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The Neurobiology of Sleep–Wake Systems: An Overview

Pierre-Hervé Luppi and Patrice Fort

[AU1]

1

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

21 Ach Acetylcholine
22 ADA Adenosine

BF	Basal Forebrain	23
CTb	Cholera toxin b subunit	24
DPGi	Dorsal paragigantocellular reticular nucleus	25
dDpMe	Dorsal deep mesencephalic reticular nucleus	26
DRN	Dorsal raphe nucleus	27
Fos+	Dorsal raphe nucleus	28
	Neuron immunoreactive for Fos	29
GAD	Glutamate decarboxylase	30
GiA	Alpha gigantocellular nucleus	31
GiV	Ventral gigantocellular reticular nucleus	32
		33
		34
		35

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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

65 Following studies of patients with postinfluenza
66 encephalitis, the neuropathologist von Economo
67 reported that inflammatory lesions of the preoptic
68 area (POA) were often associated with insomnia
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76 [5]. Finally, putative sleep-promoting neurons
77 displaying an elevated discharge rate during SWS
78 compared to waking (W), diffusely distributed
79 within a large region encompassing the horizon-

tal limb of the diagonal bands of Broca and the 80
lateral preoptic area-substantia innominata were 81
recorded in freely moving cats [6]. Altogether, 82
these studies indicate that the POA is a unique 83
brain structure containing neurons that directly 84
promote sleep. 85

This simplicity highly contrasts with the complex 86
network responsible for W, involving redundant 87
neurotransmitter systems with populations of 88
neurons disseminated from the upper brainstem, 89
caudal hypothalamus to basal forebrain, such as 90
neurons containing acetylcholine, norepinephrine, 91
serotonin, histamine, and recently discovered 92
hypocretin (Fig. 1). Collectively, through their 93
widespread ascending projections to the cortical 94
mantle and with their firing activity specific to 95
W, these systems form the ascending reticular 96
activating system (ARAS) that controls arousal 97
with a low-voltage, high-frequency, cortical acti- 98
vation [7]. During the transition to sleep, the hyp- 99
nogenic center would inhibit the multiple arousal 100
systems of the ARAS via a sustained and coordi- 101
nated inhibition (Fig. 2). The sleep pressure asso- 102
ciated with drowsiness is believed to be regulated 103
by the interaction of a homeostatic and a circa- 104
dian processes, both able to modulate the hyp- 105
nogenic center's activity to control the timing and 106
duration of sleep [8]. 107

Then, researchers identified specific sleep- 108
active neurons by using Fos as a marker of neu- 109
ronal activation in rats that had slept for a long 110
period before sacrifice [9]. These neurons are dis- 111
tributed diffusely in the POA but are more densely 112
packed in the median preoptic nucleus (MnPn) 113
and the ventrolateral preoptic nucleus (VLPO). 114
These studies showed that the number of Fos+ 115
neurons in the VLPO and MnPn positively 116
correlated with sleep quantity and sleep consoli- 117
dation during the last hour preceding sacrifice. 118
Numerous Fos+ neurons were also observed in 119
sleep-deprived rats in the MnPn but not in VLPO 120
[9, 10]. It appears, therefore, that VLPO neurons 121
would be primarily responsible for the induction 122
of sleep while MnPn neurons may also have a 123
homeostatic role in sleep control. It was later 124
demonstrated that VLPO and the suprachiasmatic 125
nucleus (SCN) have synchronized activity [11]. 126
Considering that both areas are interconnected 127

