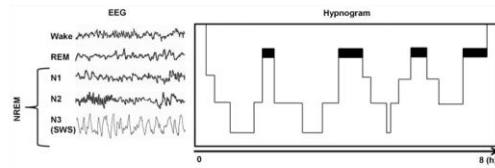


# Sleep

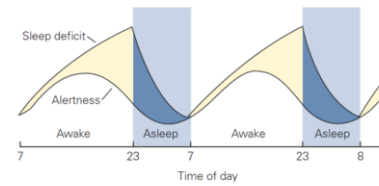
NEU/MOL 502A: **From Molecules to Systems to Behavior**

# Overview

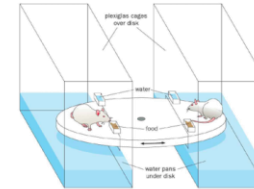
## Stages of Sleep



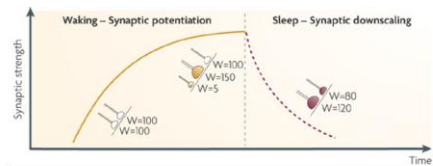
## Sleep Regulation



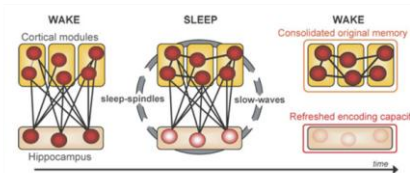
## Why do we need sleep?



## Synaptic Homeostasis Hypothesis

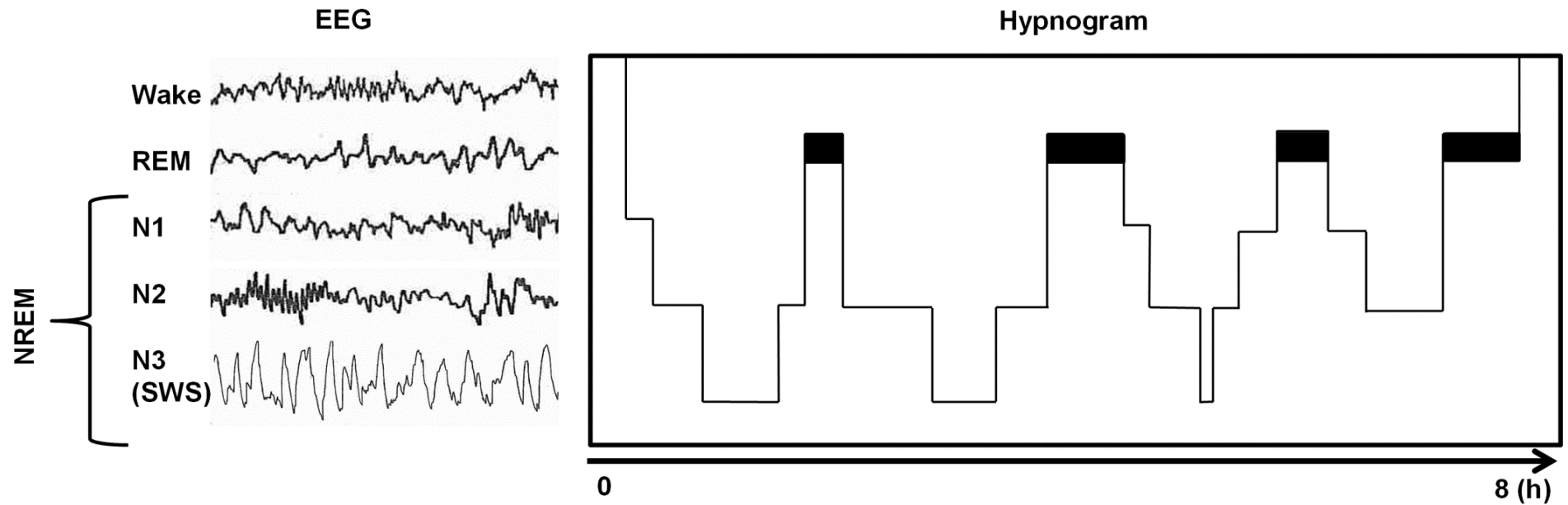


## Consolidation of Memories Hypothesis



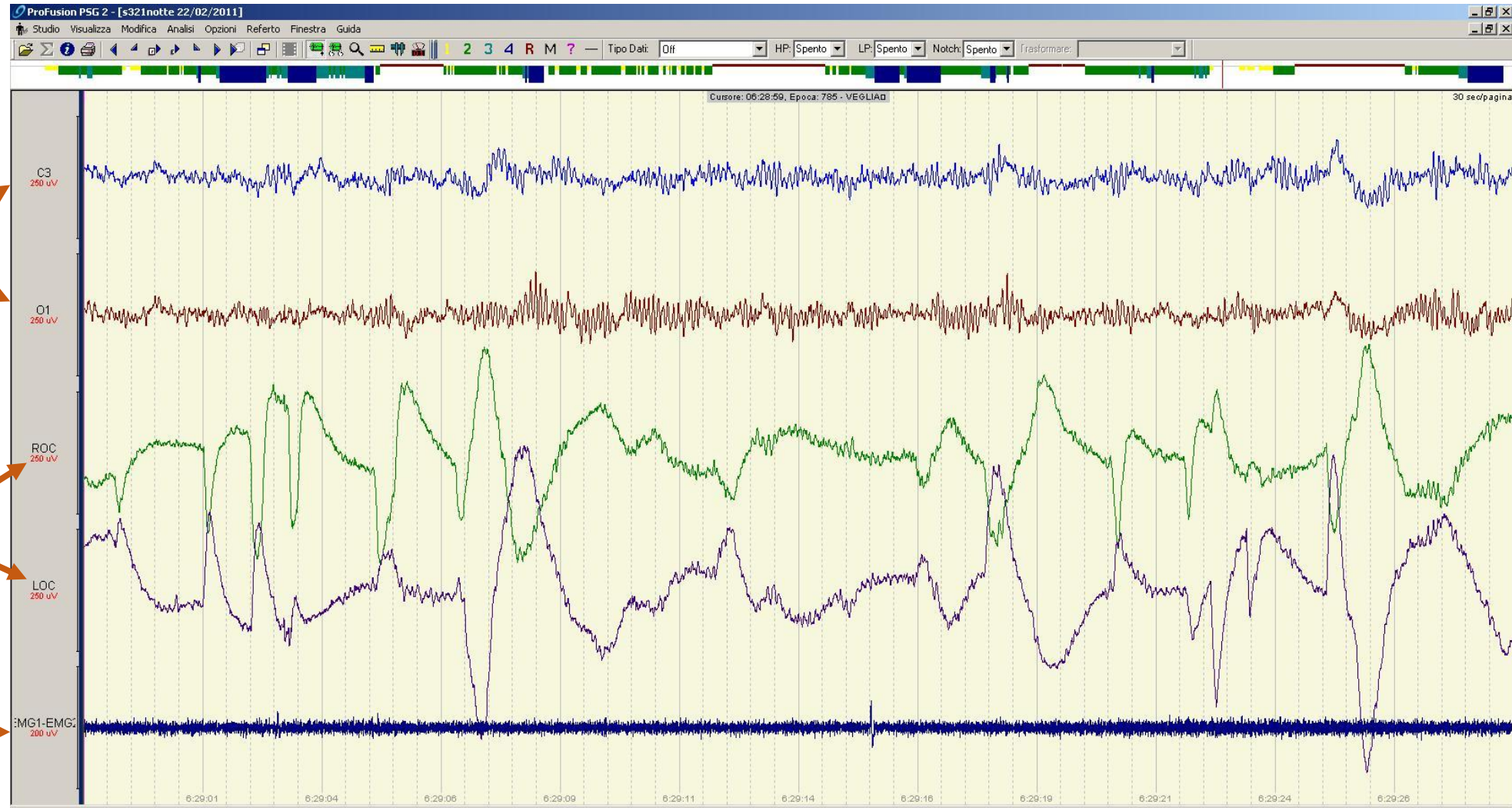
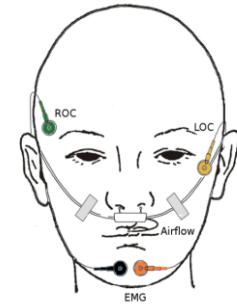
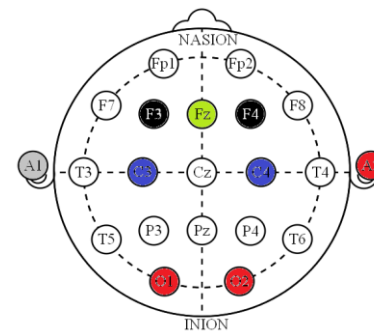
## Summary

# Stages of Sleep

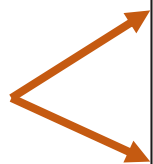


# Stages of Sleep – Wakefulness

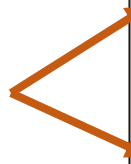
EEG: electroencephalogram  
EOG: electrooculogram  
EMG: electromyogram



Synchronization is low and fairly localized when awake.



Eye movements are regular (~4 Hz) and punctuated by large saccades.

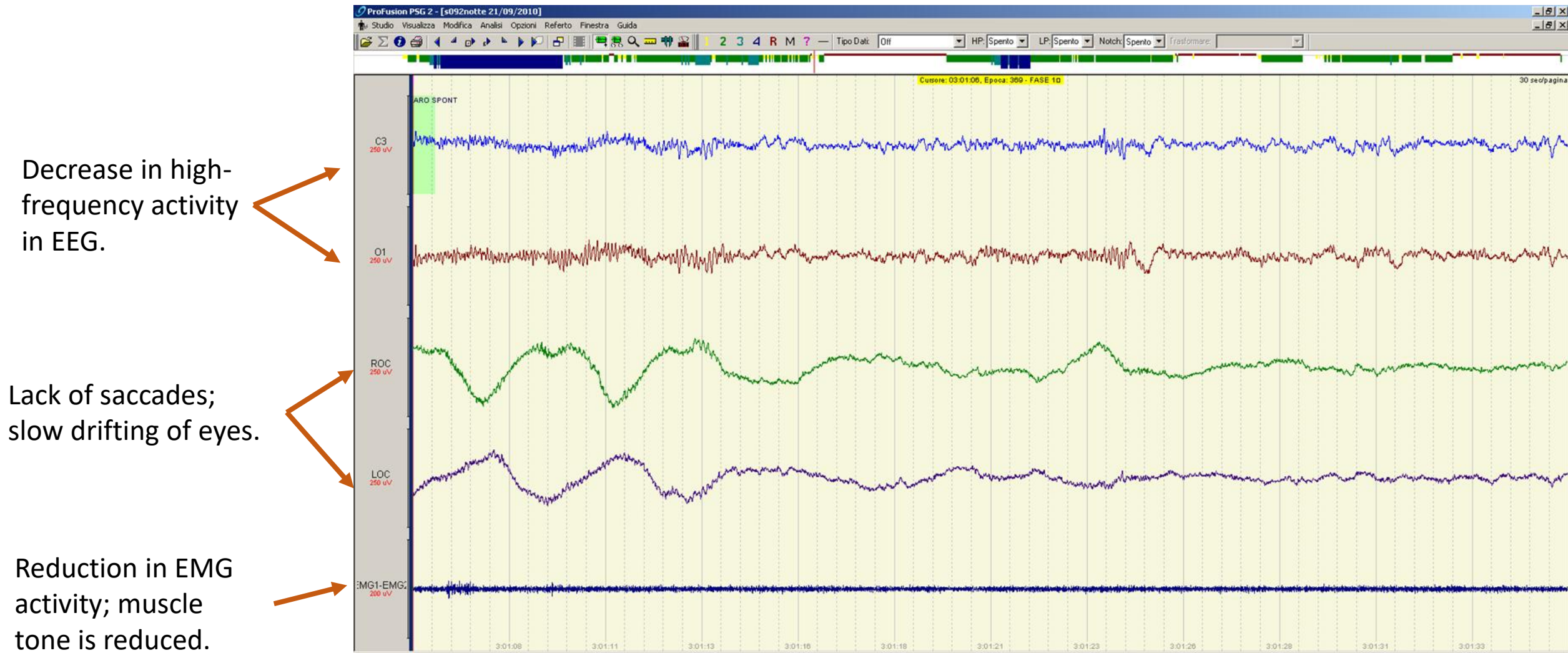


High EMG reflects movement.



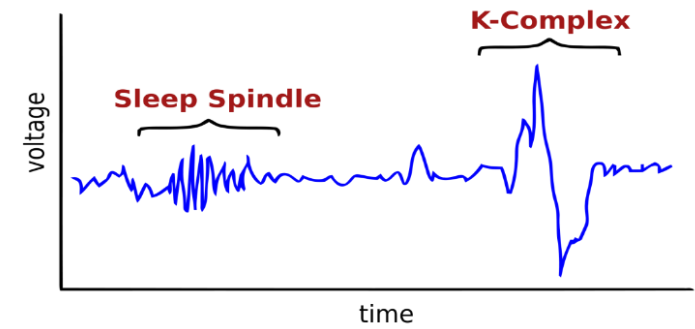
# Stages of Sleep – Non-REM

There are four stages of non-REM sleep. Stage 1 is the transition from awake to sleeping.



# Stages of Sleep – Non-REM

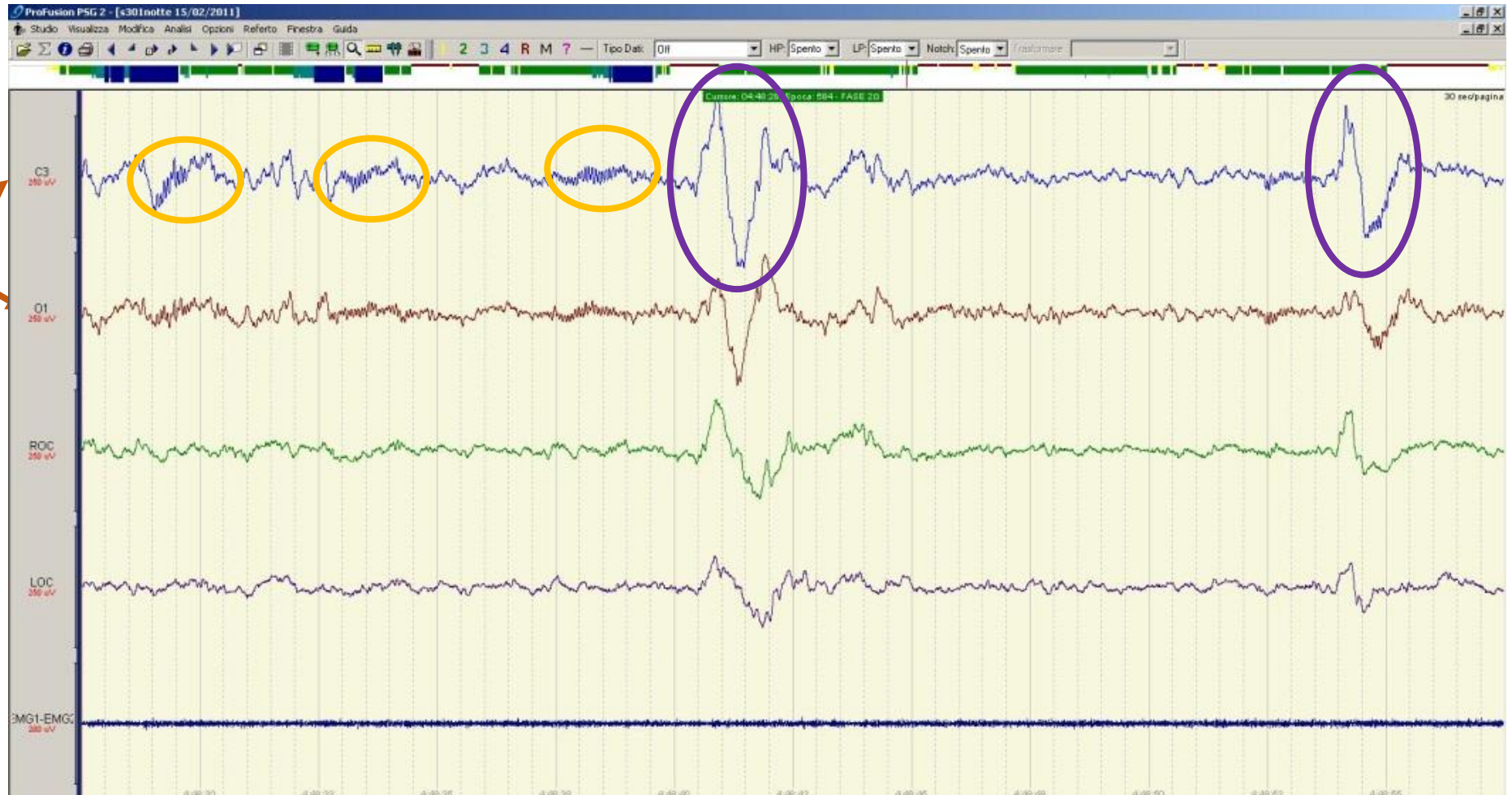
There are four stages of non-REM sleep. Stage 2 is the first stage of true 'sleeping'.



Further decrease of high-frequency activity. Emergence of **sleep spindle** and **K-Complex** waveforms.

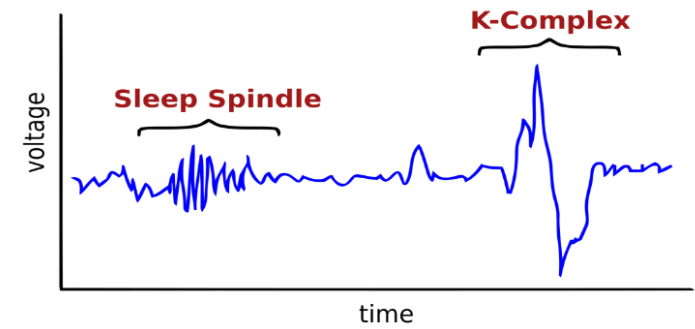
Reduced eye movements; slow drifting of eyes.

Very low muscle activity.



# Stages of Sleep – Non-REM

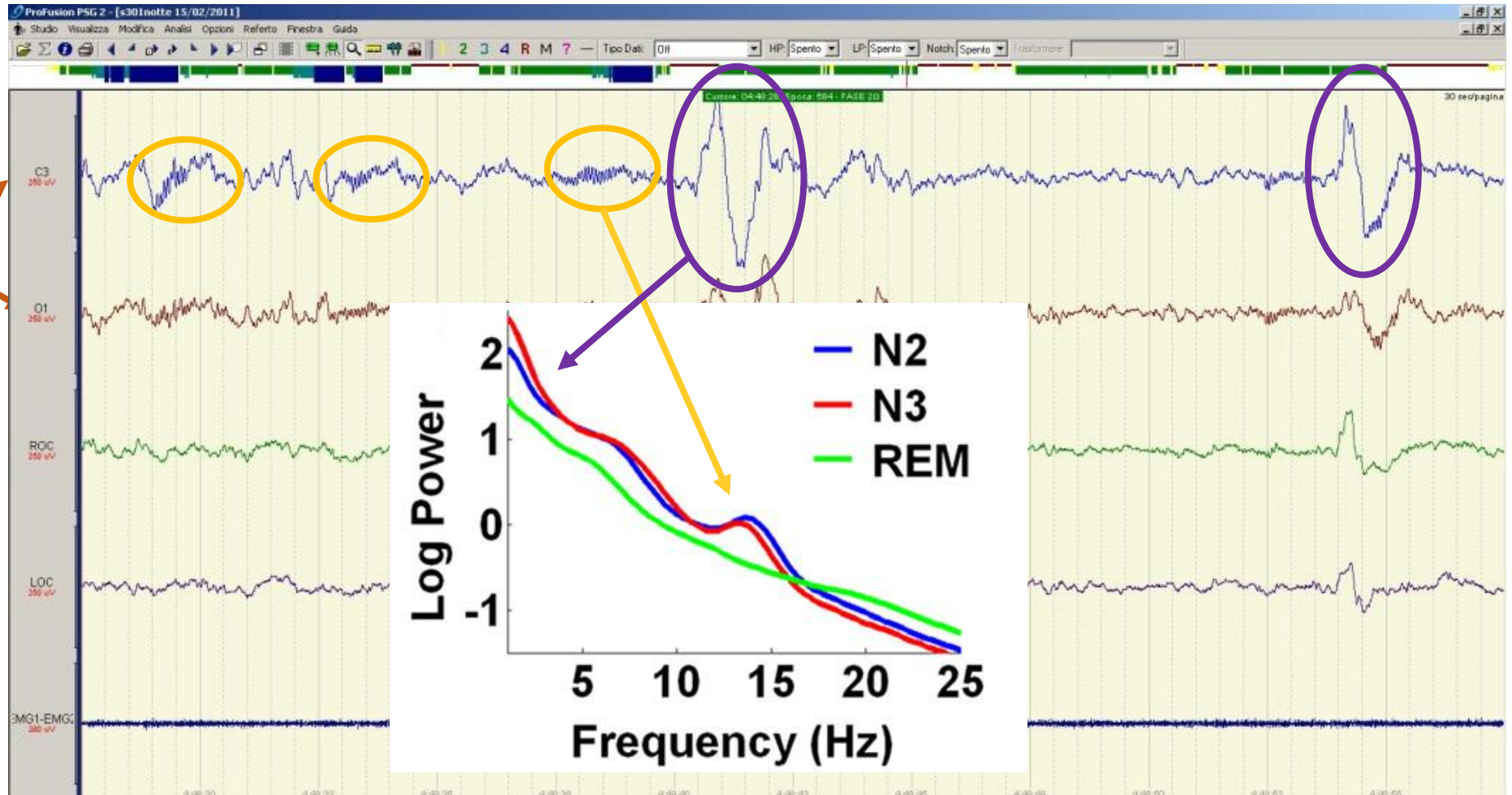
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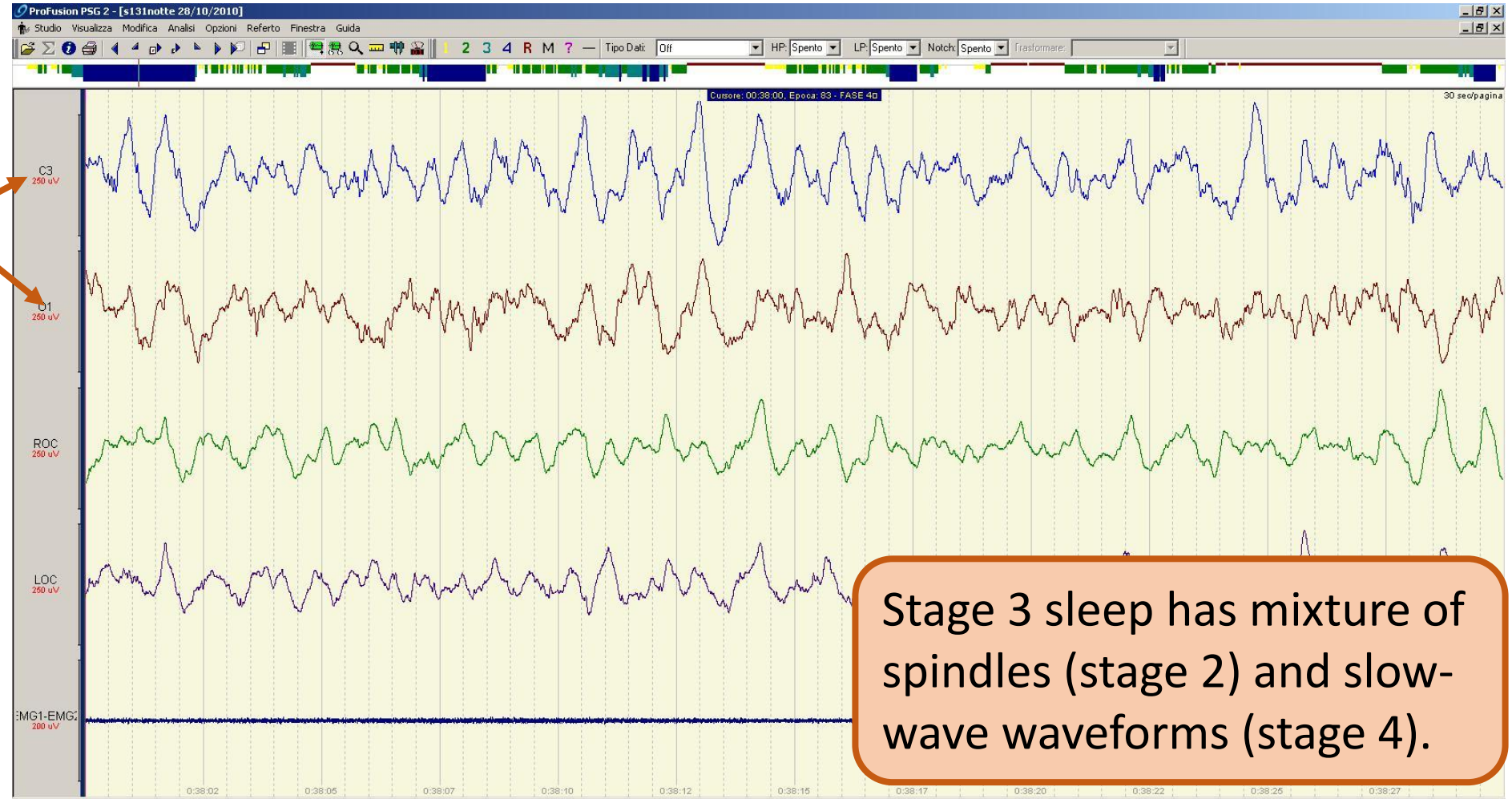
# Stages of Sleep – Non-REM

There are four stages of non-REM sleep. Stage 4 is the deepest stage sleep. It is often referred to as **Slow-wave sleep (SWS)**.

