Physiological Regulations In Sleeping Mice

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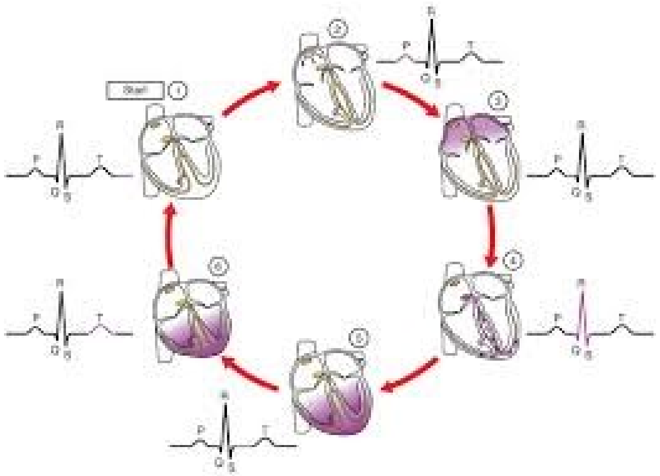
Scheme of this talk

- Electrical signals in animals
- What is sleep and why do we care?
- Mice as models of physiology and pathology
- Detection and analysis of physiological variables
- Ongoing projects

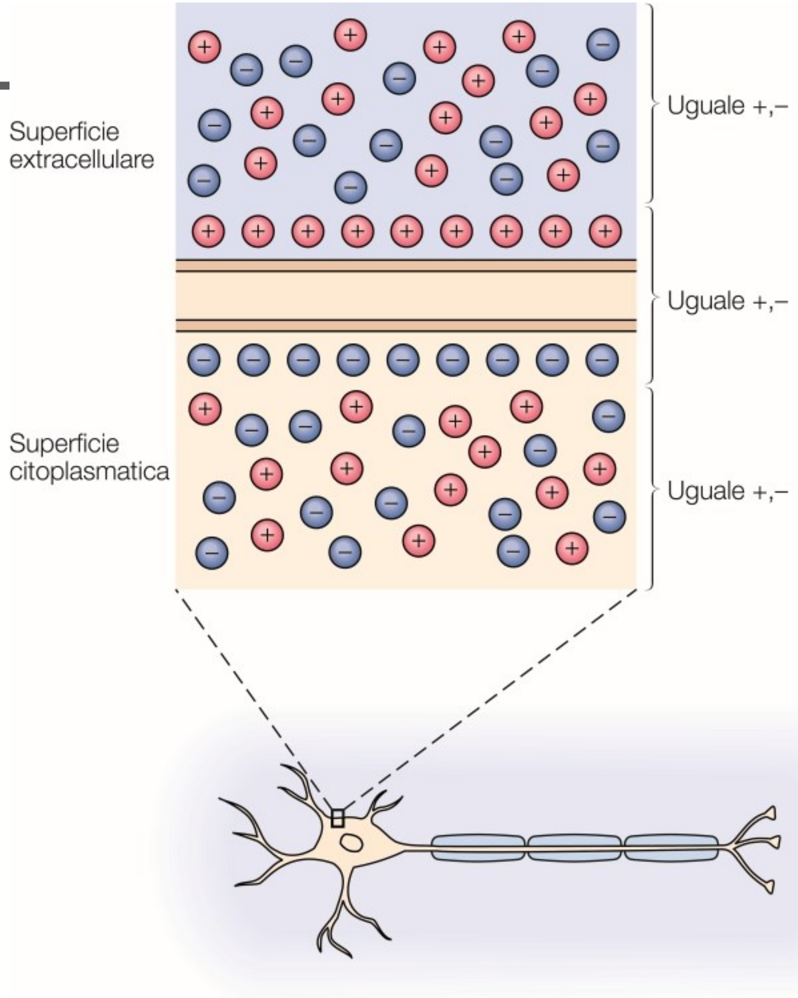
Living systems can produce electricity

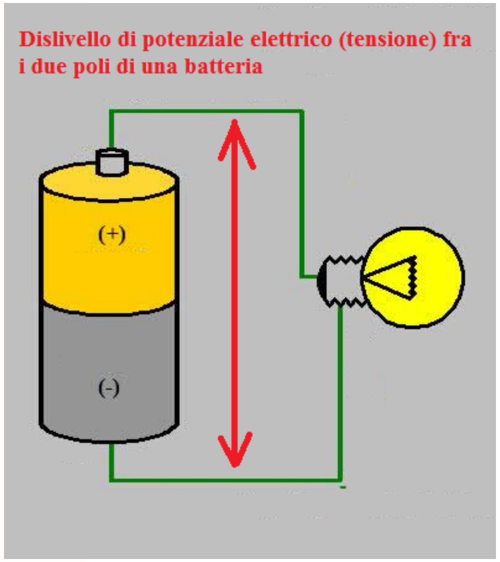


Neurons communicate with each other, forming the basis of our thoughts, behaviors, and perceptions.

Muscle cells generate electrical signals before they contract

Across the cellular membrane there is an excess of negative charges inside the cell and positive charges outside of it





Just like a battery

Action Potential Propagation

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Sleep

- *Sleep is one of the most important human behaviors, indeed it occupies about a third of our lives.*
- *The main characteristic is the reversible reduction of the response threshold to external stimuli*
- *It is a universal phenomenon found in all animals studied (from insects to mammals), therefore highly conserved in the evolutionary arc*

How can we study sleep behavior?

The electroencephalogram (EEG) is a tool that detects the electrical activity of the brain

Only through the use of EEG we can actually be sure that an individual is sleeping since brain activity changes significantly between wakefulness and sleep.

SLEEP

- <u>Stereotyped behavioral state</u>
- <u>Reduced relationship with the environment</u>

<u>NON-REM</u>

<u>(Non-Rapid Eye Movement)</u>

<u>Sleep</u>

<u>REM</u>

<u>(Rapid Eye Movement)</u>

<u>Sleep</u>

<u>REM sleep is present only in mammals and some birds.</u>

Neurons work differently between wakefulness and sleep

WAKEFULNESS

Neurons work differently between wakefulness and sleep

SLEEP

EEG rhythms

Wakefulness: fast and desynchronized rhythms

Slow Wave Sleep stage 1: light sleep

Slow Wave Sleep stage 2: synchronization of EEG

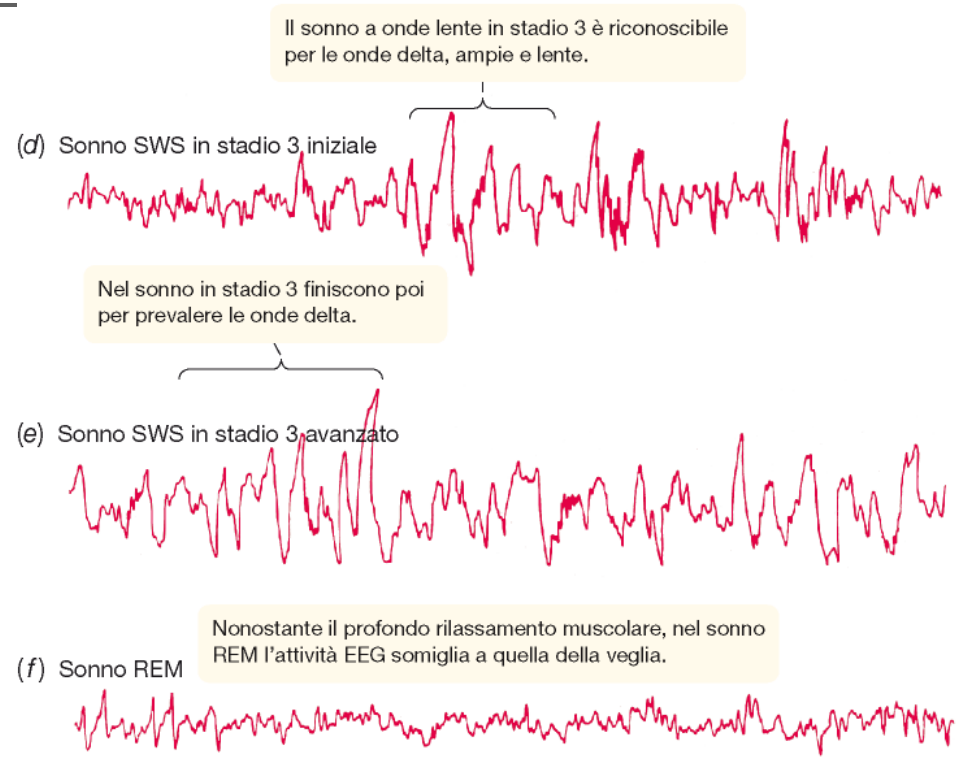
Rhythms:

