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## The neuroanatomy and neurochemistry of sleep-wake control

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### Abstract

Sleep-wake control is dependent upon multiple brain areas widely distributed throughout the neural axis. Historically, the monoaminergic and cholinergic neurons of the ascending arousal system were the first to be discovered, and it was only relatively recently that GABAergic and glutamatergic wake- and sleep-promoting populations have been identified. Contemporary advances in molecular-genetic tools have revealed both the complexity and heterogeneity of GABAergic NREM sleep-promoting neurons as well as REM sleep-regulating populations in the brainstem such as glutamatergic neurons in the sublateral nucleus.

The sleep-wake cycle progresses from periods of wakefulness to non-rapid eye movement (NREM) sleep and subsequently rapid eye movement (REM) sleep. Each vigilance stage is controlled by multiple neuronal populations, via a complex regulation that is still incompletely understood. In recent years the field has seen a proliferation in the identification and characterization of new neuronal populations involved in sleep-wake control thanks to newer, more powerful molecular genetic tools that are able to reveal neurophysiological functions via selective activation, inhibition and lesion of neuroanatomically defined sub-types of neurons that are widespread in the brain, such as GABAergic and glutamatergic neurons.<sup>1,2</sup>

### Keywords

sleep-wake circuitry; basal forebrain; dorsal raphe; laterodorsal and pedunculopontine tegmental nuclei; lateral hypothalamus; locus coeruleus; nucleus accumbens; parabrachial nucleus; parafacial zone; rostromedial tegmental nucleus; sublateral nucleus; tuberomammillary nucleus; ventrolateral periaqueductal gray; ventrolateral preoptic area; ventral medulla; ventral tegmental area; zona incerta

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Conflict of Interest Statement

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## Wakefulness

In the wakeful state, the brain is highly active and the cerebral cortex electroencephalogram (EEG) is desynchronized. Cortical desynchronization results from excitatory pre-synaptic inputs from subcortical wake-promoting neuronal populations. In early experiments performed in the 1940s, Maruzzi and Magoun first discovered that stimulation of the area between the pons and the midbrain produced wakefulness. They named this the reticular activating system. Since then, most of these neuronal populations and their ascending projections as well as neurochemical signatures have been extensively studied and are reviewed in multiple review articles.<sup>3–7</sup> They include the cholinergic basal forebrain (BF),<sup>8</sup> the histaminergic tuberomammillary nucleus (TMN),<sup>9</sup> the orexinergic lateral hypothalamus,<sup>10</sup> the dopaminergic ventrolateral tegmental nucleus (VTA),<sup>11</sup> the cholinergic laterodorsal and pedunculopontine tegmental nuclei (LDT/PPT),<sup>12</sup> the glutamatergic parabrachial nucleus (PB)<sup>13</sup> and the noradrenergic locus coeruleus (LC).<sup>14</sup> Generally, the glutamatergic, cholinergic, noradrenergic and dopaminergic neurons of the reticular formation project via two main pathways. One is a dorsal pathway to the thalamus, which then relays the arousal signals through thalamocortical neurons. The second is ventral pathway, via the basal forebrain (BF), posterior and lateral hypothalamus (LH), and the medial forebrain bundle<sup>15</sup> which contain neurons projecting to the cortex as well (Figure 1).

Different wake-promoting neurotransmitters seem to be specialized in promoting different aspects of wakefulness. For instance, acetylcholine is involved in cognitive functions, while noradrenaline is involved in salient experiences such as novelty and stress, as well as being involved in cognitive functions.<sup>16,17</sup> Similarly, orexin is responsible for both consolidated wakefulness—narcolepsy being the result of the absence of this neurotransmitter—while it is also involved in motivated behaviors such as eating<sup>18</sup> and drug seeking.<sup>19</sup> And dopamine, which is also involved in a large number of motivated behaviors, has wake-regulatory activity, particularly during presentation of salient stimuli.<sup>20,21</sup>