Waking

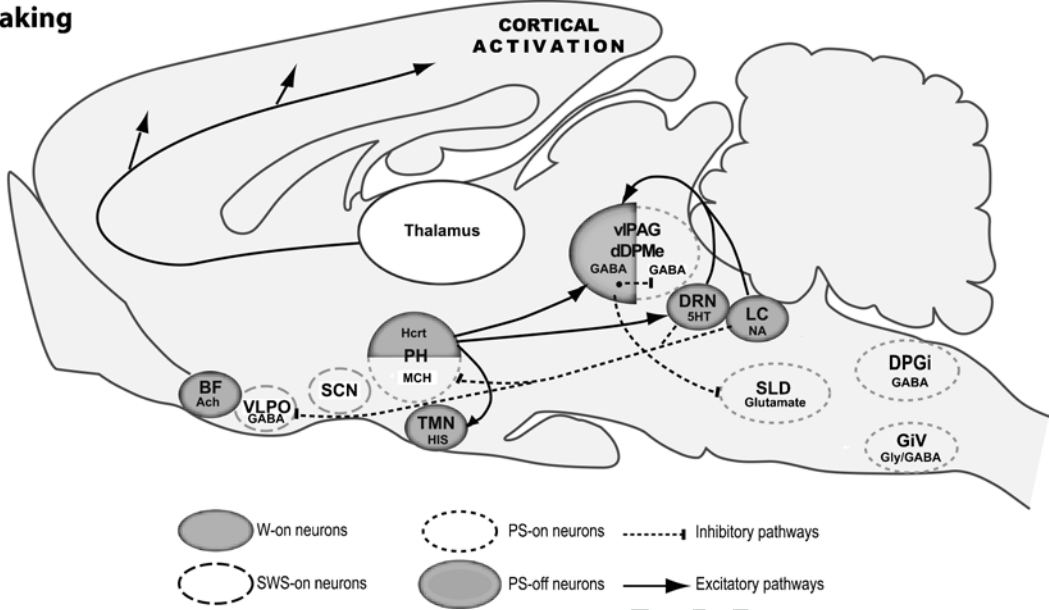


Fig. 1 Model of the network responsible for promoting wake. *Shaded nuclei* help promote wake and inhibit SWS and PS. Excitatory pathways are indicated by *solid lines*, and inhibitory pathways by *dashed lines*. Not shown on

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

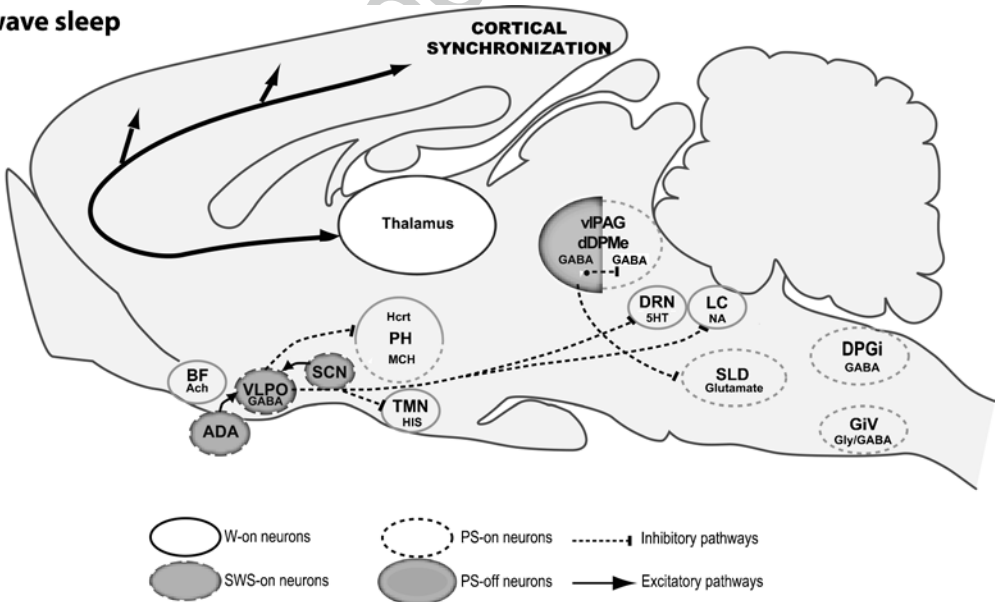


Fig. 2 Model of the network responsible for slow-wave sleep. During SWS, GABAergic neurons of the VLPO inhibit many wake-promoting regions. The VLPO neurons may be activated by adenosine or other homeostatic

signals and by circadian signals from the SCN. Other GABAergic neurons in the vIPAG and dDPMc 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⁺ 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 α_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 D₂ and adenosine have been functionally implicated in sleep, although their neuronal targets and mechanisms of action remain largely unknown (see Chap. "Prostaglandin D₂: An Endogenous Somnogen" by Urade). In this context, we showed that application of an adenosine A_{2A} receptor (A_{2A}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_{2A}R. Indeed, infusion of an A_{2A}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 A₁ receptors (A₁R) promote sleep through inhibition of the wake-promoting neurons, in particular, cholinergic and hypocretin

