Archives Italiennes de Biologie, 142: 379-396, 2004.

PARADOXICAL REM SLEEP PROMOTING AND PERMITTING NEURONAL NETWORKS

B.E. JONES[1]

Department of Neurology and Neurosurgery, McGill University, Montreal Neurological Institute, Montreal, Quebec H3A 2B4, Canada

INTRODUCTION

Deemed to be discovered in the 1950's, the state of rapid eye movement sleep (REMS) was first identified and so-named for the occurrence of rapid eye movements during behavioral sleep in humans by Aserinsky and Kleitman (2). Subsequently identified by Dement (10) and by Jouvet (46) in cats, this state was called paradoxical sleep (PS) by Jouvet to capture its essence, being the apparent contradictory association of central activation, evidenced by rapid eye movements and fast cortical activity, with peripheral inactivation, evidenced by behavioral sleep and neck muscle atonia. Indeed despite the cortical activation, this state was found to be the deepest state of sleep in terms of the thresholds for sensory-motor reflexes, responses and arousal. Its paradoxical nature was further brandished by assigning it other contradictory names of "active sleep" for the rapid eye movements, muscular twitches, and cortical activation, on the one hand or "deep sleep" for the profound sensory-motor inhibition on the other. It was during this state that dreaming was reported to occur by Dement in humans (9) and evidenced to occur by Jouvet in cats (48). As the state of dreaming when mental images or behaviors are internally generated, REMS or PS may have first been described as a unique state in the 2nd millennium B.C. within the texts of the Upanishads dated back to that period (71). The challenge of the 2nd millennium A.D. has been the electrophysiological apprehension and comprehension of the neuronal networks that are involved in promoting and permitting this paradoxical state as will be reviewed in this chapter.

IDENTIFICATION BY TRANSECTIONS AND LESIONS OF THE ESSENTIAL PS PROMOTING BRAIN REGIONS

In the 1950's and 60's, Jouvet and colleagues searched for the source of the low voltage fast cortical activity on the electroencephalogram (EEG) and the neck muscle atonia on the electromyogram (EMG) that reflect the essential paradoxical association of cortical or central activation with body muscle or peripheral inactivation

[1] Corresponding Author: Dr. Barbara E. Jones, Montreal Neurological Institute, 3801 University Street, Montreal, Quebec, Canada H3A 2B4 - Tel. 514 398 1913 - Fax 514 398 5871 - E-mail: barbara.jones@mcgill.ca

(45, 46). As depicted upon a sagittal diagram from the rat brain (Fig. 1), these phenomena were found to emanate from the brainstem, since they were differentially affected by transections through different levels of the brain (47). Following isolation of the cerebrum from the brainstem by a transection through the rostral mesencephalon (Fig. 1, RM) representing the cerveau isolé preparation in cats, spontaneously occurring cortical activation could no longer be identified during periods of sleep in the forebrain indicating that PS forebrain activation depended upon the brainstem (47, 92). On the other hand, the intact brainstem behind the transection at the rostral mesencephalic level was capable of generating rapid eye movements with muscle atonia indicative of REMS or PS. Indeed, even when the entire forebrain was removed from the cranium in front of a section that left intact the brainstem and a small portion of the hypothalamus (Fig. 1, RM), the brainstem continued to generate PS in chronic preparations (47). According to these results, PS was considered by Jouvet to represent a rhombencephalic state of sleep for which the brainstem was both necessary and sufficient (47).

Within the brainstem, the pons was subsequently shown to be the most important for the generation of PS in cats (47). First, after transections through the rostral pons (Fig. 1, RP), PS persisted in the periphery marked there by the periodic occurrence of neck muscle atonia, though without rapid eye movements which depended upon the integrity of the mesencephalon together with the pons. Interestingly, in earlier experiments in dogs, a total transection through the rostral pons was also reported by Keller (55) to be followed by periods of generalized muscle atonia and areflexia. In contrast, periodic muscle atonia or peripheral PS no longer occurred after transections through the caudal pons in cats (Fig. 1, CP) (47). It was clear from these experiments that the pons contained neurons critical for the generation of PS and the generalized motor inhibition integral to that state.

Within the pons, the reticular formation was found to contain the essential neuronal networks for PS. Following large electrolytic lesions centered in the caudal pontine reticular formation (RF PnC, Fig. 1), all signs of PS were found to be eliminated by Jouvet (47). Electrolytic lesions centered more rostrally in the oral pontine reticular formation (RF PnO, Fig. 1) were on the other hand claimed by Carli and Zanchetti (6) to be more effective in eliminating the state. In subsequent studies employing better controlled thermal lesions (produced by radio frequency instead of direct current), it was established that the most effective lesions were not those which destroyed the gigantocellular field centered in the medial caudal pontine reticular formation but instead those which destroyed fields located in the more intermediate and rostrally located oral pontine reticular fields (16). Thus the neuronal systems critical for generating the state of PS were localized to the oral pontine reticular formation.

Whereas very large lesions were necessary for the elimination of the state, including both phasic rapid eye movements or their central corollary ponto-geniculo-occipital (PGO) spikes and neck muscle atonia in association with cortical activation, small localized lesions in the pons were found by Jouvet to selectively affect the neck muscle atonia and motor inhibition of PS (48). Restricted lesions, which left intact major proportions of the PS generating cell population and thus allowed

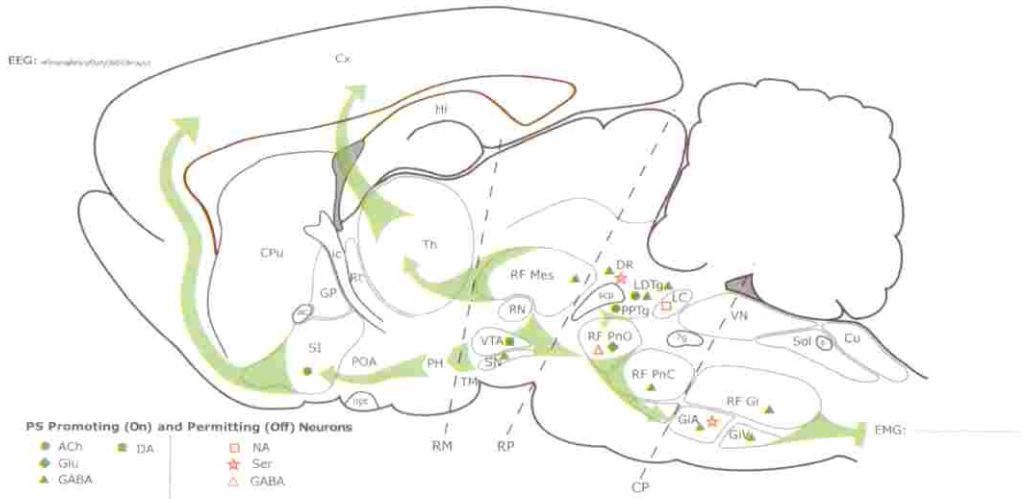

Fig. 1. *Schematic sagittal view of rat brain showing the major neuronal systems and their (excitatory, ending as arrows, and inhibitory, ending as blocks) pathways involved in promoting the EEG fast activity (upper left) and EMG muscle atonia (lower right) characteristic of rapid eye movement or paradoxical sleep (REMS or PS).*

