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EXECUTIVE MECHANISMS OF PARADOXICAL SLEEP

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INTRODUCTION

Although it by no means implies that the lower brain stem has not any important influence on the upper brain stem in terms of sleep and wake regulation and vice versa, it is now well established that the lower brain stem is responsible for the generation of paradoxical sleep (PS) as forebrain structures are responsible for slow wave sleep (SWS) and wakefulness (W) cycle (for reviws, see refs. 12, 13, 19, 23, 32). Indeed, a cyclic alternation of cortical synchronization and desynchronization — or slow wave sleep-like and waking-like states — is observed in the chronically isolated forebrain (chronic cerveau isolé) (2, 7, 31, 36), while a state of PS, characterized by postural atonia, rapid eye movements and cardiovascular changes is found periodically even in pontine cats with a total removal of all brain structures rostral to the pons (11). Recent experimental evidence from anatomical, lesion, stimulation and single unit recording studies has revealed that major tonic and phasic events of PS are generated not by a single, but by several distinct brain stem neuronal groups (9, 23, 24, 35). The main purpose of the present study is to demonstrate that not only the tonic and phasic phenomena or subsystems of PS, but also the generation of PS per se is mediated not by a single but by several highly localized and specific brain stem neuronal populations that we call «PS-on» cells which are characterized by tonic discharge selective to PS and involved in the cholinergic, cholinoceptive and monoaminoceptive mechanisms.

NEURONS RESPONSIBLE FOR THE GENERATION OF PS: PS-ON CELLS.

Our recent single unit recording studies in freely moving cats disclosed the existence of highly localized and state-specific neuron groups showing tonic activation selective to PS called «PS-on» cells (see refs. 23, 23, 26 for reviews). The PS-on cells consist of several different populations of neurons in terms of discharge properties. Although the PS-on cells, in a strict sense of the word, are those showing a tonic and selective discharge just prior to and throughout the periods of PS like a unit illustrated in Fig. 1B, the majority of PS-on cells also exhibited a low frequency, but still significant tonic discharge both during light and deep

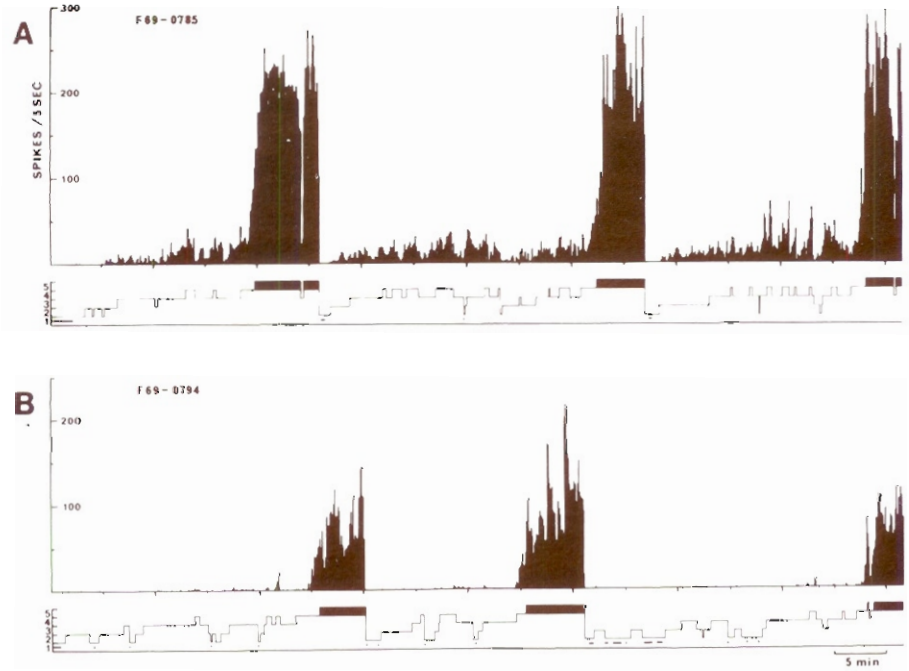

Fig. 1. — *Single unit discharge activity of two medullary PS-on cells recorded in the nucleus reticularis magnocellularis (Mc) over multiple sleep-waking cycles.*

Each vertical line represents the number of spikes in 5 sec. Different levels of vigilance are indicated at the bottom: 1) waking (W) (bars indicate active waking movements); 2) light slow wave sleep (SWS); 3) deep SWS; 4) SWS with PGO waves; and 5) paradoxical sleep (PS).

slow wave sleep, and exhibited a tremendous increase in firing rates during the periods of PS (Fig. 1A). At the present time, I also call «PS-on» cells a subset of neurons showing a significant, but only short lasting phasis discharge during active waking, otherwise presenting exactly the same characteristics as the specific types of PS-on cells, i.e., 1) a total suppression of discharge during quiet waking; 2) a significant increase in discharge rates priori to the onset of PS; 3) a sustained tonic discharge throughout the PS episode; and 4) a complete cessation of discharge during transition from PS to SWS or from PS to W. These PS-on cells, therefore, satisfy three basic criteria necessary for being PS-generator neurons responsible for the initiation and maintenance of this state of sleep: 1) the tonic PS-latency criterion; 2) the tonicity criterion; and 3) the selectivity criterion.

The PS-on cells were recorded so far in the mediodorsal pontine tegmentum, on the one hand, and in the ventromedial and lateral reticular formation of the medulla, on the other (24, 26). The localizations of "specific" and "non-specific" types of pontine and medullary PS-on cells are illustrated in Fig. 2 and Fig. 3B, respectively.