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

227 **An Updated Overview of the Neuronal**
 228 **Network Responsible for SWS**

229 A line of evidence indicates that both the VLPO
 230 and the MnPn contain neurons responsible for
 231 sleep onset and maintenance. These neurons
 232 would be inhibited by noradrenergic and cholin-
 233 ergic inputs during waking. The majority of them
 234 would start firing at sleep onset (drowsiness) in
 235 response to excitatory, homeostatic (adenosine
 236 and serotonin), and circadian drives (suprachias-
 237 matic inputs). These activated neurons, through
 238 the reciprocal GABAergic inhibition of all wake-
 239 promoting systems, would be in a position to
 240 suddenly unbalance the “flip-flop” network, as
 241 required for switching from W or drowsiness to
 242 SWS. Conversely, the slow removal of excitatory
 243 influences would result in a progressive firing
 244 decrease in VLPO neurons and therefore an acti-
 245 vation of wake-promoting systems leading to
 246 awakening [18, 25].

247 **Neuronal Network Responsible**
 248 **for Paradoxical Sleep**

249 **The Pontine Generator of PS**
 250 **and the Cholinergic Hypothesis**

251 In 1959, Jouvet and Michel discovered PS in cats,
 252 a sleep phase characterized by a complete disap-
 253 pearance of muscle tone, paradoxically associ-
 254 ated with cortical activation and rapid eye
 255 movements (REM) [26]. Rapidly, they demon-
 256 strated that the brainstem is necessary and suffi-
 257 cient to trigger and maintain PS in cats (pontine
 258 cat preparation). By using electrolytic and chemi-
 259 cal lesions, it was then found that the dorsal part
 260 of the pontis oralis (PnO) and caudalis (PnC)
 261 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 fir-
 ing selective to PS (called PS-on neurons) [29].
 Two types of PS-on neurons were identified. The
 first ones were inhibited by carbachol, an indica-
 tion 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 carba-
 chol and projected caudally to the nucleus reticu-
 larismagnocellularis (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 iontopho-
 resis into the rat sublateralodorsal tegmental
 nucleus (SLD), a very small area of the dorsolat-
 eral pontine tegmentum corresponding to the
 cat’s peri-LC alpha, induces W with increased
 muscle activity [34]. Other studies using carba-
 chol administration in rats described either a
 moderate PS enhancement or no effect [35–38].
 It was first shown that the number of pedunculo-
 pontine (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 gen-
 esis although unit recording combined with

310 juxtacellular labeling is required to draw a more
311 definitive conclusion.

312 **SLD Neurons Triggering PS** 313 **Are Glutamatergic**

314 As described above, SLD neurons activated dur-
315 ing PS are not cholinergic. We recently showed
316 that they are not GABAergic, as well. Indeed, the
317 small number of Fos+/GAD+ neurons in the SLD
318 did not increase in rats displaying a PS rebound
319 compared to control or PS-deprived rats [41]. It is
320 more likely that the Fos+ neurons observed in the
321 SLD specifically after PS recovery are glutamater-
322 gic since they express the vesicular glutamate
323 transporter, vGlut2 [42]. Further, it has been
324 shown that the SLD sends efferent projections to
325 glycinergic neurons from the ventral and alpha
326 gigantocellular nuclei (GiV and GiA, correspond-
327 ing to the cat Mc) known to generate atonia dur-
328 ing PS by direct projections to cranial and spinal
329 motoneurons in the cat [12, 33]. These glycin-
330 ergic neurons express Fos during a PS-like state
331 induced by the disinhibition of the SLD with
332 bicuculline or gabazine (GABA-A receptor antag-
333 onists) [34]. Further, glutamate release in the GiV
334 and GiA increases specifically during PS and
335 injection of non-NMDA glutamate agonists in the
336 GiV and GiA suppresses muscle tone. In contrast,
337 an increased tonus is induced during PS after the
338 lesion of these medullary areas in cats. Altogether,
339 these results strongly suggest that the SLD neu-
340 rons triggering PS are glutamatergic [12, 33].

