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The Role of Hcrt/Orx and MCH Neurons in Sleep-Wake State Regulation

Commentary on: Blouin et al. Human hypocretin and melanin-concentrating hormone levels are linked to emotion and social interaction. Nat Commun 2013;4:1547; Konadhode et al. Optogenetic stimulation of MCH neurons increases sleep. J Neurosci 2013;33:10257-63; and Jego et al. Optogenetic identification of a rapid eye movement sleep modulatory circuit in the hypothalamus. Nat Neurosci 2013 Sep 22. doi: 10.1038/nn.3522. [Epub ahead of print].

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Three articles have appeared in 2013 with important information concerning the potential role of peptides in sleep-wake state regulation. They come with new data emerging from new technical approaches, one in humans examining release of hypocretin/orexin (Hcrt/Orx) and melanin concentrating hormone (MCH) using microdialysis, the other two in transgenic mice examining the effect of selective stimulation of MCH neurons using optogenetics. Collectively, they support the notion that these peptides can selectively modulate different waking behaviors and/or sleep-wake states.

The article by Blouin et al.[1] with Siegel as senior author claims in the title that "human hypocretin and melanin-concentrating hormone levels are linked to emotion and social interaction." This formidable study measured levels of Hcrt/Orx in dialysates of extracellular fluid from the amygdala in epileptic patients under study for temporal lobe resection. Most simply, the data show higher release of Hcrt/Orx with several waking behaviors as compared to sleeping, and conversely higher release of MCH with sleeping as compared to most waking behaviors. The data are further interpreted to conclude that maximal release of Hcrt/Orx occurs with particular waking behaviors or emotions, notably social interaction and positive emotion, and minimal release with other waking conditions or behaviors, notably pain, along with eating. Conversely, maximal release of MCH occurs during waking with eating. Nonetheless, the results appear to largely corroborate what was found in rodent studies, first showing by c-Fos expression that Hcrt/Orx neurons are maximally active in association with waking behaviors, whereas MCH neurons are maximally active in association with sleep.[2,3] Electrophysiological studies subsequently confirmed that Hcrt/Orx neurons discharged maximally during active waking, whereas MCH neurons discharged maximally during sleep, particularly REM or paradoxical sleep (PS).[4-6] Their respective discharge rates were inversely correlated with postural muscle tone recorded from EMG of the neck muscles (Figure 1).[6] However, whether Hcrt/Orx neurons are selectively active with positively rewarding situations or positive emotions, as suggested in the current article along with previous rodent studies[5,7,8] or in association with fear or other conditions of arousal and stress, as suggested by other rodent studies[9,10] remains to be established. This point is obviously important when considering whether narcolepsy with cataplexy is normally prevented by the discharge of Hcrt/Orx neurons and release of their peptide predominantly in association with positive emotions. With regard to MCH, the present study suggests that MCH neurons might discharge during waking, notably during and after eating. This finding would conform to certain rodent studies involving effects of MCH administration as well as gene expression in MCH neurons in association with eating.[11] Thus the apparent role of MCH neurons in promoting sleep would appear to be consistent between the current human and rodent studies, yet the precise role of those neurons in sleep and potentially in eating remains to be further examined.

One of the problems in specifying the role of Hcrt/Orx and MCH neurons is that they lie intermingled with other neurons in the hypothalamus where they represent < 10% of the neurons. Assessment of their activity in correlation with behavior and sleep-wake states has thus necessitated selective recording and identification employing the juxtacellular labeling technique in head-fixed rats.[4,6] Fully assessing their role in behavior and states further necessitates their selective manipulation using a causative, in addition to correlative, approach. This approach has recently been made possible by the development and application of the optogenetic technique, entailing insertion of a gene for a light sensitive channel selectively into the Hcrt/Orx or MCH neurons. First applied by Adamantidis et al.[12] with de Lecea as senior author, it was demonstrated that selective activation of Orx neurons in transgenic mice increased the probability of awakening from sleep. In two recent articles, the optogenetic approach has now been applied to study the effect of selective activation of MCH neurons upon sleep-wake states.

The article by Konadhode et al.[13] with Shiromani as senior author claims in the title that "Optogenetic stimulation of MCH neurons increases sleep." Most impressively, they show that optic stimulation of the MCH neurons can increase NREM and REM sleep during the night when the mice are normally awake the majority of the time and when arousal systems, including Hcrt/Orx neurons would be active. Moreover, they show an increase in the amplitude of delta EEG activity, which serves as an index for intensity and depth of slow wave sleep (SWS). The authors conclude the MCH neurons can promote sleep, including both NREM with slow waves and REM sleep.

