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The role of the hypothalamic Lhx6 GABAergic neurons in REM sleep control

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A large body of data showed that although the brainstem is necessary and sufficient to generate REM sleep, the lateral hypothalamic area (LHA) and the ventral zona incerta (vZI) contain GABAergic and MCH neurons controlling the state by descending projections (1, 2). Indeed, a very large number of cFos-positive, REM-active cells was observed in both regions after REM sleep rebound in rats (3). Around 75% of these cFos-labeled cells express GAD67 mRNA and are likely GABAergic (2). Interestingly, it was then shown that 45% of the GABAergic neurons located in the mouse vZI express the LIM homeodomain factor (Lhx6) and are distinct from the REM-active MCH and the wake-active hypocretin neurons (4). It was also shown that Lhx6 vZI neurons increased cFos expression at lights on compared to lights off (4). We then more specifically showed that around 10% of the Lhx6 neurons in vZI are activated during REM sleep rebound occurring after deprivation in mice where they constitute one-third of the cFos-labeled neurons (5). Only 2% of the Lhx6 neurons in vZI were activated during wake. Altogether, these results suggested the involvement of vZI Lhx6 neurons in REM sleep but a direct functional demonstration was needed. In the paper published in this SLEEP issue, Vidal-Ortiz et al. (6) filled this gap by confirming that Lhx6 neurons are active during REM sleep and by demonstrating that they may play a role in REM sleep control. In the first part of their study, Vidal-Ortiz et al. (6) recorded specifically the activity of Lhx6 neurons in vZI during the sleep-waking cycle by using calcium imaging in Lhx6-cre mice. They found that 45,5% of the neurons recorded were maximally active during REM sleep and started firing 15s before bouts onset. Around 30% were maximally active during Wake, 11% during both Wake and REM sleep and 9% during NREM sleep. Interestingly, NREM neurons were also activated 15s before sleep onset. These results fit with previous findings that a large proportion of vGAT-positive, likely GABAergic neurons in vZI and LHA discharge maximally during REM sleep and that they anticipate REM sleep onset, indicating that they could play a role in triggering the state (7, 8).

In the second part of their study, Vidal-Ortiz et al. used unilateral optogenetic stimulations of Lhx6 neurons within vZI with a specific protocol of 1 min blue light on and 4 min off?? (6). The 4h stimulation at 1Hz and 5Hz?? induced significant increases in both REM sleep quantities and bouts numbers. These results indicate that even though the population of Lhx6 neurons in vZI is functionally heterogeneous, their global optogenetic activation significantly facilitates the expression of REM sleep, without any apparent impact on Wake and NREM.

In partial agreement with the present study, it has been previously shown that mice with a conditional deletion of *Lhx6* gene restricted to the hypothalamus show an increase in Wake time and a reduction of both NREM and REM sleep (4). The relative?? decrease of REM sleep was greater than that of NREM sleep. In the same study was further reported that chemogenetic activation of vZI Lhx6 neurons strongly increased REM sleep, slightly increased NREM and decrease Wake during 12h following CNO injection (4). Importantly, effects on NREM and W but not that on REM sleep were abolished after injection of suvorexant, an antagonist of hypocretin receptors (4) et alors ??????. In contrast, chemogenetic inhibition of Lhx6 neurons during the light phase induced a decrease in NREM and REM sleep and an increase in W (4). The optogenetic data of Vidal-Ortiz et al. reporting effects only on REM sleep partly fit with the chemogenetic ones (4, 6). WHY ???

In summary, the present paper combined with previous data convincingly shows that Lhx6 GABAergic neurons within vZI are implicated in REM sleep control. The open question is how they influence REM sleep and what is their specific role, compared for example to the contingent of MCH neurons within vZI and neighboring LHA.