At the level of the pons, the majority of PS-on cells are located in the rostral

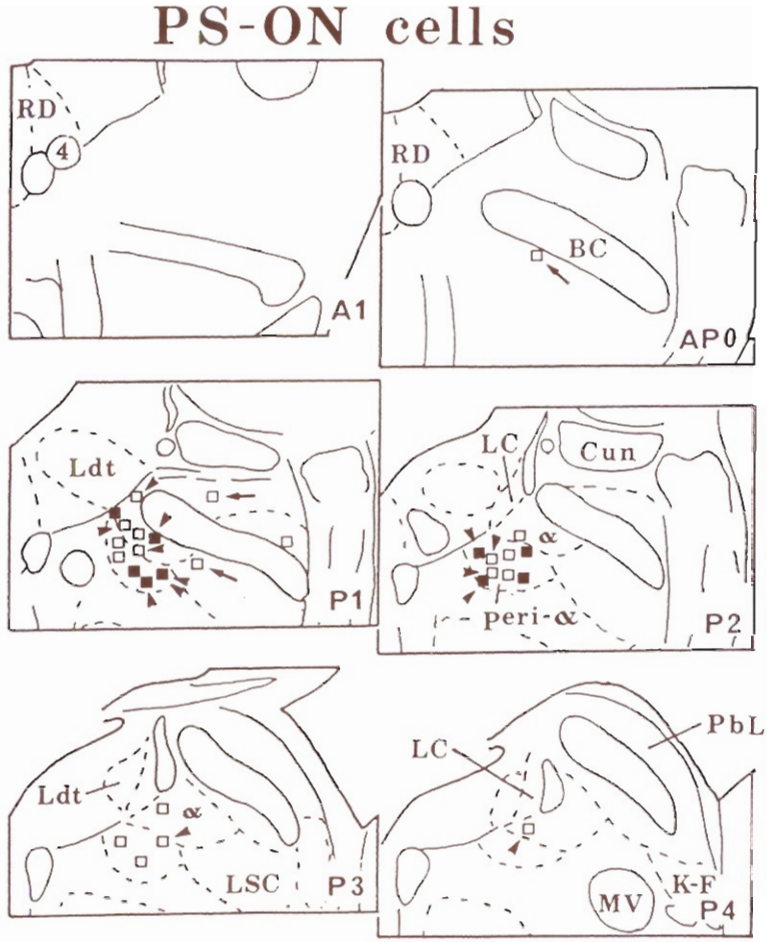

Fig. 2. — *Topographic localizations of pontine PS-on cells.*

Closed and open squares indicate "specific" and "non-specific" types of PS-on cells, respectively. Cuneiforms indicate neurons antidromically activated by the stimulation of the n. reticularis magnocellularis (Mc) of the medulla, while arrows indicate those identified by the antidromic stimulation of the intralaminar thalamic nuclei.

Abbreviations: 4, trochlear nucleus; α, nucleus locus coeruleus α; BC, brachium conjunctivum; cun, cuneiform nucleus; K-F, Kölliker-Fuse nucleus; LC, nucleus locus coeruleus; Ldt, nucleus laterodorsalis tegmenti; LSC, nucleus locus subcoeruleus; MV, motor trigeminal nucleus; peri-α, peri-locus coeruleus α; PbL, nucleus parabrachialis lateralis; RD, nucleus raphe dorsalis.

part of the nucleus locus coeruleus (LC) alpha (LCα) and adjacent peri-LCα. At the level of the medulla, PS-on cells are localized either in the ventromedial medulla corresponding to the nucleus reticularis magnocellularis (Mc) and adjacent raphe magnus (RM) or in the lateral medullary reticular formation corresponding to the nucleus reticularis parvocellularis (Pc) and adjacent nucleus paragigantocellularis lateralis (PGCL). The mean and median firing rates of these pontine and

medullary PS-on cells are shown in Fig. 4. It should be emphasized that medullary PS-on cells discharge two to three times higher than pontine PS-on cells during the periods of PS.

Using the antidromic invasion technique, we have found that the majority of pontine PS-on cells gives rise to direct descending projections to the Mc and that only the minority of the PS-on cells projects directly to the thalamic (e.g. the intralaminar thalamic nuclei) and hypothalamic (e.g. the preoptic area and the ventrolateral posterior hypothalamus) structures (cf. Fig. 2). The mean conduction velocity of the descending pontine PS-on cells was 5.5. ± 2.0 m/s (mean and SD; n = 12), while that of the ascending pontine PS-on cells was 1.6 ± 0.4 m/s

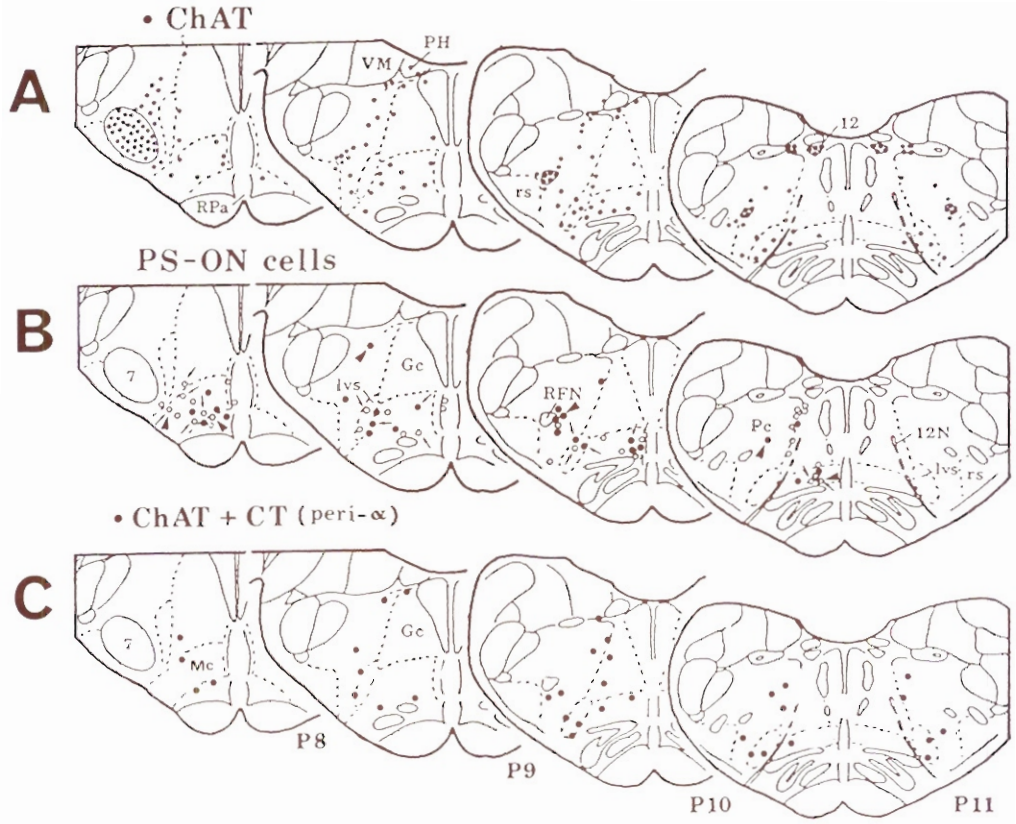

Fig. 3. — *Topographic localization of choline acetyltransferase (ChAT)-immunoreactive neurons (A), medullary PS-on cells (B) and medullary cholinergic neurons projecting directly to the peri-LC α as determined by ChAT immunohistochemistry combined with cholera toxin (CT) retrograde tracer technique (C).*

Closed and open circles in B indicate specific and non-specific types of PS-on cells, respectively. Arrows represent their descending projections to the spinal cord, while cuneiforms represent their ascending projections to the peri-LCα as identified by the antidromic invasion technique.

Abbreviations: 7, facial nucleus; 12, 12N, hypoglossal nucleus and nerve, respectively; Gc, nucleus reticularis gigantocellularis; lvs, direct lateral vestibulospinal tract; Mc, nucleus reticularis magnocellularis; Pc, nucleus reticularis parvocellularis; PH, nucleus praepositus hypoglossi; RFN, retrofacial nucleus; RPa, nucleus raphe pallidus; rs, rubrospinal tract; VM, medial vestibular nucleus.