Highly synchronized, very low-frequency oscillations across the brain (1-2 Hz).

Little eye movement; any eye movements are slow.

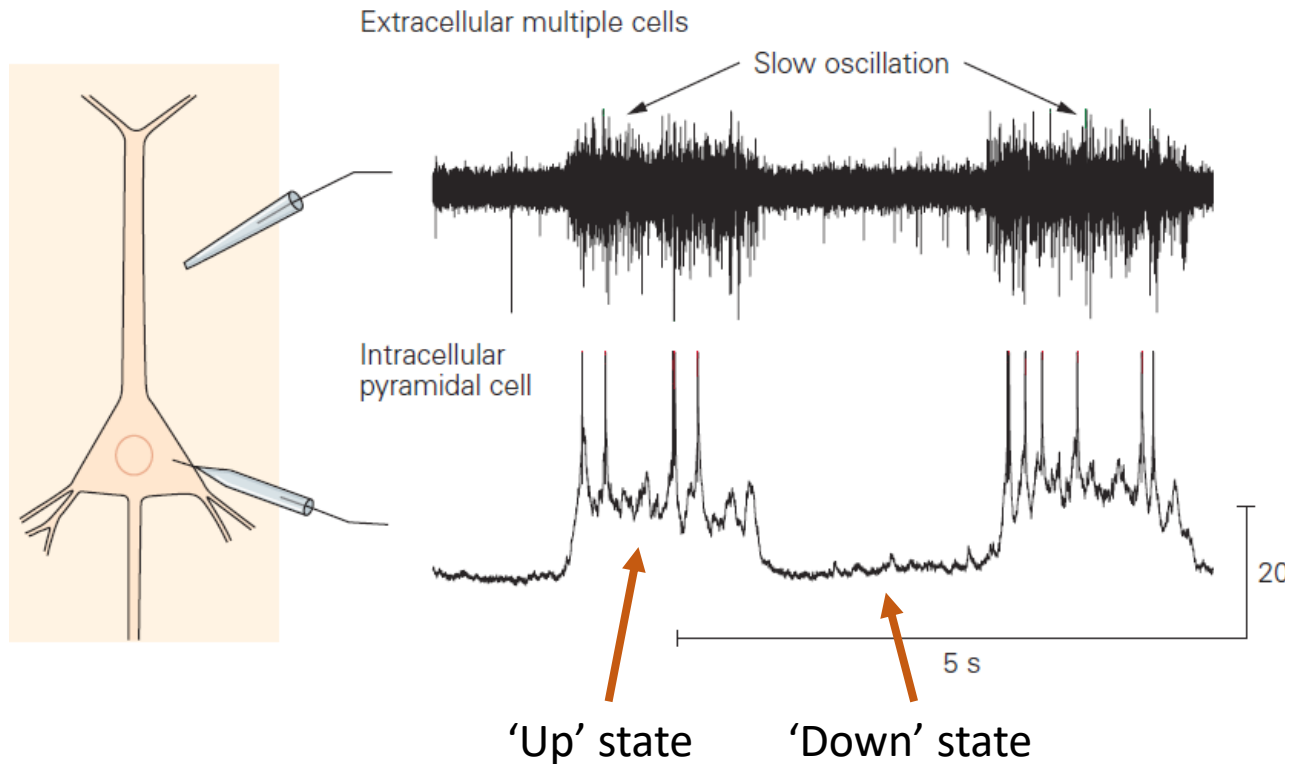
Complete loss of muscle tone.



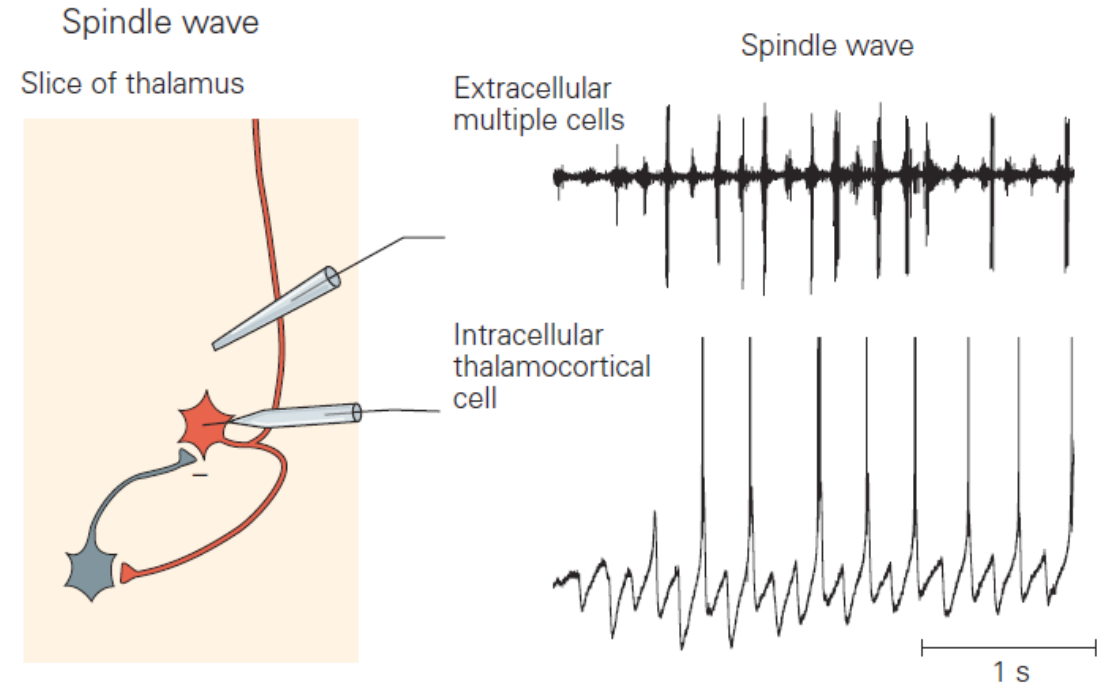
Stage 3 sleep has mixture of spindles (stage 2) and slow-wave waveforms (stage 4).

# Stages of Sleep – Non-REM

Isolated sections of cortex will show slow-wave oscillations in the 1-2 Hz range. Similar patterns are observed during anesthesia and during awake, quiescent periods.



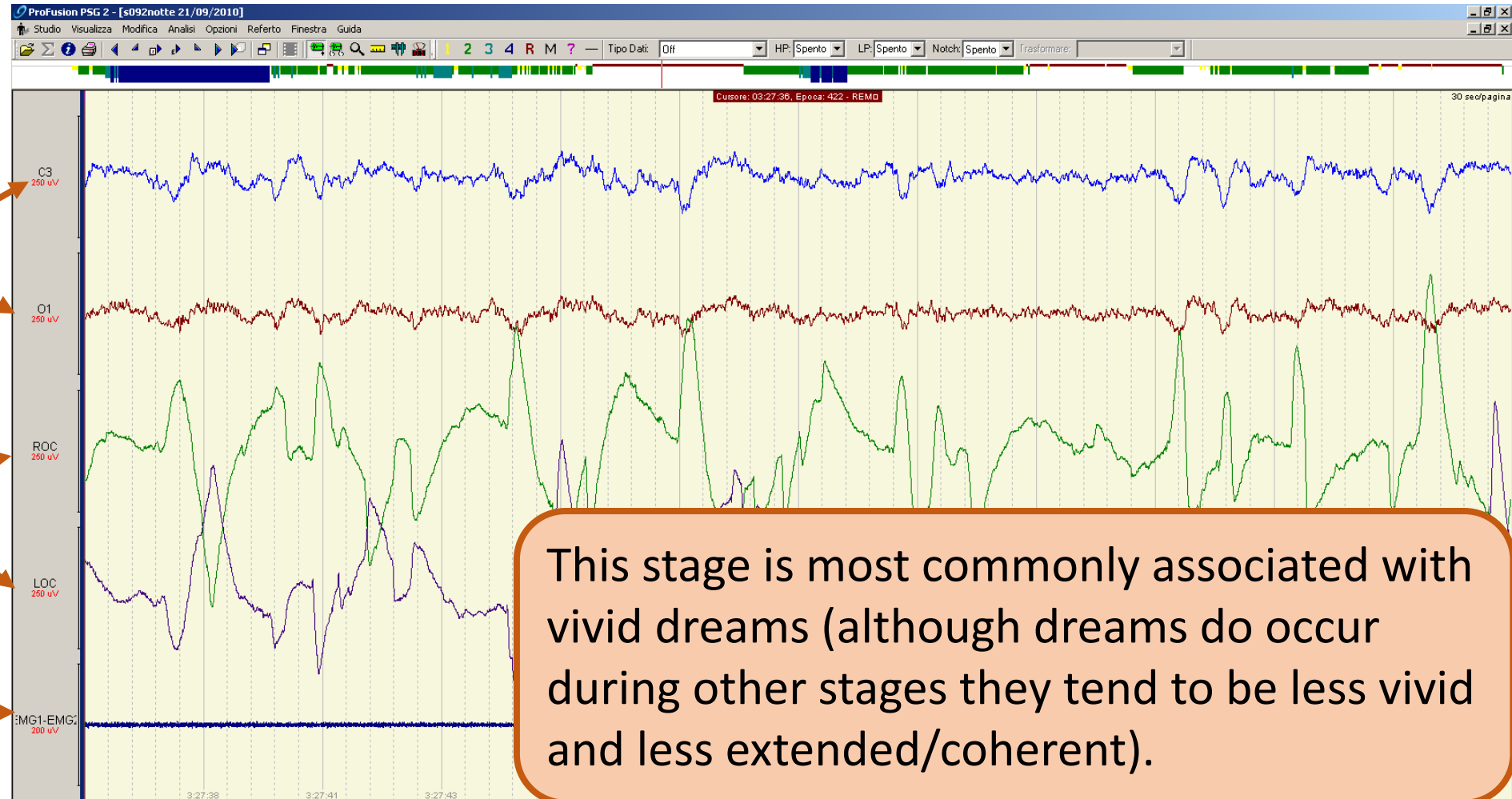
Spindles arise from excitatory-inhibitory loops in the thalamus that resonate at the 7-12 Hz frequency (~alpha range; also exist when awake!).



Suggests that the rhythms observed during non-REM may be the 'default' rhythms of the brain.

# Stages of Sleep – REM

The last stage of sleep is the **Rapid Eye Movement (REM)** stage. It is characterized by physiological similarity to awake state.



Return to desynchronized, high-frequency state, similar to awake.

Large, rapid eye movements occurring about every 3-4 seconds.

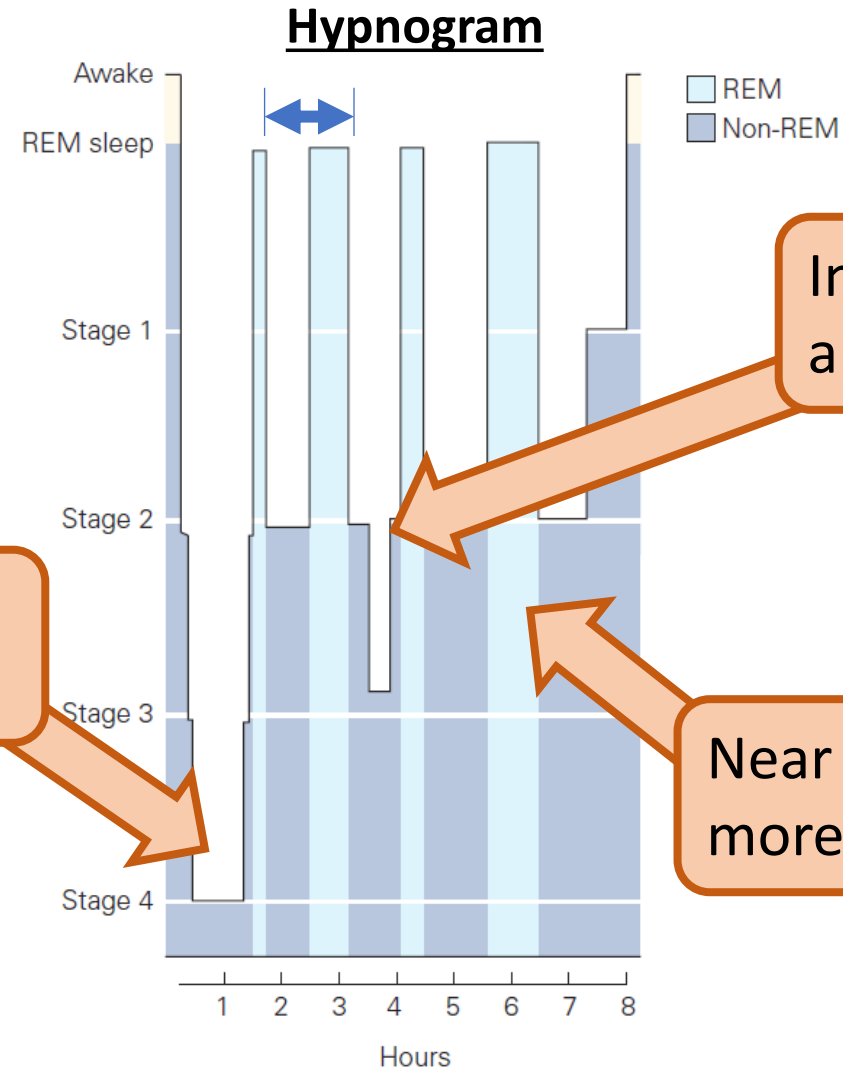
Complete loss of muscle tone in other regions of the brain due to descending inhibition of spinal cord.

This stage is most commonly associated with vivid dreams (although dreams do occur during other stages they tend to be less vivid and less extended/coherent).

# The Sleep Cycle

Sleep occurs in cycles, progressing through the different stages of non-REM sleep, reaching the deepest stage 4. It then returns to the lighter stages of sleep, but instead of returning to awake you enter REM sleep.

An average sleep cycle from stage 1/REM, through stage 4, and back to stage 1/REM lasts about **90 minutes**.



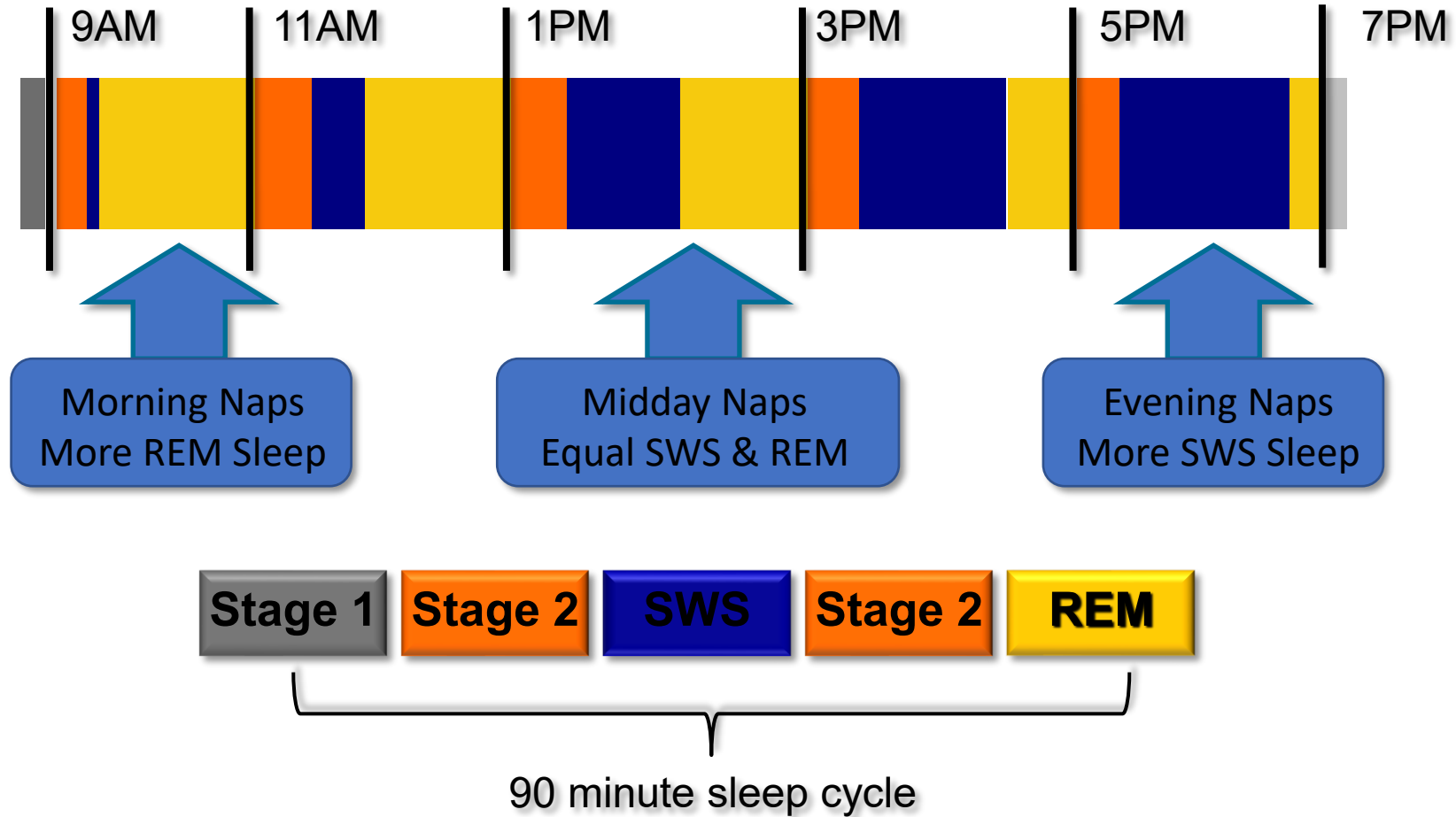
In the middle of the night there is about equal SWS and REM sleep.

Early in the night, you spend more time in SWS sleep.

Near morning you spend more time in REM sleep.

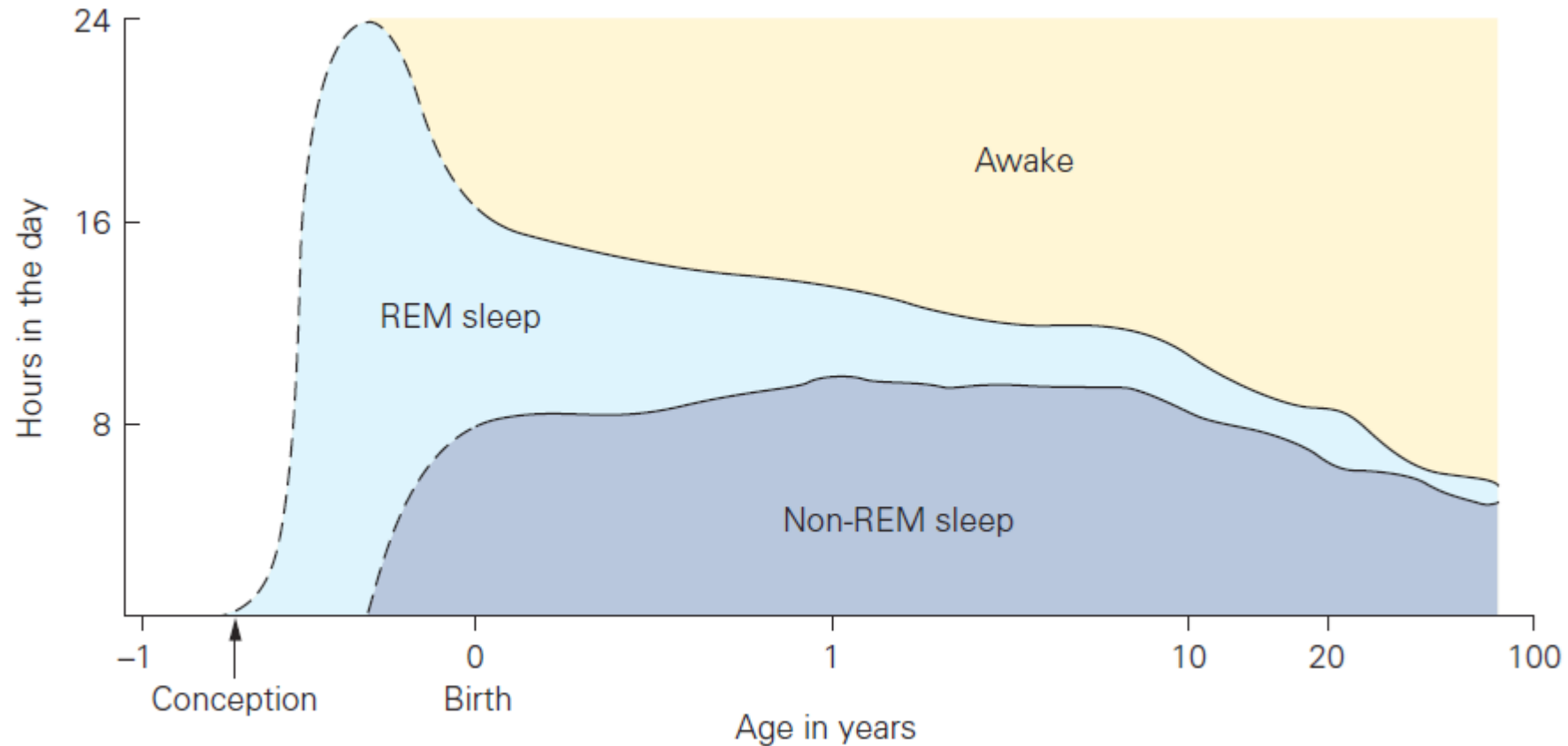
# The Nap Cycle

Sleep occurs in cycles during napping as well. The amount of time you spend in each stage of sleep is the inverse of nighttime sleep – you spend more time in REM early in the day and SWS late in the day.

