Archives Italiennes de Biologie, 149: 325-347, 2011.

Brainstem neurons responsible for postural, masseter or pharyngeal muscle atonia during paradoxical sleep in freely-moving cats

K. SAKAI, P.-C. NEUZERET

INSERM U1028, CNRS UMR5292, Neuroscience Research Center, University Lyon 1, Integrative Physiology of Brain Arousal System, Lyon, France

ABSTRACT

In this mini review, we summarize our findings concerning brainstem neurons responsible for the postural, masseter, or pharyngeal muscle atonia observed during paradoxical sleep (PS) in freely moving cats. Both the pons and medulla contain neurons showing tonic activation selective to PS and atonia, referred to as PS/atonia-on neurons. The PS/atonia-on neurons, characterized by their most slow conducting property and located in the peri-locus coeruleus alpha (peri-LCα) and adjacent LCα of the mediodorsal pontine tegmentum, play a critical executive role in the somatic and orofacial muscle atonia observed during PS. Slow conducting medullary PS/atonia-on neurons located in the nuclei reticularis magnocellularis (Mc) and parvocellularis (Pc) may play a critical executive role in the generation of, respectively, antigravity or orofacial muscle atonia during PS. In addition, either tonic or phasic cessation of activity of medullary serotonin neurons may play an important role in the atonia of pharyngeal muscles during PS via a mechanism of disfacilitation.

Key words

Disfacilitation • Inhibition • Motoneurons • REM sleep • Serotonin

Introduction

In 1959, Jouvet, Michel and Courjon have discovered, during sleep episodes in the cat, a state characterized by total disappearance of neck muscle activity (atonia), in conjunction with desynchronized neocortical electroencephalogram (EEG), rapid eye movements (REMs) and cardiovascular irregularities, the state being called by the authors a paradoxical phase of sleep, or paradoxical sleep (PS) (Jouvet et al., 1959). Since then efforts were directed at identifying brain structures and elucidating mechanisms critical for the generation of the atonia, one of the most characteristic tonic events of PS. It has been shown in the cat that during PS, i) there is suppression of both monosynaptic and polysynaptic spinal reflexes, the suppression disappearing after the section of the spinal cord (Gassel et al., 1964; Kubota et al., 1965; Baldissera et al., 1966); ii) there is a tonic decrease in excitability of both alpha and gamma motoneurons (Gassel et al., 1965; Gassel and Pompeiano, 1965; Morrison and Pompeiano, 1965; Kubota and Kidokoro, 1965; Kubota and Tanaka, 1966); iii) stimulation of the ventromedial part of the medullary reticular formation (RF), which corresponds to the medullary inhibitory center first defined by Magoun and Rhines (1946), elicits inhibitory postsynaptic potentials (IPSPs) in both extensor and flexor motoneurons, presumably via inhibitory interneurons impinging upon spinal motoneurons (Giaquinto et al., 1964; Jankowska et al., 1968);

Corresponding Author: Kazuya Sakai, U1028/UMRS5292, Université Claude Bernard, Lyon 1, 8 Avenue Rockefeller, 69373 Lyon Cedex 08, France - Tel.: +33 478777122 - Fax: +33478777151 - Email: sakai@univ-lyon1.fr

and iv) such an effect of stimulation is suppressed by the lesion of the ventral quadrant of the spinal cord, particularly the ventrolateral funiculus corresponding to the ventrolateral reticulospinal pathway (Giaquinto et al., 1964; Jankowska et al., 1968; for review see Pompeiano, 1976). In 1978, using intracellular recording techniques, Glenn et al. (1978) and Morales and Chase (1978) have demonstrated that hyperpolarization of spinal lumbar motoneuron membrane occurs during PS, supporting the assumptions that during PS, there is a tonic decrease in excitability of alpha and gamma motoneurons via either a mechanism of inhibition, or a mechanism of disfacilitation, or a combination of both, and that this phenomenon is under the control of supraspinal structures.

In parallel to these electrophysiological studies, Jouvet (1962), Carli and Zanchetti (1965), Zanchetti (1967) and Henley and Morrison (1974) have demonstrated using electrolytic brain lesion techniques in the cat that the bilateral lesioning of the pontine tegmentum, in particular the caudal part of the neucleus pontis oralis (Poo) and the rostral part of the nucleus pontis caudalis (Poc), results in a total disappearance of the atonia during PS and induced a state of PS without atonia. These findings lead to a general hypothesis that excitation of a supraspinal inhibitory system causes a tonic postsynaptic inhibition of spinal motoneurons during PS. Jouvet (1972) first proposed that noradrenergic neurons in the nucleus locus coeruleus (LC) of the dorsolateral pontine tegmentum are critical for the generation of the atonia, while Hobson, McCarley and co-workers (1975) proposed that a population of cholinergic gigantocellular neurons in the pontine RF have executive function in the generation of the atonia. We have then hypothesized that during PS, some neurons of the mediodorsal pontine tegmentum exert an excitatory influence on neurons in the medullary RF, which in turn exert a generalized inhibition of the spinal motoneurons via the ventrolateral reticulospinal pathway (Sakai, 1980; Sakai et al., 1981; see Fig. 1A). If this hypothesis is correct, both the pontine and medullary neurons should discharge tonically and selectively during PS (PS-on or atonia-on neurons). Furthermore, the pontine atonia-on neurons should be excited antidromically by electrical stimulation of the medullary inhibitory area, while the medullary atonia-on neurons should be invaded antidromically by stimulation of the ventrolateral reticulospinal pathway, and excited orthodromically by stimulation of the pontine supraspinal structure. Our experimental findings confirmed this hypothesis and further determined the localization of ponto-medullary neurons responsible for the generation of the neck muscle atonia observed during PS. In the present paper, we will describe these and other our findings concerning brainstem neurons responsible for the postural, masseter, or pharyngeal muscle atonia observed during PS in freely moving cats.

Anatomical substrates for postural atonia mechanisms

Afferent projections to the spinal cord

As described, lesions of the ventrolateral funiculus (VLF) abolish the inhibitory actions on spinal motoneurons induced by electrical stimulation of the medullary RF in the cat. What is the medullary source of the descending fibers coursing in the VLF? In order to determine the medullary neurons, we have used in cats a retrograde transport technique of horseradish peroxidase (HRP), that can be taken up by both axon terminals and axons *en passant* and transported retrogradely to their parent cell bodies (La Vail and La Vail, 1972). We found that the ventromedial medullary RF, referred to as the nucleus reticularis magnocellularis (Mc; see Sakai, 1980; Sakai et al., 1981) [also called nucleus reticularis giagantocellularis, pars alpha or ventralis, or magnocellular tegmental field (FTM) by Berman (1968)] is the major medullary source of the descending fibers coursing ipsilaterally in the VLF. This medullary reticulospinal tract appears to correspond to the ventrolateral reticulospinal tract (vlRST) first described by Pitts (1940). In line with the observations by Kuypers and Maisky (1977), we also found that the nucleus reticularis gigantocellularis (Gc), located just dorsal to the Mc, is a main medullary source of the spinal projections in the ipsilateral anterior funiculus (AF) and that the spinal cord is poorly innervated by the nucleus reticularis parvocellularis (Pc) neurons (Tohyama et al., 1979a). It should also be noted that the medial pontine RF (Poo, Poc and Gc) neurons give rise to direct descending fibers to the spinal cord mainly via the

AF (medial reticulospinal tract, mRST) (Tohyama et al., 1979a), whereas the LC complex [LC, LCα and locus subcoeruleus (LSC); see Sakai 1991] neurons together with neighboring RF cells, but not the peri-LCα neurons (see below), give rise to direct projections to the spinal cord via the VLF (Tohyama et al., 1979b; Sakai et al., 1979b).

Afferent projections to the medulla oblongata

HRP injections into the Mc, particulary its caudal and lateral two thirds, resulted in specific labeling of the most medial part of the LCα and medially adjacent region, referred to as the peri-LCα or peri-α (Sakai, 1980; Sakai et al., 1981) (Fig. 1A and B). The descending projections arising from the peri-LCα seemed to terminate in the Mc, as HRP injections into the medulla caudal to the Mc as well as into the spinal cord did not result in a significant labeling of the peri-LCα neurons (Sakai et al., 1979b; Tohyama et al., 1979b). The course of the descending pathway, as demonstrated by the HRP technique (Sakai et al., 1979b), is illustrated in the right frontal sections of Fig. 1B. This pathway seems to correspond to the lateral tegmentoreticular tract, first described by Russel (1955) with the March degeneration technique.

Effects of brain stem lesions on postural atonia

Electrolytic lesion study

Effects of bilateral brain stem lesions on the postural atonia during PS are summarized in Fig. 1B. As shown in the left side of the frontal sections (Fig. 1B), bilateral electrolytic lesions including the peri-LCα and the medial part of the LCα completely suppressed the neck muscle atonia during PS (Fig. 1C) throughout survival periods of 1 to 7 months (Sastre et al., 1979). The same effect was also obtained when such bilateral lesions were performed at various levels of the lateral tegmentoreticular pathway. Unlike these lesions, bilateral destruction (as far caudal as P9) of the medial RF of the pons and medulla (Poo, Poc, and Gc), called together gigantocellular tegmental field (FTG) by Berman (1968), had no effect on the atonia. These results strongly suggest that: i) the FTG neurons located in the pons and medulla do not play any essential role in the generation of the neck muscle atonia during PS, and thus the medullary supraspinal inhibitory structure should be located in the Mc; and ii) the peri-LCα and adjacent LCα neurons and their descending pathway are critically involved in the generation of the atonia during PS.

Chemical lesion study

These hypotheses were further confirmed by our chemical lesion experiments with microinjections of kainic acid that allowed us to destroy selectively neuronal cell bodies receiving glutamatergic inputs without affecting axons of passage or nerve terminals. Indeed, the complete bilateral destruction of the FTG neurons by the kainic acid does not affect the generation of the postural atonia during PS. In contrast, bilateral excitotoxic destruction of the peri-LCα and adjacent medial LCα neurons completely suppress the atonia throughout the survival periods lasting 4 months (Sakai et al., 1981; Sastre et al., 1981). These findings indicate that the neurons of the peri-LCα and adjacent medial LCα, together with their descending pathway (lateral tegmentoreticular tract), are critically involved in the generation of the postural atonia of PS, whereas the FTG neurons play no essential role in the mechanisms of the atonia.

Single unit recording experiments

PS-specific neurons in the pontine RF

If the neurons of the peri-LCα and adjacent medial LCα are responsible for the generation of the atonia of PS, they should exhibit tonic activation highly selective to PS. Furthermore, if the information is relayed by the Mc neurons, the pontine PS/atonia-specific neurons should be invaded antidromically by stimulation of the Mc, but not by stimulation of the structures located caudal to the Mc. On the other hand, the neighboring RF, in particular FTG, neurons sending axons to the spinal cord should exhibit different firing patterns during the sleep-waking cycle compared to the pontine PS/atonia-specific neurons.

Using a microwire bundle technique, we recorded single units in the pons of 11 freely moving cats, with stimulation electrodes implanted in the Mc,

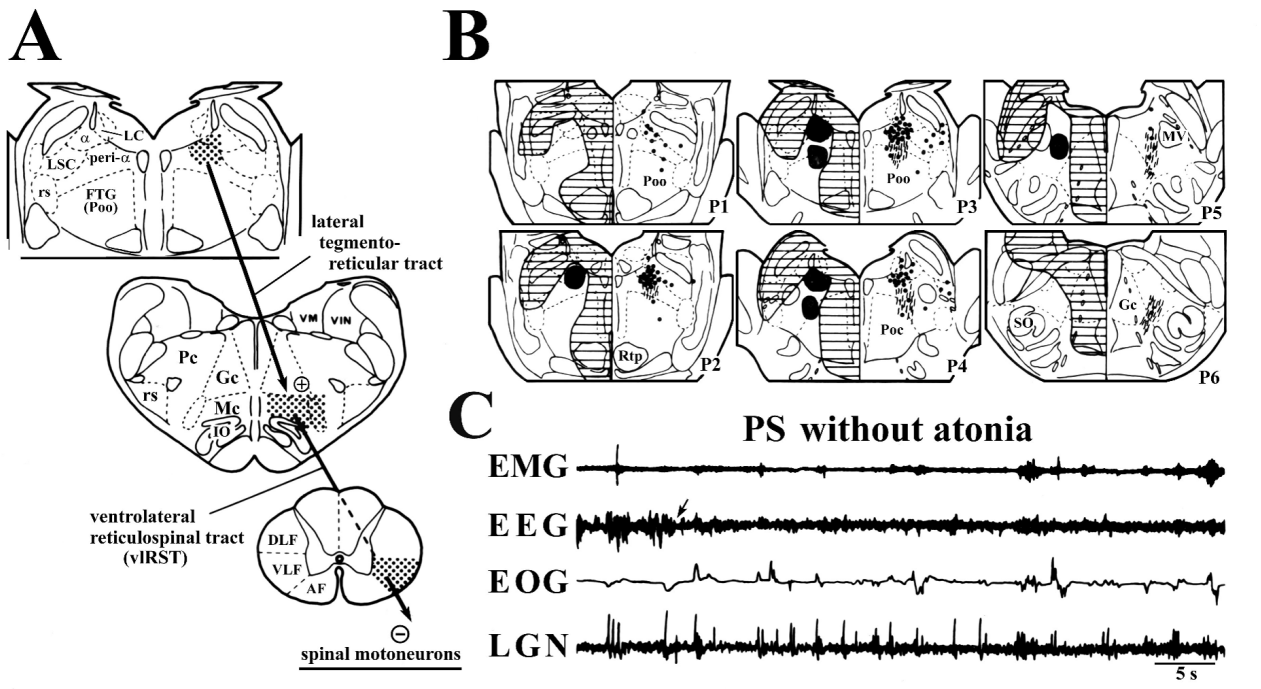

Fig. 1. - (A) Schematic illustration of the present hypothesis regarding brainstem mechanisms of the neck muscle atonia during PS. During PS, neurons of the peri-LCα and adjacent LCα exert, via the lateral tegmentoreticular tract, an excitatory influence on Mc neurons, which in turn exert a generalized inhibitory influence on spinal motoneurons via the ventrolateral reticulospinal tract. (B) Summary of effects of bilateral lesions on the atonia (left side of the frontal planes) and the location of cell bodies (dots) and their descending pathway to the Mc as determined by the HRP technique (right side). Blackened areas indicate that bilateral lesions of the structures permanently abolished atonia during PS, whereas lesions of areas with horizontal lines had no effect. (C) An example of polygraph recording showing PS without atonia. Abbreviations: α = locus coeruleus alpha; AF = anterior funiculus; DLF = dorsolateral funiculus; EEG = electroencephalogram; EMG = electromyogram; EOG = electrooculogram; FTG = gigantocellular tegmental field; Gc = nucleus reticularis gigantocellularis; IO = inferior olivary complex; LC = nucleus locus coeruleus; LGN = dorsal lateral geniculate nucleus EEG showing ponto-geniculo-occipital (PGO) waves; LSC = nucleus locus subcoeruleus; lvs = lateral vestibulospinal tract = Mc, nucleus reticularis magnocellularis; MV = motor trigeminal nucleus; Pc = nucleus reticularis parvocellularis; peri-α = nucleus peri-locus coeruleus alpha; Poc and Poo = nuclei pontis caudalis and oralis, respectively; rs = rubrospinal tract; Rtp = nucleus reticularis tegmenti pontis; SO = superior olivary complex; VIN = inferior vestibular nucleus; VLF = ventrolateral funiculus; VM = medial vestibular nucleus (modified from Sakai et al., 1981).