(n = 3). As far as axonal trajectories of the medullary PS-on cells are concerned, we have found that about 40% of Mc PS-on cells give rise to direct descending projections to the spinal cord via the ventrolateral reticulospinal tract with the mean conduction velocity of 11.1 ± 5.9 m/s (n = 14). Five of 38 Mc PS-on cells had ascending projections to the peri-LCα or to the intralaminar thalamic nuclei (cf. Fig. 3B). Their mean conduction velocity was 2.5 ± 1.4 m/s. None of Pc PS-on cells projected directly to the spinal cord, but 3 of 18 Pc PS-on cells have been identified to project directly to the peri-LCα with the mean conduction velocity of 1.9 ± 1.2 m/s (Fig. 5). It should be also noted that two of Mc PS-on cells and two of Pc PS-on cells responded antidromically to the stimulation of the motor trigeminal nucleus (cf. ref. 26).

On the whole, our findings revealed the "heterogeneity" of the ponto-medullary PS-on populations and the slow conduction velocity charactreristic of axons originating in the ascending ponto-medullary PS-on cells. Finally, it should be pointed out that the distribution of ponto-medullary PS-on cells are in parallel with that of choline acetyltransferase (ChAT)-immunoreactive neurons (10, 24, 27, 37; cf. Fig. 3), suggesting the cholinergic properties of the PS-on cells (see Discussion).

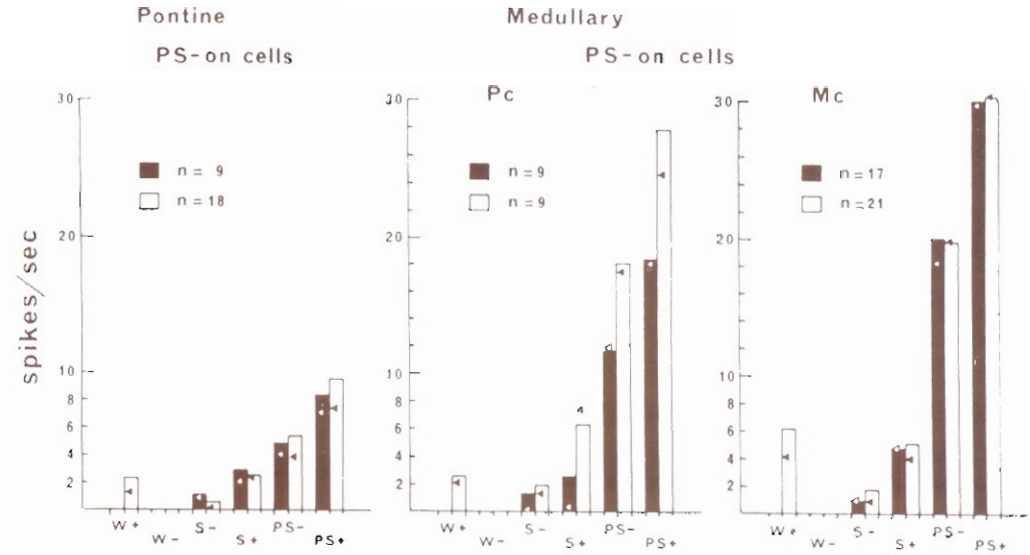

Fig. 4. — *Pooled mean (columns) and median (arrows) discharge rates of pontine and medullary PS-on cells.*

W+, maximum discharges in waking (W) periods with movements; W—, quiet W; S—, slow wave sleep (SWS) with high voltage slow neocortical EEG; S+, SWS with PGO waves; PS—, paradoxical sleep (PS) with poor PGO waves and rapid eye movements; PS+, PS with PGO waves and rapid eye movement bursts. Note the difference in discharge properties between pontine and medullary PS-on cells.

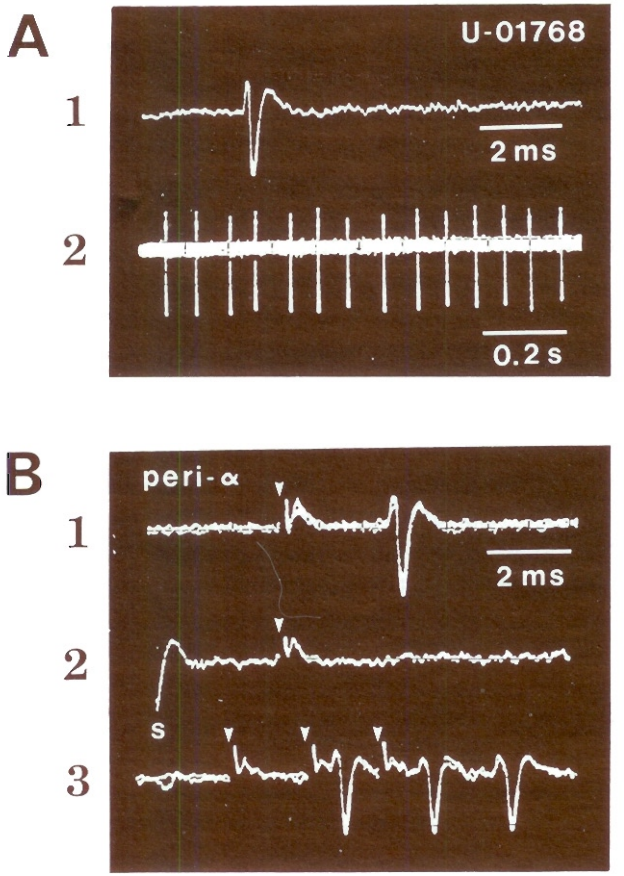

Fig. 5. — *Example of a PS-on cell recorded in the nucleus reticularis parvocellularis (Pc).*

A: this neuron was invaded antidromically by the stimuli applied to the peri-LCα. B: fixed latency (1), collison with spontaneous spikes (2) and ability to follow high frequency stimulation (3).

Carbachol microinjection experiments.

It has been reported repeatedly that microinjections of cholinergic agents such as carbachol into the dorsal pontine tegmentum lead to the induction of PS (1, 5, 18, 28, 30). However, the critical or best sites for cholinergic elicitation of PS in the dorsal pontine tegmentum has not yet been fully determined. In order to elucidate the critical sites for cholinergic induction of PS, we have conducted a series of carbachol microinjection experiments in freely moving, thirteen adult cats. A relatively small dose and volume (0.4 μg in 0.2 μl saline) of carbachol was locally applied to more than 120 sites in the lower brain stem structures.

