

SLEEPING UNDER THE STARS: ASTROCYTE CONTRIBUTIONS TO SLEEP

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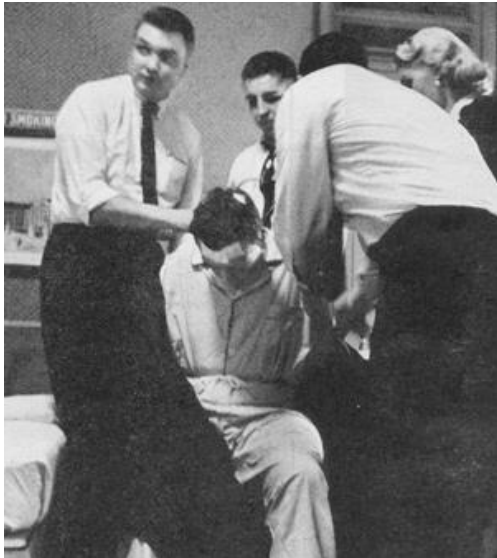
Outline

- Background on sleep and glia
- Evidence for a role for astrocytes in sleep regulation
- Conclusions and implications
- Next questions

Q: Why Sleep? A: Peter Tripp



- Radio DJ who did a wake-a-thon for March of Dimes in 1959
 - 201 hours (8 days)
- Experienced hallucinations
- Became hostile
- Long-term effects
 - Lost his job
 - Got divorced
 - Psychotic symptoms continued



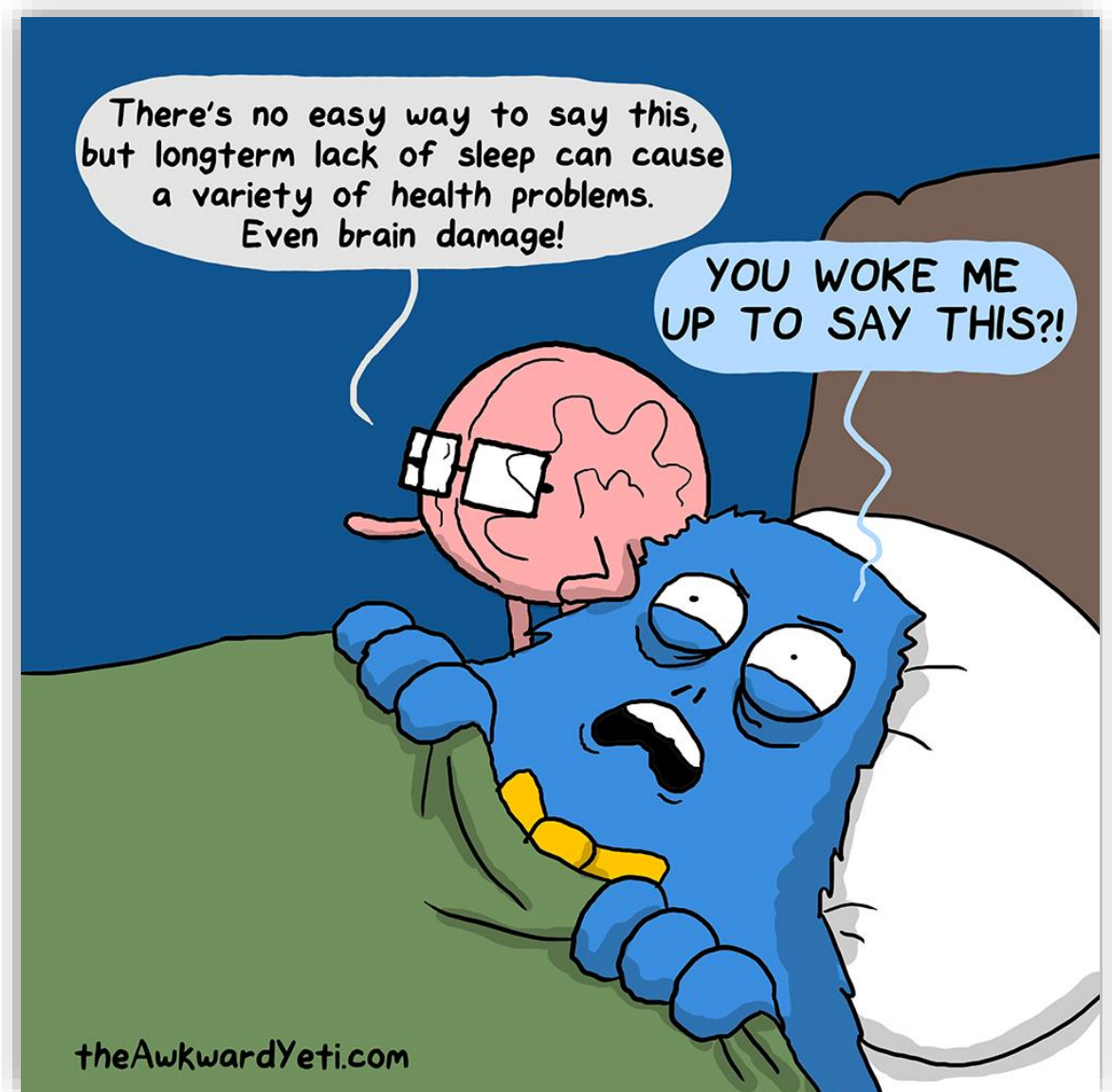
Sleep is an unsolved mystery

- Sleep is evolutionarily conserved across species, but why?
- Greatest impact on central nervous system
- Sleep is homeostatically regulated, but how?



Insufficient sleep is a prevalent health issue

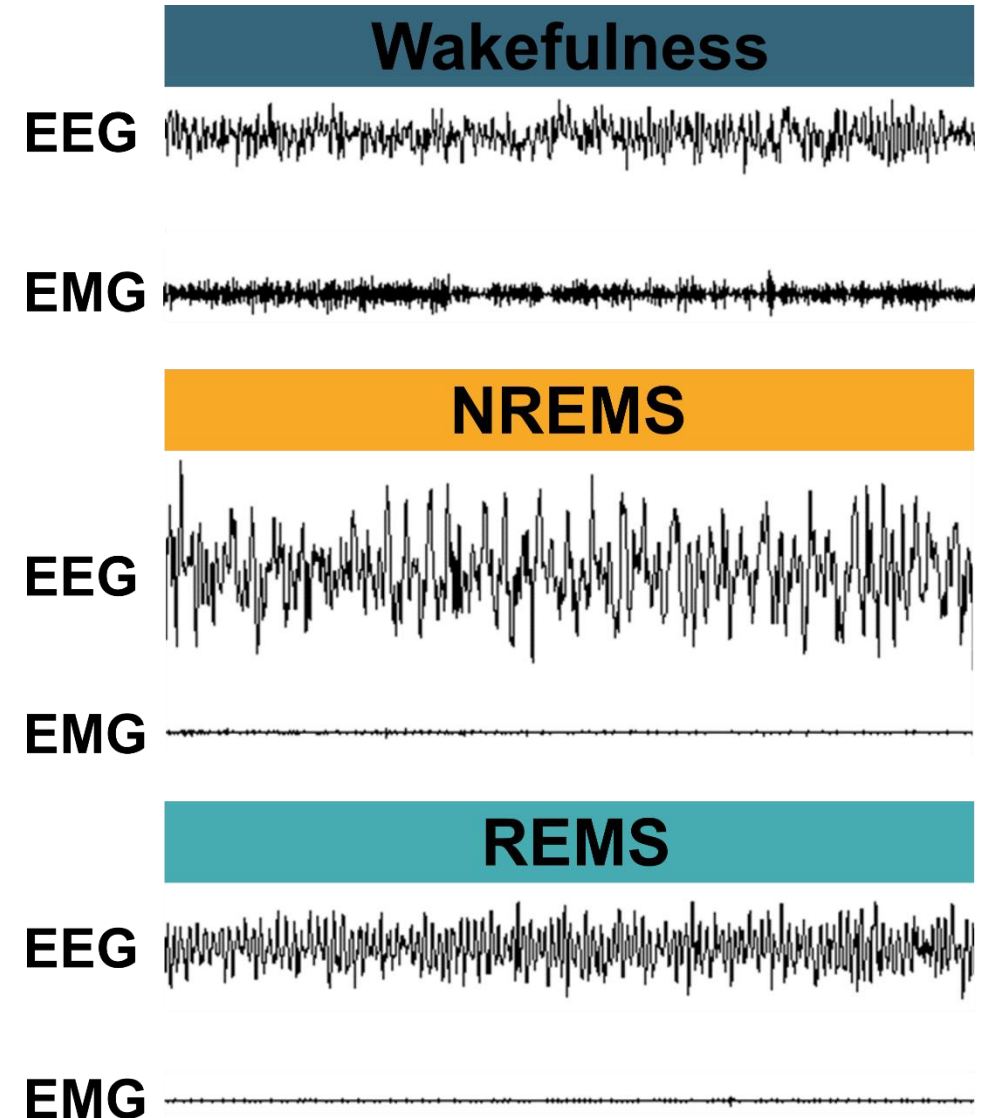
- 50 – 70 million American adults have a chronic sleep disorder¹
- >\$100 billion is spent annually treating sleep disorders¹
- Poor sleep is associated with:
 - Impaired cognition
 - Impaired immune function
 - Metabolic disorders
 - Neurodevelopmental & neurodegenerative disorders
 - CNS injury
 - Pain
 - Cancer