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

345 **SLD Neurons Triggering PS Are** 346 **Tonically Excited by Glutamate**

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

352 induction of a PS-like state. Further, application of
353 kynurenate, a glutamate antagonist, reversed the
354 PS-like state induced by bicuculline [34]. These
355 results suggest that SLD PS-on neurons are under
356 a permanent glutamatergic barrage throughout the
357 sleep-waking cycle, unmasked at the onset of PS
358 by the removal of tonic GABAergic inputs. The
359 best candidate structure for containing the gluta-
360 matergic neurons permanently activating SLD is
361 the lateral and ventrolateral periaqueductal gray
362 (vlPAG), containing numerous non-GABAergic,
363 likely glutamatergic, efferent neurons to the SLD
364 [44, 45]. Although Jouvet established that structures
365 sufficient for PS are restricted to the brainstem,
366 numerous non-GABAergic neurons projecting to
367 the SLD located in the primary motor area of the
368 frontal cortex, the bed nucleus of the stria termina-
369 lis or the central nucleus of the amygdala could
370 also contribute to the activation of the SLD neurons
371 during PS [45].

372 **SLD Glutamatergic Neurons Are** 373 **Inhibited by GABAergic Neurons During** 374 **W and SWS**

375 By early 2000, we observed that a long-lasting
376 PS-like hypersomnia can be pharmacologically
377 induced with a short latency in head-restrained
378 rats by iontophoretic applications of bicuculline
379 or gabazine into the SLD. Our results have been
380 reproduced in freely moving rats and cats with
381 pressure injection of bicuculline [46, 47]. During
382 these experiments, we were also able to record
383 neurons within the SLD that are specifically
384 active during PS and excited following bicucul-
385 line or gabazine iontophoresis [48]. Taken
386 together, our data indicate that the activation of
387 SLD neurons is mainly due to the removal of
388 GABAergic tone present during W and SWS.
389 Combining retrograde tracing with cholera toxin b
390 subunit (CTb) injected in SLD and immunostain-
391 ing for glutamic acid decarboxylase 67 (GAD67,
392 a synthetic enzyme for GABA), we thus identi-
393 fied neurons at the origin of these GABAergic
394 inputs mainly within the pontine (including SLD
395 *itself*) and the dorsal deep mesencephalic reticu-
396 lar nuclei (dDpMe) also named the lateral

397 pontine tegmentum (LPT) [45, 49]. Supporting
 398 the contribution of local GABAergic neurons in
 399 the inhibition of PS-on neurons during SWS and
 400 W, a significant increase in PS is produced by the
 401 administration of antisense oligonucleotides
 402 against GAD67 mRNA centered to the cat nuc-
 403 leus peri-LC alpha [47]. In rats, the number of
 404 GABAergic Fos+ neurons in the rostral pontine
 405 reticular nucleus decreased following PS rebound,
 406 suggesting they are active during W and SWS
 407 and inactive during PS [50]. However, we recently
 408 demonstrated that the ventrolateral part of the
 409 PAG (vIPAG) and the dDpMe are the only brain-
 410 stem structures containing a large number of
 411 Fos+ neurons expressing GAD67 mRNA after
 412 PS deprivation [41]. Further, injections of musci-
 413 mol (a GABA-A receptor agonist) in the vIPAG
 414 and/or the dDpMe induce strong increases in PS
 415 in cats and rats [41, 51]. These congruent experi-
 416 mental data led us to propose that GABAergic
 417 neurons within the vIPAG and the dDpMe gate
 418 PS by tonically inhibiting PS-on neurons from
 419 the SLD during W and SWS.