vlRST and cervical segment of the spinal cord (C2-C3). We found, in the pons, units showing tonic activation highly selective to PS, referred to as PS-on neurons. Within the pontine RF, these neurons were found almost exclusively in the peri-LCα and in the medial part of the LCα (Sakai, 1980, 1988; Sakai et al., 1981, 2001). Two types of PS-on neurons were distinguished: one, exhibiting no discharge activity during both quiet and active wakefulness (W) characterized by various motor activities; and another exhibiting a significant discharge during active W, usually when the cats sat down to take a sleeping posture, suggesting that the unit firing was related to postural change or relaxation of the postural muscles. Otherwise, both types of neurons showed quite similar firing rates and patterns during the sleep-waking cycle as follows: i) they were silent during quiet W, as well as during light slow-wave sleep (SWS) characterized by sleep spindles; ii) during deep SWS with high voltage slow EEG, these units began to exhibit a significant, but still intermittent discharge; iii) usually concomitant with the appearance of ponto-geniculo-occipital (PGO) waves, they began to fire tonically at a gradually increasing rate; iv) during PS, they exhibited a high level of tonic activity throughout the episode. Acceleration of firing rate, however, was observed concomitant with PGO and REM bursts; and v) they completely stopped firing just prior to the end of PS (Fig. 2A).

The mean discharge rates of these two types of pontine neurons during the sleep-waking cycle are given in Fig. 2C. As shown in Fig. 2B and D, 5 of 9 PS-specific cells and 7 of 18 cells exhibiting also a significant discharge in relation to postural change responded antidromically to stimulation of the Mc. Except for one LCα unit, they did not respond antidromically to stimulation of the vlRST or spinal cord, indicating that these peri-LCα and LCα cells terminate mostly in the Mc. As shown in Fig. 2D-3, they had long latency antidromic responses, the mean (± SD) conduction velocity being 5.5 ± 2.1 m/s for the 12 identified neurons.

Characteristics of pontine reticulospinal neurons as identified antidromically by stimulation of the vlRST at P15-P16 are shown in Fig. 3 (see Sakai, 1980; Sakai et al., 1981). As shown in Fig. 3A, they were located in the FTG region (Poo and Poc) ventral to the peri-LCα. Two different classes of units were identified: a) one showing a tonic firing pattern throughout the sleep-waking cycle (tonic units) (Fig. 3C, columns with oblique lines); and b) another showing a phasic increase in firing rate during both active W and PS, usually concomitant with PGO and REM bursts (phasic units) (Fig. 3C, white columns). These pontine reticulospinal neurons, therefore, did not satisfy either the selectivity or the tonicity criteria needed for the postural atonia executive neurons during PS. As shown in Fig. 3B, the great majority of the pontine reticulospinal neurons had a high conduction velocity (> 25 m/s), in sharp contrast to the descending peri-LCα neurons having a low conduction velocity (< 10 m/s).

Medullary reticular formation Mc neurons

Single unit recordings were made in the Mc and Gc using a microwire bundle method in 14 freely moving cats (Kanamori et al., 1980; Sakai, 1980; Sakai et al, 1981). As predicted, we found a cluster of units showing a tonic activation selective to PS in the Mc (n = 18; Figs. 4A and C, black columns), but not in the Gc. In addition, we found 20 other units that presented a tonic increase in firing rate during PS as compared to W and SWS (Fig. 4C, white columns), although they exhibited a significant discharge activity during active W in relation to postural change, like the subset of peri-LCα and medial LCα PS-on neurons described above. Otherwise the characteristics of these two clusters of Mc neurons were the same, and they were as follows. The Mc units exhibited little or no firing during both W and light SWS with spindles. During SWS with high voltage slow EEG, the Mc units began to show a significant, but still intermittent discharge, from about 10 to 20 s prior to the occurrence of PGO waves (SWS-). During SWS with PGO waves (SWS+) and from 5 to 20 s prior to the onset of PS, they discharged tonically at a gradually increasing rate. During PS, all units exhibited a high level of tonic activity throughout the episode. Intense acceleration of firing, however, was noticed in every unit in association with PGO and REM bursts, whereas deceleration or even complete cessation of firing was noted concomitant with slowing of neocortical EEG with or without accompanying slight tonicity of neck muscle EMG. At the end of PS, all the units completely stopped firing (see Sakai et al., 1981). An example of the firing pattern of a Mc PS-specific cell over a sleep-waking cycle including three PS episodes is illustrated in Fig. 4A. The location and mean discharge rates of the PS-specific and non-specific units are shown in Fig. 4B and C, respectively.

As shown in Fig. 4D, 7 of the 18 PS-specific and 11 of the 20 PS-non-specific Mc units were invaded antidromically by stimulation of the vlRST at the medullo-spinal junction (P15-P16) and/or at the cervical segment (C2-C3) of the spinal cord. Overall conduction velocity was estimated as 5 to 24 m/s, the PS-specific Mc units having particularly low conduction velocities (8.3 ± 1.4 m/s, mean ± SD). On the other hand, stimulation of the peri-LCα elicited synaptic excitation (latency 2.0-4.5 ms) in 6 of 11 Mc PS-specific units (Kanamori et al., 1980; Sakai et al., 1981).

Medullary reticular formation Pc neurons

Pc neurons do not represent the main medullary source of descending projections to the spinal cord. Instead, they are one of the main medullary sources of afferent projections to the motor trigeminal (MV), facial and hypoglossal nuclei (see for the cat, Mizuno et al., 1988; Takada et al., 1984; Landgren et al., 1986; Fort et al., 1989, 1991; Ono et al., 1994). As seen with the spinal lumbar motoneurons, Nakamura et al. (1978) have demonstrated the hyperpolarization of masseter motoneurons occurring during PS.

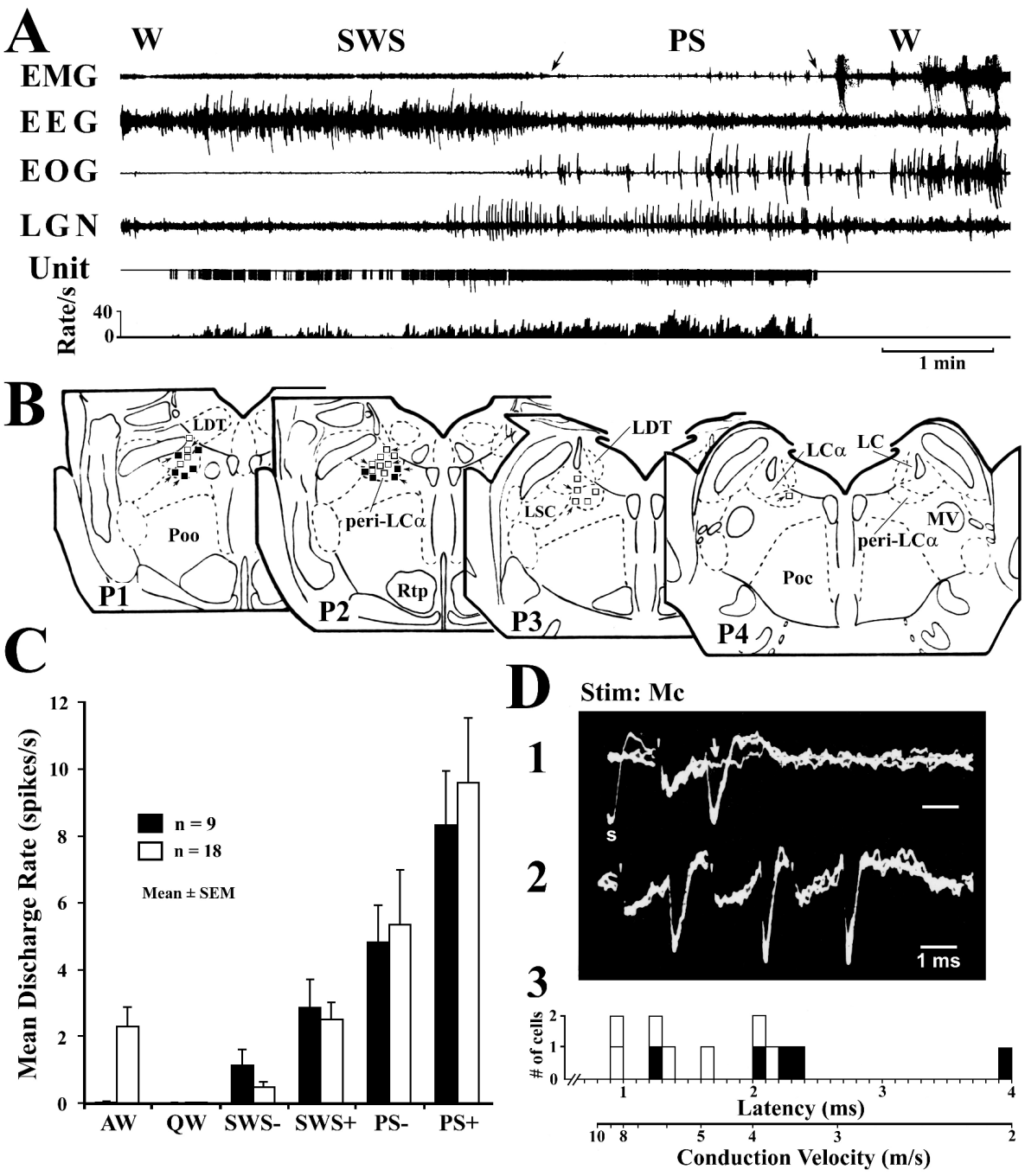

Fig. 2. - (A) Polygraph showing activity of a peri-LCα PS-on cell across wake-sleep states. (B) Localization of pontine PS-specific cells (closed squares) and those showing also a significant discharge during active W (squares), otherwise displaying the same discharge pattern as the PS-on cells. (C) mean (± SEM) discharge rates of the PS-specific (black bars) and PS-non-specific (white bars) units during the sleep-waking cycle. (D) Examples of antidromic responses of a peri-LCα PS-on cell to stimulation of the Mc (1,2) and the antidromic invasion latency histogram and their conduction velocity (3). Note the fixed latency (D1) and collision with a spontaneous spike (s) (D1, indicated by an arrow) and the ability to follow high frequency stimulation (2). Note also the low conduction velocity characteristic of the descending pontine PS-on cells (D3). Abbreviations: AW, QW = active and quiet waking, respectively; SWS-, SWS+ = slow-wave sleep without or with PGO waves, respectively; PS-, PS+ = PS without or with bursts of PGO waves and REMs, respectively (A: modified from Sakai, 1980 and Sakai et al., 1981; B, C, and D: modified from Sakai et al., 1981).