- Alfa = 8-13 Hz (Eyes closed)
- Beta = 14-30 Hz (Wake)
- Sigma = 12-14 Hz (spindles)

Correlate with memory consolidation

EEG rhythms

Slow Wave Sleep stage 3: deep sleep, EEG highly synchronized

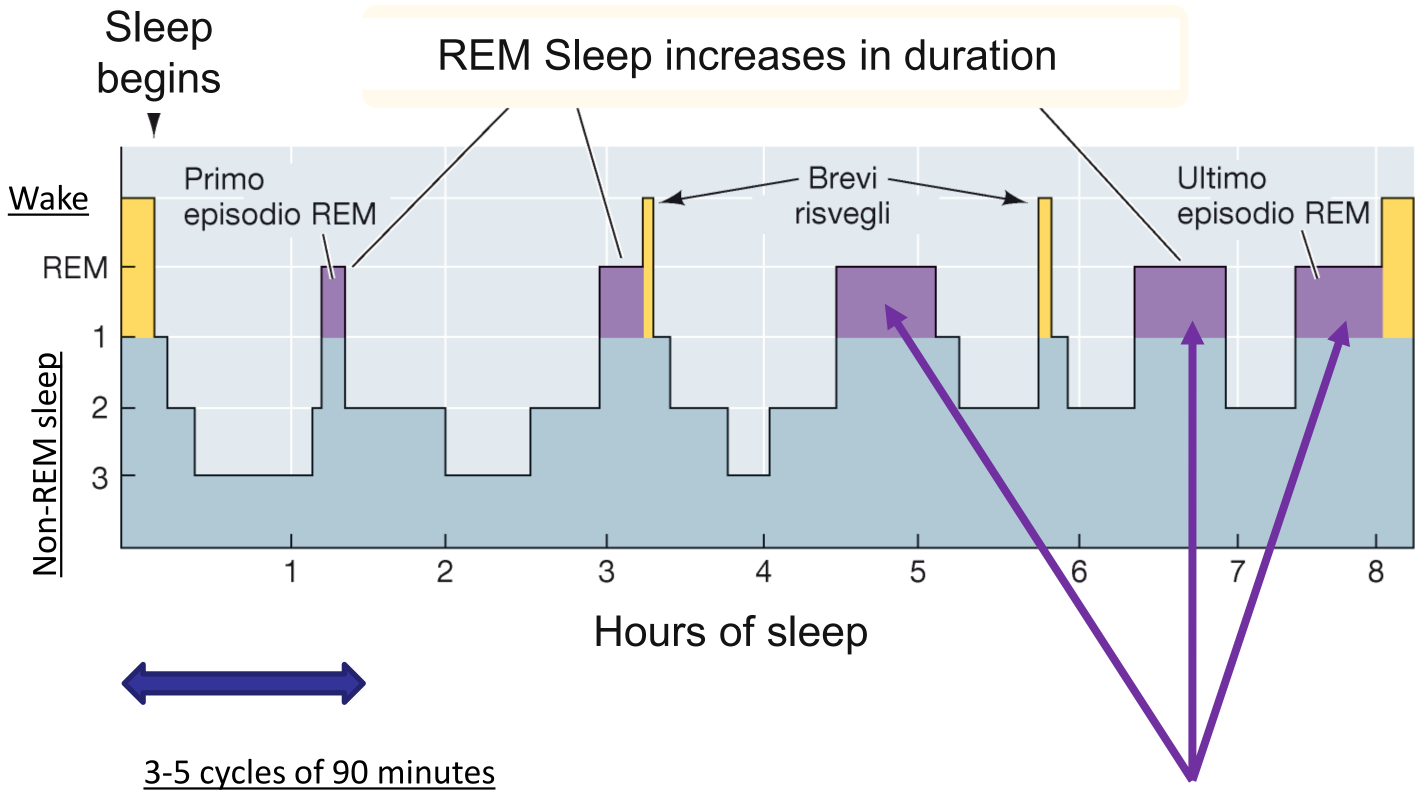


REM: fast and desynchronized rhythms

Rhythms:

- Delta = 0,5-4 Hz (slow waves)
- Theta = 4-7,5 Hz (W and REM)

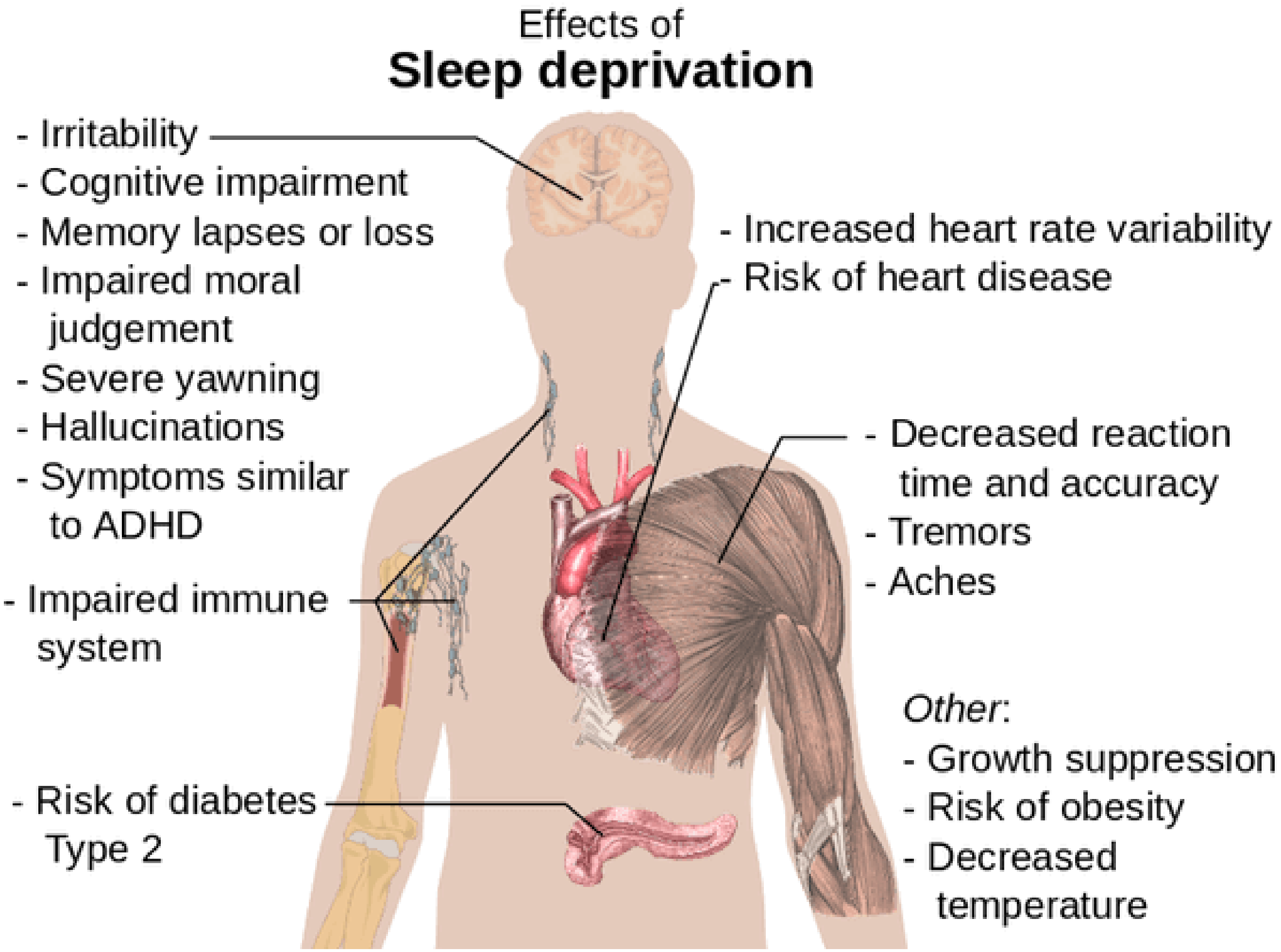
Hypnogram



3-5 cycles of 90 minutes

Here is where we dream

Why do we care about sleep?