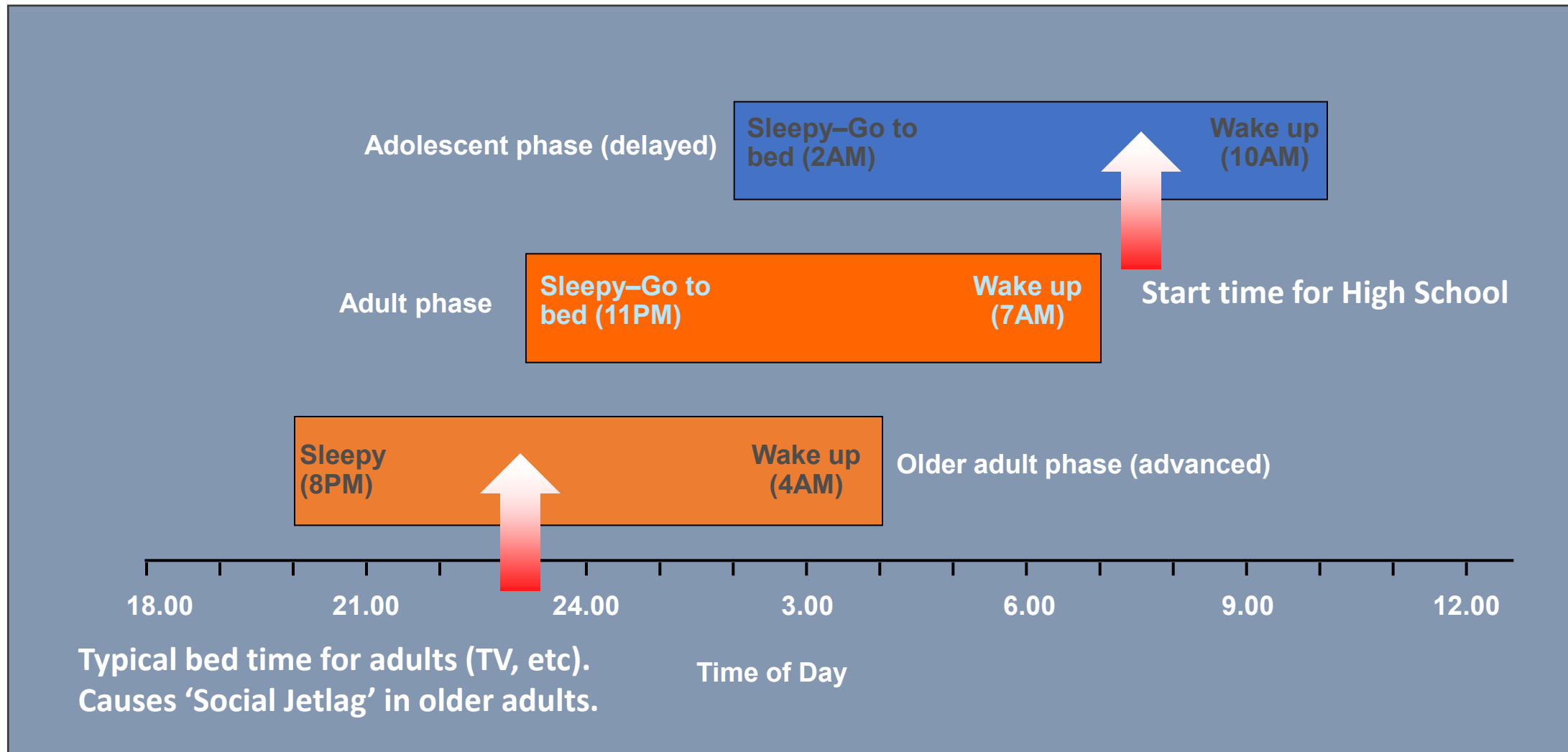


# Sleep Stages through Development

Overall time asleep decreases through development. In addition, the time spent in different stages also changes through development. Adults spend about 20% of their time asleep in REM – this is higher when younger; babies spend 50-80% of sleep in REM (even higher in premature infants, suggesting REM may be almost 100% *in utero*)

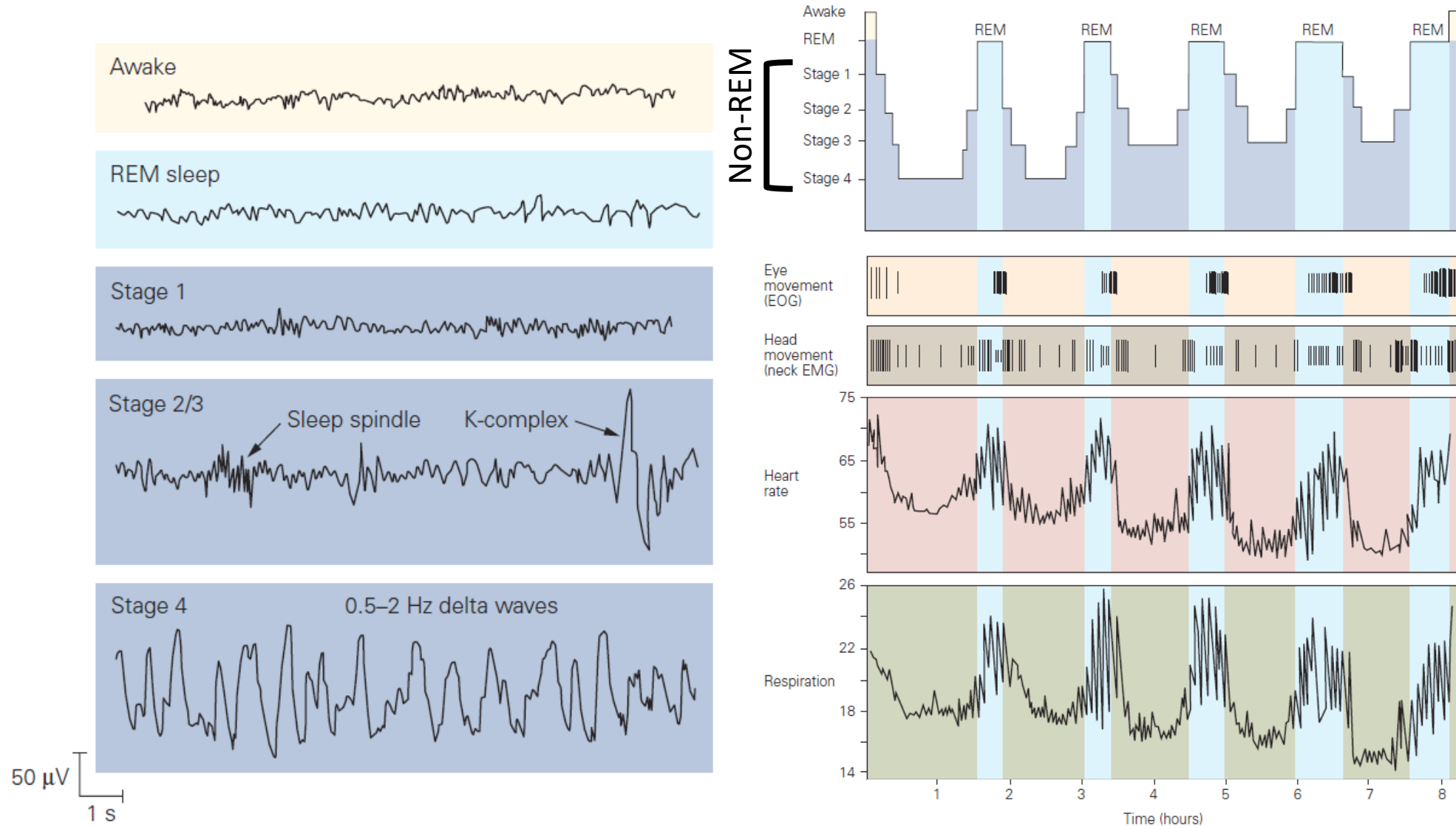


# The Sleep Cycle Through Aging – Bedtime and Waketime Changes Through Development

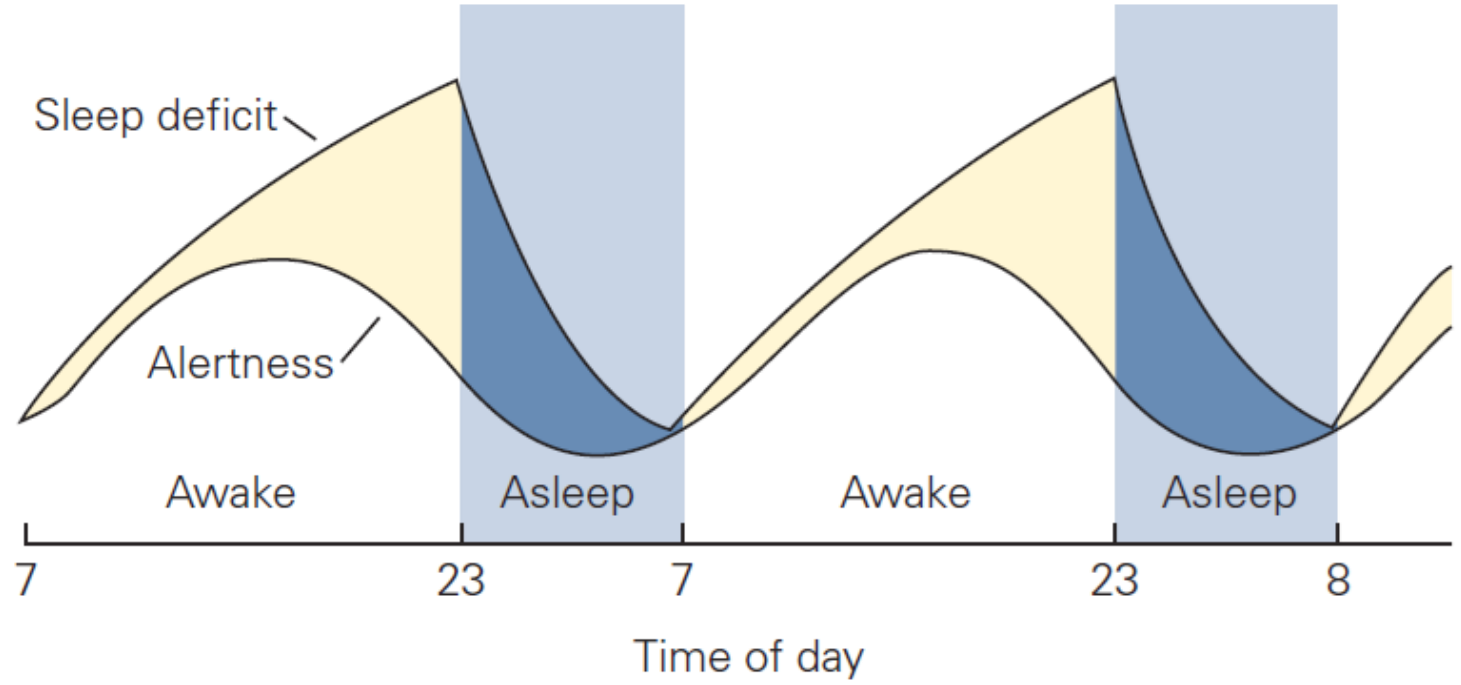


# The Sleep Cycle – General Physiology

Gross physiological markers, such as heart rate and respiration rate also change across the different stages of sleep with the slowest rates during the deepest sleep and the highest rates during awake/REM.

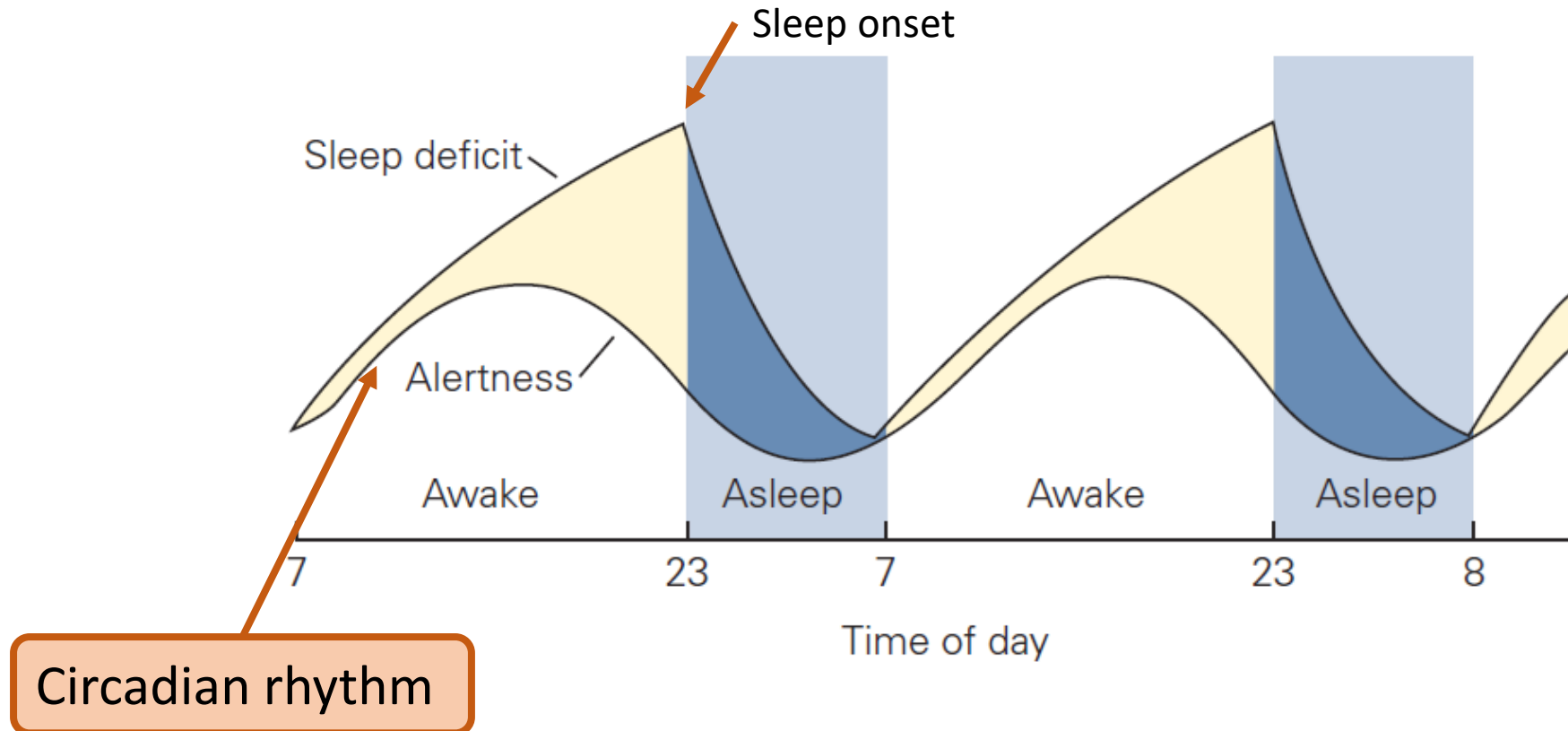


# Sleep Regulation

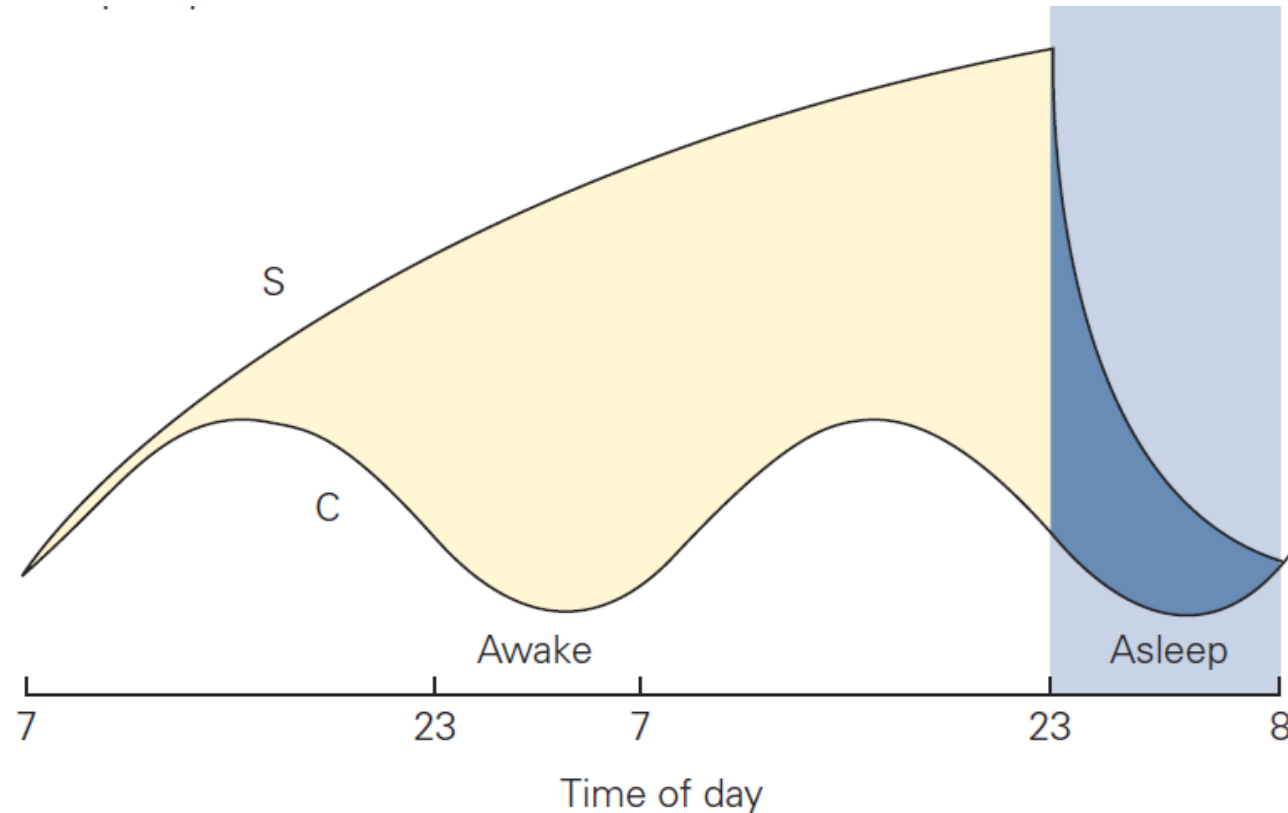


# Two-Process Model of Drive to Sleep

Sleepiness (or the 'drive for sleep') seems to depend on two factors: 1) time since last sleep and 2) time during the day. This has led to a two-process model being proposed for sleep initiation.

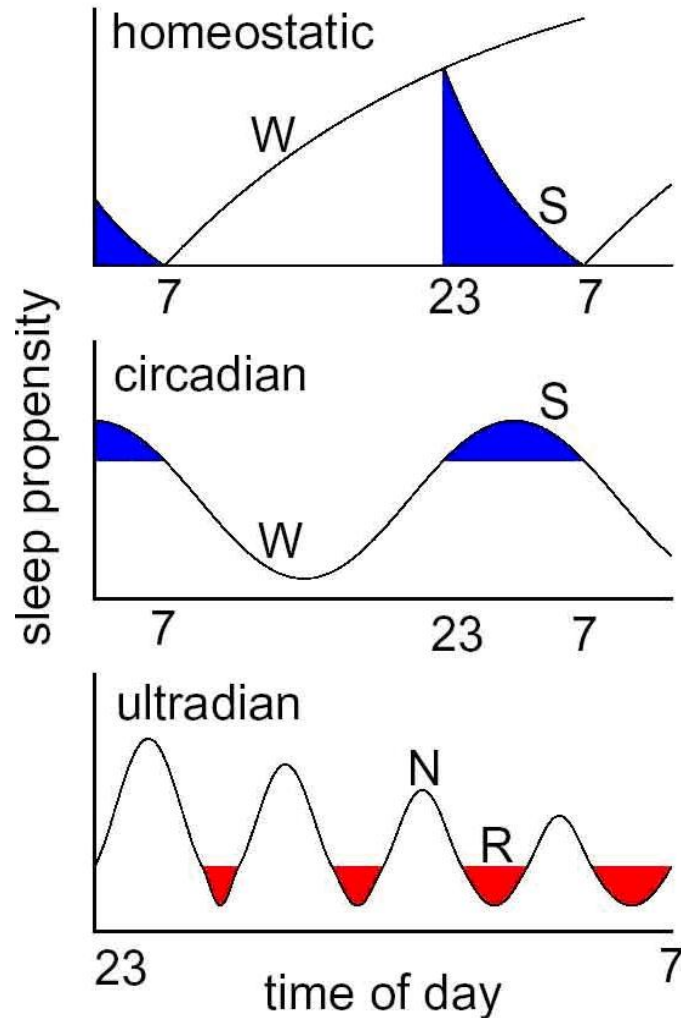


# Two-Process Model of Drive to Sleep



This model makes testable behavioral predictions, such as predicting when you will fall asleep when missing a night of sleep. When this happens, the sleep drive continues to accumulate, meaning that it becomes extremely difficult to stay awake the next night around 3-4 am, when the circadian rhythm has dropped.

# Third 'Ultradian' Process Determines Type of Sleep



A faster, nested oscillator (~90 min) that drives the transition between sleep stages.

The oscillator decays through the night, explaining the relative increase in time spent in REM sleep.

This also explains the increase in NREM following sleep deprivation.

