Neuroscience 336 (2016) 102–113

MELANIN-CONCENTRATING HORMONE NEURONS SPECIFICALLY PROMOTE RAPID EYE MOVEMENT SLEEP IN MICE

RAMALINGAM VETRIVELAN,[a]* DONG KONG,[b] LORIS L. FERRARI,[a] ELDA ARRIGONI,[a] JOSEPH C. MADARA,[c] SATHYAJIT S. BANDARU,[a] BRADFORD B. LOWELL,[c] JUN LU[a] AND CLIFFORD B. SAPER[a]*

[a] *Department of Neurology, Beth Israel Deaconess Medical Center and Division of Sleep Medicine, Harvard Medical School, Boston, MA 02215, United States*

[b] *Department of Neuroscience, Tufts University School of Medicine, Programs of Neuroscience and Cellular, Molecular and Development Biology, Tufts Sackler School of Graduate Biomedical Sciences, Boston, MA 02111, United States*

[c] *Division of Endocrinology, Diabetes and Metabolism, Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA 02215, United States*

Abstract—Currently available evidence indicates that neurons containing melanin-concentrating hormone (MCH) in the lateral hypothalamus are critical modulators of sleep-wakefulness, but their precise role in this function is not clear. Studies employing optogenetic stimulation of MCH neurons have yielded inconsistent results, presumably due to differences in the optogenetic stimulation protocols, which do not approximate normal patterns of cell firing. In order to resolve this discrepancy, we (1) selectively activated the MCH neurons using a chemogenetic approach (Cre-dependent hM3Dq expression) and (2) selectively destroyed MCH neurons using a genetically targeted diphtheria toxin deletion method, and studied the changes in sleep–wake in mice. Our results indicate that selective activation of MCH neurons causes specific increases in rapid eye movement (REM) sleep without altering wake or non-REM (NREM) sleep. On the other hand, selective deletions of MCH neurons altered the diurnal rhythm of wake and REM sleep without altering their total amounts. These results indicate that activation of MCH neurons primarily drives REM sleep and their presence may be necessary for normal expression of diurnal variation of REM sleep and wake.

Key words: lateral hypothalamus, sleep–wake, paradoxical sleep, feeding, chemogenetics, conditional deletion.

INTRODUCTION

Neurons containing melanin-concentrating hormone (MCH), localized in the lateral hypothalamus (LH), have long been implicated in sleep–wake regulation (Verret et al., 2003; Hanriot et al., 2007; Willie et al., 2008; Peyron et al., 2009, 2011). For example, mice with deletions of the *MCH* gene or MCH neurons have been reported to display an increase in wakefulness with corresponding decrease in non-rapid eye movement (NREM) sleep, suggesting that MCH neurons may promote NREM sleep(Willie et al., 2008; Tsunematsu et al., 2014). Other evidence indicates that the MCH neurons may be specifically linked to regulation of rapid eye movement (REM) sleep. For example, more than 60% of MCH neurons express cFos during REM hypersomnia following selective REM deprivation (Verret et al., 2003). MCH, when injected intracerebroventricularly, produces a robust (~200%) increase in REM sleep (Verret et al., 2003) while infusion of an MCH receptor antagonist causes a significant reduction in REM sleep in rats (Ahnaou et al., 2008). MCH neurons heavily innervate the dorsolateral brainstem regions implicated in REM generation including the sublaterodorsal nucleus (SLD) and ventrolateral periaqueductal gray matter (vlPAG), (Hanriot et al., 2007; Peyron et al., 2009; Sapin et al., 2010; Clement et al., 2012) and local injection of MCH into the nucleus pontis oralis (in the subcoeruleus region which is considered to be an SLD-equivalent in cats) produces a significant increase (~70%) in REM sleep and reduces the latency to REM sleep (Torterolo et al., 2009). Juxtacellular recording studies show that MCH neurons are maximally active during REM sleep, silent during wake, and occasionally active in NREM sleep (Hassani et al., 2009), suggesting that they promote REM sleep.

On the other hand, recent studies using optogenetic activation of MCH neurons have found contradictory results. Brief periods of stimulation of MCH neurons during NREM and REM sleep increased NREM-to-REM transitions and REM sleep bout durations respectively whereas stimulation during wake had no effect on either

*Corresponding authors. Address: Department of Neurology, Beth Israel Deaconess Medical Center, 3 Blackfan Circle, Center for Life Science # 717, Boston, MA 02215, United States.
E-mail addresses: vramalin@bidmc.harvard.edu (R. Vetrivelan), csaper@bidmc.harvard.edu (C. B. Saper).

Abbreviations: AAV, adeno-associated viral vectors; ACSF, artificial cerebrospinal fluid; CI, circadian index; CNO, clozapine-N-oxide; Cre, Cre recombinase; DT, diphtheria toxin; EEG, electroencephalogram; EMG, electromyogram; iDTR, inducible diphtheria toxin receptor; IP, intraperitoneal; ir, immunoreactive; LDT, laterodorsal tegmentum; LH, lateral hypothalamus; LMA, locomotor activity; LPT, lateral pontine tegmentum; MCH, melanin-concentrating hormone; NREM, non-rapid eye movement sleep; PC, precoeruleus; PPT, pedunculopontine tegmentum; REM, rapid eye movement; SCN, suprachiasmatic nucleus; SLD, sublaterodorsal nucleus; Tb, body temperature; TMN, tuberomammillary nucleus; vlPAG, ventrolateral periaqueductal gray matter; vM, ventrolateral medulla; WT, wildtype.

http://dx.doi.org/10.1016/j.neuroscience.2016.08.046
0306-4522/

wake–sleep transitions or bout durations (Jego et al., 2013). Long-term stimulation of MCH neurons (1 min out of 5, for 6 or 24 h), however, was reported to cause increases in both NREM and REM sleep (Konadhode et al., 2013). A third study using intermediate duration (3 h) continuous stimulation of MCH neurons found an increase in REM sleep with a significant reduction in NREM sleep (Tsunematsu et al., 2014). However, in the same study, genetically driven deletion of MCH neurons caused reduced NREM sleep (Tsunematsu et al., 2014).

