OPTRODE RECORDING OF P-WAVE GENERATING PONTINE NEURONS

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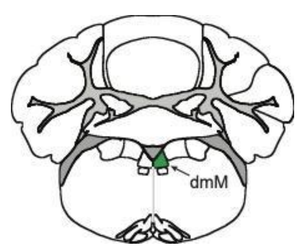
Introduction

- Rapid eye movement (**REM**) sleep plays an important role in memory consolidation, potentially mediated by phasic electrical events known as **P-waves** (Datta et al., 2004, Karashima et al., 2005))
- Previous research from the Weber lab suggests that **medullary CRH+ neurons** promote REM sleep in mice, and exhibit increased activity during P-waves in the subcoeruleus

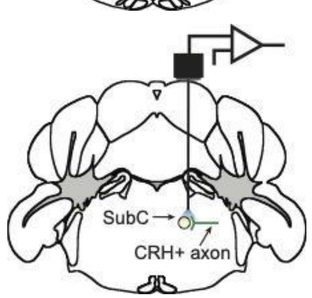
Research Questions

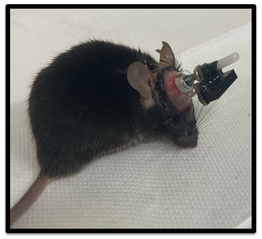
- What are the endogenous firing patterns of pontine neurons that generate P-waves?
- Do these neurons receive input from dmM CRH+ neurons?

Methods



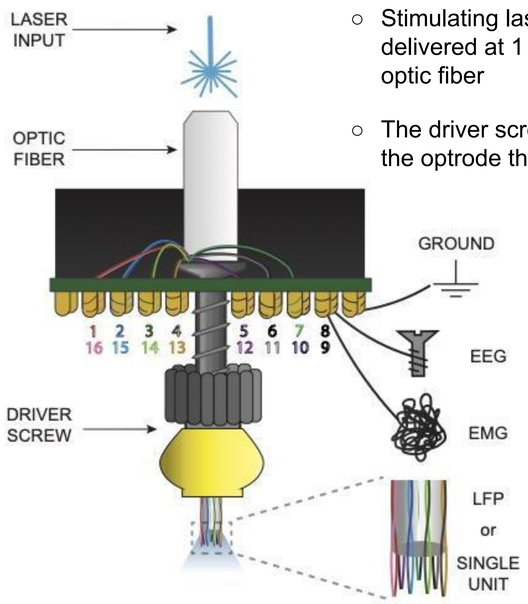
- Simultaneous single-unit recording of pontine neurons and optogenetic stimulation of local dmM CRH+ axons





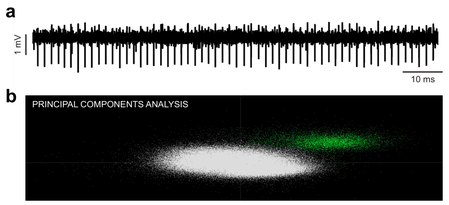
The Optrode

- We used a dual optical and electrical probe to identify and record from pontine neurons receiving direct projections from dmM CRH+ neurons

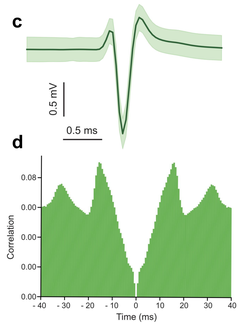


- Stimulating laser pulses (5ms, 5mW) were delivered at 1 minute intervals via the central optic fiber
- The driver screw allows for vertical movement of the optrode through the full depth of the pons
- To determine the brain state, EEG signals were collected via a skull screw above the hippocampus
- EMG activity was measured from a wire implanted in he neck muscles
- 7 twisted microelectrode pairs extracellularly recorded both single unit and multi-unit (LFP) activity

