



# The Neurobiology of sleep-wake systems: an overview

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# The Neurobiology of Sleep–Wake Systems: An Overview

Pierre-Hervé Luppi and Patrice Fort

[AU1]

## Abstract

Following studies of patients with postinfluenza encephalitis, the neuropathologist von Economo reported that inflammatory lesions of the preoptic area (POA) were often associated with insomnia and therefore proposed that the POA was critical for the production of normal sleep [1]. Then, Ranson in monkeys, Nauta in rats, and McGinty in cats showed that POA lesions induce a profound and persistent insomnia [2–4]. It was later shown in cats that POA electrical stimulation induces EEG slow-wave activity and sleep (SWS) [5]. Finally, putative sleep-promoting neurons displaying an elevated discharge rate during SWS compared to waking (W), diffusely distributed within a large region encompassing the horizontal limb of the diagonal bands of Broca and the lateral preoptic area-substantia innominata were recorded in freely moving cats [6]. Altogether, these studies indicate that the POA is a unique brain structure containing neurons that directly promote sleep.

## Keywords

Sleep–wake regulation • Forebrain • Ventrolateral preoptic area • Hypothalamus • Brainstem • Orexin • hypocretin • Monoamines • Acetylcholine • GABA

## Abbreviations

	BF	Basal Forebrain	
	CTb	Cholera toxin b subunit	
	DPGi	Dorsal paragigantocellular reticular nucleus	
	dDpMe	Dorsal deep mesencephalic reticular nucleus	
	DRN	Dorsal raphe nucleus	
	Fos+	Neuron immunoreactive for Fos	
	GAD	Glutamate decarboxylase	
	GiA	Alpha gigantocellular nucleus	
	GiV	Ventral gigantocellular reticular nucleus	
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36	Gly	Glycine
37	Hcrt	Hypocretin
38	His	Histamine
39	LC	Locus coeruleus
40	LDT	Laterodorsal pontine nucleus
41	LHA	Lateral hypothalamic area
42	Mc	Nucleus reticularis
43		magnocellularis
44	MCH	Melanin-concentrating
45		hormone
46	MnPn	Median preoptic nucleus
47	NA	Norepinephrine
48	PeF	Perifornical hypothalamic area
49	peri-LC alpha	Peri-locus coeruleus alpha
50	PH	Posterior hypothalamus
51	PnC	Pontis caudalis nucleus
52	PnO	Pontis oralis nucleus
53	POA	Preoptic area
54	PPT	Pedunculopontine nucleus
55	SCN	Suprachiasmatic nucleus
56	SLD	Sublaterodorsal nucleus
57	TMN	Tuberomamillary nucleus
58	vIPAG	Ventrolateral periaqueductal
59		gray
60	VLPO	Ventrolateral preoptic nucleus
61	ZI	Zona incerta

62 **Neuronal Networks Responsible**  
63 **for Sleep Onset and Maintenance**

64 **The Forebrain Sleep Center**

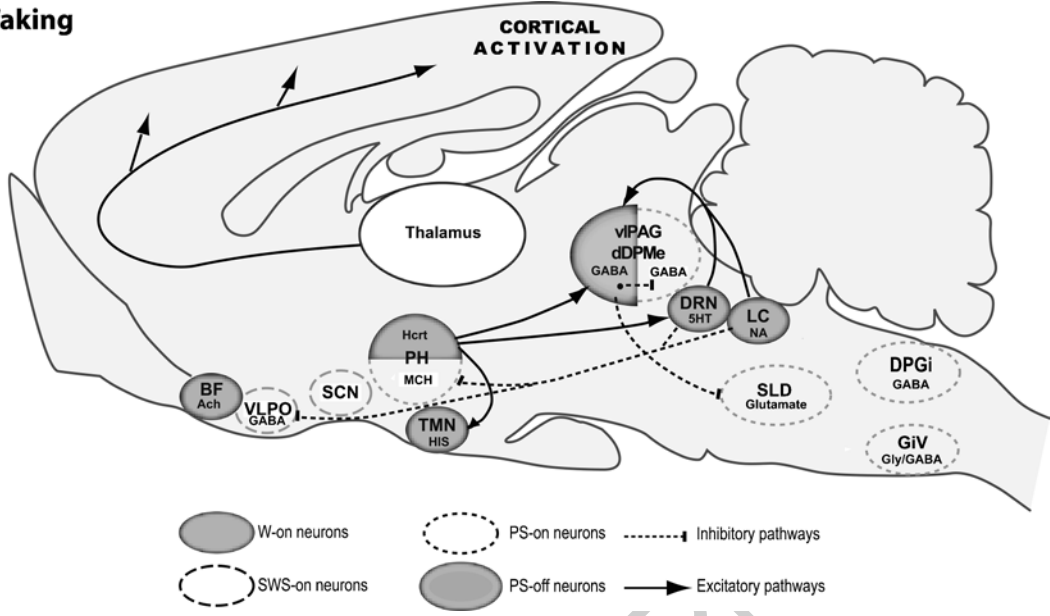
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This simplicity highly contrasts with the com-  
plex network responsible for W, involving redun-  
dant neurotransmitter systems with populations of  
neurons disseminated from the upper brainstem,  
caudal hypothalamus to basal forebrain, such as  
neurons containing acetylcholine, norepinephrine,  
serotonin, histamine, and recently discovered  
hypocretin (Fig. 1). Collectively, through their  
widespread ascending projections to the cortical  
mantle and with their firing activity specific to  
W, these systems form the ascending reticular  
activating system (ARAS) that controls arousal  
with a low-voltage, high-frequency, cortical acti-  
vation [7]. During the transition to sleep, the hyp-  
nogenic center would inhibit the multiple arousal  
systems of the ARAS via a sustained and coordi-  
nated inhibition (Fig. 2). The sleep pressure asso-  
ciated with drowsiness is believed to be regulated  
by the interaction of a homeostatic and a circa-  
dian processes, both able to modulate the hyp-  
nogenic center’s activity to control the timing and  
duration of sleep [8].