List of Sleep Problems

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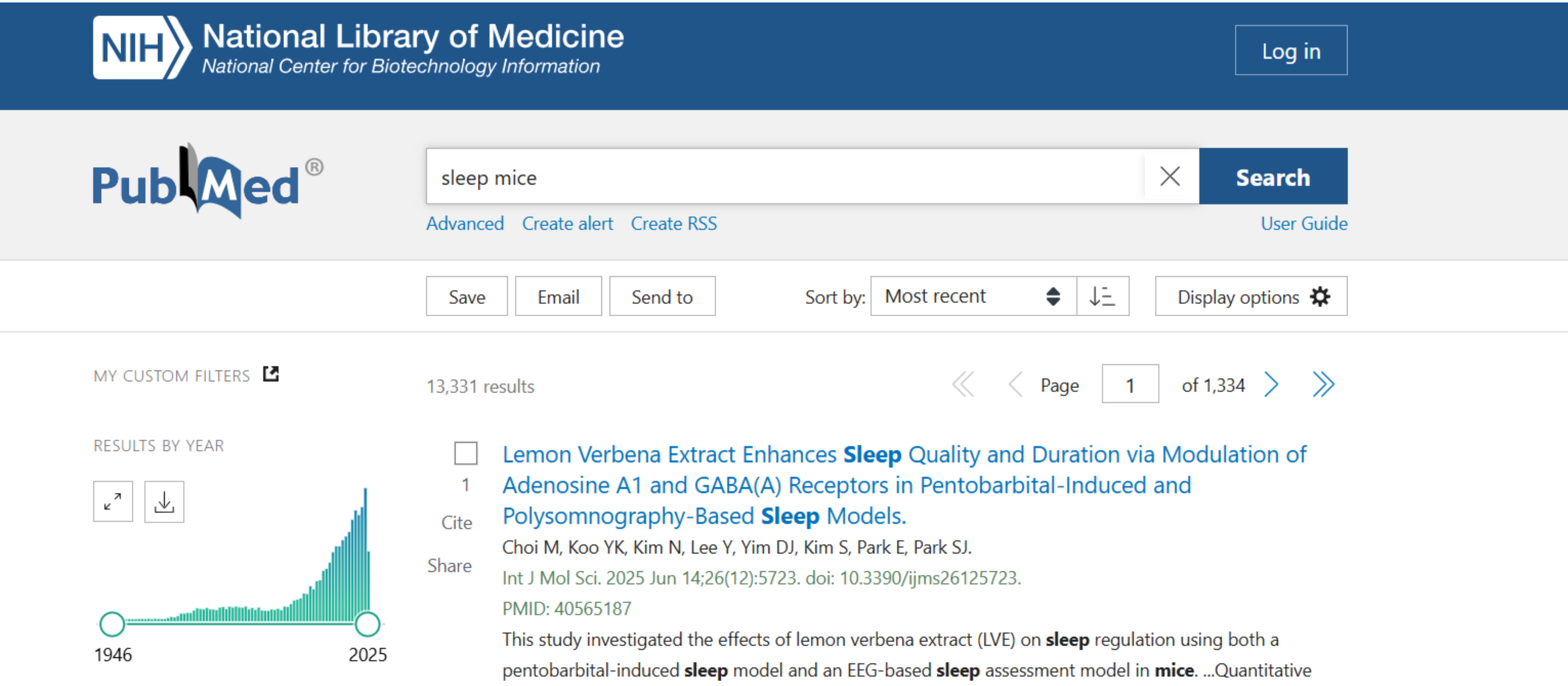
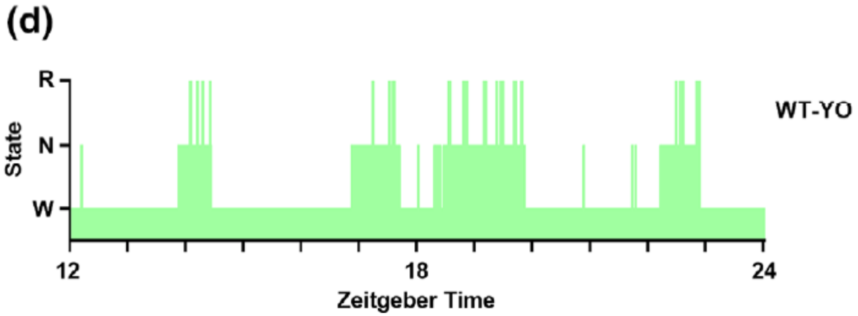
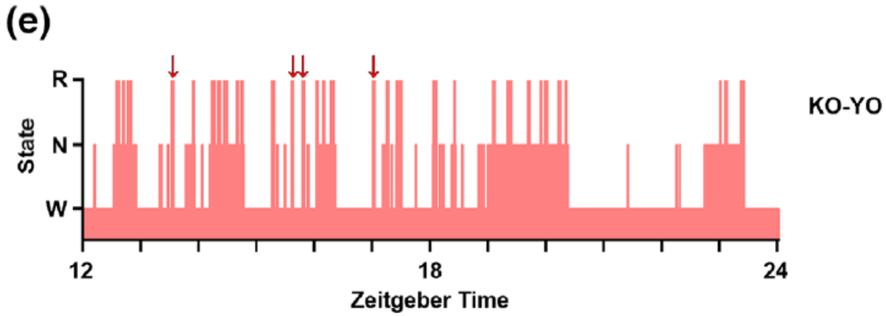
The mouse is the species of choice for studies on functional genomics… and also on sleep



SLEEP IN RODENTS

Rodents: nocturnal animals
polyphasic sleep
fragmented wake-sleep cycle
more NREM than humans
NREM sleep not divided in stages





Just an example…

Wakefulness fragmentation

Reduced REMS latency

Cataplexy

Undetectable levels of brain Orexins

Narcoleptic phenotype

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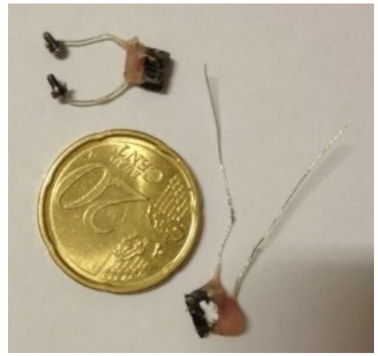
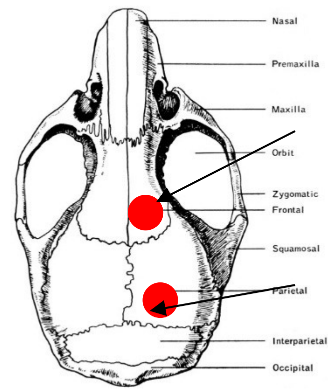
SCORING AT *PRISM lab*

EEG/EMG minielectrodes



EEG: frontal-parietal, filterd 0.3–100 Hz
EMG: neck muscles, filterd 100–1000 Hz

Cable transmission; signals sampled at 1024 Hz



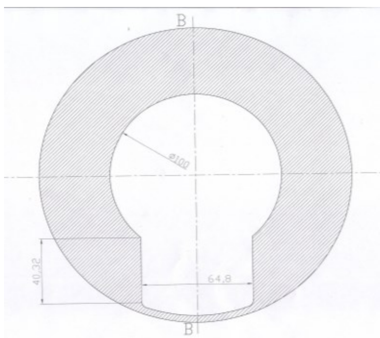
1 mm anterior and 1 mm lateral to bregma

1 mm anterior and 1 mm lateral to lambda

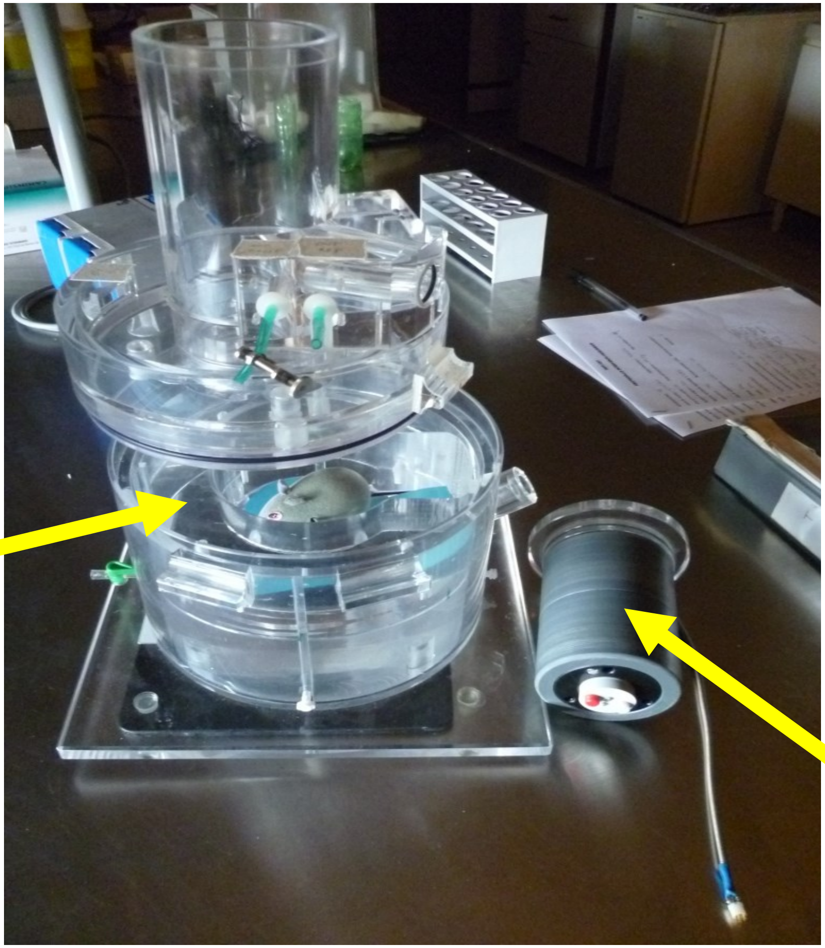
Blood pressure and heart rate telemetric transducer

catheter insertion: femoral artery
catheter tip: abdominal aorta
transducer body: subcutaneous pouch on flank