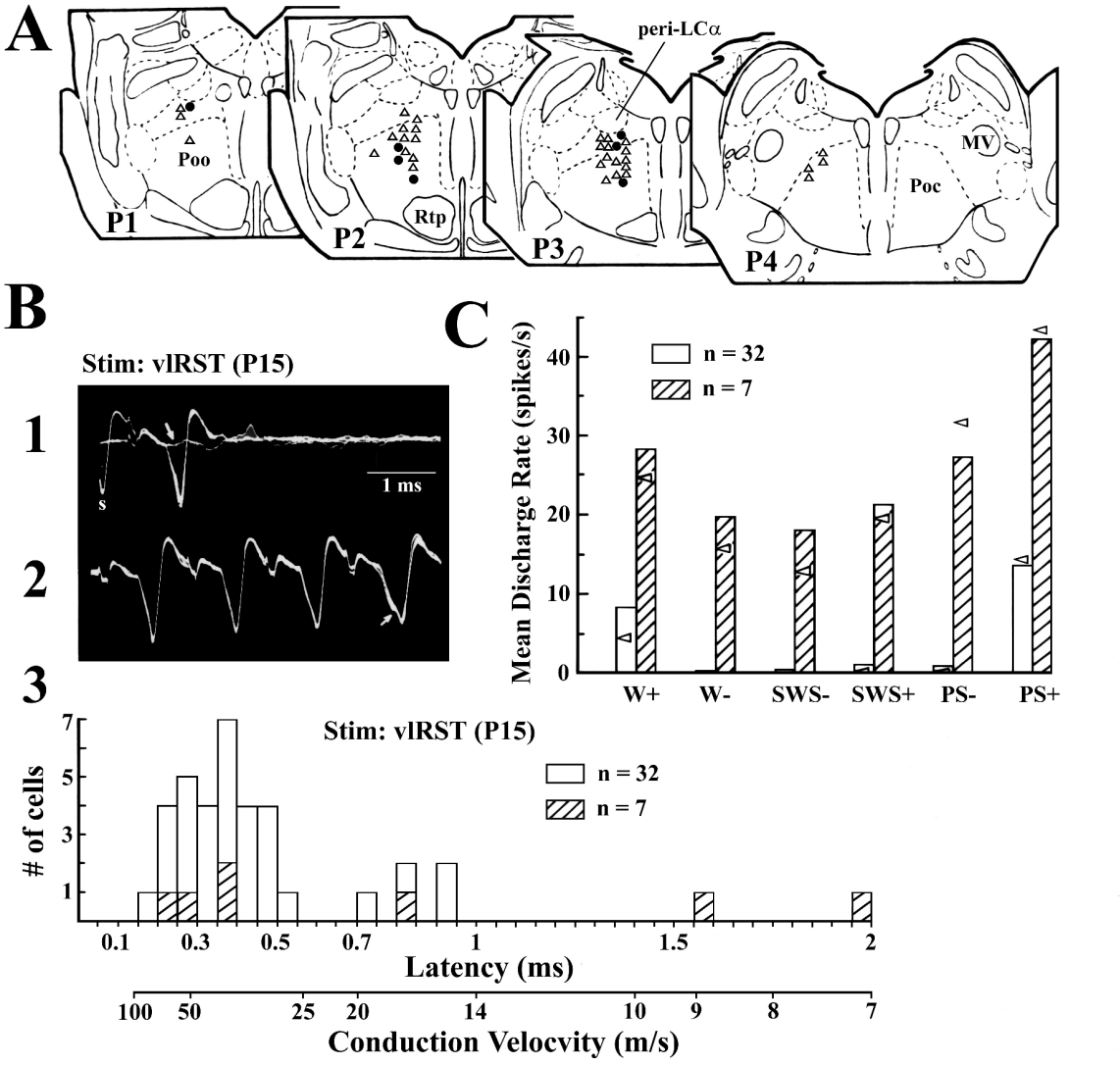

Fig. 3. - Characteristics of the pontine reticulospinal neurons identified by antidromic invasion following stimulation of the vlRST at P15. Their location and examples of the antidromic responses are shown in A and B, respectively. In A, dots indicate those units showing a tonic firing pattern throughout the sleep-waking cycle (tonic units, n = 7), while triangles indicate those showing a phasic increase in firing rate during both AW and PS, usually concomitant with bursts of PGO waves and REMs (phasic units, n = 32). The mean (columns) and median (arrows) rates of these tonic and phasic units are shown in C. Antidromic invasion latency and conduction velocity of these two types of units are shown in D. Note the fast conduction velocity of most cells. For abbreviations, see Fig. 2 (modified from Sakai et al., 1981).

In five cats, bipolar stimulation electrodes were implanted bilaterally in the MV, and we (Sakai, unpublished data) recorded single units in the medullary RF using the microwire bundle method. As shown in Fig. 5C and D (black columns), we found 9 neurons discharging tonically and selectively during PS. In addition, we found other 11 neurons showing a tonic discharge similar to those of the Pc PS-specific neurons although discharging during active W as well, possibly in relation to feeding behavior (Fig. 5D, white columns). The discharge patterns of these two classes of Pc neurons are quite similar to those of the PS-specific and PS-non-specific Mc neurons described above. As shown in Fig. 5A, these Pc neurons were located dorsally near the Gc, and ventrally close to, or in, the lateral vestibulospinal tract (lvs), or the most lateral part of the Mc (n = 2; see Fig. 5A, P9). Two Mc and 4 of

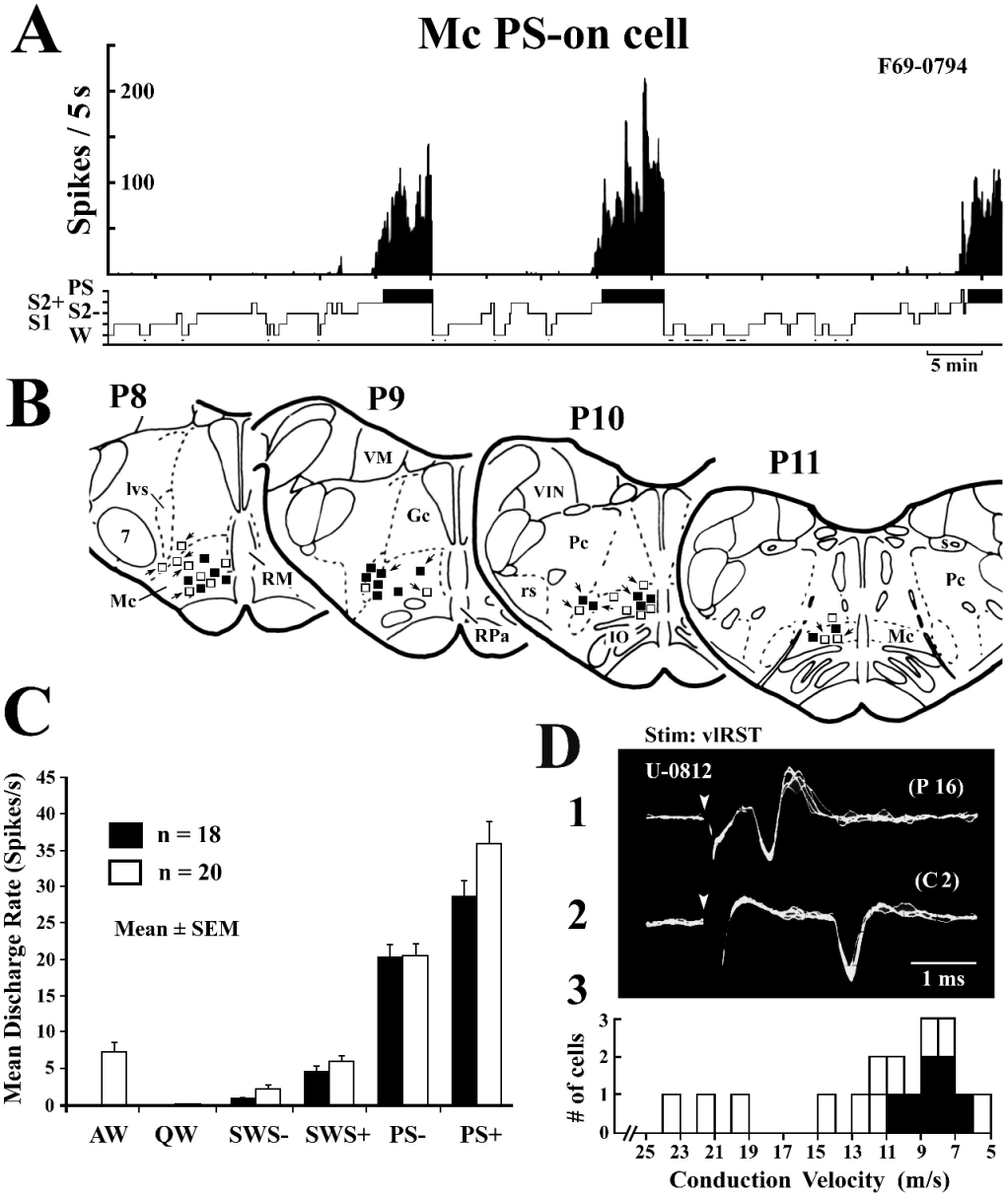

Fig. 4. - Characteristics of Mc PS-on cells. (A) PS-selective discharge pattern of a MC PS-on cell over the sleep-waking cycles, including three PS episodes. (B) Location of the Mc PS-specific (black squares) and PS-non-specific (white squares) cells. Arrows indicate those antidromically identified by stimulation of the vlRST at P15-P16 and/or at the cervical segment of the spinal cord (C2-C3). (C) Mean (± SEM) discharge rates of the two types of Mc units across the wake-sleep states. (D) Examples of antidromic responses of a Mc PS-specific unit elicited by stimulation of the vlRST (1 and 2) and their conduction velocity (3). Note the particularly slow conduction velocity of the PS-specific Mc neurons (black bars), compared to that of the PS-non-specific Mc neurons (white bars) (modified from Sakai et al., 1981).

9 PS-specific Pc neurons were excited antidromically by stimulation of the MV, together with one PS non-specific Pc neuron (Fig. 5A, indicated by arrows). For the MV-projecting PS-specific Pc units (n = 6), the mean (± SD) conduction velocity was 1.8 ± 1.1 m/s, the value being significantly lower ($p < 0.01$, Mann-Whitney test) than that of the descending Mc PS-specific units (8.3 ± 1.4 m/s). In addition, we found another 11 units that were invaded antidromically by the MV stimulation with short antidromic latencies (Fig. 5A, indicated by triangles and Fig. 5B). They were characterized by

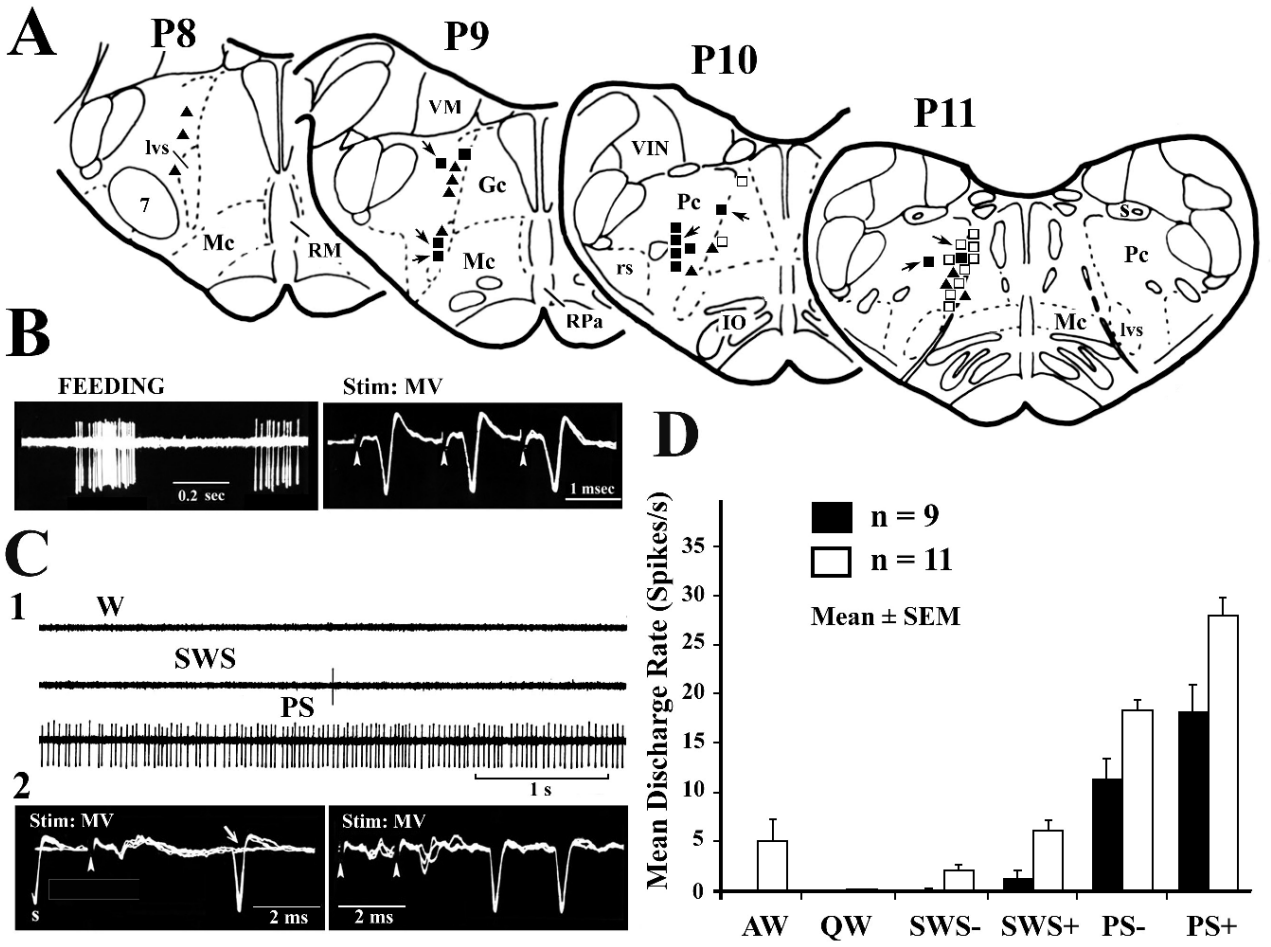

Fig. 5. - Characteristics of tonically or phasically discharging Pc cells. (A) Location of tonic Pc cells discharging either specifically during PS (black squares) or discharging during AW as well (white squares), and phasic Pc cells (black triangles). (B) High frequency, phasic discharges of a Pc cell during feeding (left trace) and its antidromic responses to stimulation of the MV (right trace). (C) Discharge activity of a Pc PS-on cell across wake-sleep states (1) and its antidromic responses to the MV stimulation (2). (D) Mean (± SEM) discharge rates of the PS-specific (black bars) and PS-non-specific (white bars) Pc cells during the wake-sleep cycle (Sakai, unpublished data).

a high frequency phasic discharge activity during feeding (Fig. 5B), little or no discharge during both quiet W and SWS, and a phasic discharge activity during PS, often in association with PGO and REM bursts. The mean conduction velocity of these 11 phasic units was 10.9 ± 2.4 m/s, the value significantly higher ($p < 0.01$, Mann-Whitney test) that that of the PS-specific tonic Pc units. These findings indicate that the Pc PS-on neurons sending axons to the MV may represent inhibitory premotor neurons to trigeminal motoneurons and play a critical role in the atonia of jaw-closer muscles during PS (see Discussion).

