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

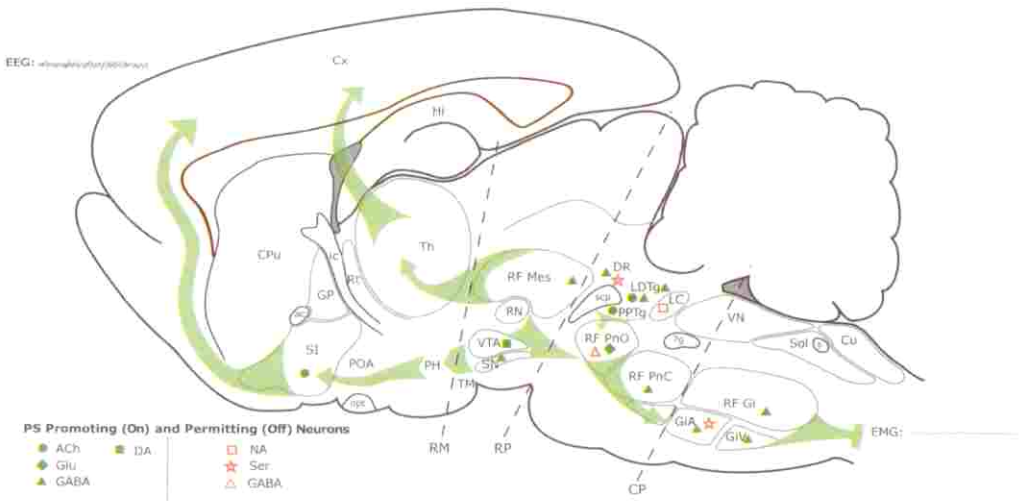

Fig. 1. *Schematic sagittal view of rat brain showing the major neuronal systems and their (excitatory, ending as arrows, and inhibitory, ending as blocks) pathways involved in promoting the EEG fast activity (upper left) and EMG muscle atonia (lower right) characteristic of rapid eye movement or paradoxical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

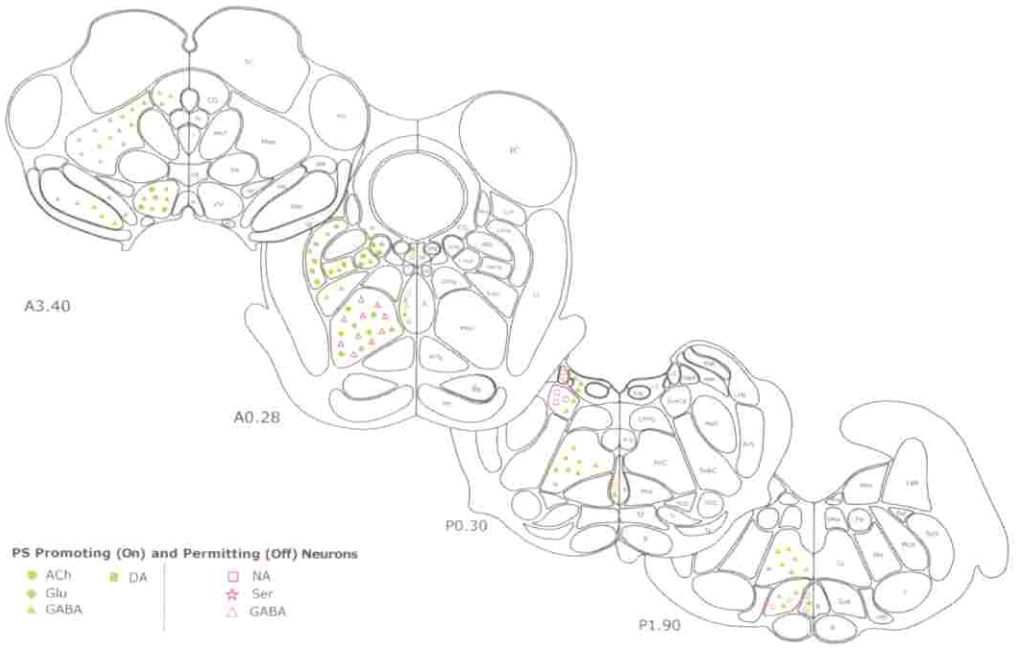

Fig. 2. *Composite of coronal sections through the mesencephalon (A3.40), oral pons (A0.28), caudal pons (P0.30) and rostral medulla (P1.90) of the rat brain showing PS-promoting (On) and PS-permitting (Off) neurons.*

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

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

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).

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.

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.

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-