We found that the mediodorsal pontine tegmentum is the only brain stem site from which PS is induced pharmacologically with short latencies less than five minutes. As shown in Fig. 6, the injection sites corresponded either to the peri-LCα

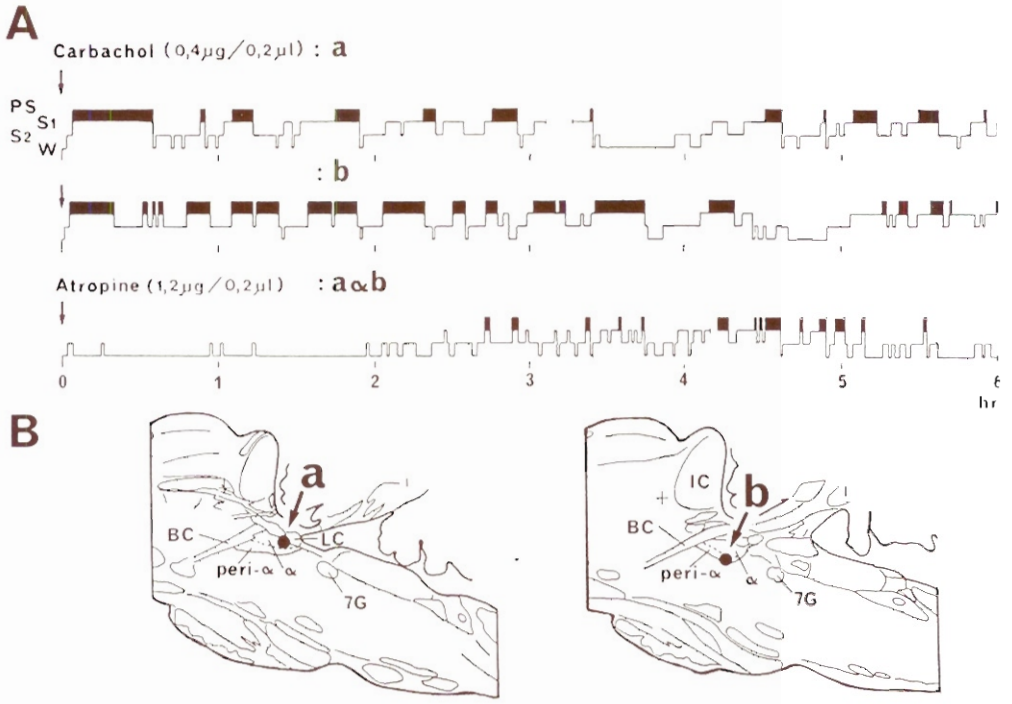

Fig. 6. — *Effects of microinjections of carbachol into the mediodorsal pontine tegmentum on the induction of paradoxical sleep (PS).*

Unilateral microinjections of carbachol (0.4 µg/0.2 µl) into the nucleus locus coeruleus alpha (LCα) or into the peri-LCα (B) induced PS with short latencies, while bilateral injections atropine sulfate into the same structures suppressed the occurrence of PS (A).

Abbreviations: 7G, genu of the facial nerve; BC, brachium conjunctivum; IC, inferior colliculus; LC, nucleus locus coeruleus.

or to the LCα. In these cases, carbachol microinjections led to the virtually immediate appearance of ponto-geniculo-occipital (PGO) waves, postural atonia, neocortical EEG desynchronization, hippocampal theta waves with high voltage and high frequency and rapid eye movements, and subsequently, the appearance of a state of PS which is indistinguishable, behaviorally and polygraphically, from normally occurring PS (cf. Fig. 8).

No remarkable effects on PS generation were obtained after carbachol microinjections into the LC proper or into the gigantocellular tegmental field (FTG). On the other hand, injections of carbachol into the medullary reticular formation or pontine reticular formation just rostral or ventral to the mediodorsal pontine tegmentum usually resulted in the increase in waking and the decrease in PS (Vanni-Mercier and Sakai, unpublished data).

The present findings are therefore in favor of the hypothesis that PS is generated by highly localized populations of neurons and suggest that pontine PS-on cells might represent a "cholinoceptive" PS-generator neuronal population.

Effects of carbachol microinjections on PS-on and PS-off cells.

In addition to cholinergic neurons, the LCα and peri-LCα contain noradrenergic and, to a lesser extent, serotonergic neurons (10, 24, 38). Accumulating evidence indicates that these monoaminergic neurons cease firing selectively during the periods of PS: these neurons are called "PS-off" cells (4, 9, 17, 21, 23). In the mediodorsal pontine tegmentum, therefore, PS-on and PS-off cells are intermingled such that cholinergic and monoaminergic neurons are intermingled in this region of the tegmentum (23, 24).

It has been reported previously that iontophoretic applications of muscarinic agonists excite noradrenergic LC neurons of anesthetized rats (6). In addition, according to the 'reciprocal interaction hypothesis' postulated by Hobson and McCarley (8, 9), putatively mono-aminergic 'REM-off' cells are inhibitory to putatively cholinergic 'REM-on' cells, but REM-on cells are excitatory to REM-off cells. The cessation of discharge of REM-off cells disinhibits REM-on cells, thereby triggering the REM sleep episode. In this model, monoaminergic REM-off cells play the role of trigger or prime mover for the production and maintenance of PS. In other words, arrest of firing by monoaminergic neurons is critical for initiating the periods of PS.

In contrast to the reciprocal interaction model proposed by Hobson and McCarley, I have postulated previously the existence of a mutual inhibitory interaction between PS-on and PS-off cells, since there is an exactly inverse relationship in unitary activity between these two groups of neurons throughout the sleep-waking cycle (23, 24). According to the mutual inhibitory interaction hypothesis, putatively monoaminergic PS-off cells are inhibitory to putatively cholinergic/cholinoceptive PS-on cells, but PS-on cells are also inhibitory to PS-off cells. PS can occur, therefore, either by direct excitation of PS-on cells or by direct inhibition of PS-off cells. Although our findings on the induction of PS by carbachol microinjections into the mediodorsal pontine tegmentum seem to support our hypothesis that PS can be generated by direct excitation of cholinoceptive PS-on cells, several basic questions arise: 1) Do local injections of carbachol really excite cholinoceptive PS-on cells?; 2) Do carbachol microinjections lead to the excitation of PS-off cells? and 3) If it is the case, do the PS-off cells remain active during the pharmacologically induced PS-like state?

In order to answer these questions, the effects of unilateral microinjections of carbachol were examined on fourteen PS-off cells and three PS-on cells recorded in the mediodorsal pontine tegmentum. For this series of experiments, single units were recorded through chronically implanted Formvar insulated stainless steel wires with diameter of 25 or 32 μm. These microelectrodes were assembled into bundles of six wires. A microdrive consists of two inner cannula (24 gauge, separated 2 mm) which could be lowered through two outer guide cannula (21 gauge). In addition, a stainless steel guide cannula (23 gauge) was incorporated into the microdrive assembly between the two microelectrode bundles and a solution of 0.2 μg of carbachol or muscarinic agonist bethanechol was applied locally through

the guide cannula by means of 1.0 µl Hamilton seringe (7001 N). The location of cholinomimetics microinjection loci and single unit recording sites were represented in Fig. 7.

