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# Which structure generates paradoxical (REM) sleep: The brainstem, the hypothalamus, the amygdala or the cortex?

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## ABSTRACT

Paradoxical or Rapid eye movement (REM) sleep (PS) is a state characterized by REMs, EEG activation and muscle atonia. In this review, we discuss the contribution of brainstem, hypothalamic, amygdalar and cortical structures in PS genesis. We propose that muscle atonia during PS is due to activation of glutamatergic neurons localized in the pontine sublaterodorsal tegmental nucleus (SLD) projecting to glycinergic/GABAergic pre-motoneurons localized in the ventro-medial medulla (vmM). The SLD PS-on neurons are inactivated during wakefulness and slow-wave sleep by PS-off GABAergic neurons localized in the ventrolateral periaqueductal gray (vPAG) and the adjacent deep mesencephalic reticular nucleus. Melanin concentrating hormone (MCH) and GABAergic PS-on neurons localized in the posterior hypothalamus would inhibit these PS-off neurons to initiate the state. Finally, the activation of a few limbic cortical structures during PS by the claustrum and the supramammillary nucleus as well as that of the basolateral amygdala would also contribute to PS expression. Accumulating evidence indicates that the activation of these limbic structures plays a role in memory consolidation and would communicate to the PS-generating structures the need for PS to process memory. In summary, PS generation is controlled by structures distributed from the cortex to the medullary level of the brain.

## 1. Introduction

REM (Paradoxical) sleep (PS) characterized by EEG activation and rapid eye movements (REMs) was discovered in 1953 by Aserinsky and Kleitman in humans. It was shown to correlate with dream activity [1,2]. In 1959, Jouvet and Michel discovered in cats that PS is also characterized by a disappearance of muscle tone, paradoxically associated with cortical activation and REMs [3,4]. Soon after, it was shown that a state characterized by muscle atonia and REMs persists following decortication, cerebellar ablation or brainstem transections rostral to the pons and in the "pontine cat", a preparation in which structures rostral to the pons have been removed [5]. These results indicate that the brainstem is necessary and sufficient to generate PS. However, in the following sixty years, it has been proposed that neuronal systems upstream to the pons might modulate PS expression in particular the posterior hypothalamus or more recently the amygdala and the cortex. The goal of the present review is to examine each of these possibilities.

## 2. Brainstem glutamatergic and GABAergic neurons trigger PS

Early transection studies in cats indicated that the brainstem is necessary and sufficient to trigger and maintain the state of PS. Electrolytic and chemical lesions then demonstrated that the dorsal part of pontis oralis (PnO) and caudalis (PnC) nuclei contain the neurons responsible for PS onset [5]. It was thought for decades that these neurons are cholinergic since injection of carbachol (a cholinergic agonist) in this cat pontine region induced PS with a short latency [6,7]. However, we did not confirm such effect in rats [8] and it was shown that cholinergic neurons of the laterodorsal tegmental nucleus and the pedunculopontine nucleus are active during both waking (W) and PS [9]. Further, in rats, most of the neurons activated in this pontine region (as cFos-labeled) after 2,5 h of PS hypersomnia induced by 72 h of PS deprivation using the flowerpot method are localized in the sublaterodorsal tegmental nucleus (SLD) and express the vesicular glutamate transporter 2 (vGlut2) indicating they are glutamatergic in nature [10]. Fur-

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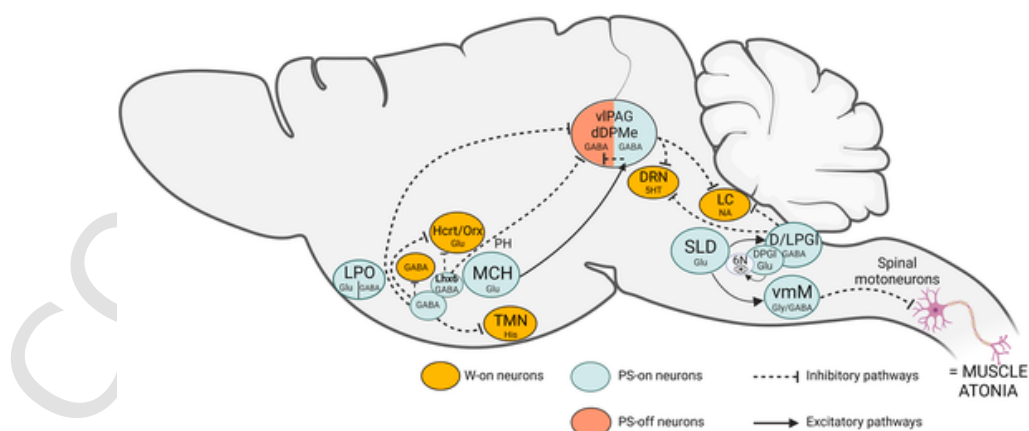
ther, a large number of the glutamatergic neurons recorded in the SLD are specifically active during PS [9] although other pontine glutamatergic neurons are W and PS-active indicating the heterogeneity of the region and its potential role in W. It is also unlikely that the GABAergic neurons of the SLD are involved in controlling PS since they do not specifically express cFos after PS deprivation or rebound [11]. Further, we showed that inactivation of glutamatergic transmission in SLD neurons using genetic method induced a 30 % decrease of PS quantities and removal of muscle atonia with abnormal and violent motor activity during the state like in REM sleep behavioral disorder (RBD) [12]. Conversely, chemogenetic activation of SLD glutamatergic neurons induced muscle atonia whereas their inactivation induced RBD [13].

