outlasted the stimulation duration suggested a secondary direct or indirect deactivating influence on the activating reticular formation rather than a direct influence on the thalamic synchronizing processes. 1, Marker; 2, left fronto-frontal cortex; 3, left fronto-temporal; 4, left temporo-occipital; 5, right fronto-frontal; 6, right parieto-temporal; 7, right temporo-occipital; 8, EMG. Modified from Ref. [188], with permission.

steady potential becomes more positive during the transition from waking to sleep. Correlative brain stem unit recordings showed a "marked activation at the onset of the positive DC shift. The histological controls revealed a concentration of recording points around the nucleus of the solitary tract" (p. 217).

Hernandez-Peon [98], in his paper at the same meeting, described in cats a forebrain descending cholinergic sleep system. Although he did not work on the medulla, he stated that "it is likely that the descending forebrain hypnotic pathway may join the ascending bulbo-pontine structures at pontine level in order to inhibit directly the mesodiencephalic vigilance system" (pp. 75–76).

Also at the Lyon's symposium, Pompeiano [188] delivered a synthesis of previously published papers [189–191]. The review partly concerned the ascending influences of somatic afferents. In chronic cats, "low rate stimulation of group II cutaneous afferents was capable of eliciting EEG synchronization (Fig. 10). On the other hand, high rate stimulation of group II fibers, as well as low or high rate stimulationof group III cutaneous fibers induced EEG and behavioral arousal...Touch receptors are essentially confined to the group II range, hair and pressure receptors account for a large proportion of both group II and III cutaneous afferents, while fibers belonging to temperature-sensitive and pain receptors appear to be restricted to the group III range of myelinated fibers" [190] (pp. 363–364). Microelectrode recordings of the brain stem showed that stimulation of group II afferents activated particularly the neurons of the M.O. nucleus reticularis gigantocellularis. As shown in Fig. 6 [191], the neurons of the solitary tract seemed to be fairly unaffected. In contrast, low rate muscular afferent stimulation did not induce synchronization. The only observed effect was an arousal reaction. Pompeiano [188] discussed the point that it was difficult to determine whether the synchronizing influence acted directly on the thalamic synchronizing structures or inactivated the activating reticular formation by acting at M.O. level on inactivating processes, with a secondary release of thalamic ones. However, the fact that EEG synchronization often outlasted the stimulation was an argument for the indirect influence of M.O. on the activating reticular core. These results have to be related to synchronizing influences of the spinal level as identified by unilateral novocaine injection which induces bilateral activation of the EEG in the flaxedilized cat [103].

Naquet *et al.* [172], in 1966, in turn undertook a study in chronic cats to determine the influence of cooling of several central levels from the M.O. up to the thalamus. The medulla was cooled at the obex and reticular levels. The thermode was first maintained at a temperature between 0 and 10°C. This did not modify the EEG and the pupil state. Between −5 and −20°C, the cooling induced either no change or EEG activation. For still lower temperatures, between −20 and −50°C, there was clear EEG activation, behavioral arousal with whiskers and jaw movements but without changes to the pupils. All these effects were transitory and disappeared prior to the arrest of the prolonged cooling. These results confirmed those of Berlucchi *et al.* [23, 24] obtained in encéphale isolé preparations. At the M.O. level, the new finding was the limited duration of the effect.

Also in 1966, Rosina and Mancia [205] in chronic cats clamped the basilar artery in order to inject thiopental specifically at rostro-pontine, mid-pontine and caudo-pontine levels. When the barbiturate was injected at caudo-pontine level during waking there was no change in the animal's state. When it was injected during slow wave sleep, it frequently induced paradoxical sleep, generally 4–6 sec after the beginning of the injection and the episodes lasted for *ca* 60 sec (Fig. 11). "When the same drug was introduced during a spontaneous episode of paradoxical sleep, this natural phase was not generally interrupted. Occasionally the bursts of eye movements appeared to be slightly increased" (p. 160). "In all our preparations the concentration attained by thiopental in the blood supplying the

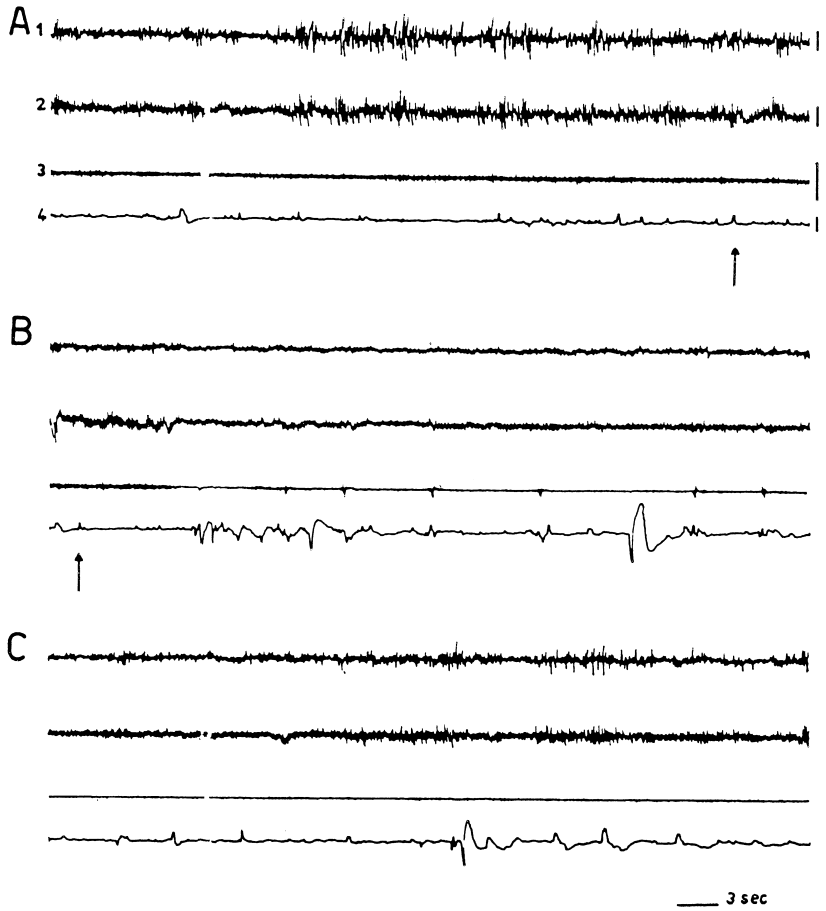

Fig. 11. Rosina and Mancia (1966) confirmed in cats the crucial role of the M.O. in paradoxical sleep inducing processes by barbiturate injection (between the two arrows) in the vertebral artery irrigating only the posterior hindbrain structures. Indeed, they could induce paradoxical sleep when the injection was performed during slow wave sleep. Moreover, injection during paradoxical sleep or during waking did not interrupt these behavioral stages. 1 and 2, Right and left fronto-parietal EEG; 3, EMG; 4, eye movements. From Ref. [205], with Elsevier's permission.

lower brain stem was, for a short time at least, presumably very high, as suggested by the bilateral disappearance of the corned reflex, which is known to be very resistant even to anaesthetic doses of barbiturate" (p. 163). The authors concluded that during slow wave sleep "an inhibitory influence arising in the lower brain-stem is acting on the more rostral placed neurons which are considered responsible for the appearance and maintenance of paradoxical sleep" (p. 164). The same effect during slow wave sleep injection was observed at midpontine level, while it induced waking at rostro-pontine level.

The same year, Koella and Czicman [126] studied the EEG-synchronizing action of 5-HT in the lower brain stem of sedated cats (Fig. 12). Injection in the carotid artery of 0.2–5.0 mg kg^{-1} of 5-HT induced a biphasic effect: first, a short-lasting EEG activation and inhibition of cortical recruiting waves induced by medial thalamic stimulation, then a long-lasting increase of slow waves and recruiting, while peripherally a myosis was observed. After midpontine transection, the synchronizing effect disappeared. Ten milligrams of 5-HT injected in the fourth ventricle in sedated normal animals increased slow waves and spindles. Then, the authors cauterized the area postrema placed side by side with the nucleus of the solitary tract. The synchronizing effects were always reduced and in a few cases were completely eliminated after intracarotid injection. The same effects were observed after local applied 5-HT blocking agents (LSD, methysergide). Finally, "topical application of 5-HT to the posterior region of the fourth ventricle induced an increase in the recruiting responses, an increase (or appearance) of spindle bursts and an increase in slow wave output often lasting as long as 15 minutes" (p. 931). Direct application to the area postrema also induced a hypersynchronization effect. Among the comments, the authors mentioned previous results showing the narrow functional relationship of the area postrema and the nucleus of the solitary tract [159] and the fact that the area postrema is surrounded by 5-HT nervous elements [81]. Moreover, it was established that the blood–brain barrier is reduced at this high vascular structure level. The general conclusion of the two last papers is that serotonin is involved at M.O. level in thalamocortical EEG synchronizing

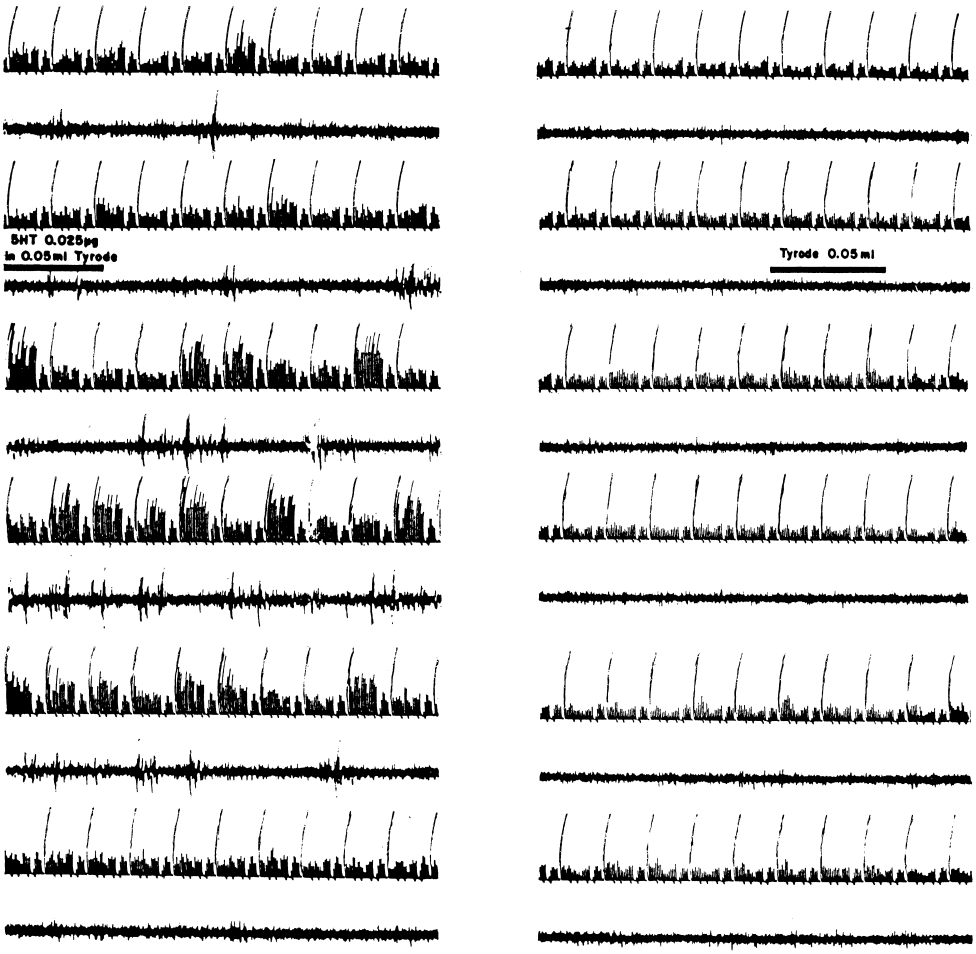

Fig. 12. Koella and Ciczman (1966) injected serotonin in the fourth ventricle of rabbits (left tracings). This rapidly induced cortical slow waves as shown by the EEG recording and frequency analysis (there are 10 sec between two large signals and, between them, from left to right, the frequency increases from 1.5 to 30 Hz). The effect lasted *ca* 6–7 min. On the right, control injection of tyrode. After cauterization of the area postrema, contiguous to the solitary tract nucleus, the effect disappeared. From Ref. [126], with permission.

processes. Some years later, Roth *et al.* [207] reached similar conclusions for rats. They cannulated "the posterior inferior cerebellar artery branch to the area postrema and adjacent medulla" and injected serotonin (1–50 mg ml^{-1}). Forty-three out of 49 trials induced cortical hypersynchronization patterns. In contrast, a noradrenaline injection (20 mg ml^{-1}) induced activation of the EEG while acetylcholine (20 mg ml^{-1}) and lactic acid produced no changes. The authors recalled that "the area postrema contains neural elements and has nerve fiber connections to the nucleus of the solitary tract which in turn projects to the dorsal vagal nucleus and the dorsal lateral bulbar reticular formation. Serotonergic nerve terminals and cell bodies have been demonstrated in the area postrema of the rat" (p. 231), as shown by Fuxe and Owman [81].

In 1966 also, Ledebur and Tissot [128] studied the effects of 5-hydroxytrytophane (5-HTP) and dihydroxyphenylalanine (DOPA) micro-injections in the brain stem on sleep and waking. This paper on monoamine precursor influence is still not based on the new findings of brain distribution of monoamines [57, 80, 81], but on previous research into sleep modulation by compounds given intravenously, intra-arterially or intraperitoneally. The two authors injected 5-HTP and DOPA either at medulla level (because of Moruzzi's and Bonvallet's findings, see above) or at pontine level. The injection of 0.1 mg of 5-HTP in what the authors call "Moruzzi's structure" of rabbits was in fact principally situated in the nucleus reticularis parvocellularis and nucleus reticularis gigantocellularis instead of the nucleus of the solitary tract and the solitary tract, as the authors wanted. 5-HTP induced "EEG picture of sleep or somnolence, slow waves and numerous spindles" (p. 380) and slow irregular activity in the hippocampus. The responsiveness to the researcher was decreased, the cardiac and respiratory frequencies remaining unchanged. In contrast, 0.1 mg injection of DOPA induced an activation of the EEG and theta rhythm in the hippocampus. Responsiveness to the researcher was present but decreased, while cardiac and respiratory frequencies were unchanged. In the discussion, the authors stated that they preferred to inject precursors since

they induced a more long-lasting effect, the transmitter itself being rapidly metabolized. The effects are parallel to those observed with compounds given intraperitoneally or systemically. Indeed, 5-HTP at least, induces slow waves in the cat under reserpine [154] while noradrenaline and adrenaline induces activation of the EEG [31, 207, 208].

In 1967, Carli *et al.* [42–44] published three papers which apparently solved the electrophysiological problem of somatic transmission processes in the M.O. during sleep–waking cycle. They first recorded, in the lemniscus medialis of 11 unrestrained and unanesthetized cats, the responses induced by stimulation of the controlateral superficial radial nerve [42]. The response was made of a first component with a latency of *ca* 3.7 msec.and a second component with 7.5–8 msec latency which increased in amplitude with stimulation intensity. This late response was related either to "cuneate-thalamic relay neurons and interneurons which responded with high frequency repetitive discharges to single shock stimulation of a peripheral nerve...or to the asynchronism of the monosynaptic bombardment of cuneate neurons by afferent impulses of the dorsal columns, a consequence of the wide range of conduction velocity" (pp. 35–37). The stimulation intensity was chosen to avoid EEG arousal reactions. Clear-cut EEG arousal induced from slow wave sleep by a whistle provoked a depression of the lemniscal response; "during transition from quiet waking to synchronized sleep there was no significant change in the amplitude of either the early or the late component of the orthodromic lemniscal response...nor was any significant difference...by comparing the spindle periods with the interspindle lulls...No tonic changes of lemniscal response can be detected during the transition from synchronized sleep to paradoxical sleep, nor at the end of the episode, when EMG activity reappears in the cervical muscles. The orthodromic lemniscal responses, however, are phasically depressed during the bursts of REMs (rapid eye movements)" (p. 39). The amplitude of both components was reduced, the late response being more attenuated. "This phasic depression could be related to occlusion phenomenon since the clonic twitches which accompany the bursts of REM can give rise to exteroceptive and proprioceptive volleys ascending along the dorsal column. This effect is not abolished, however, by a bilateral section of the dorsal half of the lateral funiculus, although the clonic twitches disappear after this operation" (p. 47).

In the second paper, Carli *et al.* [43] stimulated the cuneate nucleus by microelectrodes and recorded both in the contralateral lemniscal tract (to study postsynaptic responsiveness) and in the ipsilateral superficial radial nerve (to quantify the antidromic response and detect presynaptic processes). During a spontaneous or induced orienting reaction, a short-lasting increase of antidromic volley could be observed. The antidromic responses were not modified during relaxed wakefulness, synchronized sleep and paradoxical sleep. "A phasic enhancement of these responses occurred, however, during the bursts of REMs. This increased excitability of the central endings...is taken to indicate presynaptic inhibition" (p. 80). The orthodromic cuneo-lemniscal response was composed of a brief positive response (α-spike) the latency of which (0.7–0.8 msec) corresponds to the direct stimulation of cuneate neurons or their axons. A more prolonged and complex positive response followed (β-spike), the latency of which (1.14–1.16 msec) is "just adequate for monosynaptic transmission...Both these responses changed during arousal reactions. In some instances...a short-lasting depression of the responses occurred at the beginning of the induced arousal" (p. 68). "The excitability of the cuneate relay neurons did not change during relaxed wakefulness, synchronized sleep or paradoxical sleep. Only during the REM a depression of the direct excitability...occurred, an effect which is attributed to postsynaptic inhibitory action" (p. 80). Consequently, there is both cuneate presynaptic and postsynaptic inhibition during the REM of paradoxical sleep.

In the third paper, Carli *et al.* [44] showed that the specific lesion of the medial and descending nuclei of the vestibular complex suppresses both REMs and the phasic depression of cuneate transmission (Fig. 13). Moreover, the bilateral ablation of the sensory-motor cortex suppresses the de-

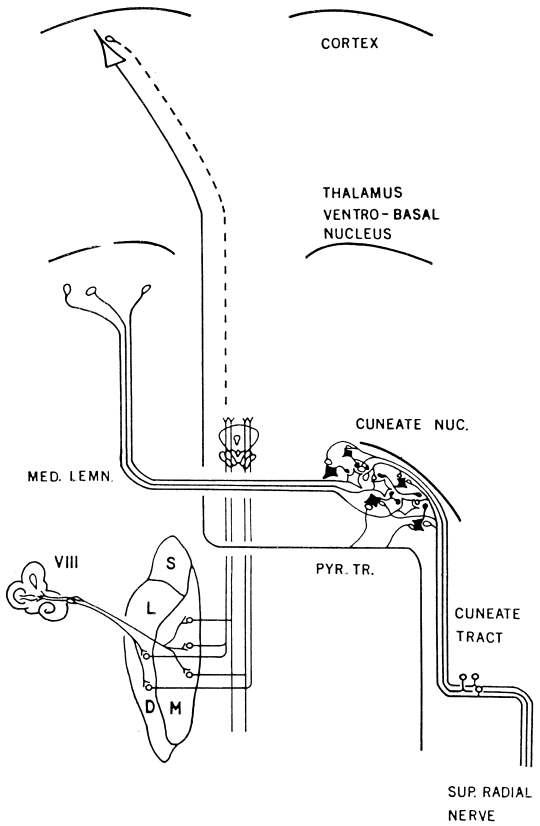

Fig. 13. Carli *et al.* (1967) observed that a lesion of the medial and descending nuclei of the vestibular complex suppresses the REMs of paradoxical sleep and the concomitant phasic depression of cuneate nucleus transmission. Moreover, the ablation of the sensory-motor cortex also suppressed this M.O. phasic depression, although the REMs were maintained. Thus, there seems to be a vestibulo-cortical loop controlling cuneate nucleus transmission. From Ref. [44], with permission.

pression of cuneate transmission during the REMs which are maintained. Consequently, the two vestibular nuclei are responsible for the phasic depression during the REMs by "roundabout" action on the cortex which, via its pyramidal neurons, controls transmission in the cuneate nucleus. These pyramidal tract inhibitory influences seem to act both pre and postsynaptically, as confirmed by Walberg [261] by electron microscopy. Carli *et al.* [44] concluded that "while sleep has been assumed for years to be due to a process of passive deafferentation, our experiments show that some aspects of sleep are associated also with a phenomenon of active deafferentation due to mechanisms of presynaptic and postsynaptic inhibition" (p. 97).

Still in 1967, Morest [160] undertook a neurohistological study of the projections of the area postrema and solitary tract nucleus. The terminals of the area postrema neurons were shown to end "in the solitary tract nucleus especially in its posterolateral part". There were reciprocal relations. The author recalled that the induction of EEG synchronization by serotonin at this level [126] (see above) could be due to action on the solitary tract nucleus. The projections of this last nucleus are directed toward the dorsal and M.O. lateral reticular formation which itself seems to have "secondary projections to the (thalamic) intralaminar and ventromedial nuclei...The present study has failed unequivocally to demonstrate a direct projection from the posterior solitary tract nucleus to the diencephalon...It does seem possible that efferent signals from the solitary tract nucleus may be relayed to the diencephalon after one or more synaptic interruptions. This could be accomplished by neurons of the dorsal or lateral reticular formation of the medulla" (p. 290). To announce further data, it is worth adding that the author found solitary tract nucleus projections toward the prepositus hypoglossi nucleus. This paper completed a previous description of the area postrema [159] in which the same author had described, in the area postrema, dendritic arborizations of neurons situated in the solitary tract nucleus. The area postrema was shown to have a very rich vasculature, and to contain neurons the axon of which could not be followed further than the solitary tract nucleus.