Then, researchers identified specific sleep-  
active neurons by using Fos as a marker of neu-  
ronal activation in rats that had slept for a long  
period before sacrifice [9]. These neurons are dis-  
tributed diffusely in the POA but are more densely  
packed in the median preoptic nucleus (MnPn)  
and the ventrolateral preoptic nucleus (VLPO).  
These studies showed that the number of Fos+  
neurons in the VLPO and MnPn positively  
correlated with sleep quantity and sleep consoli-  
dation during the last hour preceding sacrifice.  
Numerous Fos+ neurons were also observed in  
sleep-deprived rats in the MnPn but not in VLPO  
[9, 10]. It appears, therefore, that VLPO neurons  
would be primarily responsible for the induction  
of sleep while MnPn neurons may also have a  
homeostatic role in sleep control. It was later  
demonstrated that VLPO and the suprachiasmatic  
nucleus (SCN) have synchronized activity [11].  
Considering that both areas are interconnected

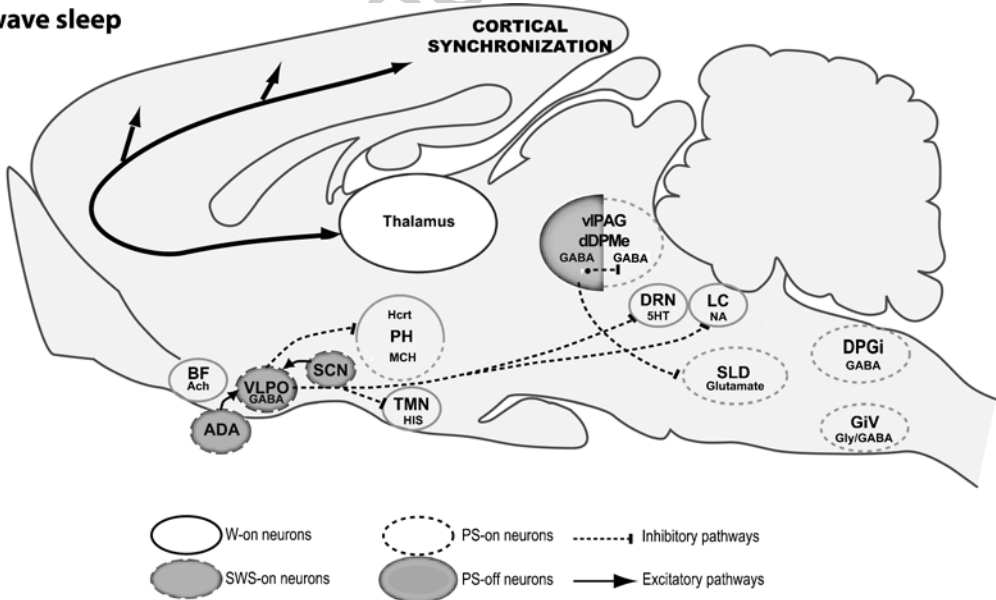
Waking



**Fig. 1** Model of the network responsible for promoting wake. *Shaded nuclei* help promote wake and inhibit SWS and PS. Excitatory pathways are indicated by *solid lines*, and inhibitory pathways by *dashed lines*. Not shown on

this figure are direct projections from monoaminergic and cholinergic nuclei to the cortex that may also contribute to the production of wake

Slow wave sleep



**Fig. 2** Model of the network responsible for slow-wave sleep. During SWS, GABAergic neurons of the VLPO inhibit many wake-promoting regions. The VLPO neurons may be activated by adenosine or other homeostatic

signals and by circadian signals from the SCN. Other GABAergic neurons in the vPAG and dDPM inhibit PS-promoting systems

and receive inputs from the retinal ganglion cells, it is thus possible that circadian- and photic-linked information may be conveyed to modulate VLPO activity [12] (Fig. 2).