Whole-body plethysmography



internal plexiglas
chamber

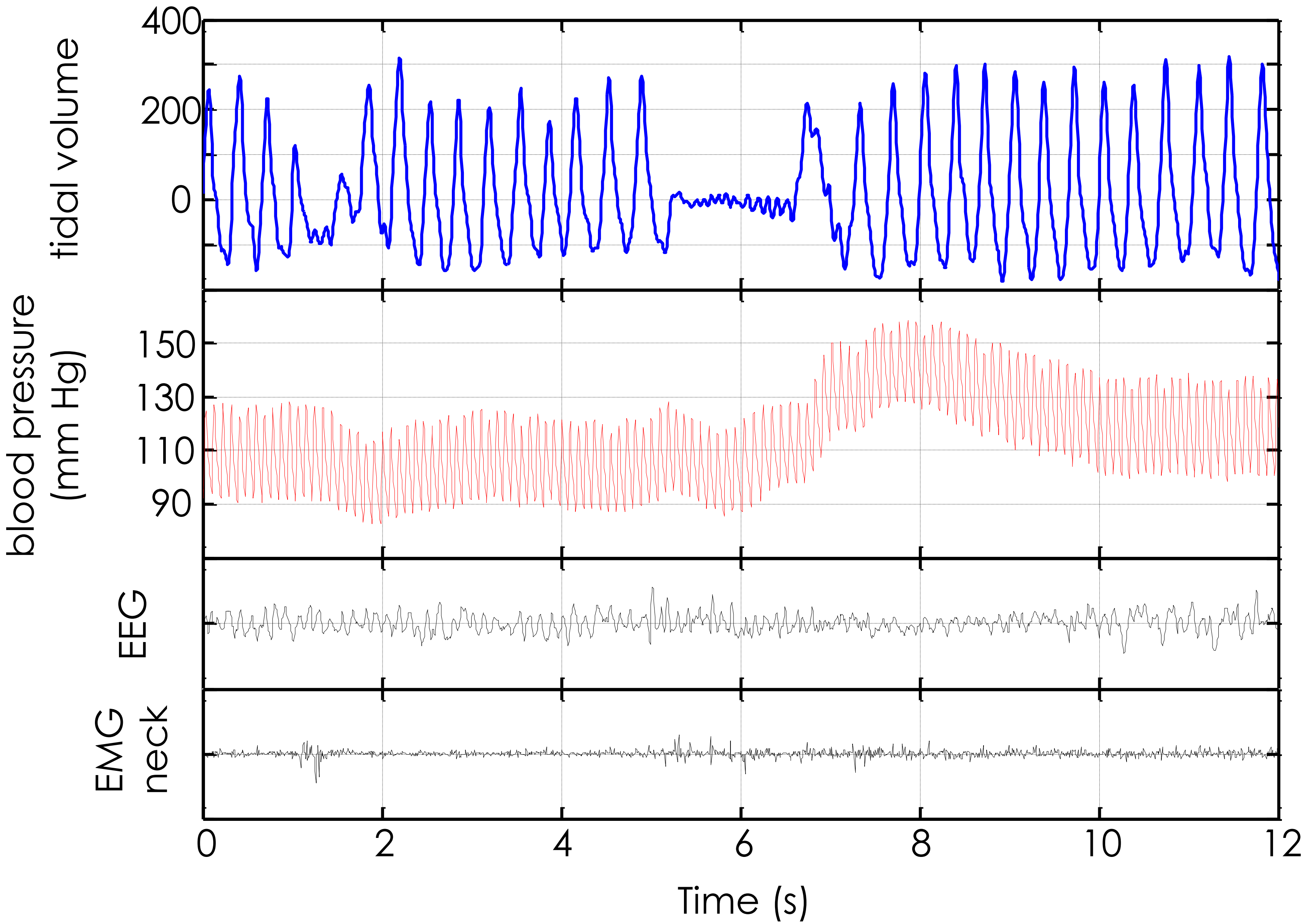


modified whole-body
plethysmograph

high-sensitivity
differential
pressure transducer

plastics plug
with electrical swivel

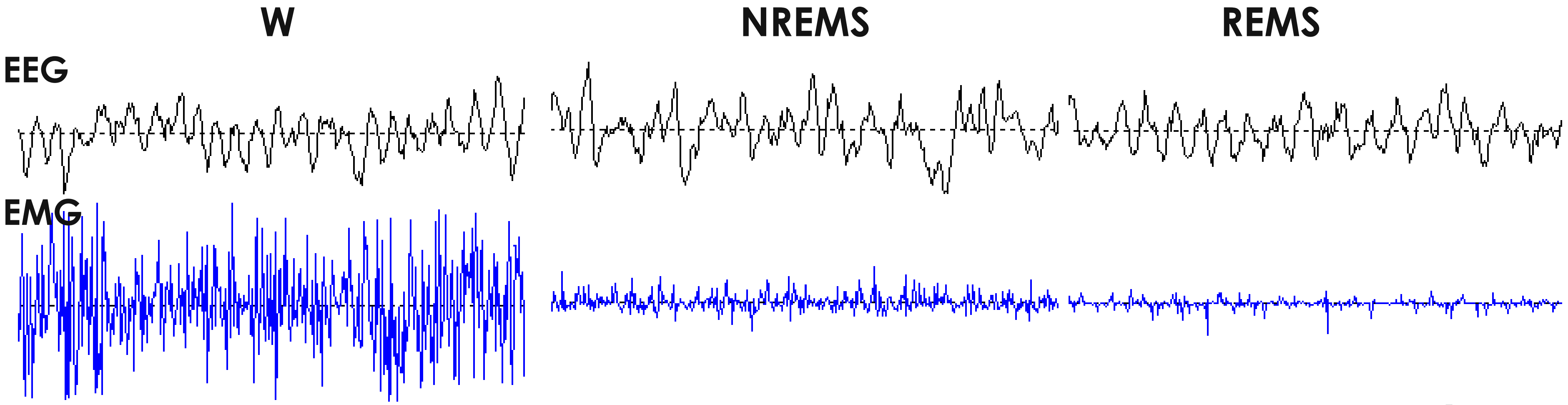
Simultaneous measurement of breathing and blood pressure