2.2.2. *1968–1977*

In 1968, Bueno *et al.* [41] confirmed in acute rabbits that M.O. influences antagonize brain stem waking inducing processes. After mid-medullary transection, they observed continuous cortical EEG activation and theta rhythm in the hippocampus. The same effect was observed after bulbar injection of carbocaine.

In 1969, Mancia [151] undertook splitting experiments at different levels of the brain stem. Those made at M.O. level were conducted in spontaneously breathing cats. With midline splits, which destroyed many neurons belonging to the M.O. gigantocellular nucleus and totally destroyed the pesterior raphe nuclei, there was no changes in sleep–waking cycle of animals recorded up to 2 weeks. However, during paradoxical sleep, "the nuchal EMG often did not drop to zero" (p. 499).

Based on the work of Moruzzi's and Bonvallet's groups, Bronzino [40] in 1972 also studied the relationship between the midbrain reticular formation and the nucleus of the solitary tract. This experiment was performed on intact cats, but also in mesencephalic and split preparations to study the bilateral projections of the reticular formation and the solitary tract nucleus. He induced reciprocal evoked potentials in the two structures and showed that there are potential influences from the reticular formation to the solitary tract nucleus which in turn is responsible for antagonistic influences on the reticular activating core. Consequently, he identified electrophysiologically the basis of a feedback circuit between these two brain levels.

In 1972, Moruzzi [168] published a major review of sleep–waking mechanisms. It was a complete state-the-of-the-art analysis of the data obtained by classic neurophysiological methods. In relation to the topic of the present paper, Moruzzi reviewed all data concerning lower brain stem ascending influences. The review was coupled with the other major review by Jouvet [114] on monoaminergic and cholinergic processes involved in sleep and waking.

In 1973, Dell's group (Puizillout *et al.*) [198] showed that vago-aortic stimulation not only induces slow waves but can also induce "un sommeil phasique à ondes lentes" (SPOL) (phasic slow wave sleep) and paradoxical sleep. SPOL was first described by Thomas and Benoit [246]. It characterizes the sleep stage occurring spontaneously just prior to paradoxical sleep, at which high amplitude spindles (also described by Ursin [251]) coexist with ponto-geniculo-occipital (PGO) waves. The first part of the experiment was undertaken with encéphale isolé preparations transected at C_2 or Th_2 level (in this case with spontaneous respiration). Bilateral or unilateral vago-aortic stimulation induced SPOL with concomitant myosis (Fig. 14). These SPOL episodes were accompanied neither by arterial pressure nor by cardiac and respiration variations. Usually, the episodes of SPOL were followed by waking at the end of stimulation. However, in some cases, a prolonged period of stimulation could induce first SPOL then paradoxical sleep. In the second part of the experiment, the authors studied semi-chronic encéphale isolé preparations. The animals with a transection at the Th_2 level, were fixed without any painful stimulation by screws in the cement of the head. The animals were kept alive for 3–4 days. The following percentage of stages could be obtained: waking 46.5%, light slow wave sleep 28.7%, deep slow wave sleep 11.8%, SPOL 4.4%, paradoxical sleep 8.6%. In 91.8% of cases, paradoxical sleep was preceded by SPOL but SPOL was followed by paradoxical sleep only in 45.1% of cases. The inducing of paradoxical sleep after SPOL by vago-aortic stimulation was easier to obtain in this semi-chronic preparation. In the sleep deprived animal prior to transection, it was still easier to induce these two stages. However, there was a refractory period. The authors did not discuss the M.O. involved processes. A second detailed paper with similar results was published a year later [78].

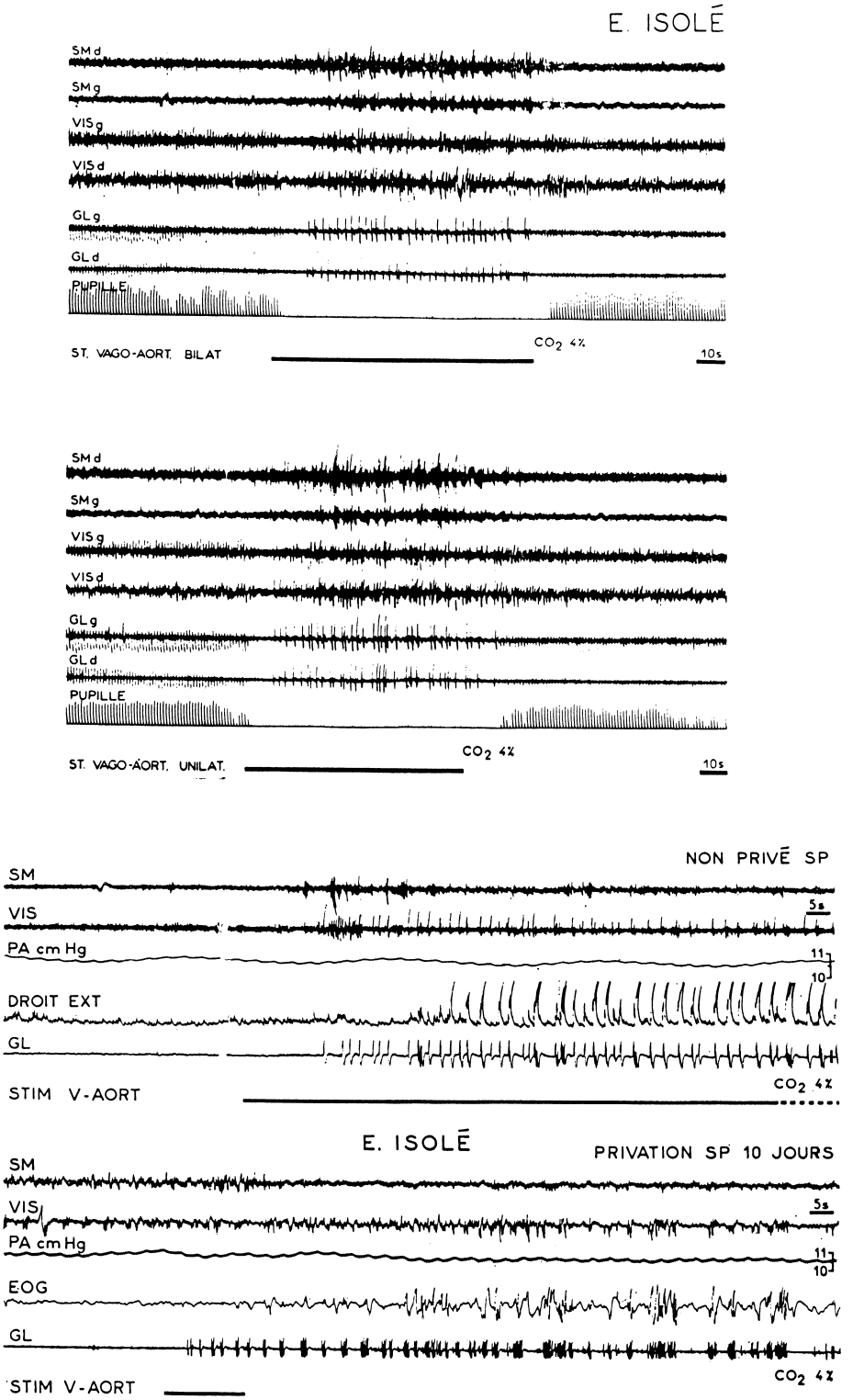

Fig. 14. Puizillout *et al.* (1973) was able to induce in cats the preliminary phase of paradoxical sleep (phasic slow wave stage) by unilateral and bilateral stimulation of vago-aortic afferents (top recordings). Bottom, The same stimulation also induced paradoxical sleep, an effect more easily obtained after 10 days specific deprivation (lower recordings). SMd, SMg, right and left somato-motor cortex; VISg, VISd, left and right visual cortex; GLd, GLg, right and left geniculate nuclei; pupille, pupil; PA, arterial pressure; Droit ext, external right ocular muscle. From Ref. [198], with permission of Masson Ed.

In 1973 also, Dell [60], at the jubilee devoted to Alfred Fessard, made a synthesis of the central influences of baroreceptor afferents. He recalled the fact that vago-aortic afferent stimulation induces synchronization even in the very awake animal and that the cortical waves are accompanied by an increase of discharges in the anterior part of the nucleus of the solitary tract [183]. Stimulation was able to induce all successive stages of sleep up to paradoxical sleep and could even induce direct entrance into paradoxical sleep, thus inducing a narcoleptic-like episode. Although stimulation of the solitary tract could induce EEG phasic synchronization, the fact that all successive stages could be induced (i.e. true sleep) and the refractory period previously shown, implies that "wet" influences like those described in Jouvet's review [110] are involved. For Dell, the results also suggested that direct paucysynaptic connections exist between the nucleus of the solitary tract and the pontine pacemaker of paradoxical sleep. From the general point of view, he stated that, during the "appetitive" phase of primary physiological needs, intense reticular activation allows muscle and energy expenditure requiring marked cardiovascular overloading. However, the baroreceptive influences bridle reticular activation and limit muscular facilitation to circulation abilities, thus acting as a protection reflex. The "consumption" act is accompanied by the abrupt disappearance of these reticular activating factors, leading to reticular active and passive deactivation favorable to sleep-inducing processes provided that the monoaminergic conditions are present.

Puizillout and Ternaux continued the Dell group research by studying the influence of vago-aortic stimulation on M.O. They first showed [194] in 1974 that this stimulation induces tonic influences on M.O. neurons recorded by semi-microelectrodes (50 m diameter). Tonic depressions were observed during cortical EEG synchronizations particularly at the level of the raphe obscurus, cuneate, parvocellular reticular and lateral reticular nuclei. Stimulation induced evoked potentiels in the nucleus of the solitary tract and in the ventral and lateral reticular formation. Tonic cell activations were more seldom observed. However, tonic depression and phasic activations were observed in several structures such as the cuneate and gracilis nuclei, the lateral reticular nucleus and the raphe obscurus and pallidus. In the following paper published the same year, Puizillout and Ternaux [195] showed that these phasic activations disappeared after diencephalic transection and after pyramidal tract lesion. In the cuneate nucleus, the descending activations would induce a presynaptic inhibition mediated by interneurons. This experiment shows a bulbo–cortico–bulbo loop which involves the nucleus of the solitary tract, a reticular deactivation process, the thalamic synchronizing system, the motor cortex, the pyramidal tract and finally the medulla neuron population.

In the same Dell group, in 1974, Gahery and Vigier [82] showed, in curarized and anesthetized cats, that vago-aortic stimulation reduced cuneate nucleus transmission processes. Since the antidromic response induced in the superficial radial nerve by cuneate stimulation was increased in amplitude, this indicated that the influence is presynaptic. The authors did not discuss by which mechanism, cortical [4] or reticular [47] this occurs.

Finally, the same year, Puizillout *et al.* [199] studied the effect of serotoninergic influences on SPOL induced by vago-aortic stimulation. The cats were transected at C_1 (encéphale isolé preparation) or D_2 (low spinal transection). The lesion of the posterior raphe nuclei (pontis, pallidus, magnus) did not induce a 'pseudo-reserpinic' syndrome (i.e. the appearance of continuous PGO spikes) as is the case with lesions of the dorsal and medial raphe nuclei. Stimulation of the vago-aortic afferents in nine of these cats induced cortical synchronization but without correlative PGO spikes of SPOL. In three chronic animals with the same lesion, there was an almost complete insomnia which disappeared after 3–4 days. At the end of the insomnia period, the cats were transected at the C_1 level. In that case, vago-aortic stimulation also induced cortical synchronization but without PGO spikes. Moreover, the continuous PGO spikes induced by 0.5 mg kg^{-1} of reserpine were not suppressed by the lesion of posterior raphe nuclei. The same results were obtained by medial (sagittal) brain stem transection which destroyed the raphe nuclei [196]. This did not prevent EEG synchronization and myosis. Injections of *p*-chlorophenylalanine (PCPA) (200 and 300 mg kg^{-1} given at a 24 h interval) given alone or after transection did not prevent the synchronization induced by vago-aortic stimulation. "The lower the level of reticular activity by minute doses of Nembutal, the easier it was to obtain vago-aortic synchronizations with raphe system destroyed and pretreated with PCPA" (p. 20).

In 1974, Mancia *et al.* [153] published a full paper on the intrareticular reciprocal connections between the M.O. and the midbrain which extended the findings of a preliminary paper written 3 years previously [152]. In the first research, intracellular recording experiments were performed on encéphale isolé cerebellectomized cats which were curarized and artificially ventilated. The authors first stimulated the gigantocellular bulbopontine nucleus and most often recorded midbrain short-latency EPSP sometimes followed by spikes. However, one-third of the neurons responded by an IPSP. The M.O. neurons responded to midbrain stimulation by IPSPs in half the cases, the other half giving mixed responses which included both excitatory and inhibitory components. This experiment showed a clear relationship between the midbrain and the M.O. reticular formation. In the paper in question [153], "in order to avoid co-stimulation of fibers passing the brain stem, experiments were also performed in cats with chronic bilateral lesion of the dorsal column nuclei and hemisection of the spinal cord at C_3 (in those experiments in which the M.O. was stimulated), and in cats with chronic unilateral lesion of the rostral midbrain (in those experiments in which the mesencephalon was stimulated)" (p. 42). In that case, M.O. stimulation only induced inhibition of midbrain neurons while M.O. neurons responded to midbrain stimulation by EPSPs followed by IPSPs. Convergence phenomena could be observed: mesencephalic neurons were postsynaptically influenced by

intralaminar thalamic, hypothalamic and M.O. stimulations. Facilitatory M.O. influences were induced by thalamic and midbrain stimulation. These results show that the M.O. reticular regions "exert a short-lasting and powerful inhibitory action on midbrain reticular neurons. Since mesencephalic reticular stimulation produces a disfacilitation of intralaminar thalamic neurons, bulbar activation might influence the thalamic mechanism through a suppression of disfacilitation, that is, disinhibition. The short-latency ascending effect follows mono or pauci-synaptic pathways as indicated by anatomical findings" (p. 49). The authors concluded that "the prevalent descending excitatory effect from the midbrain upon bulbar neurons and the prevalent ascending inhibition from the bulb indicate that an intrareticular negative feed-back is operating in the brain stem. The midbrain facilitation of bulbar neurons, which in turn inhibits the mesencephalic one, seems to favour the hypothesis that the activation of bulbar inhibition is a necessary step in the homeostatic control of the arousal mechanism. The consequent attenuation of the activating mechanism by the deactivating reticular structures may then counteract the tendency towards excessive arousal" (p. 49).

Also in 1974, by means of immunohistochemical studies using antibodies, Hökfelt *et al.* [104] showed the existence of adrenaline body cells in the M.O. of rats (Fig. 15). Indeed, by "the Falck–Hillarp technique it is difficult to differentiate between noradrenaline and adrenaline, since they have the same excitation and emission spectra...The levels of mammalian brain adrenaline are only 5–10% of the total noradrenaline and adrenaline levels in the brain" (p. 236). Consequently, these neurons are not numerous. The first nerve cell group, called C_1, was situated within the area of the reticularis lateralis in the rostral part of the noradrenergic cell group A_1. The group C_2 was situated near the group A_2, in the dorsal part of the M.O., some cell bodies being immediately ventromedial to the nucleus of the solitary tract. The ascending fibers of these two nuclei coursed in the reticular formation along the ventral noradrenergic tract. Hökfelt *et al.* [104] indicate that among the principal targets, several concern structures involved in sleep–waking processes. This is particularly the case of the high density terminals in the nucleus of the solitary tract and the moderate terminals in the locus coeruleus. They suggest that these last terminals could be responsible for the alerting action of adrenaline which could activate the coerulo-cortical noradrenergic pathway which is of importance for maintenance of wakefulness. However, medium and low density terminals rise up to the hypothalamus and subthalamus, respectively.

In 1975, Palkovitz and Jacobowitz [184] established a topographic atlas for noradrenaline and acetylcholine-containing neurons in the hindbrain. At the M.O. level, in relation to possible sleep–waking concerned structures, there were some cholinergic neurons and fibers in the reticular nuclei with little evidence of terminals in the solitary tract nucleus, and noradrenergic neurons in the A_1 and A_2 nuclei with high content of terminals in the solitary tract nucleus. There were also noradrenergic terminals in the local raphe nuclei.

In 1977, Key and Mehta [120] bilaterally perfused serotonin (10^{-5}–2.5×10^{-4} M) and D-lysergic acid diethylamide (LSD) in the solitary tract nucleus and adjacent reticular formation in 42 encéphale isolé cats. "The primary response induced by the (push–pull) perfusion (at a rate of 120 ml min^{-1}) of 5-HT into the region of the solitary tract was the initiation, or an increase in the synchronization of EEG activity and the induction of behavioral sleep. These effects were correlated with a significant attenuation in the duration of arousal responses induced both by sensory stimulation and by electrical stimulation of the midbrain reticular formation. Thus, they may be interpreted not as the result of a direct effect of 5-HT on sleep-inducing mechanisms, but rather as a consequence of the decreased responsiveness of arousal systems to incoming sensory stimuli" (p. 103). With higher doses of 5-HT, there was first synchronization, followed by prolonged

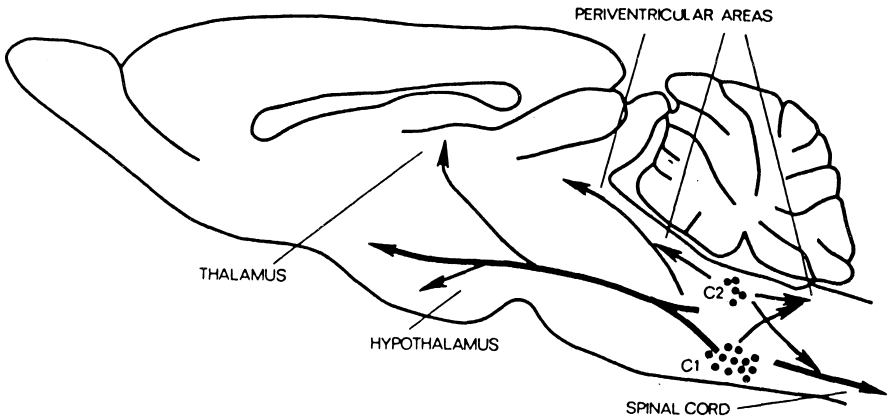

Fig. 15. Hökfelt *et al.* (1974) identified in rats adrenergic cell bodies (C_2 zone) near the A_2 noradrenergic area in the dorso-lateral M.O. Another group of adrenergic neurons (C_1) was found at ventro-lateral level, contiguous to the A_1 noradrenergic area. Later on, a third group of adrenergic neurons (C_3) was identified at dorso-medial level in the prepositus hypoglossi nucleus (see Refs [13, 70, 186]). All these neurons send ascending projections. From Ref. [104], with Elsevier's permission.

periods of desynchronization and increased responsiveness. The 5-HT antagonist "LSD induced alerting or produced varying degrees of electrocortical desynchronization when perfused in the sleeping animal" (p. 99). Key and Mehta [120] mention that the EEG synchronization effects confirm previous results [126] obtained in neighbouring structures. One of the new facts is that the effect was also observed in neighbouring reticular regions (2–3 mm). The authors think that this is a true effect, not due to diffusion of the transmitter.

Puizillout and Foutz [193], also in 1977, studied the characteristics of the sleep states induced by vago-aortic stimulation. The research was undertaken in encéphale isolé preparations. The stimulation effects were analyzed in control and in 72 hr paradoxical sleep deprived animals. The results clearly showed that, when compared to control recordings, the stimulation rapidly induced slow wave sleep patterns with short latency appearance of slow wave sleep with PGO spikes (SPOL) followed by paradoxical sleep. "The vago-aortic stimulation precipitated the onset of the different episodes of the sleep cycle" which were shortened (p. 556). In some cases, much more numerous after paradoxical sleep deprivation, phasic slow wave sleep and paradoxical sleep were directly induced. Consequently, there was a narcoleptic-like state, as already shown by Foutz *et al.* [78]. The authors admit that the deactivation of the reticular activating system which precipitates slow wave sleep could led to a second deactivation responsible for the occurrence of paradoxical sleep short latency.

The same year, Sakai *et al.* [215] studied the afferent projections to the cat locus coeruleus, as visualized by the horseradish peroxydase technique. In the M.O., they found retrograde labelled cells in the area surrounding the lateral reticular nucleus which projects to the locus coeruleus α, the subcoeruleus nucleus, the nucleus parabrachialis lateralis and the oral and caudal pontine reticular nuclei. They also found strong projections from the nucleus of the solitary tract to the locus coeruleus α, and also significant projections toward the locus subcoeruleus and the parabrachial nuclei. There were also some projections from the vagal nucleus towards the locus coeruleus α, the locus subcoeruleus and the parabrachial nuclei. None of these structures send ascending influences to the locus coeruleus itself. The authors particularly ruled out projections from A_1 and A_2 noradrenergic areas.

Also in 1977, Netick *et al.* [175] identified M.O. neurons specifically activated during paradoxical sleep. Cats were paradoxical sleep-deprived for 12 hr prior to recording. The microelectrodes were implanted in the lateral tegmental field (FTL), in some cases between the FTL and the gigantocellular tegmental field. The animals were head-restrained. Among the studied cells, three neurons were followed over two complete sleep–wakefulness cycles. Figures 2 and 3 show tonic discharges occurring only during paradoxical sleep. Phasic increases were observed during the rapid eye movements and the irregular respirations associated with them. "This irregular respiration at times consisted of rapid swings of small amplitude around zero inflow" (p. 205). In the discussion, the authors mention that the tonic regular mode of neuron functioning suggests "pacemaker-like discharge patterns".