The article by Jego et al. with Adamantidis as senior author[14] claims in the title, "Optogenetic identification of a rapid eye

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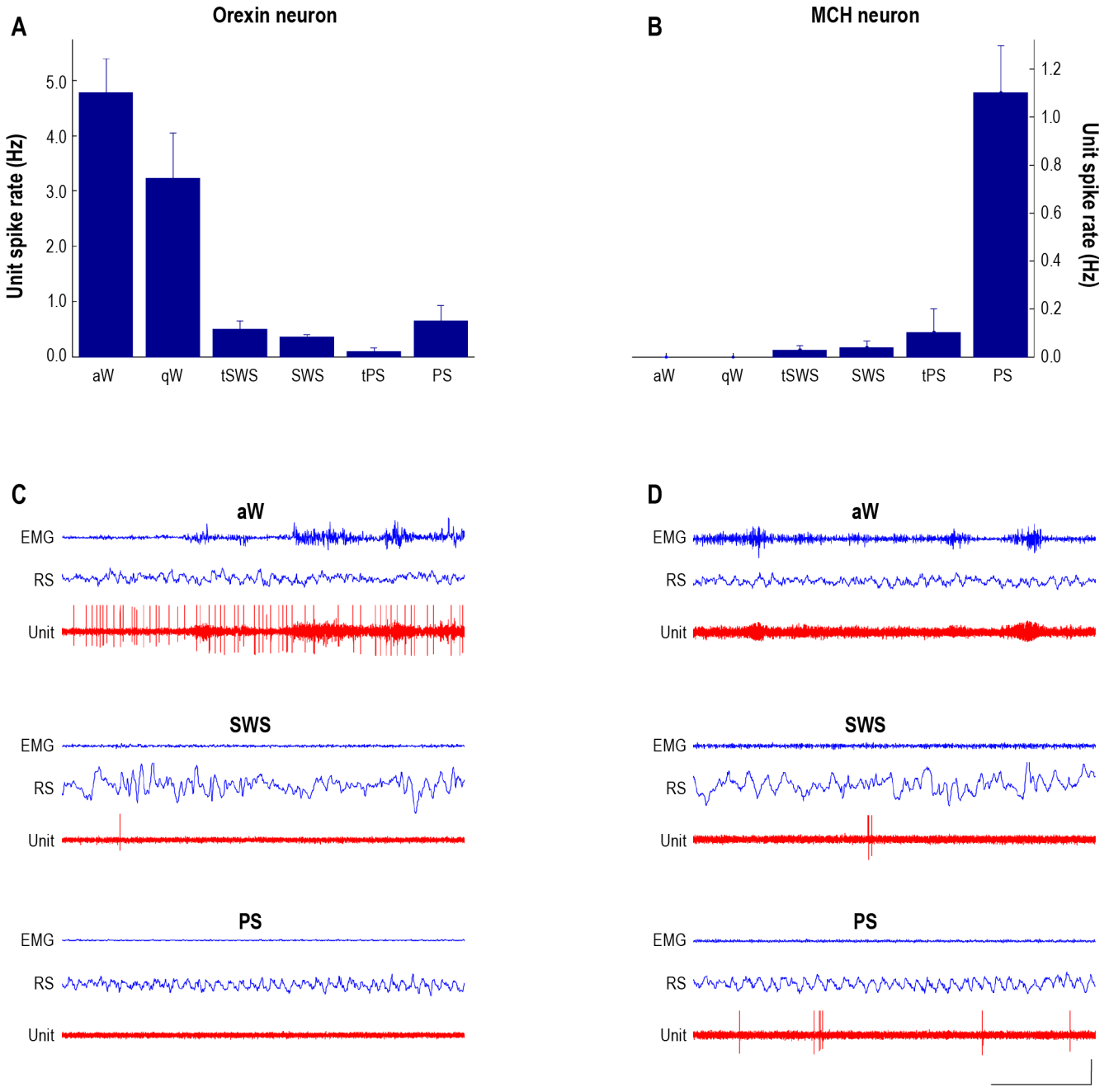