420 **Role of the Monoaminergic Neurons**
 421 **in the Control of SLD Glutamatergic**
 422 **Neurons**

423 An important discovery in the course of the iden-
 424 tification of PS mechanisms was the finding that
 425 serotonergic and noradrenergic neurons cease fir-
 426 ing specifically during PS, i.e., show a PS-off fir-
 427 ing activity reciprocal to that of PS-on neurons
 428 [12, 33]. Recently, it was shown that histaminer-
 429 gic and hypocretin neurons within PH also have a
 430 PS-off firing pattern [52–54]. These electrophysi-
 431 ological data were the basis for the classical
 432 “reciprocal interaction” model suggesting that PS
 433 onset is gated by reciprocal inhibitory interactions
 434 between PS-on and PS-off neurons [29, 55, 56].
 435 Supporting this model, drugs enhancing serotonin
 436 and noradrenergic transmission (monoamine oxi-
 437 dase inhibitors and serotonin and norepinephrine
 438 reuptake blockers) specifically suppress PS.
 439 Further, applications of noradrenaline, adrenaline,
 440 or benoxathian (an alpha2 agonist) into the peri-
 441 LC alpha inhibit PS, but serotonin has no effect

[57, 58]. In addition, noradrenaline via alpha2- 442
 adrenoceptors inhibits the noncholinergic PS-on 443
 neurons but has no effect on the putative cholin- 444
 ergic PS-on neurons from the peri-LC alpha while 445
 serotonin has no effect on both types of neurons 446
 [59]. Importantly, our recent data combining 447
 tyrosine hydroxylase and Fos staining after PS 448
 deprivation and recovery suggest that LC nora- 449
 drenergic neurons are likely not involved in the 450
 inhibition of PS particularly during PS depriva- 451
 tion. Nevertheless, a substantial number of nora- 452
 drenergic neurons from A1 and A2 cell groups 453
 displayed Fos after PS deprivation indicating that 454
 these medullary noradrenergic cell groups might 455
 contribute to PS inhibition [60]. 456

457 **GABAergic Neurons Responsible**
 458 **for the Inactivation of Monoaminergic**
 459 **Neurons During PS**

460 According to the “reciprocal interaction” model, 460
 the inactivation of monoaminergic neurons at PS 461
 onset is due to a powerful inhibitory drive origi- 462
 nating from cholinergic PS-on cells from peri-LC 463
 alpha [29, 56]. However, peri-LC alpha neurons 464
 do not send efferent projections to monoaminer- 465
 gic nuclei; acetylcholine excites noradrenergic 466
 LC neurons and is only weakly inhibitory on 467
 serotonergic DRN neurons [61, 62]. It has there- 468
 fore been suggested that inhibitory amino acids 469
 such as GABA or glycine may be more powerful 470
 than acetylcholine [63, 64]. To test this hypothe- 471
 sis, effects of iontophoretic applications of bicu- 472
 culline and strychnine (a glycine antagonist) were 473
 studied on the activity of LC and DRN cells in 474
 the head-restrained rat. Bicuculline or strychnine, 475
 applied during SWS or PS when monoaminergic 476
 neurons are silent, induces tonic firing in both 477
 types of neurons [65–67]. During W, the antago- 478
 nists produce a sustained increase in discharge 479
 rate compared to control. These results indicate 480
 the existence of tonic GABA and glycinergic 481
 inputs to the LC and DRN that are active during 482
 all vigilance states. Importantly, when the strych- 483
 nine effect occurred during transitions from PS to 484
 W, the discharge rate further increased at W 485
 onset. In the same situation with bicuculline, the 486

487 discharge rate remained unchanged at the transi- 535
 488 tion from PS to W. These distinctive electrophys- 536
 489 iological responses strongly suggest that an 537
 490 increased GABA release is responsible for the 538
 491 PS-selective inactivation of monoaminergic neu- 539
 492 rons. This hypothesis is well supported by 540
 493 microdialysis experiments in cats measuring a 541
 494 significant increase in GABA release in the DRN 542
 495 and LC during PS as compared to W and SWS 543
 496 but no detectable changes in glycine concentra- 544
 497 tion [68, 69]. It seems therefore that monoamin- 545
 498 ergic cells are under a tonic GABAergic inhibition 546
 499 with a gradually increased strength from W to PS 547
 500 resulting in their inactivation during sleep. 548
 501 GABAergic neurons located in the VLPO and 549
 502 MnPN areas may be involved in this inhibition 550
 503 during SWS while those located in the extended 551
 504 VLPO have been implicated in this inhibition 552
 505 during PS [70]. However, we could not confirm 553
 506 that Fos-positive neurons located in the VLPO 554
 507 and extended VLPO after PS rebound project to 555
 508 the LC [71]. Further, it is likely that the PS-related 556
 509 inhibition involves primarily GABAergic neu- 557
 510 rons within the brainstem, directly projecting to 558
 511 monoaminergic neurons and “turned on” specifi-
 512 cally at the onset of and during PS. Indeed,
 513 PS-like episodes spontaneously occur in pontine
 514 cats and when pharmacologically induced by car-
 515 bachol in decerebrate cats, PS episodes are
 516 still associated with the silencing of serotonergic
 517 neurons [72].