The essential neurons for PS were localized to the brainstem following transections (dashed lines) since the state persisted in the brainstem and not the forebrain following transections at the level of the rostral mesencephalon (RM). The most important population of neurons was localized to the pontine tegmentum since periods of muscle atonia persisted in the periphery after transections at the rostral pons (RP) but not after those at the caudal pons. Within the pons, neurons within the oral pontine reticular formation (PnO) were found to be critical since their destruction by lesions resulted in the elimination of the state. They together with other neurons (below) send ascending projections along the major ascending pathways of the activating system to relay in the thalamus and basal forebrain where other neurons project to the cortex to stimulate cortical activation. They also send descending projections to the spinal cord, in part directly and in part via relays in the alpha and ventral gigantocellular fields (GiA and GiV) of the medullary reticular formation, through which the motor inhibition is effected. Neurons containing different transmitters segregated or intermingled in different brainstem cell groups serve to promote PS by discharging prior to and during PS (On, shown by filled symbols), whereas others serve to permit PS by ceasing discharge prior to and during PS (Off, shown by empty symbols). Cholinergic neurons that release acetylcholine (ACh) and are located in the laterodorsal and pedunculopontine tegmental nuclei (LDTg and PPTg) are critically involved in promoting PS through their projections locally into the brainstem reticular formation and particularly the PnO, where they may inhibit local GABAergic neurons and directly excite glutamatergic neurons, as well as rostrally into the forebrain. Noradrenergic (NA) locus coeruleus (LC) and serotonergic (Ser) dorsal raphe (DR) neurons that directly inhibit the cholinergic neurons permit PS to occur by ceasing their discharge. The arrest of their discharge is effected by local GABAergic PS-On neurons, which may be excited by ACh. GABAergic neurons in the caudal pontine (PnC) and medullary reticular formation (Gi, GiA and GiV) may also inhibit local reticulo-spinal and serotonergic raphe-spinal neurons to effect a disfacilitation of motor neurons. In addition, GABAergic (and glycinergic, not shown) neurons in the ventral medullary reticular formation (GiA and GiV) that project to the spinal cord may directly inhibit motor neurons. One unique group of GABAergic neurons in the PnO ceases discharge during PS to disinhibit PnO glutamatergic neurons that propagate the ascending and descending correlates of the state. In the ascending pathways, dopaminergic (DA) neurons of the ventral tegmental area (VTA), which are excited by the cholinergic neurons, are also active during PS (On) and may stimulate activation of the limbic system. The cholinergic basal forebrain neurons (SI and septum) directly stimulate limbic (hippocampus, Hi) and neo-cortex (Cx) during PS to produce theta and gamma EEG activity. Modified from (43).

rapid eye movements with PGO spikes to occur with cortical activation, were associated with the release of REMS behavior or acting out of dreams in cats (24, 81). This same phenomenon was more recently discovered to occur with restricted brainstem lesions in humans and called REMS behavior disorder (82). The most effective lesions in cats were located in regions of the intermediate reticular fields of the pons (16, 78), what is referred to in the rat brain as the subcoeruleus including importantly the dorsally located alpha part situated immediately ventral to the locus coeruleus nucleus (42) (Fig. 2, SubC and SubCA).

Despite evidence for atonia-promoting neurons being concentrated in the intermediate oral pontine reticular fields including the subcoeruleus region, results also indicated that neurons involved in the initiation and relay of atonia form a larger network including both the pontine and medullary reticular formation. In the pons, more extensive lesions in the reticular formation were necessary to eliminate muscle atonia using neurotoxins, which destroy nerve cell bodies without damaging fibers of passage, than those using electrolytic or thermal techniques, indicating involvement of a relatively large population of neurons (94). From the early studies of Magoun and Rhines (63), employing electrical stimulation, it had been known that the medullary reticular formation also has the capacity to effect motor inhibition. The ventral portion of the medullary gigantocellular field was found to be most important for this action (Fig. 1, GiA and GiV). Interruptions of the descending pathways from the pontine tegmentum to the ventral medulla by small ventrally placed knife cuts at the level of the caudal pons (Fig. 1, CP) eliminated muscle atonia of PS (93), confirming the importance of the ponto-medullary projection for the initiation of atonia. Neurotoxic lesions of the neurons in the ventral medullary reticular formation, like those in the pons, diminished muscle atonia and motor inhibition (26), confirming the role of medullary neurons as a relay for atonia. Yet, there was recovery and a less marked loss of neck muscle atonia following medullary cellular lesions as compared to pontine cellular lesions (94), indicating that even though it is part of a larger ponto-medullary network, the pontine tegmentum must still be considered to be the most important for the initiation and effectuation of muscle atonia.

IDENTIFICATION BY DRUGS, LOCALIZATION AND LESIONS OF THE CHEMICALLY SPECIFIC PS PROMOTING CELL GROUPS

In the 1960's and 70's, pharmacological studies clearly revealed the importance of acetylcholine (ACh) for cortical activation, primarily during waking and secondarily during PS (49). Diminishing the synthesis of ACh by blocking the high affinity uptake of choline (with hemicholinium-3) decreased waking (23). Cortical activation was prevented and replaced by cortical slow waves by blocking muscarinic receptors (with atropine or scopolamine) (61). Enhancing levels of ACh by inhibiting its degradative enzyme, acetylcholinesterase (AChE, with physostigmine), stimulated cortical activation and waking but not PS in intact animals (11). Similarly, administration of nicotine or muscarinic agonists (arecoline or pilocarpine) stimu-

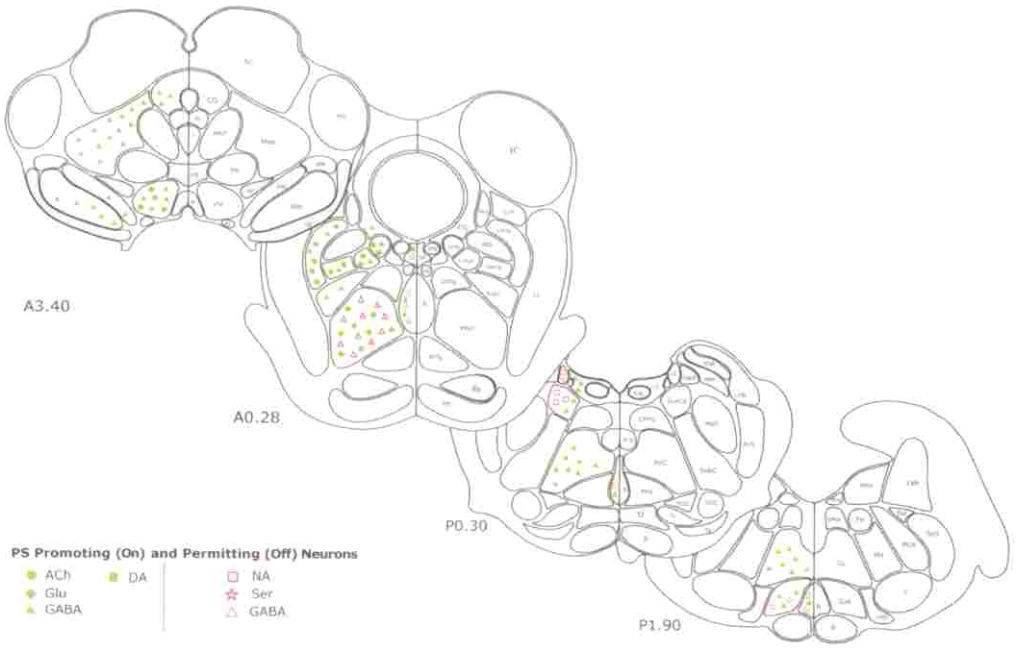

Fig. 2. *Composite of coronal sections through the mesencephalon (A3.40), oral pons (A0.28), caudal pons (P0.30) and rostral medulla (P1.90) of the rat brain showing PS-promoting (On) and PS-permitting (Off) neurons.*