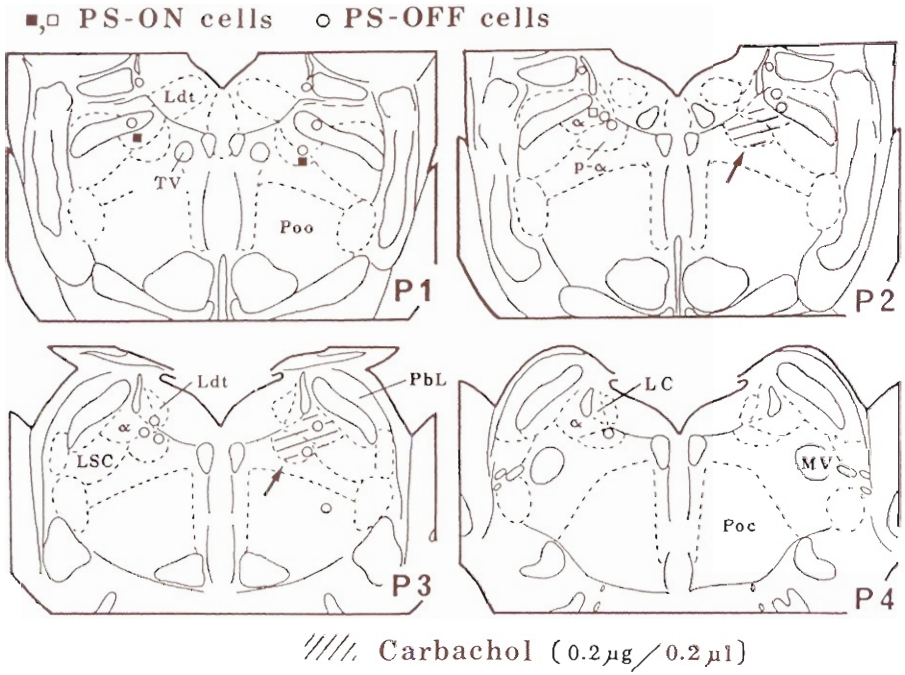

Fig. 7. — *Anatomical localization of carbachol microinjection sites (shaded area) and PS-on (closed and open squares) and PS-off (circles) cells.*

Closed and open squares indicate specific and non-specific types of PS-on cells, respectively.
Abbreviations: Poo, Poc; nuclei reticularis pontis oralis and caudalis, respectively; TV, ventral nucleus of Gudden.

Of fourteen PS-off cells, ten cells were identified as noradrenergic according to their long latency antidromic responses to the dorsal ascending noradrenaline bundle (conduction velocities of 0.5-2.5 m/s) (23); 2) inhibition by intramuscular injections of α_2-autoreceptor agonist clonidine (10-25 µg/kg) (21, 22); and/or 3) absence of responses to systemic administration of serotonin autoreceptor agonist 5-methoxy-N, N-dimethyltryptamine (50-100 µg/kg, i.m.) (33).

Without exception, microinjections of carbachol or bethanechol into the peri-LCα or LCα resulted in the induction of PS and induced the excitation of PS-on cells and the inhibition of PS-off cells within the first few minutes. A typical example of the effects of carbachol microinjections on simultaneously recorded PS-on and PS-off cells is shown in Fig. 8. As shown in this figure, there was a mirror image or a completely inverse relationship in terms of cellular discharge between the PS-on and PS-off cells prior to and during PS-like state induced

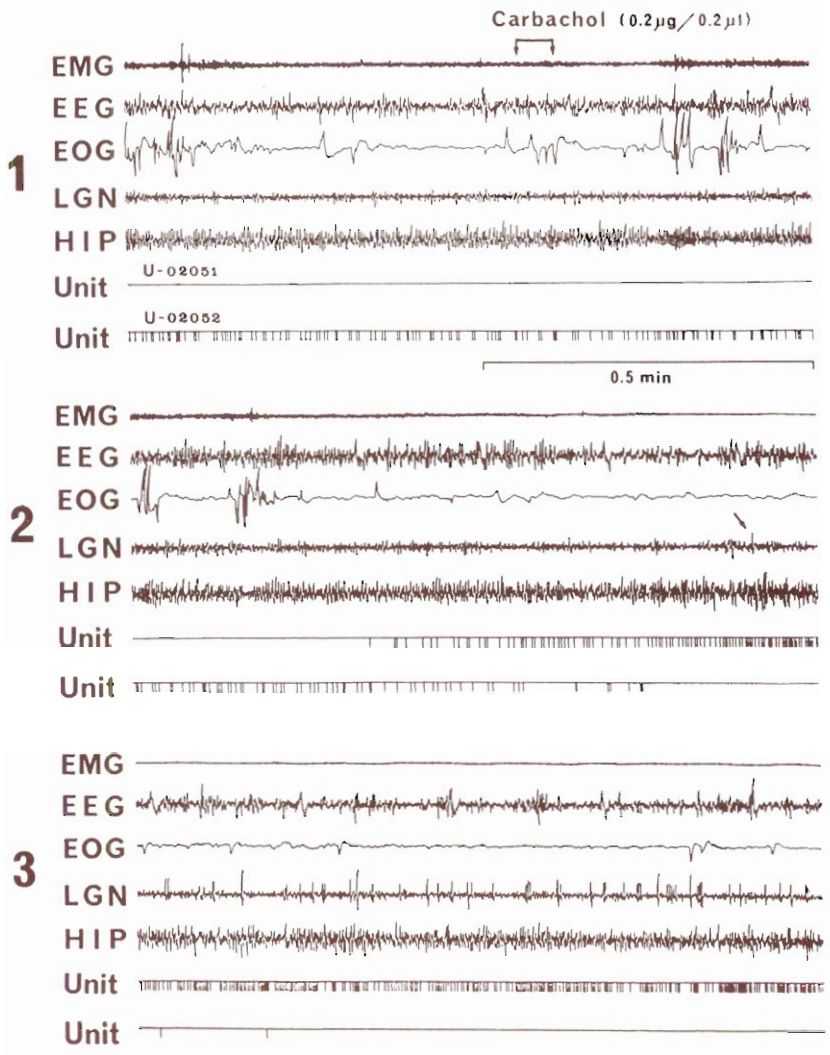

Fig. 8. — *Effects of carbachol microinjections on simultaneously recorded pontine PS-on and PS-off cells.*

Carbachol (0.2 μg/0.2 μl) was locally applied to the peri-LCα, and a PS-on cell and a PS-off cell were recorded in the ipsilateral peri-LCα and in the contralateral LCα, respectively. Note that carbachol microinjection led to the excitation of the PS-on cell and the inhibition of the PS-off cell within the first minute. An arrow indicates the appearance of the first PGO wave.

by carbachol microinjection. Just before the appearance of the first PGO wave, the PS-off cell completely stopped firing, and at the same time, the PS-on cell increased its discharge frequency and maintained its tonic discharge pattern throughout the PS-like state and subsequent PS (Fig. 9).

