Sleep and Metabolism in the Adult Brain
The Statistics about Sleep

77% of students (nationally) report not getting enough sleep.

70 million Americans suffer from a sleep problem.

Lack of sleep increases risk of heart attack by 45%.

Sleeping five or fewer hours a night may increase mortality risk by as much as 15%.

At least 25 million adults have obstructive sleep apnea.

About 7 in 10 people with type two diabetes also have obstructive sleep apnea.

Drowsy driving causes more than 30,000 motor vehicle accidents each year.
What is Circadian Rhythm?

**Circadian Rhythm:**

“Circa diem”: about a day
Body’s clock.
Physical, mental, and behavioral changes that follow a roughly 24 hour cycle.

Responds primarily to light and darkness.

![Diagram showing circadian rhythm with various activities and physiological changes throughout the day](image)
How does your Circadian Rhythm work?

SCN: Suprachiasmatic Nucleus (of the anterior hypothalamus)
- Controls the biological clock that governs some circadian rhythms

RHT: Retinohypothalamic tract
How does your Circadian Rhythm work?

Normal circadian sleep rhythm. Sleep urge is greatest at night with a small increase at mid day. Sleep need increases throughout the waking hours and is replenished during sleep.
Why does lack of sleep impair brain function?

Sleep deprivation: is a condition of not having enough sleep. It can be chronic or acute.

Effects: Fatigue, daytime sleepiness, clumsiness, higher risk of stroke, heart attack, heart disease, diabetes, obesity, depression, anxiety, and hypertension.

Affects the brain and cognitive functioning.
The Sleep and Biology Basics

Correlation between the lack of sleep and the production of ghrelin and leptin.

Ghrelin: the “hunger” hormone

Leptin: the “you’re full, stop eating” hormone

People who are sleep deprived produce more ghrelin and not enough leptin.

Sleep deprivation impairs your ability to process glucose.

Lack of sleep also triggers stress response (cortisol and norepinephrine), which are associated with insulin resistance.
High Fat Diets Lengthen Circadian Rhythms

Examine the effects of a high-calorie diet in mice on patterns of activity, feeding, and hormone production and the cycling of clock genes.
High Fat Diets Lengthen Circadian Rhythms

Mice were fed regular chow for one week before being transferred to constant darkness for two weeks. Before being transferred into darkness, the mice had a 12:12 light dark ratio.

- Showed a characteristic free-running rhythm of activity, about 23.6 hours.
  - Free Running: duration of time between the major activity periods on consecutive days.

Mice that were switched to a high fat (HF) chow showed an increase in the free running period as early as 1 week after starting the HF diet.

- The effect continued through weeks 2-6 on the diet.

Black bars: Regular Chow
Grey bars: High Fat

* Week 0 represents the second week of constant darkness when all animals were receiving RC.
High Fat Diets Changes Awake Feeding Behavior

- As early as the first week on the HF diet, mice consumed a higher percentage of daily food intake during the rest period.
- The altered feeding rhythm was due to an increase in the amount of food consumed in the light period and a decrease in the dark period (mice are nocturnal).

Altered feeding rhythm = altered circadian rhythm
What does this all mean?

- Sleep deprivation
- More Ghrelin, Less Leptin
- Dysregulation of feeding behavior
- Dysregulation of Circadian Rhythm
- High Fat Diet
- Obesity, Diabetes, Heart Disease, Stroke, etc.
The Not So Basic Biology . . .

Neurodegenerative diseases:

- β-Amyloid - Alzheimer's
- α-Synuclein - Parkinson's
- Tau - Alzheimer's/Parkinson's

All produced by metabolic processes in the brain, but where do they go?
Lymphatic system

- In the periphery we have the lymphatic system
- “Second Circulatory system”
- Used by the immune system to monitor abnormalities in blood and tissue as well as clear waste and maintain fluid balance
Glympathic system

- “Glia” - Helper cells within the brain, outnumber neurons 3.7:1
- Glia + Lymphatic = “Glympathic”
- Astrocytes make up the BBB - Decide what goes into or comes out of the brain
- AQP4- astrocyte aquaporin channel 4
- Deletion of aquaporin reduces metabolic clearance
- Concentration of Aβ is higher in awake rats - wakefulness is associated with increased Aβ production
The Hypothesis

Amyloid beta clearance is increased during sleep

The sleep-wake cycle regulates glymphatic clearance
Experimental Techniques

In vivo two-photon imaging:

- Injected dye fluoresces when struck by photon (laser)
- Good resolution and low toxicity in living tissue
- Up to ~ 1 mm depth
Experimental Techniques

Fluorescent tracers: Real-time assessment of CSF tracer movement

- Inserted into Subarachnoid CSF via Cannula (tube) into Cisterna Magna
- Volume and rate adjusted to avoid behavior or Electrocorticography (ECoG)
Experimental Techniques

State of brain activity:

- **Electrocorticography (ECoG)**
  - Records brain waves
  - Like EEG, but electrodes placed directly on nervous tissue (cortex)
  - Awake: high frequency patterns
  - Deeper sleep stages: slow wave brain patterns

- **Electromyography**
  - Neck muscles
The Experiment

Tracer inserted while asleep (naturally)

“Robust influx” of CSF to deeper cortical tissue layers (Green)
- Along arteries - “Periarterial Spaces”
- Into brain tissue - “Parenchyma” (ICF)

What Happens when mice are gently (and respectfully) awakened???

The Experiment

Arousal sharply reduced tracer influx (Yellow) by ~95% to deeper layers of cortical tissue
The Experiment

Does the state of brain activity control CSF influx?

New cohort - all awake

CSF influx largely absent (yellow)

Slowly reached superficial cortical layers

Then, animals were anesthetized...
The Experiment

(Red) tracer administered in anesthetized mice

CSF rapidly flushed into deeper cortical tissue layers
- Periarterial spaces and brain Parenchyma (ICF)
The Experiment

Rate comparable to naturally sleeping mice

Rate dependant upon degree of slow wave sleep activity

The state of brain activity controls CSF influx!

(- decreased delta wave activity with age and some medications)
The Experiment

Jeff Iliff (one of the authors of our paper) describes an animation of CSF influx in the awake vs sleeping brain...

https://www.youtube.com/watch?v=MJK-dMlATmM
7:10 - 7:32
The Conclusion

Sleep LITERALLY clears your mind!!!
What drives the movement of the CSF?

- CSF Influx is driven in part by arterial pulse waves

- HOWEVER! This does not explain why CSF influx does not increase during exercise
The more likely possibility is that interstitial volume changes!
The Test

- TMA is administered to a rat brain via an iontophoretic electrode
- A TMA sensitive recording electrode is placed 150µm away
- A higher concentration of TMA recorded means less TMA diffused along the interstitial space, indicating a smaller interstitial volume
# The Results

<table>
<thead>
<tr>
<th></th>
<th>Awake</th>
<th>Sleeping</th>
<th>Anaesthetised (Ketamine/xylazine)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interstitial Volume Fraction</td>
<td>14%</td>
<td>23%</td>
<td>23%</td>
</tr>
<tr>
<td>Tortuosity</td>
<td>No Change</td>
<td>No Change</td>
<td>No Change</td>
</tr>
<tr>
<td>Slow wave activity</td>
<td>Low</td>
<td>High</td>
<td>High</td>
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Efficiency of Metabolic Clearance During Sleep

\[
\text{C-inulin} + ^{125}\text{I-}\text{A}\beta_{1-40}
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Circadian Rhythm

Sleep-wake state
Noradrenaline

Locus Coeruleus
(major wakefulness-promoting nucleus)

Peripheral tissues
(Kidney and Heart)

Membrane transporters

Cell volume
Cocktail of adrenergic receptor antagonists or vehicle (aCSF).

The resulting rates of CSF influx was comparable to those during sleep rather than an awake state.
Effect of adrenergic inhibition on Interstitial Volume

- Antagonists were applied directly to cortex
- Interstitial volume significantly greater than awake littermates
- Increase in power of slow waves
- Awake mice are induced to have more sleep-like, though less regular, profile
Summary

- Buildup of toxic waste products can trigger neuronal degradation
- Similar to lymphatic system, the glymphatic system acts as the brain’s waste removal
- ISF decreases with age, increased amyloid-beta buildup
- Metabolic clearance is triggered by sleep, noradrenaline can impede process
- Cortical interstitial space increases by 60% during sleep
I can't sleep... would it help if I start calculating the maximum amount of sleep we can get every few minutes?

Hey just FYI before you get to sleep, that stupid thing you said earlier will resonate with the people who heard it forever and ever.

k nite

I promise if you hit snooze again, THIS nine minutes will make all the difference.