Electrophysiology experiments in behaving rats have shown that neurons recorded in the VLPO and MnPn are active during SWS, generally anticipating its onset by several seconds. Further, their firing rate is positively correlated with sleep depth and duration. Some of these neurons are also active during PS with a higher firing frequency than during the preceding SWS [13]. In addition, VLPO and MnPn neurons display a firing pattern reciprocal to the wake-active neurons (see below). Functionally, bilateral neurotoxic destruction of VLPO is followed by a profound and long-lasting insomnia in rats [14]. Retrograde and anterograde tract-tracing studies indicate that VLPO and MnPn neurons are reciprocally connected with wake-active neurons such as those containing histamine in the tuberomammillary nucleus (TMN), hypocretin in the perifornical hypothalamic area (PeF), serotonin in the dorsal raphe nuclei (DRN), noradrenaline in the locus coeruleus (LC), and acetylcholine in the pontine (LDT/PPT) and basal forebrain nuclei. In these wake-promoting areas, extracellular levels of GABA increase during SWS compared to W. It has also been shown that Fos<sup>+</sup> neurons in the VLPO express galanin mRNA and 80% of VLPO neurons projecting to the TMN contain both galanin and GAD, the GABA-synthesizing enzyme. Finally, electrical stimulation of the VLPO area evokes a GABA-mediated inhibition of TMN neurons, suggesting that VLPO and MnPn efferents to the wake-promoting systems are inhibitory [12].

## Neurotransmitters Regulating the Activity of Sleep-Promoting Neurons in the VLPO

Electrophysiological recordings of VLPO neurons in rat brain slices showed that it contains a homogeneous neuronal group with specific intrinsic membrane properties, a clear-cut chemomorphology and an inhibitory response to the major

waking neurotransmitters. Their high proportion matching that of cells active during sleep and their pharmacological profile represent convincing arguments about their status as presumed sleep-promoting (PSP) neurons [15]. It was further shown that PSP neurons are GABAergic and galaninergic in nature, multipolar triangular shaped, and endowed with a potent low threshold calcium potential. These neurons are always inhibited by noradrenaline (NA), via postsynaptic  $\alpha_2$ -adrenoceptors. The wake-promoting drug modafinil was shown to increase the NA-mediated inhibition of VLPO neurons, perhaps by blocking NA reuptake by local noradrenergic terminals [16]. Interestingly, NA-inhibited neurons are also inhibited by acetylcholine, through muscarinic postsynaptic and nicotinic presynaptic actions on noradrenergic terminals. In contrast, histamine and hypocretin did not modulate PSP neurons [15, 17]. Finally, serotonin showed complex effects inducing either excitation (50%, Type 2) or inhibition (50%, Type 1) of the PSP neurons [12, 18].

Among processes that are likely to modulate the activity of PSP neurons, homeostatic mechanisms, involving natural sleep-promoting factors accumulating during waking, had long been thought to play a crucial role in triggering sleep. Among these factors, prostaglandin D<sub>2</sub> and adenosine have been functionally implicated in sleep, although their neuronal targets and mechanisms of action remain largely unknown (see Chap. "Prostaglandin D<sub>2</sub>: An Endogenous Somnogen" by Urade). In this context, we showed that application of an adenosine A<sub>2A</sub> receptor (A<sub>2A</sub>R) agonist evoked direct excitatory effects specifically in Type 2 PSP neurons that also activated by serotonin [12, 18]. Other results also suggested that adenosine may directly activate VLPO neurons at sleep onset via an action on postsynaptic A<sub>2A</sub>R. Indeed, infusion of an A<sub>2A</sub>R agonist in the subarachnoid space rostral to the VLPO increases SWS and induces Fos expression in VLPO neurons [19] (Fig. 2).