Several results further indicate that SLD PS-on glutamatergic neurons generate muscle atonia via descending projections to medullary GABAergic/glycinergic neurons [8,12,14,15](Fig. 1). Indeed, it has been shown that the SLD sends direct efferent projections to glycinergic neurons in the ventromedial medulla (vmM that includes the ventral (GiV) and alpha gigantocellular nuclei (Gia) and the nucleus raphe magnus (RMg) [12]. In addition, glycinergic vmM neurons express cFos after induction of PS by bicuculline (a GABAa antagonist) injection in the SLD [8] or PS hypersomnia [15]. Further, glycinergic neurons of vmM monosynaptically project to lumbar spinal motoneurons [14,15]. Finally, glycinergic interneurons in the spinal cord do not express cFos [15]. These results suggest that vmM glycinergic neurons hyperpolarize motoneurons during PS. It is likely that these neurons are also GABAergic since a large majority of the cFos-labeled vmM neurons after PS hypersomnia express mRNAs for the synthesizing enzyme for GABA [11]. Further, it has been shown that combined microdialysis of bicuculline, strychnine and phaclophen (a GABAb antagonist) in the trigeminal motor nucleus restored muscle tone in masseter muscles during PS [16,17]. Finally, genetic inactivation of glycinergic and GABAergic vmM neurons induced a state of PS closed to RBD [15,18]. Altogether, these results indicate that the pre-motoneurons responsible for muscle atonia of PS are localized in the vmM and co-release GABA and glycine.

Besides, several results indicate that two other subsets of medullary PS-active GABAergic neurons located laterally to the vmM neurons in the lateral paragigantocellular nucleus (LPGi) and or dorsally to them in the dorsal paragigantocellular reticular nucleus (DPGi) are ascending

and play a crucial role in the generation of PS by means of their inhibition of wake-active serotonergic and noradrenergic neurons [19]. Indeed, the application of bicuculline during slow-wave sleep (SWS) or PS on dorsal raphe nucleus (DRN) serotonergic and noradrenergic locus coeruleus (LC) neurons restores a tonic firing [19,20]. By combining retrograde tracing with cholera toxin B subunit (CTb) and GAD immunohistochemistry in rats, we found that the LC and DRN receive GABA and glycinergic inputs from the ventrolateral periaqueductal gray (vlPAG) and the LPGi and DPGi [19,21]. We then demonstrated by using cFos that these nuclei contain numerous LC-projecting neurons selectively activated during PS rebound following PS deprivation [22]. Further, the LPGi/DPGi contain numerous PS-on neurons that are silent during waking and SWS and fire tonically during PS [23–27]. In addition, inactivation of the DPGi with muscimol (a GABAa agonist) induced wakefulness and cFos expression in LC, A1, A2 and A5 noradrenergic neurons suggesting that GABAergic neurons of the DPGi inhibit all of these noradrenergic groups during PS. Interestingly, a subpopulation of Calbindin-D28K- glutamatergic neurons located in the DPGi are also active during PS, project to the three contralateral eye-muscle nuclei and increase their firing prior to the onset of REMs during PS. Optogenetic activation of these neurons triggers eye movements selectively during PS, while their genetic ablation or optogenetic silencing suppresses them [28]. None of these perturbations led to a change in the duration of PS episodes. In contrast, optogenetic and chemogenetic activation and inactivation of DPGi corticotropin releasing factor (CRH) neurons coexpressing glutamate increased and decreased PS quantities respectively [29]. Calcium imaging of these neurons showed that they are specifically active during PS, with a phasic activation just before the occurrence of P-waves. The authors proposed that they generate P-waves by means of their projection to the P-wave generator localized in the SLD. It is very likely that the DPGi CRH and calbindin neurons belong to the same population since they are glutamatergic. Further, their activity is linked with REMs and P-waves i.e. phasic REMs. Altogether, these results indicate that the DPGi contains both inhibitory GABAergic and excitatory glutamatergic PS-active neurons. In addition, it has been shown that optogenetic stimulation during SWS of GABAergic LPGi neurons or of their ascending fibers located in the vlPAG/dDPMe induced PS [25]. However, combining CTb injection into

**PS onset and maintenance**



**Fig. 1.** PS onset and maintenance.

The onset of PS would be due to the activation by intrinsic “clock-like” mechanisms of PS-on MCH + /Glutamatergic and/or MCH-/GABAergic neurons located in the posterior hypothalamus. A subpopulation of the zona incerta GABAergic neurons co-expresses Lhx6. All or a subpopulation of the PH neurons could also be responsible of the rebound of PS occurring after its deprivation. The PH PS-on neurons would inactivate directly (GABA neurons) or indirectly (MCH neuron) the PS-off vlPAG/dDPMe GABAergic neurons and the histaminergic and orexinergic neurons. The inhibition of the PS-off vlPAG/dDPMe GABAergic neurons would induce the disinhibition of the PS-on glutamatergic SLD neurons. These neurons would directly excite glycinergic/GABAergic premotor neurons in the vmM which in turns hyperpolarize spinal and cranial motoneurons. The SLD neurons also project to and excite DPGi and LPGi ascending GABAergic neurons responsible for inhibiting the dorsal raphe serotonergic neurons and the locus coeruleus noradrenergic neurons. The SLD neurons also excite the glutamatergic neurons of the DPGi which project to oculomotor nuclei and induce REMs. The role of the PS-on glutamatergic and GABAergic neurons of the lateral preoptic area still needs to be determined.

the vlPAG/dDpMe with cFos staining after PS hypersomnia, we did not find double-labeled cells in the LPGi [30] suggesting that the effect obtained is rather due to stimulation of axons on their way to the DRN or LC. In summary, a large body of data indicates that GABA/glycinergic PS-on neurons localized in the vlPAG and the LPGi/DPGi hyperpolarize the serotonergic and noradrenergic neurons during PS. Another set of DPGi neurons is glutamatergic and is involved in phasic REM sleep and might also play a role in controlling the state itself (Fig. 1).

What are the mechanisms triggering the activation of the LPGi and DPGi neurons? We previously showed that after injecting CTb in the LPGi, cFos/CTb labeled neurons were in their vast majority localized in the SLD [23,24]. Anterogradely labeled fibers and cFos-labeled neurons were localized in the DPGi and LPGi after injection of the anterograde tracer Phaseolus leucoagglutinin (PHA-L) in the SLD and induction of PS hypersomnia [8]. In summary, it is likely that the activation of the LPGi and DPGi PS-on ascending neurons is due to an excitatory projection from the SLD.