Neurochemical properties of pontine atonia-executive neurons

In the pons, PS-on neurons are found almost exclusively in the peri-LCα, as described above. The rostral part of the peri-LCα contains a dense population of cholinergic neurons sending axons to the thalamus and/or hypothalamus, whereas the caudal peri-LCα contains mainly non-cholinergic and non-monoaminergic descending neurons (Sakai, 1991). Two different types of PS-on neurons are distinguished (Sakai and Koyama, 1996). One is characterized by a broad action potential, a slow conduction velocity, and an inhibitory response to iontophoretically-

applied carbachol, a potent cholinergic agonist, referred to therefore as Carb-I PS-on neurons and considered as cholinergic. The other is characterized by a short action potential, fast conduction velocity, and an excitatory response to applied carbachol, referred to therefore as Carb-E PS-on neurons and regarded as non-cholinergic, possibly glutamatergic. The Carb-I PS-on neurons are located exclusively in the cholinergic rostral peri-LCα, whereas the Carb-E PS-on neurons are located mostly in the non-cholinergic caudal peri-LCα. Both types of PS-on neurons are excited by iontophoretically applied glutamate (Glu), a well-known, excitatory amino acid, or bicuculline, a $GABA_A$ receptor antagonist. Although serotonin (5-HT) has no effect, microiontophoretic application of noradrenaline (NA) to Carb-E PS-on neurons results in inhibition of either their spontaneous tonic discharge during PS or their tonic discharge induced by carbachol application during W or SWS, but has no effect on Carb-I PS-on neurons. These data suggest that both cholinergic and glutamatergic mechanisms are involved in the executive mechanisms of PS and that both adrenergic and GABAergic mechanisms are implicated in the inhibitory mechanisms of PS generation in general (see Sakai et al., 2001). The pontine PS-on neurons that are invaded antidromically by stimulation of the Mc of the medulla all display non-cholinergic unitary characteristics, while stimulation of the peri-LCα results in synaptic excitation of Mc PS-on neurons, suggesting that the pontine atonia-executive neurons use an excitatory neurotransmitter, possibly glutamate. Our pharmacological experiments using microinjection or reverse microdialysis techniques further allowed us to determine within the pons a critical region for the generation of the atonia of PS.

Cholinergic mechanisms

Previous microinjection studies in the cat demonstrated that carbachol induces atonia and a dramatic increase of PS duration when injected into the peri-LCα (Vanni-Mercier et al., 1989; Yamamoto et al., 1990). It should be first mentionned that the LC and LCα, and to a lesser extent peri-LCα, contain noradrenergic neurons. The noradrenergic neurons are called "PS-off" or "REM-off" cells, as they cease firing during PS (see Jacobs 1985 for review), and the PS-off cells are found in all pontine structures containing noradrenergic neurons (see Sakai, 1980, 1991). We found that microinjections of carbachol into the peri-LCα induced excitation of the PS-on cells and inhibition of PS-off cells located near the injection site and resulted in induction of atonia and PS, suggesting that in addition to the excitation of the PS-on neurons, inhibition of the PS-off neurons may play a role in the induction of PS in general and in the induction of atonia in particular (see Sakai et al., 2001).

In agreement with the microinjection studies, we found that microdialysis application of carbachol to the peri-LCα, especially its caudal part, results in a dose-dependent increase in PS (Sakai and Onoe, 1997), and that this PS-inducing effect is antagonized by 4-DAMP, an M_1/M_3 muscarinic receptor antagonists, but not by pirenzepine, an M_1 muscarinic receptor antagonist, or methoctramine, an M_2 muscarinic receptor antagonist. Importantly, single application of 4-DAMP, especially to the caudal peri-LCα, produced a great reduction of PS and a PS without atonia (Figs. 6B and 7A) (Sakai and Onoe, 1997). These findings indicate that M_3 muscarinic receptors located in the caudal part of the peri-LCα play a crucial role in the generation of the atonia during PS. The PS- and atonia-inducing effect of carbachol is not antagonized by co-applications of GAMS, a preferential kainate (KA) receptor antagonist, even at high concentrations (Onoe and Sakai, 1995). This, and the finding that atropine does not block the PS-inducing effect of KA (see below), indicate that two distinct (cholinergic and glutamatergic) mechanisms are involved in the executive mechanisms underlying the generation of PS and atonia.

Glutamatergic mechanisms

Microdialisis application of 5 to 50 μM KA, an excitatory amino acid agonist, to the middle or caudal peri-LCα induces atonia in parallel with a marked increase in PS in a dose-dependent manner. KA application to other brain pontine structures has no effect or, on the contrary, has a PS-suppressing effect (see Sakai et al., 2001). The atonia- and PS-inducing effects of KA appear to be mainly due to activation of KA receptors, since application of AMPA (5-100 μM) or NMDA (50-500 μM) has little or no effect, and the PS-inducing effect of KA is completely blocked by GAMS, a preferential KA receptor antagonists, but not by AP-5 or MK-801, selective NMDA receptor antagonists (Onoe and

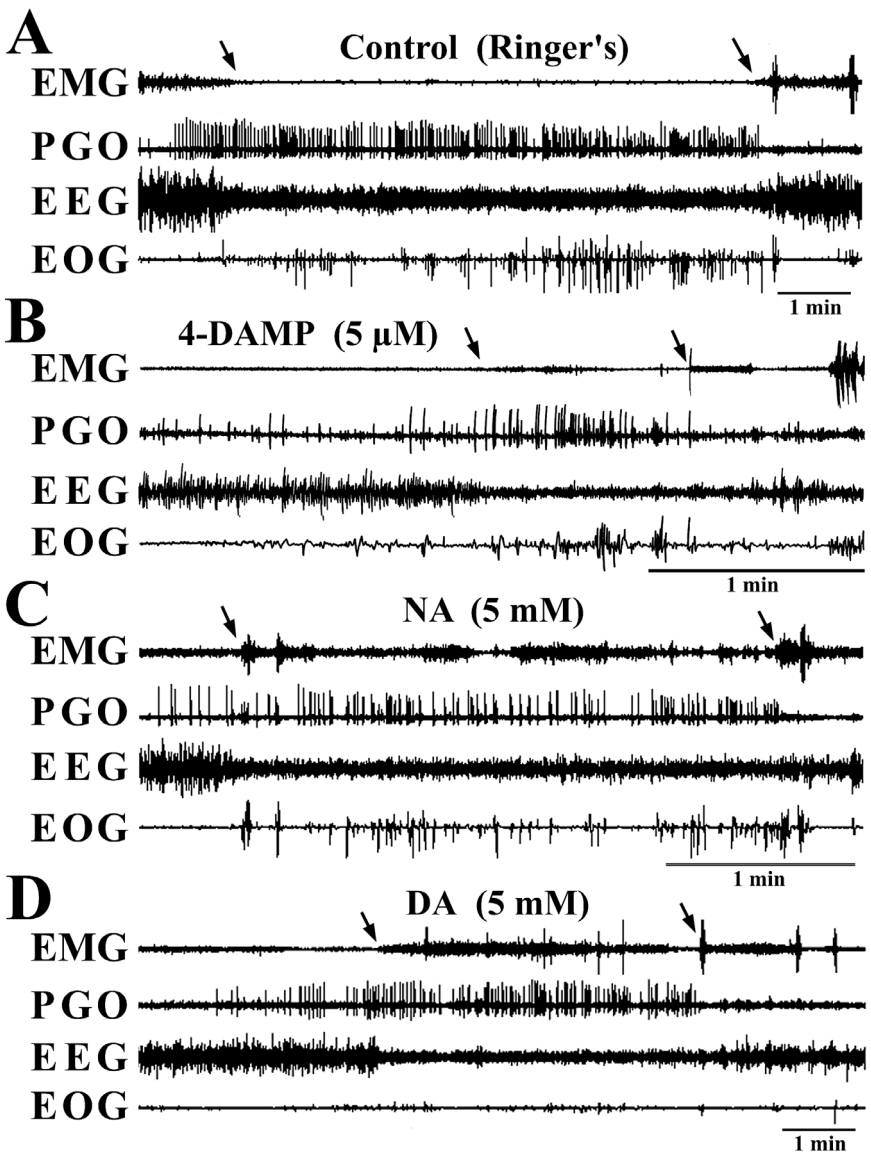

Fig. 6. - Polygraphs showing PS episodes seen before (control, Ringer's) and after application of 4-DAMP (B), NA (C) or DA (D). Note the complete suppression of neck muscle EMG activity (atonia) in control, and the suppression of the atonia after the drug applications. Arrows indicate the onset and end of PS (modified from Crochet and Sakai, 1999, 2003; and Sakai and Onoe, 1997).

Sakai, 1995). As noted, atropine, a selective muscarinic receptor antagonist, also does not block the KA effect. These data again point to an important role played by the caudal peri-LCα in the mechanisms of PS in general, and those of the atonia during PS in particular.

Adrenergic and dopaminergic mechanisms

Application of 5-HT or histamine (HA) in the caudal peri-LCα has no PS- and atonia-inhibiting effects. In sharp contrast, as shown in Figs. 6C and D and 7B and C, application of NA, adrenaline (A) or dopamine (DA) causes a marked decrease in PS without affecting other behavioral states, and induces PS without atonia (Crochet and Sakai, 1999a, b; 2003). PS/atonia inhibition by NA and A in the caudal peri-LCα is mediated by α_2-adrenoceptors, as the PS/atonia-inhibiting effect is completely blocked by co-application of rauwolscine or RX 821002, specific α_2-adrenoceptor antagonists. Furthermore, microdialy-

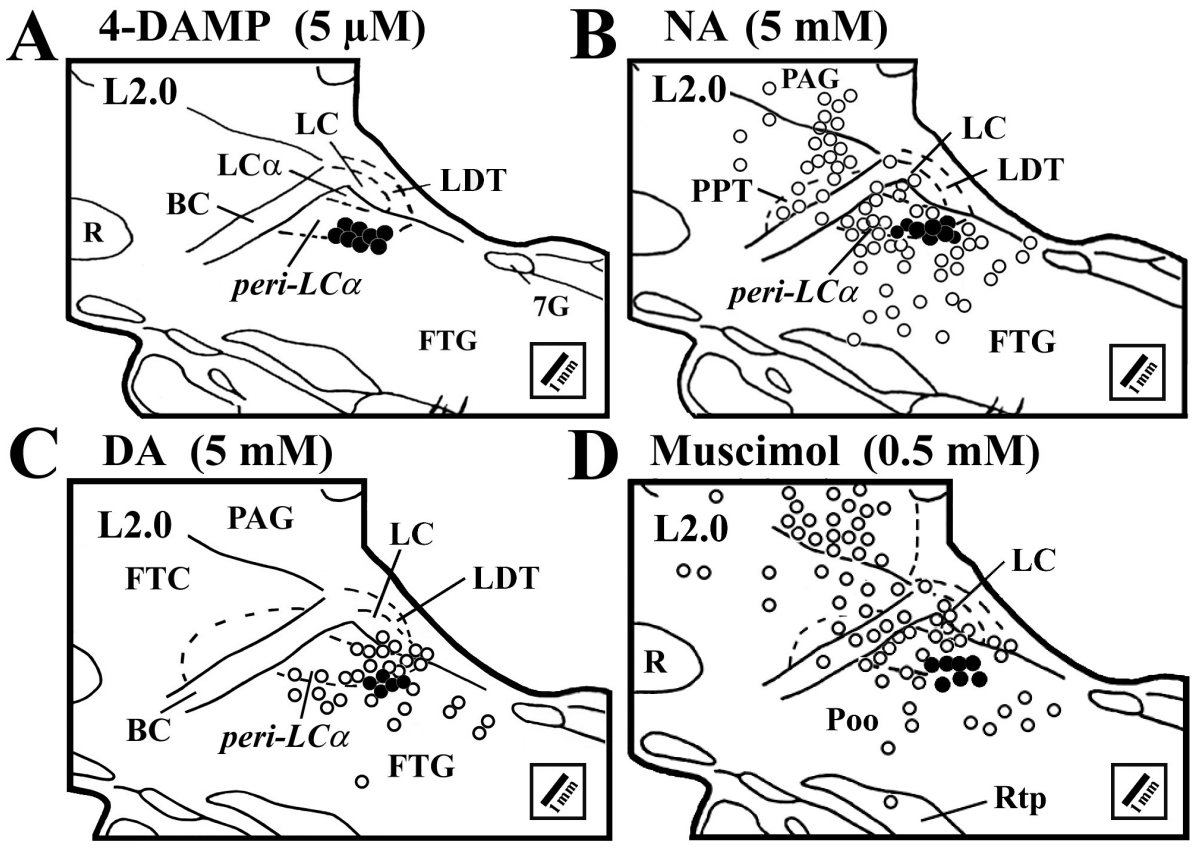

Fig. 7. - Effects of microdialysis application of 4-DAMP (A), NA (B), DA (C) or muscimol (D) on neck muscle EMG during PS. Drawings of a sagittal section are made at L2.0 with drug infusion sites (tip of the microdialysis probe; see box for the probe size). Dots indicate sites at which the drugs induced PS without atonia, whereas circles designate those at which the drugs had no effect on the EMG activity during PS (modified from Sakai and Onoe, 1997; Crochet and Sakai, 1999, 2003; Crochet et al., 2006).

sis application of clonidine, a specific α_2-adrenoceptor agonist, mimics the effect of NA and A, whereas application of methoxamine, an α_1-adrenoceptor agonist, or isoproterenol, a β-adrenoceptor agonist, does not (Crochet and Sakai, 1999b). Similarly, PS/atonia inhibition by DA in the caudal peri-LCα is also mediated by α_2-adrenoceptors, because the effects were not mimicked by SKF-81297, a selective D_1-like dopamine receptor agonist, or selective D_2-like agonists such as quinelorane, quinpirole, and 7-OH-DPAT. The effects of DA were mimicked, however, by application of clonidine and blocked by co-application of RX821002, a selective antagonist of α_2 adrenoceptors (Crochet and Sakai, 2003). The PS/atonia-inhibiting effect of NA and A seen in the caudal peri-LCα appears to result from direct inhibition of non-cholinergic, presumed glutamatergic, PS-on neurons via α_2-adrenoceptors, in the light of our iontophoretic study showing inhibition of non-cholinergic PS-on neurons by NA or A (Sakai and Koyama, 1996).