Recent studies have identified GABAergic and glutamatergic wake-promoting systems. Within the BF, it was recently shown that cholinergic neurons are not wake-promoting per se but inhibit cortical synchronization.<sup>22,23</sup> Instead, GABAergic BF neurons are responsible for cortical desynchronization and wakefulness.<sup>22,24</sup> Similarly, it has recently been shown that in the LDT/PPT, cholinergic neurons inhibit cortical slow oscillations during NREM sleep and glutamatergic neurons are primarily responsible for wakefulness.<sup>12</sup> An elegant series of studies has revealed that arousal from hypercapnia, as during apneic events, is accomplished via glutamatergic projections to the BF, central nucleus of the amygdala (CeA) and LH, from neurons in the lateral PB expressing calcitonin gene-related peptide.<sup>13</sup> The posterior hypothalamus also contains GABAergic wake-promoting neurons.<sup>25,26</sup> Within the LC, GABAergic interneurons could actively inhibit the wake-promoting noradrenergic neurons, when activated by prefrontal cortex neurons.<sup>27</sup> Yet the cortex also promotes wakefulness through descending, likely glutamatergic, projections to the LC,<sup>28</sup> which is similar to what is found in the neighboring PB.<sup>29</sup> The reciprocal projections between the cortex and these, and possibly other wake-promoting areas, suggest that the cortex itself can exert executive control over wakefulness.

GABAergic projections to the VTA, from the nucleus accumbens (NAc)<sup>30</sup> and the superior colliculus,<sup>31</sup> play a role in the wake-promoting action of VTA dopaminergic neurons. The VTA also contains glutamatergic neurons that promote wakefulness via projections to the LH and the NAc.<sup>32</sup> And finally, GABAergic neurons located in the bed nucleus of the stria terminalis promote wakefulness via orexinergic mechanism.<sup>33</sup> Since these circuits all ultimately converge on the dopaminergic reward system, they provide a pathway for both wake-promoting drugs of abuse such as methamphetamine as well as the narcoleptic treatment modafinil.

The role of the thalamus in wakefulness is controversial. Large cell body lesions of the thalamus do not affect sleep-wake quantity.<sup>34</sup> However, high frequency optogenetic stimulation of dorsomedial thalamus (DMT) calretinin-expressing neurons, paraventricular thalamic glutamatergic neurons and ventromedial thalamic neurons induces wakefulness and locomotor activity.<sup>35–37</sup> Nonetheless, most common general anesthetics suppress cortico-thalamo-cortical activity,<sup>38</sup> and this circuit plays a role in emergence from anesthesia.<sup>39</sup>

## NREM sleep

In the sequential order of sleep-wake cycles, the first to appear following wakefulness is NREM sleep, a state of cortical EEG synchronization and low sub-cortical activity.<sup>40,41</sup> Originally, it was believed that NREM sleep results in the cessation of sub-cortical excitatory inputs to the cortex, yet the recent discovery of many sub-cortical systems promoting NREM sleep, defined as being necessary for normal NREM sleep amount and sufficient to enhance NREM sleep, has changed our understanding of sleep control (Figure 2).

Most of the sub-cortical sleep-promoting systems are GABAergic, and like the wake-promoting systems they too are distributed throughout the brain. The ventrolateral preoptic area (VLPO), located in the anterior hypothalamus, was the first known NREM sleep-promoting node. It contains inhibitory GABAergic/galaninergic neurons that are active during sleep,<sup>42,43</sup> and project to and inhibit wake-promoting systems.<sup>44</sup> Chemogenetic activation of VLPO galaninergic neurons increases NREM sleep amount and decreases REM sleep amount, while also significantly attenuating body temperature.<sup>45</sup> The intersection of body temperature and sleep promotion is not uncommon for GABAergic neurons of the preoptic area in general,<sup>46</sup> which suggests that these neurons may underlie the decrease in body temperature associated with NREM sleep.

Cortical EEG activity characteristic for each vigilance stage has long been believed to be driven by sub-cortical inputs. However, recent findings indicate that the cortex itself could also actively contribute to sleep regulation. Cortical interneurons expressing neuronal nitric oxide synthase (nNOS) control both NREM amount and slow wave activity (SWA, a marker of NREM sleep depth). Interestingly, parvalbumin- and somatostatin-expressing cortical interneurons have recently been shown to be responsible for the propagation of slow waves in the cortex.<sup>47,48</sup> Finally, pyramidal neurons project to and regulate sub-cortical sleep-wake systems,<sup>27</sup> including the thalamus to promote cortical synchronization.<sup>49</sup> Thus, the cortex may also exert executive control over sleep, similar to that described above for wakefulness.