In contrast, a number of studies showed that adenosine A<sub>1</sub> receptors (A<sub>1</sub>R) promote sleep through inhibition of the wake-promoting neurons, in particular, cholinergic and hypocretin

neurons [20, 21]. However, data using transgenic mice demonstrated that the lack of  $A_1R$  does not prevent the homeostatic regulation of sleep while the lack of  $A_{2A}R$  does and blocks waking effect of caffeine, suggesting that the activation of  $A_{2A}R$  is crucial in SWS [22–24].

## An Updated Overview of the Neuronal Network Responsible for SWS

A line of evidence indicates that both the VLPO and the MnPn contain neurons responsible for sleep onset and maintenance. These neurons would be inhibited by noradrenergic and cholinergic inputs during waking. The majority of them would start firing at sleep onset (drowsiness) in response to excitatory, homeostatic (adenosine and serotonin), and circadian drives (suprachiasmatic inputs). These activated neurons, through the reciprocal GABAergic inhibition of all wake-promoting systems, would be in a position to suddenly unbalance the “flip-flop” network, as required for switching from W or drowsiness to SWS. Conversely, the slow removal of excitatory influences would result in a progressive firing decrease in VLPO neurons and therefore an activation of wake-promoting systems leading to awakening [18, 25].

## Neuronal Network Responsible for Paradoxical Sleep

### The Pontine Generator of PS and the Cholinergic Hypothesis

In 1959, Jouvet and Michel discovered PS in cats, a sleep phase characterized by a complete disappearance of muscle tone, paradoxically associated with cortical activation and rapid eye movements (REM) [26]. Rapidly, they demonstrated that the brainstem is necessary and sufficient to trigger and maintain PS in cats (pontine cat preparation). By using electrolytic and chemical lesions, it was then found that the dorsal part of the pontis oralis (PnO) and caudalis (PnC) nuclei contain the neurons responsible for PS

onset [27]. Furthermore, large bilateral injections of a cholinergic agonist, carbachol into the PnO and PnC promotes PS in cats [28]. Later, PS induction with the shortest latencies was obtained with carbachol injection restricted to the dorsal area of the PnO and PnC, coined the peri-locus coeruleus alpha (peri-LC alpha). It was then shown by unit recordings in freely moving cats that many peri-LC alpha neurons show a tonic firing selective to PS (called PS-on neurons) [29]. Two types of PS-on neurons were identified. The first ones were inhibited by carbachol, an indication that they might be cholinergic. They were restricted to the rostro-dorsal peri-LC alpha and projected to forebrain including intralaminar thalamic nuclei, posterior hypothalamus, and basal forebrain. The second ones recorded over the whole peri-LC alpha were excited by carbachol and projected caudally to the nucleus reticularis magnocellularis (Mc) within the ventromedial medullary reticular formation [29, 30]. It has been then proposed that (1) the ascending PS-on neurons are cholinergic and are responsible for the cortical activation during PS and (2) the descending PS-on neurons are not cholinergic and generate muscle atonia during PS through excitatory projections to medullary glycinergic pre-motoneurons [12, 31–33].

In contrast to data in cats, carbachol iontophoresis into the rat sublateralodorsal tegmental nucleus (SLD), a very small area of the dorsolateral pontine tegmentum corresponding to the cat’s peri-LC alpha, induces W with increased muscle activity [34]. Other studies using carbachol administration in rats described either a moderate PS enhancement or no effect [35–38]. It was first shown that the number of pedunclopontine (PPT) and laterodorsal (LDT) cholinergic neurons expressing Fos increases in rats during PS recovery following its selective deprivation by the flower-pot technique [39]. However, in our recent study reproducing these experiments (75 h PS deprivation; 3 h sleep recovery), we observed that only occasional cholinergic neurons stained for Fos in the PPT/LDT nuclei [40]. In conclusion, our results in rats are strongly against a role of pontine cholinergic neurons in PS genesis although unit recording combined with



juxtacellular labeling is required to draw a more definitive conclusion.

## SLD Neurons Triggering PS Are Glutamatergic

As described above, SLD neurons activated during PS are not cholinergic. We recently showed that they are not GABAergic, as well. Indeed, the small number of Fos+/GAD+ neurons in the SLD did not increase in rats displaying a PS rebound compared to control or PS-deprived rats [41]. It is more likely that the Fos+ neurons observed in the SLD specifically after PS recovery are glutamatergic since they express the vesicular glutamate transporter, vGlut2 [42]. Further, it has been shown that the SLD sends efferent projections to glycinergic neurons from the ventral and alpha gigantocellular nuclei (GiV and GiA, corresponding to the cat Mc) known to generate atonia during PS by direct projections to cranial and spinal motoneurons in the cat [12, 33]. These glycinergic neurons express Fos during a PS-like state induced by the disinhibition of the SLD with bicuculline or gabazine (GABA-A receptor antagonists) [34]. Further, glutamate release in the GiV and GiA increases specifically during PS and injection of non-NMDA glutamate agonists in the GiV and GiA suppresses muscle tone. In contrast, an increased tonus is induced during PS after the lesion of these medullary areas in cats. Altogether, these results strongly suggest that the SLD neurons triggering PS are glutamatergic [12, 33].