Atonia-executive structures, as determined by local application of muscimol

We then determined atonia-executive structures using microdialysis application of muscimol, a potent $GABA_A$ receptor agonist that inhibits virtually all neurons in the central nervous system (Johnston, 1978). Five hundred µM muscimol significantly inhibits PS and induces PS without atonia, especially when applied to the caudal peri-LCα, whereas muscimol application to other pontine structures including the Poo and Poc does not (Sakai et al., 2001; Crochet et al., 2006). The atonia-suppressing sites by muscimol application correspond to those seen with 4-DAMP, NA, A or DA (Fig. 7D).

Disfacilitatory mechanisms in the generation of atonia during PS

It has been shown in the cat that both spinal cord and trigeminal motoneurons are inhibited during PS as a result of postsynaptic inhibition (Glenn et al., 1978; Nakamura et al., 1978; Morales and Chase, 1978, 1981), mediated mainly by glycine, an inhibitory amino acid neurotransmitter (Chase and Morales, 1990, 2005). Recent data suggest, however, that not all motoneurons within the brainstem are postsynaptically inhibited during PS by inhibitory amino acids, and that hypoglossal motoneurons innervating the genioglossus muscles (GG) may be inhibited during PS as a result of a disfacilitation mechanism (Kubin et al., 1993, 1996). In support of this assumption, Kubin et al. (1993, 1996) showed in decerebrate cats that microinjections into the hypoglossal motor nucleus (HMN) of either strychnine, a glycinergic receptor antagonist, or bicuculline, a $GABA_A$ receptor antagonist, have little effect on the carbachol-induced atonia of the upper airway muscles. The authors suggested that the suppression of GG muscle activity during PS might be due to the withdrawal of serotonergic excitatory inputs to hypoglossal motoneurons. (Kubin et al., 1994; Lai et al., 2001), as the HMN receives dense serotonergic inputs (Kubin et al., 1996), and when applied to the HMN *in vivo*, 5-HT attenuates the decease in hypoglossal nerve or GG muscle activity seen during carbachol-induced atonia in decerebrate cats (Kubin et al., 1993). In addition to 5-HT neurons, the HMN is innervated by both NA and HA neurons which, like 5-HT neurons, cease firing during PS (Sakai, 1980; Sakai et al., 1990). The decease in GG muscle activity during PS might therefore be caused by withdrawal of excitatory inputs not only from 5-HT neurons, but also from NA and HA neurons. Using power spectral analysis in freely moving cats, we investigated in freely moving cats the effect on GG muscle activity of the microdialysis application of 5-HT, NA or HA to the HMN across the wake-sleep cycle (Neuzeret et al., 2009).

The drugs were applied unilaterally for 1 hour using a microdialysis probe (1 mm in length, 0.24 mm in diameter, and a 6000-DA Cut-off, CMA/11) to either rostral or caudal part of the HMN that, in the cat, extends 4-5 mm rostrocaudally. As shown in Fig. 8B, application of 5-HT or HA, but not NA, to the HMN resulted in a marked increase in GG muscle activity in all wake-sleep states, that sharply contrasted with the decrease in GG muscle activity seen during sleep states in control (Ringer's alone) (Fig. 8A). During the application of 5-HT or HA, there was no significant reduction in GG muscle activity from quiet W to SWS, although a small, but significant reduction in GG muscle activity was still observed during PS when compared with the preceding SWS with PGO waves (S-PGO) (Figs. 8B and 9B). It should be mentioned, however, that when PS episode occurred shortly after the beginning of 5-HT or HA application, as shown in Fig. 9A, GG muscle activity maintained the same high level of tonic activity during the transition from S-PGO to PS and throughout the PS episode. In spite of this tonic increase in GG muscle activity during PS, a pronounced phasic reduction in GG muscle activity was still seen in association with PGO and REM bursts (Fig. 10). These findings suggest both disfacilitatory and inhibitory mechanisms underlying both the tonic and phasic processes of the atonia of GG muscles during PS. It should be mentioned that, as recently confirmed in mice (Takahashi et al., 2006; 2010), both cat NA and HA neurons display a waking-specific discharge profile with virtually no discharge activity during both SWS and PS (Sakai et al., 1990; Sakai and Crochet, 2003). These data are thus at variance with the idea that the atonia of GG muscle activity seen during PS is due to the withdrawal of noradrenergic or histaminergic excitatory inputs. Unlike the NA and HA neurons, the majority of cat 5-HT neurons, in particular those in the medulla exhibit a complete cessation of discharge activity, selectively during the period of PS and atonia as well, as illustrated in Fig. 11A. In addition to this population of neurons referred to as "complete type", we found another group of 5-HT neurons in the caudal raphe nuclei and ventrolateral medulla, referred to as "incomplete type", because they did not completely cease firing during PS, but significantly reduced their tonic activity during this state (Fig. 11B; for details see Sakai, 2008). Interestingly, this latter group of neurons completely cease firing not tonically, but phasically in association with PGO and REM bursts (Fig. 11B, indicated by arrowheads). The slight reduction in GG muscle activity seen during PS under 5-HT application may result from the fact that the HMN motoneurons are only

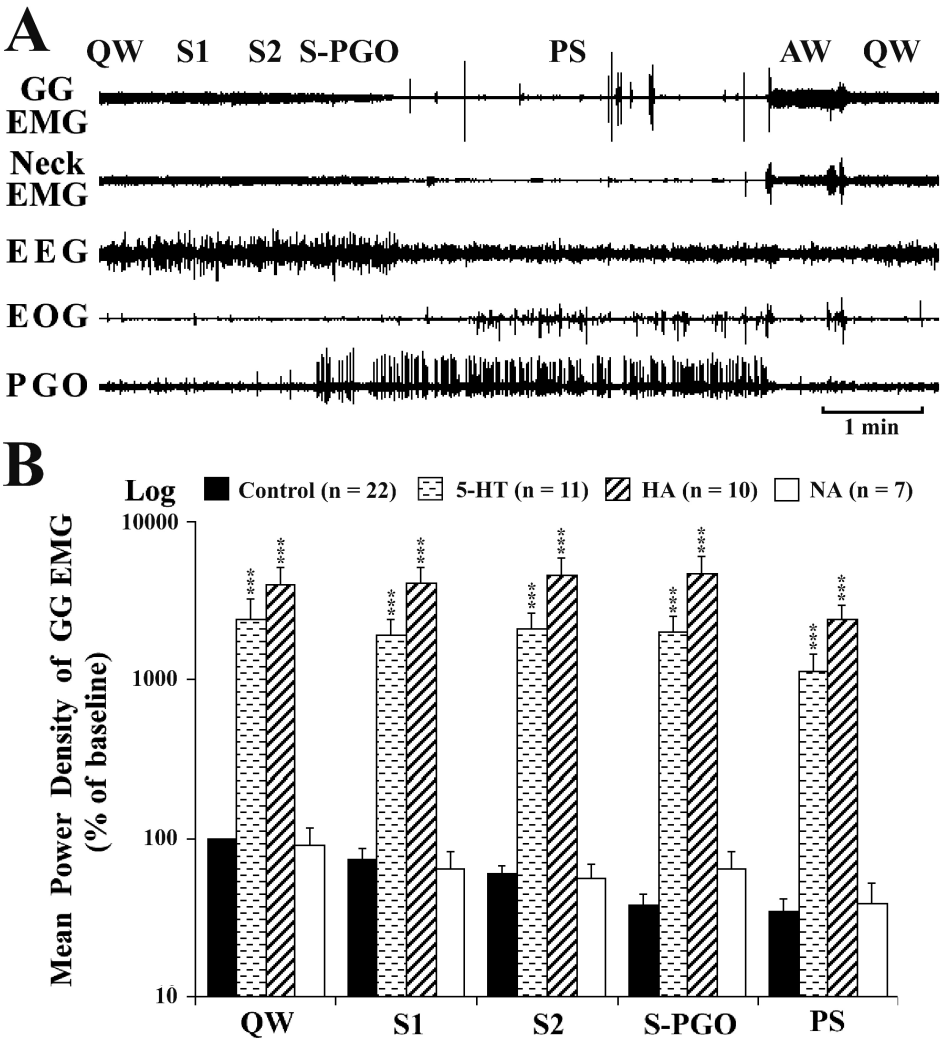

Fig. 8. - (A) Polygraph showing change in GG muscle activity (GG EMG) across the sleep-waking cycle observed in the cat without drug application. (B) Change in GG activity during wake-sleep states before (control, black bars, n = 22) and after application of 5-HT (horizontally dashed bars, n = 11), HA (hatched bars, n = 10) or NA (white bars, n = 7). Values are expressed as the percent mean power density compared with the baseline values in QW (modified from Neuzeret et al., 2009).

partially stimulated and thus they are still under the control of medullary serotonergic excitatory inputs (see Discussion).

Discussion

The present study gives evidence for the existence of two supraspinal structures responsible for the generation of the neck muscle atonia during PS: the peri-LCα and adjacent LCα in the pons and the Mc in the medulla. We demonstrated that, during PS, a group of peri-LCα and adjacent medial LCα neurons, in conjunction with a group of Mc neurons, exhibit a tonic discharge selective to PS/atonia (PS/atonia-on neurons). We suggested that, during PS, the peri-LCα and adjacent LCα neurons exert, via the lateral tegmentoreticular tract, an excitatory influence on the Mc neurons, which in turn exert a generalized inhibitory influence on spinal motoneurons via the ventrolateral reticulospinal tract. In the medulla, we also demonstrated the presence of PS-specific neurons innervating the motor trigeminal nucleus and suggested that they may play a critical role in the

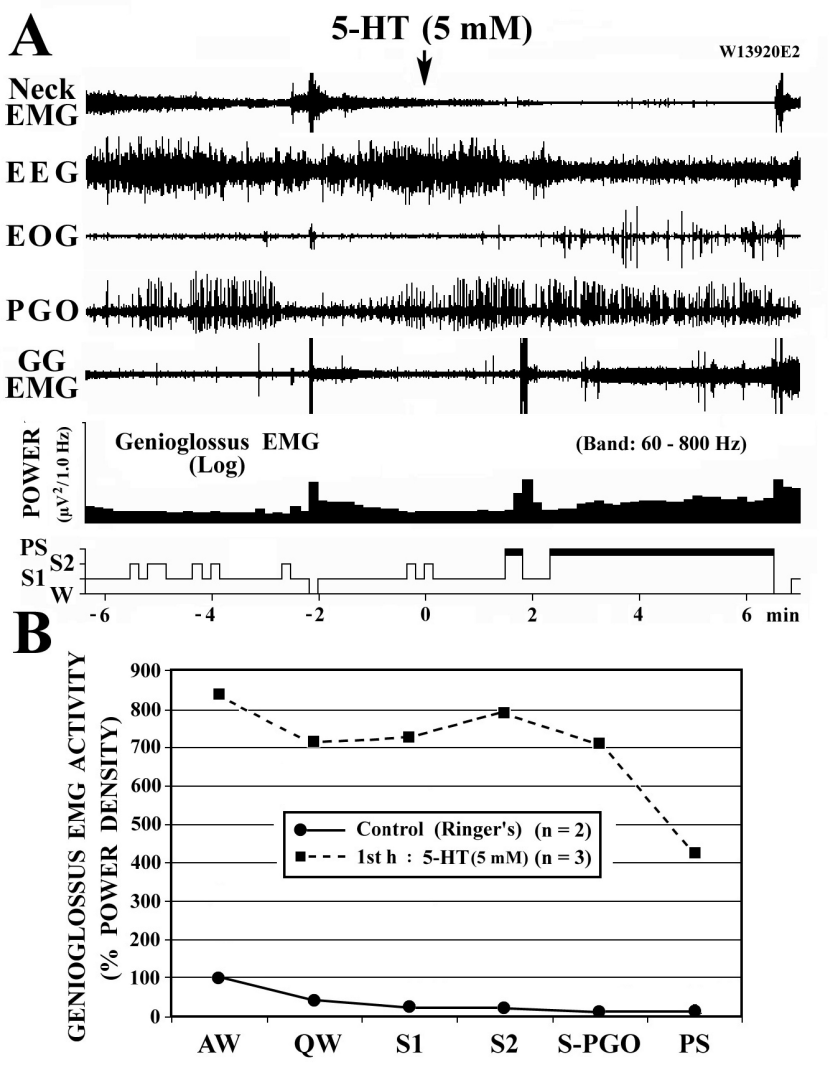

Fig. 9. - (A) Tonic activation of genioglossus (GG) muscle activity during application of 5-HT to the hypoglossal nucleus. The activation occurred a few minutes after the start of 1-hour 5-HT application period (indicated by the arrow above the top trace) and continued throughout application, leading to complete blockade of the GG muscle atonia normally seen during PS. Note that the power density of GG EMG is expressed on a $\log_{10}$ scale because of the very high values seen during AW with movements. (B) Change in the GG EMG activity across the wake-sleep cycle observed during the first hour of 5-HT application. The values are expressed as the mean percent power density with respect to the mean baseline values (Ringer's) obtained during AW without phasic discharges. The numbers in parantheses indicate the number of wake-sleep cycles during the analysis period (modified from Neuzeret et al., 2009).

inhibition of masseter motoneurons during PS. In addition, we have suggested that the tonic or phasic cessation of activity of medullary serotonergic neurons during PS play an important role in the atonia of pharyngeal muscles during PS.