Figure 1—Discharge profiles of identified Orx and MCH neurons. Bar graphs showing the average spike rates (spikes per sec in Hz) of neurobiotin-labeled immunohistochemically identified Orx **(A)** and MCH **(B)** neurons along with (5 sec) periods of unit recordings with EEG and EMG (**C** and **D**, respectively). **A** and **C**: The Orx+ unit (C32u10) discharged maximally during active wake (aW) (average rate of 4.85 Hz). From aW, it decreased firing during quiet wake (qW), more significantly during transition to slow wave sleep (tSWS), to fire minimally during slow wave sleep (SWS), transition to paradoxical sleep (tPS) and paradoxical sleep (PS). Although the Orx unit was silent for long periods of PS (as illustrated), including periods of phasic twitching, it increased its firing with intervening periods of increased postural muscle tone and within several seconds prior to awakening (not shown). During maximal firing, it tended to fire in trains of spikes (with a mode interspike interval of 93.02 ms and corresponding instantaneous firing frequency of 10.75 Hz). **B** and **D**: The MCH+ unit (C134u01) discharged maximally during PS (1.1 Hz). It was silent during both aW and qW, fired at very low rates during tSWS, SWS, and tPS and at its highest (albeit still low) rate during PS. During maximal firing, it tended to fire very phasically with doublets or clusters of spikes (a mode interspike interval of 17.01 ms and corresponding instantaneous firing frequency of 58.75 Hz). The discharge rates of the Orx and MCH units were respectively (and reciprocally) positively and negatively correlated with EMG amplitude across the sleep-waking cycle. Calibrations in **C** and **D**: horizontal, 1 sec; vertical, 1 mV (EEG, EMG), 2 mV (unit). Adapted with permission from Lee[4] and Hassani.[6]

movement sleep modulatory circuit in the hypothalamus." Employing an impressive array of channel opsins for both the activation and silencing of the MCH neurons, they show that optic stimulation of the MCH neurons during NREM sleep results in increased transitions to REM sleep and that stimulation during REM sleep results in increased duration of REM sleep prior to awakening. They also show that inactivation of the MCH neurons decreases the frequency of theta activity

during REM sleep. They then go on to show similar effects on REM sleep duration by stimulation of the terminal fibers of the opsin expressing neurons in the tuberomammillary nucleus (TMN), as well as the medial septum, where the MCH neurons project. They conclude that through their various projections and influence upon arousal systems, MCH neurons can promote REM sleep.

Jego et al. go much further to consider how MCH neurons act upon their target neurons, examining in vitro the projection from the MCH neurons to nearby histamine neurons in the TMN. They show that optic stimulation of the MCH neurons produces inhibitory postsynaptic currents (IPSCs) in the TMN neurons, which are both fast and blocked by bicuculline, an antagonist of $GABA_A$ receptors and thus undoubtedly elicited by release of GABA. What of the MCH effect? The authors found in another double transgenic and knockout (KO) mouse lacking MCH1 receptors that the postsynaptic effect of optic stimulation is still present and not reduced in amplitude. However, the frequency of IPSCs on the TMN neurons is reduced, suggesting there is normally a presynaptic facilitation of GABA release by MCH.

Collectively, these studies raise several interesting points and questions.

First, do MCH neurons play a role in waking behavior, notably eating? Whereas the release of MCH appears to be somewhat higher during eating in Siegel's study, stimulation of MCH neurons was not reported to elicit eating in the predominantly awake animals at night in Shiromani's study, nor in the predominantly sleeping animals during the day in Adamantidis's study. Perhaps the increased MCH release occurring during and more particularly after eating in Siegel's study is associated with a drowsy state leading to sleep. In unit recording studies in rats in our lab, no units which discharged during waking with cortical fast activity (n = 49) were found to be MCH+ neurons, and conversely all MCH+ neurons (n = 7) discharged only during sleep (Figure 1).[6,15]

Second, do MCH neurons promote NREM sleep with slow waves? From Shiromani's results, it would appear that MCH neurons have this capacity. The increase in NREM sleep and delta power during the night, when the mice are normally awake the majority of the time, provides a strong argument for this role. Perhaps the Adamantidis study did not report such effects because the stimulation was done during the day, when the mice are already sleeping the majority of the time and for which time no effect of sleep augmentation was reported in the Shiromani study. In unit recording studies in our lab, the MCH neurons were found to discharge at a very low rate during SWS (Figure 1).[6] If the total population of MCH neurons (~6000 in the rat) fires in this manner during SWS, they could presumably produce an increase in MCH release through the brain, which might be adequate to promote SWS.