As illustrated in Fig. 10, locally applied cholinomimetics such as carbachol and bethanechol led to the cessation of firing of PS-off cells located either ipsilaterally

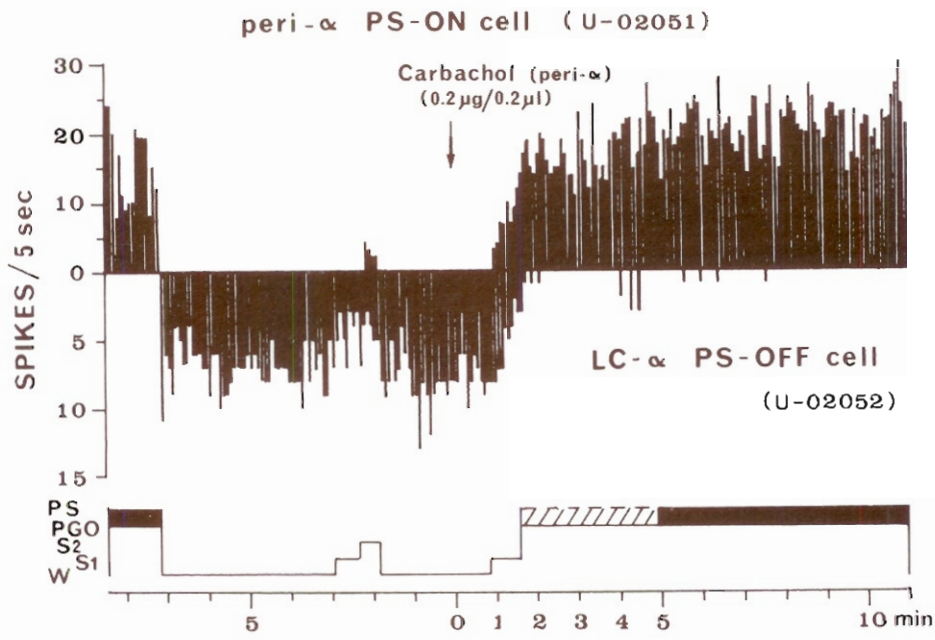

Fig. 9. — *Single unit discharge rates of a PS-on and PS-off cells before and after carbachol microinjection into the peri-LCα.*

Same neurons as in Fig. 8. Note that their changes in unitary activity are inversely correlated before and after microinjection of carbachol. A PS-like state during transition from waking to PS is illustrated by oblique lines on hypnogram.

or contralaterally to the injection sites. The complete arrest of cellular discharge was strictly correlated to the appearance of PGO waves: virtually all PS-off cells stopped firing about 20 sec before the occurrence of the first PGO wave. No significant difference in the time course of the arrest of the cellular discharge was found between the PS-off cells ipsi- and contralateral to the injection sites. It should be also noted that the effects were readily reversed by systemic administration of atropine sulfate (0.25-0.5 mg/kg, i.m.).

The present observations thus point out that locally applied cholinomimetics exert an excitatory influence on PS-on cells, and exert a consistent and powerful 'inhibitory' influence upon noradrenergic dorsal tegmental PS-off cells.

Although no dissociation occurred between the sustained tonic discharge of PS-on cells and the state of PS, the cessation of discarghe of PS-off cells was not strictly related to PS such that, shortly after carbachol injections, the great majority of PS-off cells remained virtually silent even in quiet and attentive wakefulness elicited by external stimuli, even though the units were able to respond phasically to sudden auditory stimuli and to respond safely to high frequency antidromic stimulation (Fig. 11). In such a case, therefore, the mirror image indicative of a mutual inhibitory interaction between PS-on and PS-off cells was no more present.

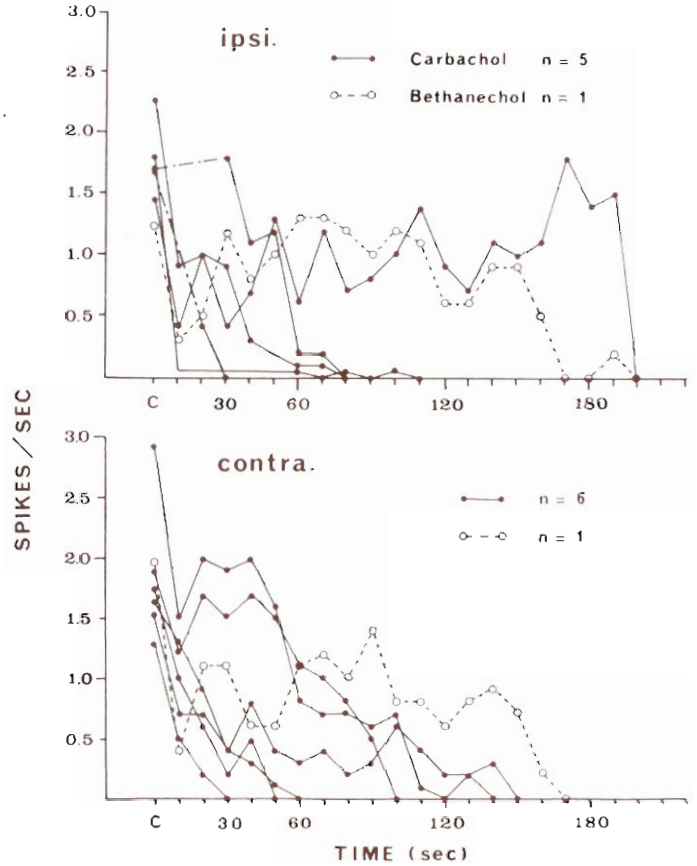

Fig. 10. — *Effects of carbachol or bethanechol microinjections on pontine PS-off cells recorded ipsilaterally or contralaterally to the injection sites.*

The cholinomimetics were applied locally either to the peri-LCα or to the LCα (cf. Fig. 7). Note that all the PS-off cells completely stopped firing within the first few minutes prior to the occurrence of PGO waves and subsequent PS.

The present data suggest that: 1) the activation of PS-on cells is more critical in the PS generation than the cessation of PS-off cells; and 2) in addition to possible inhibitory influences exerted by PS-on cells, there might be some other inhibitory or disfacilitatory mechanisms leading to the suppression of discharge of PS-off cells.

In summary, the present study support the hypothesis that the state of PS can be elicited by a direct excitation of highly localized "cholinoceptive" PS-on cells located in the mediodorsal pontine tegmentum.