# Most animals have a behavioral cycle tied to the daily light cycle

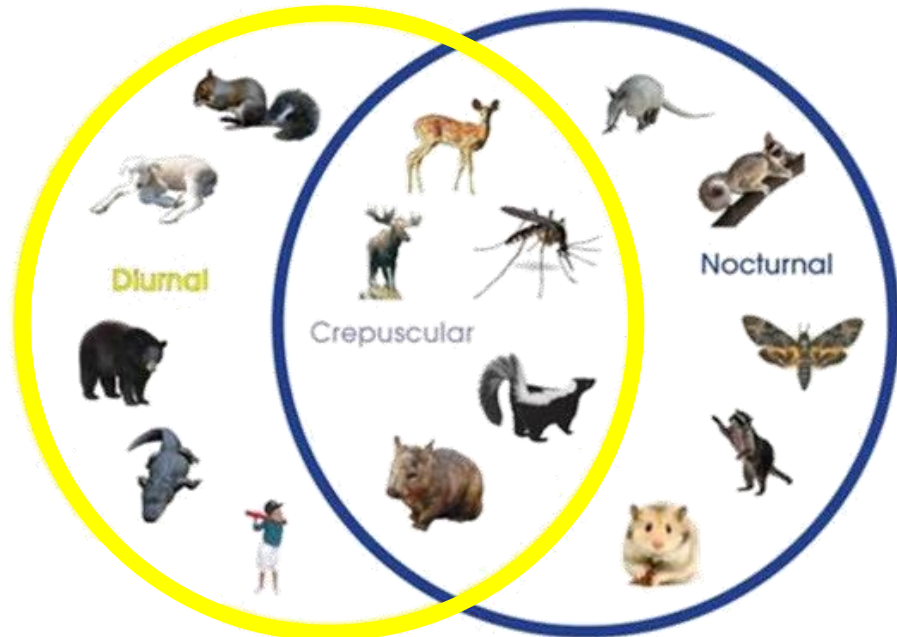
Animals can be classified by what times during the day they are active:

**diurnal**

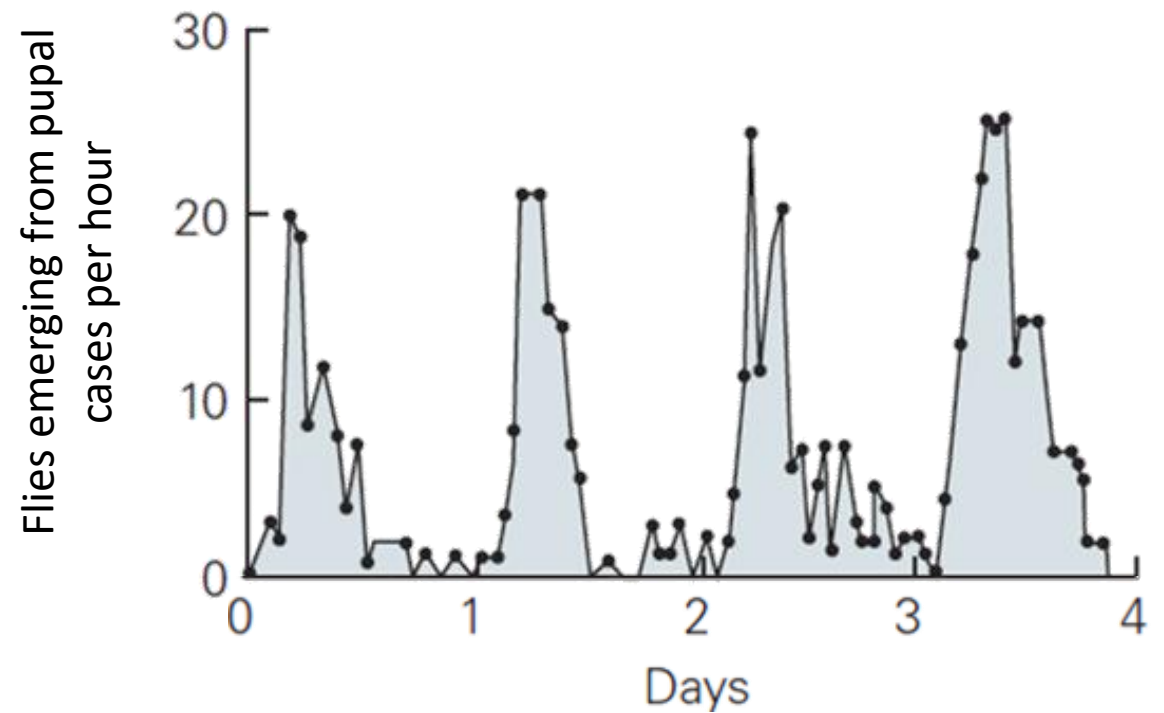
**nocturnal**

dawn day dusk night

**crepuscular**



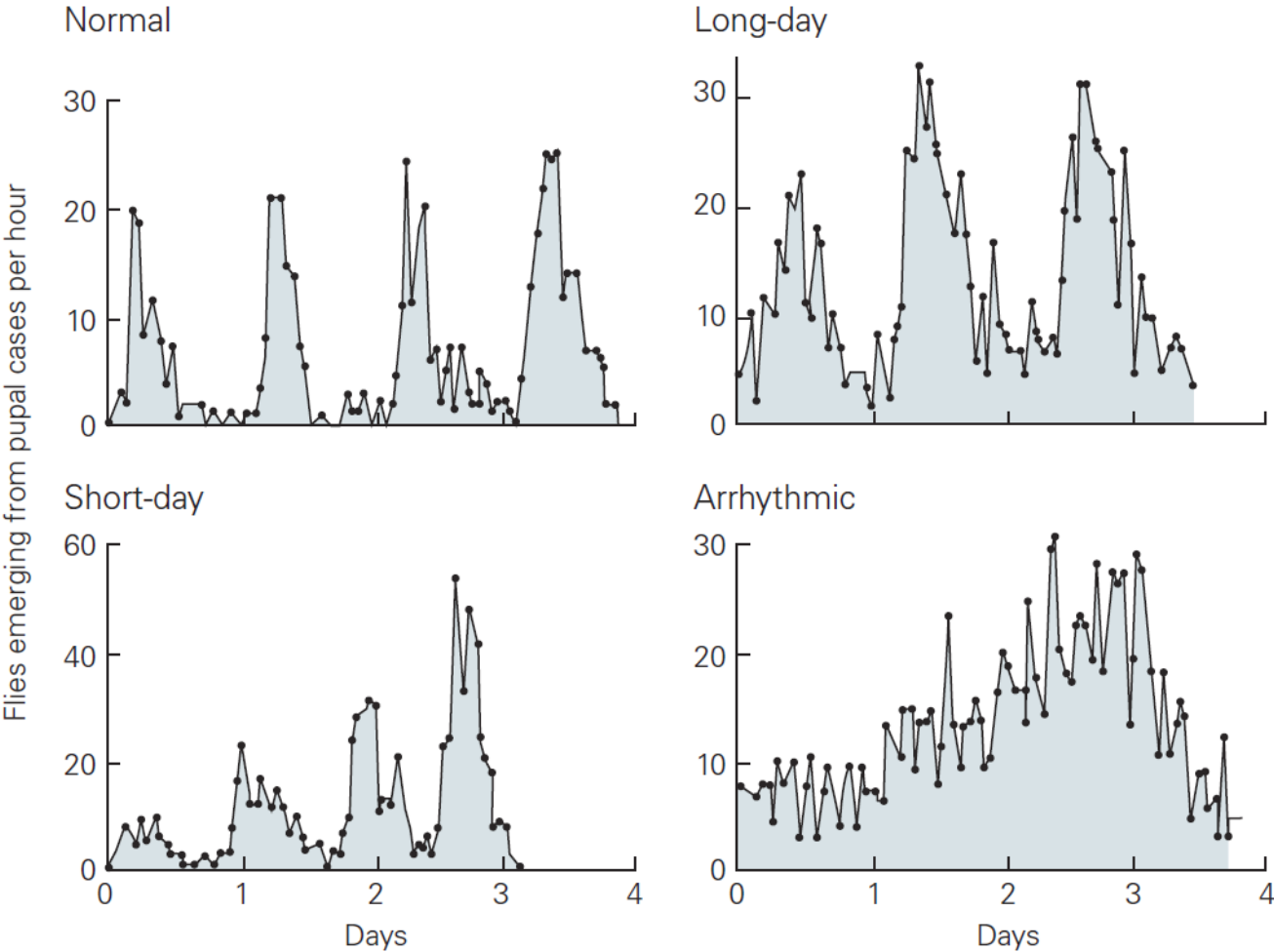
Almost all animals have a behavioral cycle:



# Oscillations in gene expression regulate circadian rhythm

Seymour Benzer used random mutagenesis to determine which genes regulated the sleep/wake cycle in flies. They found mutations in the *period* (*per*) gene disrupted the cycle of flies housed in complete darkness.

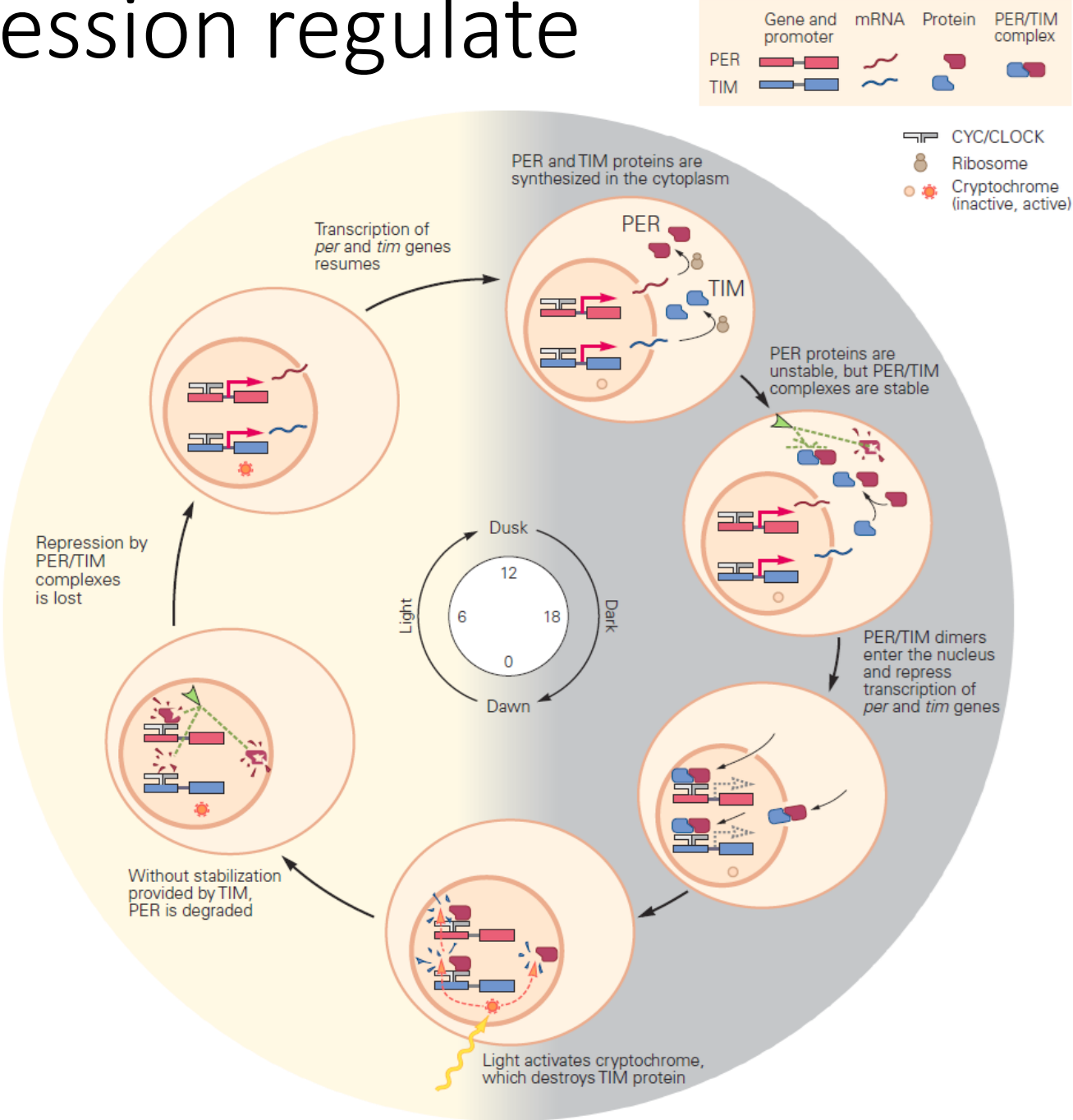
A partner *tim* (timeless) gene was later found to work together with *period*.



# Oscillations in gene expression regulate circadian rhythm

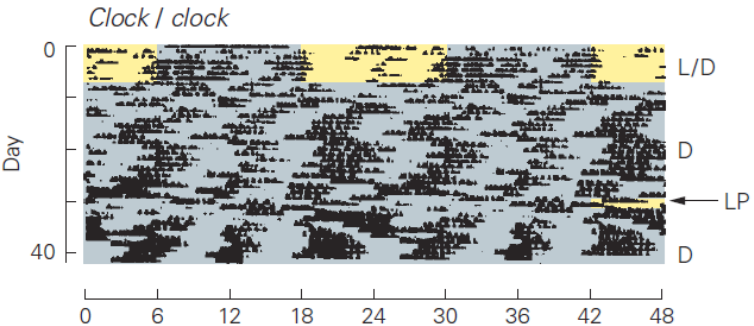
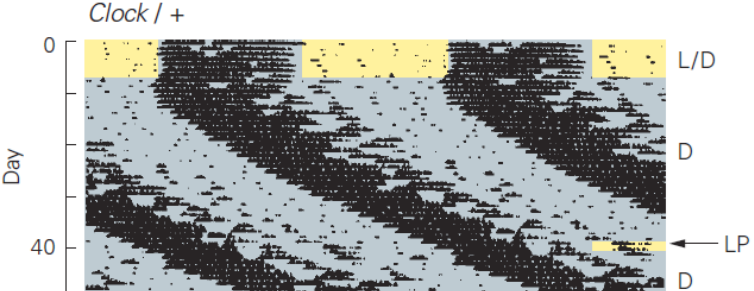
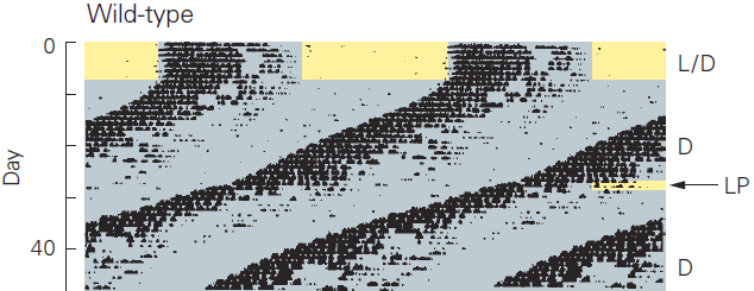
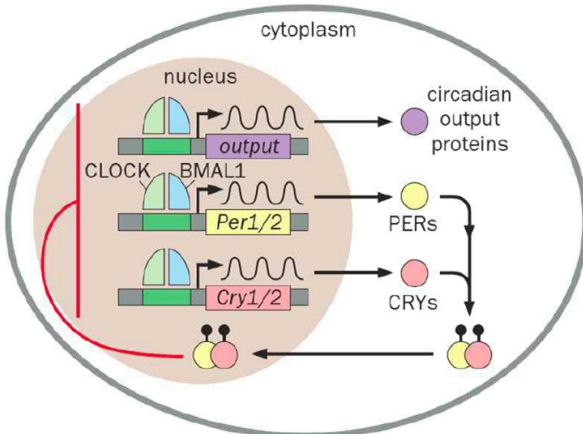
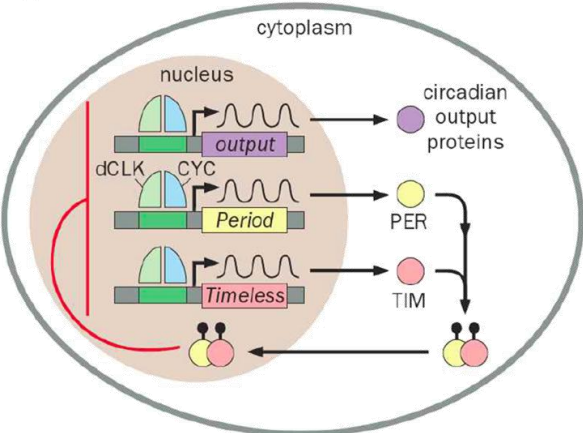
Oscillations result from mutual inhibition between two sets of proteins: the CLOCK/CYCLE proteins that are high during the day and the PER/TIM proteins that are high at night.

Regulation can be reset by light, as blue light activates 'cryptochromes' which degrade TIM proteins, helping to increase production of CLOCK/CYC.



# Oscillations in gene expression regulate circadian rhythm

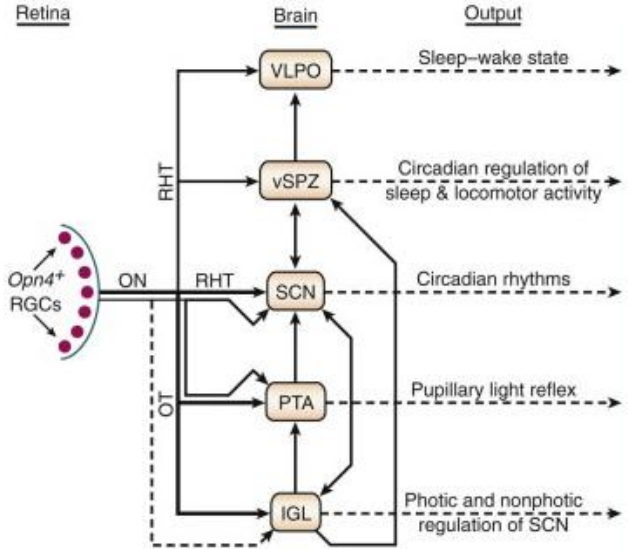
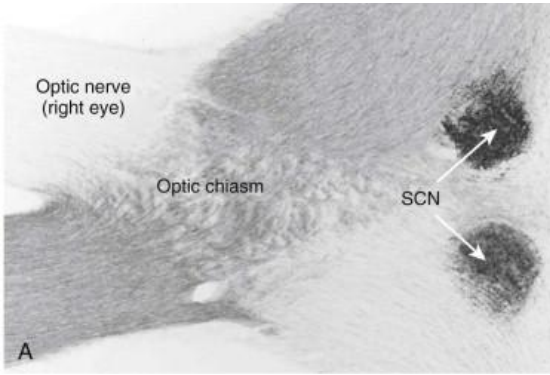
Studies in mice found complementary genes and similar mechanisms:



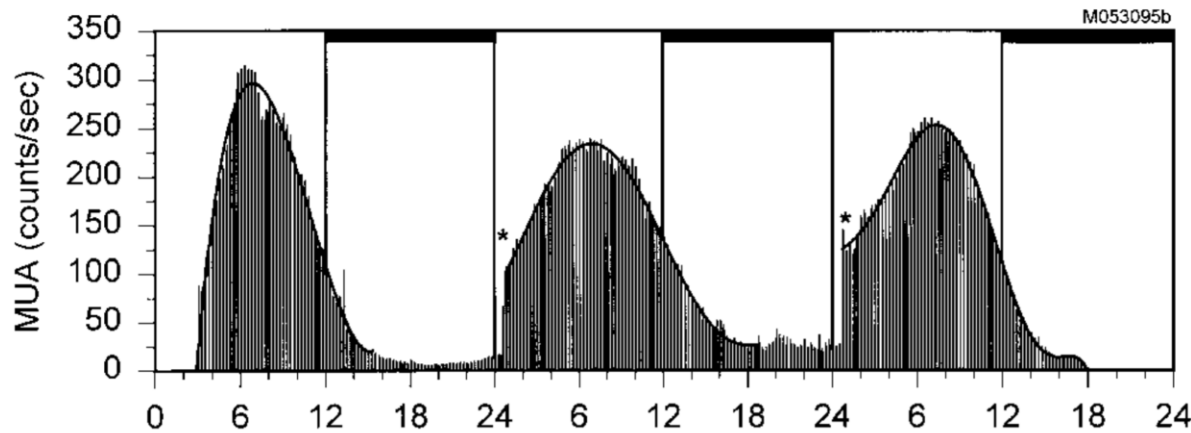
These studies were the earliest direct link between behavior and a specific gene (here, a single gene).

# Light-sensitive neurons in the SCN regulate circadian rhythm in mammals

The suprachiasmatic nucleus (SCN) is the master circadian regulator. As with drosophila, this oscillator is reset by light; in mammals via a special sub-class of RGCs.



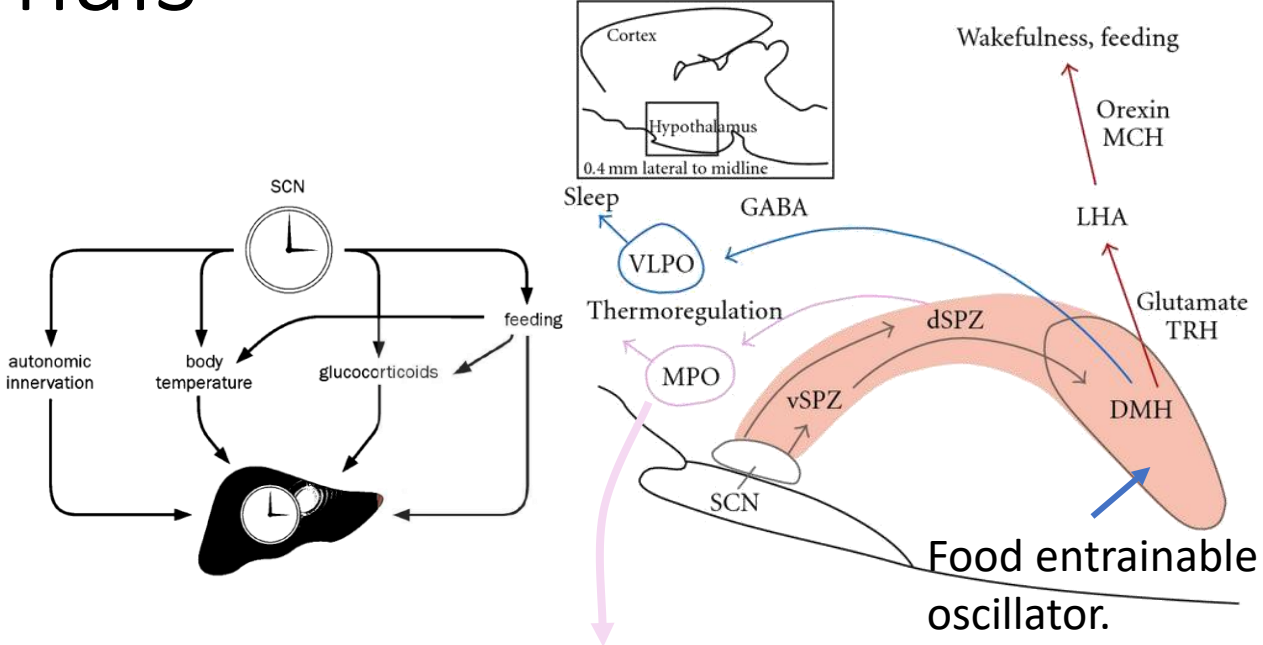
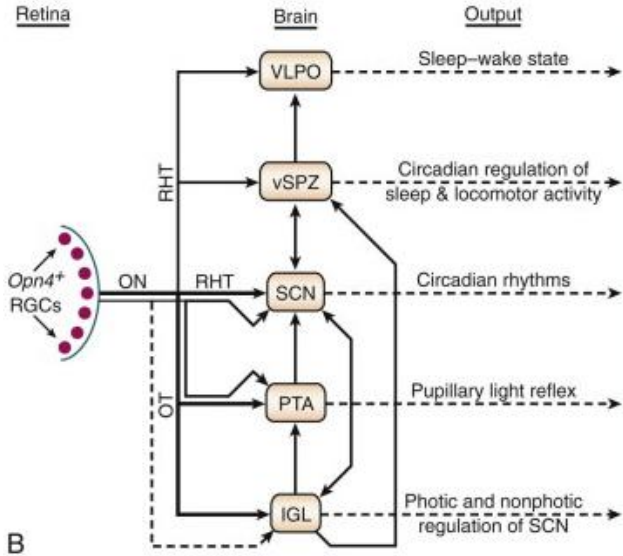
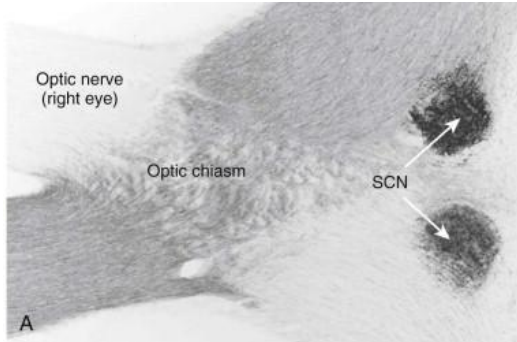
Neurons in the SCN intrinsically oscillate over the 24 hour cycle (will continue to do so when isolated *in vitro*).