The parafacial zone (PZ) was the second sub-cortical system discovered to be involved in NREM sleep control.<sup>50</sup> GABAergic PZ neurons (PZ<sup>GABA</sup>) are both necessary and sufficient to induce deep NREM sleep, so called slow-wave-sleep (SWS) which is characterized by high amplitude cortical delta activity (Fig. 3B), a marker of cortical synchronization and sleep depth. Chemogenetic activation of PZ<sup>GABA</sup> rapidly induces SWS at the expense of both wakefulness and REM sleep.<sup>51</sup> Importantly, with SWS episodes being significantly longer in the 3–5 hour period after chemogenetic activation than during control conditions, the significant increase in SWS amounts can be attributed to the substantial increase in SWS consolidation (Figure 3). Moreover, the evoked SWS resembles SWS under control conditions in two key aspects. Firstly, it is possible to awaken the animals (Figure 3C). Secondly, unlike chemogenetic activation of VLPO galaninergic neurons,<sup>45</sup> animals exhibit body temperature that one would normally find during SWS (Figure 3A–B). Thus, PZ<sup>GABA</sup> activation induces a state that is neither akin to anesthesia nor torpor. Beyond that, increased SWS amount is associated with dramatically enhanced SWA, indicating a higher SWS quality,<sup>51</sup> and PZ<sup>GABA</sup> are so powerfully SWS-promoting that they can counteract the wake-promoting action of psychostimulants.<sup>52</sup> Finally, intermingled glutamatergic neurons, the only other known cell type in this region thus far, seem to not play any role in sleep control.<sup>53</sup>

As mentioned above, the VTA was classically believed to be a wake-promoting center via its dopaminergic projections. However, a series of recent studies have revealed a more complex role of VTA in sleep-wake control. VTA dopaminergic projections to the dorsal striatum seem to be specialized in sleep promotion whereas VTA dopaminergic projections to the ventral striatum promote wakefulness.<sup>54</sup> The VTA also contains GABAergic neurons that are both necessary and sufficient for sleep. Lesion and chemogenetic inhibition of VTA GABAergic neurons result in insomnia, while chemogenetic and optogenetic activation of these neurons promotes NREM sleep. The sleep-promoting action of VTA GABAergic neurons is mediated by local projections inhibiting VTA wake-promoting neurons and projections to the LH.<sup>32,55,56</sup>

Adenosine is a sleep factor which promotes NREM sleep via inhibition of wake-active neurons and also via activation of a subpopulation of VLPO sleep-promoting neurons.<sup>57</sup> More recently, a key neuronal population mediating adenosine's sleep-promoting action has been identified in the core region of the NAc. These neurons are inhibitory and express the adenosine receptor A<sub>2A</sub> (A<sub>2A</sub>R). They are not only necessary, but also sufficient to promote NREM sleep.<sup>58</sup> These neurons enhance NREM sleep via inhibitory projections to the ventral pallidum (VP).<sup>58</sup> A<sub>2A</sub>R-expressing neurons located in the rostral striatum are also NREM sleep-promoting via inhibition of parvalbumin-expressing neurons located in the external globus pallidus.<sup>59</sup>

The posterior hypothalamus not only contains wake-promoting GABAergic neurons (described above) but also neighboring NREM sleep-promoting GABAergic neurons, in the zona incerta (ZI).<sup>60</sup> ZI GABAergic neurons expressing the transcription factor Lhx6 are both sufficient and necessary for normal NREM sleep and REM sleep amount. They could control sleep by inhibiting the wake-promoting orexinergic neurons.

Known for its REM sleep inhibitory action,<sup>61,62</sup> ventrolateral periaqueductal gray region (vlPAG) GABAergic neurons likely stabilize NREM sleep. The vlPAG contains neurons specifically active during NREM sleep.<sup>63</sup> Optogenetic activation of vlPAG GABAergic neurons increases NREM sleep episode durations and decreases REM sleep episode durations.<sup>64</sup> It has recently been shown that rostromedial tegmental nucleus (RMTg) GABAergic neurons are involved in NREM sleep control.<sup>65</sup> Lesioning or inhibition of RMTg neurons decreases NREM sleep amount whereas their activation increases NREM sleep amount. This effect could be mediated by inhibition of dopaminergic neurons located in the substantia nigra compacta (SNc) and in the VTA.