2.2.3. *1978–1987*

In 1979, Siegel *et al.* [235] recorded M.O. neurons in the lateral, magnocellular and gigantocellular tegmental field of unrestrained behaving cats. They found three kinds of neurons. The first type discharged during movements of waking. The second discharged tonically during waking and the two stages of sleep, with an increase during paradoxical sleep. The third type of neurons "were spontaneously active at low rates in quiet waking and slow wave sleep and, like type two cells, increased their discharges during both active waking and paradoxical sleep" (p. 53). "Clearly, most medullary reticular formation cells do not discharge specifically in relation to the atonia (and cortical activation) of paradoxical sleep. If paradoxical sleep selective cells exist in this area they must have virtually no waking discharge and be relatively rare" (p. 57). The authors hypothesize that the results of Netick *et al.* [175] were obtained because the animals were head restrained: although they observed neurons with increased discharges during paradoxical sleep, Netick *et al.* [175] were unable to observe the neurons during waking movements.

Vigier and Portalier [258], again in 1979, studied in rats the efferent projections of the area postrema by injecting labelled amino acids into the latter, and observed the targets attained by the axonal transport. The main targets potentially related to sleep involved structures were the nucleus of the solitary tract and the locus coeruleus. However, the authors suggest that the efferents could be implicated in cardiovascular regulation. Afferents and efferents of the area postrema were further studied in rats by Vigier and Rouvière [259] using horseradish peroxydase injection. There were both afferent and efferent connections with the nucleus of the solitary tract and the dorsal vagal nerve nucleus. The authors suggested that some afferents of these two nuclei might have originated from from the C_2 adrenergic nucleus. From the physiological point of view, the authors favor the implication of the area postrema in the control of circulation and in a neurosecretory role.

In 1979, Cespuglio *et al.* [49], of Jouvet's team, undertook cooling and stimulation experiments in semi-chronic cats with spinal and bilateral brachial plexus transections. Cooling ($+10°C$) of the raphe magnus nucleus induced EEG and behavioral arousal whatever the sleep–waking stage. This waking stage lasted the duration of short-term cooling (1–3 min). However, when the cooling was of longer duration (20–40 min) alternation of sleep stages reappeared. Cryocoagulation ($-24°C$ for 3 min) induced strong waking which lasted at least 5 hr. Unexpectedly, the stimulation of raphe magnus also induced waking in all cases. The cooling of raphe pallidus nucleus induced waking too, although in some cases it also produced light slow wave sleep. The difference with dorsal raphe nucleus cooling, which induces slow wave sleep, has to be empha-

sized while stimulation induces waking. In the discussion, the authors estimate that the stimulations are not reliable, since serotoninergic and non-serotoninergic neurons are involved and passing fibers are also concerned. This experiment of M.O. cooling confirms the results of Berlucchi *et al.* [23] and Naquet *et al.* [172].

The following year (1980), Kanamori *et al.* [117], in free-moving cats, recorded neurons during sleep–waking cycle in the M.O. gigantocellular, parvocellular and magnocellular nuclei. In the magnocellular nucleus, they found neurons, silent during active and quiet waking, which began to fire 10–20 sec prior to the SPOL PGO-spikes with an increase of firing during the PGO period. During paradoxical sleep, there was substantial tonic firing with a phasic increase during the PGO waves and the REMs. The authors showed that these neurons were antidromically activated by stimulation of the reticulospinal tract, and two of them were activated by locus coeruleus α stimulation. Consequently, with the knowledge available at that time, Kanamori *et al.* [117] concluded that these neurons are only implicated in postural atonia of paradoxical sleep.

Eguchi and Satoh [65, 66], in 1980, published two papers on the cell activity of the cat solitary tract during the sleep–waking cycle. In the first [65], they observed that more than the half of the neurons increased their firing rate during steady slow wave sleep with already some increase during the transition from waking to slow wave sleep, as slow waves were already present. In contrast, the neurons situated outside this nucleus discharged during slow wave sleep at a rate comparable to that of waking. During paradoxical sleep, many neurons increased their discharges but others decreased their discharge rate or showed no change. The stimulation of the midbrain reticular formation increased the neuron discharges during slow wave sleep.

In the second more detailed paper [66], the authors identified in the solitary tract nucleus ten neurons which increased their firing rate during established slow wave sleep (group 1), two neurons which decreased (group 2) and eight neurons which did not change their firing rate. In the neighbouring structures, 12 neurons increased, nine decreased and 26 did not change their firing rate. "This observation... supports the hypothesis that this region (the solitary tract nucleus) plays an important role in the generation of slow wave sleep. However, the behavior of these group 1 neurons did not provide any suggestion about the triggering mechanism of slow wave sleep, because the increase of discharge rate during slow wave sleep in these neurons lagged always behind the development of the EEG slow waves" (p. 341). Moreover, "During the transitional phase from slow wave sleep to paradoxical sleep the discharge rate was markedly altered in almost all neurons; the reduction being predominating in number over the augmentation. A bursting discharge correlated with the occurrence of rapid eye movements during paradoxical sleep was observed in about a half of neurons... There were no units (inside or outside the solitary tract nucleus) which responded with a reduction in activity" (p. 337). Finally, during paradoxical sleep, the solitary tract nucleus neurons were less sensitive to the stimulation of the midbrain reticular formation. For this last point concerning the relationships between brain stem structures during the sleep–waking cycle, and as a complement to Mancia *et al.* [152, 153], the reader can also consult Satoh and Kanamori [220] who found reciprocal electrophysiological influences between the M.O. part of the reticular gigantocellular nucleus and the midbrain reticular formation, the locus coeruleus and the raphe pallidus. Also consult Satoh *et al.* [221] who found by post-stimulus time histograms reciprocal activating and inhibitory influences between the raphe magnus and the midbrain reticular formation.

It has been known since Moruzzi and Magoun's [169] findings that the brain stem reticular formation induces EEG activation patterns. It was also shown [41] that mid-medullary transections, which suppress ascending waking antagonist influences, induce hippocampal theta rhythm. However, Winson [265], in 1981, showed that stimulation of the M.O. reticular formation induces a state-dependent increase of gyrus dentatus transmission processes. Indeed, a gigantocellular nucleus stimulation consisting of three pulses, when delivered prior to a perforant pathway pulse, increased in rats the evoked potential amplitude induced in granule cells when delivered during slow wave sleep. The authors did not indicate whether the reticular stimulation induced a slight arousal reaction, but this latter can be hypothesized since muscular movements were simultaneously observed. No change of transmission was induced during alert behavior. These effects, which occurred after stimulation of broad areas of the M.O. reticular formation, disappeared after administration of anesthesia. The authors concluded that, during waking, the gigantocellular nucleus may "effect tonic changes in the characteristics of neural transmission in the hippocampus that are suitable for the processing of afferent information. During paradoxical sleep and slow wave sleep the reticular formation may also act to adjust the pattern of information flow through the hippocampus to suit the requirements of a particular sleep state" (p. 49).

At the symposium on 'The serotoninergic neuron' held in Marseille in 1981, Puizilloux *et al.* [197] presented a paper on the serotoninergic mechanisms related to sleep processes. After a summary and a discussion of data then available, particularly those linked to the solitary tract nucleus, they described the existence of serotoninergic neurons in the nodose ganglia which project to the solitary tract nucleus. They further showed that "a minute injection of exogenous serotonin (25 ml 10^{-5} M) in the area of the nodose ganglia triggers immediate myosis followed 10 to 20 sec later by a generalized cortical synchronization mimicking the vago-aortic sleep... These effects persist after caudal section of the vago-aortic trunk, but are suppressed after a rostral section. These results suggest that serotonin can induce sleep by acting on peripheral nervous structures belonging to the afferent vagal system located in the nodose ganglia" (p. 419). Although the authors mention that serotonin applied to the nodose ganglia induces depolarization of local neurochemically unidentified neurons, current

knowledge could perhaps suggest an action on 5-HT_{1A} autoreceptors inducing a decrease of serotonin release at the target level, thus a disinhibition of the solitary tract nucleus which would be in agreement with later results obtained for paradoxical sleep [178] (see below). Taken together, all previous results devoted to vago-aortic stimulation show that peripheral interoceptive afferents are able to favor the activity of the solitary tract nucleus, thus have sedative effects.

Tissot [247], also in 1981, studied the effect of delta sleep inducing peptide (DSIP) when injected in different brain structures of the rabbit. The infusion of 7.5 and 15 nmol in the solitari tract nucleus induced a dose-dependent increase of slow wave sleep with spindles over 2 hr. In the median thalamus the effect was suppressed by naloxone. The author hypothetizes that there could be an interaction between DSIP and morphine receptors.

In 1983, Sakai *et al.* [216] recorded cellular activities principally from the M.O. magnus and pallidus raphe nuclei in freely moving cats (Fig. 16). There was a regular decrease of firing from active waking to paradoxical sleep. "Seventeen of the 24 PS-off neurons were characterized by a complete cessation of discharges during paradoxical sleep. These (PS-off) neurons were located either laterally around the direct lateral vestibulospinal tract or medially in the nuclei raphe magnus and pallidus, as well as in the magnocellular nucleus adjacent to the raphe nuclei" (p. 312). The other seven neurons showed some discharges which persisted during paradoxical sleep with a further decrease during the REMs. They were situated in the raphe pallidus which confirms previous findings by Trulson and Trulson [250]. Indeed these authors found in the raphe pallidus a progressive decrease of firing from active waking (5.3 spikes sec^{-1}) to paradoxical sleep (1.2 spikes - sec^{-1}). However, "there was no significant change during active waking or slow wave sleep, as compared to quiet waking" (p. 234). Consequently, the unit activity during paradoxical sleep was not suppressed but was significantly lower ($P < 0.05$) than that observed during the other stages. It has to be added that Sheu *et al.* [230], in 1974, and Cespuglio *et al.* [48], in 1981, previously found in the raphe magnus nucleus an increase of neuron firing during paradoxical sleep, particularly during the PGO waves. However, the authors underlined that only 15% of local neurons are serotoninergic. Consequently, the neurons recorded by Sakai216 were serotoninergic, while those recorded by Sheu *et al.* [230] and Cespuglio *et al.* [48] were functioning with other transmitter(s) (Fig. 17).

George *et al.* [85] had already shown that a cholinergic mixed agonist (carbachol) when injected in the pontine reticular formation of cats, induces a paradoxical sleep-like state. Baghdoyan *et al.* [14], in 1984, studied in cats the effects of carbachol when administered at midbrain, pontine and M.O. reticular levels (Fig. 18). In the M.O., the locus of injection (0.5 ml of carbachol solution) was situated

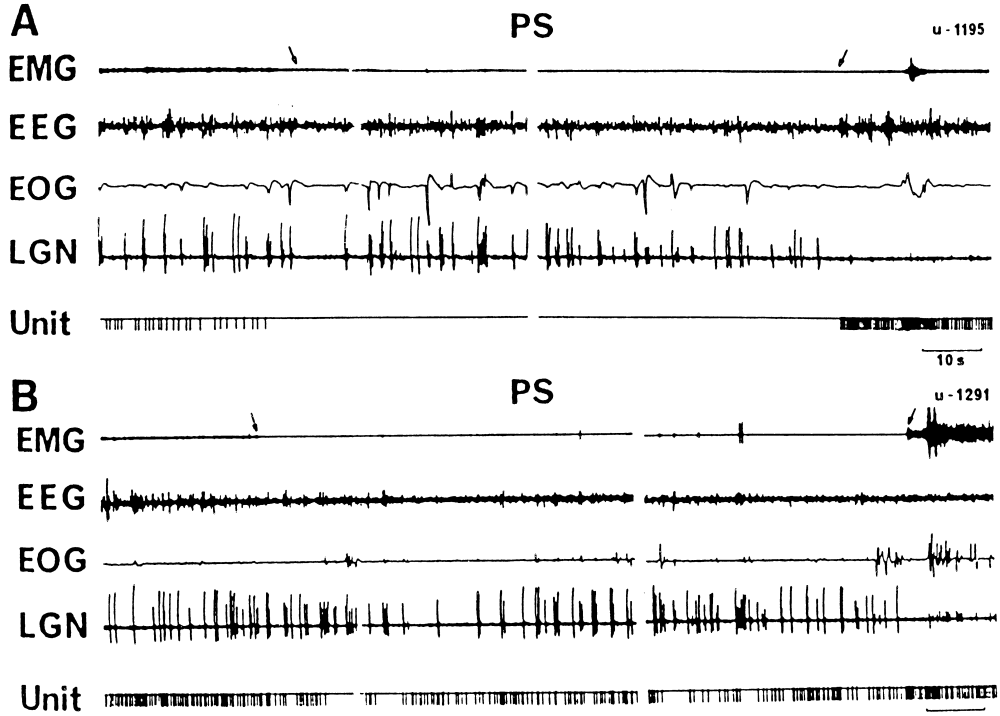

Fig. 16. Sakai *et al.* (1983) recorded serotoninergic identified neurons of raphe magnus and pallidus nuclei during sleep–waking cycle in cats. The first group (A) became silent during paradoxical sleep (PS-off neurons). Some of the second group (B) only decreased their firing rate as compared to waking and slow wave sleep. This last result confirmed the finding of Trulson and Trulson [250]. The arrows show the transition from slow wave sleep to paradoxical sleep and from paradoxical sleep to waking. From Ref. [216], with permission.

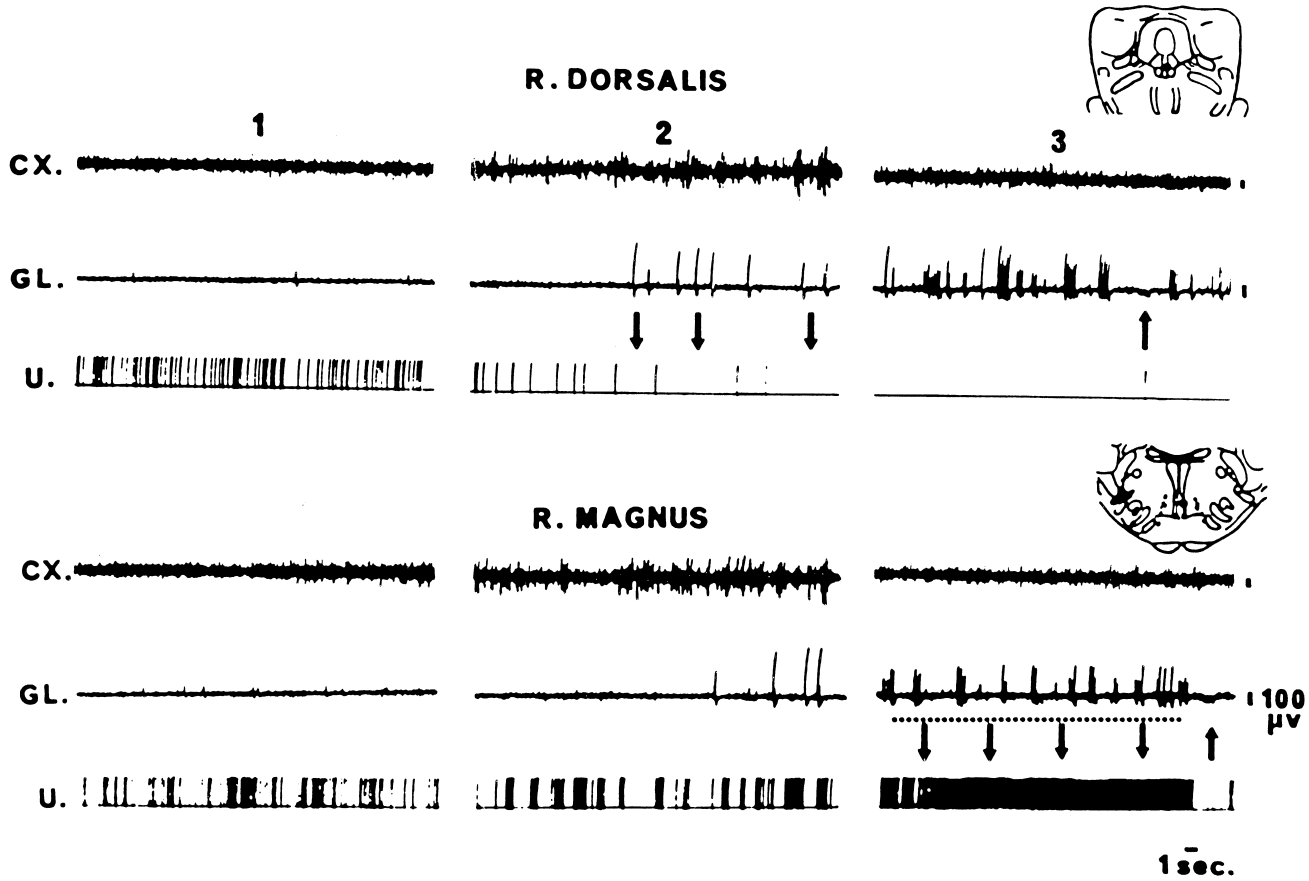

Fig. 17. Cespuglio *et al.* (1981) compared the neuron activity of the dorsal and magnus raphe nuclei during the sleep–waking cycle in cats. The cells of the dorsal raphe decreased their firing during slow wave sleep (2) as compared to waking (1) and became silent during paradoxical sleep (3). Moreover, there seemed to be an antagonism between the neuron discharges and PGO waves (see arrows). In contrast, the neurons of raphe magnus increased their firing during paradoxical sleep, particularly during PGO spikes. However, the authors recall that 70% of the dorsal raphe neurons are serotoninergic, while this is the case for only 15% of cells in the magnus nucleus. Consequently, the authors hypothesized that the recorded neurons did not contain serotonin. Similar discharge modalities were shown previously by Sheu *et al.* [230]. From Ref. [48], with Elsevier's permission.

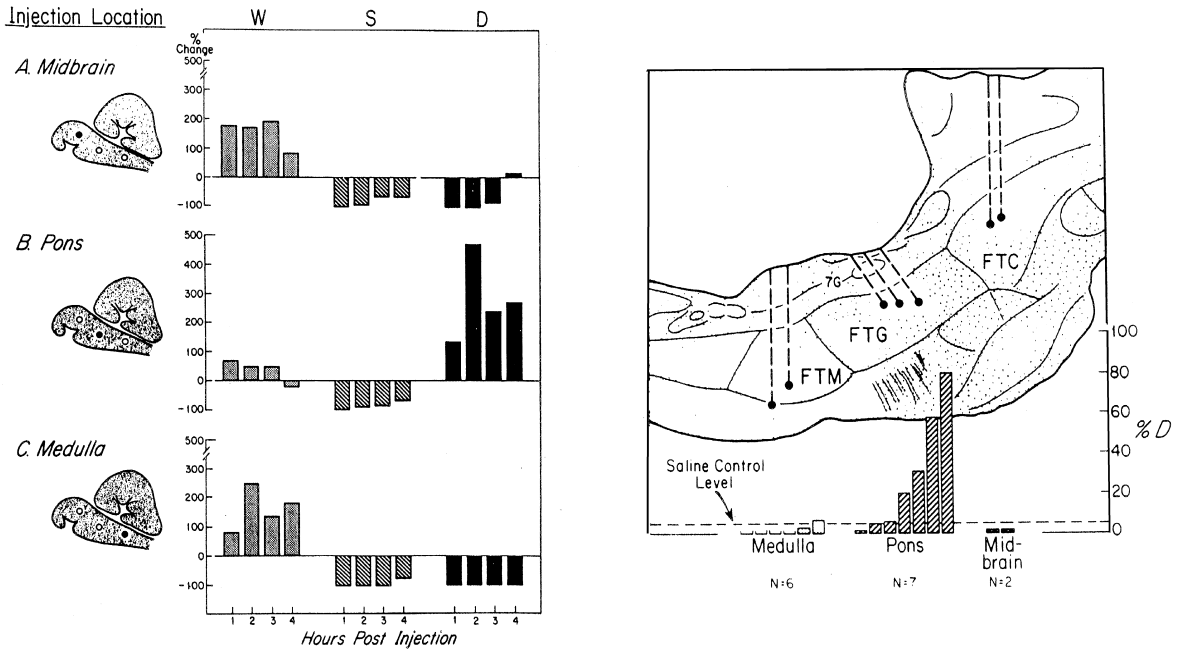

Fig. 18. Hobson's team (1983, 1984) studied the influence of a mixed nicotinic and muscarinic agonist (carbachol) and a true muscarinic agonist (bethanechol) on sleep–waking cycle in cats, when injected at different levels of the brain stem. At M.O. level, carbachol (left) increased waking while slow wave sleep and paradoxical sleep were almost suppressed. The true muscarinic agonist (right) also suppressed paradoxical sleep during the first 4 hr. Notice the opposite effects at pontine level. Left from Ref. [14], right from Ref. [101], both with Elsevier's permission.

between the rostral part of the lateral reticular nucleus and the caudolateral tegmental field. The more dorsal point was the level of the solitary tract and down the ventral part of the inferior olive. The results showed that waking accompanied by ataxia and motor activities was increased, while slow wave sleep was decreased (up to 77% during hour 4). Paradoxical sleep was suppressed. There was no increase of slow wave sleep after M.O. injection. The authors supposed that either the injections were not truly performed in the solitary tract nucleus or that this structure is not cholinoceptive or cholinergic. They recalled a previous paper [101] showing that a true muscarinic compound (bethanechol) when injected in the M.O. reticular formation, also increased waking with marked motor abnormalities, and completely suppressed paradoxical sleep in four of six cats for 6 hr (in two animals it was unchanged) without affecting slow wave sleep. Consequently, the nicotinic component of carbachol could be responsible for the decrease in slow wave sleep in the present study. Similarly, 2 years later, Gnadt and Pegram [88] injected cabachol at different brain stem levels in chronic rats. They were unable to increase paradoxical sleep by M.O. reticular injections.