In addition to the PS/atonia-on neurons, we found, in the peri-LCα and LCα, as well as in the Mc, a cluster of neurons showing tonic activation and high selectivity of firing for PS over SWS and quiet W, but exhibiting a significant increase in discharge rate during active W in relation to postural change. At present, it is unclear whether the PS-selective and PS-non-selective neurons have similar, but different functional roles or whether the supraspinal systems responsible for the postural atonia during PS could be also active during active W and serve for the control and coordination of postural muscles as well. It should be noted that the ponto-medullary postural atonia-executive system that we found is composed of neurons having a slow conduction velocity (< 10

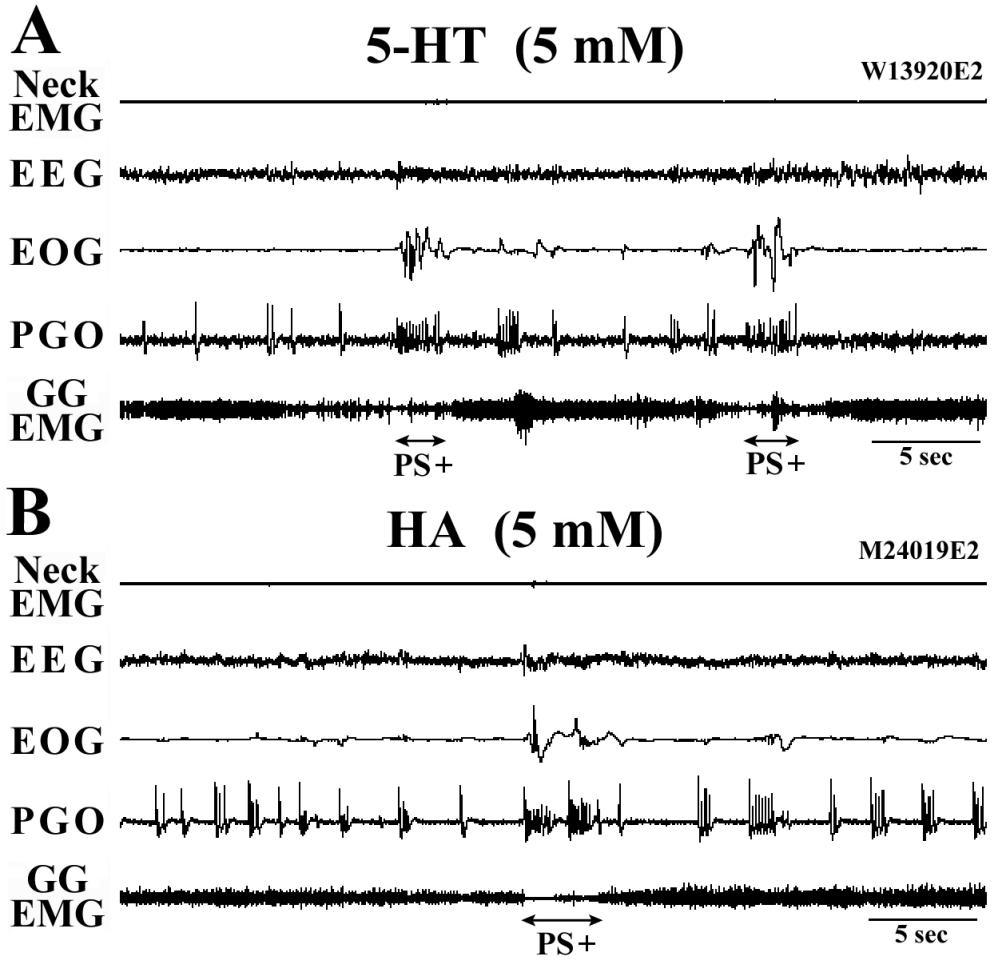

Fig. 10. - Change in GG EMG activity during PS with application of 5-HT (A) or HA (B) to the hypoglossal nucleus. Note the transient suppression of the sustained GG muscle activity during the PS episodes with bursts of PGO waves and REMs (PS+) (unpublished data from the study of Neuzeret et al., 2009).

m/s). This slow conducting system differs considerably from the fast-conducting reticulospinal systems (> 10 m/s), as described in the cat by Peterson et al. (1978) for neck motoneurons and by Chase et al. (1986), Mori (1987), and Takakusaki et al. (1989, 2001) for lumbar hindlimb motoneurons. According to Peterson et al. (1978), stimulation of the nucleus reticularis ventralis (which includes our Mc) and the dorsal part of the Gc evoked short latency (< 1.3 ms) monosynaptic IPSPs in neck motoneurons. They suggested that the inhibitory projection from the Gc is involved in vestibular and visuomotor reflexes, while the inhibitory reticulospinal neurons in the nucleus reticularis ventralis may be involved in producing the atonia of neck muscles during PS. On the other hand, Takakusaki et al. (1989) have reported that medullary reticulospinal neurons exert postsynaptic inhibitory effects, via inhibitory interneurons, upon α-motoneurons innervating cat hindlimb muscles. Using the spike-triggered averaging technique in decerebrate cats, Takakusaki et al. (1994) also reported that medullary reticulospinal neurons producing suppression of hindlimb motoneuronal activity conducted at 80-100m/s and were located in the dorsal medulla (Gc). More recently, Kohyama et al. (1998) have reported both slow- (conduction velocity < 22.8 m/s) and fast- (conduction velocity > 22.8 m/s) conducting reticulospinal neurons producing the inhibition of both neck and hindlimb muscles in the Gc and Mc, but they did not find reticulospi-

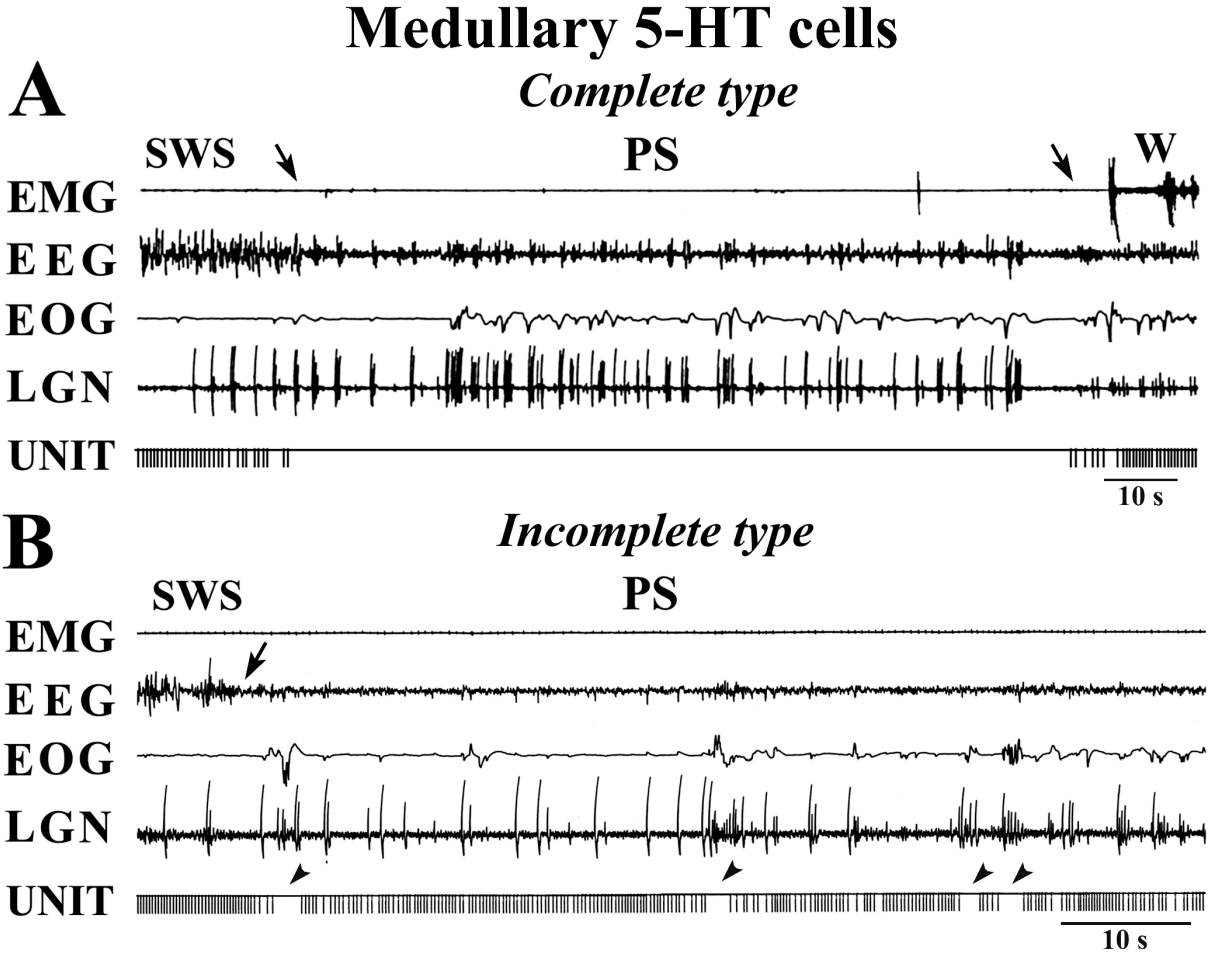

Fig. 11. - Unit activity of two medullary serotonergic neurons during different sleep-wake states. Note that there is either a complete cessation of discharge (A) or a transient suppression of sustained discharge in association with bursts of PGO waves and REMs (B) (modified from Sakai and Kanamori, 1999).

nal neurons with conduction velocity of < 10 m/s. These findings indicate that several reticulospinal systems are involved in suppression of cervical and lumbar motoneurons. The medial pontine and medullary RF (Poo, Poc, and Gc) are found to play an essential role in the fast conducting reticulospinal systems (Chase and Morales, 1990; Kohyama et al., 1998; Takakusaki et al., 1994, 2001), which may be functionally related to the rapid control of motor activities such as vestibular and visuomotor reflexes, as proposed by Peterson et al. (1982), or postural fixation and locomotion as proposed by Mori et al. (1995). On the other hand, we propose that the slow-conducting ponto-medullay descending systems that we found in the peri-LCα and LCα in the pons and in the Mc in the medulla are functionally related to the neck muscle atonia during PS, although the functional significance of this atonia during PS per se remains largely unknown. This peculiar tonic phenomenon, however, appears to play an important role in the maintenance of PS, because a drastic decrease in PS duration is observed in the cat exhibiting PS without atonia, the decrease being mainly ascribed to the appearance of stereotyped behaviors during the PS episode (Sastre and Jouvet, 1979). If the atonia during PS is not a simple epiphenomenon resulting from the activation of ponto-medullary neurons responsible for the relaxation of postural muscles during W, then it may have an active role such as reduction of thermal loss or energy conservation by minimizing muscular activity (see Zepelin and Rechtschaffen, 1974).

Our studies have suggested glutamatergic nature of the pontine atonia-executive neurons sending axons to the Mc. In support of this, it has been reported that glutamate agonist injection into the Mc elicits muscle atonia in chronic cats (Lai and Siegel, 1988) and that there is an increase in glutamate release during PS in the Mc of freely moving cat (Kodama et al., 1998). In addition, injection of the glutamate antagonist γ-D-glutamylglycine into the Mc reverses the atonia induced by pontine carbachol injection (Lai and Siegel, 1988), the finding also supporting our hypothesis that pontine regulation of muscle activity is mediated through the Mc. It should be also emphasized that total destruction of the pontomedullary Gc neurons by either electrolytic or cytotoxic lesions has no effect on the atonia during PS (Sastre et al., 1979, 1981). Furthermore, if the brainstem transection at the pontomedullary junction is incomplete so that the ventrally located lateral tegentoreticular tract to the Mc remains intact, no effect can be observed on the atonia and the sleep-waking states (Sakai, 1985; Webster et al., 1986; Vanni-Mercier et al., 1991). These findings further support our hypothesis that the Mc, but not the dorsally located Gc, plays a critical role in the generation of neck muscle atonia during PS. Holmes and Jones (1994) have previously suggested, however, that the medial medullay RF neurons contribute to the generation and maintenance of PS and to the associated inhibition of postural muscles, but are not necessary for the appearance of that state, because despite extensive cytotoxic destruction by neurotoxin quisqualic acid of the medullary neurons, the full sleep-wake cycle reappeared and the loss of the atonia recovered within a week after surgery. It should be mentioned, however, that their cytotoxic lesions were limited to the medial medullary RF, and, as also mentioned by the authors, did not included ventrolateral most portion of the Mc, which receives major afferent projections from the peri-LCα and contains many PS-on neurons (see Fig. 4). It is worth noting that all our attempts at bilateral and total destruction of the Mc neurons by either electrolytic or excitotoxic lesions were unsuccessful, the lesions always leading to the death of the animals by arrest of respiration (Sakai, 1985). In sharp contrast, Petitjean (1981) reported that electrolytic lesions of the medial medullary RF including the caudal raphe nuclei (raphe magnus and pallidus), and adjacent medial part of the Gc and Mc had no effects on the generation of the atonia, although such lesions produced a marked decrease in PS amount.