¹Institute of Medicine. Sleep Disorders and Sleep Deprivation: An Unmet Public Health Problem. Washington, DC: The National Academies Press; 2006

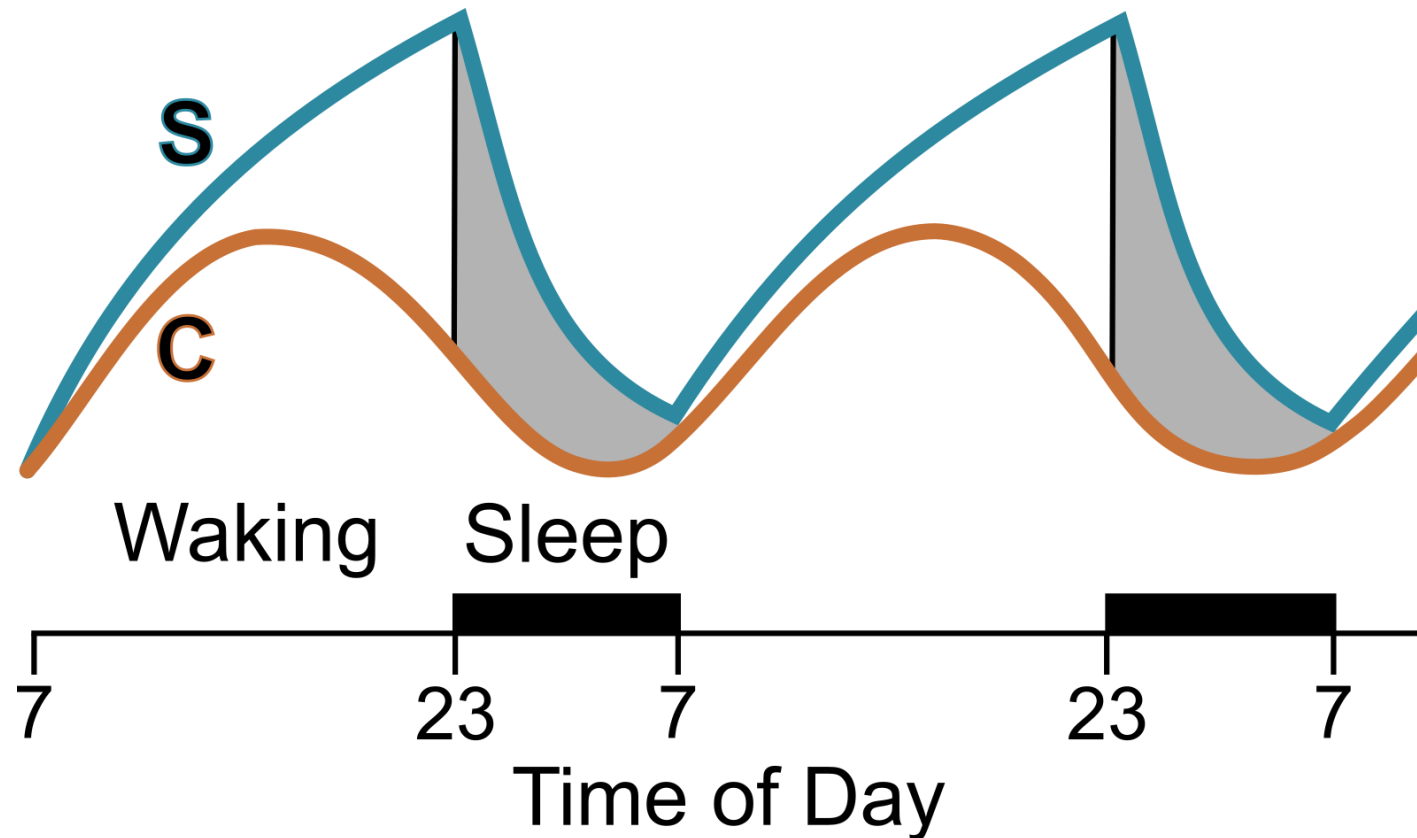
Sleep is a quantifiable and modifiable behavior

- Electrophysiologically defined
 - Electroencephalography (EEG)
 - Electromyography (EMG)
- 3 arousal states
 - Wakefulness
 - Non-rapid eye movement sleep (NREMS)
 - Rapid eye movement sleep (REMS)
- Quantifiable
 - How much
 - Organization
 - How deep or intense
 - NREM delta power
- Modifiable



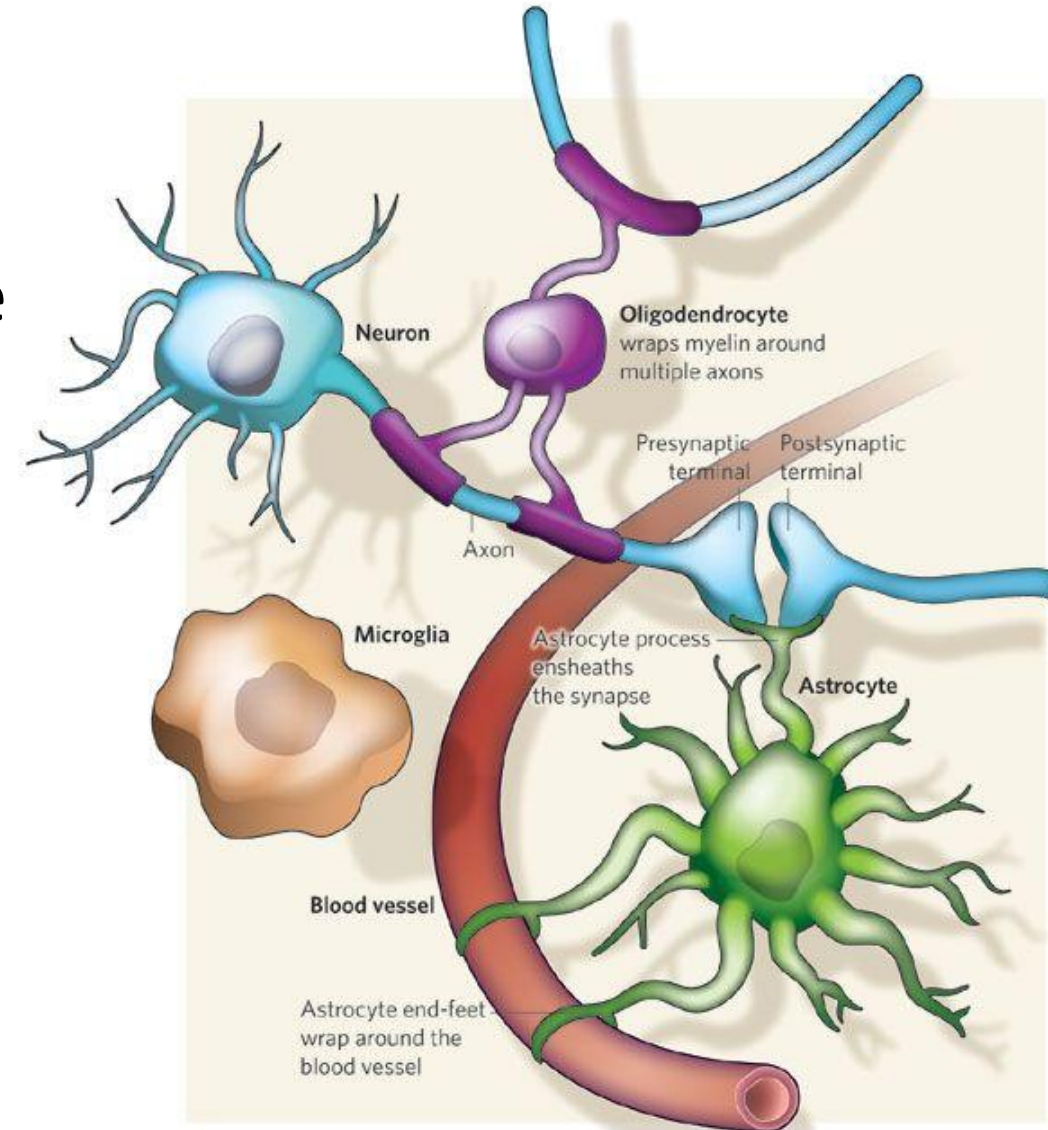
Sleep is a homeostatically regulated process

- Two-process model of sleep regulation
 - **Process C**: when to sleep
 - **Process S**: how much sleep we need (and how deeply)



Glia: more than glue...