Interestingly, though most of the NREM sleep-promoting neuronal populations are GABAergic/inhibitory, including a recently discovered population of GABAergic neurons co-expressing neurotensin in the CeA,<sup>66</sup> two new glutamatergic NREM sleep-promoting neuronal populations were recently described. First, mainly REM sleep- or NREM/REM sleep-active neurons were identified in the deep mesencephalic nucleus (DpMe).<sup>63</sup> Specific chemogenetic activation of DpMe glutamatergic neurons increases NREM sleep amounts and decreases REM sleep amounts.<sup>67</sup> Second, perioloculomotor glutamatergic neurons promote NREM sleep via projections to the preoptic area and to the ventromedial medulla.<sup>68</sup>

Finally, the rostro-ventral medullary reticular formation contains neurons that are specifically active during NREM sleep and could be involved in wakefulness to NREM sleep and NREM sleep to REM sleep transitions.<sup>69</sup> The neurochemical identity, however, remains to be determined and necessity and sufficiency need to be confirmed using lesion/inhibition and activation of these neurons.

## REM sleep

REM sleep was first described in humans as a stage of sleep associated with rapid eye movements (REM).<sup>70</sup> Soon after, REM sleep was characterized in cat as the stage in which one observes both an active cortical EEG and muscle atonia and was therefore also named “paradoxical sleep” (PS).<sup>71</sup> Most of the neurons involved in REM sleep control are located in the brainstem and comprise both REM-on (PS-on) and REM-off (PS-off) neurons (Figure 4).<sup>61,72</sup> REM-on neurons are located in the sublaterodorsal nucleus (SLD; also called per-LC $\alpha$  in cat). The SLD contains glutamatergic neurons that actively promote all features of REM sleep<sup>53</sup> and can be divided into two populations, one projecting rostral to the forebrain and involved in cortical activation and hippocampal theta activity, the other projecting caudally to the ventromedial medulla (VM) and spinal motoneurons to actively promote muscle atonia. The SLD seems to also contain GABAergic neurons projecting to and inhibiting REM-off neurons.<sup>73</sup> REM-off neurons are located in the vlPAG (GABA), lateropontine tegmentum (GABA), LDT/PPT (acetylcholine), LC (noradrenaline) and dorsal raphe (DR; serotonin), and each of these populations project to and actively inhibit REM-on neurons. These findings were recently confirmed using more advanced molecular genetic tools.<sup>62</sup> Further, a hypothalamic regulation of REM sleep has recently been suggested. It involves GABAergic/galaninergic neurons from the dorsomedial hypothalamus<sup>74</sup> and melanin-concentrating hormone (MCH) from the lateral hypothalamus.<sup>75</sup> Finally, the VM seems to actively control REM sleep but the findings point to open

questions to be investigated. For instance, in the rat, complete lesioning of VM neurons or chronic inactivation of inhibitory neurotransmission specifically results in REM sleep without atonia.<sup>76,77</sup> Whereas in mice, the VM contains REM-on neurons and optogenetic activation of GABAergic neurons induces REM sleep.<sup>78</sup>

## Conclusion

Sleep-wake control is extremely complex, involving neuronal populations spread throughout the brain. Moreover, each vigilance stage is regulated by multiple brain areas and distinct neurochemical populations. The challenge for sleep research is now to understand how all of these neuronal populations interact and are synchronized to control sleep-wake cycle, under the homeostatic and circadian processes.<sup>3</sup>

## Abbreviations list

<b>A<sub>2A</sub>R</b>	adenosine receptor A <sub>2A</sub>
<b>BF</b>	basal forebrain
<b>CeA</b>	central nucleus of the amygdala
<b>CNO</b>	clozapine N-oxide
<b>DMT</b>	dorsomedial thalamus
<b>DpMe</b>	deep mesencephalic nucleus
<b>DR</b>	dorsal raphe
<b>EEG</b>	electroencephalogram
<b>LC</b>	locus coeruleus
<b>LDT</b>	laterodorsal tegmental nucleus
<b>LH</b>	lateral hypothalamus
<b>MCH</b>	melanin-concentrating hormone
<b>MN</b>	motoneuron
<b>NAc</b>	core region of the nucleus accumbens
<b>nNos</b>	neuronal nitric synthase
<b>NREM</b>	non rapid eye movements sleep
<b>NTS</b>	nucleus of the solitary tract
<b>PB</b>	parabrachial nucleus
<b>PPT</b>	pedunculopontine tegmental nucleus
<b>PS</b>	paradoxical sleep