Converging evidence from unit recording and c-Fos activation, which allows immunohistochemical identification of active cells, indicates that cholinergic LDTg and PPTg neurons are active during PS and may excite glutamatergic reticular neurons of the PnO reticular formation which are also active and generate both ascending and descending processes respectively involved in cortical activation and peripheral muscle atonia. Also excited by ACh and active during PS are DA-containing neurons of the VTA which send ascending impulses to the limbic system. GABAergic PS-On neurons are located in the central gray where they may promote PS by inhibiting the NA- and Ser-containing neurons, which are PS-Off and permitting since they inhibit the PS-On cholinergic cells. GABAergic PS-On neurons are also distributed through the reticular formation where they presumably inhibit other reticulo-spinal and raphe-spinal neurons or project directly to the spinal cord from the medulla to effect a respective disfacilitation and inhibition of motor neurons. Finally, critical PS-permitting GABAergic PS-Off neurons are located in the oral pontine reticular formation (PnO) where they hold the glutamatergic neurons under inhibition during W and permit them to discharge during PS to effect the ascending and descending components of the state.

lated cortical activation and waking (49, 53). However, in the rhombencephalic preparation (above), physostigmine was found to elicit PS complete with rapid eye movements, pontine spikes and muscle atonia (47, 67). Atropine prevented this effect. Most dramatic of all, it was discovered that local injection of the mixed muscarinic/nicotinic agonist, carbachol, into the oral pontine reticular formation elicited PS in intact cats (18). This induction of PS by carbachol has been studied by many groups in cats and rats over many years to substantiate and localize the most effective sites of injection in the pons (4, 17, 20, 28, 90, 91). In general, the effective sites

were found within the region where lesions also eliminated the state of PS and across groups have included the entire oral pontine reticular formation. Some groups, nonetheless, maintain that the dorsal subcoeruleus alpha (and peri-alpha) region is most effective (90). However, large populations of pontine reticular neurons are likely involved in propagating the ascending forebrain activation, including limbic and neo-cortical theta and gamma activity, and the descending peripheral muscle atonia. Large numbers of neurons in the oral pontine reticular field give rise to ascending forebrain or descending spinal and/or brainstem projections, and small numbers even to both ascending and descending projections, such as to have the collective capacity to mediate both ascending and descending components of PS (34) (Fig. 1).

By histochemical staining for the degradative enzyme, AChE, Shute and Lewis documented in the 1960's the existence of putative cholinergic neurons in the brainstem and forebrain (84). From their results, it was long believed that the neurons of the brainstem reticular formation were cholinergic (68). Only years later by immunohistochemical staining for the synthetic enzyme, choline acetyltransferase (ChAT), was it apparent that only limited groups of neurons in the pontine tegmentum were cholinergic (1, 35). These are located in the laterodorsal and pedunculopontine tegmental nuclei (LDTg and PPTg, Fig. 1). They are situated in a region that lies behind the most caudal transection that allowed the persistence of PS in the cat (Fig. 1, above) (39). They give rise to projections into the reticular formation, including the oral pontine reticular formation where carbachol induces PS (38). They also give rise to ascending projections, most particularly to the thalamus and the nuclei of the nonspecific thalamo-cortical projection system through which they stimulate cortical activation (22, 36, 37). Some fibers pass ventrally through the extra-thalamic pathway up to the forebrain where they reach the cholinergic cell group in the basal forebrain. The basal forebrain cholinergic neurons project to the cerebral cortex to directly stimulate cortical activation, including theta and gamma activity during waking and PS (44) (Fig. 1).

Neurotoxic lesions of the cholinergic LDTg and PPTg neurons resulted in a loss of PS in cats (94). After 3 weeks during which some PS and PGO spikes reappeared in some animals, the percent PS and number of PGO spikes were positively correlated with the number of remaining ChAT-immunopositive (ChAT+) neurons in the pons. These results clearly demonstrated the importance of the brainstem cholinergic neurons in the generation of PS.

In addition to ACh, the excitatory amino acid glutamate is important in PS. The state of PS was induced by local delivery of the glutamate agonist, kainate, into the pontine tegmentum and particularly into the subcoeruleus alpha region (5, 74). Thus, glutamatergic input to the pontine reticular formation can elicit PS. According to high concentrations of glutamate and also phosphate-activated-glutaminase, its synthetic enzyme, the major population of neurons that form the reticular formation utilize glutamate as a neurotransmitter (42, 50). These include the large reticular neurons of the oral pontine reticular formation. Thus it is not surprising that as the neurotransmitter of the reticular formation, glutamate, would be important in eliciting and generating PS.

IDENTIFICATION BY DRUGS, LOCALIZATION AND LESIONS OF THE CHEMICALLY SPECIFIC PS PERMITTING CELL GROUPS

Early pharmacological evidence in the 1970's indicated that the monoamines (MA) could play a reciprocal role to that of ACh such as to prevent the appearance of PS. Such a relationship could explain why enhancement of ACh with physostigmine enhanced only waking when administered alone (above), but elicited PS when administered following depletion of the MA with reserpine in rats (51). By itself, reserpine also resulted in a release of PS phasic phenomena, PGO spikes, into the waking state in cats (49). Moreover, enhancing levels of monoamines by preventing their catabolism with monoamine oxidase (MAO) inhibitors eliminated PGO spiking and PS for prolonged periods (31, 49).

By histochemical techniques, serotonin (Ser, or 5-hydroxytryptamine, 5-HT)- and catecholamine (CA)-containing neurons were respectively localized to the midline and intermediate regions of the brainstem in rats and cats (8, 75). Lesions of the Ser-containing raphe neurons led to the release of PGO spikes into waking, while PS was diminished along with slow wave sleep (SWS) (49). As only later understood to be due to destruction of adjacent or overlapping pontine reticular and cholinergic neurons, lesions of the noradrenaline (NA)-containing locus coeruleus neurons produced decreases in PS, associated however with a release of PGO spikes into SWS (33, 49, 94). In contrast, lesions of the dopamine (DA)-containing neurons did not appear to affect PS in any fundamental way (32). So, results of lesions must be interpreted in light of collateral damage to adjacent structures but nonetheless corroborate a restraining influence by serotonergic and noradrenergic neurons upon the appearance of the phasic phenomena of PS evident as PGO spikes in SWS and waking that are normally confined to PS. Pharmacological and lesion studies thus revealed an inhibitory role for Ser and NA that was subsequently to be called 'permissive' for PS (25).

Most recently an important permissive role of GABA in the generation of PS has also become apparent through the effects of blocking GABAergic transmission by local injections of the $GABA_A$ receptor antagonist bicuculline into the oral pontine reticular formation of the cat (97). Bicuculline, which would prevent the GABAergic inhibition of the reticular neurons, elicited PS. The same effect has now been obtained in rats by other groups (5, 76, 80). These results reiterate the importance of the pontine reticular neurons in generating PS but also indicate that GABAergic input to these reticular neurons may normally hold them under inhibition such as to prevent the appearance of the muscle atonia and other parameters of this state intruding into waking. Such GABAergic neurons could also be considered permissive for PS since they would have to cease firing prior to PS to lift the inhibition from the PS generating reticular neurons. GABAergic neurons are predominantly small neurons distributed through the reticular formation of the brainstem, including the midbrain, pons and medulla (14, 41, 42). Although some are long projecting neurons, most appear to be locally projecting neurons (27). Different groups

of GABAergic neurons may play different roles in generating PS and its ascending or descending components as considered below.