At present, the neurochemical nature of Mc and Pc atonia-executive neurons remains unclear. It remains also unclear whether the hyperpolarization of spinal and trigeminal motoneurons during PS is induced monosynaptically by excitation of inhibitory premotor reticular neurons or di- or polysynaptically by excitation of excitatory premotor neurons impinging upon inhibitory spinal or trigeminal interneurons. The Mc, Pc and Gc all contain glutamatergic, GABAergic and glycinergic neurons (Jones et al., 1991; Fort et al., 1993; Holms and Jones, 1994; Holstege, 1996; Stornetta and Guyenet, 1999; Vetrivelan et al., 2009). In the Pc, we found a cluster of PS-specific neurons projecting directly to the MV and suggested that they may play a role in the atonia of jaw-closer muscles during PS. Recently, Morales et al. (2006) reported that glycinergic Pc neurons do not contribute to the suppression of masseter motoneuron activity during PS, as no glycinergic Pc cells expressed c-fos during carbachol-induced PS. These findings suggest that the Pc PS-specific neurons projecting directly to the MV may be not glycinergic, though the atonia of masseter muscles during PS is thought to be due to glycinergic postsynaptic inhibition, mediated by monosynaptically projecting medullary neurons (Chase and Morales, 2005; Chase, 2008). Further studies are thus needed to determine the neurochemical nature the Pc PS-specific neurons and their synaptic connectivity with trigeminal motoneurons and interneurons.

Recently it has been reported that stimulation of the Mc in decerebrate cats increased both glycine and GABA release and decreased both NA and 5-HT release in the ventral horn, while its glutamate level remained unchanged (Kodama et al., 2003; Lai et al., 2001, 2010). This increase in glycine and GABA may come from reticulospinal glycinergic and GABAergic inhibitory premotor neurons as supposed by Chase and Morales (2005), or spinal interneurons containing these inhibitory neurotransmitters (Todd and Sullivan, 1990), as proposed earlier by Jankowska et al. (1968), and more recently by Takakusaki et al. (1989, 2001) in decerebrate cats.

In the Mc, we found PS-specific and PS-non-specific, although discharging selectively during PS over quiet W and SWS, descending neurons. They had significantly different mean (± SD) conduction velocities of, respectively, 8.3 ± 1.4 m/s and 13.3 ± 5.9 m/s, suggesting that they may use different neurotransmitters and have different functions. Further studies combining electrophysiology and single cell labeling in chronic cats are necessary to identify the neurochemical nature of these Mc cells.

Finally, we will briefly discuss the serotonergic disfacilitation hypothesis on GG muscle atonia during PS (Kubin et al., 1994, 1996). The HMN receives not only monoaminergic excitatory inputs, but also glycinergic and GABAergic inhibitory inputs from neurons located in the Pc (Li et al., 1997; see also Neuzeret et al., 2009). Recently, Lai et al. (2001) and Kodama et al. (2003) have reported that stimulation of the mesopontine RF produced a suppression of both postural and respiratory muscle tones, and simultaneously caused a significant reduction in NA and 5-HT release and a significant increase in glycine and GABA of similar magnitude in both the HMN and the ventral horn of spinal cord. Although our findings on the carbachol-induced suppression of LC neuronal activity are in favor of a disfacilitatory action of NA neurons on GG muscles atonia during carbachol-induced PS, NA neurons may not contribute to the selective suppression of GG muscle activity during naturally occurring PS, because NA neurons cease firing as early as light SWS in the cat (Sakai and Crochet, 2003) or prior to sleep onset in the mouse (Takahashi et al., 2010). NA application to the HMN had no excitatory effect on GG muscle activity in the cat. This may be due to preferential activation of α_2-adrenoceptors in the cat (see Neuzeret et al., 2009). Like the NA neurons, HA neurons display waking-selective discharge activity in both cats and mice (Sakai et al., 1990; Takahashi et al., 2006). The GG muscle atonia-blocking effect of HA may thus be pharmacological, but not physiological. The findings, however, might open up new pharmacological approaches to increasing airway muscle tone during sleep.

On the other hand, the effect of 5-HT does not appears to be simply pharmacological, since unlike the HA and NA neurons, change in discharge activity of medullary 5-HT neurons, that project to the HMN, is in parallel with that in GG muscle activity across the wake-sleep states. During the application, however, a slight, but still significant tonic decrease in GG muscle activity was observed, together with phasic suppression of activity in association with PGO and REM bursts. This decrease or suppression of GG muscles may be due to the fact that HMN motoneurons are only partially stimulated and thus they are still under the control of serotonergic excitatory inputs. Alternatively, these changes in GG muscle activity may be due to decrease in activity of GABAergic and/or glycinergic inhibitory premotor neurons projecting to the HMN, as suggested by Lai et al. (2001) and Kodama et al. (2003). At present, nothing is known about the discharge activity of these inhibitory premotor neurons across wake-sleep states. In light of the anatomical findings (Li et al., 1997, 1996), it seems likely that a cluster of PS-specific Pc neurons that we have described play a role of this inhibitory premotor neurons, and a cluster of phasic Pc neurons discharging phasically during PS in association with PGO and REM bursts may play a role in phasic suppression of GG muscle activity during PS. Further studies are needed to examine these hypotheses.

Summary

Our studies support the existence of multiple slow conducting and fast conducting systems in the brainstem that control the activity of spinal and orofacial motoneurons across wake-sleep states. We propose that the PS/atonia-on neurons characterized by their most slow conducting property and located in the peri-LCα and adjacent LCα of the mediodorsal pontine tegmentum play a critical executive role in the somatic and orofacial muscle atonia observed during PS. Slow conducting medullay PS/atonia-on neurons located in the Mc and Pc may play a critical executive role in the generation of, respectively, antigravity or orofacial muscle atonia during PS. In addition, either tonic or phasic cessation of activity of medullary 5-HT neurons may play an important role in the atonia of GG muscles during PS via a mechanism of disfacilitation.

Acknowledgements

This study was supported by INSERM U1028, CNRS UMR5292, and Claude Bernard University.

References

Baldissera F., Broggi G., Mancia M. Monosynaptic and polysynaptic spinal reflexes during physiological sleep and wakefulness. *Arch. Ital. Biol.*, **104**: 112-133, 1966.

Berman A.L. *The Brain Stem of the Cat. A Cytoarchitectonic Atlas with Stereotaxic Coordinates*. Madison, Wisconsin, University of Wisconsin Press, 1968.

Carli G. and Zanchetti A. A study of pontine lesions suppressing deep sleep in the cat. *Arch. Ital. Biol.*, **103**: 751-788, 1965.

Chase M.H., Morales F.R., Boxer P.A., Fung S.J., Soja, P.J. Effect of stimulation of the nucleus reticularis gigantocellularis on the membrane potential of cat lumbar motoneurons during sleep and wakefulness. *Brain Res.*, **386**: 237-244, 1986.

Chase M.H. and Morales F.R. The atonia and myoclonia of active (REM) sleep. *Ann. Rev. Psychol.*, **41**: 557-584, 1990.

Chase M.H. and Morales F.R. Control of motoneurons during sleep. In: Kryger M.H., Roth T., Dement W.C. (Eds.) *Principles and practice of sleep medicine*, Philadelphia, WB Saunders: 154-168, 2005.

Chase M.H. Confirmation of the consensus that glycinergic postsynaptic inhibition is responsible for the atonia of REM sleep. *Sleep*, **31**: 1487-1491, 2008.

Crochet S. and Sakai K. Alpha-2 adrenoceptor mediated paradoxical (REM) sleep inhibition in the cat. *Neuroreport*, **10**: 2199-2204, 1999a.

Crochet S. and Sakai K. Effects of microdialysis application of monoamines on the EEG and behavioural states in the cat mesopontine tegmentum. *Eur. J. Neurosci.*, **11**: 3738-3752, 1999b.

Crochet S. and Sakai K. Dopaminergic modulation of behavioral states in mesopontine tegmentum: a reverse microdialysis study in freely moving cats. *Sleep*, **26**: 801-806, 2003.

Crochet S., Onoe H., Sakai, K. A potent non-monoaminergic paradoxical sleep inhibitory system: a reverse microdialyis and single unit recording study *Eur. J. Neurosci.*, **24**: 1404-1412, 2006.

Fort P., Sakai K., Luppi P.H., Salvert D., Jouvet M. Monoaminergic, peptidergic and cholinergic afferents to the cat facial nucleus as evidenced by a double-immunostaining method with unconjugated cholera toxin as a retrograde tracer. *J. Comp. Neurol.*, **301**: 262-275, 1989.

Fort P., Luppi P.H., Sakai K., Salvert D., Jouvet M. Nuclei of origin of monoaminergic, peptidergic and cholinergic afferents to the cat trigeminal motor nucleus: a double-labeling study with cholera-toxin as a retrograde tracer. *J. Comp. Neurol.*, **301**: 262-275, 1991.

Fort P., Luppi P.H., Jouvet M. Glycine-immunoreactive neurons in the can brain stem reticular formation. *Neuroreport*, **4**: 1123-1126, 1993.

Gassel M.M., Marchiafava P.L., Pompeiano O. Tonic and phasic inhibition of spinal reflexes during deep, desynchronized sleep in unrestrained cats. *Arch. Ital. Biol.*, **102**: 471-499, 1964.

Gassel M.M., Marchiafava P.L., Pompeiano O. An analysis of supraspinal influences acting on motoneurons during sleep in the unrestrained cat. Modification of the recurrent discharge of the alpha motoneurons during sleep. *Arch. Ital. Biol.*, **103**: 25-44, 1965.

Gassel M.M. and Pompeiano O. Fusimotor function during sleep in unrestrained cats. *Arch. Ital. Biol.*, **103**: 347-368, 1965.

Giaquinto S., Pompeiano O., Somogyi I. Descending inhibitory influence on spinal reflexes during natural sleep. *Arch. Ital. Biol.*, **102**: 282-307, 1964.

Glenn L.L., Foutz A.S., Dement W.C. Membrane potential of spinal motoneurons during natural sleep in cats. *Sleep*, **1**: 199-204, 1978.

Henley K. and Morrison R. A re-evaluation of the effects of lesions of the pontine tegmentum and locus coeruleus on phenomena of paradoxical sleep in the cat. *Acta. Neurobiol. Exp.*, **34**: 215-232, 1974.

Hobson J.A., McCarley R.W., Wyzinski P. Sleep cycle oscillation: reciprocal discharge by two brainstem neuronal groups. *Science*, **189**: 55-58, 1975.

Holmes C.J. and Jones B.E. Importance of cholinergic, GABAergic, serotonergic and other neurons in the medial medullary reticular formation for sleep-wake states studied by cycotoxic lesions in the cat. *Neuroscience*, **62**: 1179-1200, 1994.

Holstege J.C. The ventro-medial medullary projections to spinal motoneurons: ultrastructural, transmitters and functional aspects. *Prog. Brain Res*, **107**: 159-181, 1996.

Jacobs B.L. Overview of the activity of brain monoaminergic neurons across the sleep-wake cycle. In: Wauquier A., Gaillard J.M., Monti J.M., Radulovacki M. (Eds.) *Sleep: Neurotransmitters and Neuromodulators*, New York, Raven Press: 1-14, 1985.

Jankowska E., Lund S., Lundberg A., Pompeiano O. Inhibitory effects evoked through ventral reticulospinal pathways. *Arch. Ital. Biol.*, **106**: 124-140, 1968.

Johnston G.A.R. Neuropharmacology of amino acid inhibitory transmitters. *Annu. Rev. Pharmacol. Toxicol.*, **18**: 269-289, 1978.

Jones B.E., Holmes C.J., Rodriguez-Veiga E., Mainvill L. GABA-synthesizing neurons in the medulla: their relationship to serotonin-containing and spinally projecting neurons in the rat. *J. Comp. Neurol.*, **313**: 349-367, 1991.

Jouvet M., Michel F., Courjon J. Sur un stade d'activité électrique cérébrale rapide au cours du sommeil physiologique. *Compt. Rend. Soc. Biol.*, **153**: 1024-1028, 1959.

Jouvet M. Recherches sur les structures nerveuses et les mécanismes responsables des différentes phases du sommeil physiologique. *Arch. Ital. Biol.*, **100**: 125-206, 1962.

Jouvet M. The role of monoamines and acetylcholine-containing neurons in the regulation of the sleep-waking cycle. *Ergebn. Physiol.*, **64**: 166-307, 1972.

Kanamori N., Sakai K., Jouvet M. Neuronal activity specific to paradoxical sleep in the ventromedial medullary reticular formation of unrestrained cats. *Brain Res.*, **189**: 251-255, 1980.

Kodama T., Lai Y.Y., Siegel J.M. Enhanced glutamate release during REM sleep in the rostromedial medulla as measured by *in vivo* microdialysis. *Brain Res.*, **780**: 178-181, 1998.

Kodama T., Lai Y.Y., 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.

Kohyama J., Lai Y.Y., Siegel J.M. Reticulospinal systems mediate atonia with short and long latencies. *J. Neurophysiol.*, **80**: 1839-1859, 1998.

Kubin L., Kimura H., Tojima H., Davies R.O., Pack A.L. 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.

Kubin L., Reignier C., Tojima H., Taguchi O., Pack A.I. Changes in serotonin level in the hypoglossal nucleus region during carbachol-induced atonia. *Brain Res.*, **645**: 291-302, 1994.

Kubin L., Tojima H., Reignier C., Pack A.I., Davies R.O. Interaction of serotonergic excitatory drive to hypoglossal motoneurons with carbachol-induced, REM sleep-like atonia. *Sleep*, **19**: 187-195, 1996.