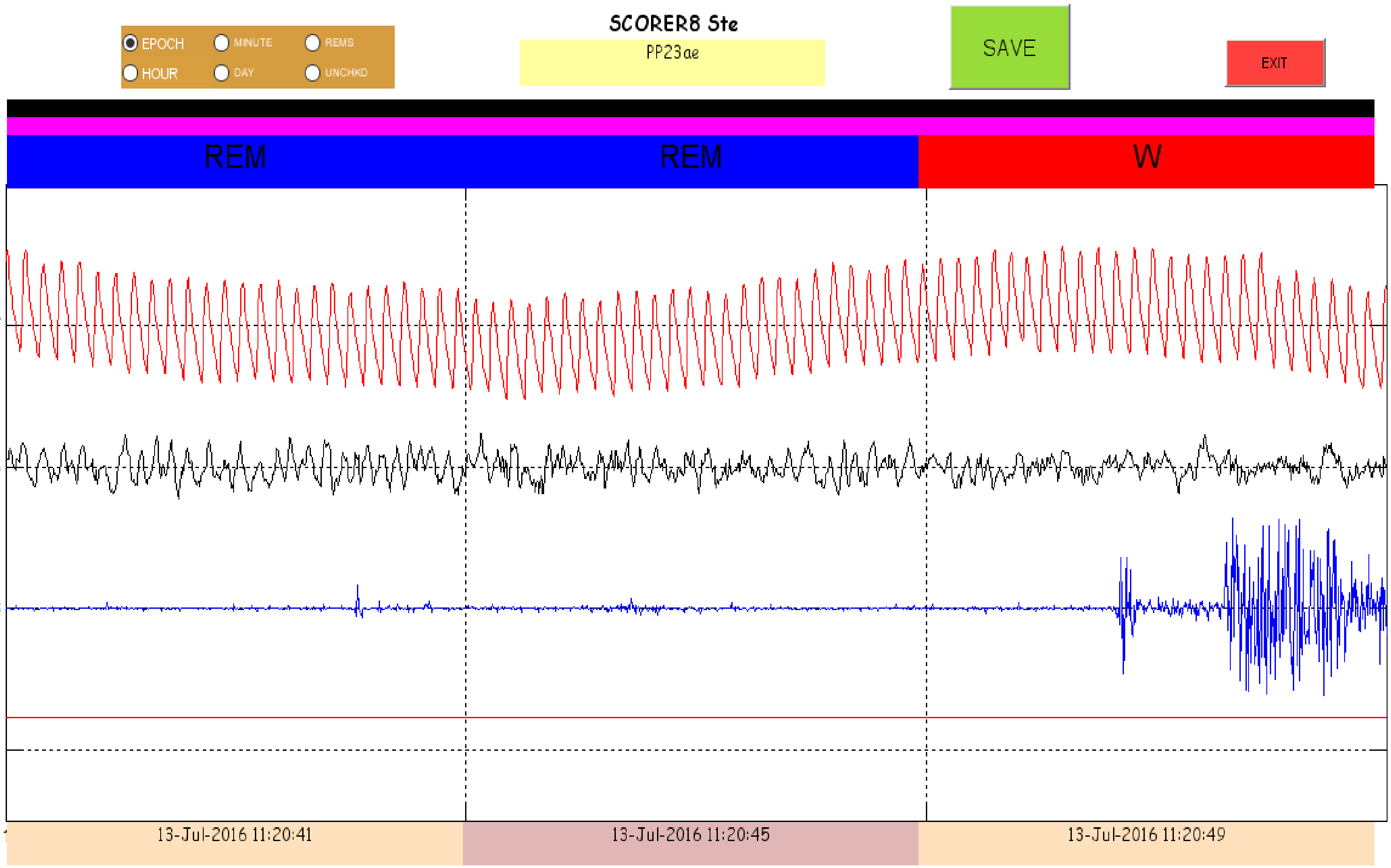
SCORING AT *PRISM lab*

Scoring performed on <u>4 s epochs</u>, based on EEG/EMG signals

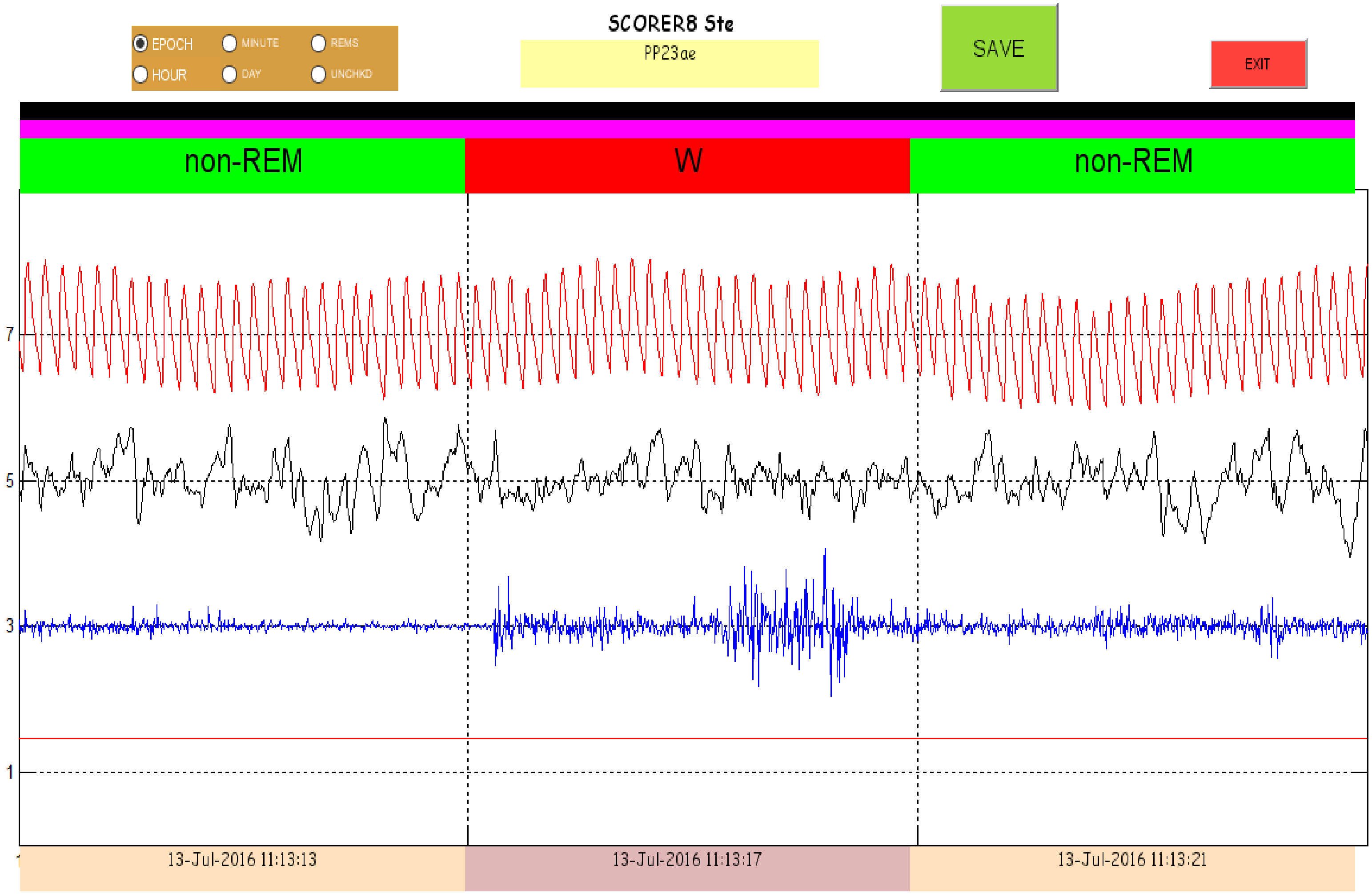
W: EMG: high tone
EEG: low voltage, possible θ(6–9 Hz) components
NREMS: EMG lower than in W
EEG: high voltage, prominent δ (0.5–4 Hz) components
REMS: EMG muscle atonia, occasional muscle twitches
EEG: low voltage, predominant θ components



SCORING SLEEP/AROUSALS IN RODENTS



SCORING SLEEP/AROUSALS IN RODENTS



Power spectrum analysis of EEG to highlight wake-sleep cycle differences

knockout, WT, wild type, YO, young.

