Since the discovery of the MCH peptide\(^1\) and the identification of its receptor(s),\(^2\) experimental and clinical studies have demonstrated a role for the MCH peptide in many hypothalamic functions, from energy homeostasis and stress response to learning and memory, stress/anxiety, LTP, and sleep.\(^3\) This heterogeneity of functions is consistent with the broad distribution of MCH terminals\(^4\) and MCH receptors\(^5\) throughout the brain and suggests a modulatory mode of action. Whether this heterogeneity of brain functions results from the existence of subpopulations of MCH neurons or distinct terminal domains remains unknown.

Over the last decade, experimental studies based on correlational, lack- or gain-of-function evidence led to the consensus that MCH peptide facilitates NREM and REM sleep, suggesting a "MCH hypnogenic hypothesis." Importantly, juxtacellular recordings showed that MCH neurons are silent during wakefulness, while their discharge rate progressively increases during NREM sleep to be maximal during REM sleep.\(^6\) Unfortunately, genetic deletion models partially confirmed the hypnogenic hypothesis (see below), leaving the question about the precise role of MCH neurons (or MCH peptide) in regulating NREM and REM sleep unanswered. Thus, we and other have started to investigate the mechanisms underlying the control of sleep states by MCH neurons by combining optogenetic with electrophysiology and genetic engineering in mice.

The four commentaries\(^7\)-\(^10\) included in this critical forum all converge to four major points of discussion. Those include (1) the causality of the targeting networks in promoting or maintaining particular sleep-wake states, (2) the dynamics of peptide vs. transmitters release and their implication in sleep behavior, (3) the functional wiring of a single sleep circuit, and (4) its functional relevance in the complex sleep-wake networks of the brain. Those points are discussed below according to the three papers included in this forum, as well as experimental studies reported over the last years.

**MCH Neurons Action on Wake, NREM and REM Sleep**

**Wake**

It is assumed that neurons that discharge before or after the onset of a specific state participate in the onset or the maintenance, respectively, of that particular state (e.g., wake, NREM or REM). According to this view, MCH neurons, which are silent during wakefulness, progressively increase their discharges rate during NREM sleep and become maximally active during REM sleep\(^6\) suggesting that they would promote and maintain REM, rather than NREM sleep.

However, although silent during wakefulness, MCH peptide is implicated in energy homeostasis. Indeed, Blouin et al.\(^7\) found that the content of MCH peptide in the extracellular space of the human amygdala increased during and after eating, as well as at sleep onset. This result is consistent with MCH-induced dampening of energy expenditure\(^11\),\(^12\) and is in agreement with the MCH hypnogenic hypothesis. However, its level during stable sleep (i.e., in between behavioral transitions) remained similar to those during quiet wakefulness. Although it is still higher than during hyperarousal (emotion, pain, etc.), it contrasts with the activity of MCH neurons selectively during REM sleep.

Those differences could be partly explained by the microdialysis sample collection rate (every 15 minutes), which does not allow sufficient temporal resolution to differentiate a role in NREM versus REM sleep, as well as the short recovery period after surgical procedure and the possible influence of postoperative drugs on general behavior (sleep, mood, hedonia etc.).

**NREM vs REM Sleep**

The MCH hypogenic hypothesis was tested by Konadhode et al.\(^13\) using chronic optogenetic activation of MCH neurons randomly across the sleep-wake cycle and by ourselves using optogenetic stimulation that were time-locked to the onset of NREM or REM sleep state and terminated at the next transition (typically REM or wake). Konadhode et al. started their stimulation at the onset of the dark period and found an increase of both NREM and REM sleep associated with a decrease of wakefulness, supporting the general hypnogenic hypothesis. In contrast, our acute sleep-state specific optogenetic stimulation showed that MCH neurons have no effect on NREM sleep per se, but increase the number of NREM-to-REM sleep transitions and extend the duration of REM sleep. This was confirmed by SSFO (stabilized step-function opsin) experiments suggesting that our results were not due to an overstimulation of MCH system.