<b>PZ</b>	parafacial zone
<b>PZ<sup>GABA</sup></b>	parafacial zone GABAergic neurons
<b>REM</b>	rapid eye movements sleep
<b>RMTg</b>	rostromedial tegmental nucleus
<b>SNe</b>	substantia nigra compacta
<b>SWA</b>	slow wave activity
<b>SWS</b>	slow-wave-sleep
<b>TMN</b>	tuberomamillary nucleus
<b>vlPAG</b>	ventrolateral periaqueductal gray region
<b>VLPO</b>	ventrolateral preoptic area
<b>VM</b>	ventromedial medulla
<b>VTa</b>	ventral tegmental area
<b>ZI</b>	zona incerta

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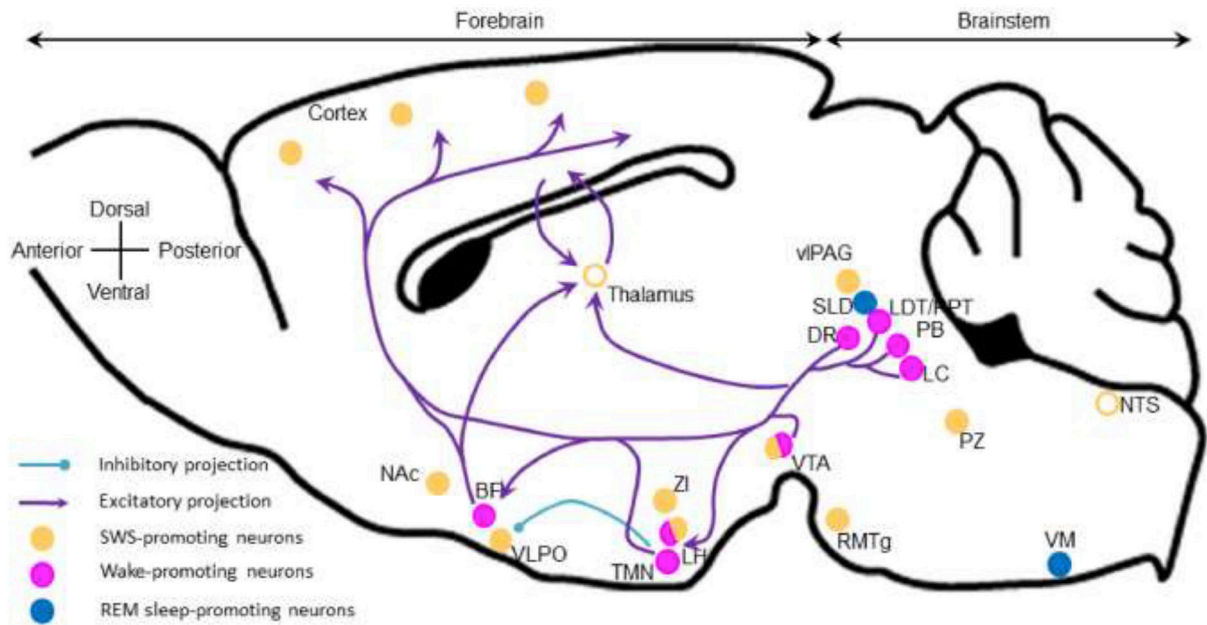


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with the early transection experiments in animals. More importantly, parafacial zone GABAergic neurons are critically involved in NREM sleep promotion and maintenance because it is one of the two neuronal populations able to induce consolidated NREM sleep and enhance cortical slow-wave activity. This study also provides a powerful model of slow-wave-sleep enhancement.