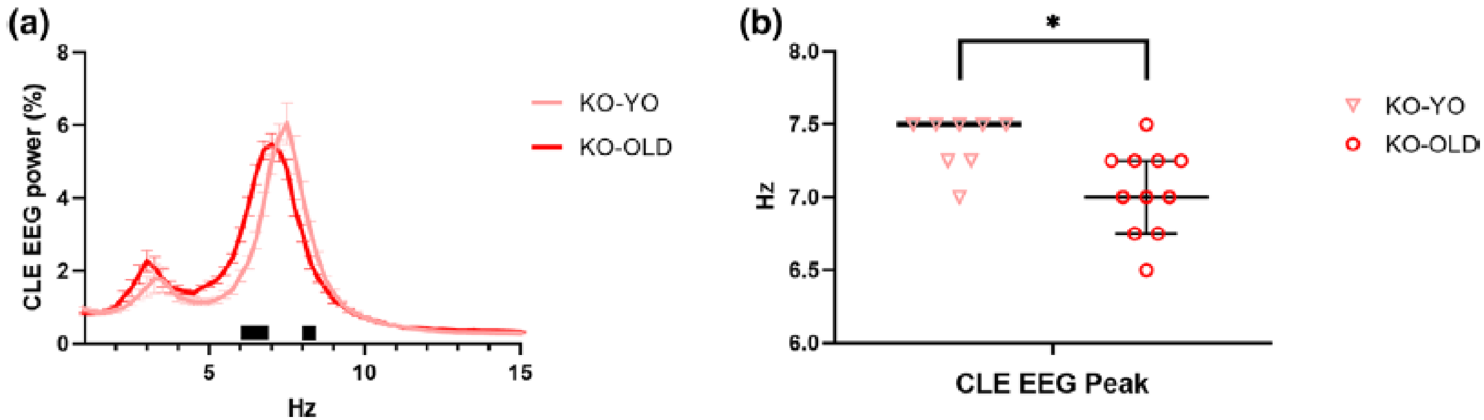
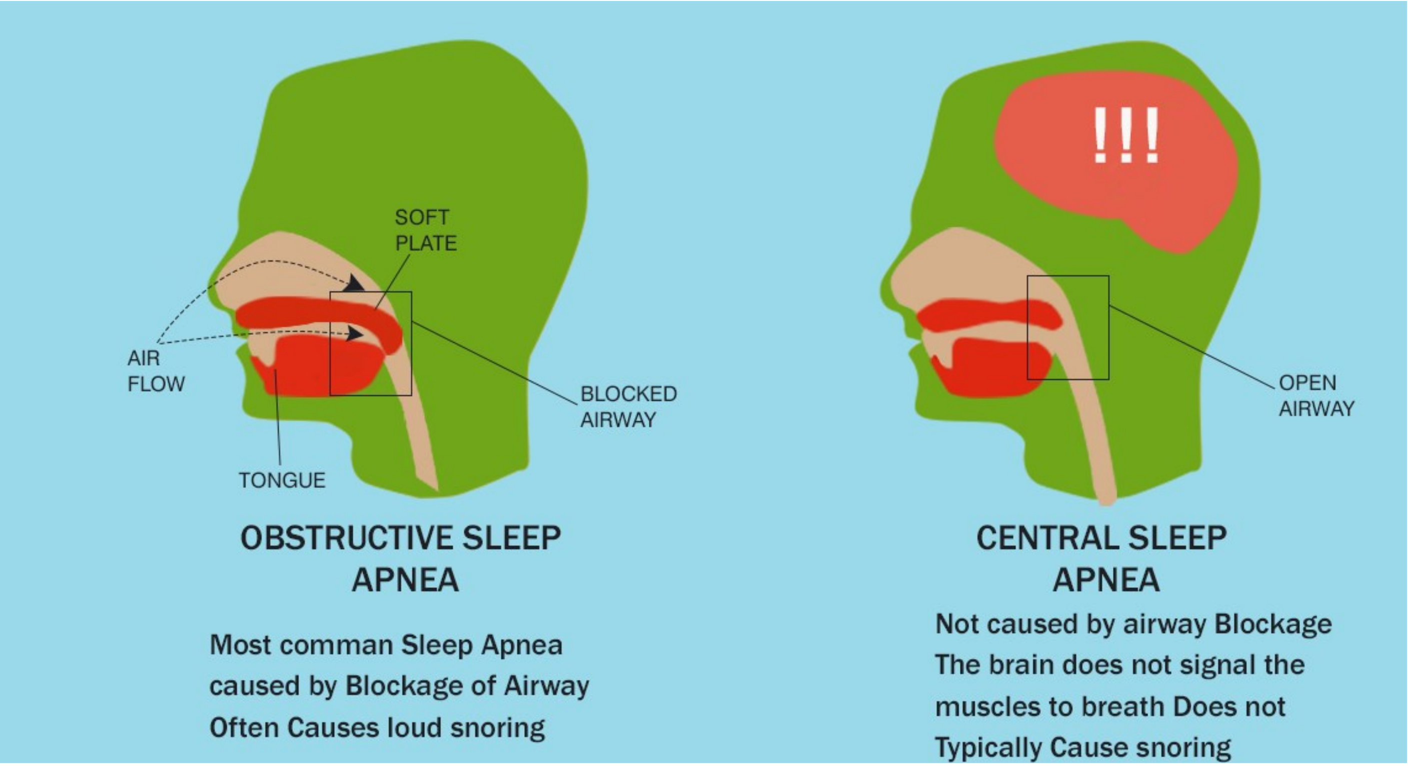
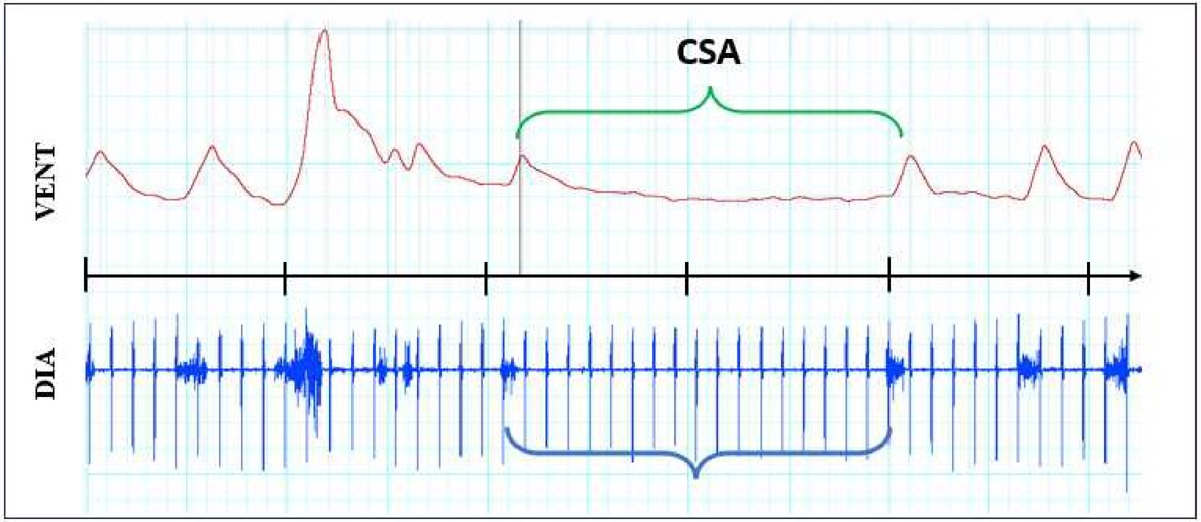


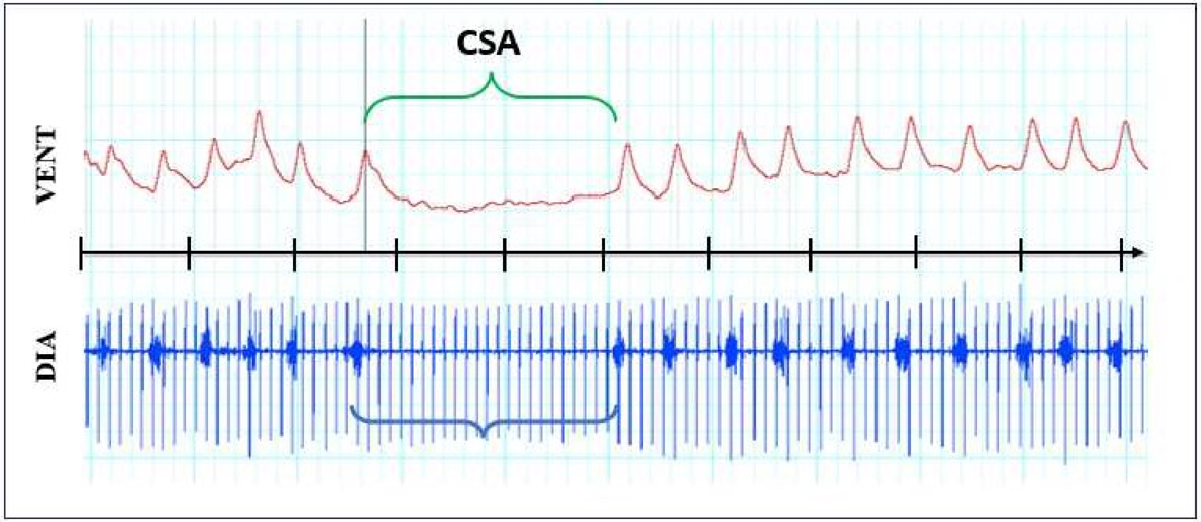
FIGURE 4 Electroencephalogram spectral analysis during cataplexy-like episodes. Electroencephalographic (EEG) spectral power (expressed as mean ± SEM of the percentage of total EEG spectral power) and peak (median with the 95% confidence interval) during cataplexy-like episodes (CLE) are shown for OLD and young (YO) orexin-knockout (KO). **p* < 0.05 for Mann–Whitney test with the false discovery rate correction, with significant Kruskal-Wallis test. Black bars indicate *p* < 0.05 for the post-hoc corrected comparison between KO-OLD and KO-YO mice with significant age × EEG frequency interaction.