518 By combining retrograde tracing with CTb 535
 519 and GAD immunohistochemistry in rats, we 536
 520 found that the LC and DRN receive GABAergic 537
 521 inputs from neurons located in a large number of 538
 522 distant regions from the forebrain to medulla 539
 523 [67]. Two brainstem areas are common inputs to 540
 524 DRN and LC since they contain a substantial 541
 525 number of GABAergic/CTb+ neurons, and are 542
 526 thus candidates for mediating the PS-related inhi- 543
 527 bition of monoaminergic neurons: the vIPAG and 544
 528 the dorsal paragigantocellular nucleus (DPGi). 545
 529 Furthermore, we demonstrated by using Fos that 546
 530 both nuclei contain numerous LC-projecting neu- 547
 531 rons selectively activated during PS rebound fol- 548
 532 lowing PS deprivation [71]. Since the DPGi has 549
 533 not previously been considered in sleep, we stud- 550
 534 ied the firing activity of DPGi neurons across the

535 sleep–wake cycle in head-restrained rats. In full 536
 537 agreement with our Fos data, the DPGi contains 538
 539 numerous PS-on neurons; they are silent during 539
 540 W and SWS but fire tonically during PS. They 540
 541 start discharging approximately 15 s before PS 541
 542 onset and become silent around 10 s before EEG 542
 543 signs of arousal. This singular electrophysiological 543
 544 pattern suggests their contribution to the gener- 544
 545 ation of PS [73]. Indeed, local application of 545
 546 bicuculline blocked the DPGi-evoked inhibition 546
 547 of LC neurons and electrical DPGi stimulation 547
 548 induces an increase in PS quantities in rats [74, 548
 549 75]. Taken together, these data support the 549
 550 presence within the DPGi of neurons responsible 550
 551 for the inactivation during PS of LC neurons. 551
 552 A contribution from the vIPAG in this inhibitory 552
 553 mechanism is also likely. In rat brainstem slices, 553
 554 iontophoretic NMDA application in the vIPAG- 554
 555 induced bicuculline-sensitive inhibitory postsyn- 555
 556 aptic potentials in serotonergic neurons [76]. 556
 557 Moreover, an increase in Fos+ GAD immunore- 557
 558 active neurons has been reported in the vIPAG 558
 after PS rebound induced by deprivation in rats [39, 41].

559 **Role of the Posterior Hypothalamus,** 559 560 **in Particular MCH Neurons in PS** 560

561 As reported above, numerous Fos+ cells were 561
 562 observed in structures implicated in PS, such as 562
 563 SLD, vIPAG, or DPGi in rats allowed to recover 563
 564 from a 75-h PS-selective deprivation. Surpris- 564
 565 ingly, we also observed a very large number of 565
 566 Fos+ cells in the PH including zona incerta (ZI), 566
 567 perifornical area (PeF), and the lateral hypothal- 567
 568 amic area (LHA) [77]. Only a few experimental 568
 569 results already support the notion that the PH 569
 570 contributes to PS regulation. Indeed, bilateral 570
 571 injections of muscimol in the cat mammillary 571
 572 and tuberal hypothalamus induced a drastic 572
 573 inhibition of PS [78]. Further, neurons specifi- 573
 574 cally active during PS were recorded in the PH 574
 575 of cats or head-restrained rats. By using double- 575
 576 immunostaining, we further showed that half of 576
 577 Fos+ cells (76% in the PeF) in PH are positive 577
 578 for the neuropeptide melanin-concentrating 578
 579 hormone (MCH) while almost none of the 579