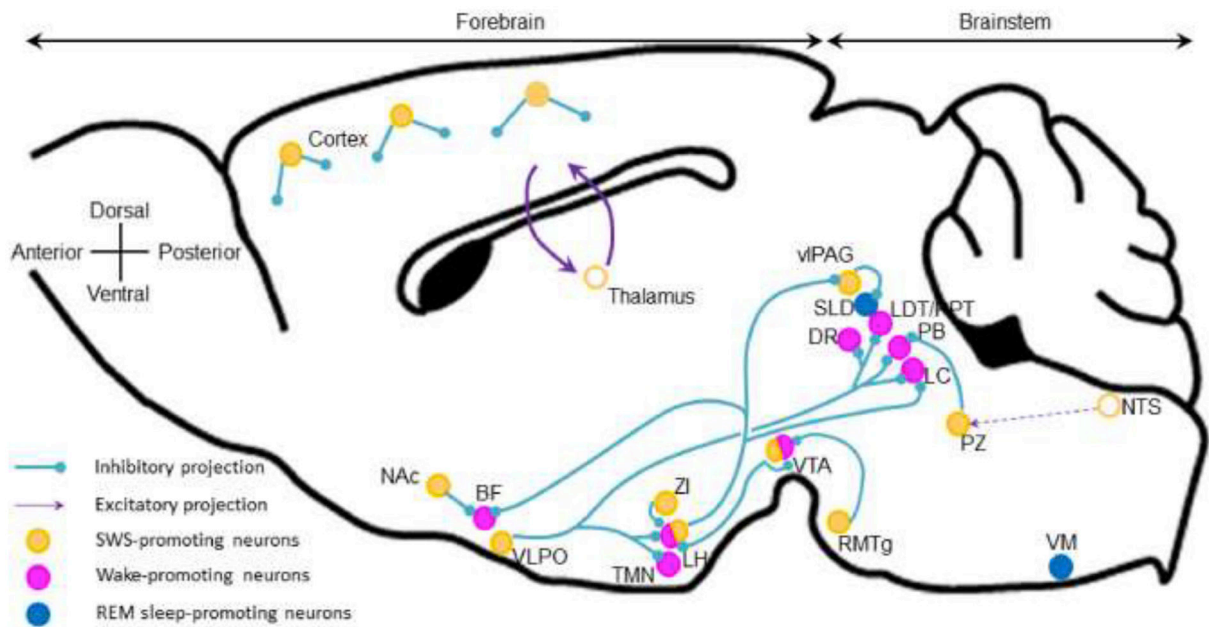
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**Figure 1: Wake-promoting nuclei and projections. Sagittal plane of a mouse brain.**