### 3. GABAergic PS-off neurons in the vlPAG gate the onset of PS

A long-lasting PS-like episode can be pharmacologically induced with a short latency in head-restrained unanesthetized rats by iontophoretic applications of bicuculline or gabazine, two GABA<sub>A</sub> receptor antagonists, specifically into the SLD [8]. Neurons at the origin of the GABAergic innervation of SLD are localized in the vlPAG and the dorsal deep mesencephalic reticular nuclei (dDpMe) [31]. Further, these two structures contain a large number of cFos-positive neurons expressing GAD67 mRNA after 72 h of PS deprivation [11]. Compared to the PS-on neurons also localized in the vlPAG, the PS-off neurons seem to be located more ventrally at its ventral border adjacent to the dDpMe [11]. Injection of muscimol in the vlPAG and/or the dDpMe or their neurochemical lesion induce a strong increase in PS quantities [11,32,33]. Further, optogenetic or chemogenetic stimulation of PS-off GABAergic neurons within vlPAG/dDpMe almost completely suppresses PS by shortening the duration of PS episodes [25,26,34]. Conversely, their pharmacogenetic inhibition increases PS, mainly by increasing the number of PS episodes [34]. Unit recording and calcium imaging revealed that most vlPAG GABAergic neurons start to be active at the end of PS episodes and decrease gradually their activity until the next PS episode. To our knowledge, such progressive decrease of activity has never been reported for PS-off neurons like the monoaminergic ones.

These results suggest that vlPAG GABAergic neurons powerfully gate PS by tonically inhibiting PS-on glutamatergic neurons from the SLD and may contribute to the ultradian rhythm of PS and the SWS/PS alternation [26]. It will be crucial in the future to identify the intrinsic mechanisms responsible for the slow decrease of activity of these neurons between subsequent PS episodes (Fig. 2).

### 4. The hypothalamus controls PS occurrence

In addition to the brainstem, a very large number of cFos-positive cells was also unexpectedly observed in the posterior hypothalamus (PH) including zona incerta (ZI), perifornical area and lateral hypothalamic area (LHA) after PS hypersomnia in rats [35]. Around 75 % of these cFos-labeled cells express GAD67 mRNA and are likely GABAergic [36]. Besides, one third of the cFos-GAD67 neurons co-express melanin concentrating hormone (MCH) [35] and nesfatin-1 [37]. Unit recordings in head-restrained rats confirmed that MCH neurons fire mostly during PS [38]. Recent calcium imaging data showed that these neurons are indeed less active during SWS and quiet waking compared to PS [39–41]. The authors also reported that a large majority of MCH neurons are activated specifically during the exploration of new objects during active waking [39–41]. Intracerebroventricular administration of MCH induced a strong dose-dependent increase in PS and, to a minor extent SWS quantities in rats [35]. Such sleep increase was also obtained using chronic (24 h) optogenetic activation of MCH neurons [42]. Their acute optogenetic activation at the onset of SWS epoch did not increase SWS duration but increased the probability of SWS-to-PS transitions. This effect was reproduced by using a chemogenetic approach to modulate the firing activity of MCH neurons [43]. When similar optogenetic stimulations were applied at PS onset, the duration of PS episodes was significantly prolonged [44]. It was also recently shown that the increase in PS quantities induced by a rise in ambient temperature was abolished when inactivating MCH neurons by optogenetic manipulation [45]. Based on the finding that MCH neurons express GAD67 mRNAs, it was first thought that they are GABAergic and thus inhibitory. However, three independent molecular studies demonstrated that they do not express vGAT but vGlut2, the vesicular glutamate transporter 2 [46,47] and that they indeed release glutamate to excite neurons, as in lateral septum [48].

Besides, it has been shown that a large population of MCH-negative vGAT-positive neurons in PH discharge maximally during PS [49].

State of PS network during wakefulness

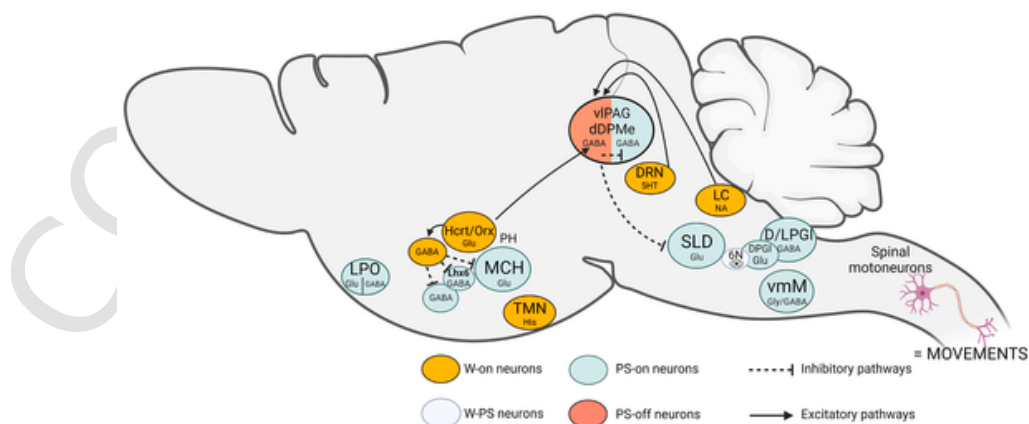


Fig. 2. State of PS network during wakefulness.

The exit from PS would be due to the activation of the PS-off GABAergic neurons and the neuronal systems responsible for inducing wakefulness. During PS, the PS-off GABAergic neurons located in the vlPAG and dDpMe would progressively increase their activity until they will reach a level high enough to induce the inhibition of the SLD glutamatergic neurons. The activity of the PS-off GABAergic neurons would be reinforced by monoaminergic and hypocretin/orexin inputs. Their activity will gradually decrease between two PS episodes releasing at the end the SLD from inhibition. The inhibition of the MCH and GABAergic neurons of the PH would be due to the activation of local Wake-active GABAergic neurons excited by the hypocretin/orexin neurons.