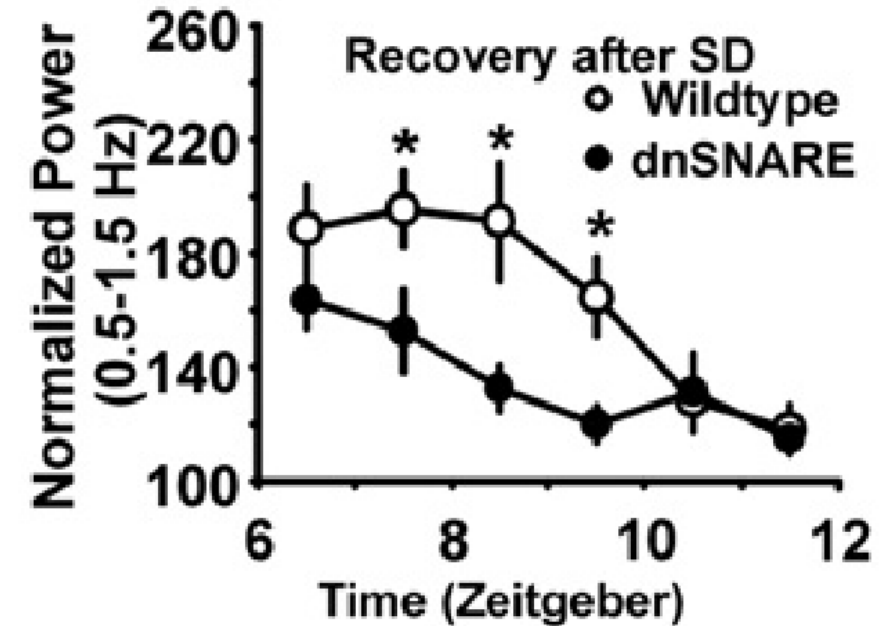
- Neurons do not exist in isolation
 - 1:1 - 50:1 ratio of glia:neurons
- Astrocytes poised to detect and integrate neural signals
 - Tile the brain
 - Surround synapses
 - Have receptors for neurotransmitters
- Astroglial roles in:
 - Immune function
 - CNS injury recovery
 - Development
 - Aging
 - Cognition
 - Sleep



Evidence for a role for astrocytes in sleep

Astrocytes release sleep-promoting substances¹

Astrocytes respond to sleep-promoting substances to promote sleep²



¹Halassa et al., Neuron, 2009

²Ingiosi et al., Brain Behav Immun, 2015

Challenges for studying astrocytes



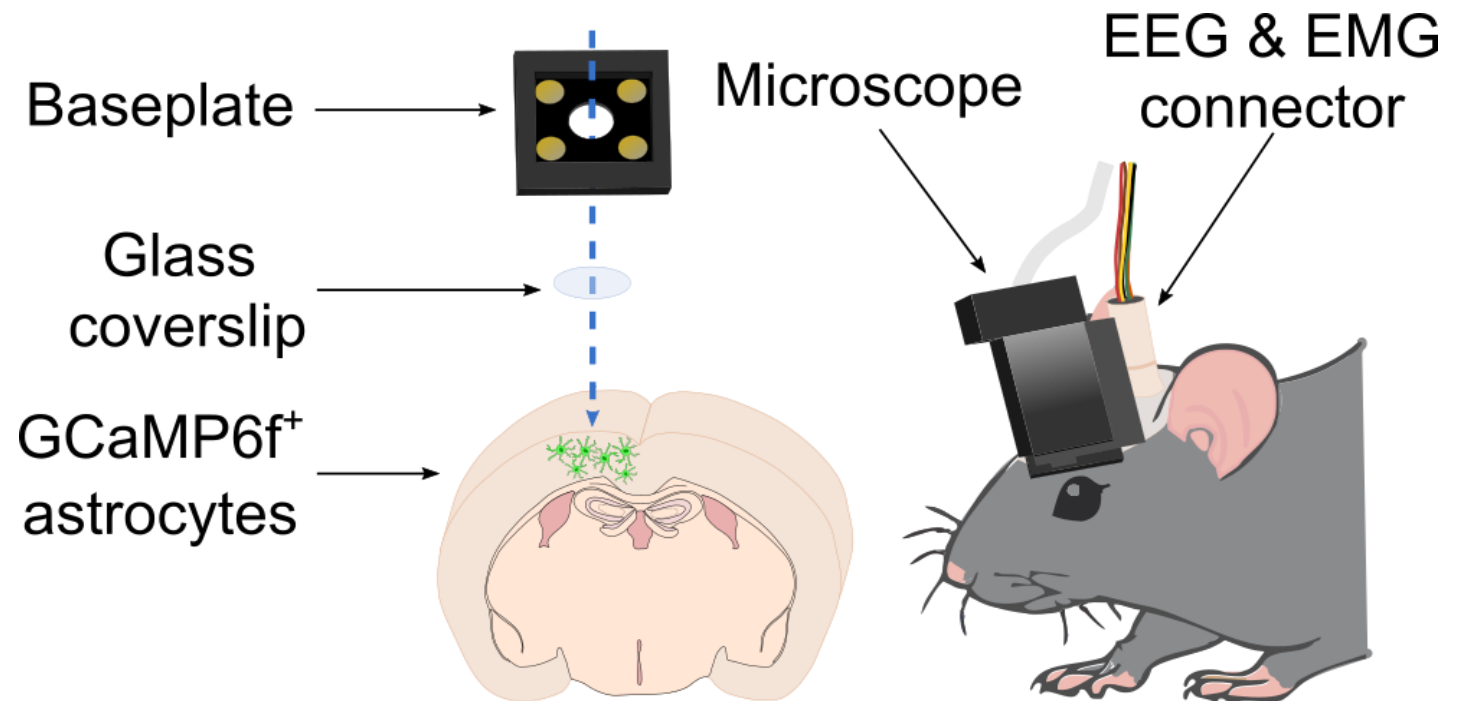
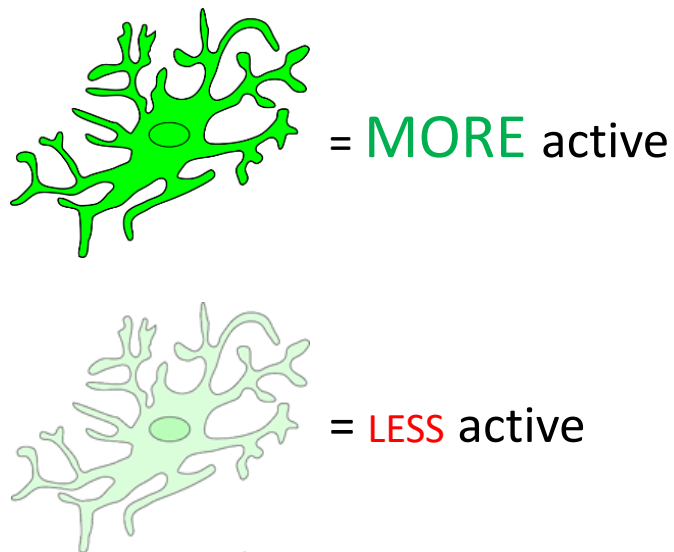
- Limited tools to study astrocytes
- Not electrically excitable like neurons
- Astrocytes use calcium (Ca^{2+}) to mediate their functions¹
- Astroglial Ca^{2+} is important for releasing sleep-promoting substances²

My research aims to determine a role for astroglial Ca^{2+} in sleep

- Does astroglial Ca^{2+} activity change dynamically across the sleep-wake cycle?
- Does astroglial Ca^{2+} change with sleep need?
- Is astroglial Ca^{2+} important for sleep homeostasis?

In vivo Ca²⁺ imaging of astrocytes

- GCaMP6f expressed selectively in astrocytes of frontal cortex
- Head-mountable epifluorescent miniscope & two-photon microscopy with EEG/EMG recording
 - **Miniature microscope:** Okay spatial resolution but animal moves freely
 - **Two-photon microscope:** Good spatial resolution but animal is restrained
- Imaged *unanesthetized* mice naturally cycling through arousal states



Astroglial Ca^{2+} activity measures

A. AMPLITUDE

Determined by intensity changes of fluorescent Ca^{2+} indicators as a proxy for changes in Ca^{2+} concentration

B. EVENT FREQUENCY

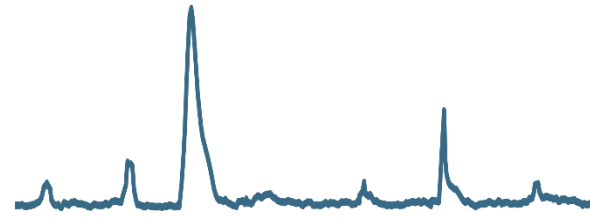
Deflections of Ca^{2+} activity from baseline levels can be defined as events based on a set of criteria or a threshold and tallied across time

C. SYNCHRONY

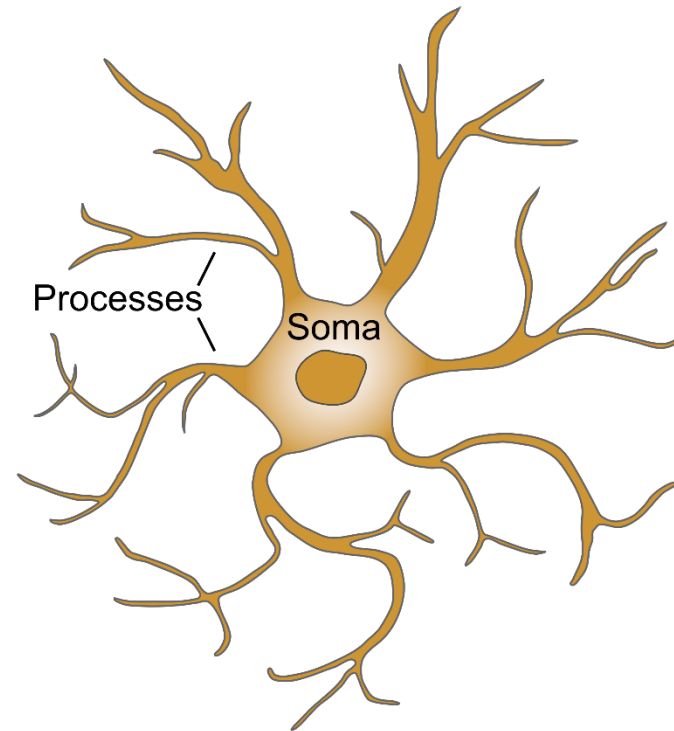
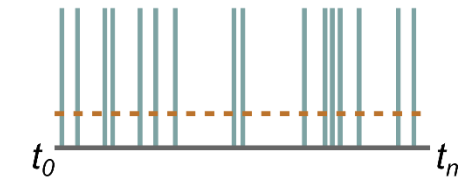
Determined by comparing patterns of Ca^{2+} activity across astrocytes, within individual astrocytes, or with a different cell type (e.g., neurons)