IDENTIFICATION BY UNIT RECORDING, C-FOS EXPRESSION AND TRANSMITTER RELEASE OF THE CHEMICALLY SPECIFIC PS PROMOTING AND PERMITTING CELL GROUPS

From early unit recording studies, it appeared that the majority of neurons in the pontine reticular formation increased their rate of discharge during PS and could thus be involved in promoting that state (85). Interestingly, however, a minority of neurons decreased or ceased their discharge during PS and could thus be involved in normally preventing that state in what was considered to be a permissive role. The challenge in understanding the relative roles of different neurons has been to establish the neurotransmitter identity of the recorded cells. For most transmitter cell types that are intermingled with other transmitter cell types in the tegmentum, it has not been possible to identify them up to now. Only by application of juxtacellular labeling of neurons recorded during sleep-wake states in head-fixed animals will this identification be possible as has only recently been performed in the basal forebrain (59). In the mean time, expression of c-Fos has been employed as a reflection of neuronal discharge and combined with immunohistochemical staining for neurotransmitter identity of the c-Fos expressing neurons. Combined with knowledge of specific neurotransmitter release during PS, the chemically specific neurons have accordingly been identified that serve to promote or permit PS.

CHOLINERGIC PS-PROMOTING NEURONS

Neurons in the region of the LDTg and PPTg, where cholinergic neurons reside, were found to discharge at high rates during PS as well as waking, relative to SWS and thus considered to be W/PS-On cells (13, 54, 86, 87). Interestingly, some neurons discharged at particularly high rates during PS and at minimal rates during waking and were thus considered to be selective PS-On neurons. The discharge rate of all PS-On cells increased in the transition period leading up to PS. Certain PS-On neurons discharged in bursts in association with PGO spikes. Thus, 'possibly' cholinergic neurons could discharge in association with the cortical activation of PS and waking as W/PS-On neurons, or selectively in association with the muscle atonia or PGO spiking of PS, as selective PS-On neurons involved in promoting that state. However, proof was lacking from the electrophysiological experiments that the active neurons were cholinergic, since cholinergic neurons are co-distributed with a large number of non-cholinergic neurons, including GABAergic neurons, in the LDTg and PPTg (14).

Recent studies employing c-Fos in combination with immunohistochemistry for ChAT showed that the numbers of c-Fos expressing neurons that were ChAT-

immunopositive (+) were greatly increased following rebound of PS (65). Moreover, the number of c-Fos+/ChAT+ neurons was positively correlated with the% PS across PS rebound, deprived and control conditions. These results supported the contention that cholinergic brainstem neurons are active during PS (Figs. 1, 2). Since there were more c-Fos+/ChAT+ cells in the PS rebound than in the PS deprived group, the data would also suggest that some cholinergic neurons are selectively active during PS, perhaps in addition to others that are also active during waking. From the electrophysiological and c-Fos results, it is thus supposed that many cholinergic ponto-mesencephalic neurons are active selectively during PS and some are active during both PS and waking. These different cell groups could potentially play specific roles in muscle atonia or PGO spiking or in W/PS cortical activation.

The release of ACh in the thalamus, to where ponto-mesencephalic cholinergic neurons project, is high during both waking and PS relative to SWS (96), providing evidence that the cholinergic neurons are predominantly W/PS-On cells. Yet, release of ACh in the pontine reticular formation, where carbachol injections elicit PS (above), is selectively higher during PS than during both W and SWS (56). These results would indicate that different groups of cholinergic cells with forebrain vs. brainstem projections may be differentially active in waking and PS with one group being selectively active in PS to promote that state.

MONOAMINERGIC PS-PERMITTING NEURONS

That serotonergic and noradrenergic neurons could suppress the appearance of PS as indicated by pharmacological evidence (above) was supported by the profile of their firing across states in the early recording studies within the dorsal raphe and locus coeruleus. Possibly serotonergic neurons were found to cease their discharge prior to and during PS (69, 70) and possibly noradrenergic neurons were found to do the same (25, 68). The reciprocal changes in discharge rate between the putative noradrenergic locus coeruleus neurons and the pontine reticular neurons suggested that a reciprocal interaction between these cell groups could underlie the cyclic appearance of PS.

Although neither serotonergic nor noradrenergic neurons have been identified as such in recording studies in the cat or the rat (3, 30, 89), it has become generally accepted that these neurons are active during waking, decrease their rate of firing during SWS and cease firing during PS. This supposition has recently been substantiated by dual-immunostaining of c-Fos expressing neurons for Ser or tyrosine hydroxylase (TH), the synthetic enzyme for catecholamines (65). Whereas the number of c-Fos+/ChAT+ neurons was greater in PS rebound than PS deprivation (above), the numbers of c-Fos+/Ser+ and c-Fos+/TH+ neurons were lesser in PS rebound than PS deprivation and their numbers were negatively correlated with the% PS across conditions and groups in the same animals (Figs. 1, 2). On the other hand, dopaminergic neurons of the ventral tegmental area (VTA) appear to be active during PS and perhaps through their ascending projections into the limbic system may contribute to the emotional tenor of this state (64) (Figs. 1, 2).

Certain pharmacological data has provided further support for the notion of reciprocal interaction between serotonergic/noradrenergic PS-Off neurons and cholinergic PS-On neurons. In vitro studies have indeed shown that most identified cholinergic LDTg/PPTg neurons are hyperpolarized and inhibited by serotonin and NA (60, 62, 95). Yet, the activity of possibly cholinergic W/PS-On neurons and the equivalent release of ACh during waking and PS from the thalamus (above) indicate that some cholinergic neurons may also be active in parallel or even tandem with the monoaminergic neurons. This paradox could be explained by different subgroups of cholinergic cells, some being selectively PS-On and others W/PS-On that could be differentially modulated by the monoamines. In recording studies combining microdialysis of drugs in the LDTg, it was found that neurons which were selectively active during PS were inhibited by the serotonin ($5\text{-}HT_{1A}$) receptor agonist, 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT), whereas those which were active during both waking and PS were not inhibited by 8-OH-DPAT (88), suggesting different putative cholinergic cell groups might be differentially affected by Ser and thus comprise PS-On and W/PS-On neurons. Recently, in examining adrenergic receptors on cholinergic LDTg/PPTg neurons in the rat, it was discovered that whereas the majority of ChAT+ neurons bear α_2-adrenergic receptors, which are associated with hyperpolarizing or inhibitory actions of NA, others bear α_1-adrenergic receptors, which are associated with depolarizing or excitatory actions of NA (29), suggesting that in their response to NA some cholinergic neurons would be selectively PS-On whereas others would be W/PS-On.

Whereas presumed selective PS-On cholinergic neurons are inhibited by NA, all noradrenergic neurons have been found to be excited by ACh (12). Accordingly, they could not be turned off during PS by a direct action of ACh upon them in a direct reciprocal manner. Their inhibition could instead occur via the intermediary of local GABAergic neurons (40).

GABAERGIC PS-PROMOTING NEURONS

In studies employing c-Fos activation, it also appeared that many GABAergic neurons in the periventricular and periaqueductal gray region surrounding or intermingled with the serotonergic and noradrenergic neurons were active during PS (65) (Figs. 1, 2). The number of c-Fos expressing neurons that were immunopositive for glutamic acid decarboxylase (GAD), the synthetic enzyme for GABA, was greatly increased with PS rebound. The number of c-Fos+/GAD+ neurons was also positively correlated with the % PS in a parallel manner to that of the c-Fos expressing cholinergic neurons and an inverse manner to that of the c-Fos expressing monoaminergic neurons. Increased activity by the GABAergic neurons innervating the monoaminergic cell groups during PS was also evident by measurement of increased GABA release during PS in the dorsal raphe and locus coeruleus (72, 73). And antagonism of the $GABA_A$ receptor with bicuculline in the locus coeruleus could both prevent the cessation of discharge by the locus coeruleus neurons and disrupt the occurrence of PS (19, 52).