 Interestingly, Konadhode et al.\(^13\) confirmed previous pharmacological studies showing an increase of REM, but also NREM sleep,\(^14\)\(^-\)\(^18\) but their chronic optogenetic experiments made it difficult to assess the role of MCH neurons selectively in NREM or REM sleep. In such experimental conditions, it is possible that sustained activation of MCH neurons led to the accumulation of transmitters/modulators release in several brain areas that eventually increased NREM and REM sleep.
Whether this reflects the endogenous release of GABA, MCH, and other peptide released by MCH neurons during wakefulness is unclear.

Interestingly, the NREM effect in Konadhode et al., study suggests the existence of a subset of MCH cells (located in the dorsomedial hypothalamus) that may regulate NREM sleep. It is possible that this effect was masked in our experiments in which the vast majority (~90%) of MCH neurons in the lateral hypothalamus area was genetically targeted with opsins and activated by sufficient light power (calculation and test were based on previous optogenetic studies\(^\text{19,20}\)). Furthermore, the lack of NREM sleep effect is consistent with the idea that MCH neurons are minimally active during NREM sleep—\(^\text{6}\)—a result that was confirmed by our SSFO experiments—and may result from the gating/filtering of MCH neuronal hypnogenic signals by post-synaptic cells during NREM sleep.

In regard to the commentary by McGinty and Alam,\(^\text{9}\) activation of temperature-sensitive cells in the hypothalamus upon optogenetic stimulation is a valid point of discussion. However, we control for this possible bias by optically stimulating animals expressing YFP, but not opsin, protein. The absence of sleep quantities and mean duration changes in each of our control conditions ruled out a possible thermal effect.

**MCH Peptide vs GABA**

MCH neurons communicate through the release of GABA and presumably MCH, MCH-related peptide (NEI, NGE, and MGOP), Nesfatin-1, CART, and possibly unknown peptides. This question—What do MCH neurons release upon optogenetic stimulation?—was raised in all commentaries. One can assume that peptides are synaptically released upon burst of action potentials, whereas neurotransmitters are released upon slower, tonic activity.\(^\text{21}\) Thus, it is important to understand their respective actions on MCH neuron targets and ultimately their effect on sleep-wake states.

Although the firing rates of MCH neurons during REM sleep are low when averaging spike number over the total duration of REM sleep episode, interspike intervals (ISI) analysis revealed that MCH neurons can discharge at high-frequency rate (up to 60 Hz) during doublets (see Hassani and Jones commentary). This supports the use of burst mode optogenetic stimulation in our experimental procedures and further validates the use of SSFO to induce non-synchronous bursting discharges of MCH neurons. Whether “burst mode” firing induces MCH peptide release at the synapse remains unclear since technologies for measuring peptide release with high temporal resolution are not available.

Surprisingly, we found that genetic deletion of MCH receptor 1 had no effect on REM sleep prolongation, suggesting that GABA or peptides other than MCH are involved in acute modulation of REM sleep. Although this contrasts with previous pharmacological studies, it is consistent with the slow and long lasting action of peptide-activated G protein coupled receptors.\(^\text{22}\)

Importantly, our in vitro experiments suggest that high frequency (20 Hz) activation of MCH neurons induces the release of MCH peptide that act presynaptically on MCH terminals to potentiate GABA transmission, while low frequency (1 Hz) had no effect. Additional experiments are now required to verify how much MCH peptides is released upon optogenetic activation.

As noted above, chronic optogenetic stimulation of a subset of MCH neurons, as performed by Konadhode et al., may result in a progressive accumulation of MCH peptides, GABA and possibly other peptides at the synaptic cleft. Note that such circadian accumulation may occur during the active period to “prepare” sleep onset by lowering the sleep threshold, as suggested for the hypocretin/orexin system.\(^\text{23}\) Note that overstimulation may also result in desensitization of target receptors or spillover/diffusion of those molecules to neighboring cells, which may explain the increase in NREM sleep seen in chronic optogenetic conditions and pharmacological studies.\(^\text{13,18}\)

**Functional Wiring of MCH Neurons**

MCH neurons may promote REM sleep by direct inhibition of arousal centers or feed-forward disinhibition (net activation) of NREM and/or REM sleep nuclei, or both.