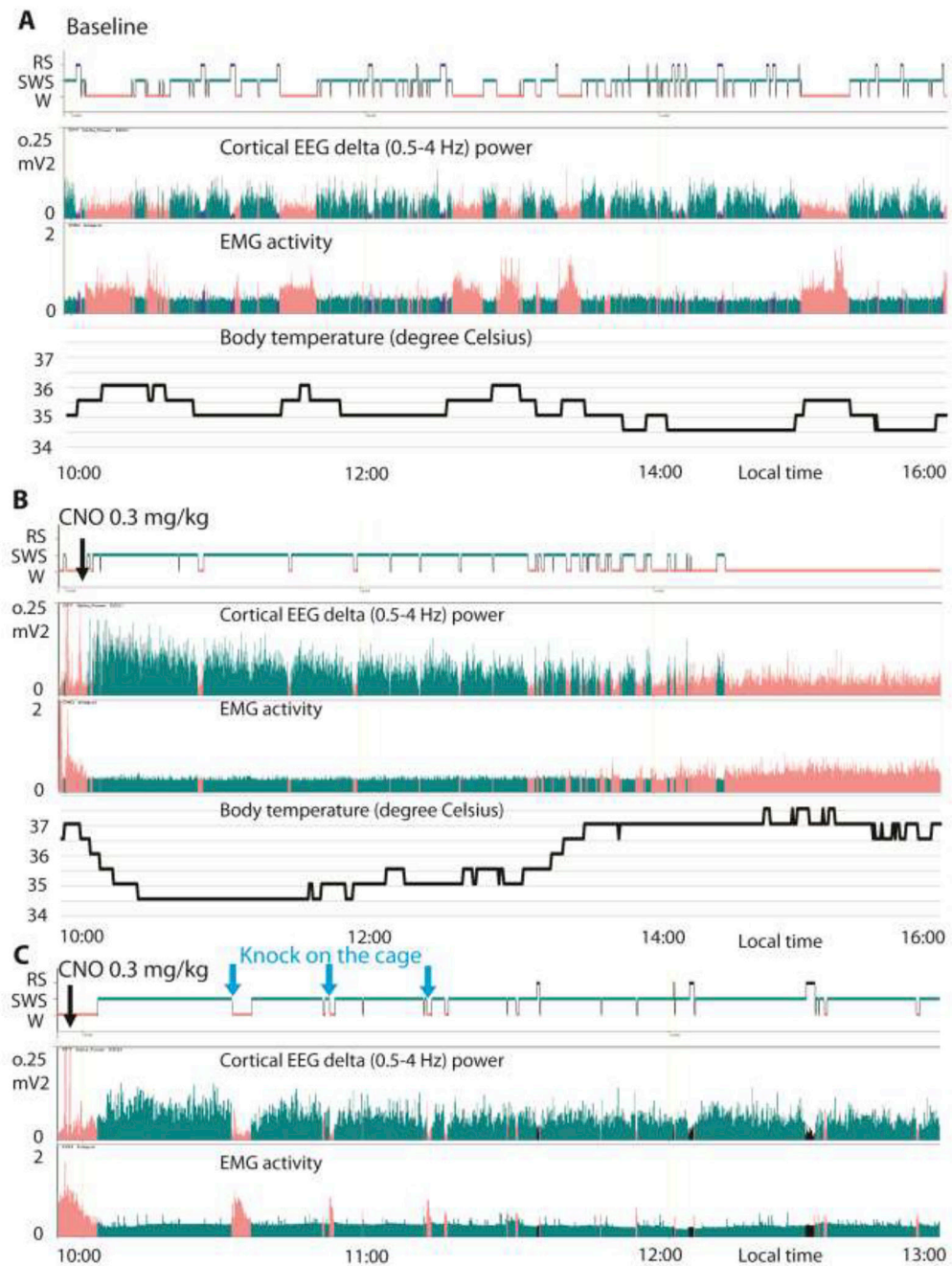
Wake-promoting neurons are located in the brainstem and in the forebrain, and project to the cortex to actively promote cortical activation and wakefulness. Histaminergic neurons located in the TMN actively inhibit VLPO sleep-promoting neurons. Open circles, brain areas contributing to sleep control but not sleep-promoting per se. BF, basal forebrain; DR, dorsal raphe; LDT/PPT, laterodorsal and pedunculopontine tegmental nuclei; LH, lateral hypothalamus; LC, locus coeruleus; NAc, nucleus accumbens; NTS, nucleus of the solitary tract; PB, parabrachial nucleus; PZ, parafacial zone; RMTg, rostromedial tegmental nucleus; SLD, sublaterodorsal nucleus; TMN, tuberomammillary nucleus; vPAG, ventrolateral periaqueductal gray; VLPO, ventrolateral preoptic area; VM, ventral medulla; VTA, ventral tegmental area; ZI, zona incerta.



**Figure 2: NREM sleep-promoting nuclei and projections.**

NREM-promoting neurons are distributed along the neural axis and also in the cortex. They are mainly GABAergic and inhibit wake-promoting systems. Cortical interneurons and the thalamo-cortico-thalamic feedback loop actively promotes cortical synchronization. Open circles, brain areas contributing to sleep control but not sleep-promoting per se. BF, basal forebrain; DR, dorsal raphe; LDT/PPT, laterodorsal and pedunculopontine tegmental nuclei; LH, lateral hypothalamus; LC, locus coeruleus; Nac, nucleus accumbens; NTS, nucleus of the solitary tract; PB, parabrachial nucleus; PZ, parafacial zone; RMTg, rostromedial tegmental nucleus; SLD, sublateralodorsal nucleus; TMN, tuberomamillary nucleus; vIPAG, ventrolateral periaqueductal gray; VLPO, ventrolateral preoptic area; VM, ventral medulla; VTA, ventral tegmental area; ZI, zona incerta.