In 1984 also, Steriade *et al.* [239] studied in cats the M.O. projections to the thalamus which were thought to participate in the cortical EEG activation of paradoxical sleep (Fig. 19). By antidromic stimulation, they identified projections from the gigantocellular and magnocellular fields to the ventromedial and intralaminar nuclei and midbrain reticular formation. Only 3% (or 6%?) of the neurons showed bifurcating axons with projection also to the spinal cord. As shown in Fig. 19, the neurons of the magnocellular field showed tonic activation during overall paradoxical sleep while phasic activation related to PGO waves or REMs and motor activities of waking was seen in neurons localized in the gigantocellular as well as the magnocellular field. There was already "an increased firing rate of thalamic projecting bulbar reticular neurons prior to the EEG activation at paradoxical sleep onset but it was not related to PGO waves" (p. 472). These ascending influences of M.O. neurons were of an activating nature both at mesencephalic and thalamic level.

The same year, Vertes [256] wrote a review on the "brainstem control of the events of paradoxical sleep" (p. 248). A paragraph was devoted to M.O. influences. Vertes concluded that this brain level is "probably not involved in the genesis of paradoxical sleep but may participate in certain paradoxical sleep-associated events: namely, muscle atonia, cortical EEG activation and respiratory fluctuations" (p. 274).

Siegel *et al.* [233], also in 1984, undertook chronic transections at the pontomedullary junction. The experiment was performed in three cats. Expired CO_2 levels were maintained without assistance in the normal range except in one cat which showed only transient hypercapnia. The rectal temperature was controlled and nutrition given by gavage. The transection passed dorsally between the rostral and caudal portions of the abducens nucleus. Ventrally, it passed through the trapezoid body caudal to the basilar pons...The transections were quite complete with a few filaments of the trapezoid body spared in

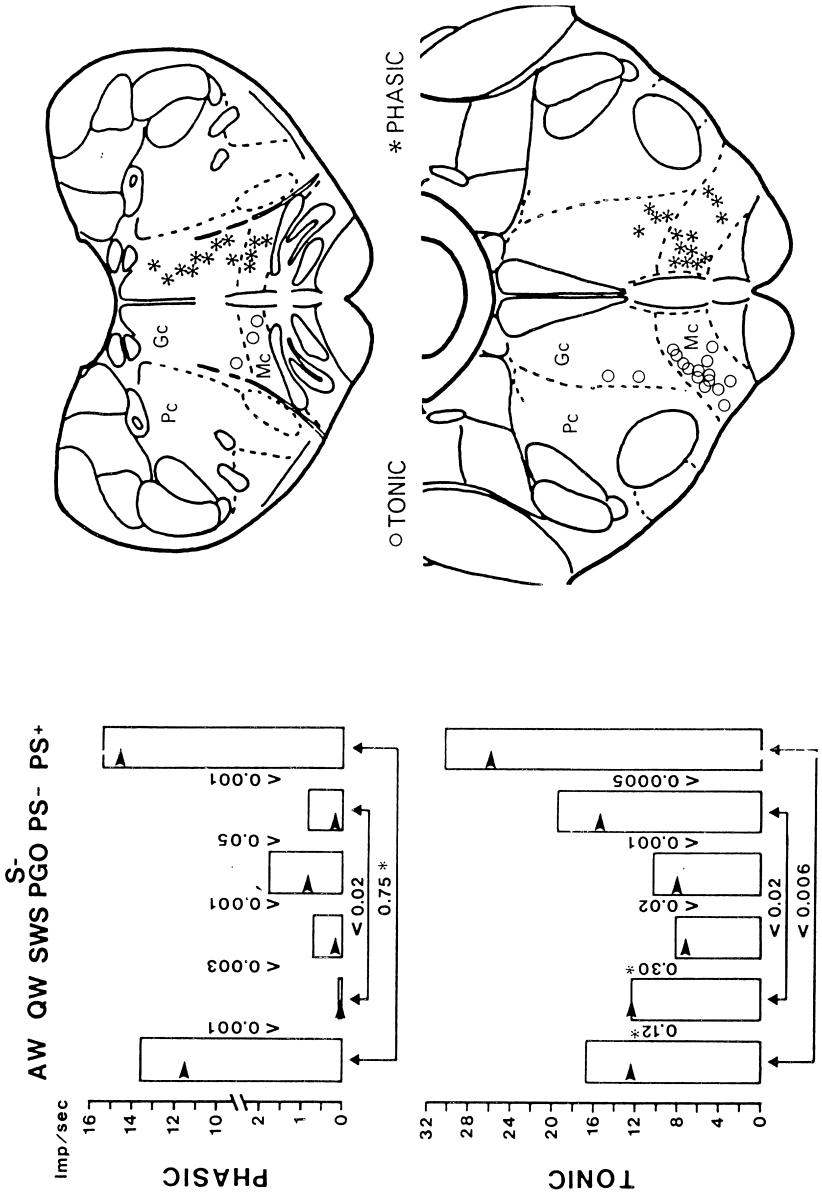

Fig. 19. Steriade *et al.* (1984) studied in cats the thalamic and midbrain ascending influences of medulla reticular neurons during sleep and waking. They distinguished neurons located in the magnocellular nucleus which fired tonically during paradoxical sleep, from those those discharging phasically (during motor activities of waking, PGO waves and REMs) which were located in the gigantocellular as well as in the magnocellular nucleus. This study shows the participation of the M.O. in paradoxical sleep generating mechanisms. AW, Active waking; QW, quiet waking; SWS, slow wave sleep; S-PGO, slow wave sleep with PGO spikes; PS −, paradoxical sleep without phasic activities; PS +, paradoxical sleep with phasic activities. From Ref. [239], with permission.

one cat. Results in this cat did not differ from the two with complete transections (p. 242). "By post-transection day two, a state with EEG and olfactory bulb desynchrony, PGO spikes and PGO spike bursts and hippocampal theta rhythm was observed in all three cats. This state closely resembled the paradoxical sleep state seen during baseline recordings and recurred throughout the survival period. Rapid eye movements were concentrated in this state but could also be seen in other states. States similar to waking and non-paradoxical sleep were also observed...The cycle length for paradoxical sleep-like state was significantly shorter after transection than in the baseline period ($P < 0.03$). Mean state duration was not significantly different after transection...All transected cats showed some paradoxical sleep-like states with duration exceeding 25 minutes, far longer than any paradoxical sleep state seen in intact cats" (pp. 242–243). The authors concluded that the M.O. is not required to generate this sleep stage but "may play a critical role in regulating the timing of paradoxical sleep state" (p. 243). These data show clearer results than those of Jouvet [113] who made transections at the same level (see above). Moreover, this research suggested that the M.O. ascending influences on thalamocortical activating processes [239] and on hippocampal formation for theta rhythm inducing influences [265] did not seem to be crucial for paradoxical sleep forebrain activities.

In 1985, Sakai [210] wrote a synthesis on paradoxical sleep mechanisms. As far as the M.O. is concerned, Sakai thought that the ventromedial medulla, particularly the magnocellular nucleus with its 'paradoxical sleep-on neurons' (PS-on), corresponds, like pontine structures, to 'paradoxical sleep-executive' neurons. The pontine structures were thought to receive cholinergic influences from the medulla, as shown by Kimura *et al.* [121], and noradrenergic paradoxical sleep-off influences from A_1 and A_2 nuclei [57]. Indeed, electrolytic lesions of the reciprocal pathways interconnecting the peri-locus coeruleus α and the M.O. magnocellular nucleus induce a complete or almost complete suppression of paradoxical sleep. In the same way, the M.O. "magnocellular nucleus (PS-on neurons) receives presumed cholinergic afferents from the peri-locus coeruleus α and receives serotoninergic afferents from the dorsal raphe and noradrenergic afferents from the locus coeruleus complex" (p. 129). An interesting new hypothesis advanced by Sakai is that, in contrast to Hobson *et al.*'s [102] theory of reciprocal discharges of PS-on and PS-off neurons, there could be a mutual inhibition between PS-on and PS-off neurons.

Sastre *et al.* [219], also in 1985, injected bovine pituitary extracts (5, 10, 20, 30 mU) in the IVth ventricle or bilaterally in the M.O. magnocellular nucleus, area postrema and nucleus of the solitary tract. The intact cats were $< 400\ mg\ kg^{-1}$ PCPA. With a latency of 79 ± 35 min, the intracerebroventricularly (icv) injection induced paradoxical sleep for a while. The injection in the magnocellular nucleus induced a transient reappearance of paradoxical sleep with a latency of 58 ± 26 min. No changes were triggered by an injection in the M.O. dorsal structures (solitary tract nucleus and area postrema). The serotonin injection in the ventricle induced paradoxical sleep with a latency which was double that of pituitary extract (130 min vs 70 min). Moreover, serotonin first induced the two foregoing slow wave sleep stages whereas the pituitary extract induced paradoxical sleep directly after the first slow wave sleep stage. Thus, the effect seemed to be independent of serotonin. The authors concluded that there are paradoxical sleep executive structures in the M.O.

In 1986, the participation of the M.O. in paradoxical sleep generating processes was also emphasized by Webster *et al.* [263]. These authors undertook transections of the reticular formation at the pontomedullary junction in 13 cats. The animals were recorded for 3 days in baseline condition and for 21 days after transection. The transection of the entire reticular formation suppressed paradoxical sleep, as characterized by cortical low voltage activity, PGO spikes or muscle atonia. Slow wave sleep was reduced and the EEG amplitude was also diminished, which agrees with the Moruzzi group results (see above). After transection of the dorsal half of the reticular formation, paradoxical sleep was still present though with a smaller number of PGO spikes. The same modifications of slow wave sleep were observed after this partial transection. After transection of the ventral half of the reticular formation, paradoxical sleep was present, as shown by cortical low voltage activity and PGO spikes, at a reduced rate. Muscular tonus did not disappear. The amount of paradoxical sleep was reduced. This was also the case for slow wave sleep. In the discussion, the authors stated that "the pontine tegmentum, when (totally) disconnected from the medullary reticular formation, was not sufficient to generate paradoxical sleep in the forebrain, as would have been evident by PGO spiking in association with low-amplitude EEG" (p. 14). The decrease of PGO spikes after dorsal and ventral transection shows that the phasic activities require recruitment of a large population of reticular neurons. They concluded by emphasizing that "neither the pons nor the medulla alone is sufficient for paradoxical sleep" (p. 21).

The same year, Sakai *et al.* [214] determined the localization of cholinergic neurons in the lower brain stem of cats. They used a monoclonal antibody against choline acetyltransferase, the acetylcholine synthetizing enzyme. At M.O. level, cholinergic neurons were situated only in the medial and caudal part of the magnocellular nucleus and adjacent gigantocellular nucleus. These results confirmed those of Kimura *et al.* [121] also obtained from cats.

In 1986 also, an extensive study of ascending projections from the M.O. reticular formation was undertaken by Vertes *et al.* [257] in rats. They used an autographic method with (^{3}H)leucine labelling of fibers after injection in the reticular nuclei. "Nucleus gigantocellularis injections produced heavy labelling in the pontomesencephalic reticular formation...the pontine and midbrain central gray...the ventral midbrain tegmentum...the centromedian–parafascicular complex...the rostral intralaminar nuclei and

the lateral hypothalamic area. Nucleus gigantocellularis projections to the rostral forebrain were sparse...The projections of the ventral reticular nucleus, like those from the gigantocellular nucleus, ascend largely in the tract of Forel and were distributed toward the pontomedullary reticular core...the pontine nuclei and the dorsolateral pontine tegmentum including the locus coeruleus and the parabrachial complex. Although projections diminished significantly rostral to the pons, labelling was still demonstrable in several mesodiencephalic nuclei'' (p. 873). The magnocellular nucleus, that the authors called gigantocellular-pars alpha nucleus, projected in part to the same brain stem sites innervated by the gigantocellular nucleus. There were projections toward the pontomedullary reticular formation, the locus coeruleus, the pontine nuclei, the dorsolateral tegmental nucleus but also to the raphe pontis and adjacent regions of the pontine grey. ''At mesencephalic levels, labelling was present in the rostral raphe nuclei (dorsal, median and linearis), the mesencephalic gray...the ventral tegmental area...as well as the mediodorsal and reticular nuclei of the thalamus. Injections of the parvocellular reticular nucleus labelled axons which coursed through the lateral medullary tegmentum to heavily innervate lateral regions of the medullary and caudal pontine reticular formation and the parabrachial complex. Parvocellular projections to the medial pontomedullary reticular formation were scarce and few ascended rostrally beyond the mid-pons. Labelled fibers from reticular formation injections that included the A_1 noradrenergic area coursed laterally through the brainstem and distributed significantly to the locus coeruleus, the parabrachialis complex, the dorsolateral tegmental nucleus and mid-lateral regions of the pontine gray. Labelling was light throughout the core of the pontomedullary reticular formation. Labelling in the diencephalon was pronounced. Several groups of the hypothalamus were heavily labelled. In the thalamus, light labelling was observed in the rostral intralaminar nuclei and midline nuclei...significant numbers of labelled fibers reached the rostral forebrain and distributed to part of the amydaloid complex...as well as to the medial septum and the diagonal band nucleus '' (p. 873). ''In the forebrain, most of A_1 fibers coursed within the medial forebrain bundle, giving off projections to several nuclei en route'' (p. 896). The authors concluded that the anterior projections of the M.O. reticular neurons are connectionally heterogenous in the rat. ''It seems reasonable to predict that such heterogeneity will be manifested by differences in function'' (p. 896).

Shiromani and Fishbein [231], in 1986, infused cholinergic interacting compounds in the pons, the M.O. and the fourth ventricle of rats. At the medulla level, the continuous injections (days 2 through 5) were situated on the midline, above the raphe magnus nucleus, at the level of the solitary tract nucleus and laterally in the magnocellular nucleus. Midline infused scopolamine ($9.0\ mg\ ml^{-1}\ hr^{-1}$), a muscarinic blocker agent, induced a mean decrease of total sleep time and paradoxical sleep during the total recording infusion duration, the changes occurring only during the day-time. Midline carbachol infusion ($0.5\ mg\ ml^{-1}\ hr^{-1}$) induced an increase of paradoxical sleep at night. During the first day of injection, scopolamine induced a decrease of total sleep and an increase of paradoxical sleep during the day-time and an increase of slow wave sleep at night. During the same first day, midline infusion of carbachol induced an increase of total sleep, slow wave sleep and paradoxical sleep during the day-time and an important increase of paradoxical sleep during the night. In the magnocellular nucleus, tested only under carbachol, there was a decrease of paradoxical sleep during the first daytime period and an increase of total sleep and slow wave sleep during the night period. The discussion was principally centered on the pontine results. However, the authors mentioned the 30% increase of paradoxical sleep amount after infusion in the M.O.. This increase was related to the enhanced number of phases. Consequently, this paper confirms the possible participation of the M.O. in paradoxical sleep inducing processes mediated by cholinergic influences.

The same year, Puizilloux [192] wrote a review in French on the effects of vago-aortic stimulation on slow wave sleep and paradoxical sleep. He analyzed the serotoninergic processes regulating the functioning of the solitary tract nucleus. He interestingly recalled that Galien (*ca* 131–201 AD) had observed that compression of a neck vessel induces sleep signs and that he called the artery the carotid (gr. karôtides) from karoûn: to make drowsy. Moreover, Puizilloux [192] described several examples of clinical data showing the involvement of the vago-aortic afferences in the induction of sleep symptoms.

In 1986 once again, Astier *et al.* [9] studied the influence of the M.O. C_2 adrenergic neurons on the noradrenergic neurons of the locus coeruleus of rats (Fig. 20). They made bilateral electrolytic lesions of the adrenergic nucleus and quantified *in vitro* the response of the locus coeruleus neurons. They observed an increase (+104%) in tyrosine hydroxylase activity (the synthetizing and limiting enzyme of noradrenaline) without any change of dopamine beta hydroxylase (noardrenaline synthetizing enzyme) and phenylethanolamine-*N*- methyltransferase (PNMT, adrenaline synthetizing enzyme) activities. The lesion of the M.O. noradrenergic area A_2 did not induce any change in the locus coeruleus. Consequently, the M.O. adrenergic area C_2 seems to control the locus coeruleus by inhibitory influences while noradrenergic control from area A_2 seems to be absent. This adrenergic control is of importance since the locus coeruleus is a crucial structure for sleep–waking and sensory processes [11, 12]. As shown in a subsequent paper [10], the influences ascend particularly along the ipsilateral medullary longitudinal bundle as do those of the adrenergic C_1 nucleus. Indeed, the lesion of this bundle significantly diminishes the PNMT-immunoreactive fibers of the upper situated brain stem structures. This is an another important finding suggesting possible M.O. control of sleep–waking processes.

At the same period, also in rats, Aston-Jones's group published a number of papers over a period of several years on the factors regulating the functioning of the locus coeruleus which itself had been

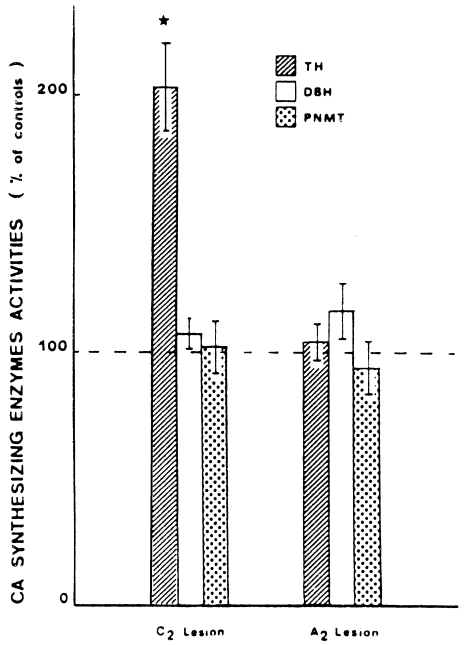

Fig. 20. Astier *et al.* (1986) examined in rats the effects of an adrenergic C_2 area lesion (C_2 is juxtaposed to the A_2 noradrenergic area) on the locus coeruleus neurochemical functioning. The lesion induced an increase in tyrosine hydroxylase (TH) activity, while the dopamine beta hydroxylase activity (DBH) and phenylethanolamine-*N*-methyltransferase (PNMT) (enzyme of adrenaline synthesis) activities remained unchanged. The lesion of the A_2 area did not induce any change. Consequently, unlike the A_2 area, the C_2 area seems to control the locus coeruleus by inhibitory influences. From Ref. [10], with Elsevier's permission.

previously studied [11, 12]. They first determined [13] in 1986 that the afferents to the locus coeruleus originate in the M.O. Using the tracer wheat germ agglutinin-conjugated-horseradish peroxidase injected in the locus coeruleus (precisely identified by single cell recordings through the injection pipette) they found retrograde labelling in the ventral paragigantocellular and dorsal prepositus hypoglossi nuclei (Fig. 21). The first projections were predominantly ipsilateral while the second group were bilateral but slightly greater contralaterally. Interestingly, this paper shows that the locus coeruleus which innervates many central structures of the brain is essentially controlled by two M.O. structures.

In a study published at the same time, Ennis and Aston-Jones [67] identified excitatory and low inhibitory inputs to the locus coeruleus induced by M.O. ventrolateral stimulation. The afferents came from the paragigantocellular nucleus. Single low intensity pulses activated 60% of the locus coeruleus recorded neurons. The latency of responses was 11.7 msec and the duration 35.4 msec. In 20% of cases, the stimulation induced a pure inhibitory response with a latency of 22.8 msec, the duration of response arrest being 404 msec. The monosynaptic activating response was blocked by kynurenic acid, an excitatory amino acid antagonist, while scopolamine, a muscarinic antagonist had no effect. These data suggest that excitatory and inhibitory responses result from two different neuron populations. "However, it is also possible that the inhibitory responses were mediated by a collateral feedback mechanism among locus coeruleus neurons" (p. 303).

In 1987, Ennis and Aston-Jones [68] further dissociated two kinds of neurons of the ventrolateral M.O. which innervate the locus coeruleus. They were situated in the paragigantocellular nucleus where 20 over 79 studied neurons were antidromically activated by locus coeruleus stimulation (this was the case with two over 44 in the lateral reticular nucleus and in one of these two cases, the neuron was near the caudal border of the paragigantocellular nucleus). One kind of spontaneously active neuron was preferentially located in the medial half of the nucleus while the other, which was non-spontaneously active, was distributed throughout the nucleus. The conduction velocity was higher in the first kind of neuron which could correspond to myelinated neurons while the non-spontaneous locus coeruleus projecting neurons seemed to be non-myelinated. These latter neurons could be adrenergic neurons from the C_1 area. Although the paragigantocellular nucleus regulates locus coeruleus functioning during the sleep–waking cycle [69] (see below), the authors mentioned that the paragigantocellular neuron activity varies with blood pressure, respiratory and cardiac cycles, pCO_2 and noxious and non-noxious stimuli.