Central vs obstructive apnea during sleep

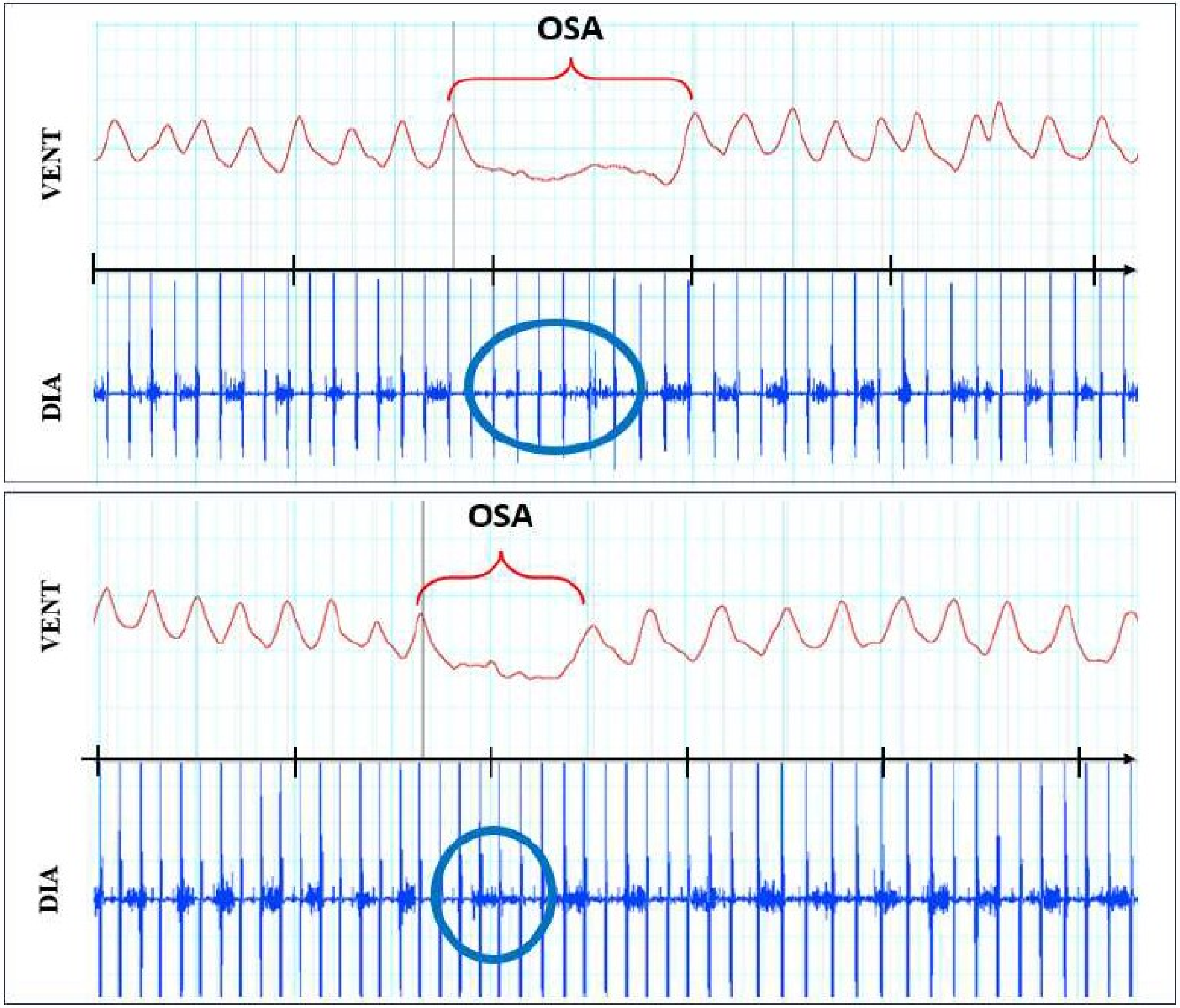


Central vs obstructive apnea during sleep





Central vs obstructive apnea during sleep



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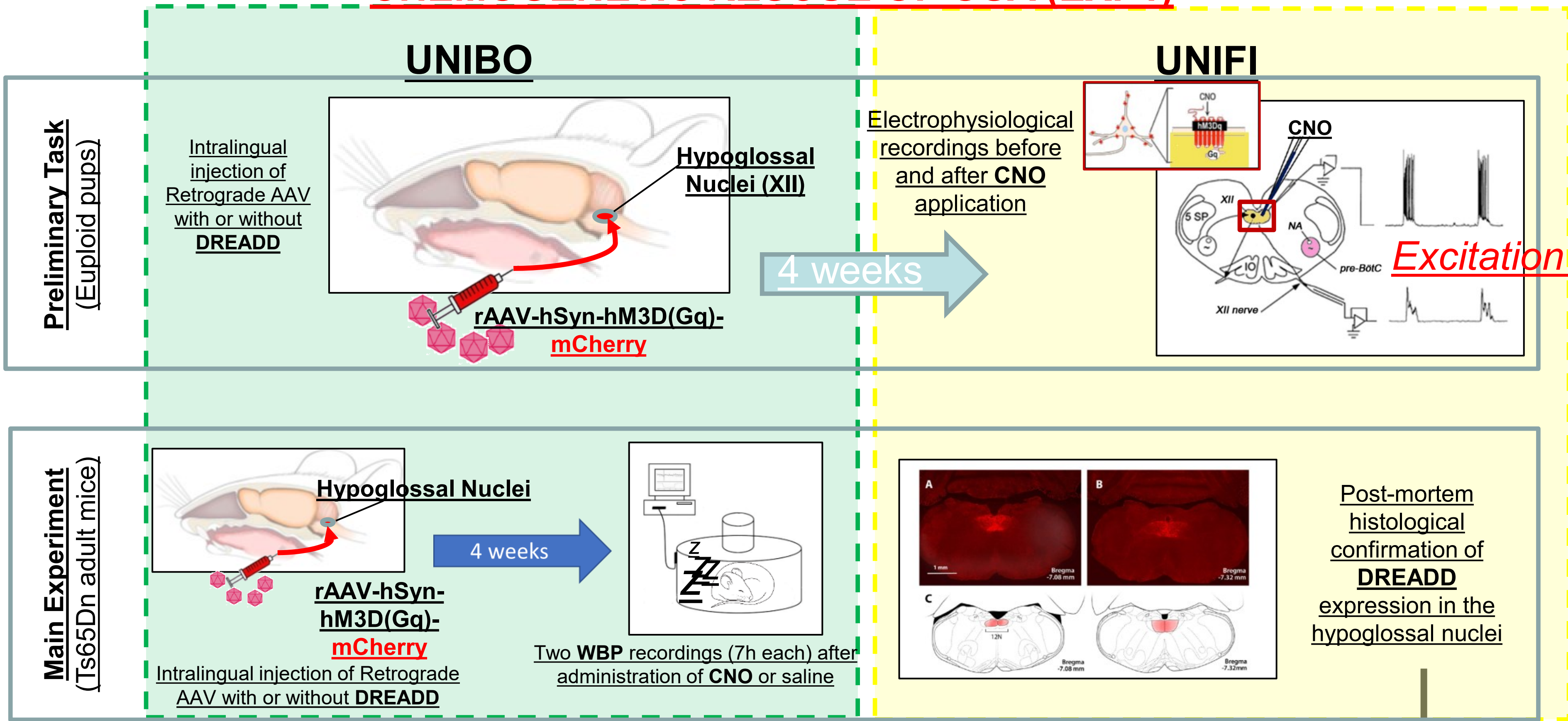
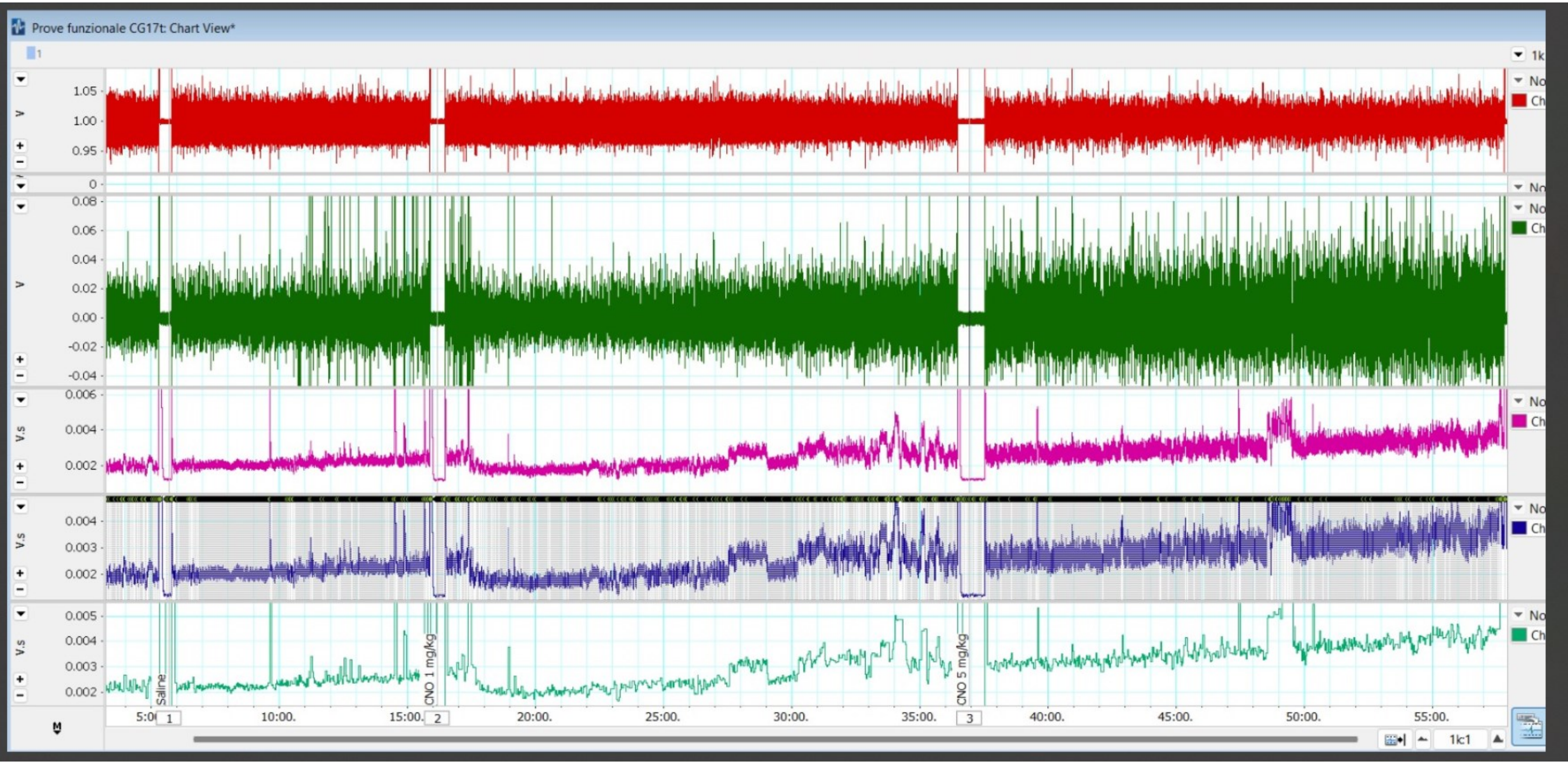
PART A