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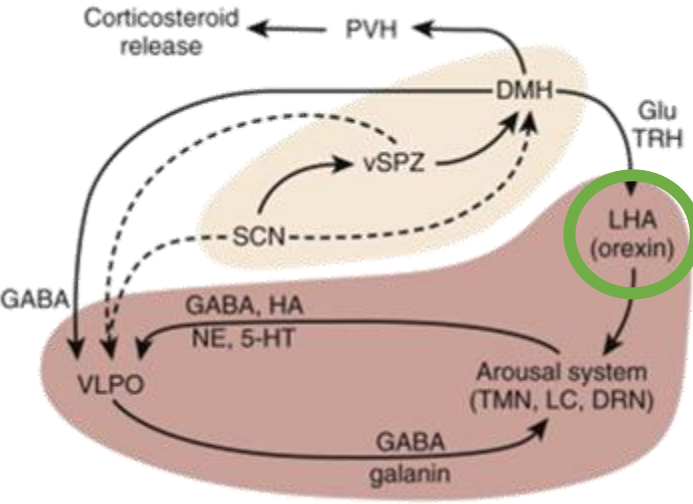


**Circadian Rhythm (Body-Temperature Cycle)**

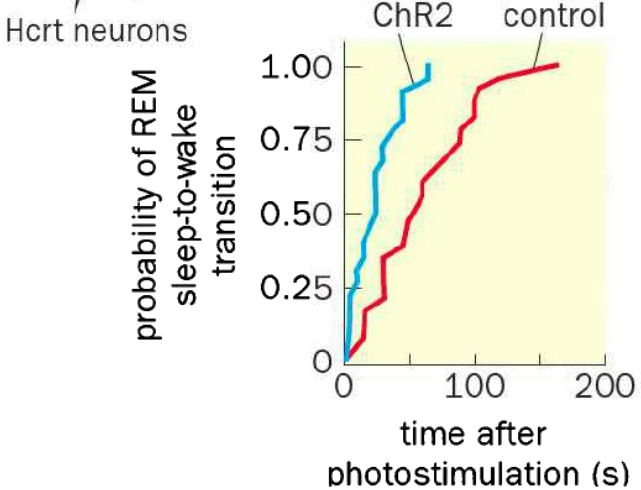
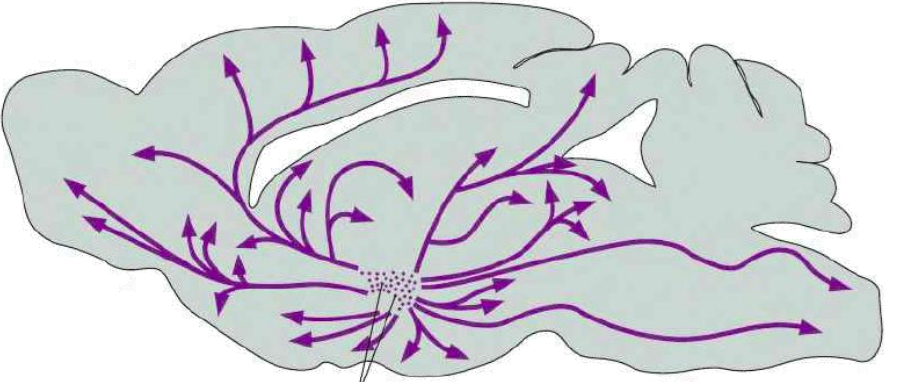


# Light-sensitive neurons in the SCN regulate circadian rhythm in mammals

Neurons in the SCN interact with two nuclei that have opposite effects on one another.

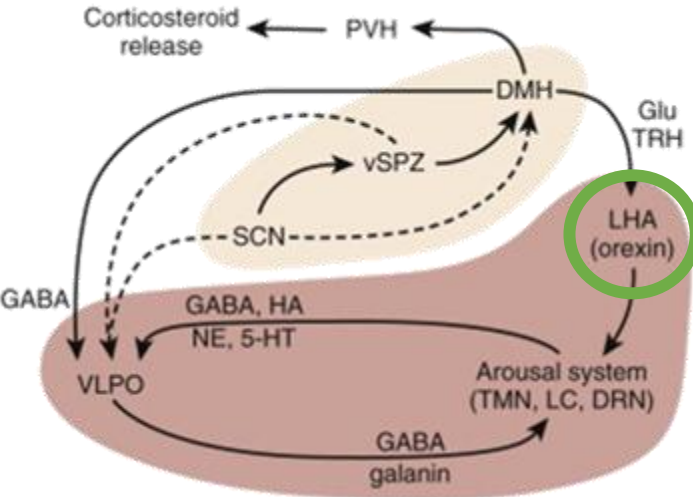


Awake promoting neurons in the lateral hypothalamic area (LHA) that express hypocretin (orexin) drive activity in a distributed network of neuromodulatory regions (ACh, 5HT, NE, DA).

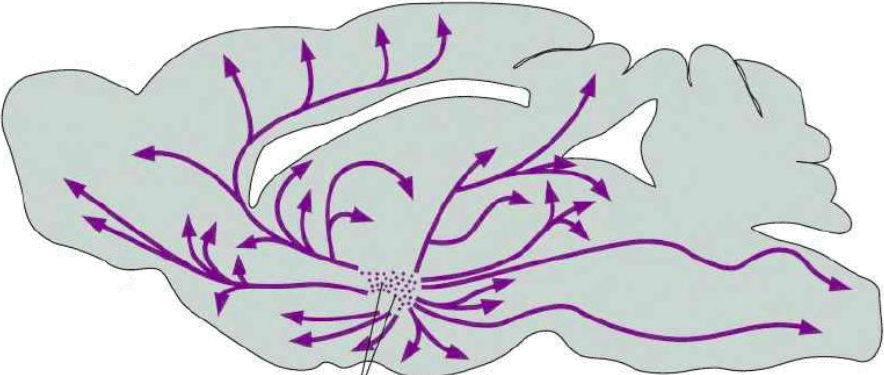


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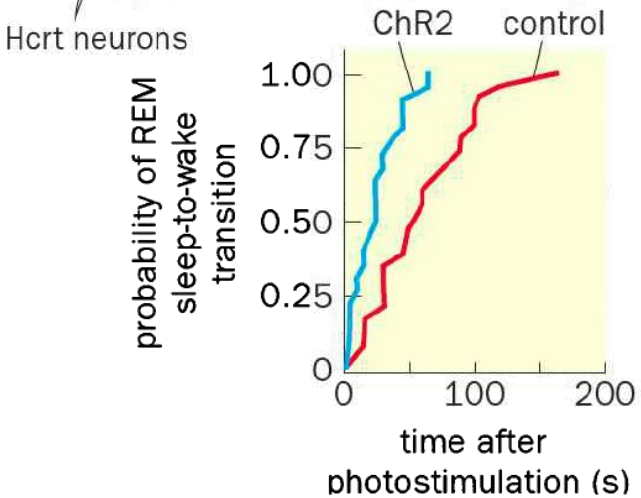
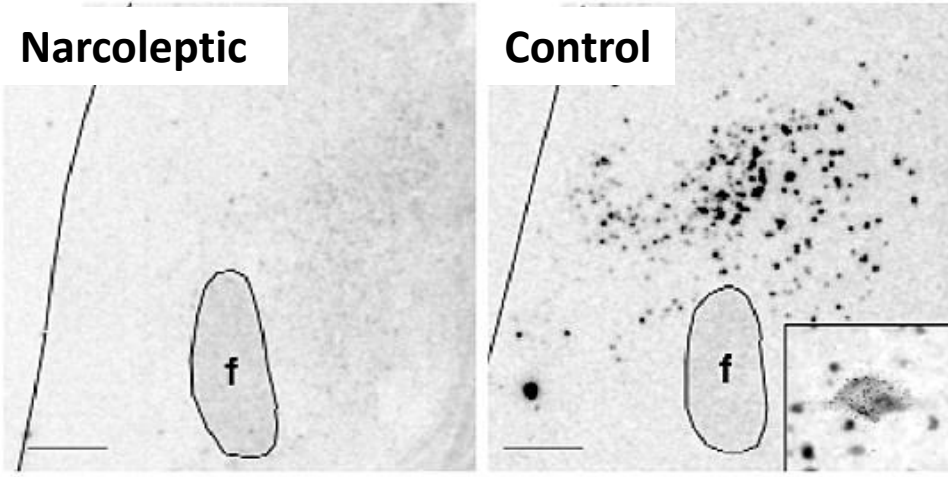
Awake promoting neurons in the lateral hypothalamic area (LHA) that express hypocretin (orexin) drive activity in a distributed network of neuromodulatory regions (ACh, 5HT, NE, DA).



Narcolepsy is a sleep disorder characterized by sleepiness during the day and abnormal onset of REM sleep. The disease affects ~1 in 2000 individuals. Causes are largely unknown but are thought to be a combination of genetic, auto-immune and environmental.

Recent work has shown that patients with narcolepsy do not express *hypocretin* in their LHA.

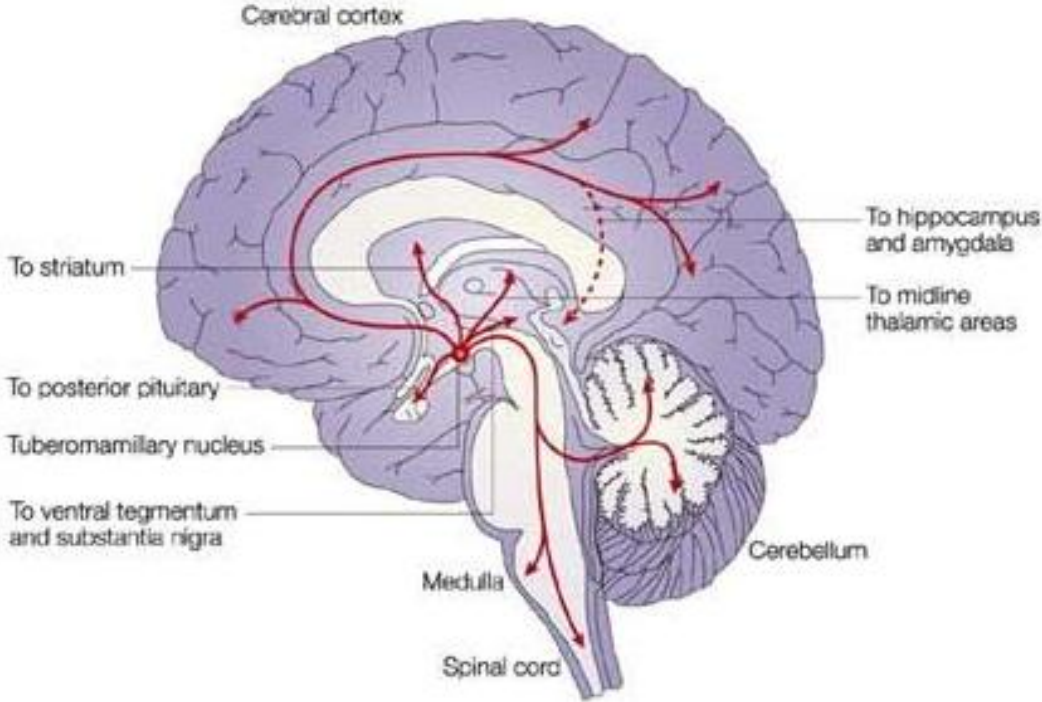
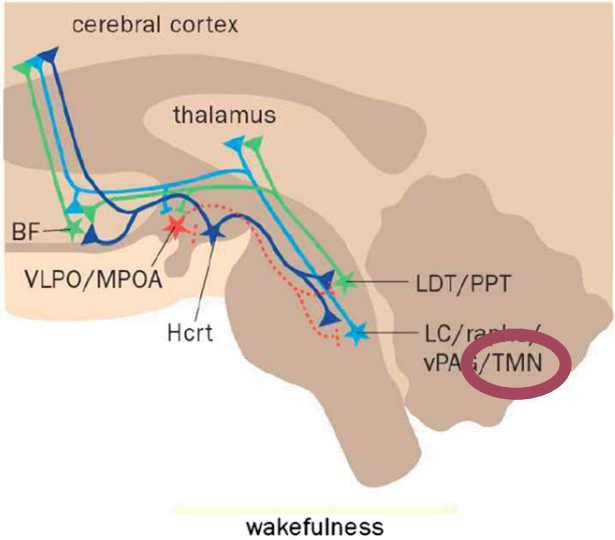
Peyron et al, *Nat Med* 2000



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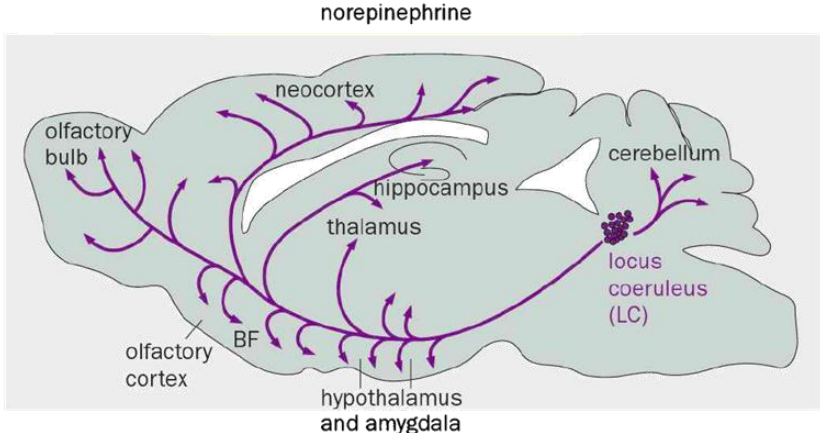
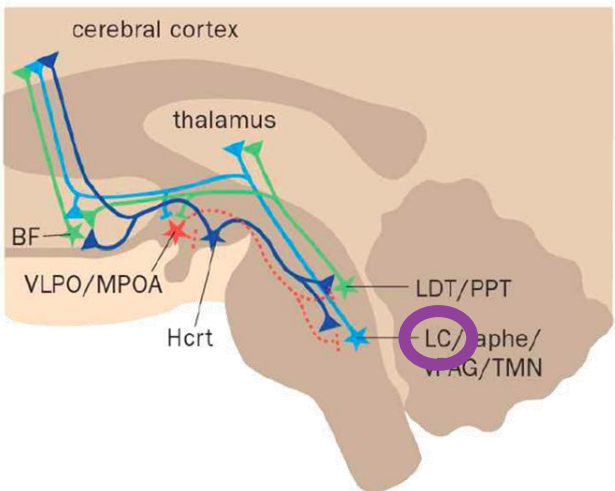
Hypocretin (orexin) neurons in the lateral hypothalamus activate all major neuromodulatory systems during wakefulness.

A major target of Hcrt neurons is the Tuberomammillary nucleus (TMN). TMN contains the only histaminergic neurons in the brain but the project extremely broadly. Histamine is thought to promote wakefulness.

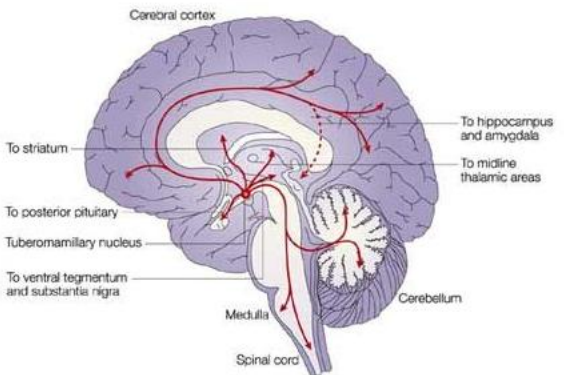


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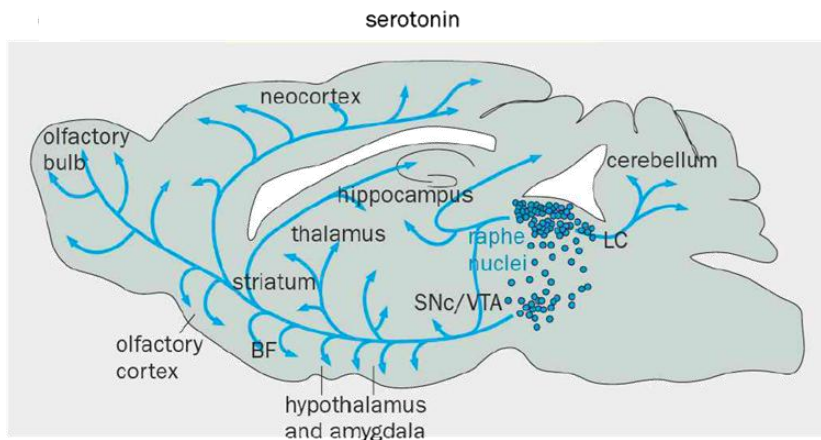
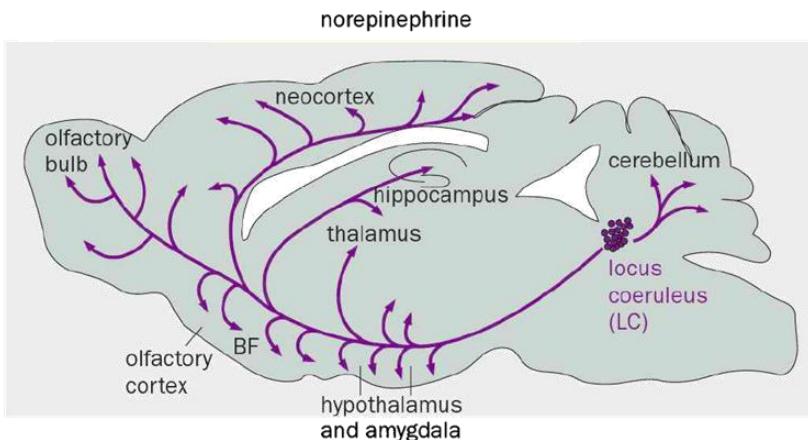
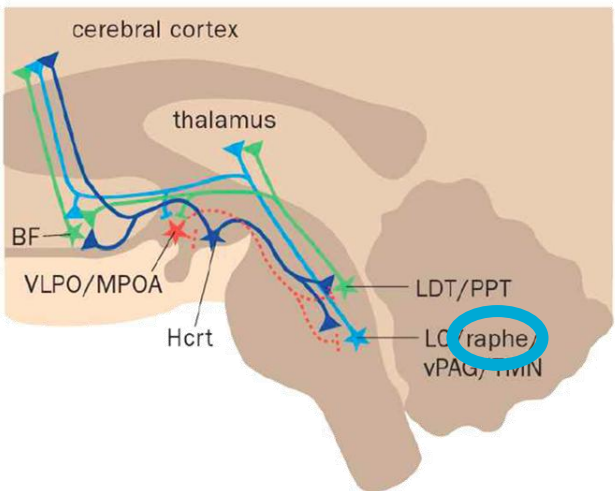


## Histamine

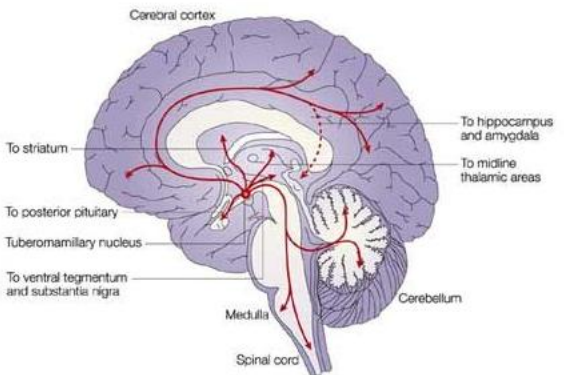


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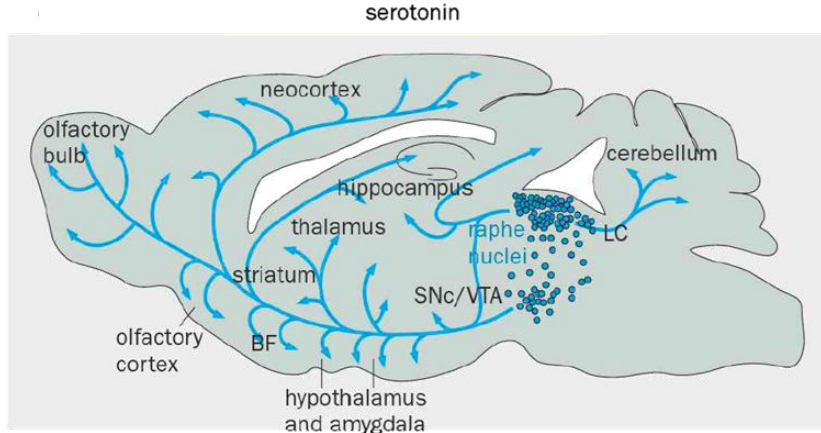
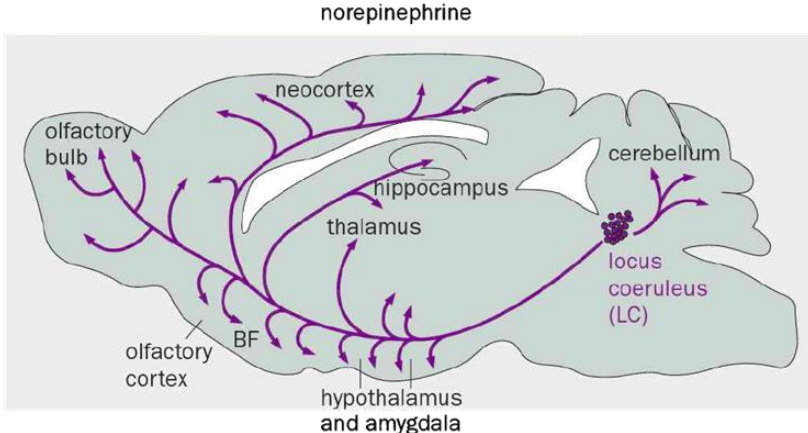
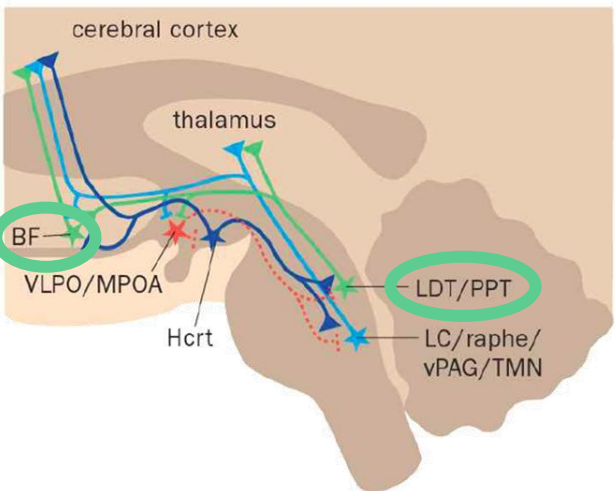