D. SIZE, DURATION, & MOVEMENT

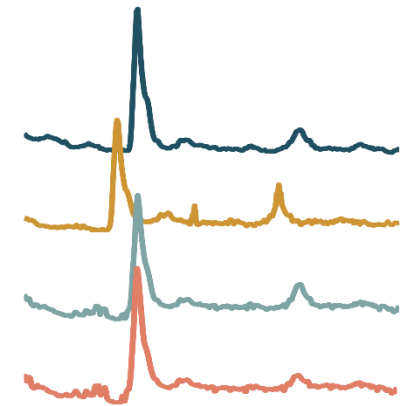
A. Amplitude



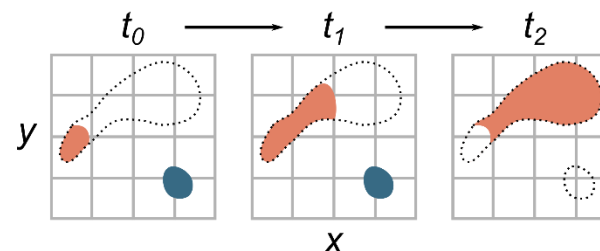
B. Event Frequency



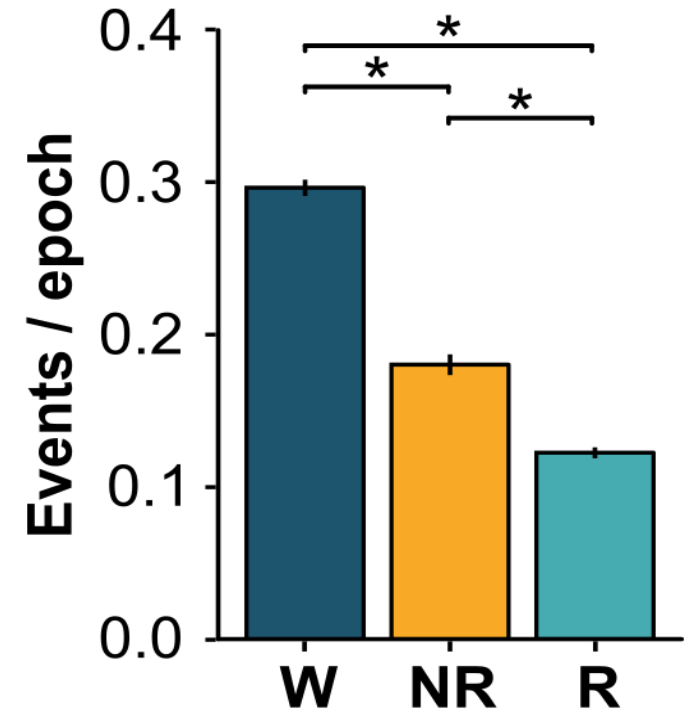
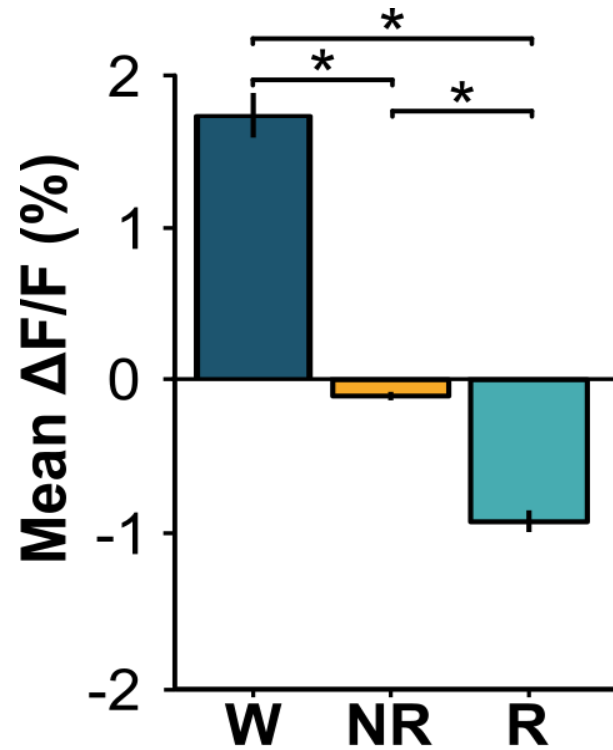
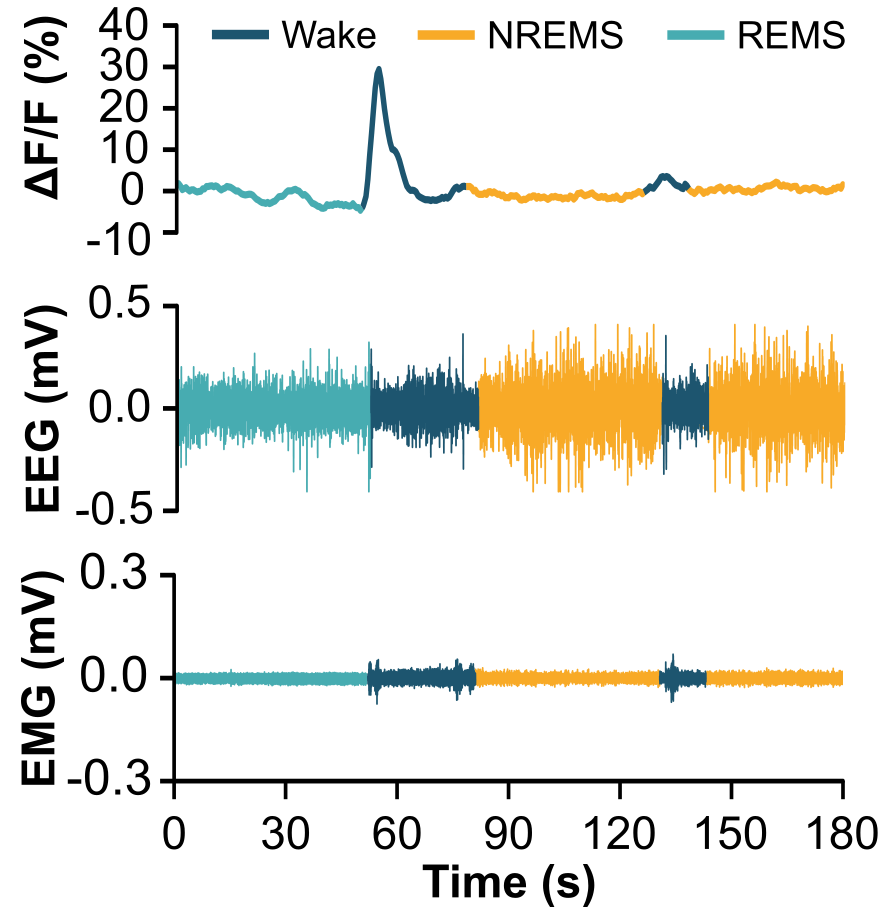
C. Synchrony