Since these neurons anticipate PS onset, they could play a role in triggering the state. GABAergic neurons co-expressing Lhx6 localized in the ZI could constitute a subpopulation of these MCH-/vGAT + PS-on neurons [50,51]. Indeed, it has been shown that chemogenetic activation of Lhx6 +/vGAT + neurons increases PS whereas their inhibition decreases it [50]. Further, it has been shown that they are activated during PS hypersomnia [51]. Finally, it has been shown with calcium imaging that 45.5 % of the Lhx6 neurons were more active during PS than the two other states. Further, unilateral optogenetic stimulation of the ZI Lhx6 neurons significantly increased the percentage of PS [52].

To determine whether hypothalamic neurons indeed play a crucial role in PS, muscimol was bilaterally injected in the rat LH [30]. It induced a total inhibition of PS and an increase in SWS quantities. Further, the vPAG/dDpMe region contained many cFos/GAD67 + and of cFos/CTb + neurons in animals with a CTb injection in the SLD [30]. These results indicate that the activation of PH neurons is a necessary step for PS to occur. They further suggest that MCH +/vGluT2 + and MCH-/vGAT + PS-on neurons control PS onset and maintenance by means of direct or indirect inhibitory projections to vPAG/dDpMe PS-off GABAergic neurons. From these results, it can be proposed that these MCH +/vGluT2 + and MCH-/vGAT + neurons of the PH constitute a “master” generator of PS that controls a “slave” generator located in the brainstem. To fit with Jouvet's hypothesis (i.e. brainstem is necessary and sufficient to generate a state characterized by muscle atonia and REMs) [5], it can be proposed that few days after removal of the forebrain, the brainstem generator is able to induce by itself a state with muscle atonia and REMs. However, the brainstem generator is under control of the PH generator in intact animals (Fig. 1).

In addition to the descending pathway to the PS-off GABAergic neurons, the MCH neurons might also promote PS by means of their projections to the histaminergic, serotonergic, noradrenergic and hypocretin/orexin PS-off neurons. Indeed, Jégo et al. [44] showed that optogenetic activation of MCH axons in the tuberomammillary nucleus (TMN) inhibits histaminergic cells through activation of GABA<sub>A</sub> receptors. It was also shown that stimulation of MCH fibers in CA1 field of the hippocampus induced IPSP in local pyramidal cells [53]. These results in line with the initial hypothesis that MCH neurons are GABAergic are contradictory to recent results showing that they are glutamatergic and thus excitatory. One possibility is that they excite local inhibitory interneurons. Additional studies are required to confirm that MCH neurons are indeed glutamatergic and that they inhibit the vPAG PS-off and other types of PS-off neurons by means of GABAergic PS-on interneurons.

The excitatory inputs at the origin of the activation of the MCH neurons at the entrance of PS remain to be identified. Many studies indicate that MCH neurons also play a key role in metabolism control and food intake [47,54]. Therefore, the activation of these neurons at the onset and during PS could be influenced by the metabolic state in addition to temperature increase as shown recently [45]. It is possible that the preoptic area which projects to the LH and contains PS-on neurons is involved. Indeed, it has been shown that lesion of the lateral preoptic area (LPO) induced a decrease in PS [55]. Further, GABAergic and glutamatergic neurons of the LPO selectively raised calcium activity in PS and this calcium entry depends on NMDA receptors. In this study, they also deleted the GluN1 NMDA receptor subunit in the LPO and obtained a marked “insomnia” phenotype with high sleep-wake fragmentation and greatly diminished PS [56]. Additional studies are necessary to determine whether the LPO controls PS by means of descending projection to PH neurons. In addition, it is possible that, yet undiscovered endogenous cellular or molecular clock-like mechanisms may play a role in the activation of the PS-on neurons and the increase in its quantity after PS deprivation, depression and acute or chronic stress. It is in fact probable that different mechanisms and pathways are involved in each case. We propose that the increase of PS after its deprivation is due to intrinsic molecular and cellular mechanisms occurring in LPO or PH PS-on neurons. The increase induced by chronic stress and depression

could be due to the activation of systems upstream of the PS-generating systems like the basolateral amygdala or the limbic cortex (see below).

Conversely, the cessation of activity of the MCH/vGluT2 + PS-on neurons and more largely of all the PS-on neurons at the end of PS episodes may be due to a different mechanism than at the entrance into the state since animals enter PS slowly from SWS, while in contrast they exit abruptly from it by a micro-arousal. The most likely hypothesis is that PS-on neurons are rapidly inhibited by the reactivation of the arousal circuits (PH GABAergic neurons, orexin, histamine, norepinephrine, serotonin, and acetylcholine). In that context, it has been shown that acetylcholine, serotonin and norepinephrine inhibit MCH neurons *in-vitro* while hypocretin/orexin surprisingly excite them [57]. Besides, it has been shown that if hypocretin/orexin neurons are mainly active during W, 7 % of them projecting to the SLD are also active during PS and contribute to muscle atonia [58]. In addition, Lhx6 + neurons receive inputs from dopaminergic neurons of the ventral tegmental area, serotonergic neurons of the dorsal raphe nucleus, and GABAergic neurons of both the ventrolateral periaqueductal gray and lateral hypothalamus [50]. From all these results, we propose that PS-on hypothalamic neurons are inhibited during W by GABAergic W-active neurons excited by hypocretin/orexin neurons and direct monoaminergic inputs (Fig. 2). The vPAG PS-off could be also be responsible for stopping PS since they are the only PS-off neurons showing a progressive increase of activity during PS episodes and a progressive decrease of activity in between two PS episodes. They might contain intrinsic molecular and cellular mechanisms inducing their progressive rise of activity during PS episodes. However, these neurons are known to control brainstem but not hypothalamic PS-on neurons and it therefore remains to be shown whether they could also induce their inactivation.