**Figure 3: PZ chemogenetic activation of PZ GABAergic neurons promotes physiologic NREM sleep.**

(A-B) Example hypnogram, fast Fourier transform (FFT)-derived delta (0.5–4 Hz) power, EMG activity and body temperature over 6 h of spontaneous sleep-wake cycles (A) or following clozapine N-oxide (CNO, designer drug, 0.3 mg per kg, intraperitoneal, 10 A.M.) administration (B) in a mouse with bilateral hM3Dq (excitatory designer receptor) expression in PZ<sup>GABA</sup> neurons. Note that, as compared with spontaneous sleep, CNO injection rapidly induced consolidated slow-wave sleep, characterized by increased slow-wave activity (delta power), minimum muscle tone and a progressive decrease of body



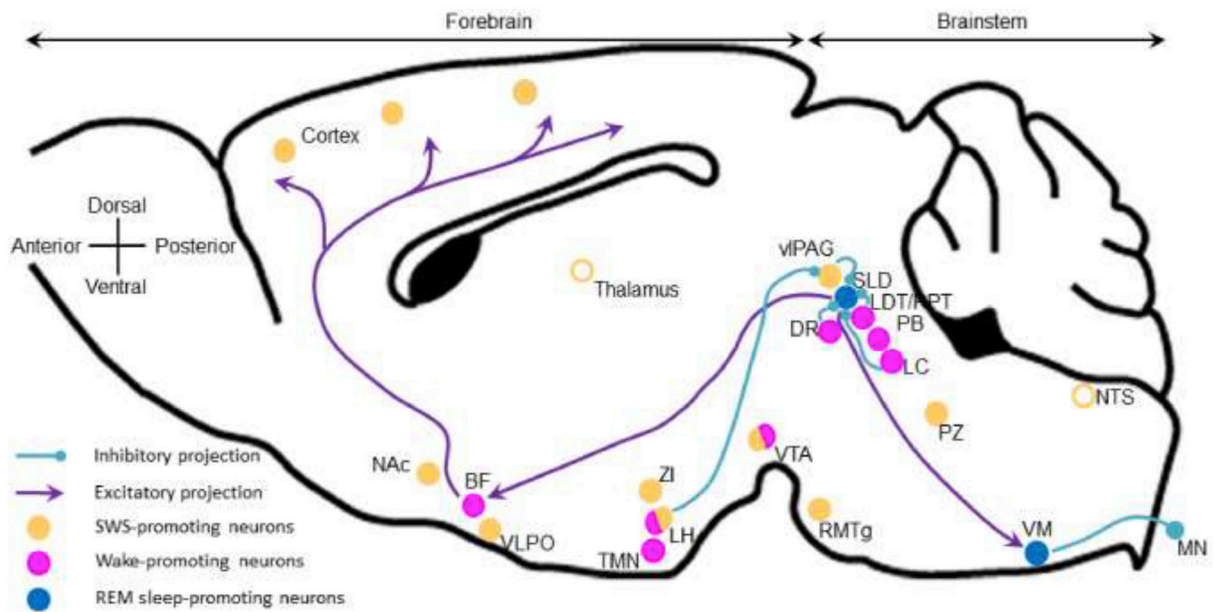
temperature down to the minimum body temperature recorded during spontaneous sleep. (C) Another mouse with bilateral hM3Dq was injected with CNO and woken up by knocking on the cage (blue arrows) three times, at 30, 50 and 70 min following CNO administration, during PZ<sup>GABA</sup> induced SWS. This example confirms the reversibility of the state, a criteria for sleep. Salmon = wakefulness (W); cyan = slow-wave-sleep (SWS); and black = REM sleep (RS).

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**Figure 4: REM sleep-promoting nuclei and projections.**

REM sleep is driven by the SLD which projects rostrally to promote cortical activation and caudally to actively drive muscle atonia. SLD REM sleep-promoting neurons are glutamatergic and receive inhibitory inputs from the pontine wake-promoting neurons. The LH contributes to REM sleep by inhibiting the vIPAG REM-off neurons. Open circles, brain areas contributing to sleep control but not sleep-promoting per se. BF, basal forebrain; DR, dorsal raphe; LDT/PPT, laterodorsal and pedunculopontine tegmental nuclei; LH, lateral hypothalamus; LC, locus coeruleus; MN, motoneurons; Nac, nucleus accumbens; NTS, nucleus of the solitary tract; PB, parabrachial nucleus; PZ, parafacial zone; RMTg, rostromedial tegmental nucleus; SLD, sublaterodorsal nucleus; TMN, tuberomamillary nucleus; vIPAG, ventrolateral periaqueductal gray; VLPO, ventrolateral preoptic area; VM, ventral medulla; VTA, ventral tegmental area; ZI, zona incerta.