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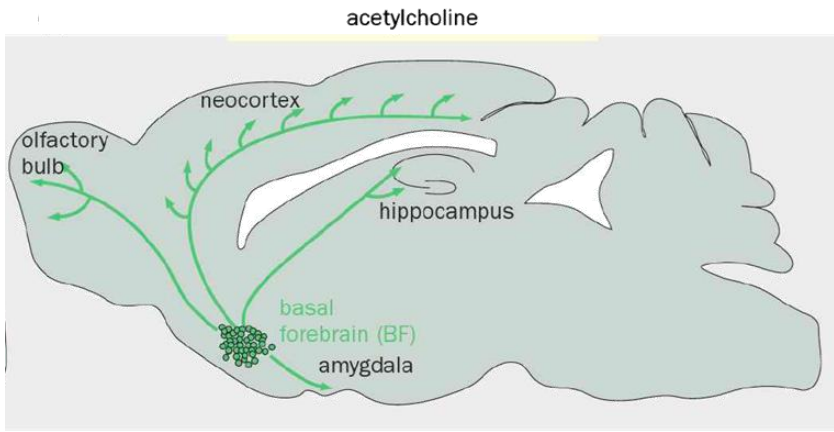
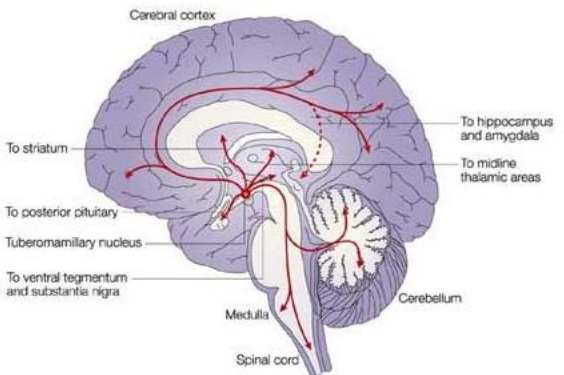


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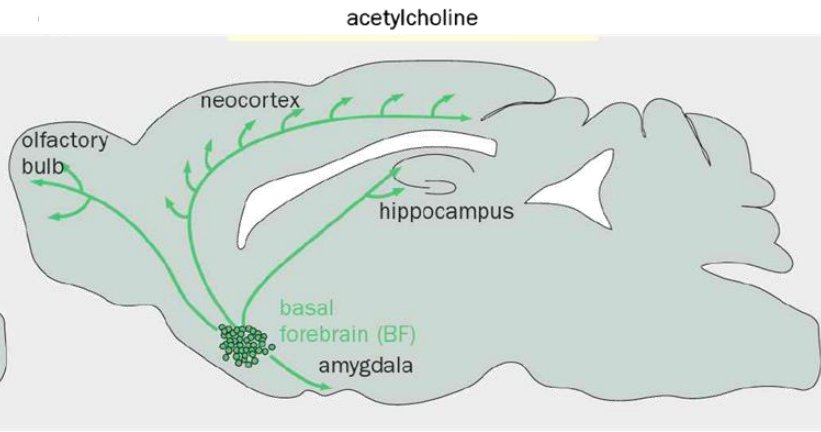
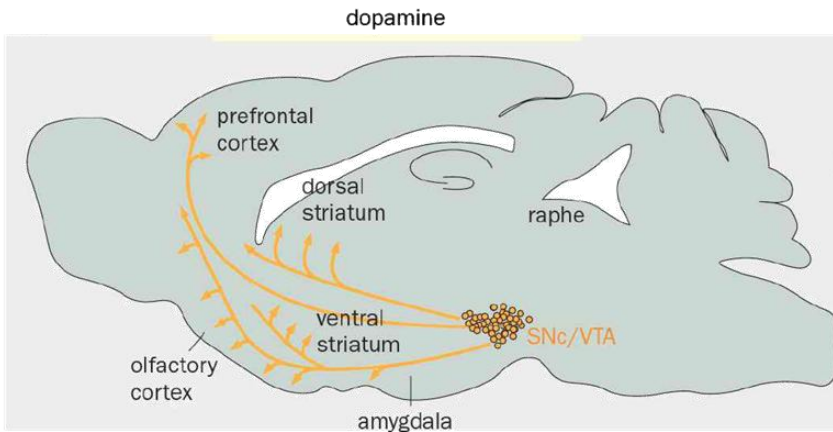
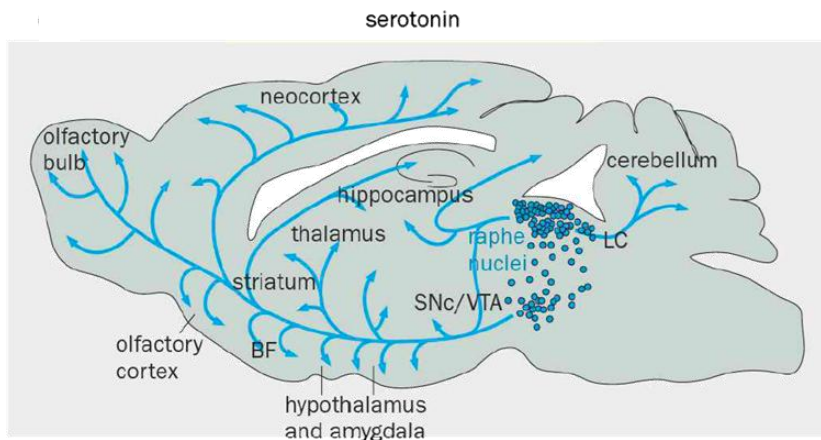
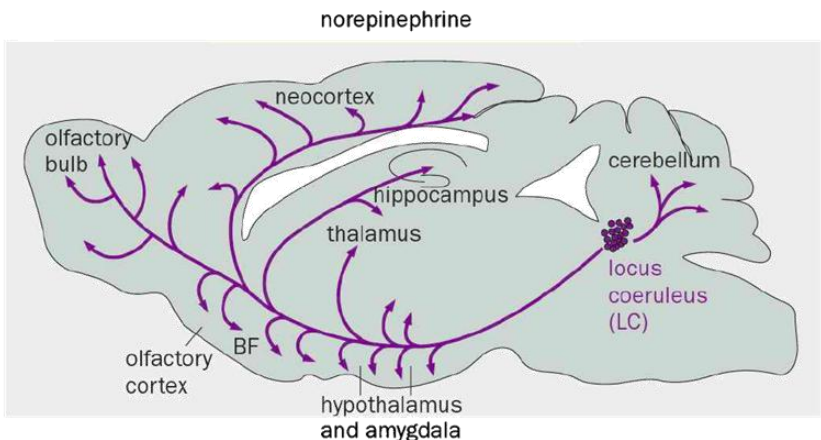
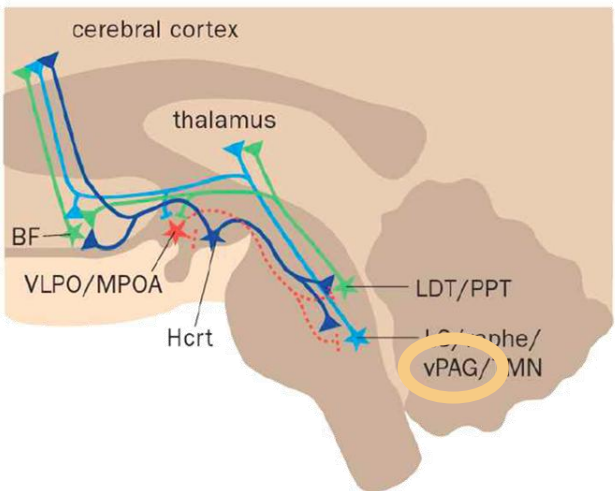


## Histamine

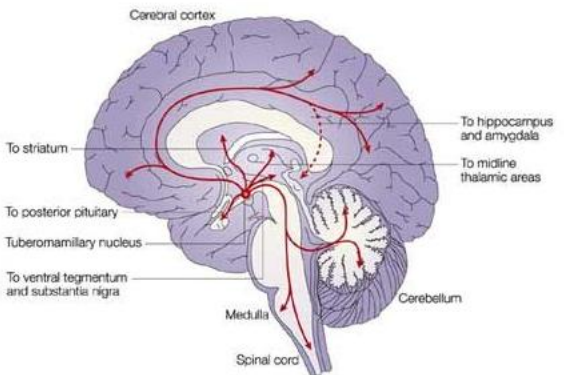


# Light-sensitive neurons in the SCN regulate circadian rhythm in mammals

Hypocretin (orexin) neurons in the lateral hypothalamus activate all major neuromodulatory systems during wakefulness.

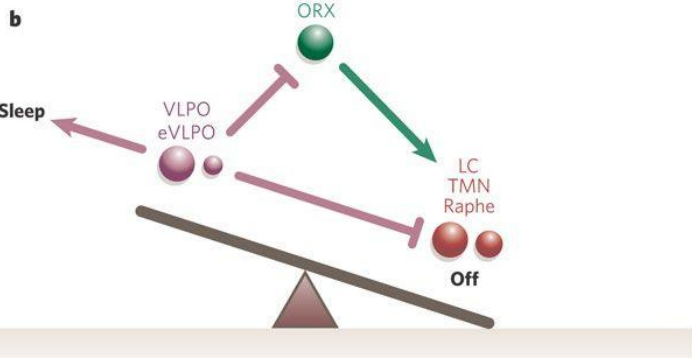
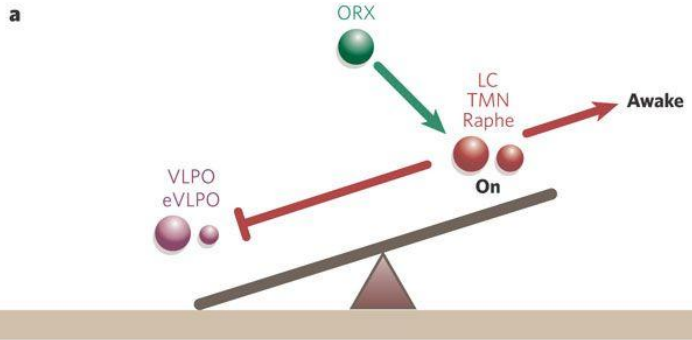
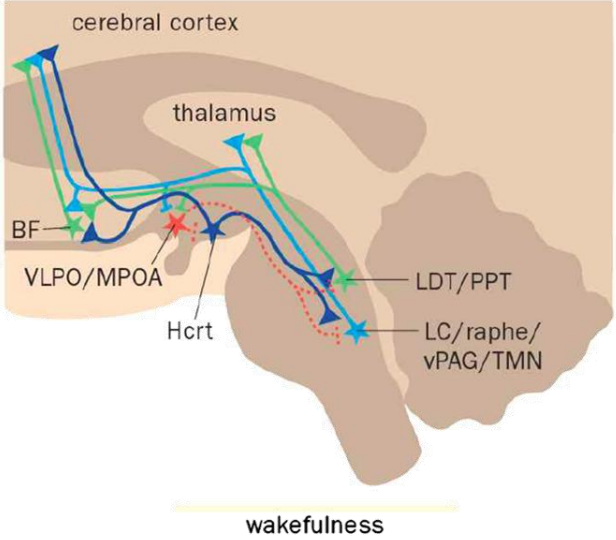
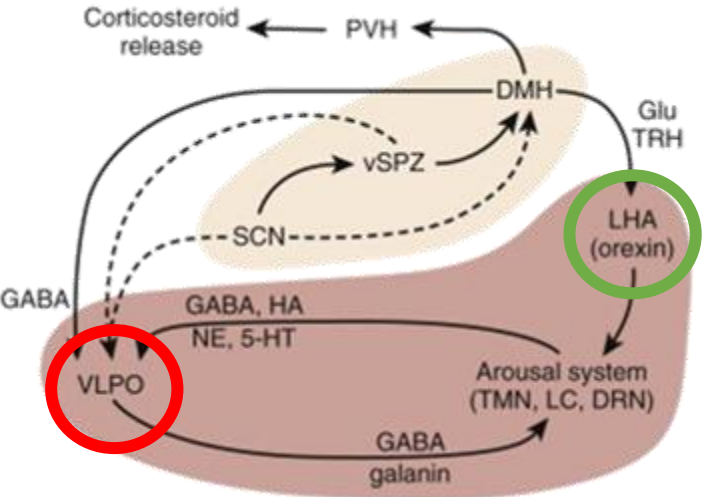
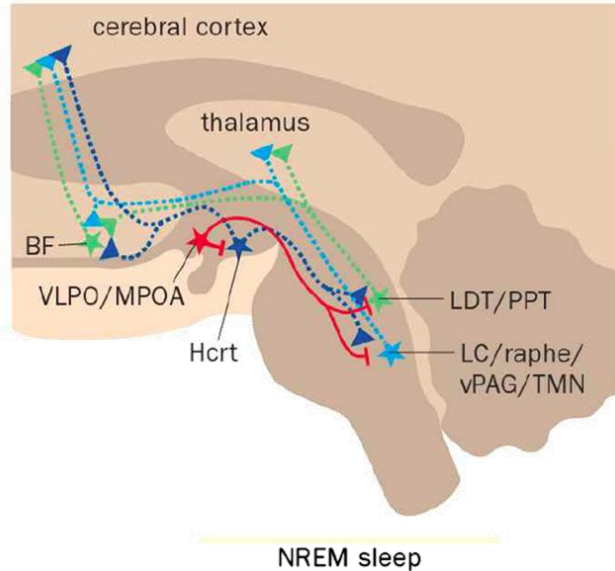


## Histamine



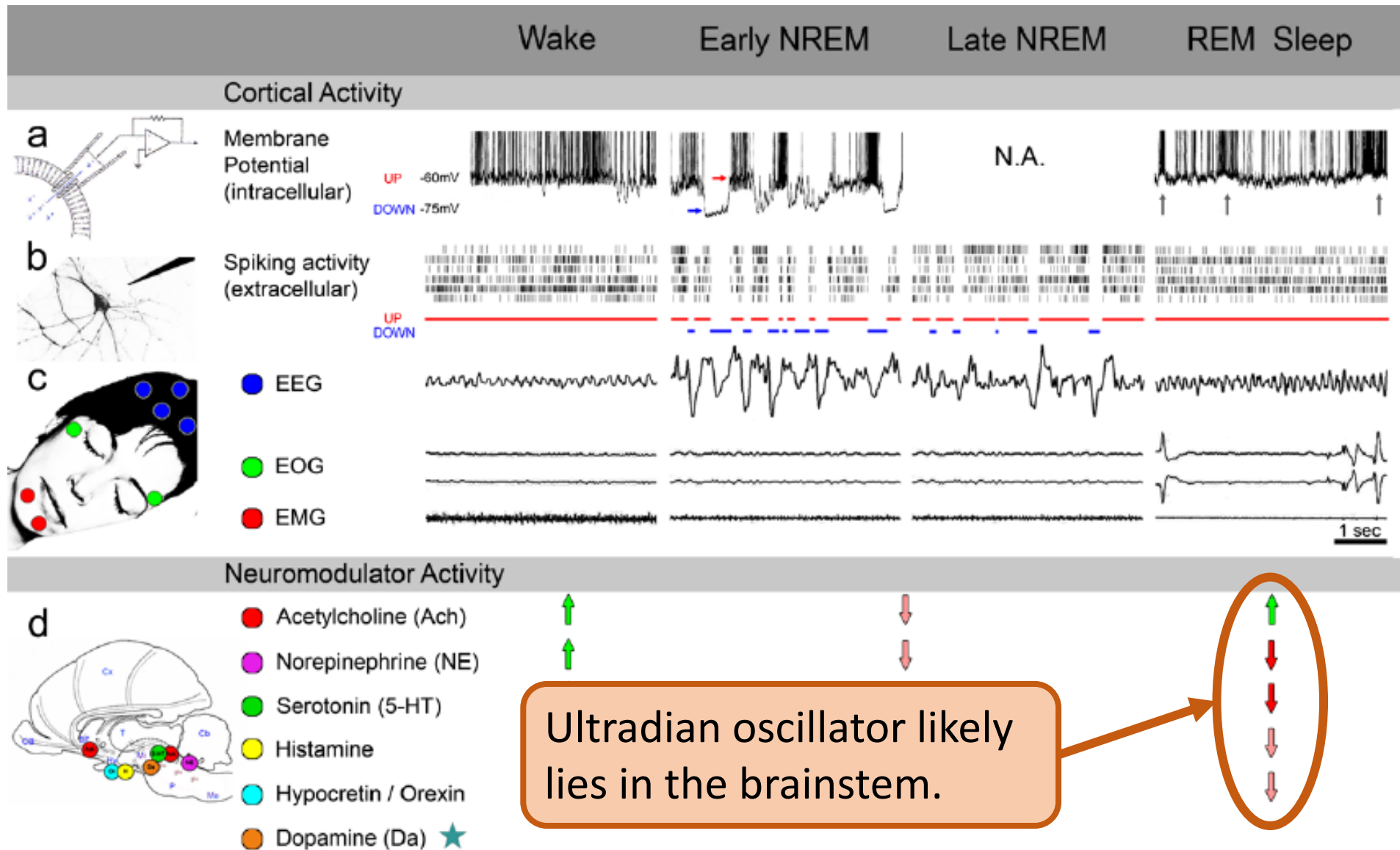
# Light-sensitive neurons in the SCN regulate circadian rhythm in mammals

Neurons in the SCN interact with two nuclei that have opposite effects on one another.

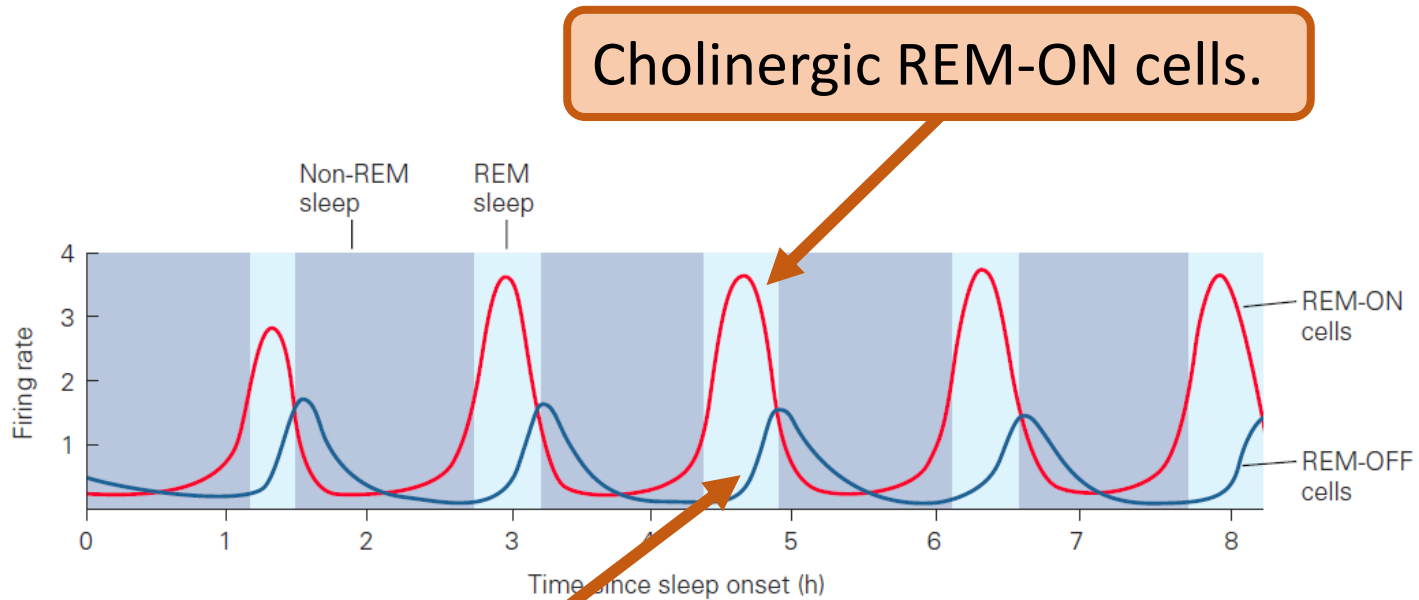


A second, 'sleep active' population of neurons is found in the ventrolateral preoptic area (VLPO). Lesioning VLPO causes a > 50% reduction in NREM and REM sleep