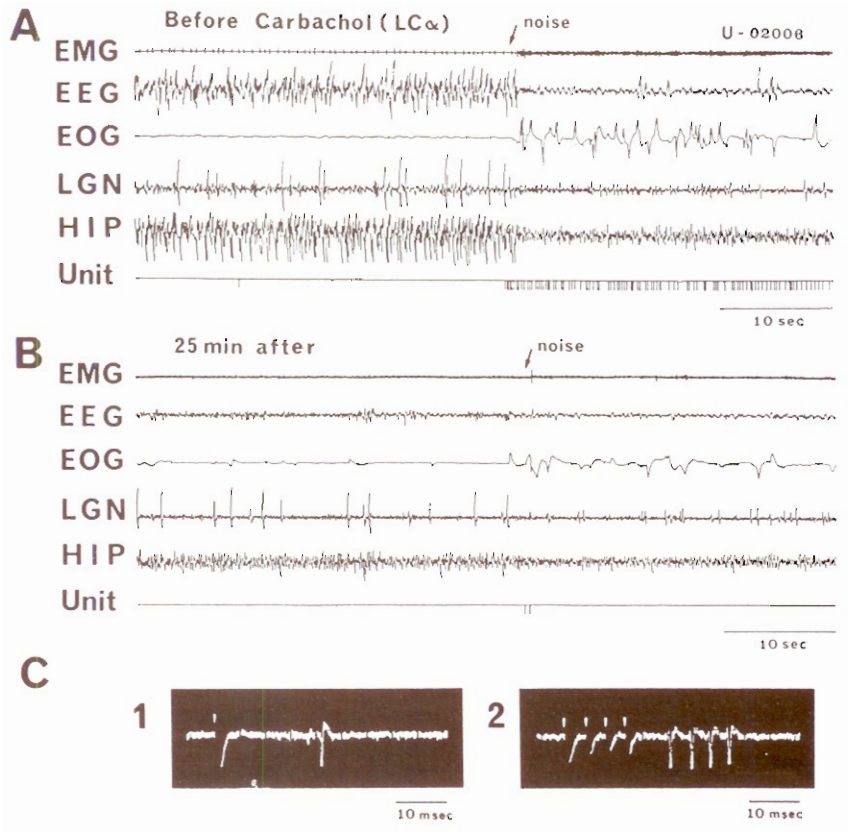

Fig. 11. — *Effects of carbachol microinjection on a pontine PS-off cell.*

After carbachol microinjection into the LCα, the PS-off cell remained silent even in waking states elicited by external stimuli. The unit, however, responded phasically to the sudden auditory stimuli (B) and responded safely to both the low and high frequency antidromic stimulation with the same stimulus intensities as control (C). Note that this unit was invaded antidromically by the stimuli applied to the dorsal ascending noradrenaline bundle as well as to the preoptic area (C).

CHOLINERGIC AFFERENTS TO THE MEDIODORSAL PONTINE TEGMENTUM.

A basic question arises concerning the cells of origin of cholinergic afferents to the cholinoceptive pontine PS-on cells. The activity of the cholinoceptive neurons should be also tonic and selective to PS. Where are these "cholinergic" PS-on cells? In order to answer the question, we have used a retrograde tracer technique with unconjugated cholera toxin B subunit (CT), that was revealed by a modified ABC method with DAB-nickel as a chromogen, in conjunction with choline acetyltransferase (ChAT) immunohistochemistry.

The CT (0.1-0.2 µl of a 1% solution) was injected into the peri-LCα and adjacent LCα where the majority of pontine PS-on cells are recorded. At the level of the pons, CT-immunoreactive and ChAT-immunoreactive double-labelled cells were

found in the dorsal pontine tegmentum such as the LCα, the peri-LCα and the peribrachial nuclei ipsi- and contralateral to the injection sites (Fig. 12). At the level of the medulla, we found that cholinergic neurons located in the Mc and Pc also project directly to the peri-LCα and adjacent LCα. In this case, the distribution of the double-labelled cells was in parallel with that of medullary PS-on cells (Fig. 3). These anatomical findings together with our antidromic invasion data showing direct projections of some Pc and Mc PS-on cells to the peri-LCα (Fig. 5), strongly suggest the existence of cholinergic PS-on cells at least in the Mc and Pc of the medulla oblongata.

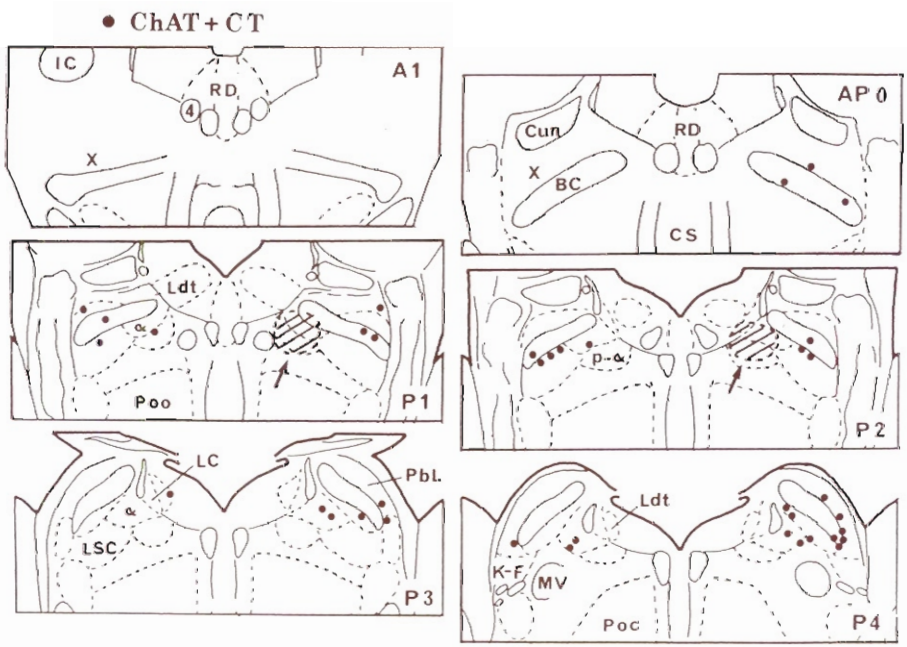

Fig. 12. — *Anatomical localization of a retrograde tracer cholera toxin (CT) injection site (shaded area) and CT-labelled and choline acetyltransferase (ChAT)-immunoreactive double-labelled neurons (closed circles) in the pontine tegmentum.*

For other abbreviations, see Fig. 2.

DISCUSSION

The present study gives evidence supporting the hypotheses that PS is generated not by a single, but by several highly localized and specialized neuronal populations that we call PS-on cells. In fact, the PS-on cells discharge tonically and selectively just prior to and throughout the periods of PS. The PS-on cells, therefore, satisfy the selectivity, tonicity and tonic PS-latency criteria necessary for being PS-generator

neurons that are responsible for the initiation and maintenance of the states of PS. The PS-on cells are found, not in a single, but in several highly localized lower brain stem structures containing ChAT-immunoreactive neurons, i.e., the nuclei LCα and peri-LCα of the mediodorsal pontine tegmentum and the nuclei reticularis magnocellularis (Mc) and parvocellularis (Pc) of the medulla. As the PS-on cells are found exclusively in these brain stem structures containing cholinergic neurons, we have suggested previously the cholinergic nature of the PS-on cells (23, 24).

Apart from motor cranial nuclei and parasympathetic preganglionic nuclei neurons, central cholinergic neurons are by no means homogeneous. At the present time, the cholinergic properties of the septal and basal forebrain neurons projecting respectively to the hippocampus and to the cerebral cortex have been demonstrated together with their role in the generation of hippocampal theta waves and neocortical EEG desynchronization, respectively (3,15). In addition, we have recently demonstrated that PGO waves are generated by ponto-mesencephalic cholinergic neurons (24, 25) and further suggested that some cholinergic tegmental neurons play a role in the processes of neocortical desynchronization (23). It raises a question of whether or not PS-on cells are also cholinergic. In the present study, we have demonstrated the heterogeneity of PS-on cells in terms of unit discharge properties, axonal trajectories and conduction velocities: a) medullary PS-on cells discharge two to three times more frequently than pontine PS-on cells; b) the majority of pontine PS-on cells give rise to descending projections to the Mc. Similarly, the majority of Mc PS-on cells have such a descending projection. The possible role of these descending PS-on cells in the generation of postural atonia during PS has been reported previously (20, 26); and c) The mean conduction velocities of descending PS-on cells are significantly faster than those of ascending ones (5.5-11.1 m/s vs 1.6-2.5 m/s). It appears, therefore, that brain stem PS-on cells consist of several distinct populations of neurons.