It has been indeed previously shown with unit recordings in head-restrained rats that the MCH neurons fire specifically during REM sleep (9). These results were later confirmed by calcium imaging data showing that MCH neurons are less active during NREM sleep and quiet Wake

compared to REM sleep (10-12). In line with these functional data, hypothalamic MCH neurons strongly express cFos after REM sleep rebound but not after sustained Wake (3). As reported above for the Lhx6 neurons (6), the chronic (24h) optogenetic activation of MCH neurons results in increased REM sleep amounts (13). In addition, their acute optogenetic activation at the onset of SWS epoch did not increase NREM duration but increased the probability of NREM-to-REM sleep transition. This effect was also obtained using the chemogenetic approach (14). When optogenetic stimulations were applied at REM sleep onset, the duration of REM sleep episodes was significantly prolonged (15). Besides, while Lhx6 neurons express vGAT and are likely GABAergic in nature, recent studies demonstrated that MCH neurons express vGlut2, the vesicular glutamate transporter 2 (16, 17) and thus glutamatergic in nature. Altogether, these results indicate the existence of at least two distinctive populations of REM sleep-active neurons within vZI and LHA. The question still open is what are the respective roles and the downstream targets of these two populations?

It has been shown (qui ??) that Lhx6 neurons project to hypocretin and GABAergic neurons within LHA, to serotonergic wake-active neurons in dorsal raphe (DRN) and to the ventrolateral periaqueductal grey (vlPAG) known to contain GABAergic neurons gating REM sleep (review in Luppi et al., 2011). These efferent projections emanating from Lhx6 neurons might subserve their role in REM sleep expression (4)(Fig. 1). Further, their GABAergic inhibitory influence on LHA hypocretin and GABAergic neurons has been directly confirmed using optogenetic stimulation of Lhx6 neurons in brain slices (4). Interestingly, Lhx6 neurons also receive inputs from Wake-active and REM sleep-inhibiting neurons such as DRN serotonergic neurons and vlPAG GABAergic neurons. They also receive inputs from cholinergic neurons of the basal forebrain and brainstem pedunculopontine nucleus. These projections could inhibit Lhx6 neurons during Wake. (references de tout ca ????).

The MCH neurons might also promote REM sleep through their descending pathways to the REM-off vIPAG GABAergic and TMN histaminergic neurons. Indeed, 40% of the neurons projecting from the LHA and vZI express MCH (1) and inhibition of MCH terminals in the vIPAG significantly reduced NREM to REM sleep transition (18). In addition, optogenetic activation of MCH axons within TMN inhibits histaminergic cells through activation of GABA_A receptors (15). Since MCH neurons are now believed to be excitatory glutamatergic neurons, they might excite local inhibitory REM-on GABAergic neurons themselves inhibiting the above REM inhibiting systems (Fig. 1).

In summary, the present work of Vidal-Ortiz et al. (6) convincingly confirms that around half of the vZI Lhx6 neurons are involved in REM sleep. These results combined with previous ones clearly show that at least two different populations of vZI and LHA neurons, the Lhx6 and the MCH neurons, promote REM sleep. The fact that Lhx6 but not MCH neurons anticipate REM sleep onset might indicate that the former but not the latter are involved in REM sleep induction. Additional studies are strongly required to confirm that the MCH and Lhx6 are respectively glutamatergic and GABAergic neurons acting therefore on different downstream systems to promote REM sleep as we proposed (Fig. 1). The upstream systems controlling the activation of these neurons during REM sleep remain also to be identified. Further, there is still room for additional REM sleep controlling populations within vZI and LHA since these two populations constitute only a portion of numerous cFos-labeled neurons in these regions after REM sleep rebound (3, 5). Finally, the results of Vidal-Ortiz et al. (6) also show that Lhx6 are not homogeneous with a minority of them being active during NREM or Wake. Such heterogeneity is difficult to interpret in term of function and requires additional exploration.

Disclosure statement:

The author has nothing to disclose

Legend of Figure 1:

Two populations of neurons localized in the zona incerta (ZI) and the lateral hypothalamic area (LHA), the Lhx6 and MCH neurons are active during REM sleep. An additional population of GABAergic neurons is activated during REM sleep. We propose that during REM sleep, Lhx6 neurons inhibit Wake-active hypocretin and GABAergic neurons of the LHA as well as the REM-off serotonergic dorsal raphe neurons and GABAergic ones localized in the ventrolateral periaqueductal gray (vlPAG) and the dorsal deep mesencephalic reticular nucleus (dDPMe). On the other hand, the MCH neurons would excite GABAergic REM-on neurons localized in the LHA and the vlPAG and by this mean might inhibit the wake histaminergic neurons of the tuberomammillary nucleus and the REM-off neurons of the vlPAG/dDPMe.

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