## 5. The potential role of the basolateral amygdala in PS control

Hasegawa et al. [59] recently observed that a phasic peak of dopamine occurred at the SWS-PS transition, specifically in the basolateral amygdala (BLA). Then, they showed that 1 s optogenetic stimulations during SWS of dopamine terminals in the BLA using a stabilized step function opsin (SSFO) causes transitions to PS after a long latency. Such stimulation every 30 min for 3 h induced a 200 % increase in PS quantities (from 10 % to more than 30 % of total time). Following the inhibition of the dopamine D2-expressing neurons in the BLA, several surrounding BLA and central amygdala neurons expressed cFos with some of them projecting to the brainstem structures generating PS. Further, the optogenetic inhibition (5 min per 25 min during 3 h) of BLA dopaminergic axons expressing vertebrate low-wavelength opsin (vLWO) was followed by a near disappearance of PS. They finally demonstrated that ventral tegmental area (VTA) dopaminergic neurons projecting to the BLA are different than those projecting to the nucleus accumbens and the LHA. These results are very surprising since the amygdala has not been previously involved in PS generation, but in cataplexy occurring during narcolepsy. Indeed, lesions of the amygdala reduced cataplexy in mice but had no effect on PS [60]. Further, amygdala lesions in monkeys did not induce a decrease in PS [61]. Finally, although VTA dopaminergic neurons have similar level of activity during wakefulness and PS [62], their chemogenetic inhibition inhibits wakefulness and induces sleep with an increase in PS quantities [63]. Conversely, optogenetic excitation of DA VTA neurons by optogenetic induced wakefulness. In addition, the fact that the induction of PS occurred after very long latencies clearly indicates that the BLA is not a core element of the PS generating network but is rather acting upstream to it. What could be the mechanisms and the neuronal network involved? It is known that D2 dopamine receptors are inhibitory, and the authors reported that some neurons of the BLA are activated following D2 receptors activation. It is therefore likely that DA induced the inhibition of GABAergic interneurons by means of D2 inhibitory receptors. Such inhibition would induce a disinhibition of the pyramidal gluta-

matergic neurons. They proposed that these neurons project to the brainstem PS-on neurons and induced PS by this means. However, this proposed mechanism is not possible since BLA neurons do not directly project to the brainstem. They project, instead, to central amygdala GABAergic neurons which are projecting to the brainstem. These neurons indeed project to vLPAG PS-off neurons and could therefore inhibit them and by this means induce a disinhibition of SLD glutamatergic neurons inducing PS. BLA neurons have also strong reciprocal connections with the ventral hippocampus, nucleus accumbens and medial prefrontal cortex. It is well accepted that these pathways play a role in emotional memory [64]. One possibility is that when an emotional memory stored in the hippocampus is reactivated at the transition between SWS and PS it induces DA release in the BLA and a subsequent increase of PS to consolidate or erase such memories. Further analyses are needed to test such hypothesis and to determine the neuronal networks activated by the activation of the BLA at the transition between SWS and PS. Whole brain cFos analysis after DA stimulation of the BLA could provide a key element in that regard.

## 6. The limited cortical activation compared to wakefulness

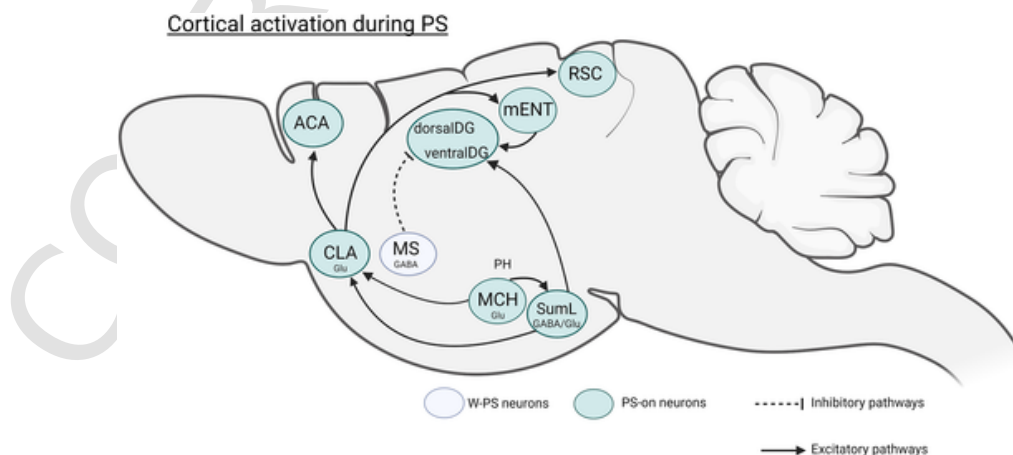
It has been shown that during PS hypersomnia compared to control and PS deprivation, the dentate gyrus (DG), claustrum, parasubiculum, presubiculum, cortical amygdaloid nucleus, anterior cingulate, medial entorhinal and retrosplenial cortices (RSC) are the only cortical structures containing neurons with an increased expression of Bdnf, cFos and/or Arc, known markers of activation and/or synaptic plasticity [65]. Among these cortical areas, DG was the only structure that contained more cFos-labeled neurons during PS hypersomnia than during wakefulness [65]. In addition, using a new type of genetically modified mice (TRAP2) allowing us to determine in the same animal whether the same neurons are activated during two states, we recently reported that different neurons are activated during W and PS in the retrosplenial and anterior cingulate cortices, claustrum and dentate gyrus *Fare clic o toccare qui per immettere il testo* [66,67]. (Fig. 3). Corroborating these results, it has been shown that the RSC contains many neurons specifically activated during PS and phase-locked with theta oscillations [68]. It was also recently confirmed using calcium imaging that the RSC is the only neocortical structure significantly more activated during PS than W and SWS [69]. Further, neurons more activated during PS were located in layer 2/3 and not layer 5 like those activated during wakefulness [69]. Finally, the inactivation of the retrosplenial cortex induced a

shortening of PS, a disappearance of facial movements and a decrease in sympathetic tone [69].