The same year, Nosjean *et al.* [178] studied in rats the role of serotoninergic influences on the solitary tract nucleus which were possibly implicated in the sleep–waking cycle. The afferents originated from the raphe nuclei and nodose ganglia. The authors destroyed the serotoninergic terminals with 5,7-dihydroxytryptamine bilateral microinjections in the nucleus of rats pretreated with desmethylimipramine to prevent noradrenergic system alteration. The lesions were either complete with injection at three levels (global commissural-intermediate lesions of the medial and commissural solitary tract nucleus) or regional from one injection. The initial effect with all lesions was an increase in slow wave sleep and a decrease in paradoxical sleep. The long-term effect was a decrease in slow wave sleep with the commissural and global lesion and an increase in paradoxical sleep with the global and obex lesion (Fig. 22). Consequently, the specific effects were a decrease in slow wave sleep with the commissural lesion and an increase in paradoxical sleep with the obex lesion. The increase in paradoxical sleep resulted from an increase in the mean duration of phases, principally during the light period. The authors also studied the associated arterial pressure variations. There was a basic increase during all behavioral stages but "the evolution during the sleep–waking cycle was the same after lesion" (p. 479). The authors hypothesized the influence of the solitary tract nucleus on the locus coeruleus to explain the increase in paradoxical sleep and concluded that "the messages coming from the visceral vagal fibres are integrated within the solitary tract nucleus in order to influence the cardio-vascular and sleep–wake control for homeostatic purposes" (p. 480). These data could be

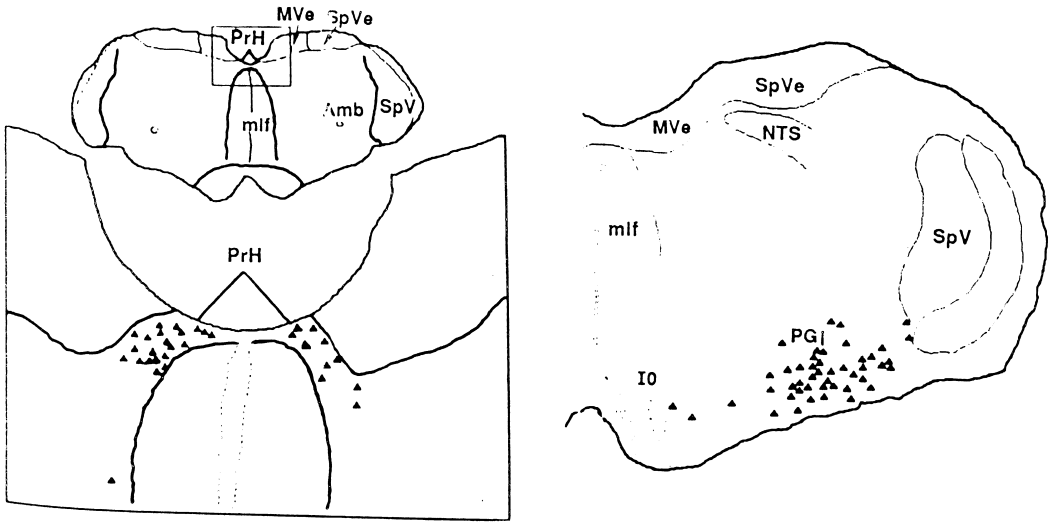

Fig. 21. Aston-Jones *et al.* (1986) studied in rats the M.O. projections toward the locus coeruleus. They were produced exclusively by the ventrolateral paragigantocellular nucleus (PGi) and the dorsal prepositus hypoglossi nucleus (PrH) containing the C_3 adrenergic area. From Ref. [13], with permission.

related to the ability to induce paradoxical sleep by vago-aortic stimulation [198] and to a possible disinhibition of the solitary tract nucleus by the nodose ganglia [197] and the raphe nuclei [216]. This hypothesis is reinforced by the more recent results of Feldman [77], showing an inhibition of firing of solitari tract nucleus neurons by serotonin.

The potential influences of the M.O. reticular formation on the pontine reticular core involved in paradoxical sleep processes were further studied by Ito and McCarley [110] in 1987. In head-restricted unanesthetized and undrugged cats, the authors quantified the intracellular activity of medial pontine reticular formation under M.O. reticular microstimulation (usually < 70 mA). The stimulation occurred principally during slow wave sleep. The pontine postsynaptic excitatory potentials appeared with a latency of < 1.2 msec. The stimulation of the magnocellular tegmental field induced depolarizing effects in 76.8% of cases and hyperpolarizing current in 12.3%. There were no differences in the rostral and middle part of the pontine reticular formation. A pontine antidromic response (one characteristic of which was to follow to a frequency usually > 300 Hz) was observed in 12.8% of the 539 neurons tested. There were no antidromic responses observed in the reticular zone just dorsal to the pontine gigantocellular tegmental field. The stimulation of the M.O. gigantocellular tegmental field induced pontine depolarizing postsynaptic current in 81.3% of cases and hyperpolarizing effects in 12.5% of cases. Pontine antidromic responses were observed in 9.4% of cases after M.O. stimulation. All were localized in the middle zone of the medial pontine reticular field. The stimulation of the M.O. lateral tegmental field induced depolarizing potentials in 90% of cases in pontine reticular neurons. No hyperpolarizing current was induced postsynaptically following M.O. stimulation. A total of 12.5% of pontine neurons gave rise to antidromic responses. This paper shows the existence of reciprocal relationships between the reticular formation of the two ascending brain stem levels and the preferential activating influences of the M.O. reticular formation on the medial pontine reticular formation. However, as observed by the authors, the antidromic activation may concern fibers of passage directed toward the spinal cord but with possible collaterals at the M.O. level.

2.2.4. *1988–1997*

In 1988, Ennis and Aston-Jones [69] stimulated neurons of the paragigantocellular nucleus. They induced activation of 73% of locus coeruleus neurons and inhibited 16% of them. After blocking the excitatory amino acid (EAA) transmission with kynurenic acid and γ-D-glutamylglycine (DGG) administered icv the stimulation of neurons only induced the inhibitory response of locus coeruleus neurons. This inhibitory response could result from adrenergic neuron activation or "activation of collaterals of other locus coeruleus neurons that are excited by the paragigantocellular nucleus" (p. 3651). The excitatory response induced by paragigantocellular nucleus stimulation was not prevented by *N*-methyl-D-aspartate (NMDA) antagonist 2-amino-7-phosphonoheptanoic acid (AP7) nor by the preferential quisqualate receptor antagonist, glutamate diethyl ester (GDEE). Consequently, the EAA could act at the kainate receptor level. Neither scopolamine nor mecamylamine, anti-muscarinic and anti-nicotinic compounds respectively, modified the excitatory responses. Thus, acetylcholine was not involved.

In 1988 also, Danguir and de Saint-Hilaire-Kafi [59] studied the influence of somatostatin antiserum on the carbachol induced increase in paradoxical sleep when injected in the solitary tract nucleus of rats. Indeed, the first author had previously shown that intraventricular injection (third ventricle) of somatostatin selectively increases paradoxical sleep [58] and there are somatostatin containing-neurons and nerve terminals in this M.O. nucleus. Here, they first injected carbachol (1.0 mg

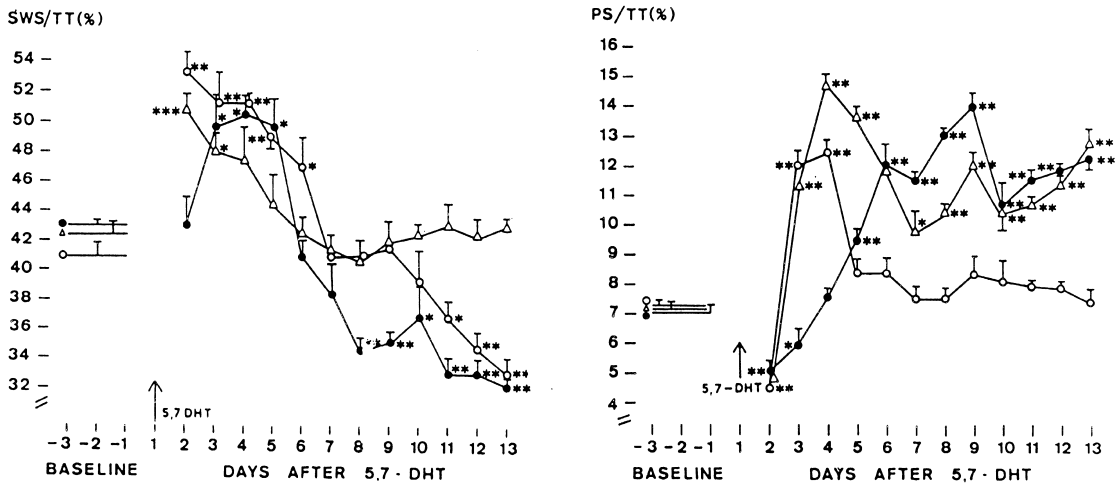

Fig. 22. Nosjean *et al.* (1987) destroyed in rats the serotoninergic afferents to the solitary tract nucleus. The global-intermediate lesion (dotted circles) decreased slow wave sleep after an initial increase (left) and increased paradoxical sleep (right). The commissural lesion alone (○) also decreased slow wave sleep after a transient increase, and did not affect paradoxical sleep (after a transient increase). The lesion at obex level (triangles) only transiently increased slow wave sleep and increased paradoxical sleep. From Ref. [178], with Elsevier's permission.

in 0.5 ml delivered over 2 min) and observed a significant increase in paradoxical sleep over the 8 hr recording sessions, when injected at 9.00 a.m (as already shown by Shiromani and Fishbein [231] at midline level, see above). There was a non-significant increase in slow wave sleep. At a second stage, they infused somatostatin antiserum just after the carbachol injection (while the carbachol group had received normal sheep serum). Not only did the increase in paradoxical sleep disappeared, but there was also a decrease vs the control recording, while slow wave sleep increased. Carbachol increased paradoxical sleep essentially by lengthening the mean duration of the episodes, the mean number of episodes remaining unchanged. The addition of somatostatin antiserum reduced both the mean duration and the mean number of episodes. Consequently, somatostatin at the solitary tract nucleus acts both on paradoxical sleep induction and maintenance. Thus, acetylcholine, at solitary tract nucleus level, seems to induce paradoxical sleep mediated by somatostatin.

The same year (1988), Rye *et al.* [209] studied in rats medullary and spinal efferents of the pedunculopontine and dorsolateral tegmental nuclei, and of the non-cholinergic adjacent mesopontine tegmentum. The results showed that the pedunculopontine nucleus has diffuse projections to the M.O. and that the axons it emits collateralize extensively along their course. Eighteen percent of the pedunculopontine nucleus neurons were labelled by horseradish peroxydase injection in the M.O. gigantocellular field. In contrast, the authors did not find significant M.O. projections from the dorsolateral tegmental nucleus. Moreover, there were descending neurons from the locus coeruleus and periventricular gray to the caudal M.O. Finally, reciprocal relations linked the solitary tract nucleus and the parabrachial nucleus. The authors hypothesized that the pedunculopontine nucleus acting at the M.O. gigantocellular field could be involved in initiating paradoxical sleep since Cordeau *et al.* [55] (see above) were able to induce paradoxical sleep by acetylcholine local injection.

Sakai [211] published a review of the "executive mechanisms of paradoxical sleep" in 1988. He again dissociated:

1. The PS-on neurons which show the significantly highest discharge level during paradoxical sleep, although there are discharges during phasic active waking and a slow increase in discharges from slow wave sleep to the transitional sleep prior to paradoxical sleep (Fig. 23). At the M.O. level, these were observed in the ventromedial and lateral reticular formation, particularly in the magnocellular, parvocellular and lateral paragigantocellular nuclei. During paradoxical sleep, these neurons discharged two to three times more than the pontine PS-on cells. Moreover, these neurons receive descending projections from the pontine PS-on neurons and although they have descending projections to the spinal level, five of the 38 neurons gave rise to ascending projections toward the peri-locus coeruleus α or to intralaminar thalamic nuclei.
2. The PS-off neurons which become silent during paradoxical sleep and correspond to the monoaminergic neurons.

Sakai [211] further developed his theory of reciprocal inhibition ("mutual inhibitory interaction") of PS-on and PS-off neurons. He showed that injection of carbachol (again, mixed cholinergic compound) and bethanechol (a purely muscarinic agonist) in the locus coeruleus α and peri-locus coeruleus α induced excitation of PS-on cells and paradoxical sleep and otherwise inhibited the PS-off cells within the first few minutes. The pontine local PS-off cells were identified (10 out of 14) as noradrenergic by their long latency and antidromic responses to the dorsal

ascending noradrenergic bundle, by their inhibition by intramuscular injection of α_2-autoreceptor agonist clonidine and/or the absence of response to systemic administration of a serotonin autoreceptor agonist. The author does not mention a possible monoaminergic PS-off control of M.O. reticular PS-on neurons and reciprocally, but this can be inferred from his team's previous research [216]. In the discussion, Sakai [211] hypothesizes that the PS-on cells of the M.O. could be cholinergic and that the PS-on process is more important than the PS-off process in inducing paradoxical sleep. An argument in favor of this hypothesis (not mentioned by the author) is that the dorsal raphe PS-off neurons [137] fire six times more than in intact cats during paradoxical sleep without atonia [249]. Moreover, this hypothesis of Sakai [211] is strongly reinforced by a recent finding of Gervasoni *et al.* [86] which shows that bicuculine, a $GABA_A$ receptor antagonist, when infused in the locus coeruleus, induces local important neuron firing during paradoxical sleep without suppressing this sleep stage.

In 1988, the afferents, specifically serotoninergic, towards the solitary tract nucleus were studied in rats by retrograde radioautoradiographic tracing and serotonin immunochemistry [222]. Schaffar *et al.* [222] found projections from the raphe magnus, the ventromedial paragigantocellularis nucleus (synonymous with the magnocellular nucleus), the other raphe nuclei pontis, medialis and dorsalis. The majority of serotoninergic afferents came from the raphe magnus and dorsalis.

A year later, Vanni-Mercier *et al.* [253] devoted an extensive paper to the cholinoceptive structures implicated in paradoxical sleep generation in cats. Regarding the M.O., they injected carbachol unilaterally in the dorsal part of the medulla reticular formation (gigantocellular and parvocellular tegmental fields). They induced "waking with neurovegetative disturbances such as polypnea, emesis, defecation and sometimes vomiting during the first two hours. Paradoxical sleep and advanced slow wave sleep were totally suppressed for the first 4 to 5 hours" (p. 151). The authors thought that the induced "wakefulness...cannot be attributed to the activation of a specific waking system (but) rather to neurovegetative syndromes due to the stimulation of cholinergic or cholinoceptive neurovegetative nuclei located in this region" (p. 158). In contrast, at a more ventral level, extending to the magnocellular tegmental field, carbachol induced waking lasting for 20–30 min, followed by sleeping position but without inducing sleep. Paradoxical sleep and advanced slow wave sleep were suppressed for 2–5 hr and for 6–7 hr, respectively. No vegetative troubles were observed. In this last case, the authors assumed to be in the lower brain stem part of the ascending reticular activating system of Moruzzi and Magoun [169]. This last result partly confirms the finding of Baghdoyan *et al.* [14]. However, as mentioned, these previous authors observed peripheral effects, unlike Vanni-Mercier *et al.* [253]. This discrepancy could result from the different doses used (4 mg/0.5 ml vs 0.4 mg/0.2 ml for the present authors). Consequently, in spite of the M.O. PS-on neurons,

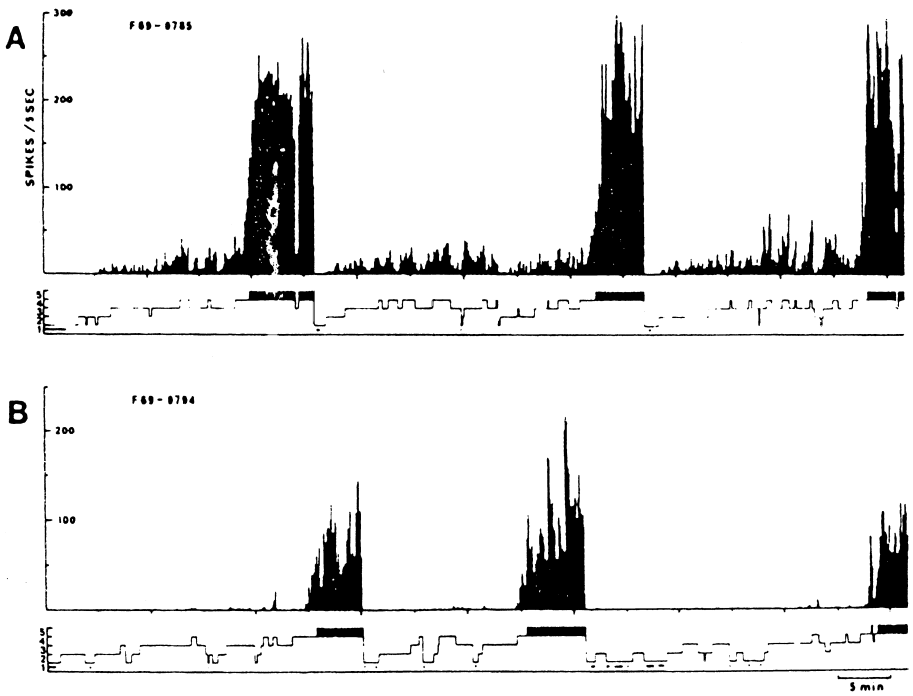

Fig. 23. Sakai (1988) recorded in cats the neurons of the M.O. reticular formation during sleep–waking stages. In the magnocellular nucleus there were highly specific paradoxical sleep activated neurons (PS-on cells). Notice that the neurons already begin to increase their firing rate just prior to paradoxical sleep, during slow wave sleep with PGO waves. (A and B) Two different cells. From Ref. [211], with per-

these results suggest that this brain stem. reticular cholinergic processes are involved in triggering waking and not in generating paradoxical sleep.

In 1989 also, Ennis and Aston-Jones [70, 71] further studied the influence of the dorsomedial rostral M.O. on the locus coeruleus. In their first paper [70], they antidromically stimulated the locus coeruleus of anesthetized rats and activated neurons were found in the nucleus prepositus hypoglossi but not in the solitary tract nucleus. Then, they stimulated the M.O. nucleus prepositus hypoglossi and induced an inhibition of 52 over 63 neurons of the locus coeruleus (Fig. 24). In contrast, only few locus coeruleus neurons were weakly activated by stimulation of the solitary tract nucleus (three out of 22): four were inhibited, 15 were unaffected. Moreover, the effects appeared with a long latency (76 msec vs 18 for prepositus hypoglossi nucleus) suggesting an indirect pathway of influence. Finally, the lesion of the solitary tract nucleus did not change the spontaneous activity of the locus coeruleus neurons. The authors hypothesized that adrenergic influences of the C_3 area of the prepositus hypoglossi nucleus [186] are responsible for locus coeruleus inhibition, although the α_2 receptor antagonist idazoxan did not alter the evoked inhibition. Indeed, Cederbaum and Agajanian [46] had already shown in rats that adrenaline (and noradrenaline) infusion in the locus coeruleus inhibits its neuron discharges.

In a second paper the same year, Ennis and Aston-Jones [71] studied the neurochemical background of the inhibition of 42 out of 47 neurons of the locus coeruleus by prepositus hypoglossi nucleus stimulation. This inhibition "was unaffected by administration of the opiate receptor antagonist naloxone or (again) the α_2-receptor antagonist idazoxan, but was substantially reduced by systemic picrotoxin, an antagonist of gamma-amino-butyric acid (GABA). The $GABA_A$ receptor antagonist, bicuculine methiodide, blocked the inhibition from the prepositus hypoglossi nucleus, whether applied by local microinfusion or by iontophoresis into the locus coeruleus" (p. 2973). The authors discussed the fact that the locus coeruleus neurons are "largely silent" during paradoxical sleep and that there is also a marked reduction in the field potentials during this state. This can only occur under the influence of extrinsic afferents. In addition, these inhibitory influences "may be responsible for the decreased activity and sensory responsiveness found for these cells during other nonvigilant behaviors such as slow-wave sleep, grooming and consumption" (p. 2980). The authors conclude that there is a $GABA_A$-receptor inhibition of the locus coeruleus neurons. However, they recall that there are also inhibitory influences originating from the paragigantocellular nucleus which seem to be adrenergic in nature. Here, also, the results with GABA influences confirm previous data from Cederbaum and Aghajanian [46] which have shown that eight of eight neurons of the locus coeruleus were inhibited by GABA local infusion. In any case, all these experiments show the crucial regulating role of the M.O. on a highly important pontine noradrenergic structure implicated in sleep–waking basic and associated processes (control of sensory information).

The same year also (1989), the group of Aston-Jones (Van Bockstaele *et al.* [252]) studied in rats the afferents of the paragigantocellular nucleus which sends principally facilitatory influences to the locus coeruleus. "The projections to the paragigantocellular nucleus arise from a wide variety of nuclei with autonomic, visceral and sensory-related functions. Major afferents with consistent and robust retrograde labelling include...the nucleus of the solitary tract, the A_1 area, the lateral parabrachialis, the periaqueductal gray...Other notable afferents, seen only after large caudal injections in the nucleus paragigantocellularis, include the lateral hypothalamus, the paraventricular nucleus of the hypothalamus and the medial prefrontal cortex. Minor afferents include the gigantocellular nucleus, the area postrema, the caudal raphe group...the A_5 area and the locus coeruleus" (p. 561). "The functional repertoire of the paragigantocellular nucleus has expanded with recent results, revealing that this area is the sole major excitatory afferent to the noradrenergic locus coeruleus. The locus coeruleus has been strongly implicated in mechanisms underlying arousal, attention and vigilance. Thus, by virtue of its strong connections to the locus coeruleus, the paragigantocellular nucleus may play an important role in behavioral state control" (p. 581).