In addition to those surrounding monoaminergic neurons, many GABAergic neurons in other regions appear from c-Fos activation to be active during PS. These include GABAergic neurons in the LDTg and PPTg that are active in parallel with the cholinergic neurons therein (65) and could correspond to thin-spike presumed non-cholinergic neurons that have been found in recording studies to be active like the presumed broad-spike cholinergic cells during PS (79) (Figs. 1, 2). They also include large numbers of c-Fos/GAD+ neurons located in the mesencephalic, caudal pontine and medullary reticular formation (66) (Figs. 1, 2). These GABAergic PS-On neurons are presumed to be PS-promoting through inhibition of neurons that must be turned off for PS to occur (Figs. 1, 2). The latter neurons would include excitatory reticulo-spinal and raphe-spinal neurons as well as motor neurons in the brainstem and spinal cord that cease discharge during PS. Such PS-Off reticular and motor neurons are turned off by both disfacilitation and inhibition, effected by decreasing facilitatory, including monoaminergic, inputs and increasing inhibitory, including GABAergic and glycinergic, inputs (7, 15, 58, 77). Indeed, during carbachol-induced PS, monoamine release is reduced and GABA in addition to glycine release is increased in the medullary reticular formation and motor nuclei (57).

GABAERGIC PS-PERMITTING NEURONS AMONG GLUTAMATERGIC PS-PROMOTING NEURONS

Whereas c-Fos+/GAD+ neurons were increased in number in most regions of the brainstem during PS rebound, they were actually decreased in number in the oral pontine reticular formation, where c-Fos+ non-GABAergic reticular neurons were increased in number (66). These results suggested that such GABAergic neurons could be PS-Off neurons and involved in disinhibiting local reticular neurons that are actively involved in generating PS. Indeed this interpretation was substantiated by pharmacological results showing that bicuculline injected into the oral pontine reticular formation could elicit PS (above) (97). The c-Fos results indicated that local GABAergic neurons would be one important source of the inhibition on PS-On, presumed glutamatergic reticular neurons during waking and perhaps also though to lesser degree, SWS (Figs. 1, 2). Input from LDTg/PPTg cholinergic PS-On neurons could be inhibitory to the local GABAergic neurons and excitatory to the glutamatergic reticular neurons in the oral pontine reticular formation through different cholinergic receptors. Indeed, a differential effect of ACh upon neurons in the pontine reticular formation was reported in vitro (21) as well as in vivo following PS-eliciting carbachol injections (83) with some neurons therein being silenced and others excited. As evidenced by the loss of PS following lesions of the oral pontine reticular formation (above) and the elicitation of PS by carbachol injections into the oral pontine reticular formation (above), the glutamatergic reticular neurons must propagate in tandem with the cholinergic neurons the forebrain activation and peripheral muscle atonia that characterize PS.

SUMMARY

Since its electrophysiological identification in the 1950's, the state of REMS or PS has been shown through multiple lines of evidence to be generated by neurons in the oral pontine tegmentum. The perpetration of this paradoxical state that combines cortical activation with the most profound behavioral sleep occurs through interplay between PS-promoting (On) and PS-permitting (Off) cell groups in the pons. Cholinergic cells in the LDTg and PPTg promote PS by initiating processes of both forebrain activation and peripheral muscle atonia. Bearing α_1-adrenergic receptors, cholinergic cells, which likely project to the forebrain, are excited by NA and active during both W and PS (W/PS-On), when they promote cortical activation. Bearing α_2-adrenergic receptors, other cholinergic cells, which likely project to the brainstem, are inhibited by NA and thus active selectively during PS (PS-On), when they promote muscle atonia. Noradrenergic, together with serotonergic, neurons, as PS-Off neurons, thus permit PS in part by lifting their inhibition upon the cholinergic PS-On cells. The noradrenergic/serotonergic neurons are inhibited in turn by local GABAergic PS-promoting neurons that may be excited by ACh. Other similarly modulated GABAergic neurons located through the brainstem reticular formation become active to participate in the inhibition of reticulo-spinal and raphe-spinal neurons as well as in the direct inhibition of motor neurons. In contrast, a select group of GABAergic neurons located in the oral pontine reticular formation and possibly inhibited by ACh turn off during PS. These GABAergic PS-permitting neurons release from inhibition the neighboring large glutamatergic neurons of the oral pontine reticular formation, which are likely concomitantly excited by ACh. In tandem with the cholinergic neurons, these glutamatergic reticular neurons propagate the paradoxical forebrain activation and peripheral inactivation that characterize PS.

Acknowledgements. - I would like to thank Elida Arriza for her assistance with the illustrations and Karen Maloney, Lynda Mainville and other members of my lab for their contribution to the research reviewed and cited in this article. The research was supported by the Canadian Institutes of Health Research (CIHR, 13458).

List of Abbreviations for Anatomical Structures

3: oculomotor nucleus; 7: facial nucleus; 7g: genu facial nerve; 7n: 7th nerve; ac: anterior commissure; CG: central grey; CnF: cuneiform nucleus; cp: cerebral peduncle; CPu: caudate putamen; Cu: cuneate nucleus; Cx: cerebral cortex; Dk: Darkschewitsch nucleus; DMTg: dorsomedial tegmental area; DPGi: dorsal paragigantocellular nucleus; DR: dorsal raphe nucleus; DTg: dorsal tegmental nucleus; Gi: gigantocellular reticular field; GiA: gigantocellular reticular field, alpha part; GiV: gigantocellular reticular field, ventral part; GP: globus pallidus; Hi: hippocampus; ic: internal capsule; IC: inferior colliculus; IMLF: interstitial nucleus of the medial longitudinal fasciculus; IRt: intermediate reticular field; LC: locus coeruleus; LDTg: laterodorsal tegmental nucleus; LDTgV: laterodorsal tegmental nucleus, ventral part; lfp: longitudinal fasciculus of the pons; LL: lateral lemniscus; LPB: lateral parabrachial nucleus; LPGi: lateral paragigantocellular nucleus; LPPTg: lateral pedunculopontine tegmental nucleus; LVe: lateral vestibular nucleus; Me5: mesencephalic trigeminal nucleus; Mes: mesencephalic reticular field; MG: medial geniculate nucleus; ml: medial lemniscus; mlf: medial longitudinal fasciculus; Mo5: motor trigeminal nucleus; mp: mammillary peduncle; MPB: medial parabrachial nucleus; MPPTg: medial pedunculo-

pontine nucleus; MVe: medial vestibular nucleus; opt: optic chiasm; p: pyramid; PCR: parvocellular reticular field; PH: posterior hypothalamic area; Pn: pontine nuclei; PnC: pontine reticular field, caudal part; PnO: pontine reticular field, oral part; PnV: pontine reticular field, ventral part; POA: preoptic area; PPTg: pedunculopontine tegmental nucleus; Pr5: principal sensory trigeminal nucleus; PrH: prepositus hypoglossal nucleus; R: raphe nuclei; RF: Reticular Formation; RN: red nucleus; Rt: reticular thalamic nucleus; RtTg: reticulotegmental nucleus of the pons; s: solitary tract; SC: superior colliculus; scp: superior cerebellar peduncle (brachium conjunctivum); SI: substantia innominata; SN: substantia nigra; SNC: substantia nigra, compact part; SNL: substantia nigra, lateral part; SNR: substantia nigra, reticular part; SOC: superior olivary complex; Sol: nucleus of the solitary tract; Sp5: spinal trigeminal nucleus; SubC: subcoeruleus; SubCa: subcoeruleus, alpha part; Th: thalamus; TM: tuberomammillary nucleus; tz: trapezoid; VN: vestibular nuclei; VTA: ventral tegmental area; vtt: ventral tegmental tract.