It is likely that the glycinergic neurons of the GiA and GiV are also GABAergic since a large majority of the Fos+ neurons localized in these nuclei after PS recovery express GAD67 mRNA [41].

## SLD Neurons Triggering PS Are Tonically Excited by Glutamate

In cats, the microdialysis administration of kainic acid, a glutamate agonist in the peri-LC alpha induces a PS-like state [43]. We reproduced these experiments in rats and also observed a firing activation of SLD neurons reliably associated with the

induction of a PS-like state. Further, application of kynurenate, a glutamate antagonist, reversed the PS-like state induced by bicuculline [34]. These results suggest that SLD PS-on neurons are under a permanent glutamatergic barrage throughout the sleep-waking cycle, unmasked at the onset of PS by the removal of tonic GABAergic inputs. The best candidate structure for containing the glutamatergic neurons permanently activating SLD is the lateral and ventrolateral periaqueductal gray (vlPAG), containing numerous non-GABAergic, likely glutamatergic, efferent neurons to the SLD [44, 45]. Although Jouvet established that structures sufficient for PS are restricted to the brainstem, numerous non-GABAergic neurons projecting to the SLD located in the primary motor area of the frontal cortex, the bed nucleus of the stria terminalis or the central nucleus of the amygdala could also contribute to the activation of the SLD neurons during PS [45].

## SLD Glutamatergic Neurons Are Inhibited by GABAergic Neurons During W and SWS

By early 2000, we observed that a long-lasting PS-like hypersomnia can be pharmacologically induced with a short latency in head-restrained rats by iontophoretic applications of bicuculline or gabazine into the SLD. Our results have been reproduced in freely moving rats and cats with pressure injection of bicuculline [46, 47]. During these experiments, we were also able to record neurons within the SLD that are specifically active during PS and excited following bicuculline or gabazine iontophoresis [48]. Taken together, our data indicate that the activation of SLD neurons is mainly due to the removal of GABAergic tone present during W and SWS. Combining retrograde tracing with cholera toxin b subunit (CTb) injected in SLD and immunostaining for glutamic acid decarboxylase 67 (GAD67, a synthetic enzyme for GABA), we thus identified neurons at the origin of these GABAergic inputs mainly within the pontine (including SLD *itself*) and the dorsal deep mesencephalic reticular nuclei (dDpMe) also named the lateral

pontine tegmentum (LPT) [45, 49]. Supporting the contribution of local GABAergic neurons in the inhibition of PS-on neurons during SWS and W, a significant increase in PS is produced by the administration of antisense oligonucleotides against GAD67 mRNA centered to the cat nucleus peri-LC alpha [47]. In rats, the number of GABAergic Fos+ neurons in the rostral pontine reticular nucleus decreased following PS rebound, suggesting they are active during W and SWS and inactive during PS [50]. However, we recently demonstrated that the ventrolateral part of the PAG (vIPAG) and the dDPMe are the only brainstem structures containing a large number of Fos+ neurons expressing GAD67 mRNA after PS deprivation [41]. Further, injections of muscimol (a GABA-A receptor agonist) in the vIPAG and/or the dDPMe induce strong increases in PS in cats and rats [41, 51]. These congruent experimental data led us to propose that GABAergic neurons within the vIPAG and the dDPMe gate PS by tonically inhibiting PS-on neurons from the SLD during W and SWS.

### **Role of the Monoaminergic Neurons in the Control of SLD Glutamatergic Neurons**

An important discovery in the course of the identification of PS mechanisms was the finding that serotonergic and noradrenergic neurons cease firing specifically during PS, i.e., show a PS-off firing activity reciprocal to that of PS-on neurons [12, 33]. Recently, it was shown that histaminergic and hypocretin neurons within PH also have a PS-off firing pattern [52–54]. These electrophysiological data were the basis for the classical “reciprocal interaction” model suggesting that PS onset is gated by reciprocal inhibitory interactions between PS-on and PS-off neurons [29, 55, 56]. Supporting this model, drugs enhancing serotonin and noradrenergic transmission (monoamine oxidase inhibitors and serotonin and norepinephrine reuptake blockers) specifically suppress PS. Further, applications of noradrenaline, adrenaline, or benoxathian (an alpha2 agonist) into the peri-LC alpha inhibit PS, but serotonin has no effect