D. Size, Duration, & Movement

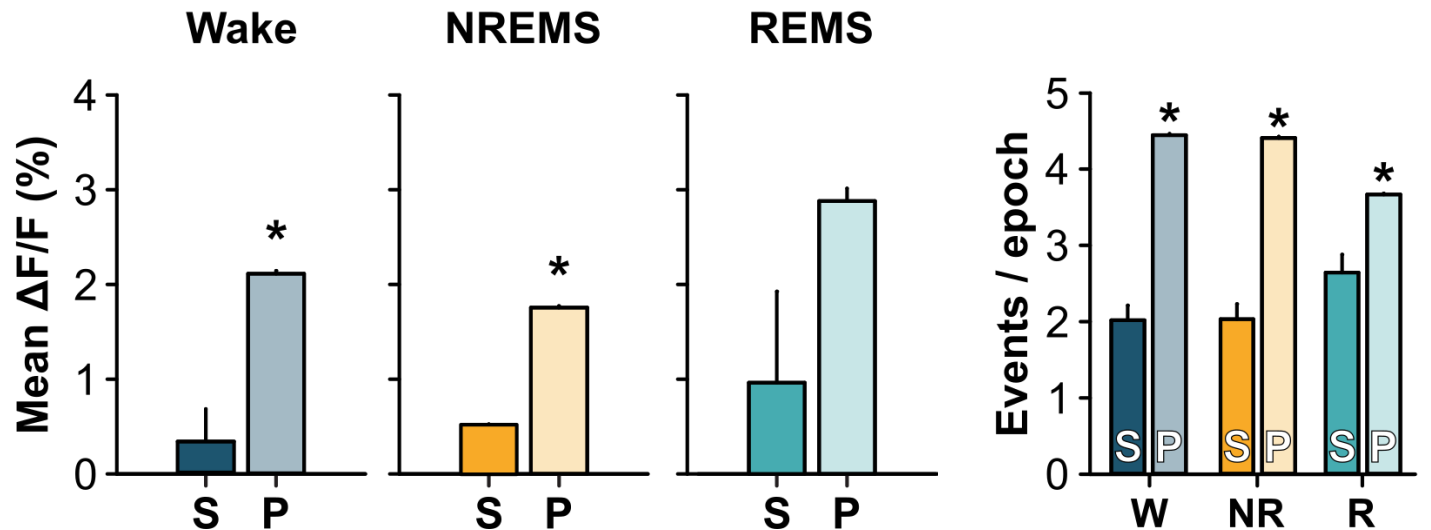
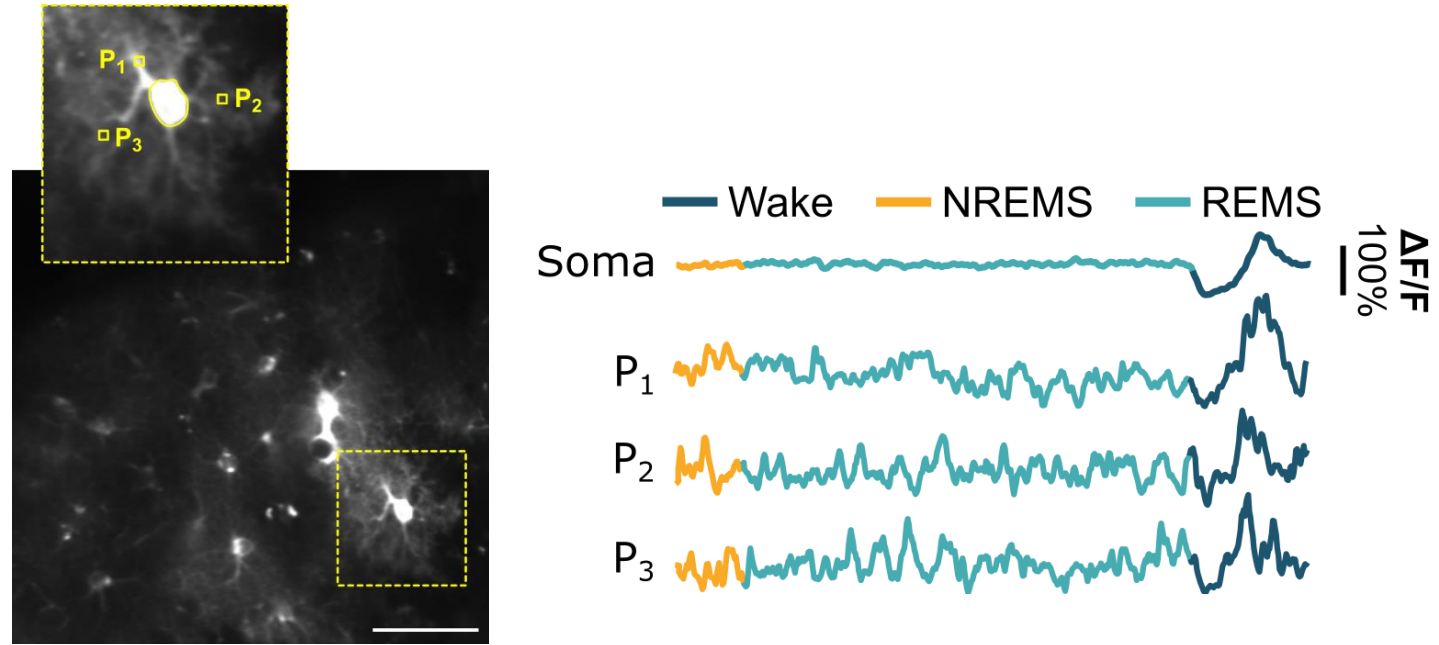


Astroglial Ca^{2+} activity is greatest during wake

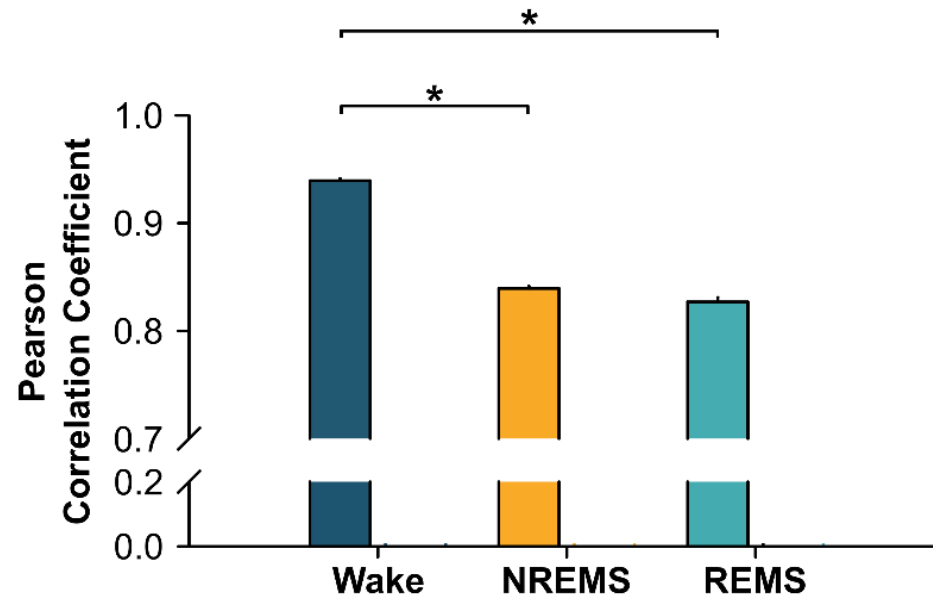
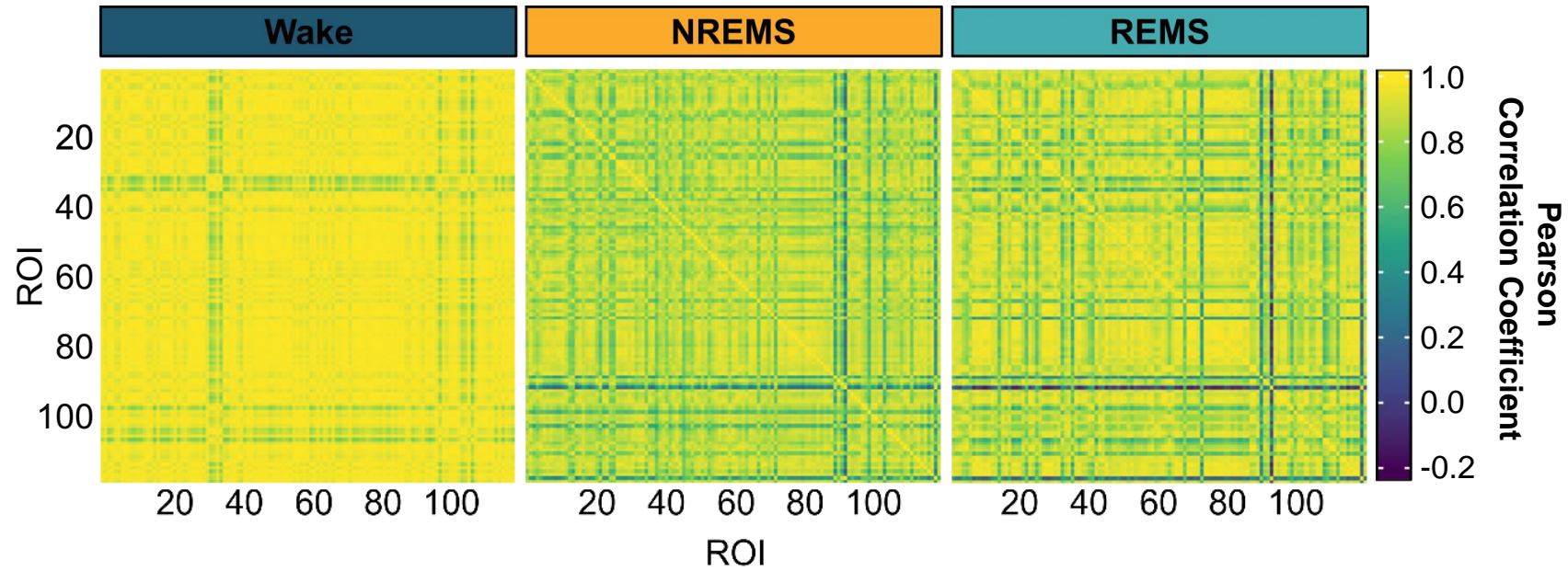


Ca²⁺ dynamics are more robust in astroglial processes

- Ca²⁺ changes are greater in processes vs. somata
- Ca²⁺ events are more frequent in the processes vs. somata



Astroglial synchrony is greatest during wake



Research questions

- Does astroglial Ca^{2+} activity change dynamically across the sleep-wake cycle?

Yes!