REFERENCES

1. Armstrong, D.M., Saper, C.B., Levey, A.I., Wainer, B.H. and Terry, R.D. Distribution of cholinergic neurons in rat brain: Demonstrated by the immunocytochemical localization of choline acetyltransferase. *J. Comp. Neurol.*, **216:** 53-68, 1983.
2. Aserinsky, E. and Kleitman, N. Regularly occurring periods of eye motility, and concomitant phenomena during sleep. *Science*, **118:** 273-274, 1953.
3. Aston-Jones, G. and Bloom, F.E. Activity of norepinephrine-containing locus coeruleus neurons in behaving rats anticipates fluctuations in the sleep-waking cycle. *J. Neurosci.*, **1:** 876-886, 1981.
4. Baghdoyan, H.A., Rodrigo-Angulo, M.L., Mccarley, R.W. and Hobson, J.A. A neuroanatomical gradient in the pontine tegmentum for the cholinoceptive induction of desynchronized sleep signs. *Brain Res.*, **414:** 245-261, 1987.
5. Boissard, R., Gervasoni, D., Schmidt, M.H., Barbagli, B., Fort, P. and Luppi, P.H. The rat ponto-medullary network responsible for paradoxical sleep onset and maintenance: a combined microinjection and functional neuroanatomical study. *Eur J Neurosci.*, **16:** 1959-1973, 2002.
6. Carli, G. and Zanchetti, A. A study of pontine lesions suppressing deep sleep in the cat. *Archives Italiennes de Biologie*, **103:** 751-788, 1965.
7. Chase, M.H., Soja, P.J. and Morales, F.R. Evidence that glycine mediates the postsynaptic potentials that inhibit lumbar motoneurons during the atonia of active sleep. *J. Neurosci.*, **9:** 743-751, 1989.
8. Dahlstrom, A. and Fuxe, K. Evidence for the existence of monoamine-containing neurons in the central nervous system. I. Demonstration of monoamines in the cell bodies of brain stem neurons. *Acta Physiol. Scand.*, **62:** 1-55, 1964.
9. Dement, W. and Kleitman, N. Cyclic variations in EEG during sleep and their relation to eye movements, body motility and dreaming. *Electroencephalogr. Clin. Neurophysiol.*, **9:** 673-690, 1957.
10. Dement, W. The occurrence of low voltage, fast, electroencephalogram patterns during behavioral sleep in the cat. *Electroencephalogr. Clin. Neurophysiol.*, **10:** 291-296, 1958.
11. Domino, E.F., Yamamoto, K. and Dren, A.T. Role of cholinergic mechanisms in states of wakefulness and sleep. *Progr. Brain Res.*, **28:** 113-133, 1968.
12. Egan, T.M. and North, R.A. Actions of acetylcholine and nicotine on rat locus coeruleus neurons *in vitro*. *Neuroscience*, **19:** 565-571, 1986.
13. El Mansari, M., Sakai, M. and Jouvet, M. Unitary characteristics of presumptive cholinergic tegmental neurons during the sleep-waking cycle in freely moving cats. *Exp. Brain Res.*, **76:** 519-529, 1989.

14. FORD, B., HOLMES, C., MAINVILLE, L. AND JONES, B.E. GABAergic neurons in the rat pontomesencephalic tegmentum: codistribution with cholinergic and other tegmental neurons projecting to the posterior lateral hypothalamus. *J. Comp. Neurol.*, **363:** 177-196, 1995.
15. FORT, P., LUPPI, P.H. AND JOUVET, M. Glycine-immunoreactive neurones in the cat brain stem reticular formation. *NeuroReport*, **4:** 1123-1126, 1993.
16. FRIEDMAN, L. AND JONES, B.E. Computer graphics analysis of sleep-wakefulness state changes after pontine lesions. *Brain Res. Bull.*, **13:** 53-68, 1984.
17. GARZON, M., DEANDRES, I. AND REINOSO-SUAREZ, F. Sleep patterns after carbachol delivery in the ventral oral pontine tegmentum of the cat. *Neuroscience*, **83:** 1137-1144, 1998.
18. GEORGE, R., HASLETT, W. AND JENDEN, D. A cholinergic mechanism in the brainstem reticular formation: induction of paradoxical sleep. *Int. J. Neuropharmacol.*, **3:** 541-552, 1964.
19. GERVASONI, D., DARRACQ, L., FORT, P., SOULIERE, F., CHOUVET, G. AND LUPPI, P.-H. Electrophysiological evidence that noradrenergic neurons of the rat locus coeruleus are tonically inhibited by GABA during sleep. *Eur. J. Neurosci.*, **10:** 964-970, 1998.
20. GNADT, J.W. AND PEGRAM, G.V. Cholinergic brainstem mechanisms of REM sleep in the rat. *Brain Res.*, **384:** 29-41, 1986.
21. GREENE, R.W., GERBER, U. AND MCCARLEY, R.W. Cholinergic activation of medial pontine reticular formation neurons *in vitro*. *Brain Res.*, **476:** 154-159, 1989.
22. HALLANGER, A.E., LEVEY, A.I., LEE, H.J., RYE, D.B. AND WAINER, B.H. The origins of cholinergic and other subcortical afferents to the thalamus in the rat. *J. Comp. Neurol.*, **262:** 105-124, 1987.
23. HAZRA, J. Effect of hemicholinium-3 on slow wave and paradoxical sleep of cat. *Eur. J. Pharmacol.*, **11:** 395-397, 1970.
24. HENDRICKS, J.C., MORRISON, A.R. AND MANN, G.L. Different behaviors during paradoxical sleep without atonia depend on pontine lesion site. *Brain Res.*, **239:** 81-105, 1982.
25. HOBSON, J.A., MCCARLEY, R.W. AND WYZINSKI, P. W. Sleep cycle oscillation: reciprocal discharge by two brainstem neuronal groups. *Science*, **189:** 55-58, 1975.
26. HOLMES, C.J. AND JONES, B.E. Importance of cholinergic, GABAergic, serotonergic and other neurons in the medullary reticular formation for sleep-wake states studied by cytotoxic lesions in the cat. *Neuroscience*, **62:** 1179-1200, 1994.
27. HOLMES, C.J., MAINVILLE, L.S. AND JONES, B.E. Distribution of cholinergic, GABAergic and serotonergic neurons in the medullary reticular formation and their projections studied by cytotoxic lesions in the cat. *Neuroscience*, **62:** 1155-1178, 1994.
28. HORNER, R.L. AND KUBIN, L. Pontine carbachol elicits multiple rapid eye movement sleep-like neural events in urethane-anesthetized rats. *Neuroscience*, **93:** 215-226, 1999.
29. HOU, Y.P., MANNS, I.D. AND JONES, B.E. Immunostaining of cholinergic pontomesencephalic neurons for alpha 1 versus alpha 2 adrenergic receptors suggests different sleep-wake state activities and roles. *Neuroscience*, **114:** 517-521, 2002.
30. JACOBS, B.L. Single unit activity of locus coeruleus neurons in behaving animals. *Progr. Neurobiol.*, **27:** 183-194, 1986.
31. JONES, B.E. The respective involvement of noradrenaline and its deaminated metabolites in waking and paradoxical sleep: a neuropharmacological model. *Brain Res.*, **39:** 121-136, 1972.
32. JONES, B.E., BOBILLIER, P., PIN, C. AND JOUVET, M. The effect of lesions of catecholamine-containing neurons upon monoamine content of the brain and EEG and behavioral waking in the cat. *Brain Res.*, **58:** 157-177, 1973.
33. JONES, B.E., HARPER, S.T. AND HALARIS, A.E. Effects of locus coeruleus lesions upon cerebral monoamine content, sleep-wakefulness states and the response to amphetamine. *Brain Res.*, **124:** 473-496, 1977.