580 neighboring hypocretin neurons were Fos+.
 581 Almost 60% of the MCH-immunoreactive neu-
 582 rons counted in PH were Fos+ [77]. In support
 583 of our Fos data, it has been recently shown in
 584 head-restrained rats that hypothalamic neurons,
 585 identified *ex vivo* as containing MCH, fire quite
 586 exclusively during PS, with a slow tonic rate or
 587 phasically in doublets or burst of spikes [79]. In
 588 addition, rats receiving ICV administration of
 589 MCH showed a strong dose-dependent increase
 590 in PS and, to a minor extent, SWS quantities
 591 [77]. Further, subcutaneous injection of an MCH
 592 antagonist decreases SWS and PS quantities,
 593 and mice with genetically inactivated MCH
 594 signaling exhibit altered vigilance state archi-
 595 tecture and sleep homeostasis [80–82]. Thus,
 596 growing evidence indicates that MCH neurons
 597 and/or MCH signaling play a key role in PS
 598 regulation/homeostasis. Since MCH is primarily
 599 an inhibitory peptide, we have proposed that
 600 MCH neurons might promote PS by inhibiting
 601 W active neurons permissive for PS like the
 602 vIPAG/dDpMe GABAergic, the monoaminergic
 603 PS-off neurons and the hypocretin neurons
 604 intermingled in the PH with them [12, 83].
 605 Supporting this hypothesis, observations with
 606 electron microscopy indicate that MCH and
 607 Hcrt neurons are interconnected [84, 85] and
 608 recent data from MCH-R1 KO mice demon-
 609 strated interactions between Hcrt and MCH sys-
 610 tems, implying that MCH may exert an inhibitory
 611 influence on Hcrt signaling [86]. In addition,
 612 GABA, present in MCH neurons, likely contrib-
 613 utes to the cessation of activity of Hcrt neurons
 614 during SWS and PS since bicuculline applica-
 615 tion in the PH-induced W and Fos expression in
 616 hypocretin neurons [87, 88].

617 **Projections of the Hypocretin Neurons**
 618 **Involved in PS Regulation**
 619 **and Cataplexy**

620 Hypocretin neurons are specifically active dur-
 621 ing W [53, 54]. ICV or local injection of hypo-
 622 cretin in the LC or the TMN neurons induces W
 623 and inhibits PS [89–91]. Narcolepsy, a sleep
 624 disorder characterized by excessive daytime

625 sleepiness and cataplexy, is caused by the lack
 626 of hypocretin (Hcrt) mRNA and peptides in
 627 humans or a disruption of the Hcrt-R2 or its
 628 ligands in dogs and mice [92–94]. Excessive
 629 daytime sleepiness is likely due to the absence
 630 of excitatory hypocretin inputs on the other
 631 waking systems such as the histaminergic and
 632 noradrenergic ones. The absence of these inputs
 633 might also be responsible for cataplexy. It is,
 634 however, more likely that the absence of an
 635 excitatory hypocretin input to the GABAergic
 636 PS-off dDpMe and vIPAG neurons tonically
 637 inhibiting SLD PS-on neurons during W is
 638 involved. Indeed, we have shown that the
 639 removal of the GABAergic inhibition of SLD
 640 neurons is sufficient to induce PS since these
 641 neurons are permanently excited by a gluta-
 642 matergic input [45]. We therefore propose that
 643 normally during strong emotions, hypocretin
 644 neurons excite the PS-off GABAergic neurons
 645 to counteract a simultaneous increase in gluta-
 646 matergic tone on SLD PS-on neurons that may
 647 be coming from the central amygala [45]. The
 648 absence of the excitatory hypocretin input on
 649 the PS-off GABAergic neurons in narcoleptics
 650 would allow the activation of the SLD PS-on
 651 neurons by the glutamate input.

652 **A Network Model for PS Onset**
 653 **and Maintenance**

654 Our model integrates all the experimental data
 655 described above. It also integrates the observa-
 656 tion that PS episodes in the rat start from SWS
 657 after a relatively long intermediate state during
 658 which the EEG displays a mix of spindles and
 659 theta activity, and then terminate abruptly, associ-
 660 ated with a short microarousal [95].