- Ca^{2+} dynamics are greatest in wake and lowest in REMS
- Ca^{2+} dynamics are more robust in the processes compared to somata
- Astroglial synchrony is highest in wake

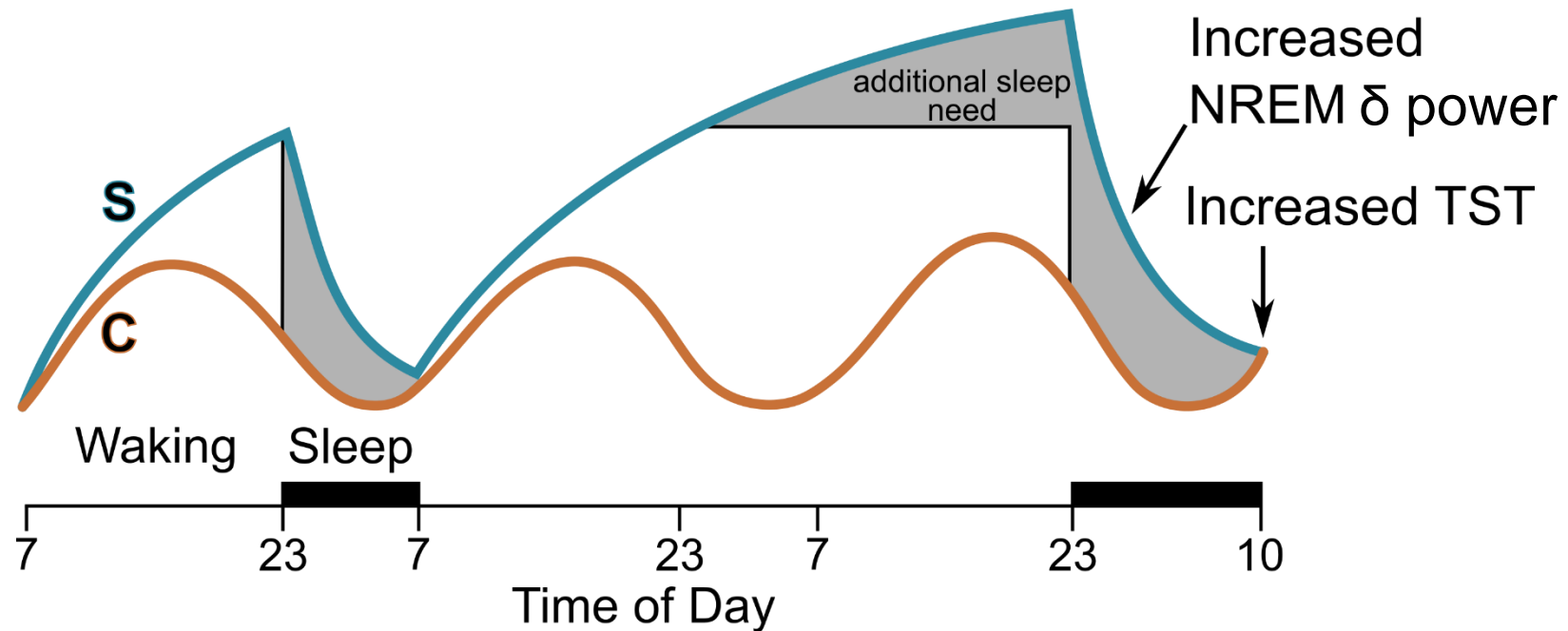
- Does astroglial Ca^{2+} change with sleep need?

Sleep deprivation increases sleep need

- Sleep need accumulates with wake & dissipates with sleep

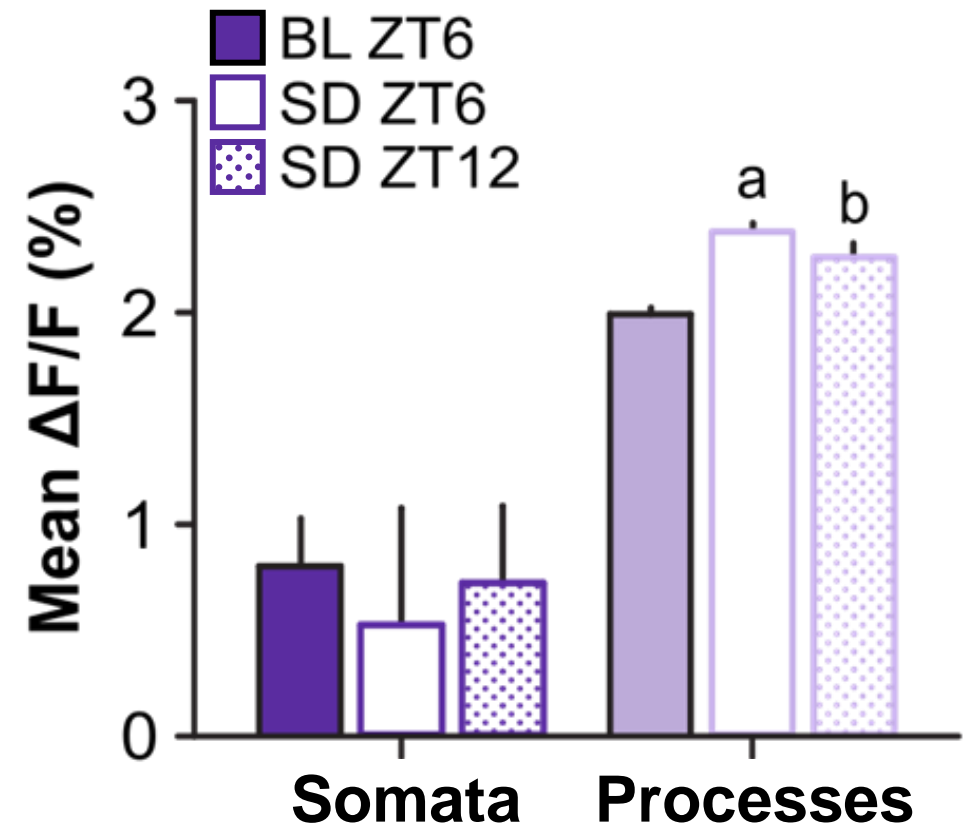
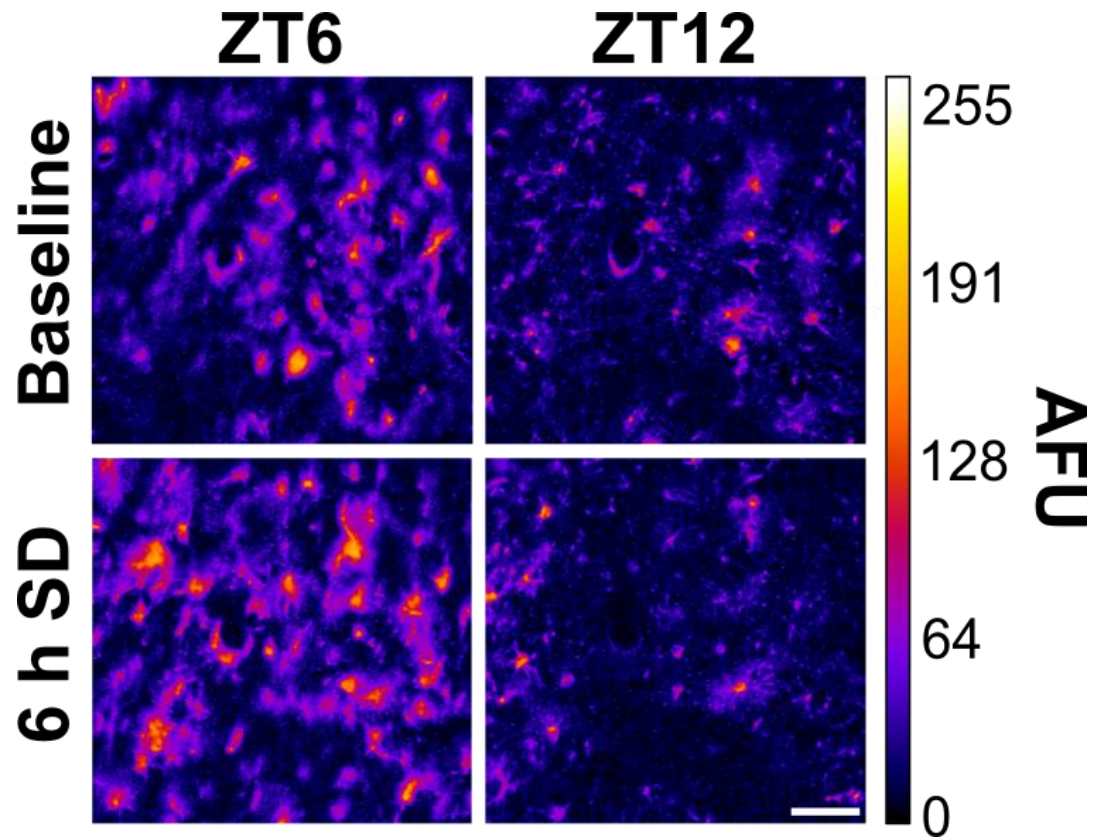
- Sleep deprivation (SD) increases sleep need

- Sleep response to SD:
 - ↑ total sleep time (TST)
 - ↑ NREM delta (δ) power
 - ? Astroglial Ca^{2+}