In contrast, the number of cFos-labeled neurons was very low in the somatosensory cortices after PS hypersomnia compared to PS deprivation and wakefulness indicating that these cortices are rather deactivated during PS compared to wakefulness [65]. These results are in line with the finding of local slow waves in the middle and the superficial layers of primary sensory and motor cortices [70,71]. Further, the activity of pyramidal cells within somatosensory cortices is decreased during PS compared to SWS and wakefulness [72]. Such decrease was accompanied by activation of parvalbumin inhibitory interneurons [72]. Interestingly in that regard, It has been also shown in mice, that the somatic activity of pyramidal neurons of the dorsal prefrontal cortex (dPFC) is decreased during PS compared to other states because their soma are inhibited by parvalbumin (PV) interneurons [73]. However, these authors further found that the dendrites of pyramidal neurons are disinhibited during PS by the activation of vasoactive intestinal peptide (VIP) interneurons inhibiting somatostatin interneurons responsible for dendritic inhibition. Further, they demonstrate that optogenetic inhibition of PS-active neurons from the central medial thalamus that project to the dPFC induces the inactivation of parvalbumin interneurons and the activation of pyramidal neurons. The origin of the activation of VIP interneurons during PS remains to be determined.

Finally, it was recently shown using calcium imaging that pyramidal neurons of the infralimbic cortex and prelimbic cortices projecting to the lateral hypothalamic area are more active during PS than the other states [75]. It was further shown that optogenetic activation of these neurons induced a 20 % increase in PS and conversely a 20 % decrease in SWS quantities. Inhibition of these neurons during PS shortened PS episodes and decreased phasic PS [75]. These results are at variance with the data showing that the soma of dPFC neurons are inhibited during PS [72]. More data is now necessary to confirm the data obtained and to extend them to other prefrontal regions.

In summary, recent results strongly suggest that only a few limbic cortical structures are strongly activated during PS whereas other cortices such as the somatosensory one are rather deactivated. It has also been shown that PFC pyramidal soma are inactivated while their dendrites are activated. Finally, the fact that RSC inhibition strongly decreases PS duration and that this structure contains neurons specifically active during PS suggest that such cortical regions could play an active role in the regulation of PS quantity and its function (see below).



**Fig. 3.** Cortical activation during PS.

Cortical activation during PS is limited to a few limbic cortices (the retrosplenial, anterior cingulate and medial entorhinal cortices and the dentate gyrus). The activation of the the retrosplenial, anterior cingulate and medial entorhinal cortices and the ventral dentate gyrus would be induced by direct and indirect projections from the claustrum. The dorsal DG would be activated by a projection from GABA/glutamatergic neurons located in the lateral supramammillary nucleus. The MCH neurons would excite and induce the activation of the neurons of the claustrum and the lateral supramammillary nucleus. In addition the GABAergic neurons of the medial septum would play a crucial role in the genesis of the theta waves recorded in the hippocampus and the retrosplenial cortex.

## 7. Identification of the subcortical structures activating the cortex during PS

It has been shown that the claustrum is the only subcortical structure containing a large number of cFos-positive neurons projecting to the anterior cingulate and RSC after PS hypersomnia [65]. In addition, the lateral supramammillary nucleus (SumL) was the only subcortical structure containing cFos-positive neurons projecting to the dorsal part of the DG [65] whereas the medial entorhinal cortex was the only structure containing many cFos-labeled neurons projecting to the ventral part of the DG [76]. After lesion of the SumL, cFos-labeled neurons were no longer visible in the dorsal, but were still visible in the ventral, DG after PS hypersomnia and theta power was decreased during PS [76]. Of particular interest, SumL neurons activated during PS hypersomnia and projecting to the DG display a dual glutamatergic and GABAergic phenotype [76,77] (Fig. 3). Finally, optogenetic activation of the SumL-DG pathway increased both the power and frequency of theta oscillations as well as gamma power in the DG during PS [78]. Based on these results, it has been proposed that SumL neurons could simultaneously hyperpolarize the soma and depolarize the dendrites of dentate granule neurons [77]. The release of glutamate on the dendrites of granule neurons during PS could activate NMDA receptors and initiate an intracellular cascade inducing the expression of genes involved in synaptic plasticity like cFos, Bdnf, Arc and Cox2. The fact that only a subset of granule cells expresses these proteins during PS hypersomnia suggests that these neurons might receive additional excitatory input during PS from other afferent structures such as the medial entorhinal cortex. It has also recently been shown that glutamatergic neurons located in the claustrum project to the medial entorhinal cortex [79]. It is therefore likely that the claustrum is also responsible for the direct and indirect activation of the medial entorhinal cortex and the ventral dentate gyrus, although it remains to be demonstrated. Besides, optogenetic silencing during PS of GABAergic neurons within the medial septum dramatically reduces theta power indicating that these neurons also contribute to PS-specific hippocampal activity [80]. Finally, it has been shown that basal forebrain acetylcholine release during PS is significantly greater than during wakefulness suggesting that it could play a role in cortical activation during PS [81]. Further, it was recently shown that the amount of cholinergic innervation is negatively correlated with the likelihood of a cortical region to exhibit synchronized activity during PS whereas the midline desynchronized cortical areas receive the strongest cholinergic innervation from the BF [71]. These results suggest that acetylcholine release is contributing to localized neocortical activation during PS.

Altogether, these results indicate that GABAergic neurons of the medial septum strongly promote theta oscillation in hippocampal neurons during PS while those of the SumL activate specifically dorsal DG granule cells and increase theta pacing. They also suggest that glutamatergic neurons of the claustrum activate anterior cingulate and retrosplenial cortices and also likely the medial entorhinal cortex during PS (Fig. 3). One important remaining question is to determine which structure(s) involved in PS generation is (are) responsible for activating the medial septum, claustrum and SumL. The MCH neurons could be responsible since they are excitatory and project to all these structures [84,85].