One reason for these varying results may be that optogenetic stimulation produces artificial, monotonous firing that may not approximate endogenous firing patterns. MCH neurons contain both GABA and glutamate, as well as several additional peptides, so the mix of neurotransmitters released at a given terminal site may be different with different firing patterns (Arrigoni and Saper, 2014). The effects of MCH and other peptides that work through G-protein-coupled receptors may also have prolonged effects on membrane potential that are difficult to predict. Thus, the long-term effects of MCH neurons on their targets *in vivo* may be different from what is measured by brief optogenetic activation of MCH neurons either *in vivo* or *in vitro* (Arrigoni and Saper, 2014). Hence, we used a chemogenetic approach, expressing genetically targeted hM3Dq mutated muscarinic acetylcholine receptors to excite MCH neurons by using the ligand clozapine-N-oxide (CNO). We also used genetically targeted diphtheria toxin (DT) to selectively ablate MCH neurons and re-examine the changes in sleep–wake.

Finally, sleep and wake occur in the context of a variety of important behaviors such as feeding and locomotor activity (LMA), and physiological functions such as regulation of body weight and temperature (Tb). Because MCH neurons have also been implicated in these functions (Qu et al., 1996; Shimada et al., 1998; Astrand et al., 2004; Whiddon and Palmiter, 2013), we studied concomitant changes in feeding, body weight, LMA and Tb during MCH neuron stimulation and deletion.

EXPERIMENTAL PROCEDURES

Animals

Two transgenic mouse lines (MCH-Cre and MCH-Cre/+; iDTR, mice) were used in this study.

MCH-Cre transgenic mice were generated as previously described (Kong et al., 2010). These mice specifically express Cre recombinase (Cre) under the MCH promoter and the eutopic expression of Cre in MCH neurons has been verified (Kong et al., 2010).

MCH-Cre/+;iDTR mice were generated by crossing the MCH-Cre mice with another transgenic mouse line, inducible diphtheria toxin receptor (iDTR) mice (Stock no. 007900; Jackson's laboratories, USA). The resultant offspring express DTR specifically in the MCH neurons (MCH-Cre/+;iDTR) and thus allow specific deletion of MCH neurons upon intraperitoneal (IP) administration of diphtheria toxin, DT.

Animal care. All mice were housed in individual cages and maintained under 12:12 light–dark cycle (lights on at 0700; 150 lux). Ambient temperature in the animal room was kept at 22 ± 1 °C. Care of the animals met National Institutes of Health standards, as set forth in the Guide for the Care and Use of Laboratory Animals, and all protocols were approved by the BIDMC Institutional Animal Care and Use Committee.

Viral vector. In order to selectively activate the MCH neurons, we injected Cre-dependent adeno-associated viral vectors (AAV) containing the stimulatory hM3Dq receptor (AAV-hSyn-DIO-hM3D(Gq)-mCherry; AAV serotype 8; University of North Carolina Vector Core, USA) into the MCH-Cre mice. The construction of this vector has been described in previous publications (Krashes et al., 2011). The ligand for the hM3Dq receptors is clozapine-N-oxide (CNO), which is administered intraperitoneally.

Experiment 1: Selective stimulation of MCH neurons

Surgery and recordings. Adult male MCH-Cre mice (n = 11) were anesthetized (100 mg/kg ketamine + 10 mg/kg xylazine; IP) and stereotaxically microinjected with AAV-hSyn-DIO-hM3D(Gq)-mCherry; AAV serotype 8 (AAV-hM3Dq; 520 nl per side) into the lateral hypothalamic MCH field (anteroposterior: −1.7 mm from bregma, ventral: 4.8 mm from duramater, lateral ±1.0 mm) bilaterally. These injections were performed using glass pipettes with a 10–20 μm diameter tip and a pressure-injection system (Scammell et al., 1998). The mice were then implanted with miniature telemetry transmitters (TLM2-F20EET; Data Science International, USA) for recording electroencephalogram (EEG), electromyogram (EMG), body temperature (Tb) and locomotor activity (LMA) (Vetrivelan et al., 2009). Baseline recording of sleep–wake (EEG and EMG), Tb and LMA from all the mice were performed for a period of 24 h at 2–3 weeks after surgery using Dataquest ART 3.1 software (Data Sciences International, USA) (Vetrivelan et al., 2009). Then the mice were IP injected with the vehicle (saline) or CNO (at a dose of 0.3 mg/kg body weight; Sigma, USA) and post-injection recordings were continued for 24 h on each occasion. Each animal received four IP injections – two saline and two CNO injections; one each at 10 AM and 7 PM. Injections were performed in a randomized crossover fashion and there was at least one week between two CNO injections in the same animal.

Histology. Three days after the final recordings, the mice were injected with CNO at 10 AM and euthanized under anesthesia by perfusion with 10% formalin 3-h after these injections. The brains were then removed and cut into three series of 40-μm sections. One series of sections were immunolabeled for cFos (as a marker of neuronal activity) and DsRed (to label hM3Dq-mCherry expressing neurons) as described previously (Anaclet et al., 2014). The following primary antibodies were used – rabbit (Rb) anti-cFos (Oncogene Sciences;