34. JONES, B.E. AND YANG, T.-Z. The efferent projections from the reticular formation and the locus coeruleus studied by anterograde and retrograde axonal transport in the rat. *J. Comp. Neurol.*, **242:** 56-92, 1985.
35. JONES, B.E. AND BEAUDET, A. Distribution of acetylcholine and catecholamine neurons in the cat brain stem studied by choline acetyltransferase and tyrosine hydroxylase immunohistochemistry. *J. Comp. Neurol.*, **261:** 15-32, 1987.
36. JONES, B.E. AND WEBSTER, H.H. Neurotoxic lesions of the dorsolateral pontomesencephalic tegmentum – cholinergic cell area in the cat. I. Effects upon the cholinergic innervation of the brain. *Brain Res.*, **451:** 13-32, 1988.
37. JONES, B.E. AND CUELLO, A.C. Afferents to the basal forebrain cholinergic cell area from pontomesencephalic – catecholamine, serotonin, and acetylcholine – neurons. *Neuroscience*, **31:** 37-61, 1989.
38. JONES, B.E. Immunohistochemical study of choline acetyl transferase-immunoreactive processes and cells innervating the pontomedullary reticular formation. *J. Comp. Neurol.*, **295:** 485-514, 1990.
39. JONES, B.E. Paradoxical sleep and its chemical/structural substrates in the brain. *Neuroscience*, **40:** 637-656, 1991.
40. JONES, B.E. Noradrenergic locus coeruleus neurons: their distant connections and their relationship to neighboring – including cholinergic and GABAergic – neurons of the central gray and reticular formation. Pp. 15-30. In: BARNES, CD. AND POMPEIANO, O. (Eds.), *Neurobiology of the Locus Coeruleus. Progr. Brain Res.* Amsterdam, Elsevier, **85:** 15-30, 1991.
41. JONES, B.E., HOLMES, C.J., RODRIGUEZ-VEIGA, E. AND MAINVILLE, L. GABA-synthesizing neurons in the medulla: Their relationship to serotonin-containing and spinally projecting neurons in the rat. *J. Comp. Neurol.*, **312:** 1-19, 1991.
42. JONES, B.E. Reticular formation. Cytoarchitecture, transmitters and projections. Pp. 155-171. In: PAXINOS, G. (Ed) *The Rat Nervous System. 2nd Ed.* New South Wales, Academic Press Australia, 1995.
43. JONES, B.E. Neurotransmitter systems regulating sleep-wake states. Pp. 1215-1228. In: D'HAENEN, D., DEN BOER, J.A. AND WILLNER, P. (Eds) *Biological Psychiatry*. John Wiley & Sons, 2002.
44. JONES, B.E. Activity, modulation and role of basal forebrain cholinergic neurons innervating the cerebral cortex. *Progr. Brain Res.*, **145:** 157-169, 2004.
45. JOUVET, M. AND MICHEL, F. Corrélations électromyographiques du sommeil chez le chat décortiqué et mésencéphalique chronique. *C.R. Soc. Biol.*, **153:** 422-425, 1959.
46. JOUVET, M., MICHEL, F. AND COURJON, J. Sur un stade d'activité électrique cérébrale rapide au cours du sommeil physiologique. *C. R. Soc. Biol.*, **153:** 1024-1028, 1959.
47. JOUVET, M. Recherches sur les structures nerveuses et les mécanismes responsables des differentes phases du sommeil physiologique. *Archives Italiennes de Biologie*, **100:** 125-206, 1962.
48. JOUVET, M. Paradoxical sleep – a study of its nature and mechanisms. *Progr. Brain Res.*, **18:** 20-57, 1965.
49. JOUVET, M. The role of monoamines and acetylcholine-containing neurons in the regulation of the sleep-waking cycle. *Ergebn. Physiol.*, **64:** 165-307, 1972.
50. KANEKO, T., ITOH, K., SHIGEMOTO, R. AND MIZUNO, N. Glutaminase-like immunoreactivity in the lower brainstem and cerebellum of the adult rat. *Neuroscience*, **32:** 79-98, 1989.
51. KARCZMAR, A.G., LONGO, V.G. AND SCOTTI DE CAROLIS, A. A pharmacological model of paradoxical sleep: the role of cholinergic and monoamine systems. *Physiol. Behav.*, **5:** 175-182, 1970.
52. KAUR, S., SAXENA, R.N. AND MALLICK, B.N. GABA in locus coeruleus regulates sponta-

neous rapid eye movement sleep by acting on $GABA_A$ receptors in freely moving rats. *Neurosci. Lett.*, **223:** 105-108, 1997.
53. KAWAMURA, H. AND DOMINO, E.F. Differential actions of m and n cholinergic agonists on the brainstem activating system. *Int. J. Neuropharmacol.*, **8:** 105-115, 1969.
54. KAYAMA, Y., OHTA, M. AND JODO, E. Firing of 'possibly' cholinergic neurons in the rat laterodorsal tegmental nucleus during sleep and wakefulness. *Brain Res.*, **569:** 210-220, 1992.
55. KELLER, A.D. Generalized atonia and profound dysreflexia following transection of the brainstem through the cephalic pons. *J. Neurophysiol.*, **8:** 275-288, 1945.
56. KODAMA, T., TAKAHASHI, Y. AND HONDA, Y. Enhancement of acetylcholine release during paradoxical sleep in the dorsal tegmental field of the cat brain stem. *Neurosci. Lett.*, **114:** 277-282, 1990.
57. KODAMA, T., LAI, Y.Y. AND SIEGEL, J.M. Changes in inhibitory amino acid release linked to pontine-induced atonia: an *in vivo* microdialysis study. *J. Neurosci.*, **23:** 1548-1554, 2003.
58. KUBIN, L., KIMURA, H., TOJIMA, H., DAVIES, R.O. AND PACK, A.I. Suppression of hypoglossal motoneurons during the carbachol-induced atonia of REM sleep is not caused by fast synaptic inhibition. *Brain Res.*, **611:** 300-312, 1993.
59. LEE, M.G., HENNY, P. AND JONES, B.E. Sleep-wake discharge properties of juxtacellularly labeled and immunohistochemically identified cholinergic basal forebrain neurons in head-restrained rats. *Soc. Neurosci. Abst. Online*, 932: 6, 2003.
60. LEONARD, C.S. AND LLINAS, R.R. Electrophysiology of mammalian pedunculopontine and laterodorsal tegmental neurons *in vitro*: implications for the control of REM sleep. Pp. 205-223. In: STERIADE, M. AND BIESOLD, D. (Eds) *Brain Cholinergic Systems*. New York, Oxford University Press, 1990.
61. LONGO, V.G. Behavioral and electroencephalographic effects of atropine and related compounds. *Pharmacol. Rev.*, **18:** 965-996, 1966.
62. LUEBKE, J.I., GREENE, R.W., SEMBA, K., KAMONDI, A., MCCARLEY, R.W. AND REINER, P.B. Serotonin hyperpolarizes cholinergic low-threshold burst neurons in the rat laterodorsal tegmental nucleus *in vitro*. *Proc. Natl. Acad. Sci.*, **89:** 743-747, 1992.
63. MAGOUN, H.W. AND RHINES, R. An inhibitory mechanism in the bulbar reticular formation. *J. Neurophysiol.*, **9:** 165-171, 1946.
64. MALONEY, K., MAINVILLE, L. AND JONES, B.E. c-Fos expression in dopaminergic and GABAergic neurons of the ventral mesencephalic tegmentum after paradoxical sleep deprivation and recovery. *Eur. J. Neurosci.*, **15:** 1-6, 2002.
65. MALONEY, K.J., MAINVILLE, L. AND JONES, B.E. Differential c-Fos expression in cholinergic, monoaminergic and GABAergic cell groups of the pontomesencephalic tegmentum after paradoxical sleep deprivation and recovery. *J. Neurosci.*, **19:** 3057-3072, 1999.
66. MALONEY, K.J., MAINVILLE, L. AND JONES, B.E. c-Fos expression in GABAergic, serotonergic and other neurons of the pontomedullary reticular formation and raphe after paradoxical sleep deprivation and recovery. *J. Neurosci.*, **20:** 4669-4679, 2000.
67. MATSUZAKI, M., OKADA, Y. AND SHUTO, S. Cholinergic agents related to para-sleep state in acute brain stem preparations. *Brain Res.*, **9:** 253-267, 1968.
68. MCCARLEY, R.W. AND HOBSON, J.A. Neuronal excitability modulation over the sleep cycle: a structural and mathematical model. *Science*, **189:** 58-60, 1975.
69. MCGINTY, D. AND HARPER, R.M. Dorsal raphe neurons: depression of firing during sleep in cats. *Brain Res.*, **101:** 569-575, 1976.
70. MCGINTY, D.J., HARPER, R.M. AND FAIRBANKS, M.K. Neuronal unit activity and the control of sleep states. Pp. 173-216. In: WEITZMAN, E.D. (Ed) *Advances in Sleep Research*. New York, Spectrum Publications, 1974.