Third, do MCH neurons promote REM sleep? From both Adamantidis's and Shiromani's studies, there is strong evidence to indicate that MCH neurons can promote REM sleep. These results and conclusions are supported by recording studies in our lab showing that MCH neurons fire at their maximal rate during PS (Figure 1).[6] Collectively, the results indicate that MCH neurons promote sleep and the full sleep cycle progressing through SWS into PS.

Fourth, do MCH neurons act upon target neurons through release of GABA alone? Adamantidis showed that stimulation of the MCH neurons produced inhibitory currents in the histamine neurons by release of GABA onto $GABA_A$ receptors and independent of MCH receptors. And it has been shown in our lab that MCH varicosities contain the vesicular GABA transporter (VGAT) and form GABAergic (gephyrin+) synapses on their target noradrenaline (NA) neurons in the locus coeruleus (LC).[16] However, only a small proportion of the MCH varicosities contain VGAT and form synapses (< 10%), indicating that the vast majority of the MCH varicosities would likely only release MCH and act in a nonsynaptic manner on receptors located on target neurons or their afferent inputs. Indeed, postsynaptic as well as presynaptic effects of MCH have been demonstrated on certain neurons in vitro,[17,18] and MCH has been shown to antagonize the excitatory effect of Hcrt/Orx on the NA LC neurons.[19] In addition, MCH suppresses glutamate release from presynaptic terminals on hypothalamic neurons, including the Hcrt/Orx neurons.[17,20] Since the first demonstration of the potential role of MCH in sleep-wake regulation was by intracerebroventricular administration of the peptide, which produced increased SWS and PS,[3] MCH neurons must thus be presumed to promote sleep by these multiple actions of the peptide, in addition to the actions of the co-transmitter, GABA.

Fifth, how can MCH neurons promote both NREM sleep with slow waves (0.5-4 Hz) and REM sleep with rhythmic theta activity (6.5-9 Hz)? Whereas the Shiromani group showed increased delta activity during NREM sleep with MCH stimulation, the Adamantidis group showed decreased rhythmic theta activity during REM sleep with MCH inactivation, suggesting that the MCH neurons would normally stimulate rhythmic theta activity during REM sleep. These results could perhaps be interpreted in a coherent manner when assuming first that MCH or MCH neurons can dampen the activity of arousal neurons, including the Orx neurons, to generally facilitate sleep, including both SWS and PS.[20] In addition, according to evidence that MCH acts to suppress glutamate release from presynaptic terminals,[17,20] it could facilitate slow waves in the cortex by increased disfacilitation.[21] The MCH neurons could also facilitate rhythmic theta activity if they facilitate GABA release, as Adamantidis's results suggest, which is critical in theta generation.[22]

Sixth, how do MCH and Hcrt/Orx neurons fire to release their peptide and/or amino acid neurotransmitter? In our in vivo recordings, we found that both Hcrt/Orx and MCH neurons tended to fire in a phasic manner, instead of continuously, the Hcrt/Orx particularly with movement and increased muscle tone, the MCH neurons with absence of movement and muscle tone (Figure 1). The Hcrt/Orx neurons tended to fire in trains of spikes, and the MCH neurons in highly spaced doublets or clusters of spikes. From analysis of interspike intervals, the average instantaneous firing frequency of the Hcrt/Orx neurons was found to be around 10 Hz (median 9 Hz, range 7 to 26 Hz, n = 6) and that of the MCH neurons around 20 Hz (median 18 Hz, range 15 to 59 Hz, n = 5). From classical studies, it appears that bursts of spikes are necessary for release of peptides, whereas single spikes are sufficient for releasing GABA or glutamate, which would be co-released from some of the Hcrt/Orx or MCH varicosities, respectively.[16,23] So, it is likely that these neurons can

release glutamate or GABA with single spikes but Hcrt/Orx or MCH, respectively, only with trains or bursts of spikes. Interestingly, in both of the optogenetic studies, no effect was obtained with stimulation frequencies of 1 Hz but only with frequencies of 10 or 20 Hz for both the Hcrt/Orx and MCH neurons.[12-14] One would thus imagine that the peptide is being released at these higher frequencies and reciprocally that the peptide release measured in humans[1] depends upon this type of phasic discharge by the Hcrt/Orx and MCH neurons during the behavior or states in which their clear-cut maximal release occurs.

Collectively, these new findings lead to the suggestion that pharmacological manipulation of peptide transmission might be effectively applied to the treatment of sleep disorders such as narcolepsy and insomnia.

CITATION

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