[57, 58]. In addition, noradrenaline via alpha2-adrenoceptors inhibits the noncholinergic PS-on neurons but has no effect on the putative cholinergic PS-on neurons from the peri-LC alpha while serotonin has no effect on both types of neurons [59]. Importantly, our recent data combining tyrosine hydroxylase and Fos staining after PS deprivation and recovery suggest that LC noradrenergic neurons are likely not involved in the inhibition of PS particularly during PS deprivation. Nevertheless, a substantial number of noradrenergic neurons from A1 and A2 cell groups displayed Fos after PS deprivation indicating that these medullary noradrenergic cell groups might contribute to PS inhibition [60].

### **GABAergic Neurons Responsible for the Inactivation of Monoaminergic Neurons During PS**

According to the “reciprocal interaction” model, the inactivation of monoaminergic neurons at PS onset is due to a powerful inhibitory drive originating from cholinergic PS-on cells from peri-LC alpha [29, 56]. However, peri-LC alpha neurons do not send efferent projections to monoaminergic nuclei; acetylcholine excites noradrenergic LC neurons and is only weakly inhibitory on serotonergic DRN neurons [61, 62]. It has therefore been suggested that inhibitory amino acids such as GABA or glycine may be more powerful than acetylcholine [63, 64]. To test this hypothesis, effects of iontophoretic applications of bicuculline and strychnine (a glycine antagonist) were studied on the activity of LC and DRN cells in the head-restrained rat. Bicuculline or strychnine, applied during SWS or PS when monoaminergic neurons are silent, induces tonic firing in both types of neurons [65–67]. During W, the antagonists produce a sustained increase in discharge rate compared to control. These results indicate the existence of tonic GABA and glycinergic inputs to the LC and DRN that are active during all vigilance states. Importantly, when the strychnine effect occurred during transitions from PS to W, the discharge rate further increased at W onset. In the same situation with bicuculline, the



discharge rate remained unchanged at the transition from PS to W. These distinctive electrophysiological responses strongly suggest that an increased GABA release is responsible for the PS-selective inactivation of monoaminergic neurons. This hypothesis is well supported by microdialysis experiments in cats measuring a significant increase in GABA release in the DRN and LC during PS as compared to W and SWS but no detectable changes in glycine concentration [68, 69]. It seems therefore that monoaminergic cells are under a tonic GABAergic inhibition with a gradually increased strength from W to PS resulting in their inactivation during sleep. GABAergic neurons located in the VLPO and MnPN areas may be involved in this inhibition during SWS while those located in the extended VLPO have been implicated in this inhibition during PS [70]. However, we could not confirm that Fos-positive neurons located in the VLPO and extended VLPO after PS rebound project to the LC [71]. Further, it is likely that the PS-related inhibition involves primarily GABAergic neurons within the brainstem, directly projecting to monoaminergic neurons and “turned on” specifically at the onset of and during PS. Indeed, PS-like episodes spontaneously occur in pontine cats and when pharmacologically induced by carbachol in decerebrate cats, PS episodes are still associated with the silencing of serotonergic neurons [72].

By combining retrograde tracing with CTb and GAD immunohistochemistry in rats, we found that the LC and DRN receive GABAergic inputs from neurons located in a large number of distant regions from the forebrain to medulla [67]. Two brainstem areas are common inputs to DRN and LC since they contain a substantial number of GABAergic/CTb+ neurons, and are thus candidates for mediating the PS-related inhibition of monoaminergic neurons: the vIPAG and the dorsal paragigantocellular nucleus (DPGi). Furthermore, we demonstrated by using Fos that both nuclei contain numerous LC-projecting neurons selectively activated during PS rebound following PS deprivation [71]. Since the DPGi has not previously been considered in sleep, we studied the firing activity of DPGi neurons across the

sleep–wake cycle in head-restrained rats. In full agreement with our Fos data, the DPGi contains numerous PS-on neurons; they are silent during W and SWS but fire tonically during PS. They start discharging approximately 15 s before PS onset and become silent around 10 s before EEG signs of arousal. This singular electrophysiological pattern suggests their contribution to the generation of PS [73]. Indeed, local application of bicuculline blocked the DPGi-evoked inhibition of LC neurons and electrical DPGi stimulation induces an increase in PS quantities in rats [74, 75]. Taken together, these data support the presence within the DPGi of neurons responsible for the inactivation during PS of LC neurons. A contribution from the vIPAG in this inhibitory mechanism is also likely. In rat brainstem slices, iontophoretic NMDA application in the vIPAG-induced bicuculline-sensitive inhibitory postsynaptic potentials in serotonergic neurons [76]. Moreover, an increase in Fos+ GAD immunoreactive neurons has been reported in the vIPAG after PS rebound induced by deprivation in rats [39, 41].