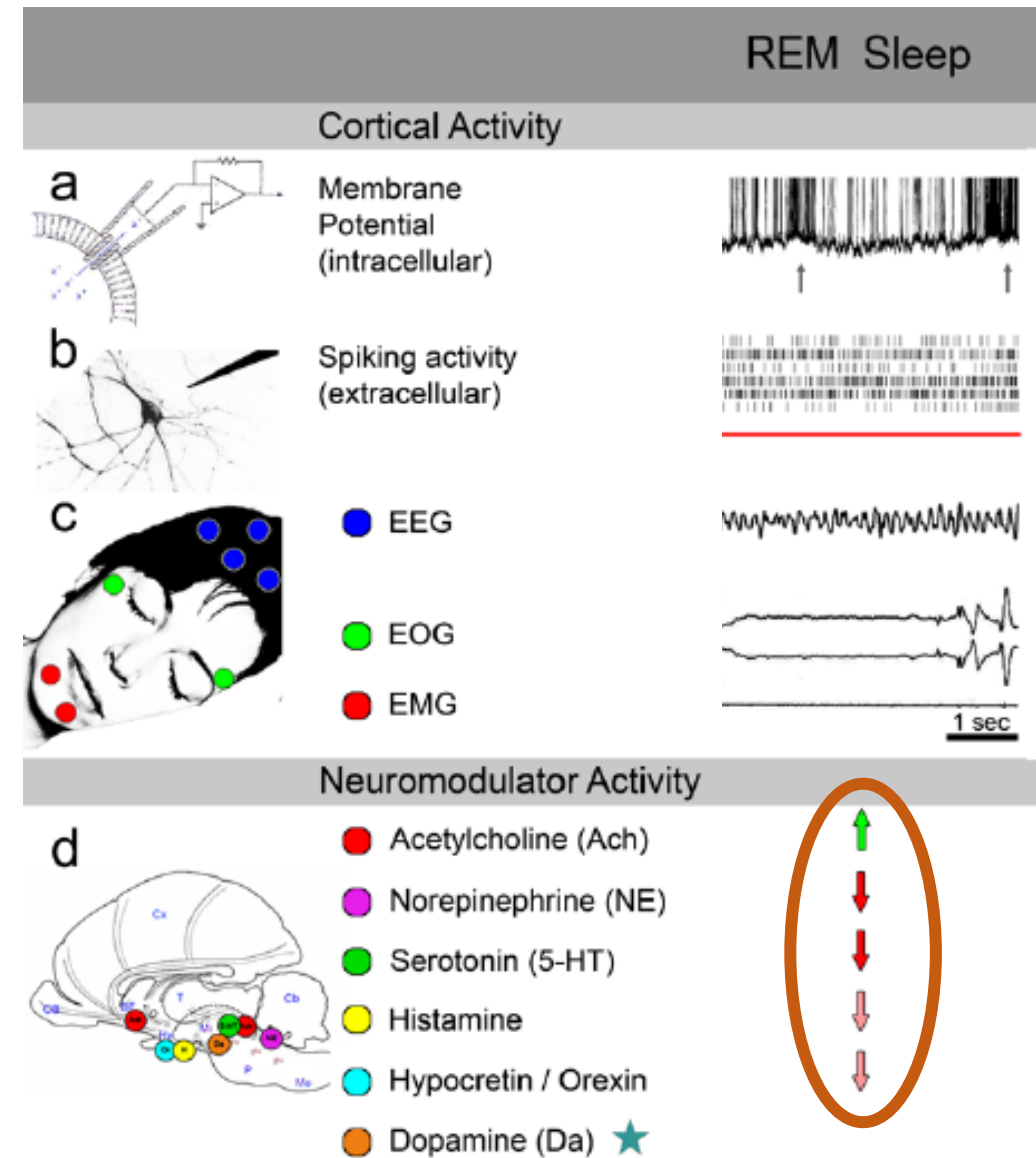
# The Sleep Cycle – Changes in Neuromodulators



# The Sleep Cycle – Changes in Neuromodulators

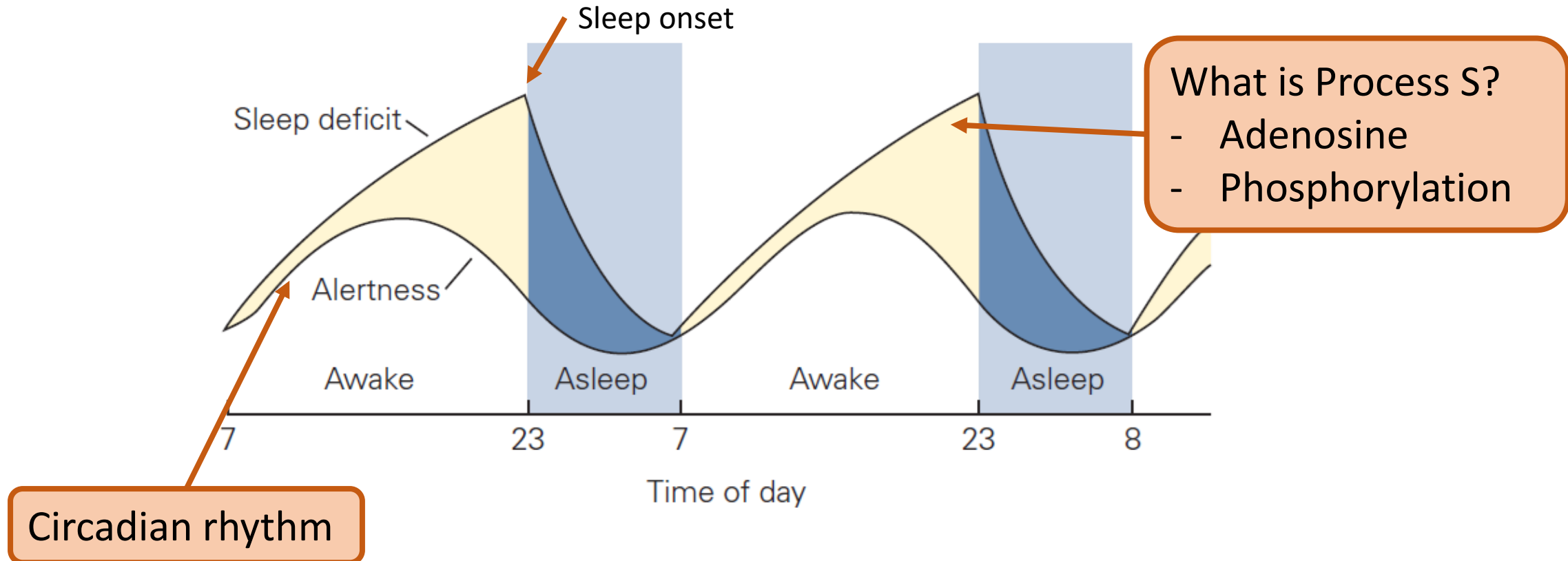


Serotonergic / Noradrenergic / Histaminergic REM-OFF cells.



# Two-Process Model of Drive to Sleep

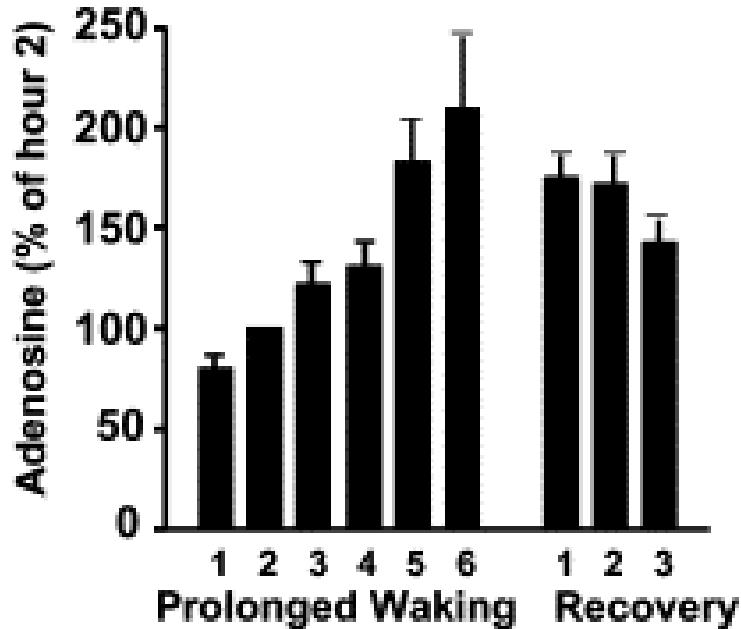
Sleepiness (or the 'drive for sleep') seems to depend on two factors: 1) time since last sleep and 2) time during the day. This has led to a two-process model being proposed for sleep initiation.



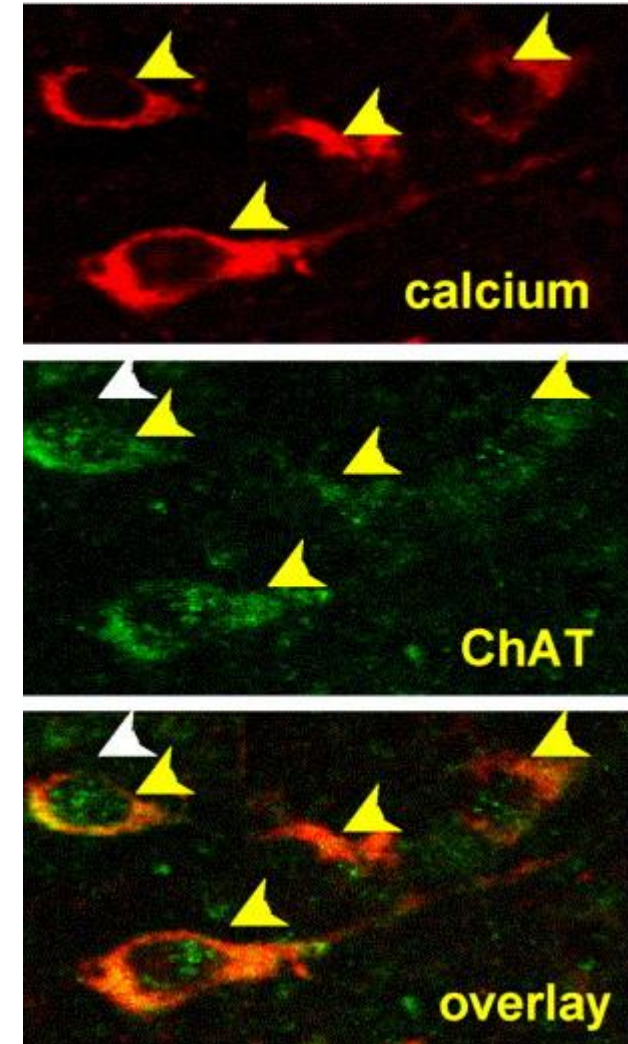
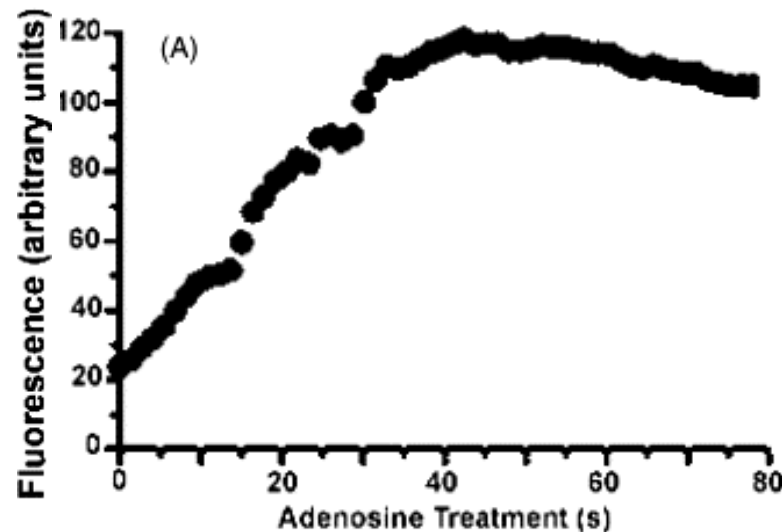


# Adenosine as the homeostatic 'Process S' variable

Adenosine aggregates during waking, but is metabolized during sleep:



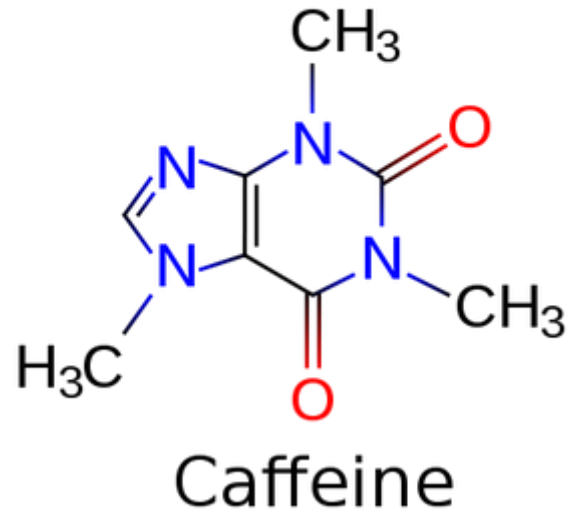
Adenosine leads to an increase in intracellular calcium, primarily in cholinergic neurons in basal forebrain.



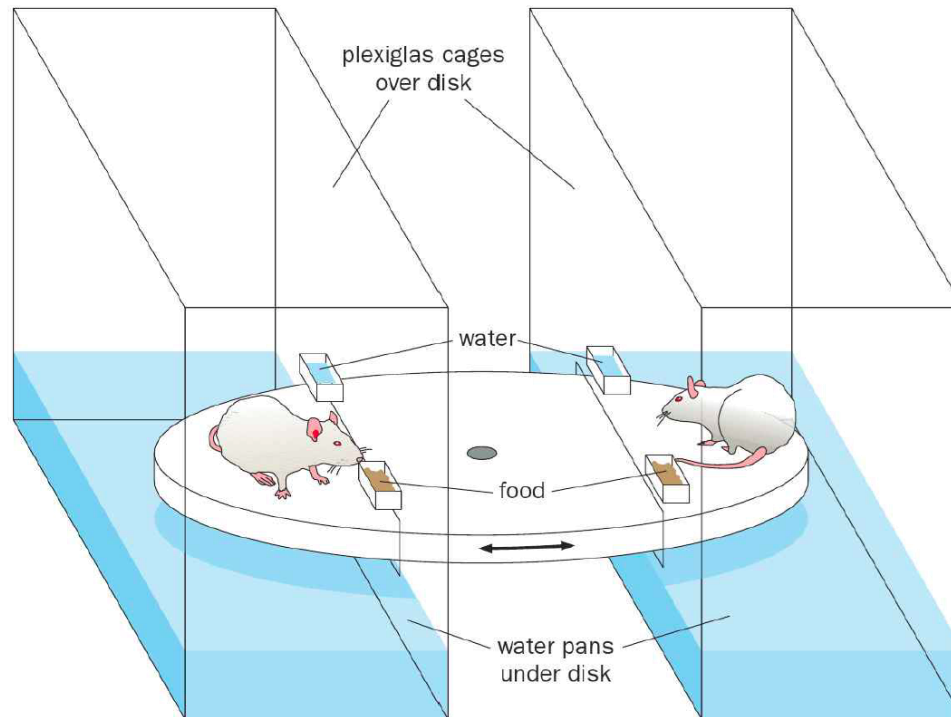
Adenosine leads to inhibition of activity in cholinergic nuclei, giving rise to its somnogenic effects.

# Adenosine as the homeostatic 'Process S' variable

A very common drug acts to competitively inhibit adenosine receptors, increasing wakefulness:

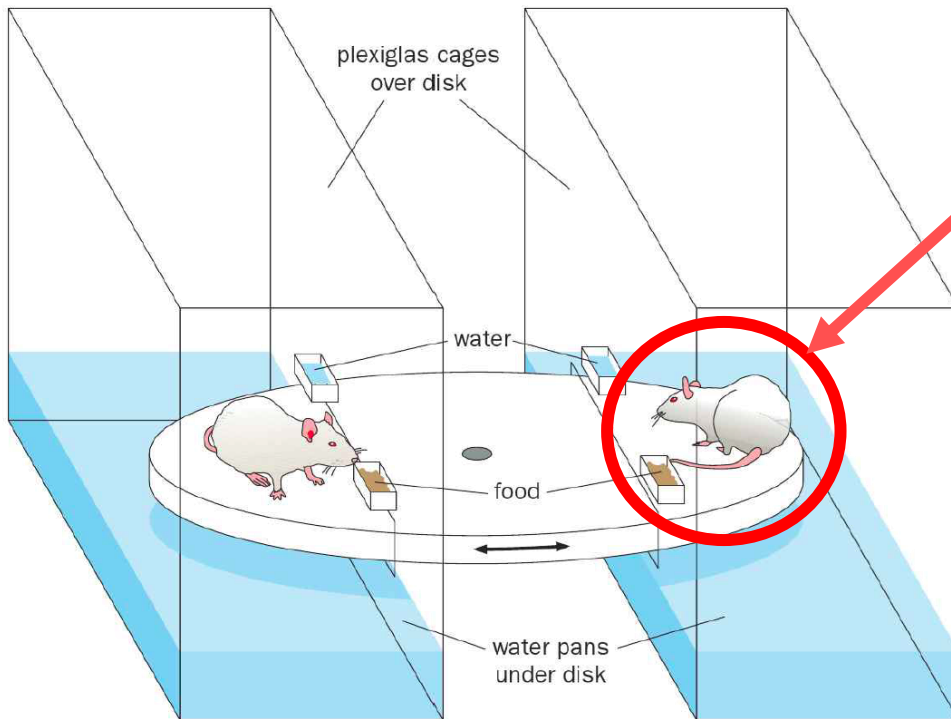


# Why do we need sleep?



# Sleep is necessary: not sleeping will kill you

Sleep was limited experimentally in rats:



EEG was recorded from both animals. Rotation of the platform was tied to one rat's EEG: if it indicated they were about to fall asleep the platform started rotating, forcing the animal awake.

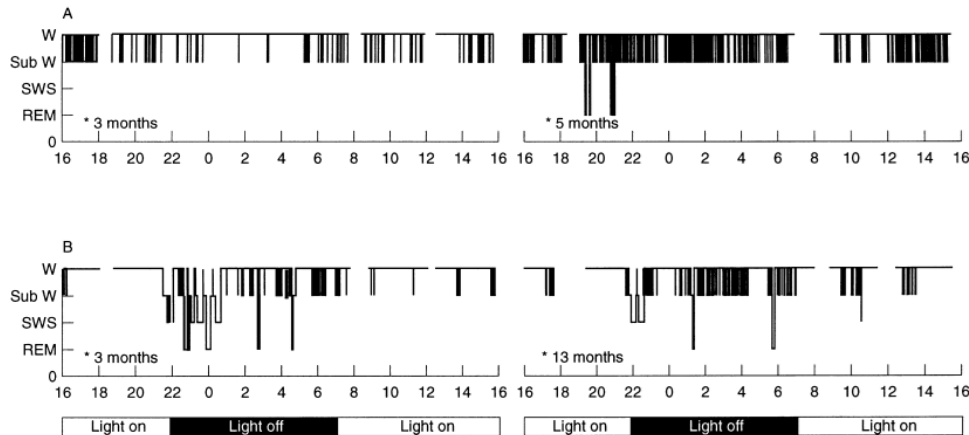
Control rat could sleep as long as the experimental rat was awake.

**After a few weeks experimental rat died; demonstrating that sleep is essential for life.**

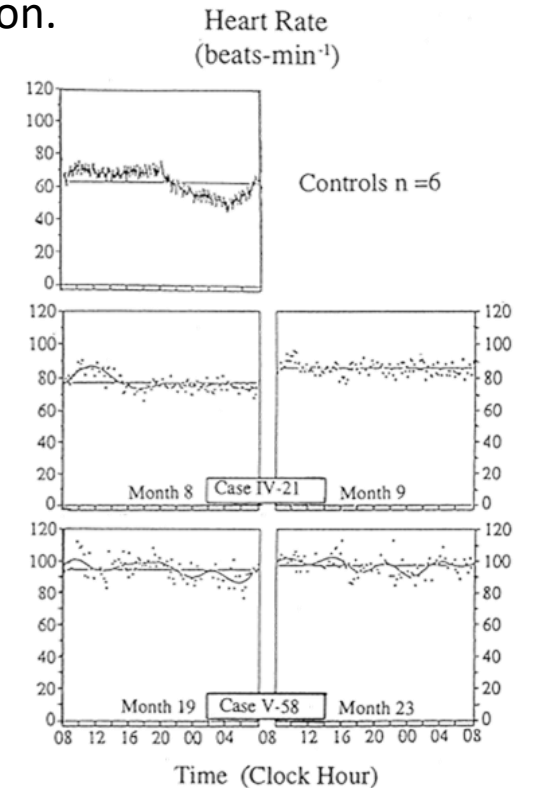
# Fatal Familial Insomnia: Lack of sleeping will kill humans

Fatal Familial Insomnia (FFI) is a **prion disease**. An autosomal dominant mutation of a prion protein.

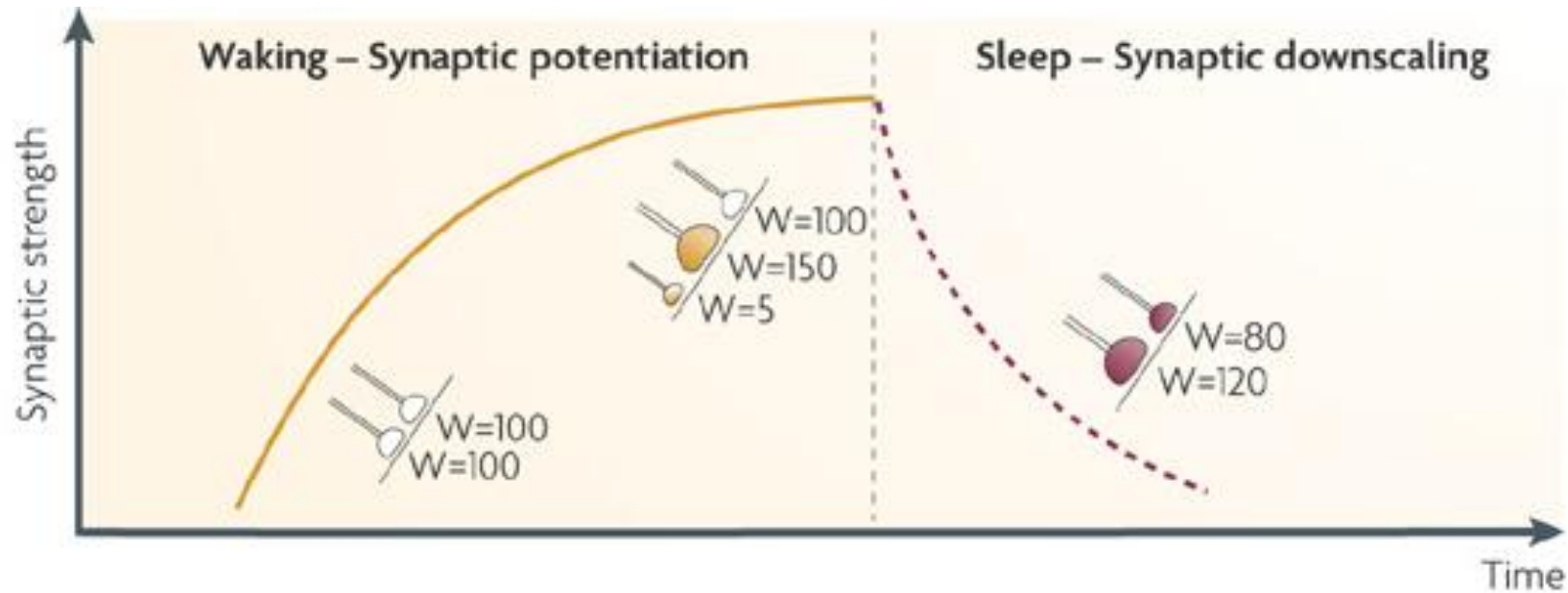
- Disease presents around 50 years of age.
- Increasingly difficult to sleep; patients do not pass beyond stage 1 of sleep:



- Patients become increasingly apathetic and ataxic. Unable to pay attention.
- Patients usually die between 12 months (+/+) and 24 months (+/-) of initial presentation.
- Disruption of circadian clock:
- Prion aggregation appears to lead to apoptosis in the thalamus. (suggests a role for the thalamus in sleep regulation?).
- Transferring human prion to mice will cause same symptoms.