- 6 h SD via “gentle handling” in mice

Astroglial Ca^{2+} tracks sleep need after SD



- Astroglial Ca^{2+} changes are most aligned with NREMS

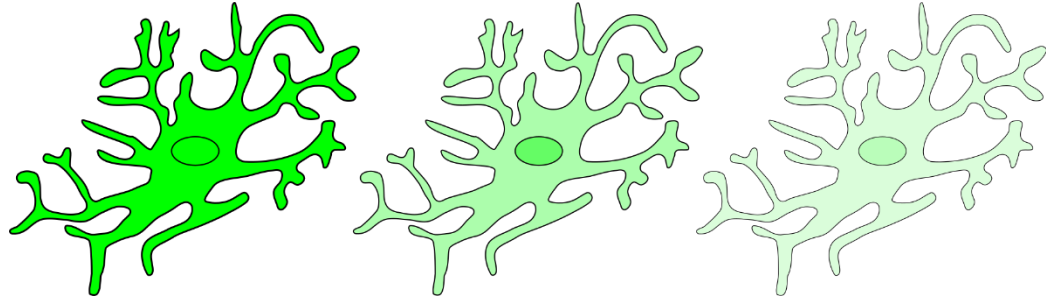
Summary of astroglial Ca^{2+} dynamics

DYNAMIC

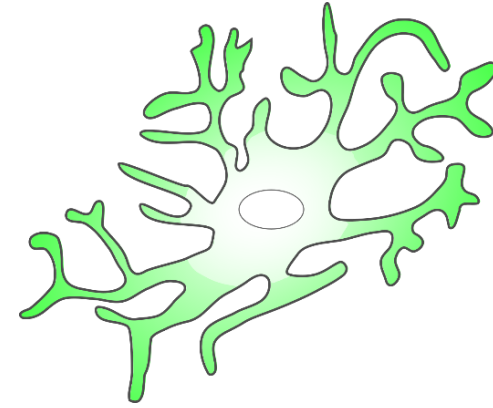
WAKE

NREMS

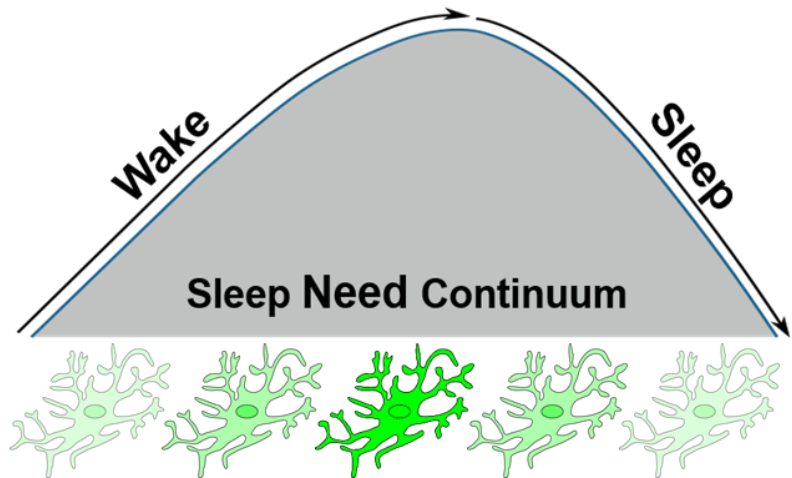
REMS



PROCESSES > SOMATA



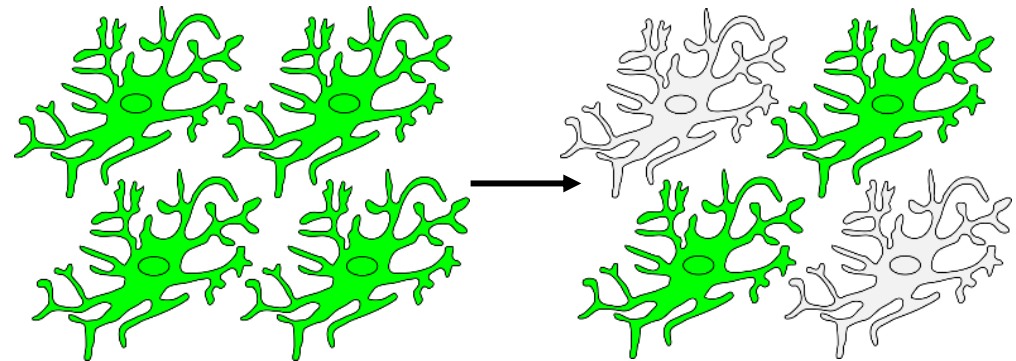
ENCODE SLEEP NEED



SYNCHRONY CHANGES WITH STATE & SD

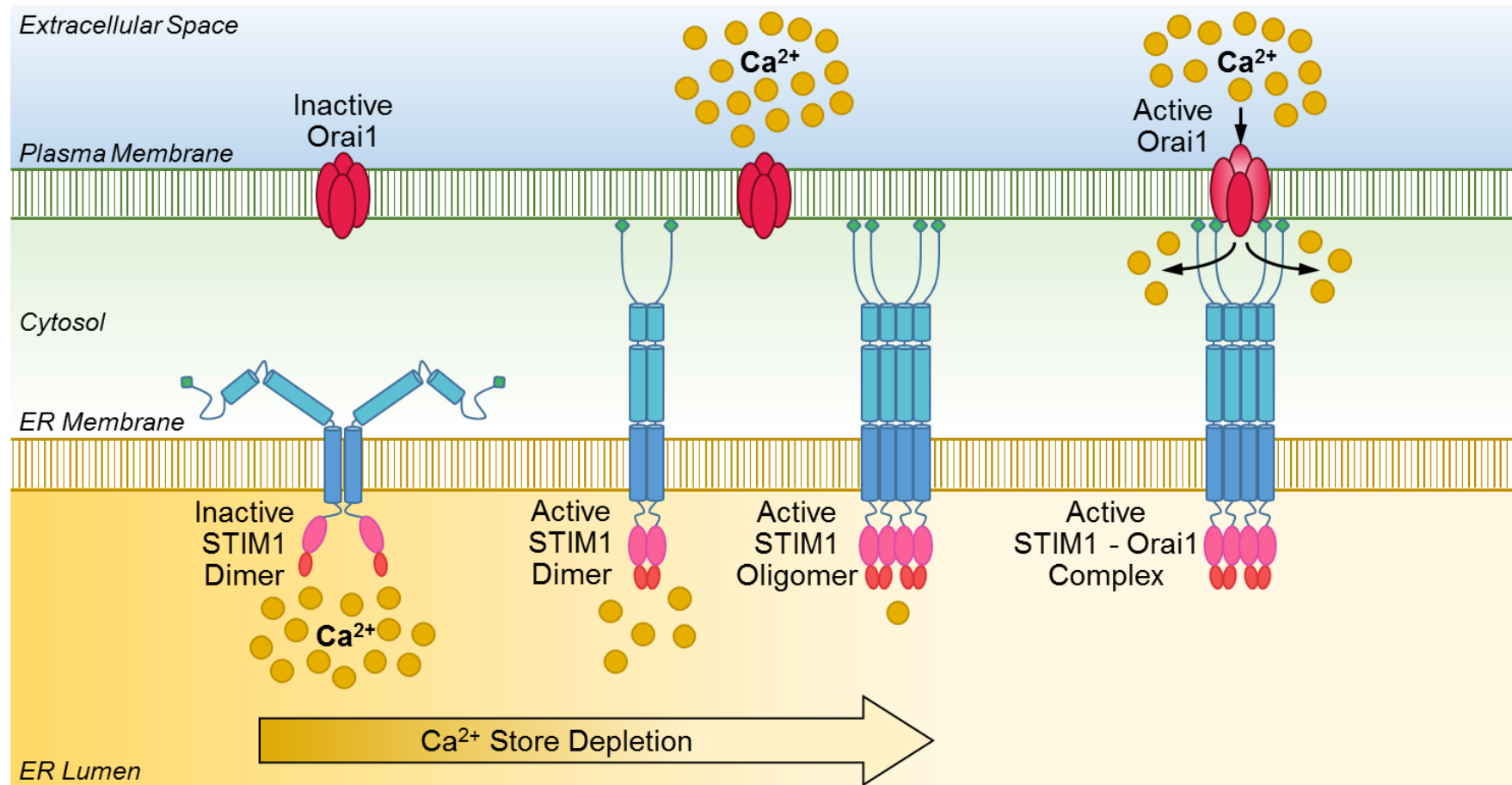
WAKE OR BL

SLEEP OR SD



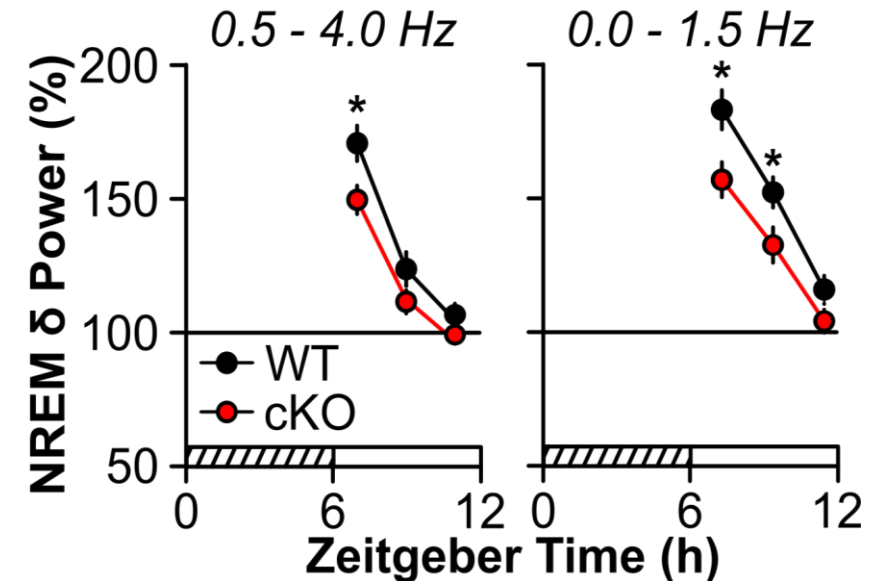
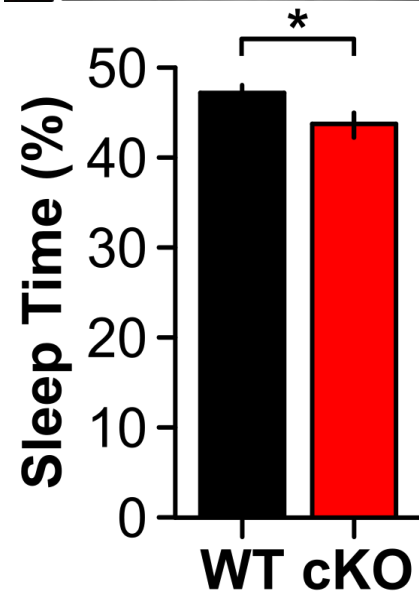
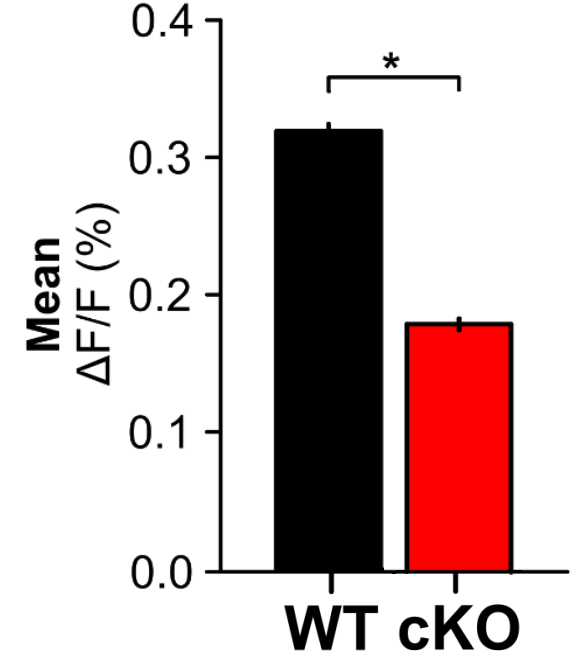
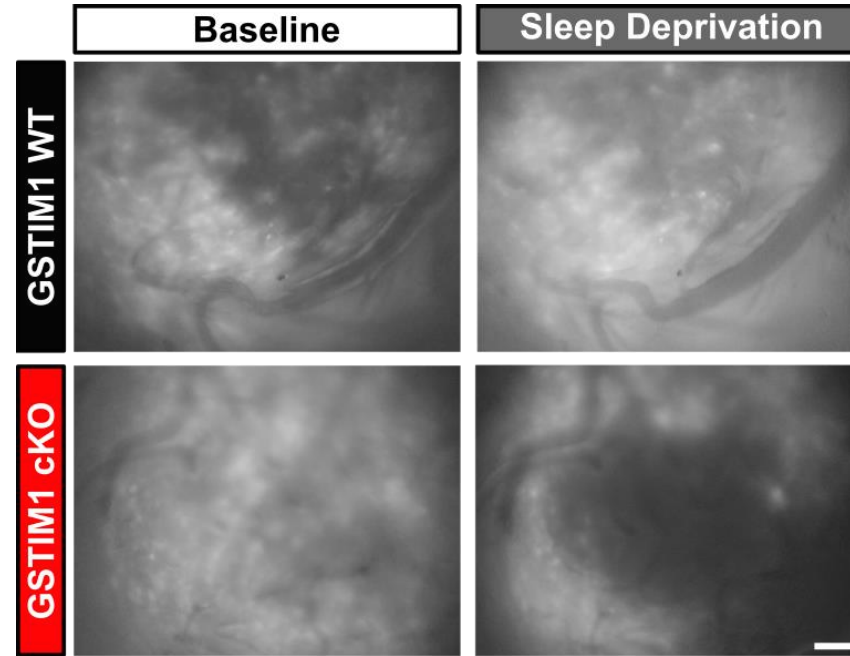
Is astroglial Ca^{2+} important for sleep homeostasis?

- Inhibit replenishment of internal Ca^{2+} stores via knockout of stromal interaction molecule 1 (STIM1)
 - GFAP-CreERT2 x STIM1^{fl/fl}



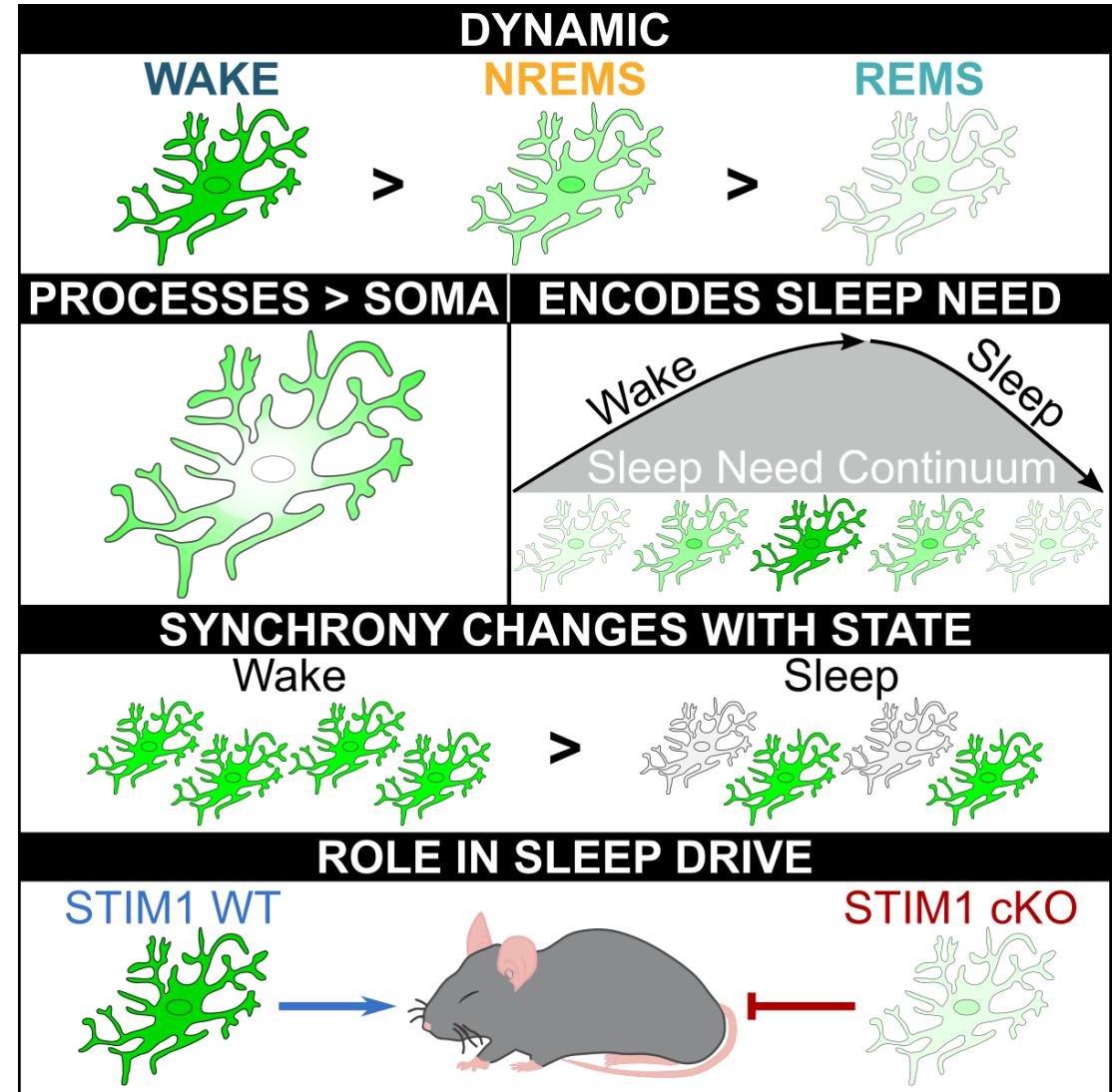
Astroglial Ca^{2+} plays a role in sleep homeostasis

- Knocking out STIM1 in astrocytes decreases:
 - Intracellular Ca^{2+}
 - Sleep time after SD
 - Sleep need/intensity after SD



CONCLUSIONS: Astroglial Ca^{2+} activity contributes to sleep regulation

- Astrocytes represent a new level of brain organization that varies dynamically across arousal states
- Astroglial Ca^{2+} activity tracks changes in sleep need
- Astroglial activity does not mirror neuronal activity
- Astroglial Ca^{2+} plays a role in regulating behavioral responses to sleep loss



What's next?

- Are astroglial Ca^{2+} dynamics uniform throughout the brain?
- How do astrocytes interact with other cells to mediate sleep-wake behavior and neurophysiology?
- Does astroglial activity change with diseases or disorders associated with abnormal sleep?

THANK YOU!

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