71. NIKHILANANDA, S. *The Upanishads*. London: Phoenix House, 1949.
72. NITZ, D. AND SIEGEL, J. GABA release in the dorsal raphe nucleus: role in the control of REM sleep. *Am. J. Physiol.*, **273:** R451-R455, 1997.
73. NITZ, D. AND SIEGEL, J.M. GABA release in the locus coeruleus as a function of sleep/wake state. *Neuroscience*, **78:** 795-801, 1997.
74. ONOE, H. AND SAKAI, K. Kainate receptors: a novel mechanism in paradoxical (REM) sleep generation. *NeuroReport*, **6:** 353-356, 1995.
75. PIN, C., JONES, B. AND JOUVET, M. Topographie des neurones monoaminergiques du tronc cerebrale du chat: etude par histofluorescence. *C. R. Soc. Biol.*, **162:** 2136-2141, 1968.
76. POLLOCK, M.S. AND MISTLBERGER, R.E. Rapid eye movement sleep induction by microinjection of the GABA-A antagonist bicuculline into the dorsal subcoeruleus area of the rat. *Brain Res*; **962:** 68-77, 2003.
77. RODRIGUEZ-VEIGA, E., HOLMES, C. AND JONES, B.E. Relationship of GABA neurons to reticulo- and raphe-spinal neurons in the medullary reticular formation. *Soc. Neurosci. Abst.*, **16:** 851, 1990.
78. SAKAI, K., SASTRE, J.-P., SALVERT, D., TOURET, M., TOHYAMA, M. AND JOUVET, M. Tegmentoreticular projections with special reference to the muscular atonia during paradoxical sleep in the cat: an HRP study. *Brain Res.*, **176:** 233-254, 1979.
79. SAKAI, K. AND KOYAMA, Y. Are there cholinergic and non-cholinergic paradoxical sleep-on neurones in the pons. *NeuroReport*, **7:** 2449-2453, 1996.
80. SANFORD, L.D., TANG, X., XIAO, J., ROSS, R.J. AND MORRISON, A.R. GABAergic regulation of REM sleep in reticularis pontis oralis and caudalis in rats. *J Neurophysiol*; **90:** 938-945, 2003.
81. SASTRE, J. AND JOUVET, M. Le comportement oneirique du chat. *Physiol. Behav.*, **22:** 979-989, 1979.
82. SCHENCK, C.H., BUNDLIE, S.R., ETTINGER, M.G. AND MAHOWALD, M. Chronic behavioral disorders of human REM sleep: A new category. *Sleep*; **9:** 293-308, 1986.
83. SHIROMANI, P.J. AND MCGINTY, D.J. Pontine neuronal response to local cholinergic infusion: relation to REM sleep. *Brain Res.*, **386:** 20-31, 1986.
84. SHUTE, C.C.D. AND LEWIS, P.R. Cholinesterase-containing systems of the brain of the rat. *Nature*, **199:** 1160-1164, 1963.
85. STERIADE, M. AND HOBSON, J.A. Neuronal activity during the sleep-waking cycle. *Progr. Neurobiol.*, **6:** 155-376, 1976.
86. STERIADE, M., DATTA, S., PARE, D., OAKSON, G. AND CURRO DOSSI, R.C. Neuronal activities in brain-stem cholinergic nuclei related to tonic activation processes in thalamocortical systems. *J. Neurosci.*, **10:** 2541-2559, 1990.
87. STERIADE, M., PARE, D., DATTA, S., OAKSON, G. AND CURRO DOSSI, R. Different cellular types in mesopontine cholinergic nuclei related to ponto-geniculo-occipital waves. *J. Neurosci.*, **10:** 2560-2579, 1990.
88. THAKKAR, M.M., STRECKER, R.E. AND MCCARLEY, R.W. Behavioral state control through differential serotonergic inhibition in the mesopontine cholinergic nuclei: a simultaneous unit recording and microdialysis study. *J. Neurosci.*, **18:** 5490-5497, 1998.
89. TRULSON, M.E. AND JACOBS, B.L. Raphe unit activity in freely moving cats: correlation with level of behavioral arousal. *Brain Res.*, **163:** 135-150, 1979.
90. VANNI-MERCIER, G., SAKAI, K., LIN, J.-S. AND JOUVET, M. Mapping of cholinoceptive brainstem structures responsible for the generation of paradoxical sleep in the cat. *Archives Italiennes de Biologie*, **127:** 133-164, 1989.
91. VERTES, R.P., COLOM, L.V., FORTIN, W.J. AND BLAND, B.H. Brainstem sites for the carbachol elicitation of the hippocampal theta rhythm in the rat. *Exp. Brain Res.*, **96:** 419-429, 1993.

92. VILLABLANCA, J. The electrocorticogram in the chronic *cerveau isole* cat. *Electroencephalogr. Clin. Neurophysiol.*, **19:** 576-586, 1965.
93. WEBSTER, H.H., FRIEDMAN, L. AND JONES, B.E. Modification of paradoxical sleep following transections of the reticular formation at the pontomedullary junction. *Sleep*, **9:** 1-23, 1986.
94. WEBSTER, H.H. AND JONES, B.E. Neurotoxic lesions of the dorsolateral pontomesencephalic tegmentum-cholinergic cell area in the cat. II. Effects upon sleep-waking states. *Brain Res.*, **458:** 285-302, 1988.
95. WILLIAMS, J.A. AND REINER, P.B. Noradrenaline hyperpolarizes identified rat mesopontine cholinergic neurons *in vitro*. *J. Neurosci.*, **13:** 3878-3883, 1993.
96. WILLIAMS, J.A., COMISAROW, J., DAY, J., FIBIGER, H.C. AND REINER, P.B. State-dependent release of acetylcholine in rat thalamus measured by *in vivo* microdialysis. *J. Neurosci.*, **14:** 5236-5242, 1994.
97. XI, M.-C., MORALES, F.R. AND CHASE, M.H. Evidence that wakefulness and REM sleep are controlled by a GABAergic pontine mechanism. *J. Neurophysiol.*, **82:** 2015-2019, 1999.