## Role of the Posterior Hypothalamus, in Particular MCH Neurons in PS

As reported above, numerous Fos+ cells were observed in structures implicated in PS, such as SLD, vIPAG, or DPGi in rats allowed to recover from a 75-h PS-selective deprivation. Surprisingly, we also observed a very large number of Fos+ cells in the PH including zona incerta (ZI), perifornical area (PeF), and the lateral hypothalamic area (LHA) [77]. Only a few experimental results already support the notion that the PH contributes to PS regulation. Indeed, bilateral injections of muscimol in the cat mammillary and tuberal hypothalamus induced a drastic inhibition of PS [78]. Further, neurons specifically active during PS were recorded in the PH of cats or head-restrained rats. By using double-immunostaining, we further showed that half of Fos+ cells (76% in the PeF) in PH are positive for the neuropeptide melanin-concentrating hormone (MCH) while almost none of the

neighboring hypocretin neurons were Fos+. Almost 60% of the MCH-immunoreactive neurons counted in PH were Fos+ [77]. In support of our Fos data, it has been recently shown in head-restrained rats that hypothalamic neurons, identified *ex vivo* as containing MCH, fire quite exclusively during PS, with a slow tonic rate or phasically in doublets or burst of spikes [79]. In addition, rats receiving ICV administration of MCH showed a strong dose-dependent increase in PS and, to a minor extent, SWS quantities [77]. Further, subcutaneous injection of an MCH antagonist decreases SWS and PS quantities, and mice with genetically inactivated MCH signaling exhibit altered vigilance state architecture and sleep homeostasis [80–82]. Thus, growing evidence indicates that MCH neurons and/or MCH signaling play a key role in PS regulation/homeostasis. Since MCH is primarily an inhibitory peptide, we have proposed that MCH neurons might promote PS by inhibiting W active neurons permissive for PS like the vIPAG/dDpMe GABAergic, the monoaminergic PS-off neurons and the hypocretin neurons intermingled in the PH with them [12, 83]. Supporting this hypothesis, observations with electron microscopy indicate that MCH and Hcrt neurons are interconnected [84, 85] and recent data from MCH-R1 KO mice demonstrated interactions between Hcrt and MCH systems, implying that MCH may exert an inhibitory influence on Hcrt signaling [86]. In addition, GABA, present in MCH neurons, likely contributes to the cessation of activity of Hcrt neurons during SWS and PS since bicuculline application in the PH-induced W and Fos expression in hypocretin neurons [87, 88].

### Projections of the Hypocretin Neurons Involved in PS Regulation and Cataplexy

Hypocretin neurons are specifically active during W [53, 54]. ICV or local injection of hypocretin in the LC or the TMN neurons induces W and inhibits PS [89–91]. Narcolepsy, a sleep disorder characterized by excessive daytime

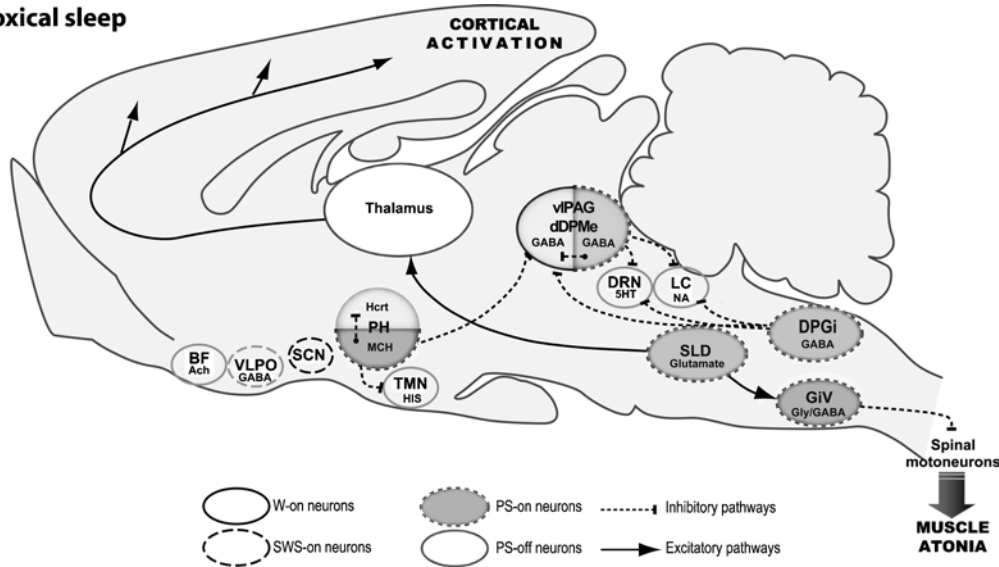
sleepiness and cataplexy, is caused by the lack of hypocretin (Hcrt) mRNA and peptides in humans or a disruption of the Hcrt-R2 or its ligands in dogs and mice [92–94]. Excessive daytime sleepiness is likely due to the absence of excitatory hypocretin inputs on the other waking systems such as the histaminergic and noradrenergic ones. The absence of these inputs might also be responsible for cataplexy. It is, however, more likely that the absence of an excitatory hypocretin input to the GABAergic PS-off dDpMe and vIPAG neurons tonically inhibiting SLD PS-on neurons during W is involved. Indeed, we have shown that the removal of the GABAergic inhibition of SLD neurons is sufficient to induce PS since these neurons are permanently excited by a glutamatergic input [45]. We therefore propose that normally during strong emotions, hypocretin neurons excite the PS-off GABAergic neurons to counteract a simultaneous increase in glutamatergic tone on SLD PS-on neurons that may be coming from the central amygdala [45]. The absence of the excitatory hypocretin input on the PS-off GABAergic neurons in narcoleptics would allow the activation of the SLD PS-on neurons by the glutamate input.