Kubota K. and Kidokoro Y. Excitability of the membrane of lumbar motor neurons and natural sleep in the cat. *Jap. J. Physiol.*, **16**: 217-226, 1965.

Kubota K. and Tanaka R. The fusimotor activity and natural sleep in the cat. *Brain Res.*, **3**: 198-201, 1966.

Kuypers H.G.J.M. and Maisky V.A. Funicular trajectories of descending brain stem pathway in the cat. *Brain Res.*, **136**: 159-165, 1977.

La Vail J.H. and La Vail M.M. Retrograde axonal transport in the central nervous system. *Science*, **176**: 1416-1417, 1972.

Lai Y.Y. and Siegel J.M. Medullary regions mediating atonia. *J. Neurosci.*, **8**: 4790-4796, 1988.

Lai Y.Y., Kodama T., Siegel J.M. Changes in monoamine release in the ventral horn and hypoglossal nucleus linked to pontine inhibition of muscle tone: an *in vivo* microdialysis study. *J. Neurosci.*, **21**: 7384-7391, 2001.

Lai Y.Y., Kodama T., Schenkel E., Siegel J.M. Behavioral responses and transmitter release during atonia elicited by medial medullary stimulation. *J. Neurophysiol.*, **104**: 2024-2033, 2010.

Landgren S., Olsson K.A., Westberg K.G. Bulbar neurons with axonal projections to the trigeminal motor nucleus in the cat. *Exp. Brain Res.*, **65**: 98-111, 1986.

Li Y.-Q., Takada M., Kaneko T., Mizuno N. GABAergic and glycinergic neurons projecting to the trigeminal motor nucleus: A double labeling study in the rat. *J. Comp. Neurol.*, **373**: 498-510, 1996.

Li Y.-Q., Takada M., Kaneko T., Mizuno N. Distribution of GABAergic and glycinergic premotor neurons projecting to the facial and hypoglossal nuclei in the rat. *J. Comp. Neurol.*, **378**: 283-294, 1997.

Magoun H.W. and Rhines R. An inhibitory mechanism in the bulbar rerticular formation. *J. Neurophysiol.*, **9**: 165-171, 1946.

Mizuno N., Yasui Y., Nomura S., Itoh K., Konishi A., Takada M., Kudo M. A light and electron microscopic study of premotor neurons for the trigeminal motor nucleus. *J. Comp. Neurol.*, **215**: 290-298, 1988.

Morales F.R. and Chase M.H. Intracellular recordings of lumbar motoneuron membrane potential during sleep and wakefulness. *Exp. Neurol.*, **62**: 821-827, 1978.

Morales F.R. and Chase M.H. Postsynaptic control of lumbar motoneuron excitability during active sleep in the chronic cat. *Brain Res.*, **225**: 279-295, 1981.

Morales F.R., Sampogna S., Rampon C., Luppi P.H., Chase M.H. Brainstem glycinergic neurons and their activation during active (rapid eye movement) sleep in the cat. *Neuroscience*, **142**: 37-47, 2006.

Mori S. Integration of posture and locomotion in acute decerebrate cats and in awake, freely moving cats. *Progr. Neurobiol.*, **28**: 785-809, 1987.

Mori S., Iwakiri H., Homma Y., Yokoyama T., Matsuyama K. Neuroanatomical and neurophysiological bases of postural control. *Adv. Neurol.*, **67**: 289-303, 1995.

Morrison A.R. and Pompeiano O. An analysis of the supraspinal influences acting on motoneurons during sleep in the unrestrained cat. Responses of the alpha motoneurons to direct electrical stimulation during sleep. *Arch. Ital. Biol.*, **103**: 497-516, 1965.

Nakamura Y., Goldberg L.G., Chandler S.H., Chase M.H. Intracellular analysis of trigeminal motoneuron activity during sleep in the cat. *Science*, **199**: 204-207, 1978.

Neuzeret P.C., Sakai K., Gormand F., Petitjean T., Buda C., Sastre J.P., Parrot S., Guidon G., Lin J.S. Application of histamine or serotonin to the hypoglossal nucleus increases genioglossus muscle activity across the wake-sleep cycle. *J. Sleep Res.*, **18**: 113-121, 2009.

Ono T., Ishiwata Y., Inada N., Kuroda T., Nakamura Y. Hypoglossal premotor neurons with rhythmical inspiratory-related activity in the cat: Localization and projection to the phrenic nucleus. *Exp. Brain Res.*, **98**: 1-12, 1994.

Onoe H. and Sakai K. Kainate receptors: a novel mechanism in paradoxical (REM) sleep generation *Neuroreport*, **6**: 353-356, 1995.

Peterson B.W., Pitts N.G., Fukushima K., Mackel R. Reticulo-spinal excitation and inhibition of neck motoneuron. *Exp. Brain Res.*, **32**: 471-489, 1978.

Peterson B.W. and Fukushima K. The reticulospinal system and its role in generating vestibular and visuomotor reflexes. In: Sjölund B. and Björklund A. (Eds.) *Brain Stem Control of Spinal Mechanisms*, Amsterdam, Elsevier: 225-251, 1982.

Petijean F., Insomnie et hypersomnie chez le chat. Thesis. Doctorat ès Sciences, Claude Bernard University, Lyon, 1981, p. 422.

Pitts R.F. The respiratory center and its descending pathway. *J. Comp. Neurol.*, **72**: 605-625, 1940.

Pompeiano O. Mechanisms responsible for spinal inhibition during desynchronized sleep: experimental study. In: Guilleminaul C., Dement W.C., Passouant P. (Eds.) *Advances in Sleep Research. Narcolepsy*, Vol. 3, New York, Spectrum: 411-449, 1976.

Russel G.V. The nucleus locus coeruleus (dorsolateralis tegmenti). *Tex. Rep. Biol. Med.*, **13**: 939-988, 1955.

Sakai K., Kanamori N., Jouvet M. Activités unitaires spécifiques du sommeil paradoxal dans la formation réticulée bulabire chez le Chat non-restreint. *C.R. Acad. Sci. (Paris)*, **289**: 557-561, 1979a.

Sakai K., Sastre J.P., Salvert D., Touret M., Tohyama M., 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, 1979b.

Sakai K. Some anatomical and physiological properties of ponto-mesencephalic tegmental neurons with special reference to PGO waves and postural atonia during paradoxical sleep in the cat. In: Hobson J.A. and Brazier M.A.B. (Eds.) *The Reticular Formation Revisited*, New York, Raven Press: 427-447, 1980.

Sakai K., Sastre J.P., Kanamori N., Jouvet M. State-specific neurons in the ponto-medullar reticular formation with special reference to the postural atonia during paradoxical sleep in the cat. In: Ajimone-Marsan C. and Pompeiano O. (Eds.) *Brain Mechanisms and Perceptual Awareness*, New York, Raven Press: 405-429, 1981.

Sakai K. Anatomical and physiological basis of paradoxical sleep. In: McGinty D.J., Drucker-Colin R., Morrison A.R., Parmeggiani L. (Eds.) *Brain Mechanisms of Sleep*, New York, Raven Press: 111-137, 1985.

Sakai K. Executive mechanisms of paradoxical sleep. *Arch. Ital. Biol.*, **126**: 239-257, 1988.

Sakai K., El Mansari M., Lin J.S., Zhang J.G., Vanni-Mercier G. The posterior hypothalamus in the regulation of wakefulness and paradoxical sleep. In: Mancia M. and Marini M. (Eds.) *The Diencephalon and Sleep*, New York, Raven Press: 171-198, 1990.

Sakai K. Physiological properties and afferent connections of the locus coeruleus and adjacent tegmental neurons involved in the generation of paradoxical sleep in the cat. In: Barnes C.D. and Pompeiano O. (Eds.) *Neurobiology of the Locus Coeruleus*, Prog. Brain Res., Vol. 88, Amsterdam, London, Oxford, Tokyo, Elsevier: 31-45, 1991.

Sakai K. and Koyama Y. Are there cholinergic and non-cholinergic paradoxical sleep-on neurons in the pons? *Neuroreport*, **7**: 2449-2453, 1996.

Sakai K. and Onoe H. Critical role for M_3 muscarinic receptors in paradoxical sleep generation in the cat. *Eur. J. Neurosci.*, **9**: 415-423, 1997.

Sakai K. and Kanamori N. Are there non-monoaminergic paradoxical sleep-off neurons in the brainstem? *Sleep Res. Online*, **2**: 57-63, 1999.

Sakai K., Crochet S., Onoe H. Pontine structures and mechanisms involved in the generation of paradoxical (REM) sleep. *Arch. Ital. Biol.*, **139**: 93-107, 2001.

Sakai K. and Crochet S. A neural mechanism of sleep and wakefulness. *Sleep Biol. Rhyth.*, **1**: 29-42, 2003.

Sakai K. Electrophysiological studies of serotonergic neurons and sleep. In: Monti J.M., Pandi-Perumal S.R., Jacobs B.L., Nutt D. (Eds.) *Serotonin and Sleep: Molecular, Functional and Clinical Aspects*, Basel, Switzerland, Birkhauser Verlag: 205-236, 2008.

Sastre J., Sakai K., Jouvet M. Persistence du sommeil paradoxal chez le chat après destruction de l'aire gigantocellulaire du tegmentum pontique par l'acide kaïnique. *C.R. Acad. Sci. (Paris)*, **289**: 959-964, 1979.

Sastre J.P. and Jouvet M. Le comportement onirique du chat. *Physiol. Behav.*, **22**: 979-989, 1979.

Sastre J.P., Sakai K., Jouvet M. Are the gigantocellular tegmental field neurons responsible for paradoxical sleep? *Brain Res.*, **229**: 147-161, 1981.

Stornetta R.L. and Guyenet P.G. Distribution of glutamic acid decarboxylase mRNA-containing neurons in rat medulla projecting to thoracic spinal cord in relation to monoaminergic brainstem neurons. *J. Comp. Neurol.*, **407**: 367-380, 1999.

Takada M., Itoh K., Yasui Y., Mitani A., Nomura S., Mizuno N. Distribution of premotor neurons for the hypoglossal nucleus in the cat. *Neurosci. Lett.*, **52**: 141-146, 1984.

Takahashi K., Lin J.S., Sakai K. Neuronal activity of histaminergic tuberomammillary neurons during wake-sleep states in the mouse. *J. Neurosci.*, **26**: 10292-10298, 2006.

Takahashi K., Kayama Y., Lin J.S., Sakai K. Locus coeruleus neuronal activity during the sleep-waking cycle in mice. *Neuroscience*, **169**: 1115-1126, 2010.

Takakusaki K., Ohta Y., Mori S. Single medullary reticulospinal neurons exert postsynaptic inhibitory effects via inhibitory interneurons upon alpha-motoneurons innervating cat hindlimb muscles. *Exp. Brain Res.*, **201**: 1-13, 1989.

Takakusaki K., Shimoda N., Matsuyama K., Mori S. Discharge properties of medullary reticulospinal neurons during postural changes induced by intrapontine injections of carbachol, atropine and serotonin, and their functional linkages to hindlimb motoneurons in cats. *Exp. Brain Res.*, **99**: 361-374, 1994.

Takakusaki K., Kohyama J., Matsuyama K., Mori S. Medullary reticulospinal tract mediating the generalized motor inhibition in cats: parallel inhibitory mechanisms acting on motoneurons and on interneuronal transmission in reflex pathways. *Neuroscience*, **103**: 511-527, 2001.

Todd A.J. and Sullivan A.C. Light microscopic study of the coexsistence of GABA-like and glycine-like immunoreactivities in the spinal cord of the rat. *J. Comp. Neurol.*, **296**: 496-505, 1990.

Tohyama M., Sakai K., Touret M., Salvert D., Jouvet M. Spinal projections from the lower brainstem in the cat as demonstrated by the horseradish peroxidase technique. I. Origins of the reticulospinal tract and their funicular trajectories. *Brain Res.*, **173**: 383-403, 1979a.

Tohyama M., Sakai K., Salvert D., Touret M., Jouvet M. Spinal projections from the lower brainstem in the cat as demonstrated by the horseradish peroxidase technique. II. Projections from the dorsolateral pontine tegmentum and raphe nuclei. *Brain Res.*, **176**: 233-254, 1979b.

Vanni-Mercier G., Sakai K., Lin J.S., Jouvet M. Mapping of cholinoceptive brainstem structures responsible for the generation of paradoxical sleep in the cat. *Arch. Ital. Biol.*, **127**: 133-164, 1989.

Vanni-Mercier G., Sakai K., Lin J.S., Jouvet M. Carbachol microinjections in the mediodorsal pontine tegmentum are unable to induce paradoxical sleep after caudal pontine and prebulbar transections in the cat. *Neurosci. Lett.*, **130**: 41-45, 1991.

Vetrivelan R., Fuller P., Tong Q., Lu J. Medullay circuitry regulating rapid eye movement sleep and motor atonia. *J. Neurosci.*, **29**: 9361-9369, 2009.

Webster H.H., Friedman L., Jones B.E. Modification of paradoxical sleep following transections of the reticular formation at the pontomedullary junction. *Sleep*, **9**: 1-23, 1986.

Yamamoto K., Mamelak A.N., Quattrochi J.J., Hobson J.A. A cholinoceptive desynchronized sleep induction zone in the anterodorsal pontine tegmentum: locus of the sensitive region. *Neuroscience*, **39**: 279-293, 1990.

Zanchetti A. Brain stem mechanisms of sleep. *Anesthesiology*, **28**: 81-98, 1967.

Zepelin H. and Rechtshaffen A. Mammalian sleep, longevity and energy metabolism. *Brain Behav. Evol.*, **10**: 425-470, 1974.