1. Research project title

Chemogenetic and Optgenetic Rescue of Sleep Apnea (CORSA) in mice

CHEMOGENETIC RESCUE OF OSA (EXP1)




Prove funzionale CG17t: Chart View*
Saline
CNO 1 mg/kg
CNO 5 mg/kg
5:0
10:00.
15:00.
20:00.
25:00.
30:00.
35:00.
40:00.
45:00.
50:00.
55:00.
1k1

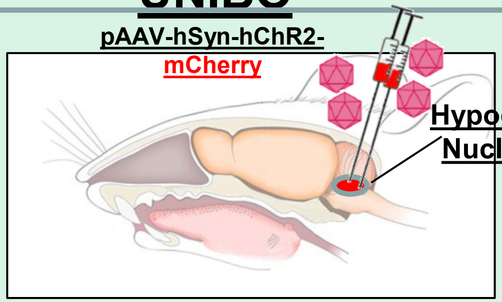
OPTOGENETIC RESCUE OF OSA (EXP2)

UNIBO

UNIFI

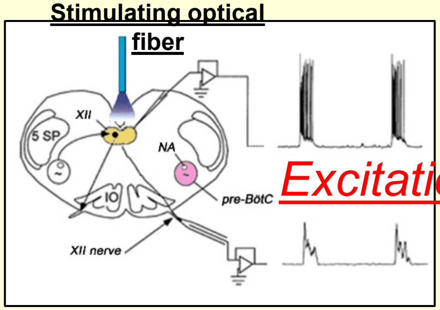
Preliminary Task (Euploid pups)

Brainstem injections of AAV with or without **light-sensitive receptors**



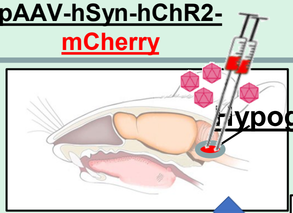
4 weeks

Electrophysiological recordings during different stimulating frequencies and pulse durations



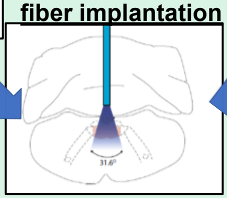
Excitation?

Main Experiment (Ts65Dn adult mice)

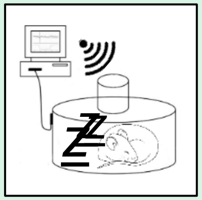


Brainstem injection of AAV with or without **light-sensitive receptors**

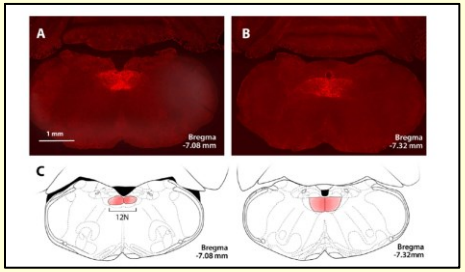
4 weeks



1 week

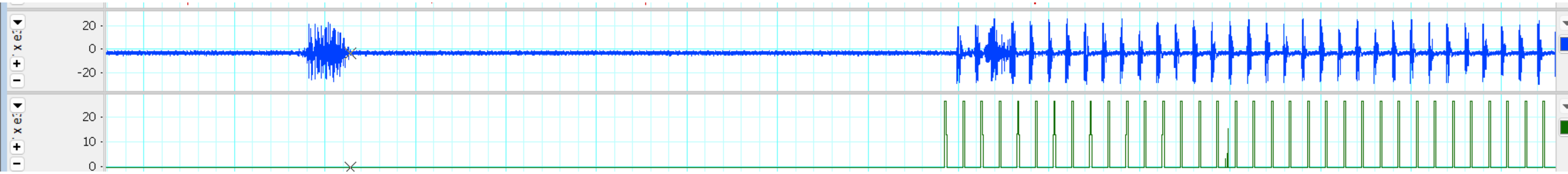


Two **WBP** recordings (7h each) with or without light stimulation during REM sleep



Post-mortem histological confirmation of **light-sensitive receptors** in the hypoglossal nuclei

Tongue EMG



Optical Stimulation

PRIN: PROGETTI DI RICERCA DI RILEVANTE INTERESSE NAZIONALE – Bando 2022 PNRR
Prot. P2022RTCCA

PART A

1. Line of intervention

Main line/Linea Principale

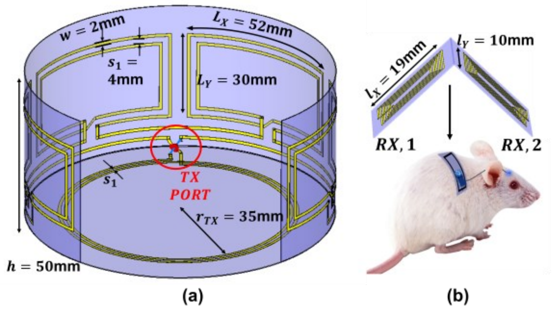
2. Research project title

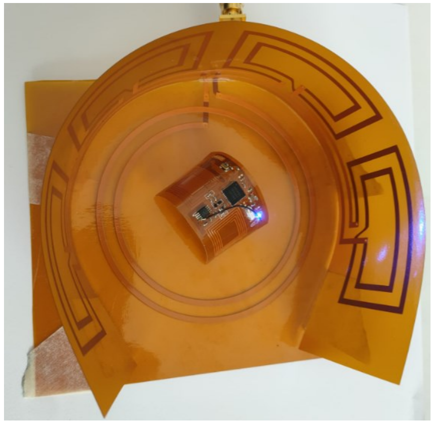
ESTROSA: Energy-autonomous System for TReatment of Obstructive Sleep Apnea

Methods

• To develop a WPT opto-stimulating system, we performed in-silico conceptualization, simulation and validation of transmitting and implantable receiving components. The design and optimization of these devices guarantees to harvest a minimum DC (Direct Current) power of 30 mW regardless of the position of the mouse inside the WBP chamber, to allow brain optogenetic stimulation using an implantable light-emitting diode (μ-ILED) directly controlled by a microcontroller unit.

Results





Schematic representation of the WPT system (left) and images of the prototype (right)

We designed and validated a transmitting multi-coil array operating at 13.56 MHz to be integrated into the walls of a circular mouse cage or WBP chamber. A pair of receiving coils with minimal size and weight were designed and validated to be implanted subcutaneously on the mouse back, together with the electronic circuitry for RF-to-DC power conversion and digital wireless control, then connected subcutaneously to a μ-ILED probe in the HN.

The in-silico validation of the proposed WPT opto-stimulating system was successful, supporting the achievement of a continuous power of ≥ 30 mW, independently of the position and orientation of the mouse inside the cage. This amount of harvested energy is sufficient for the wireless control of the implantable receiving device and to switch the μ-ILED on

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