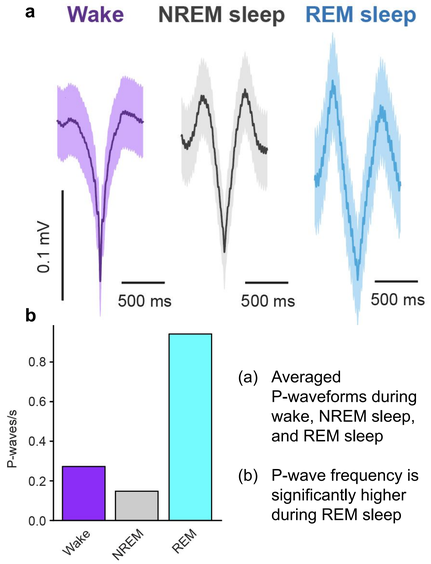
Single Unit Spikes



(a) Raw spikes from extracellular single unit optrode recording
(b) Cluster separation of spikes (green) from background (white) using principal components analysis (PCA)
(c) Averaged waveform of extracted neuron
(d) Neuronal refractory period shown in autocorrelation

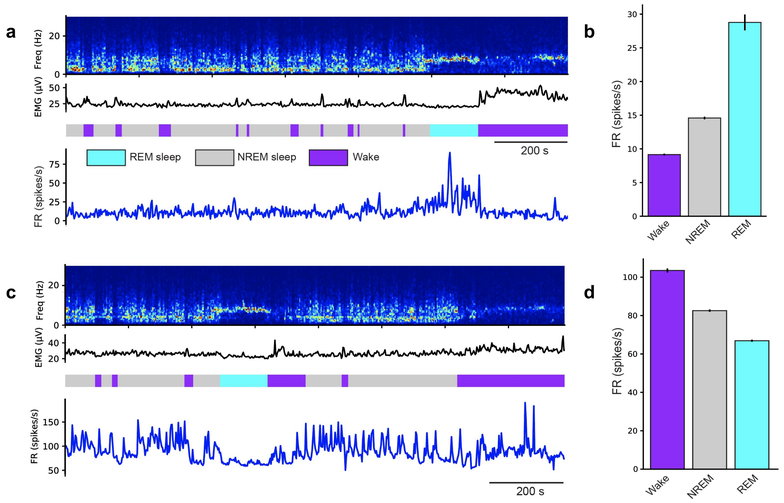


Local Field Potentials (LFPs)



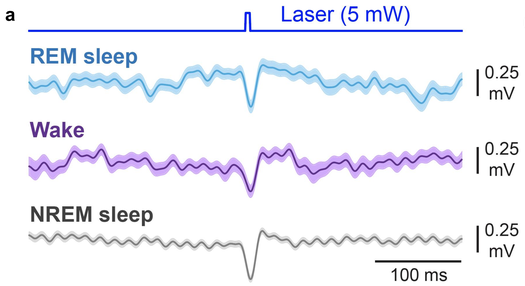
(a) Averaged P-waveforms during wake, NREM sleep, and REM sleep
(b) P-wave frequency is significantly higher during REM sleep

State-Dependent Neuronal Firing



(a) Example recording of a REM-active neuron, including spectrogram (top), EMG amplitude (middle top), brain state annotation (middle bottom) and firing rate plotted over time (bottom)
(b) Average firing rate of the neuron seen in (a) during wake, NREM sleep, and REM sleep
(c) Example recording of a REM-minimum neuron
(d) Average firing rate of the neuron seen in (c) during wake, NREM sleep, and REM sleep

Laser-Triggered LFP Events



(a) Averaged LFP signals surrounding a 5 ms laser stimulation of dmM CRH+ axons in the subcoeruleus during REM sleep, wake, and NREM sleep. Though no individual laser-triggered units have been detected, activation of dmM CRH+ terminals evokes a brief increase in multi-unit activity, producing a waveform visually similar to spontaneous P-waves observed during REM sleep.

Summary

- We successfully used the optrode to record a number of simultaneous electrophysiological signals (EEG, EMG, LFPs, and single neural spikes)
- Pontine neurons exhibit a range of state-dependent activity, and stimulation of local CRH+ axons elicits a P-wave-like LFP event
- In future experiments, we will continue recording to collect a set of laser-triggered pontine neurons, as well as recording dmM CRH+ neuron activity and P-waves to determine the order of activation