Using cholera toxin (CT) retrograde tracer technique in conjunction with ChAT immunohistochemistry, we have recently revealed the "non-cholinergic" properties of descending mediodorsal pontine tegmental and medullary Mc neurons (16). The findings lead us to conclude that the descending PS-on cells, that might be responsible for the occurrence of postural atonia (26), are not cholinergic/cholinoceptive. Relatively fast conduction velocities of the descending PS-on cells are also in line with this conclusion, since it has been reported previously that putatively cholinergic septo-hippocampal and basalo-cortical neurons have slow conduction velocities of 2-3 m/s (3,15) and that putatively cholinergic PGO-on cells possess similar slow conduction characteristic (25). On the other hand, the slow conduction velocity characteristic of ascending PS-on cells seem to support the hypothesis bearing on the cholinergic nature of these PS-on cells. In the present study, we have shown that carbachol microinjections into the pontine PS-on neuronal groups lead to the excitation of PS-on cells and to the induction of PS, suggesting the "cholinoceptive" characteristic of pontine PS-on cells and the existence of "cholinergic" afferents, that should be tonic and selective to PS, to the pontine PS-on

cells. In this context, our experimental evidence from anatomical and antidromic invasion studies strongly suggests that some Mc and Pc PS-on cells projecting directly to the peri-LCα and adjacent LCα might be cholinergic in nature. The slow conduction velocity characteristic of the ascending medullary PS-on cells also corroborates this hypothesis (Fig. 5).

We have reported previously the existence of a mirror image or an exactly inverse relationship in terms of cellular activity between the PS-on and PS-off cells throughout the sleep-waking cycle and suggested the presence of a mutual inhibitory interaction between these two neuronal populations (23, 24). According to my reciprocal interaction model, the cessation of firing of PS-off cells excites PS-on cells by disinhibition, while the excitation of PS-on cells inhibits PS-off cells. Accordingly, the state of PS can occur either by direct excitation of PS-on cells or by inhibition of PS-off cells. In this sence, among others, my mutual inhibitory interaction hypothesis is different from the reciprocal interaction model proposed by Hobson and McCarley (8, 9), in which REM-off cells are inhibitory to REM-on cells, but REM-on cells are 'excitatory' to REM-off cells. According to their model, therefore, the inactiviation of activity of putatively monoaminergic REM-off cells play a critical role in the production and maintenance of REM sleep.

In the present study, carbachol or bethanechol microinjections into the LCα and adjacent peri-LCα, where cholinergic and noradrenergic neurons are intermingled (10, 24), led to the simultaneous excitation of all PS-on cells and inhibition of all noradrenergic PS-off cells tested. Our findings are, therefore, in accord with the mutual inhibitory interaction hypothesis. Interpretatioin of these microinjection experiments is, however, complicated by the previous experimental evidence showing the excitation of noradrenergic LC neurons by iontophoretically applied cholinergic agonists in the anesthetized rats (6). In our experiments, the time course of inactivation was similar within the PS-off cell groups recorded ipsilaterally and contralaterally to the injection sites. In addition, microinjections of carbachol into the LC proper had no effects on the elicitation of PS, the induction of major tonic and phasic events of PS and the inactivation of PS-off cells. It thus appears that the arrest of discharge of PS-off cells induced by microinjections of cholinomimetics is not due to the direct action of muscarinic agonists on noradrenergic neurons, but mediated by some synaptically connected neurons. It is possible that "cholinoceptive", but not "cholinergic" pontine PS-on cells participate in this inactivation process. However, experimental evidence such as the long-lasting cessation of discharge of PS-off cells observed outside the cholinergically induced PS episode strongly suggests that it might not be only the case. In our experiments, the PS-off cells responded safely to high frequency antidromic stimulation with the same stimulus intensities before and after carbachol microinjections. This finding suggests that disfacilitatory rather than inhibitory mechanisms play an important role in the processes underlying the inactivation of PS-off cells after carbachol and bethanechol microinjections. Changes in autonomic functions such as decrease in blood pressure and sympathetic tone, as well as the occurrence of postural atonia induced by microinjections of cholinergic agonists might play

a key role in the disfacilitation of PS-off cells (14, 29, 34). In future studies, it is hoped to disclose precise synaptic inputs to PS-off cells and to determine their physiological significance. Despite these problems, it appears that, even though acetylcholine exert an excitatory influence on noradrenergic PS-off cells, this excitatory action is overcome by still unknown inhibitory and/or disfacilitatory mechanisms that function when muscarinic agonists were injected into the peri-LCα or LCα.

For a better understanding of cellular mechanisms underlying the triggering and maintenance of PS, we have to determine, in future anatomical and physiological studies, the precise input-output organization of PS-on and PS-off cells and to elucidate cellular mechanisms underlying the activation of PS-on cells and the inactivation of PS-off cells.

CONCLUSIONS

In the present study, we have demonstrated the existence of several distinct populations of neurons showing tonic discharge selective to paradoxical sleep: PS-on cells. These neurons are located either in the mediodorsal pontine tegmentum such as the nuclei locus coeruleus alpha (LCα) and peri-LCα, and in the lateral and ventromedial medullary reticular formation such as the nuclei reticularis parvocellularis (PC) and magnocellularis (Mc), respectively. We have revealed the existence of a mirror image or an exactly inverse relationship in terms of cellular discharge between the PS-on and the PS-off cells that cease firing selectively during the periods of PS, suggesting the existence of a mutual inhibitory interaction between the two distinct neuronal populations.

Carbachol microinjection experiments in freely moving cats pointed out that the peri-LCα and LCα are the critical sites for the induction of PS with short latencies. Evidence from single unit recordings in conjunction with microinjections of cholinomimetics into the mediodorsal pontine tegmentum showed that muscarinic agonists exert an excitatory influence on PS-on cells and exert an inhibitory or disfacilitatory influences on PS-off cells, supporting the mutual inhibitory interaction hypothesis and suggesting the cholinoceptive properties of pontine PS-on cells.

Using a cholera toxin retrograde tracer technique combined with choline acetyltransferase (ChAT) immunohistochemistry, we have found that ChAT-immunoreactive neurons located in the dorsal pontine tegmentum and in the Mc and Pc of the medulla give rise to direct projections to the peri-LCα and adjacent LCα. In addition, using the antidromic invasion technique, we have found that some Pc and Mc PS-on cells project directly to the peri-LCα and that these ascending PS-on cells showed the slow conduction velocity characteristic of putatively cholinergic neurons.

It is concluded that PS is generated not by a single, but by several highly localized and specific neuronal populations called PS-on cells that are involved in the cholinergic, cholinoceptive and monoaminoceptive mechanisms. It is proposed that there is a mutual inhibitory interaction between putatively choliner-

gic/cholinoceptive PS-on cells and putatively monoaminergic PS-off cells, but the tonic excitation of PS-on cells is more critical for the initiation and maintenance of PS.

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