According to the first hypothesis, we found that activation of MCH neurons terminals in the TMN, an important arousal center in the hypothalamus, inhibit TMN cells \textit{in vitro} and prolonged REM sleep duration \textit{in vivo}. It is thus possible that MCH neurons inhibit other arousal centers including the noradrenergic cells from the locus coeruleus\(^\text{24}\) to prolong REM sleep episodes (see Hassani and Jones commentary). Another putative arousal target for MCH neurons is the dorsal raphe (DR), brainstem nuclei encompassing serotonergic neurons that are active during wakefulness.\(^\text{25}\) Although pharmacological infusion of MCH in the DR promotes REM sleep,\(^\text{16,17}\) we did not replicate these findings in our optogenetic study. On the other hand, MCH neurons may inhibit GABAergic REM-off neurons in the vlPAG and other nuclei to promote REM sleep.\(^\text{26}\)

One other target for MCH neurons is the medial septum (MS), a structure involved in hippocampal theta rhythm generation during both wakefulness and REM sleep.\(^\text{27}\) We found that activation of MCH neuronal projection to the MS extends the duration of REM sleep episodes, suggesting that MCH neurons can directly modulate REM sleep through action on theta rhythm generating structures.

In regard to a possible role of MCH neurons in NREM sleep, activation of their terminals may result in a direct disinhibition of cells in the ventro-lateral preoptic area (VLPO) and promote NREM sleep as suggested by pharmacological infusion.\(^\text{14}\) This would be consistent with the increase of NREM sleep and delta activity by using a chronic optogenetic activation of MCH neurons reported by Konadhode et al., thus direct cortical MCH neuronal projections may be involved.

Besides the possibility of MCH neuron subpopulations with distinct functional projections to arousal or sleep centers as discussed above, MCH terminals may be organized in topographical domains where GABA, MCH (or other peptides) are released separately or together from MCH terminals, as hypothesized previously.\(^\text{28}\) In agreement with this hypothesis, only a small proportion of the MCH varicosities containing the vesicular GABA transporter (VGAT) form synapses in the LC.\(^\text{24}\) Although this needs to be functionally confirmed, it suggests that MCH rather than GABA modulates cellular activity in the LC. Such topographical specialization of MCH terminals may define additional subsets of MCH neurons or distinct functional
domains amongst the terminals originating from a single MCH neuron. This would multiply the tuning possibility of sleep-wake centers by MCH neurons and may explain the discrepancy regarding the NREM sleep effect in Konadhode et al. compared to our study.29

Finally, stimulation of MCH neuronal projections to the TMN and MS were sufficient to extend REM sleep duration, suggesting some level redundancy among the system, although finer tuning during specific sleep states is possible (point raised in commentary by Luppi et al.10). Silencing of MCH projections to these targets would provide a better understanding of the necessity of each of these structures.

Sufficiency vs Necessity: Modulatory Action of MCH

We showed that activation of MCH cell bodies was sufficient to promote and facilitate REM sleep, whereas their silencing induced a slowing of the theta rhythm which rapidly affected the stability of theta oscillations during REM sleep. Whether such changes should be considered as the cessation of an intact REM sleep episode remains debatable. It needs to be determined if the appearance of slow-theta during MCH neuronal inhibition is mediated directly by the MS or another structure (pons, mammillary nucleus). This is an especially important question in regard to the role of theta rhythm27 and the MCH system in cognitive functions.30

One possible interpretation of the onset of slow theta during REM sleep is that silencing of MCH neurons facilitates “back-transition” from REM to NREM sleep. Although this is purely speculative, it is consistent with the result that MCH neuron activation promotes transition from NREM to REM sleep. Clearly, the fact that animals did not show a complete transition to NREM sleep suggests that other systems are involved. Overall, these silencing results strongly suggest that MCH is necessary for stability of theta oscillation during REM sleep. One interesting point, raised by Luppi et al.10 in their commentary, would be to see if MCH neuronal silencing before REM sleep (e.g., during NREM sleep or transition state) blocks REM sleep onset or decreases REM sleep duration.

Collectively, our results29 together with results from the two other studies11,13 included in this forum strongly suggest that MCH neurons play a modulatory role on REM sleep and energy homeostasis rather than an effector system that triggers REM sleep or food intake. Further investigations would be needed to determine the role of the MCH neurons in learning and memory, and in REM sleep-dependent facilitation of memory consolidation.

CITATION

DISCLOSURE STATEMENT
This was not an industry supported study. The authors have indicated no financial conflicts of interest.

REFERENCES