## 8. Function (s) of the limbic cortical activation during PS

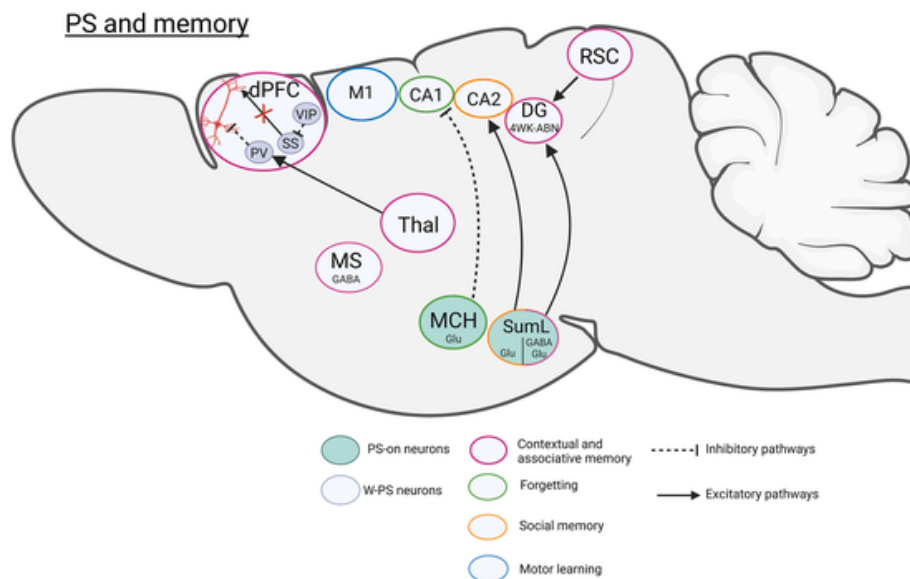
Optogenetic inhibition during PS of GABAergic neurons in the medial septum known to project to the hippocampus, medial entorhinal and RSC cortices decreased theta power and induced contextual memory impairments [80]. Besides, Kumar and colleagues [87] found that optogenetic silencing specifically of 4-weeks adult born dentate gyrus granule cells activity during PS alters the structural remodeling of spines on adult born neurons dendrites and impairs spatial memory consolidation. These results provided a causal link between the activity

of these neurons during PS and spatial memory consolidation. Recently, Qin et al. [88] also found that neurons of the supramammillary nucleus (SuM) projecting to CA2 are highly active during PS and nearly inactive during SWS or quiet wakefulness. PS-selective optogenetic silencing of these neurons impaired social memory (Fig. 4). By contrast, the silencing of PS-active SuM neurons that project to the DG impaired spatial memory but had no effect on social memory [88]. We also recently found that a training-induced modulation of causality between hippocampus and RSC during PS was correlated with spatial memory retrieval 24 h later. Retrieval was positively correlated with an increased influence of the RSC over the hippocampus during PS. Importantly, causality peaks during theta acceleration occur in synchrony with phasic PS. Altogether, these results open a window for the study of the contribution of phasic PS in hippocampo-cortical memory consolidation and suggest that causality modulation between RSC and hippocampus during PS plays a functional role in the phenomena [89]. It has been also shown that PS prunes newly formed small postsynaptic dendritic spines on pyramidal neurons in the motor cortex both during development and after exposure to a rotarod motor task and on the contrary strengthen and maintain the larger newly formed spines [90]. These authors also showed that calcium spikes within dendrites are increased during PS. Such processes of selective spine modifications could play a key role in learning and memory consolidation. Recently, Aime et al. [73] showed in mice that unit activity of dPFC pyramidal neurons decreased during PS while their dendritic activity increased. They further showed that the soma of pyramidal neurons are inhibited during PS by parvalbumin (PV) interneurons. In contrast, their dendrites are disinhibited because vasoactive intestinal peptide (VIP) interneurons inhibit somatostatin interneurons responsible for dendritic inhibition. They also found that after inhibition of VIP interneurons during PS mice were less able to discriminate conditional auditory stimuli (CS) and the AMPA/NMDA ratio was decreased in pyramidal neurons. In contrast, PV interneuron inhibition induced a significant increase in CS discrimination and an increase in the AMPA/NMDA ratio in pyramidal neurons.

Besides, induction of both long-term synaptic potentiation (LTP) and depression (LTD) in CA1 were also impaired in MCH receptor 1 knockout (R1-/-) mice [91]. It was also shown that MCH/ataxin3 mice were slow in performing a short-term memory T-maze task, displayed retardation in acquiring a reference memory task in a Morris water maze, and showed a habituation deficit in an open field task [92]. Activation or inhibition of MCH neurons impairs or improves hippocampus-dependent memories, respectively. Activation of MCH axonal terminals *in vitro* reduced firing of hippocampal pyramidal neurons by increasing inhibitory inputs, suggesting that the activation of the MCH projection to the hippocampus during PS may contribute to forgetting [53]. Altogether, these results indicate that MCH activation during PS plays a role in memory consolidation. The link between such a role and that of activation of the medial septum GABAergic neurons, dentate adult born neurons and prefrontal pyramidal neurons remains to be identified. Further, the function in memory consolidation of additional cortical structures activated during PS such as the retrosplenial and medial entorhinal cortices and the claustrum remains to be determined.