661 We propose that the induction of PS is due to
 662 the activation of glutamatergic PS-on neurons in
 663 the SLD. During W and SWS, the activity of these
 664 PS-on neurons would be inhibited by an inhibi-
 665 tory GABAergic tone originating from PS-off
 666 neurons localized in the vIPAG and dDpMe. These
 667 PS-off neurons would be activated during W by
 668 the Hcrt neurons and the monoaminergic neurons.
 669 The onset of PS would be due to the activation by

Paradoxical sleep

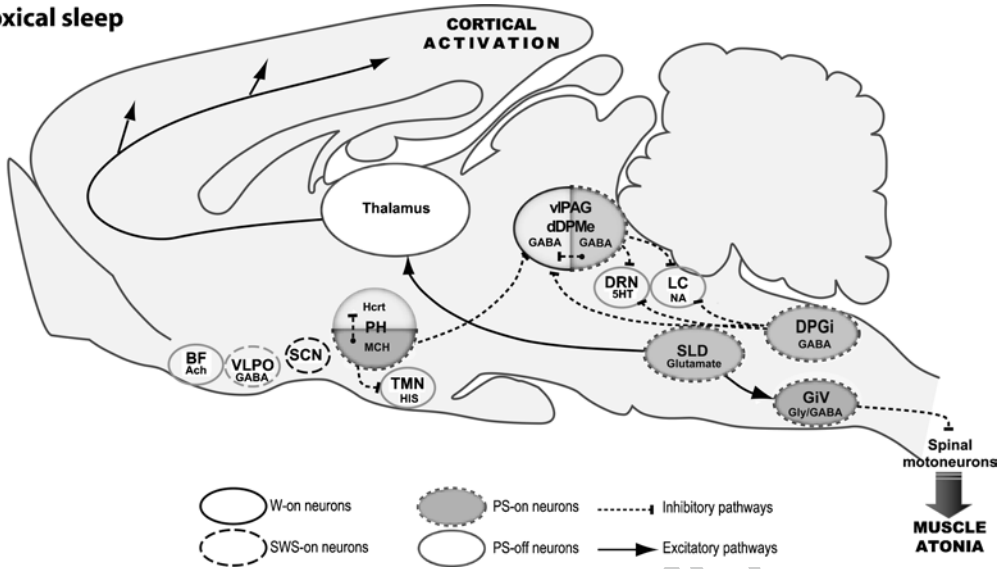


Fig. 3 Model of the network responsible for paradoxical sleep (REM sleep). During PS, glutamatergic neurons of the SLD may promote cortical activation through projections to the thalamus and promote atonia by activating

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, vIPAG, and by MCH neurons in the lateral hypothalamus

670 intrinsic mechanisms of PS-on MCH/GABAergic
 671 neurons and PS-on GABAergic neurons localized
 672 in the DPGi and vIPAG. These neurons would
 673 inactivate the vIPAG/dDPMe PS-off neurons and
 674 the PS-off monoaminergic and Hcr neurons dur-
 675 ing PS. The disinhibited ascending SLD PS-on
 676 neurons would in turn induce cortical activation
 677 via their projections to intralaminar thalamic relay
 678 neurons in collaboration with W/PS-on cholin-
 679 ergic and glutamatergic neurons from the LDT
 680 and PPT, mesencephalic and pontine reticular
 681 nuclei and the basal forebrain. Descending PS-on
 682 SLD neurons would induce muscle atonia and
 683 sensory inhibition via their excitatory projections
 684 to glycinergic/GABAergic premotoneurons local-
 685 ized in the alpha and ventral gigantocellular retic-
 686 ular nuclei and the nucleus raphe magnus. The
 687 exit from PS would be due to the activation of
 688 waking systems since PS episodes are almost
 689 always terminated by an arousal. The waking sys-
 690 tems would inhibit the MCH/GABAergic and
 691 GABAergic PS-on neurons localized in the DPGi
 692 and vIPAG. Since the duration of PS is negatively
 693 coupled with metabolic rate, we propose that the
 694 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 dDPMe and the vIPAG during emotion and a
 concomitant excitation of the SLD by gluta-
 matergic 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

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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 ₂ : An Endogenous Somnogen." Please check if appropriate.	
AU3	Please provide journal title, volume number, and page range for the reference 42.	

Uncorrected Proof