### A Network Model for PS Onset and Maintenance

Our model integrates all the experimental data described above. It also integrates the observation that PS episodes in the rat start from SWS after a relatively long intermediate state during which the EEG displays a mix of spindles and theta activity, and then terminate abruptly, associated with a short microarousal [95].

We propose that the induction of PS is due to the activation of glutamatergic PS-on neurons in the SLD. During W and SWS, the activity of these PS-on neurons would be inhibited by an inhibitory GABAergic tone originating from PS-off neurons localized in the vIPAG and dDpMe. These PS-off neurons would be activated during W by the Hcrt neurons and the monoaminergic neurons. The onset of PS would be due to the activation by

Paradoxical sleep



**Fig. 3** Model of the network responsible for paradoxical sleep (REM sleep). During PS, glutamatergic neurons of the SLD may promote cortical activation through projections to the thalamus and promote atonia by activating

GABA/glycinergic neurons in the GiV that inhibit spinal and cranial motor neurons. Monoaminergic neurons are inhibited during PS by GABAergic neurons in the DPGi, vPAG, and by MCH neurons in the lateral hypothalamus

intrinsic mechanisms of PS-on MCH/GABAergic neurons and PS-on GABAergic neurons localized in the DPGi and vPAG. These neurons would inactivate the vPAG/dDPM PS-off neurons and the PS-off monoaminergic and Hcrt neurons during PS. The disinhibited ascending SLD PS-on neurons would in turn induce cortical activation via their projections to intralaminar thalamic relay neurons in collaboration with W/PS-on cholinergic and glutamatergic neurons from the LDT and PPT, mesencephalic and pontine reticular nuclei and the basal forebrain. Descending PS-on SLD neurons would induce muscle atonia and sensory inhibition via their excitatory projections to glycinergic/GABAergic premotoneurons localized in the alpha and ventral gigantocellular reticular nuclei and the nucleus raphe magnus. The exit from PS would be due to the activation of waking systems since PS episodes are almost always terminated by an arousal. The waking systems would inhibit the MCH/GABAergic and GABAergic PS-on neurons localized in the DPGi and vPAG. Since the duration of PS is negatively coupled with metabolic rate, we propose that the activity of the waking systems is triggered to end

PS to restore competing physiological parameters like thermoregulation (Fig. 3).

Regarding PS-related pathologies in humans, cataplectic episodes in narcoleptics would be due to the absence of an increase of the hypocretin excitation of GABAergic PS-off neurons in the dDPM and the vPAG during emotion and a concomitant excitation of the SLD by glutamatergic inputs from the lateral bed nucleus of the stria terminalis and the central amygdala. Finally, phasic motor movements during RBD would be due to a degeneration or destruction of at least part of the SLD PS-on neurons and/or the phasic activation of motor systems normally occurring during PS.

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# Author Queries

Chapter No.: 10      0001263352

Queries	Details Required	Author's Response
AU1	Please check the affiliations of all authors in this chapter.	
AU2	Note that "Chap. XX" has been updated as Chap. "Prostaglandin D <sub>2</sub> : An Endogenous Somnogen." Please check if appropriate.	
AU3	Please provide journal title, volume number, and page range for the reference 42.	

Uncorrected Proof