In 1990, Sakai *et al.* [217] studied the efferents from the lower brain stem to the cat posterior hypothalamus. Using a double-immunostaining technique with cholera toxin as retrograde tracer, they found moderate projections from the magnocellular nucleus reaching the dorsolateral posterior hypothalamus. "These labeled neurons were fusiform or multipolar and medium-sized to large. A few labeled, small to medium-sized cells were also scattered in the nuclei gigantocellularis and parvocellularis. At the level of the caudal medulla, a few labeled cells were observed, in all injection cases, in the caudal lateral bulbar reticular formation around the nucleus ambiguus and lateral reticular nucleus and just around the nucleus of the solitary tract as well. These neurons were mainly medium-sized and distributed bilaterally" (pp. 443–444). The great majority of ipsilabeled neurons within and around the solitary tract nucleus reaching the dorsomedial part of the posterior hypothalamus belonged to the noradrenergic A_2 nucleus as shown by

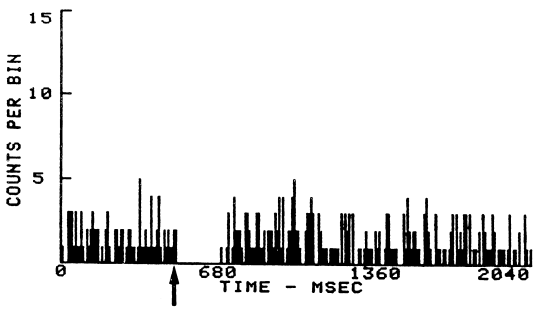

Fig. 24. Ennis and Aston-Jones (1989) showed that the prepositus hypoglossi nucleus (containing the adrenergic C_2 area) is responsible for potent inhibitory influences on the neurons of locus coeruleus. From Ref. [70], with Elsevier's permission.

tyrosine hydroxylase immunoreactivity. The labeled neurons of the most caudal part of the M.O. belonged to the A_1 area. Cells from the rostral part of the lateral medulla and projecting diffusely belonged to the adrenergic C_1 nucleus as shown by phenylethanolamine *N*-methyl transferase immunoreactivity. Finally, in the lateral and middle part of the magnocellular nucleus of the ventral medulla, there were cholinergic neurons projecting specifically into the dorsolateral posterior hypothalamus. Consequently, the posterior hypothalamus which is implicated in sleep–waking processes [130, 131, 136, 172, 173, 200, 213, 260] receives substantial influences from the M.O.

In 1991, Sakai [212] also studied the "physiological properties and afferent connections of the locus coeruleus and adjacent tegmental neurons involved in the generation of paradoxical sleep in the cat" (p. 31). After showing the anatomical situation of the different nuclei of the locus coeruleus region and related neuron functioning modalities (PS-on and PS-off cells), he studied the afferents by retrograde transport of cholera toxin, the transmitters being identified by immunostaining method. He found projections to the locus coeruleus coming from the reticular parvocellular nucleus of the rostral medulla. When the injection was directed at the noradrenergic regions of the locus coeruleus, the solitary tract nucleus contained numerous labeled neurons. The reticular magnocellular nucleus (paragigantocellular pars a in the rat) was shown to send efferents to the medial part of the locus coeruleus α. Only some afferents came from the lateral paragigantocellular and prepositus hypoglossi nuclei. Among the monoaminergic afferents, the raphe magnus and pallidus did not project to the locus coeruleus. In contrast, Sakai [212] found projections from the noradrenergic A_1 and A_2 nuclei, most often ipsilateral, when the retrograde tracer was injected into the noradrenergic regions of the locus coeruleus. Injections at the same level gave rise to retrograde labeling of the adrenergic C_1 cell group. The cholinergic part of the locus coeruleus received projections from scattered neurons in the M.O. parvocellular, magnocellular, gigantocellular and lateral paragigantocellular nuclei and few neurons were found in the prepositus hypoglossi nucleus. These neurons correspond to previously identified PS-on neurons [211]. Moreover, the neurons of the locus coeruleus and adjacent peri-locus coeruleus α send projections to the magnocellular of the ventromedial medulla which corresponds to the inhibitory reticular formation described by Magoun and Rhines [145]. Finally, there were very few ascending peptidergic projections from the paragigantocellular nucleus. Summing up, Sakai [212] observed that there are only few M.O. cholinergic afferents to the locus coeruleus whereas there are dense adrenergic and noradrenergic inputs in this region of paradoxical sleep permissive processes. In contrast, in the neighbouring cholinergic structures involved in paradoxical sleep executive mechanisms, there are dense cholinergic but few adrenergic and noradrenergic afferents. In his conclusion, Sakai [212] suggested that rather than a direct reciprocal interaction between PS-on and PS-off neurons [102], local inhibitory and excitatory interneurons could play an important role in paradoxical sleep generating processes.

Woolf [266], in 1991 also, wrote a review of the cholinergic systems in the mammalian brain and spinal cord. Several comments concern the M.O. "The prepositus hypoglossal nucleus and adjacent region of the medullary tegmentum contain a collection of scattered choline acetyltransferase-positive somata, which in the rat, cat and monkey are smaller than the forebrain cholinergic somata in each species" (p. 506). No ascending projections involving sleep–waking processes are described in the rat. However, "the cholinergic nature of other projections from this area remains to be determined" (p. 506). In the conclusion, the author discusses the influence of upper situated cholinergic systems (pedunculopontine and dorsolateral tegmental nuclei) on paradoxical sleep and waking. The possible contribution of the M.O. is not taken into account.

In an *in vitro* study performed on rabbits, Hay and Bishop [97] (1991) stimulated the area postrema and recorded unit activities in the nucleus of the solitary tract. This research is interesting since the area postrema is contiguous to the solitary tract and sends terminals to it [159, 160] and is rather devoid of blood–brain barrier, which can explain the results obtained by Koella and Czicman [126] (see above). The majority of the neurons in the solitary tract were not spontaneously active. A total of 83% of the neurons were sensitive to area postrema stimulation. In 37 out of 53 neurons, the stimulation of the area postrema induced an action potential. Multiple action potentials were evoked in the 16 other neurons. In the spontaneously active neurons, the stimulation induced an increase of firing. These activating effects were blocked by the α_2-receptor antagonist yohimbine but not by the α_1-antagonist prazosin.

Vanni-Mercier *et al.* [254], in 1991, undertook a study of the influence of the posterior pons and M.O. on paradoxical sleep generating processes (Fig. 25). "Following (complete brain stem) transections either at the caudal pontine level or at the pontomedullary junction, all the cats presented a cycle of waking and slow wave sleep…During waking the hippocampal theta rhythm was faster (4–4.5 Hz) than in the intact cat (3–4 Hz). None of them displayed behavioral or polygraphic signs of paradoxical sleep throughout their survival period (17–30 days)…Carbachol (0.4 mg/0.2 ml) unilateral injections in the locus coeruleus α and peri-locus coeruleus α level could no longer evoke paradoxical sleep in the transected cats, independently of the level of the transection" (p. 43). " Our results show that paradoxical sleep is absent in cats with caudal pontine or prebulbar transections. This indicates that the pons is insufficient for paradoxical sleep generation and thus connections between the pons and the medulla are necessary for the appearance of this state of vigilance. Furthermore, in these transected cats, carbachol is no longer able to induce paradoxical sleep by activating the cholinoceptive pontine structures located in the locus coeruleus α and peri-locus coeruleus α" (p. 44). It can be mentioned that, later on, Gottesmann *et al.* [92] made

complete brain stem transections in the posterior pons of rats (at the level of the middle of the nucleus reticularis pontis caudalis). They were unable to observe paradoxical sleep and its forerunner intermediate stage. Only waking with continuous theta rhythm, drowsiness with theta rhythm and slow wave sleep occurred in the animals recorded up to 9 days after transection.

It had already been demonstrated that the level of brain acetylcholine decreases during paradoxical sleep deprivation [33] while pontine PS-on neurons increase their discharges [150]. Consequently, Thakkar and Mallick [243], in 1991, studied the acetylcholinesterase activity during paradoxical sleep deprivation induced in rats maintained on a small platform (6.5 cm diameter) surrounded by water. They observed an increased activity in the brain stem after 4 and 8 days deprivation. In contrast, there was an increase in the whole brain only after 8 days deprivation. Probably, the cholinergic structures of the M.O. were partially involved in this brain stem increase in acetylcholine degradation, as shown by the following paper.

Indeed the same year, Mallick and Thakkar [148] observed in rats that a short-lasting paradoxical sleep deprivation (24 and 48 hr) by the flower pot method induces an increase in acetylcholinesterase activity in the medulla but neither in the midbrain nor in the pons (Fig. 26). Consequently, the lower brain stem functioning is the first area to be affected by the deprivation. The authors hypothesized that acetylcholine in the M.O. is involved in atonia and cortical EEG activation of paradoxical sleep.

The year after (1992), Mallick and Thakkar [149] continued their research into acetylcholinesterase variations during paradoxical sleep deprivation in rats (Fig. 27). They quantified midbrain, pontine and M.O. changes: on the one hand, in the membrane-bound enzyme which is mainly located in the nerve terminals and is primarily responsible for degradation of acetylcholine and, on the other hand, the free-form which is cytosolic. At the M.O. level, the free-form was decreased after 2 and 4 days platform paradoxical sleep deprivation while the bound-form was increased only after 4 days deprivation. The increase of this form may be responsible for the decrease of acetylcholine previously mentioned. The authors suggest that "the decrease of the free- or soluble-form may reflect its conversion to the bound-form" (p. 678).

Kodoma *et al.* [125] have previously shown by microdialysis methodology that acetylcholine release

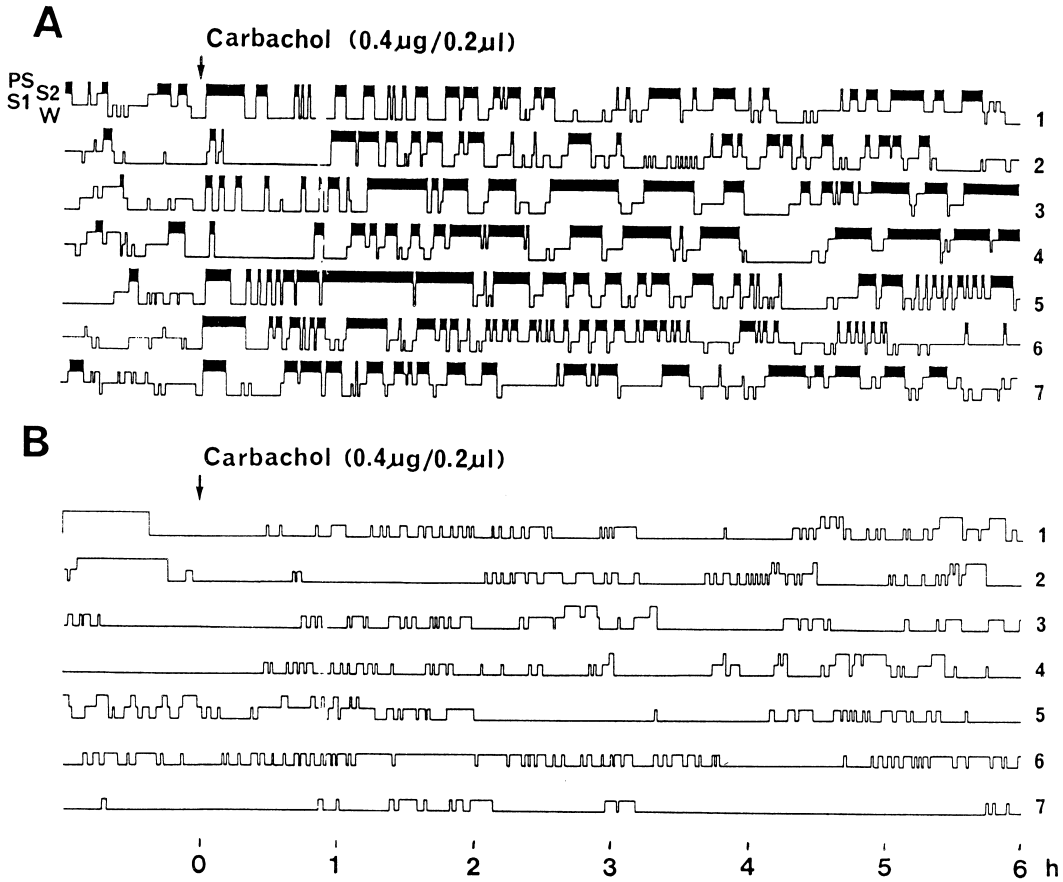

Fig. 25. Vanni-Mercier *et al.* (1991) made brain stem transections at the caudal pontine level (cats 1–5) and at the ponto-medullary junction (cats 6 and 7). In control animals, carbachol injection in the locus coeruleus α and peri-locus coeruleus α induced paradoxical sleep. After transection, at both levels, the animals displayed only waking and slow wave sleep. S1, Light slow wave sleep; S2, deep slow wave sleep; W, waking. From Ref. [254], with Elsevier's permission.

is increased prior to and during paradoxical sleep in the nucleus reticularis pontis oralis of cats. Using the same approach, in 1992 [124], they now studied the release of acetylcholine in the M.O. magnocellular and paramedian nuclei. These nuclei were shown to induce atonia by glutamate and acetylcholine infusion respectively [127]. However, since these nuclei send some ascending axons, this research on cats will be analyzed. In the paramedian nucleus, there was a significant increase of acetylcholine release during paradoxical sleep as compared to the similar levels of waking and slow wave sleep. In contrast, in the magnocellular nucleus, the release, similar during waking and paradoxical sleep, was significantly higher than during slow wave sleep. The authors principally discuss the pontine descending influences on the M.O. and the spinal influences of the M.O.

Because of the importance of the solitary tract nucleus in sleep–waking processes, it is of interest to mention the paper by Glaum *et al.* [87] in 1992 who showed that 5-hydroxytryptamine-3-receptors (5-HT_3) modulate its synaptic activity *in vitro*. The authors first recall that 5-HT_3 receptors were previously described in this nucleus and that they do not function in the metabotropic mode, like all other 5-HT receptors, but in the ionotropic mode since they are directly coupled to an ion channel with a high permeability to Na^+ and K^+. The authors showed that an agonist (2-methyl-5-HT) induced depolarization in 35 over 40 cells. The evoked activity induced by afferents stimulation was reduced by application of 2-methyl-5-HT. This result suggests the existence of both pre and postsynaptic receptors. Since the dose active for evoked activity was significantly lower than for spontaneous activity, the authors suppose that there are more presynaptic than postsynaptic 5-HT_3 receptors. These presynaptic receptors are thought to be situated, as heteroreceptors, on the synaptic terminals of glutamate- and GABA-containing neurons. Consequently, serotonin acting at the level of these receptors may control glutamate and GABA release in the solitary tract nucleus. These effects were observed throughout the nucleus of the rats.

In 1992 also, Pompeiano *et al.* [187] studied the cerebral distribution of 5-HT_{1A} receptors. Using *in situ* hybridization histochemistry to visualize the distribution of mRNA coding for 5-HT_{1A} receptors, the authors were more specifically able to distinguish the receptors localized on the cell body and dendrites. At the M.O. level, they found receptors in magnus, obscurus and pallidus nuclei in smaller quantities than in the dorsal raphe nucleus. In contrast, high levels of hybridation were found in the prepositus hypoglossal nucleus and the solitary tract nucleus. Some receptors were found in the paragigantocellular and lateral reticular nuclei. These results are to be related to the older results of Dahlstrom and Fuxe [57] and Fuxe and Owman [81] who showed serotoninergic neurons in the contiguous area postrema. The authors did not discuss the possible physiological implications for sleep–waking mechanisms.

Again in 1992, Merchant-Nancy *et al.* [157] used Fos-like immunostaining to quantify neurons and

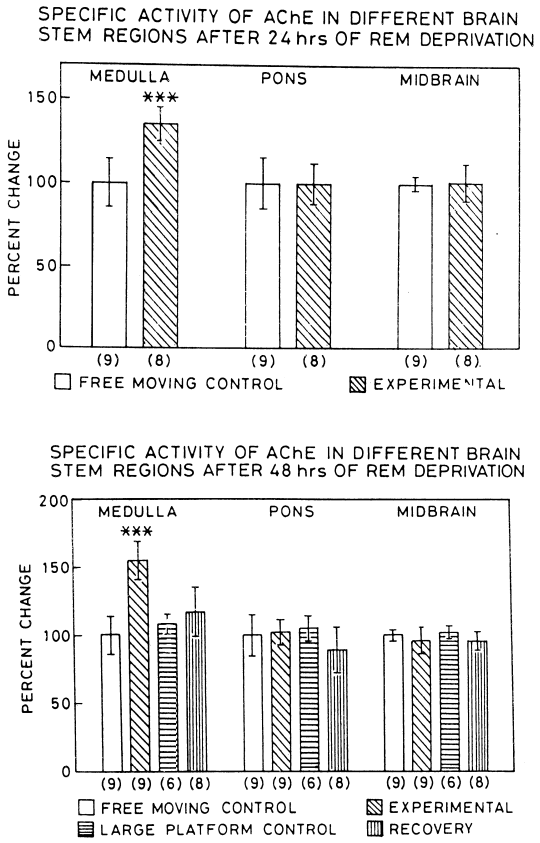

Fig. 26. Mallick and Thakkar (1991) showed in rats that 24 and 48 hr paradoxical sleep deprivation specifically increases acetylcholinesterase activity at M.O. level. From Ref. [148], with Elsevier's permission.

structures which express c-*fos* in the brain. c-*fos* proto-oncogene expression is correlated with ongoing neuronal activity. At the M.O. level, the solitary tract was activated during the paradoxical sleep increase induced by auditory stimuli and during the recovery of paradoxical sleep deprivation by the flower pot technique. Pallidus nucleus activity was increased only by sleep deprivation. The authors recall the results of Puizillout and Foutz [193] showing that stimulation of solitary tract nucleus afferents is able to induce paradoxical sleep.

On account of the major role played by mesopontine cholinergic structures in sleep–waking processes, Steininger *et al.* [238] of Wainer's group continued in 1992 their neuroanatomical study by looking for their central afferents in rats. Regarding the M.O. reticular formation, they found projections to the pedunculopontine nucleus which were less dense than those in the pontine reticular formation. "Labeled neurons were scattered bilaterally throughout the gigantocellular region and appeared more concentrated in medial regions. In addition, a cluster of large (15–35 mm) neurons was labeled in the controlateral dorsomedial reticular formation ventral to the prepositus hypoglossi. A small number of labeled neurons was observed in the rostral ventrolateral medulla" (p. 526). A moderate number of neurons was labeled in the solitary tract nucleus.

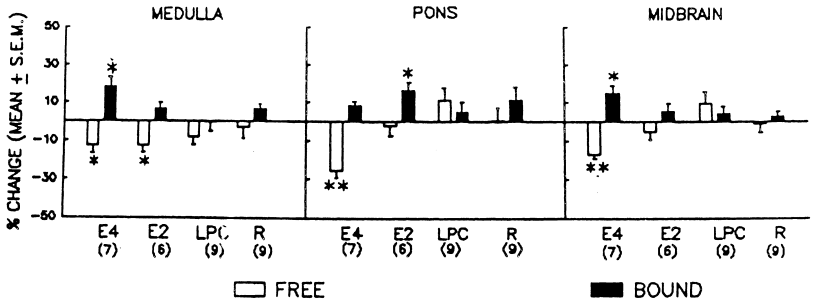

Fig. 27. Mallick and Thakkar (1992) dissociated the free and bound form of acetylcholinesterase in the brain stem of paradoxical sleep deprived rats. In the M.O., the free form was decreased after 4 (E4) and 2 (E2) days deprivation. The bound form, which could be responsible for acetylcholine level decrease in these experimental conditions, was increased after 4 days deprivation. LPC, Large platform control; R, recovery. $*P < 0.05$; $**P < 0.01$. Modified from Ref. [149], with permission.

They were concentrated in the region of the nucleus caudal to the obex and closely approximated the region occupied by the A_2 cell group. The authors also studied the projections of the medial adjacent region to the pedunculopontine nucleus. "Very little retrograde labeling was seen in the medullary reticular formation. A few labeled neurons were observed in the dorsal gigantocellular field and in the nucleus prepositus hypoglossi. A few labeled neurons were observed in the cuneate nucleus" (p. 530). Finally, the authors discussed the brain stem activating influences which can act on the pedunculopontine nucleus but they insist more on the pontine than on the M.O. influences.

In 1993, Semba [227] identified aminergic and cholinergic inputs to the pontine paradoxical sleep inducing zone by fluorescence retrograde tracing combined with immunofluorescence in the rat. They found very few afferents from the M.O. The low number of serotoninergic afferents came predominantly from the raphe magnus nucleus. The few adrenergic projections came from the C_1 adrenergic zone.

Thakkar and Mallick [244], the same year, studied the effect of 1, 2 and 4 days paradoxical sleep deprivation on the MAO, MAO-A and MAO-B activity in the whole brain, cerebrum and brain stem of rats (Table 1). In the brain stem, the authors dissociated the midbrain, the pons and the M.O. They distinguished MAO-A and MAO-B activities since "the former is primarily responsible for the breakdown of noradrenaline". The latter "is a nonspecific enzyme for the breakdown of amines in general" (p. 677). The paradoxical sleep deprivation was achieved using the flower pot technique. To avoid criticism related to induced immobilization stress, a group of rats was maintained on normal litter but of restricted size (12.5 cm diameter) for a fortnight. Regarding the brain stem, the MAO-A activity in control animals was highest in the midbrain and similar in the pons and M.O., but the differences were (apparently) not significant. There were no differences for MAO-B activity at the three levels. "After one day paradoxical sleep deprivation, the MAO-A activity increased in the medulla ($P < 0.001$) whereas it did not change significantly in the pons or midbrain. Two days deprivation significantly increased MAO-A activity in the medulla compared to the control animals. However, the activity was significantly reduced compared to the one day paradoxical sleep deprived rats" (p. 680). It did not change in the pons and midbrain. After 4 days deprivation there was only a global decrease of MAO-A activity in the whole brain stem. MAO-B activity did not change in the M.O. after paradoxical sleep deprivation. It decreased in the pons after 2 days deprivation. In the discussion, the authors pointed out that the changes in MAO-A activity support the involvement of noradrenaline mechanism in paradoxical sleep regulation. The fact that the M.O. is particularly involved in the modifications of MAO-A activity suggests the implication of this brain level in paradoxical sleep generating processes, especially by its PS-on neurons. Since noradrenaline concerns the PS-off neurons, the increased MAO-A activity in the M.O. after deprivation may constitute an attempt to reduce the noradrenaline level, and restore a normal situation.