In summary, a large body of evidence in rodents indicates that PS is involved in both spatial and social memory consolidation and forgetting (Fig. 4). Such processes have been identified in the hippocampus but also in the motor, prefrontal and retrosplenial cortices. Based on these and our results, we propose that the retrosplenial and the medial entorhinal cortices are at the origin of the replay of memories during PS. Further, we hypothesize that they may stimulate subcortical PS generating structures such as the MCH neurons to increase the quantity of PS required to consolidate or erase memories. Besides, the role of the activation of the BLA in memory consolidation and the pathways involved remain to be identified. Finally, it is also likely that activation of all these structures during PS plays a role well beyond consolidation of





**Fig. 4.** PS and memory.

During PS, learning and memory consolidation occur in the prefrontal cortex, motor cortex and retrosplenial cortex (RSC) and in the dentate gyrus (DG). The medial septum (MS) induces theta in the hippocampus and retrosplenial cortex during PS and plays a role in memory consolidation. The pathway from the supramammillary nucleus (SuM) to the DG activates all granule cells during PS. We propose that in addition the pathway from the RSC to the hippocampus more strongly activates 4-weeks adult born (4WK-ABN) neurons forming memory engrams to consolidate spatial memory. Another set of glutamatergic supramammillary neurons activates during PS the CA2 pyramidal neurons to induce memory consolidation of social memory. Besides, the MCH neurons excite local GABAergic neurons in CA1 and by this mean induce inhibition of CA1 pyramidal neurons and forgetting. Motor learning is also consolidated during PS by means of pruning newly formed small postsynaptic dendritic spines and on the contrary strengthening and maintaining the larger newly formed spines on pyramidal neurons in the primary motor cortex (M1) both during development and after exposure to a motor task. Finally, memory consolidation is also induced by reactivation of dendrites of pyramidal neurons of the dorsal prefrontal cortex (dPFC) during PS. Such reactivation is induced by the disinhibition of their dendrites by the removal of the input of somatostatin interneurons inhibited during PS by the parvalbumin interneurons excited by a pathway originating from the thalamus.

recent memories. Indeed, PS is highly expressed during development and strongly modified by stress and in mood disorders. Its role in long-lasting memory and insight has also not yet been explored. Additional works are therefore necessary to explore its involvement in such functions by examining the activity of the limbic cortices more broadly than in control PS episodes or after recent learning.

**9. General conclusion**

Considering available data, it can be concluded that as stated in the past, the brainstem is necessary and sufficient to generate a state with muscle atonia and REMs. However, growing body of evidence indicates that the posterior hypothalamus controls the brainstem and PS occurrence by descending pathways. Finally, although there is recent evidence that the cortex and the amygdala directly control PS occurrence, more data are necessary to determine whether such effects are direct or indirect and linked to the functions of the state, such as emotional control and memory consolidation.

**Practice points**

- Muscle atonia during PS is produced by a network of glutamatergic and GABA/Glycinergic neurons located in the brainstem.
- GABAergic and MCH (glutamatergic) PS-on neurons located in the posterior hypothalamus control PS expression by means of descending projections to the brainstem GABAergic PS-off structures.
- The claustrum and the lateral supramammillary nucleus activate the limbic cortex during PS.
- A limited number of cortical limbic areas control PS duration and play a role in memory consolidation during PS.

**Research agenda**

- The mechanisms responsible for PS homeostasis need to be identified. It could be taking place in the PS-off GABAergic neurons of the brainstem, the MCH or GABAergic PS-on PH neurons or the PS-on neurons of the lateral preoptic area.
- The exact role of the basolateral amygdala in PS control needs to be examined in further detail. It could be implicated in emotional memory processing during the state.
- The role played by the lateral preoptic area in PS regulation needs to be further assessed. It could control PS-on neurons of the posterior hypothalamus.
- The role of the different subpopulations of neurons in PS of the posterior hypothalamus beyond the MCH neurons like those expressing Lhx6 should be identified.
- The respective functions and links between each cortical limbic region activated during PS need to be identified.
- The role of the claustrum in the activation of the limbic neocortical structures during PS should be confirmed.
- The potential role of PS in functions other than short memory consolidation like life-long lasting memory, development and mood regulation needs to be identified

**Uncited references**

[]; []; []; [].

**Declaration of competing interest**

The authors do not have any conflicts of interest to disclose.

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## Abbreviations

|         |                                               |
|---------|-----------------------------------------------|
| 4WK-ABN | 4 weeks adult born neurons                    |
| 5-HT    | serotonin                                     |
| ACA     | anterior cingulate cortex                     |
| Ach     | acetylcholine                                 |
| BF      | Basal Forebrain                               |
| BLA     | basolateral amygdala                          |
| CLA     | claustrum                                     |
| DA      | dopaminergic                                  |
| dDG     | dorsal dentate gyrus                          |
| dPFC    | dorsal prefrontal cortex                      |
| DPGi    | dorsal paragigantocellular reticular nucleus  |
| dDpMe   | dorsal deep mesencephalic reticular nucleus   |
| DRN     | dorsal raphe nucleus                          |
| GiV     | ventral gigantocellular reticular nucleus     |
| Glu     | glutamate                                     |
| Gly     | glycine                                       |
| Hcrt    | hypocretin/orexin containing neurons          |
| His     | histamine                                     |
| LC      | locus coeruleus                               |
| LHA     | lateral hypothalamic area                     |
| LPGi    | lateral paragigantocellular reticular nucleus |
| LTP     | long-term synaptic potentiation               |
| MCH     | melanin concentrating hormone                 |
| M1      | primary motor cortex                          |
| mENT    | medial entorhinal cortex                      |
| MS      | medial septum                                 |
| PeF     | perifornical area                             |
| PH      | posterior hypothalamus                        |
| PS      | paradoxical (REM) sleep                       |
| PV      | parvalbumin                                   |
| REMs    | rapid eye movements                           |
| RSC     | retrosplenial cortex                          |
| SCN     | suprachiasmatic nucleus                       |
| SLD     | sublaterodorsal nucleus                       |
| SumL    | Lateral supramammillary nucleus               |
| SWS     | slow wave sleep                               |
| Thal    | thalamus                                      |
| TMN     | tuberomammillary nucleus                      |
| vDG     | ventral dentate gyrus                         |
| VIP     | vasoactive intestinal peptide                 |
| vIPAG   | ventrolateral periaqueductal gray             |
| VLPO    | ventrolateral preoptic nucleus                |
| VTA     | ventral tegmental area                        |
| W       | wake                                          |
| ZI      | zona incerta                                  |

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