Thakkar and Mallick [245], in 1993, continued their study of the metabolic consequences of paradoxical sleep deprivation in rats. They studied in the cerebrum, cerebellum and brain stem the evolution of hexokinase activity, the first rate-limiting enzyme in the glycolytic pathway and glucose-6-phosphatase, an enzyme involved in the conversion of glucose-6-phosphate to glucose. The authors still had carefully organized control groups (large platform, restricted normal litter cage and swimming test). The animals were deprived for 1, 2 and 4 days. "Long-term (4 days) paradoxical sleep deprivation significantly increased hexokinase activity in all areas of the brain studied...An increase in hexokinase activity...is likely to enhance metabolism of glucose and thereby induce an increase in energy production. Thus, an increase in hexokinase activity induced by paradoxical sleep deprivation supports increased energy expediture" (p. 693). Only long-term paradoxical sleep deprivation decreased the glucose-6-phosphatase activity in the brain stem. This decrease is likely to reduce glucose transfer and may be responsible for an increase in glucose metabolism and energy expediture. The authors mention that they tested hexokinase activity in different regions of the brain stem (midbrain, pons, medulla) but they found no differences after 1 and 2 days

Table 1. Thakkar and Mallick (1993) studied in rats the influence of paradoxical sleep deprivation on the brain stem activity of MAO-A, which is mainly responsible for noradrenaline degradation, and MAO-B

Groups	Specific activity of MAO-A			Specific activity of MAO-B		
	Medulla	Pons	Midbrain	Medulla	Pons	Midbrain
FMC	0.008 ± 0.003 (12)	0.009 ± 0.003 (12)	0.016 ± 0.005 (12)	0.020 ± 0.006 (12)	0.020 ± 0.003 (12)	0.025 ± 0.003 (12)
E1	0.016 ± 0.002** (12)	0.008 ± 0.002 (12)	0.018 ± 0.001 (12)	0.021 ± 0.002 (12)	0.022 ± 0.001 (12)	0.028 ± 0.002 (12)
E2	0.011 ± 0.004** (8)	0.009 ± 0.003 (8)	0.015 ± 0.003 (8)	0.018 ± 0.002 (8)	0.012 ± 0.001** (8)	0.022 ± 0.001 (8)
R	0.009 ± 0.004 (8)	0.009 ± 0.004 (8)	0.016 ± 0.005 (8)	0.019 ± 0.002 (8)	0.018 ± 0.003 (8)	0.024 ± 0.002 (8)
LPC	0.008 ± 0.003 (12)	0.011 ± 0.003 (12)	0.016 ± 0.004 (12)	0.019 ± 0.003 (12)	0.018 ± 0.006 (12)	0.023 ± 0.004 (12)

MAO-A activity was increased, in the medulla alone, after 1 (E1) and 2 (E2) days deprivation, while MAO-B activity level was unchanged. However, the increase in MAO-A activity was reduced after 2 days deprivation. From Ref. [244], reproduced with Elsevier's permission.No. of animals is shown in parentheses under respective group; *$\alpha = 0.05$; **$\alpha = 0.01$.

deprivation. They could not study reliably the glucose-6-phosphate activity at these different levels.

The same year (1993), Gulyani and Mallick [94] studied the effect of paradoxical sleep deprivation on rat brain Na–K ATPase activity (Fig. 28). Indeed, they hypothesized that the neuronal membrane-bound enzyme activity could be responsible for the enhanced central reactivity observed after this specific deprivation. The animals were deprived for 2, 4 and 8 days. To be sure that the observed effects would be related only to the deprivation and not to associated stress, the authors had the same control groups as above. Moreover, there was another control group with movement restriction. Four days deprivation increased Na–K ATPase activity in the cerebrum, cerebellum and brain stem. At this last level, the disturbance was long-lasting since it persisted after 3 days recovery but disappeared after 6 days recovery. After 2 days deprivation, there was an increase of activity in the M.O. whereas there was a decrease at pontine level. The altered enzyme activity in both regions returned to normal level after 3 days recovery. In the discussion, the authors mention that pontine and M.O. enzyme changes of activity were proportional to the length of deprivation. Moreover, they recall that the activity of other enzymes, namely acetylcholinesterase [243] (see above) and MAO-A [244] (see above) were also first affected in the medulla and the pons after paradoxical sleep deprivation, an observation which highlights the importance of the medulla in these sleep phase generating processes. The M.O. increase in Na–K ATPase activity could be related to monoaminergic PS-off cells which are maintained in activity during paradoxical sleep deprivation [150], since noradrenaline, at least, is known to activate this enzyme [241]. "It is likely that there will be an increase and/or constant supply of noradrenaline, instead of a reduction, in the areas of projection of the paradoxical sleep-off cells including the medulla, leading to an increase in enzyme activity there" (p. 49). Older results reinforce this hypothesis, since Sinha *et al.* [236] showed an increase of tyrosine hydroxylase activity ($P < 0.02$) in the lower brain stem, "by a cut just behind the colliculi, rejecting the cerebellum" (p. 1289) after 96 h paradoxical sleep deprivation.

Sherriff and Henderson [229], in 1994, studied the cholinergic projections of the paragigantocellular nucleus in rats. They were sparse. The neurons had a small cell body and did not project to the pontine cholinergic nuclei. For a possible contribution to sleep–waking processes, there were projections towards the medial medullary formation but it cannot be said whether they were cholinergic in nature. Some projections were found toward the median raphe nucleus and trigeminal motor nucleus. As already shown by Sakai *et al.* [217] a "robust pathway" reaching the forebrain was identified.

The same year, Everson *et al.* [73] studied the brain energy metabolism in total sleep deprived free moving rats. Local glucose utilization was quantified in animals maintained for 11–12 days "on a platform divided in two halves by a fixed wall which was rotated slowly for 6 sec each time sleep onset was detected by microcomputer assessment of

changes in amplitude of cortical EEG, theta and EMG signals...Sleep deprived rats were awake $90 \pm 4\%$ of total time" (p. 6771). Many central brain structures were studied with the quantitative autoradiographic 2-[^{14}C]deoxyglucose method. In many structures, there was a decrease of glucose utilization, in spite of "a marked total body catabolism". In the M.O., no difference in glucose utilization was found in the following nuclei: dorsal paragigantocellular, gigantocellular; raphe obscurus; paramedian reticular; solitary tract; and lateral reticular. Only a slight non-significant increase ($+6\%$) was found in several of these nuclei. Interestingly, the authors mention a paper by Moruzzi [167] who said that the M.O. structures have been purported not to "need" sleep.

Also in 1994, Jones's group [105, 106] published two complementary papers on the M.O. reticular formation, the first one focusing on the distribution of the cholinergic, GABAergic and serotoninergic neurons and their axon projections, the second one studying their influences on the sleep–waking cycle. In the first paper, Holmes *et al.* [106] performed immunohistochemical staining either in control cats

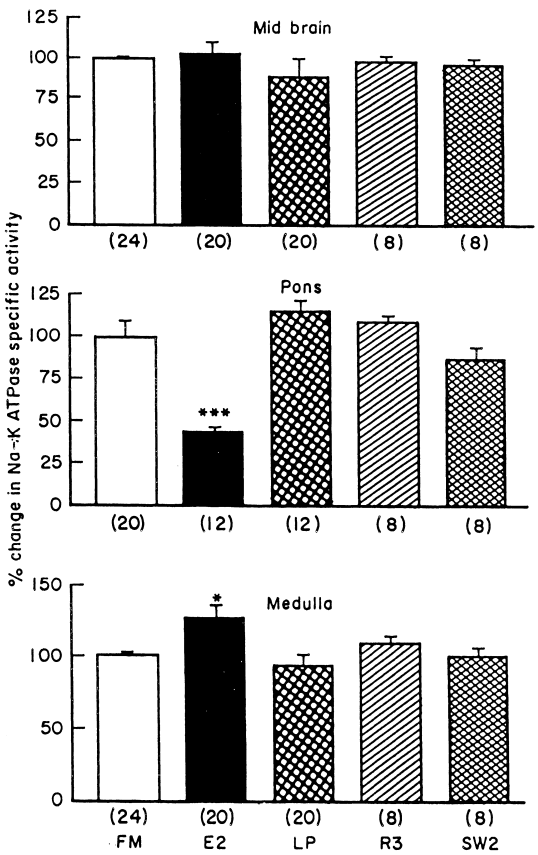

Fig. 28. Gulyani and Mallick (1993) quantified in rats the membrane-bound Na–K ATPase activity which could express neuronal excitability. This activity was increased in the M.O. after 2 days (E2) paradoxical sleep deprivation whereas it decreased in the pons. The increase at M.O. level could be related to the sustained activity of monoaminergic neurons during deprivation. FM, Free moving control; LP, large platform control; R3, after 3 days recovery. SW2, 2 hr swim test. From Ref. [94], with permission.

or in quisqualic acid lesioned animals. The lesions were concentrated in the central and ventral medullary gigantocellular and the magnocellular fields. The authors found that "choline acetyltransferase-immunoreactive neurons were unevenly and sparsely distributed, though none the less in significant numbers (estimated at ≅9080 in total), through the medial medullary reticular formation, and were present in all tegmental fields, including the gigantocellular (≅3700) and magnocellular (≅1760) rostrally and the ventral (≅3240) and paramedian (≅380) caudally, and were absent in the midline raphe. Glutamic acid decarboxylase-immunoreactive neurons are more evenly and densely distributed in large numbers (estimated at ≅18 720) through the medial medullary reticular formation, being present in the gigantocellular (≅5960), magnocellular (≅8260), ventral (≅2280) and paramedian (≅2220) tegmental fields, and are also numerous within the raphe magnus and pallidus-obscurus nuclei (≅3880). Serotonin-immunoreactive cells are sparse in the medial medullary reticular formation (estimated to total ≅1540), where they are mainly located in the magnocellular tegmental field (≅1340) and are concentrated in large numbers within the raphe nuclei (≅8060)" (p. 1155). In the M.O. gigantocellular and magnocellular fields, the cholinergic neurons represent *ca* 5%, the GABAergic *ca* 10–20% and the serotoninergic *ca* 3% of the cells. After a quisqualic acid lesion of the M.O. reticular formation, there was a 17% decrease in cholinergic fibers in the pontine lateral reticular formation and the density of terminals was positively correlated with the number of choline acetyltransferase cells located in the ventral reticular field. The terminals with varicosities in the pontine gigantocellular tegmental field and the locus subcoeruleus were reduced by 20% and the corresponding cholinergic cell bodies were located in the caudal M.O. reticular formation and paramedian reticular field. Of course, there were intramedullary cholinergic projections with unevenly oval varicosities which were shown to innervate non-cholinergic neurons. The lesion induced a decrease in M.O. GABAergic varicosities which were all of short length axons. There were non-significant ascending projections to the pons. Moreover, the highest density of serotoninergic fibres with varicosities was seen in the magnocellular tegmental field. Nevertheless, there were also terminals in the gigantocellular tegmental field, in the paramedian reticular nucleus and the ventral tegmental field. The lesion induced a decrease of fine varicose fibres in the pontine gigantocellular field, the pontine lateral reticular formation and the locus subcoeruleus. This was correlated with a lesion of the serotoninergic cell bodies in the M.O. magnocellular tegmental field. The authors suggested that the local projections of the GABAergic neurons favor modulation processes within the medulla. "They could thus be responsible for the inhibition of PS-off cells presumed to be in part serotoninergic or also the disinhibition of PS-on cells, presumed to be in part cholinergic" (p. 1175).

The second paper [105] studied the repercussion of these M.O. neurons quisqualic acid lesions on sleep–waking cycle. The cats were recorded for base-

line and up to 3 weeks after the lesion, the results of which were pooled by weeks. The reader can find a description of the acute behavior effects of the lesion. The sleep–waking study showed that following the cytotoxic lesions which affected 60% of the medullary gigantocellular and magnocellular tegmental fields, there were no significant variations of the amount of waking and slow wave sleep. However, twitching during slow wave sleep increased. "There was a significant reduction in the amount of paradoxical sleep (to a mean of 64% of baseline) during the first postlesion week, that recovered variably across cats in the second and third week. The individually variable amounts of postlesion paradoxical sleep were correlated positively with the number of surviving cholinergic cells, negatively with the number of surviving serotoninergic or GABAergic cells and positively with the ratio of surviving cholinergic or GABAergic cells to serotoninergic cells" (p. 1179). During paradoxical sleep, there was a significant increase of moving, twitching and, of course, of EMG (as was already the case during slow wave sleep). PGO spiking was not modified by the lesion. In the discussion, the authors stressed that the M.O. "cholinergic neurons may play a facilitatory role in the generation of paradoxical sleep and motor inhibition, whereas serotoninergic neurons may act as an antagonistic manner to these processes and GABAergic neurons as intermediaries between these cell populations" (p. 1193). Holmes and Jones [105] mention that their lesion did not destroy the lateral tegmental field and the ventrolateral part of the magnocellular tegmental field where there are PS-on neurons. The "significant reduction in the daily amount of paradoxical sleep in the present study (shows) that the medullary reticular neurons do normally contribute to the generation and maintenance of the state of paradoxical sleep" (p. 1194) which is in agreement with previous results obtained by the same team [263] after reticular transection at the medulla-pons limit (see above) and the results of Vanni-Mercier *et al.* [254] and Gottesmann *et al.* [92] after transections at almost the same level. "The more rostral medial reticular formation where the gigantocellular and magnocellular tegmental field are located was the more important" (p. 1195) for paradoxical sleep appearance. The paradoxical sleep positive effects of cholinergic neurons and the negative effects of the serotoninergic neurons could be influenced by the local GABAergic short-axoned neurons which could "serve as intermediaries between the cholinergic, putative PS-on cells and serotoninergic putative PS-off cells" (p. 1195). Finally, these results show that "the medullary reticular neurons may comprise a non-essential component of the ascending activating system" [169] (p. 1196) responsible for waking processes.

Osaka and Matsumura [182], in 1994, studied the influence of the locus coeruleus and the ventrolateral M.O. on the activity of the forebrain preoptic area in rats. This concerns two noradrenergic structures, since the medulla locus corresponded to the A_1 nucleus. The deep electrodes were implanted in the loci at the site where the stimulation induced a cortical EEG activation at 50 Hz. These chronic animals were stimulated in the A_1 zone by a brief train of three consecutive pulses at 200 Hz with current intensities up to 200 mA. The rats were unanesthetized, head-restrained and on some occasions, sleep-deprived for 12–14 hr. The authors principally identified preoptic area neurons which were sleep-active or waking-active. The stimulation of the medulla inhibited 54% of the sleep-active neurons and excited 67% of the waking-active neurons. $Alpha_2$ adrenoreceptors mediated the inhibitory responses as seen by preoptic injection of yohimbine, an α_2-blocker which attenuated the inhibitory effects. However, the authors think that there should be postsynaptic α_2 receptors. In contrast, the activation of waking-active neurons is possibly the result of an action at α_1 and β postsynaptic receptors acting on different neurons, as evidenced by the attenuation of responses induced by prazosin and timolol, antagonists of α_1 and β receptors, respectively. This experiment shows that the M.O. is able to modulate major forebrain sleep–waking regulating structures.

Duan *et al.* [63], in 1994, studied in rabbits the hypothalamic descending influences modulating the functioning of the solitary tract nucleus. Out of 128 recorded M.O. neurons, 59 were activated, 69 were inhibited and 40 did not respond to stimulation to what the authors call the "hypothalamic vigilance area", since it induced bradycardia, inspiratory apnea or swallow tachypnea in anesthetized rabbits and behavioral freezing in conscious animals. This area is situated at the medial edge of the dorsal portion of the lateral hypothalamus; it is lateral to the fornix and medial to the optic tract. In this paper, reference is made to several articles showing, in particular, descending influences on the solitary tract nucleus which emanate from the amygdala. This paper demonstrates that, during the sleep–waking cycle, the solitary tract nucleus is also affected by higher brain structures.

The same year, Kobayashi *et al.* [122] studied, using immunochemistry and retrograde transport techniques, the descending projections of the raphe serotoninergic neurons. They confirmed previous studies of 5-HT cell body localizations and found projections towards the M.O. reticular formation. The fiber terminals with fine varicosities surrounded both small and large reticular neurons situated in the medulla gigantocellular field. In the discussion, the authors suggest that these terminals could play a role in the sleep–waking cycle. Indeed, since these serotoninergic neurons are active during waking and silent during paradoxical sleep, they could influence M.O. reticular neurons involved in Moruzzi and Magoun's [169] ascending processes as well as permissive ones allowing PS-on neuron activation.

In 1995, Jouvet *et al.* [116] hypothesized a M.O. Zeitgeber for paradoxical sleep. The data were mainly based on the amount and distribution of paradoxical sleep in the pontine cat during hypothermia. Thirty-six degrees Celsius constitutes the absolute upper threshold for the appearance of paradoxical sleep in this preparation. At lower temperatures, this sleep stage increases, because the ultradian rhythm τ' (the duration between the beginning of two consecutive paradoxical sleep stages) decreases. The authors first concluded that there are

no data available which would explain this increase in τ' frequency by a decrease of monoaminergic permissive mechanisms and facilitation of the cholinergic executive processes of paradoxical sleep. They further recalled that in normal animals, paradoxical sleep is suppressed both by hyperthermia and hypoxia. Consequently, they hypothesized that at 36°C, in pontine preparations, glucose utilization is oriented toward the lactate anaerobic pathway while, during hypothermia, brain functioning could be oriented toward the areobic glycolysis. To verify this hypothesis, pontine cats were subjected to hyperoxia at constant central temperature (34°C). Paradoxical sleep was increased by shortening the ultradian rhythm. "This finding suggests that the genesis of paradoxical sleep involves oxidative glycolysis whereas 'waking' may occur during anaerobic glycolysis" (p. 53). Finally, the authors showed the importance of prolactin acting at M.O. level to induce paradoxical sleep. They recalled previous data showing, first, that a female cat when suckling, and thus releasing prolactin, when prepared as pontine animal with isolated hypophysis, showed an increase of paradoxical sleep; second, that "in a PCPA insomniac cat the injection of pituitary extract (thus containing prolactin) in the ventrolateral part of the medulla is able to restore the ultradian clock (of paradoxical sleep) for some hours" (p. 51) (Fig. 29). These results are described above [219]. Proceeding still further, the authors now injected ovine prolactin (150 UI) in pontine preparations without hypophysis. Indeed, prolactin seems also to favor oxydative metabolism. There was an increased frequency of paradoxical sleep. Consequently, Jouvet *et al.* [116] suggested that "there might be an oscillator responsible for the ultradian rhythm located in the ventrolateral medulla which would control the executive mechanisms of paradoxical sleep located in the pons" (p. 53). This conclusion was reinforced by two arguments advanced by the authors:

1. after a brain stem transection at the anterior limit of the M.O., paradoxical sleep is suppressed (see above [254, 263]);
2. in the same preparation, Siegel *et al.* [234] found cells discharging with a 60 min periodicity which could represent the pacemaker (these authors described a periodicity of 30–60 min, in cells in the gigantocellular nucleus. However, they said that this activity was recorded not during quiet motor periods but during motor active periods resembling those found during waking in intact cats).

It is worth mentioning that Homeyer *et al.* [107], also in 1995, described how ventrolateral M.O. influences are responsible for the suppression of olfactory bulb Ottoson's slow potential and Adrian's induced activity during paradoxical sleep. Indeed, unexpectedly, this phenomenon, observed in intact animals persists in the pontine cat without any possible centrifugal regulation. During paradoxical sleep there is a vasodilatation of nasal vessels. "A significant negative correlation ($P < 0.001$) was obtained between the increase in nasal temperature and the disappearance of olfactory bulb activity during paradoxical sleep" (p. 774). During waking, there is vasoconstriction consecutive to sympathetic influences. Indeed, lidocaine injection in the paragigantocellular nucleus induced vasodilatation by inhibition of local sympathetic tone. The sympathetic vasoconstriction processes are favored by NMDA agonists whereas the parasympathetic vasodilatation influences are mediated by acetylcholine and vasoactive intestinal peptide. Consequently, M.O. influences regulate not only nervous but also vasomotor processes inducing central specific nervous patterns.

In 1995 also, Gulyani and Mallick [95], further tried to explain the increase of Na–K ATPase activity in the paradoxical sleep deprived rat. They studied the cerebrum, the cerebellum and the brain stem. They did not distinguish between the M.O. and the other brain stem levels, but the increase of enzyme activity was previously found at M.O. level [94]. Consequently, it might be expected to be the same in this experiment. "Intraperitoneal injection of alpha-1 antagonist, prazosin or alpha-2 adrenoceptor agonist, clonidine, into the control rats reduced Na–K ATPase activity significantly. Prazosin decreased the Na–K ATPase activity by 35–42% while clonidine by 86–93% in all the areas studied. However, the beta receptor antagonist, propanolol, did not induce any significant effect on the basal enzyme activity in any of the brain regions studied. Paradoxical sleep deprivation increased Na–K ATPase activity in the clonidine treated rats" (p. 256). In a complementary *in vitro* study, noradrenaline increased Na–K ATPase activity in the whole brain homogenate of free moving rats. Prozasin reduced the enzyme activity. These results "indicate that the increase of Na–K ATPase activity after paradoxical sleep deprivation was likely to be mediated by noradrenaline acting primarily on postsynaptic alpha-1 adrenoceptors…However…the precise mechanism is to be determined" (p. 259). It can be mentioned that, just previously, the same authors [146] had found no difference in the midbrain, pons and M.O. chloride-sensitive Mg-ATPase and in chloride-insensitive Mg-ATPase after 2 days paradoxical sleep deprivation, whereas there was an increase in activity of the former in the total brain stem (also in the cerebrum and cerebellum) after 4 days deprivation: "The increase in the chloride-sensitive Mg-ATPase activity is likely to cause an increase in the extrusion of chloride ions out of the neurons, causing a reduction in the hyperpolarization of the neurons" (p. 361).

Reinoso-Barbero and de Andres [202], in 1995, studied the effects of opioid injections in the solitary tract nucleus on the sleep–waking cycle of cats. The basis of the experiment was related first to the research of Albus and Herz [2]. These authors infused morphine in the cerebral ventricles: "When morphine was allowed to spread in the anterior parts of the ventricles (lateral ventricles, 3rd ventricle) only minor changes were observed. However, when morphine was injected into the 4th ventricle, the EEG activation induced by nocipceptive and non-nociceptive stimuli as well as the nociceptive reaction were strongly inhibited…It is concluded that the deactivating effects on behavior and the EEG are mediated by structures bordering the ven-

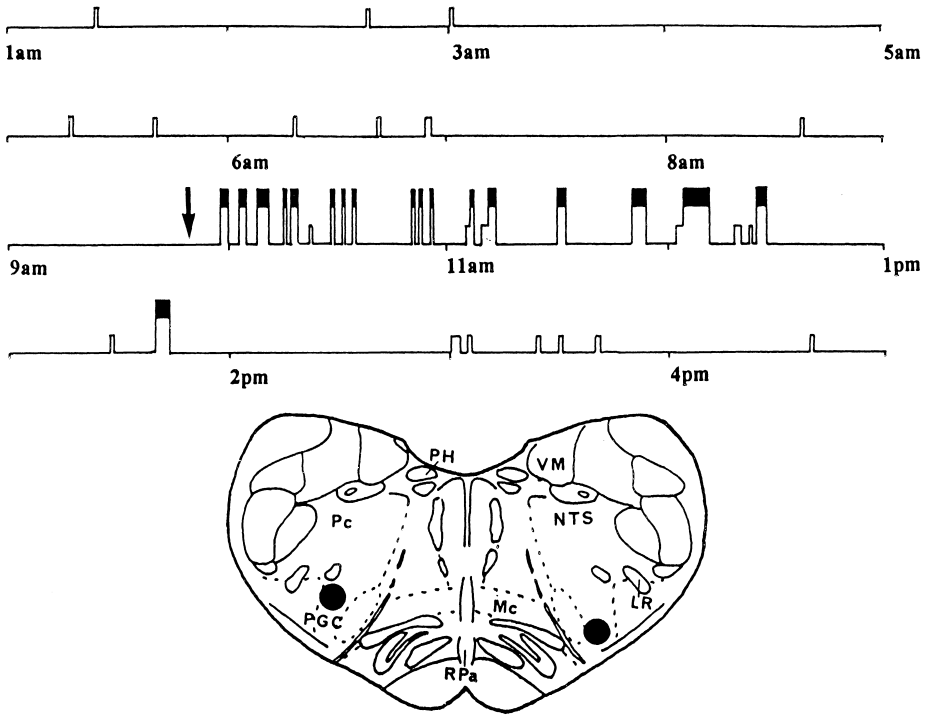

Fig. 29. In Jouvet *et al.*'s demonstration (1995) of a probable Zeitgeber for paradoxical sleep in the M.O., an important argument is the transient induction of paradoxical sleep by pituitary extract injected into the lateral paragigantocellular nucleus (PGC) of cats under PCPA. This experimental result was earlier highlighted by Sastre *et al.* (1985) (see Ref. [219]) From Ref. [116] with permission.

tricles, especially those in vicinity of the 4th ventricle" [2] (p. 588). Later on, Corpas and de Andres [56] showed that, after brain stem transections at midpontine, rostropontine and mesencephalic levels, morphine induced a full EEG activation and mydriasis in contrast with the high voltage, slow burst activity typically seen in the EEG of intact cats under the same doses of morphine (0.5, 2.0 and 3.0 mg kg^{-1}). The effects were blocked by naloxone. In a second previous paper, de Andres and Corpas [8] studied the posterior effects of these transections. Morphine, at the same doses, suppressed paradoxical sleep. After a quiet post-drug period of 10–60 min, the drug induced a long period (1–6 hr) of activated behavior. In the present research, Reinoso-Barbero and de Andres [202] microinjected opioids in the nucleus of the solitary tract. Indeed, the density of opioid receptors is very high at this location. The cats received three doses (0.8×10^{-9} M, 1.7×10^{-9} M or 2.4×10^{-9} M in 0.05 ml) of morphine and of each opioid agonist (μ, δ, κ). Slow wave sleep was increased by morphine (effect prevented by naloxone), μ and δ agonists. Paradoxical sleep was unchanged by the compounds. The κ agonist caused no changes. The positive effects were only observed when the injection site was the solitary tract nucleus. This paper shows opioid control in this nucleus, which confirms Tissot's hypothesis [247].

Because of the importance of calcium in cellular physiology, Mallick and Gulyani [147], in 1996, carried out their research into brain functioning during sleep with their study of paradoxical sleep deprivation (Fig. 30). They first analyzed in rats the synaptosomal calcium total concentrations in brain structures after flower-pots paradoxical sleep deprivation. After 2 and 4 days deprivation, there was an increase in the total brain stem. However, when distinguishing midbrain, pons and M.O. there was an increase ($P < 0.001$) only at medulla level. Then, they quantified the bound and free calcium. Bound calcium increased ($P < 0.001$) whereas free calcium decreased ($P < 0.001$) in the M.O. after 2 days deprivation. In contrast, there was only a reduction ($P < 0.001$) of free calcium in the pons while there were no changes in the midbrain. In the discussion, the authors point out that these results confirm the importance of pons and M.O. in paradoxical sleep generating processes. This conclusion is reinforced by their previous research showing that "Na K ATPase [95] (see above), acetylcholinesterase [243] (see above) and MAO-A [244] (see above) were also affected first in the pontomedullary regions" (p. 734). They also postulate a possible influence of the locus coeruleus which, though usually silent during paradoxical sleep, discharges during paradoxical sleep deprivation and has projections towards the M.O. The release of noradrenaline at M.O. level might possibly increase the calcium influx, increasing the bound form which is already the major pool of the normal cell (99% of the total intracellular pool). These results show a disturbance of M.O. functioning since the free pool which is decreased "is effectively responsible for neuronal functions" (p. 734). The authors suggest that "changes in intracellular calcium concentration could be at least one of the primary underlying mechanisms for paradoxical sleep deprivation-induced alterations in overall

physiological responses of a neuron and ultimately the functioning of the nervous system" (p. 735).

Also in 1996, Aicher *et al.* [3] showed monosynaptic projections from the solitary tract nucleus to the adrenergic C_1 nucleus. Although the authors only mention autonomic potential functions, it had already been shown that the C_1 nucleus projects to the solitary tract nucleus, the locus coeruleus and the hypothalamus [104]. Because of the importance of all these structures in sleep–waking processes, it is useful to mention this pathway.

Nitz and Siegel [177], in 1997, studied by microdialysis the release of GABA, glycine and glutamate in the locus coeruleus of cats. This research into the variations of release during sleep–waking cycle is interesting since GABA afferents to the locus coeruleus have been shown to stem at least from the M.O. prepositus hypoglossal nucleus [71] (see above). The results indicated the highest level of GABA release during paradoxical sleep, the lowest level during waking, an intermediary level of release occurring during slow wave sleep. These data reinforced the theory of permissive influences of locus coeruleus noradrenergic neurons for paradoxical sleep induction [102]. The authors found no differences for glycine and glutamate.

This result is in accordance with the research undertaken by Kaur *et al.* [118], the same year. These authors implanted bilateral guide cannulae in the locus coeruleus of rats and tested the effect of picrotoxin (a $GABA_A$ receptor antagonist) on sleep–waking stages. A total of 250 ng of picrotoxin dissolved in 250 nl ferric chloride solution were injected at 9.00 hr and the animals were recorded for 8 hr. In five randomly selected rats with baseline recording and saline injection control procedure, picrotoxin induced reduced active waking, increased light slow wave sleep and decreased paradoxical sleep. These results show that a reduction of $GABA_A$ influences on the locus coeruleus, thus disinhibition, decreases paradoxical sleep.

In a study not related to sleep mechanisms, Nishiike *et al.* [176], in 1997, showed that the rostral ventromedial part of the paragigantocellular nucleus is responsible for inhibitory influences acting on the locus coeruleus. Indeed, electrical or chemical lesions at this level suppress the inhibitory influences of caloric vestibular stimulations acting at locus coeruleus level. These effects were not mediated by the solitary tract nucleus. The transmitter involved in this inhibition is thought to be GABA. Consequently, this M.O. level probably influences the locus coeruleus during the sleep–waking cycle by inhibitory and disinhibitory processes, as also shown by Gervasoni *et al.* [86].

The same year, Perez and Benedito [185] studied in rats the MAO-A and MAO-B level of activity in the total brain stem, medulla, pons, hypothalamus and hippocampus after 96 hr paradoxical sleep deprivation by the technique using a small platform surrounded by water. The results are similar to those obtained by Thakkar and Mallick [244] for a shorter duration of deprivation. There was an increase in MAO-A activity in the M.O. while MAO-B activity was unchanged. There were no changes in the pons and an increase in MAO-A activity occurred in the total brain stem samples.

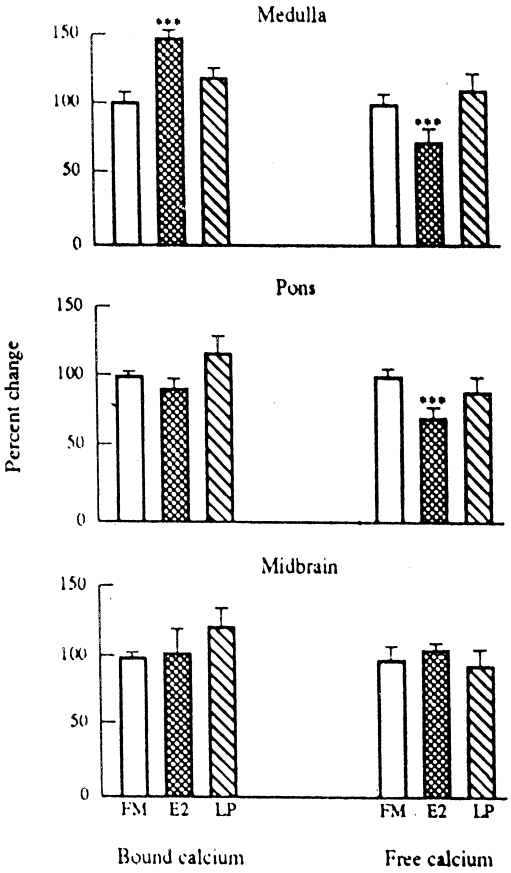

Fig. 30. Mallick and Gulyani [147] showed in the M.O. of rats that, after 2 days paradoxical sleep deprivation, there is an increase of bound calcium concentration and a decrease of free calcium ($P < 0.001$). Because of the importance of calcium in cell functioning, this result reinforces the role of the M.O. in paradoxical sleep generating processes.

Nakanishi *et al.* [171], also in 1997, found in monkeys an increase of protein synthesis in the solitary tract nucleus during deep slow wave sleep ($P < 0.02$). The authors measured the incorporation of L-[1-^{14}C]leucine in seven *Macaca mulatta* sitting in chairs during waking and sleep. There was no change in the magnocellular nucleus. This result has to be related to the increase of local cell discharges observed in cats during the same sleep stage [45, 65, 66, 183].

Kawano and Masuko [119], in 1997, showed in rats that substance P (SP) is found in the terminals impinging the neurons of the solitary tract nucleus. This finding is interesting since the authors identified be retrograde tracer that the corresponding postsynaptic neurons innervate the caudal ventrolateral medulla containing the noradrenergic A_1 and adrenergic C_1 nuclei which project to the hypothalamus, more particularly the paraventricular nucleus. These SP containing terminals were seen especially between 1.00 mm caudal and 0.5 mm rostral to the obex. Some of them, at least, originated from baroreceptor afferents. The solitary tract nucleus projec-

tions towards the catecholaminergic nuclei were bilateral with an ipsilateral dominance. The authors conclude that the hypothalamus is probably affected by the solitary tract nucleus in two ways, one direct, one indirect, the one described here. Although the hypothalamus paraventricular nucleus is not heavily involved in sleep–waking processes, other hypothalamic nuclei are innervated by the catecholaminergic nuclei. It is worth noting that the direct way could also concern some cells stemming from the next situated area postrema. Indeed, lesions principally situated in the area postrema but probably affecting also directly or secondarily the solitary tract nucleus induce neuropeptide Y changes in the hypothalamic paraventricular nucleus [64].

And finally, in 1997, Charnay *et al*. [50] studied the central distribution of 5-HT_{1A} receptors in cats. Using [^{3}H]8-OH-DPAT, a labelled 5-HT_{1A} receptor agonist, they found at M.O. level strong binding sites in the raphe magnus and obscurus nuclei and relatively high labelling in the solitary tract nucleus. These results confirm those of Pompeiano *et al*. [187] (see above) and the authors emphasize the importance of these structures in sleep–waking processes. This research could not identify whether these receptors are postsynaptic or autoreceptors, which is probably the case for the receptors located in the raphe nuclei.

3. DISCUSSION

There is no point in insisting on the neuro-anatomical situation of the M.O. Many of the abovementioned studies have shown that this lowest brain stem level has many, often reciprocal, connections with higher situated structures implicated in sleep–waking processes.

The influence of the M.O. on waking inducing processes is not easy to demonstrate because of the preponderant role of the anterior part of the brain stem as evidenced by the consequences of midpontine transections [15–18] which induce massive increase in waking. However, Moruzzi and Magoun [169] have shown that M.O. reticular stimulation induces cortical activation in light anesthetized animals. These influences favoring waking were confirmed by M.O. reticular injections of adrenaline which increased waking, sometimes without side-effects [55]. Diffusion of noradrenaline at M.O. level induced similar effects [208], and the stimulation of the noradrenergic A_1 area activates waking-active neurons and inhibits sleep-active neurons in the forebrain preoptic nucleus [182]. Acetylcholine at M.O level is also probably involved in waking processes. Indeed, acetylcholine global agonists [14], as well as true muscarinic compounds [101], when injected in the M.O. reticular formation, increase waking (although sometimes with some side-effects), a finding recently confirmed by Vanni-Mercier *et al*. [253].

Besides these probable reticular global effects, often without clearly identified targets, more precise M.O. influences could impinge upon the locus coeruleus which is heavily implicated in waking mechanisms [11, 12]. Indeed, the paragigantocellular nucleus, which sends axon terminals to this nucleus, activates the majority of its cells by action at the kainate receptor level [69]. In the same way, the prepositus hypoglossi nucleus inhibits the majority of locus coeruleus neurons [70] by $GABA_A$ postynaptic receptor targets [71] and it is probably during waking that these ascending inhibitory influences are at their lowest level [177]. Moreover, the locus coeruleus neurons are subjected to inhibitory influences stemming from the M.O. adrenergic nucleus also never previously recorded during sleep–waking cycle [9]. It is possible that all M.O. influences on the locus coeruleus could be involved in the quantitative aspects of waking [132], which seem to be considerable since the destruction of anterior reticular neurons does not significantly disturb waking [62]. However, they could also be involved in its qualitative effects [129], that is, the increase of the signal-to-noise ratio of sensory inputs [12] by the noise reduction relative to an unchanged signal [22].

Consequently, the anatomical position of the M.O. tends to conceal its functional properties when compared to the structures situated in the upper brain stem. Although currently available data do not clearly support a crucial role for waking processes, several results suggest at least a valuable contribution which need further research for better evaluation.

Numerous data since the pioneer work of Moruzzi's group [15–18, 54, 140] and Bonvallet and Bloch [26, 27] support M.O. involvement in slow wave sleep inducing processes. The main involved structure is the solitary tract nucleus. Indeed, stimulation of its vago-aortic afferents [29, 32, 52, 93, 183] as well as direct stimulation [138] induce cortical EEG synchronized activities. In contrast, the elimination of the solitary tract nucleus induces prolonged EEG activation [23–25, 28, 168]. Moreover, there is an increase in its neuron activity during established slow wave sleep [45, 65, 66, 183] and there is a concomitant increase in protein synthesis [171]. The ascending influences seem, on one hand, to act tonically on the activating reticular formation (there are reticular descending activating influences on the M.O. which trigger ascending antagonist influences [27, 152, 153]). On the other hand, they act phasically on the thalamic synchronizing structures [76, 138, 164].

In spite of a general decrease in serotoninergic neuron functioning during slow wave sleep [112, 137, 201, 216], these deactivating and synchronizing influences seem to be partly mediated by serotoninergic processes [120, 126, 128, 178, 207] although EEG synchronization can be obtained in serotonin depleted animals [196].

Less abundant data may suggest the possibly lesser importance of cholinergic component in sleep inducing mechanisms [55, 231].

Consequently, the M.O. and the forebrain preoptic area with its waking antagonistic influences [38, 53, 173, 179, 218, 240, 242, 260] are two anatomical antipodal brain levels favoring slow wave sleep inducing processes.

Numerous recent arguments show the involvement of M.O. in paradoxical sleep mechanisms, although it has been known since 1959 [115], that

the responsible structures are situated under the midbrain.

First of all, although Siegel *et al*. [233] found paradoxical sleep signs in cats transected at the pontomedullary junction, this result could not be confirmed. In contrast, Webster *et al*. [263] found no paradoxical sleep after transection of the reticular formation at the same brain stem level. Vanni-Mercier *et al*. [254] also found no paradoxical sleep after complete transection at the pontomedullary junction and could not even induce this sleep stage by carbachol injection in the pons. Finally, in rats, Gottesmann *et al*. [92] found neither paradoxical sleep nor its forerunner intermediate stage with brain stem complete transection just before the pontomedullary limit.

Older results have also shown that stimulation of vago-aortic afferents, by acting on the solitary tract nucleus, induces paradoxical sleep and its forerunner SPOL stage, with possible direct entrance into paradoxical sleep [198].

Moreover, there are PS-on neurons in the M.O. [175, 210, 211, 235, 239], discharging even more than those at the pontine level [211], although they also often discharge during active waking. However, this is also the case at pontine level where it was first thought that the discharges were paradoxical sleep specific [89, 90, 135] before the discharges during phasic motor activities of waking were described [137, 162, 232, 255]. The destruction of the majority of these reticular PS-on neurons induces a reduction in paradoxical sleep which is positively correlated to the surviving cholinergic neurons and negatively correlated to the serotoninergic and local GABAergic interneurons [105]. However, cholinergic agonists applied in the M.O. reticular formation most often inhibit paradoxical sleep [14, 101, 253].

There are also PS-off neurons in the M.O. [216]. This probably accounts for M.O. functional elimination favoring the induction of paradoxical sleep during slow wave sleep [211], and when these, partly at least, serotoninergic influences are suppressed, there is an increase in paradoxical sleep [178].

The more recent experiments performed by Mallick's group on paradoxical sleep deprivation, namely the increase of acetylcholinesterase bound form [149], of MAO_A activity [244], of synaptosomal Ca and bound Ca [147] and the decrease in Na–K ATPase activity [94], specifically in the M.O., clearly show the involvement of this brain level in these sleep stage mechanisms.

Finally, Jouvet's group study in the pontine cat, with the influence of pituitary extract and prolactine injection in the M.O., appears to show that a paradoxical sleep Zeitgeber [116] is localized there.

Surprisingly, increasing knowledge about M.O. functions has progressively demonstrated that this lowest brain is already crucially involved in sleep–waking mechanisms. It is probable that future research into the activity of cells correlated with their neurochemical support will highlight still further its importance in this fundamental neurobiological rhythm.

Acknowledgements—This research was supported by DSP/STTC grant no. 96-068. I Thank M. Rodi and M. Bordes for providing the photocopy of some papers.

Notes added in proof: Recently, in 1997, Jia *et al*. [112a] described noradrenergic ascending projections from the ventrolateral M.O. (probably A_1 area) and the solitari tract nucleus (probably A_2 area) to the central nucleus of the amygdala. Gabaergic neurons send reciprocal fibers. It could be possible that this ascending pathway, which conveys visceral information, also interferes with amygdala central nucleus influences on sleep-waking behavior [218a], particularly with P.G.O. inducing processes in cats [41a] and rats [59a,b], although serotoninergic influences are obviously concerned. Moreover, in 1998, Hipolide *et al*. [99a] studied in rats the binding to noradrenergic receptors after 96 hour paradoxical sleep deprivation. At M.O. level, they did not find any differences for α_2 receptors in the cuneate, paragigantocellular and solitary tract nuclei. The α_1, β_1 and β_2 receptors were not studied in these structures.

A paper of Aston-Jones's team was overlooked. In 1992, Ennis *et al*. [71a] showed that the facilitatory influences of the ventromedial M.O. (paragigantocellular nucleus) on the locus coeruleus [252] are mediated by excitatory amino acids acting at non-NMDA receptor level. Finally, in 1984, Watanabe *et al*. [262a] found histaminergic terminals in the solitary tract nucleus. Since the corresponding posterior hypothalamic neurons are active only during waking [252a] their arrest of firing during slow wave sleep and paradoxical sleep, could directly or indirectly favor the M.O. structures involved in these two sleep stages.

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