# Synaptic Homeostasis Hypothesis



# Synaptic Homeostasis Model

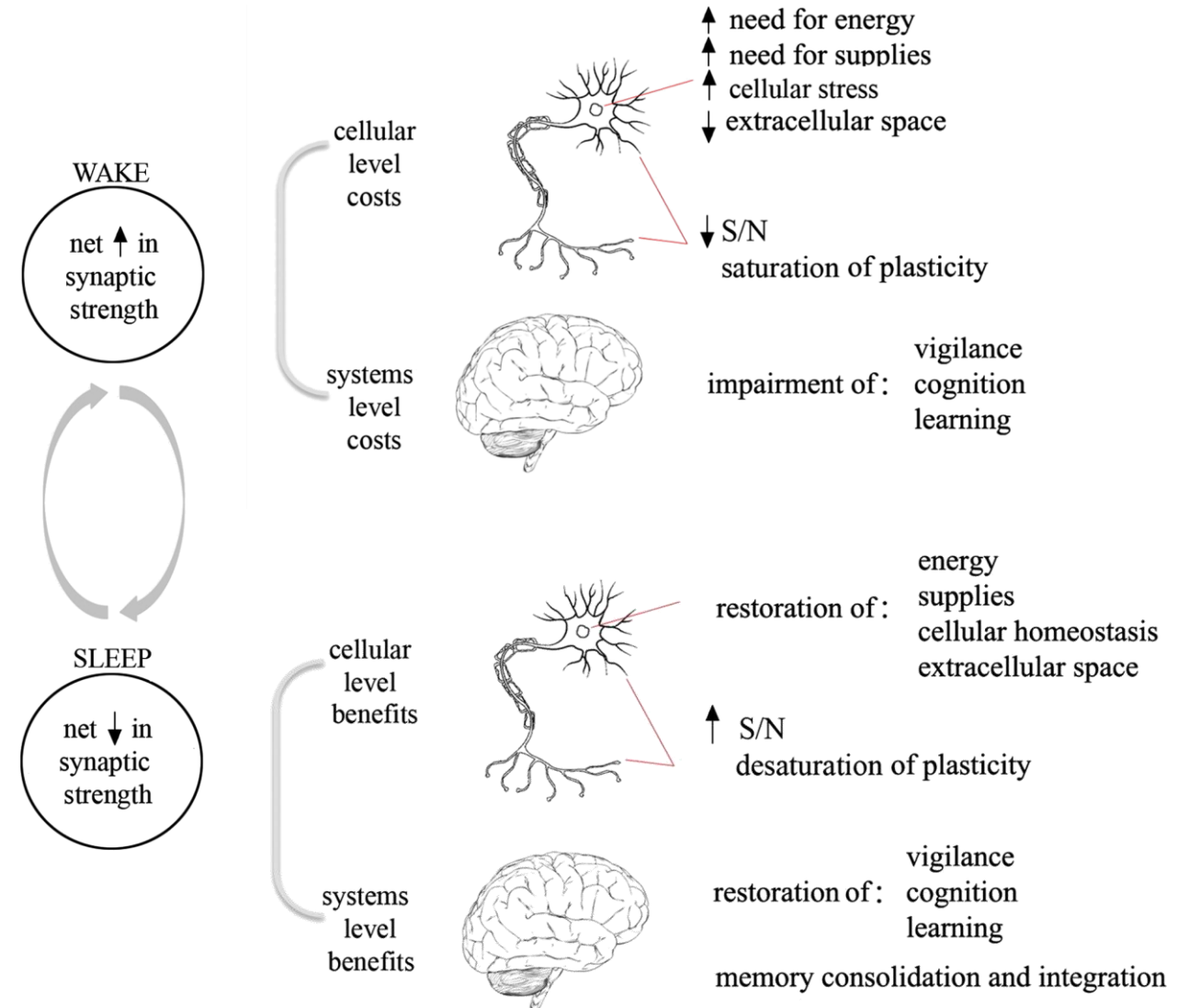
The synaptic homeostasis hypothesis (SHY) suggests there are several costs of being awake:

- Higher spiking activity leads to increased metabolic rate (e.g. re-establishing gradient, etc).
- Vesicle recycling, increased shuttling of proteins.
- High levels of synaptic plasticity.

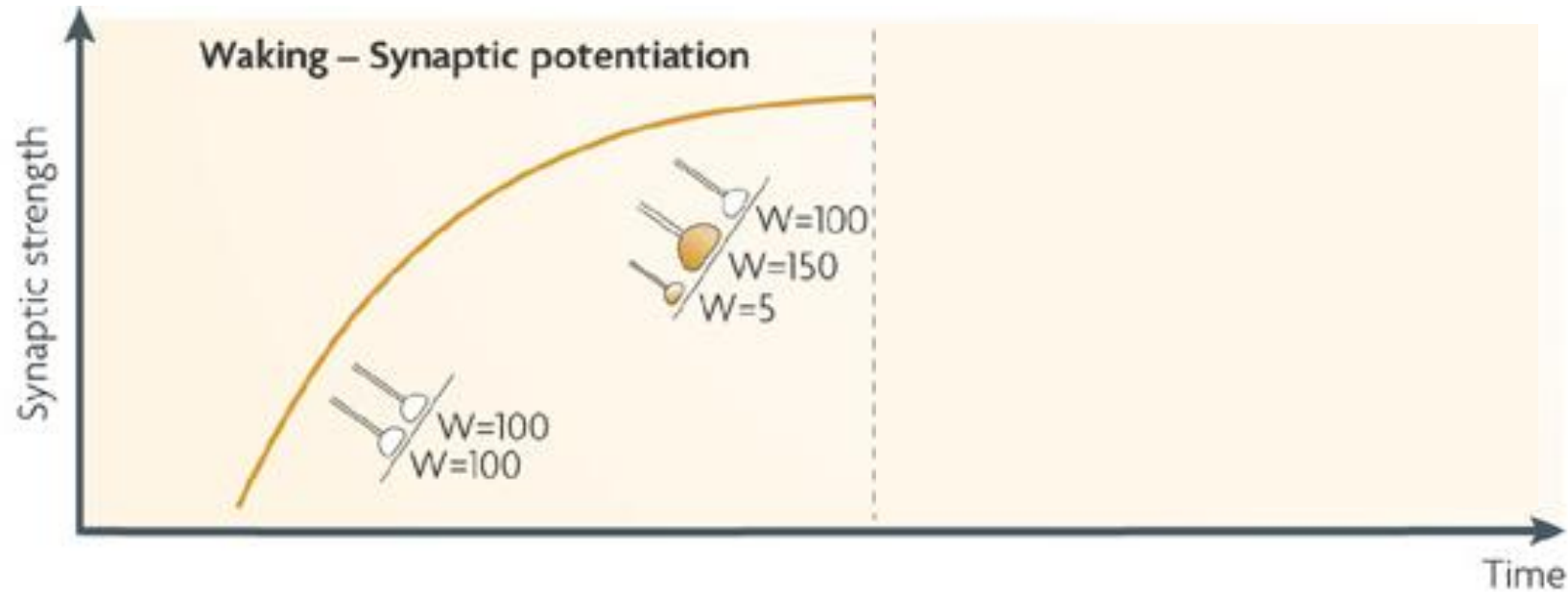
This leads to the impairment in vigilance, cognition, and learning.

Therefore, sleep exists to offset these costs:

- Neurons conserve energy by reducing firing rate and firing more sparsely.
- Reduced levels of learning – this makes sense as you shouldn't learn much from sleep.
- Rescaling of synapses.



# Synaptic Homeostasis Model



Synaptic potentiation can move synaptic weights outside an optimal range:

- 1) Too strong and you no longer integrate multiple inputs.
- 2) Too strong can cause runaway excitation.

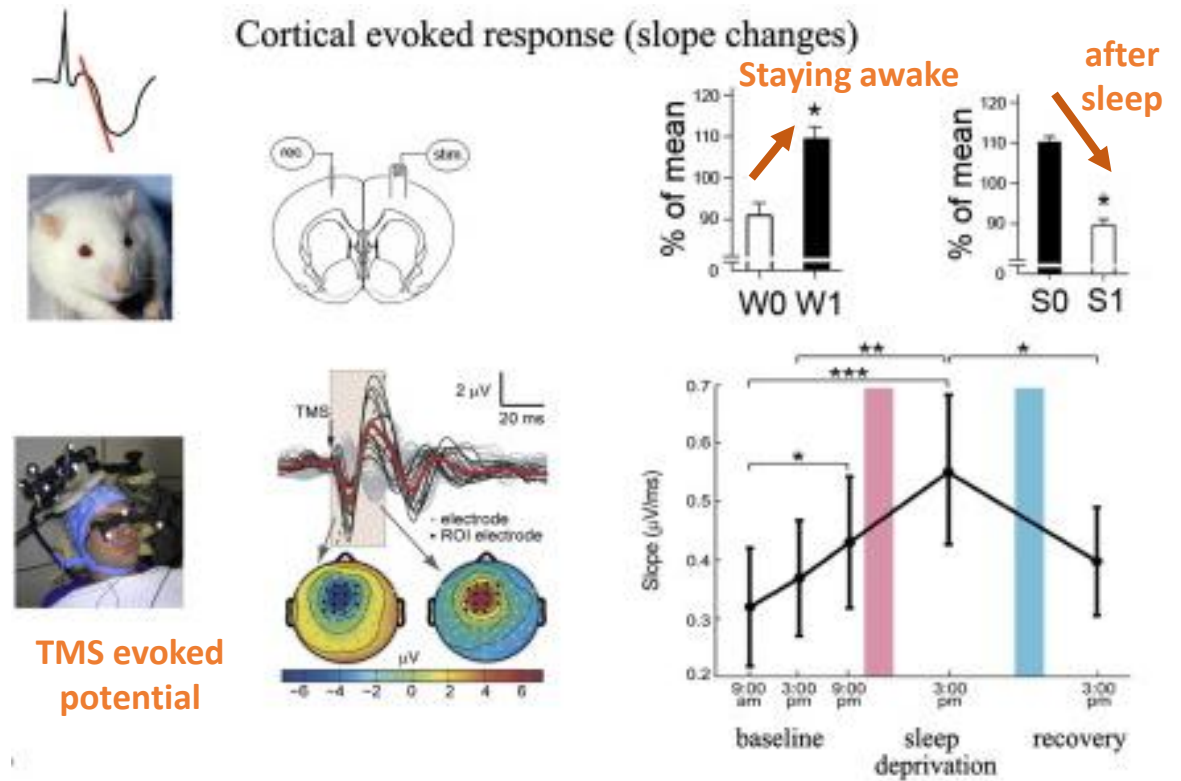
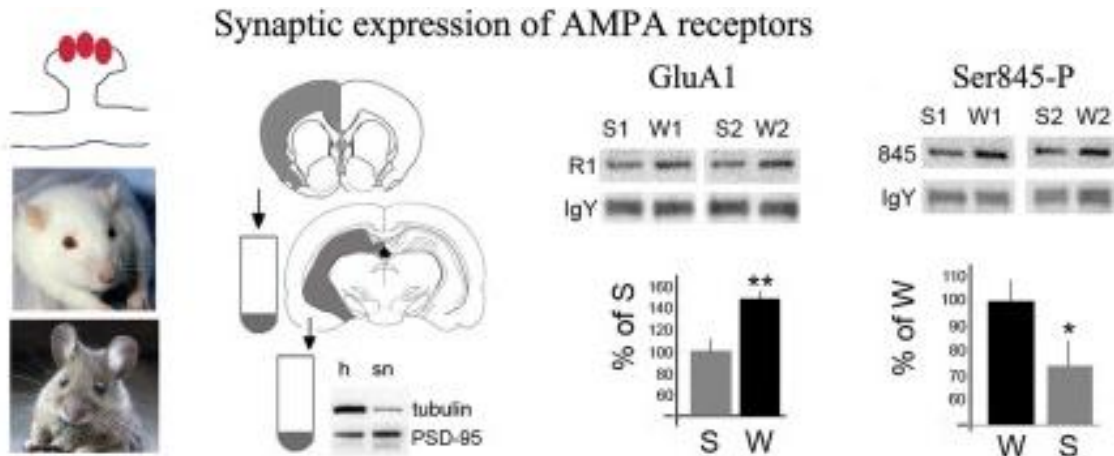
**Slow-wave sleep is hypothesized to renormalize weights.**

# Synaptic Homeostasis Model

There is evidence that synaptic weight increases during wake and that this is re-normalized during sleep.

Being awake also leads to an increase in the slope of the evoked potential, consistent with a potentiation of synapses. This is reduced after sleep.

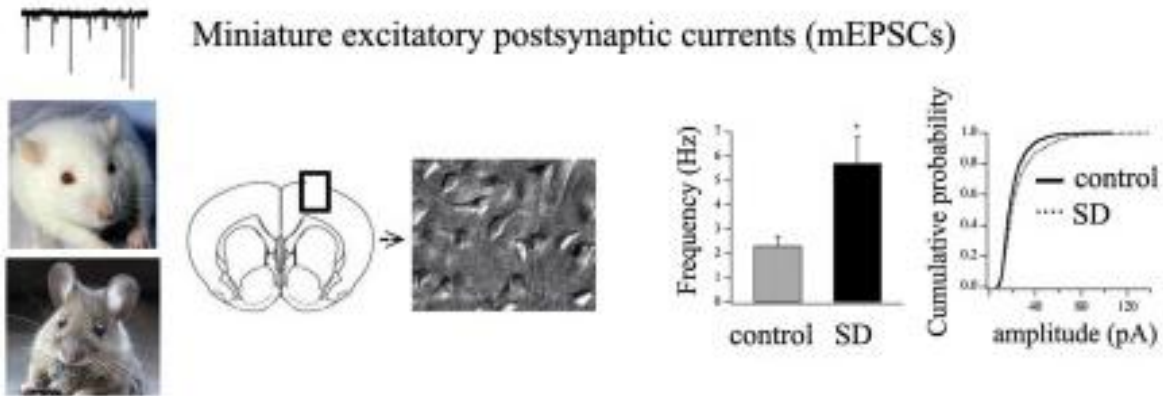
Being kept awake increases the # of AMPA receptors, as well as their phosphorylation (trafficking to membrane), relative to sleeping.



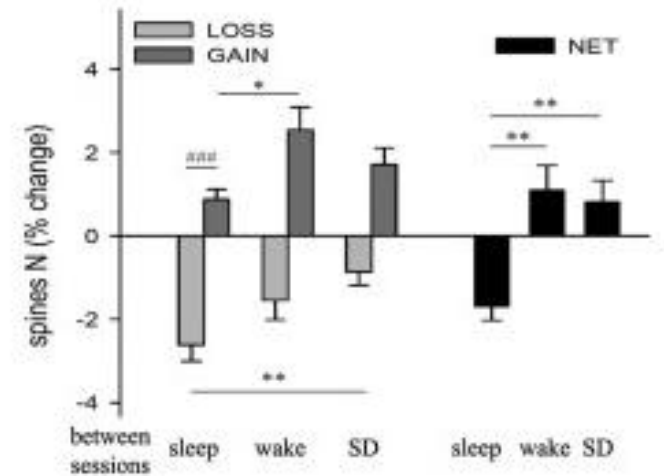
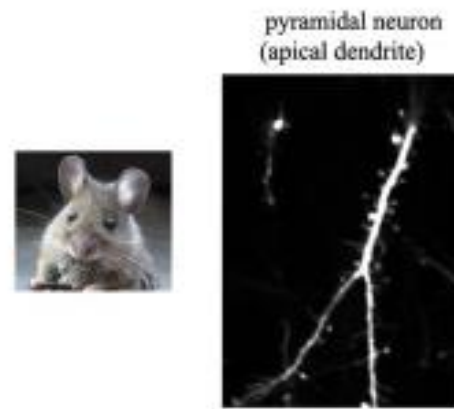
# Synaptic Homeostasis Model

There is evidence that synaptic weight increases during wake and that this is re-normalized during sleep.

Sleep deprivation increases the frequency and amplitude of mini-EPSCs.



Being awake increases the number of spines, which is then reduced during sleep.

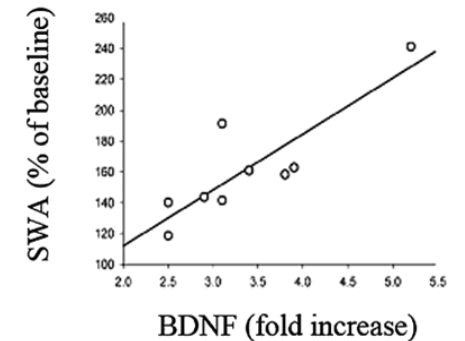
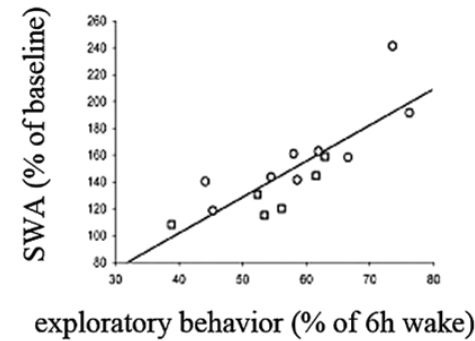
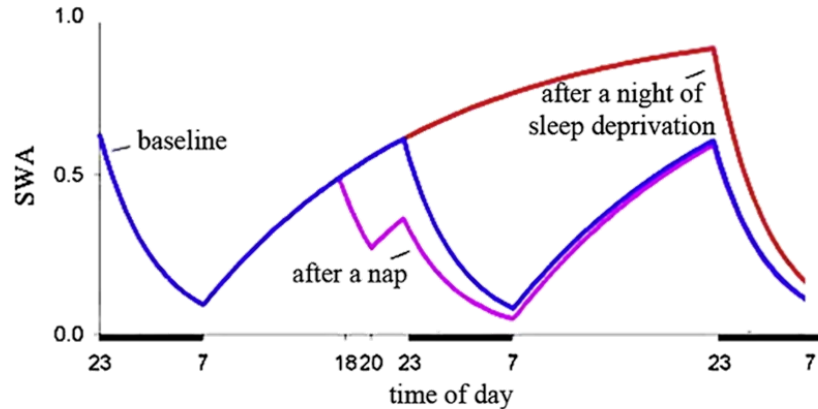


# Synaptic Homeostasis Model

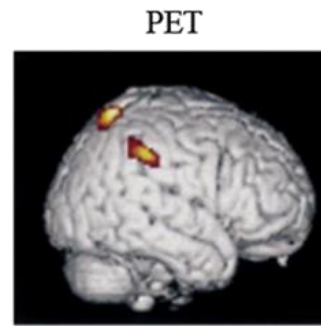
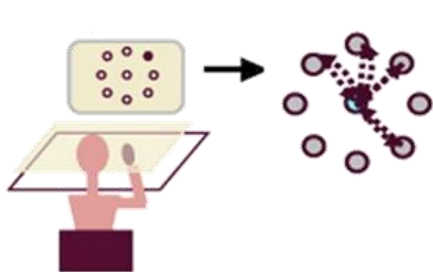
Several lines of experimental evidence provide indirect support for the idea that slow-wave sleep is critical to re-normalizing synapses.

Exposing rats to novel objects increases the amount of time they spend exploring and neural activity (reflected in BDNF expression). These are both positively correlated with the amount of subsequent SWS sleep:

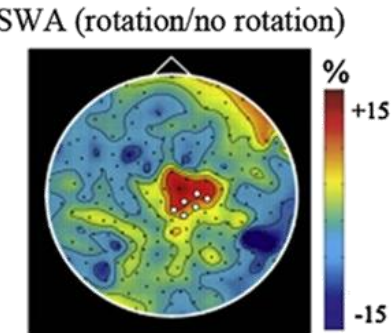
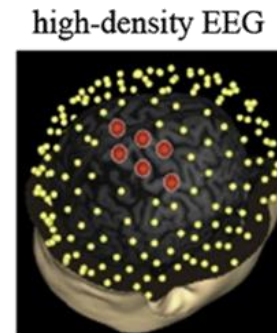
The amount of SWS is correlated with sleep pressure:



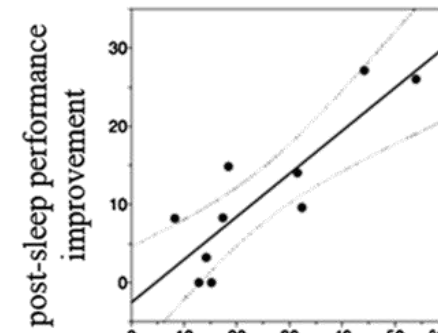
Learning a new motor skill increases SWS in the related brain region:



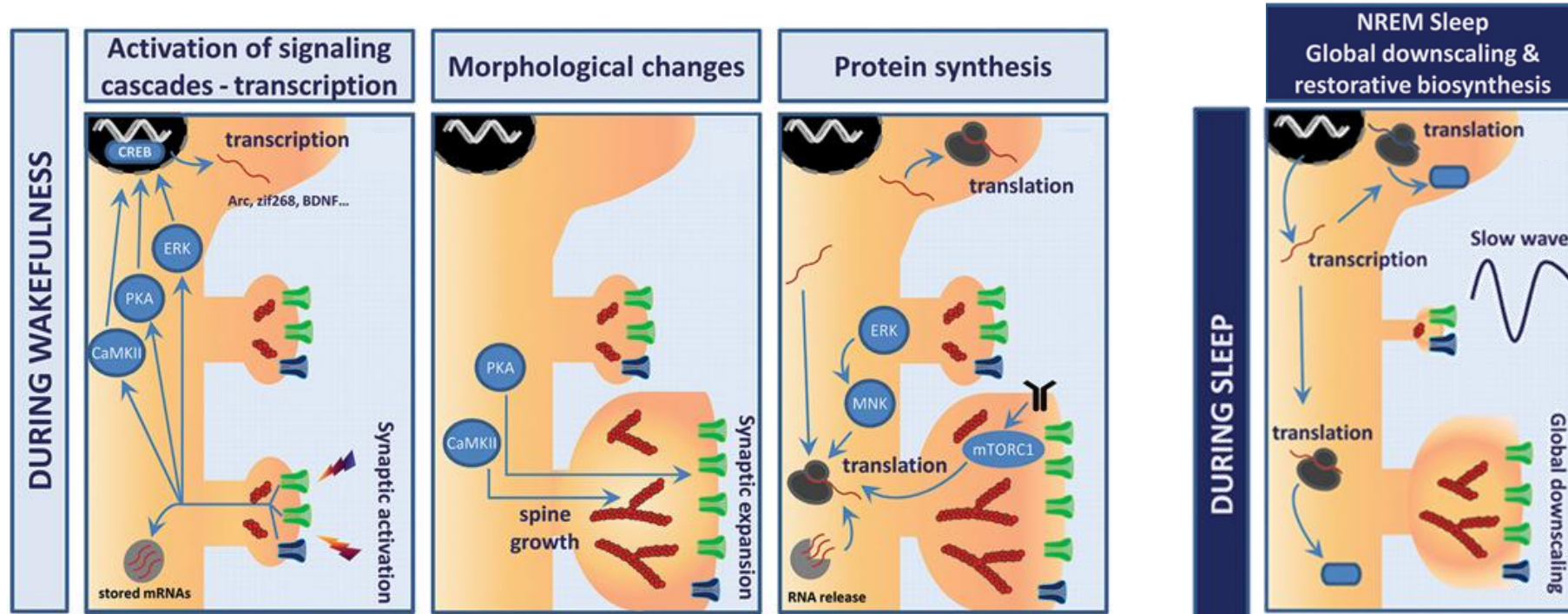
Task Activation



Change in SWS



# Summary of Synaptic Homeostasis



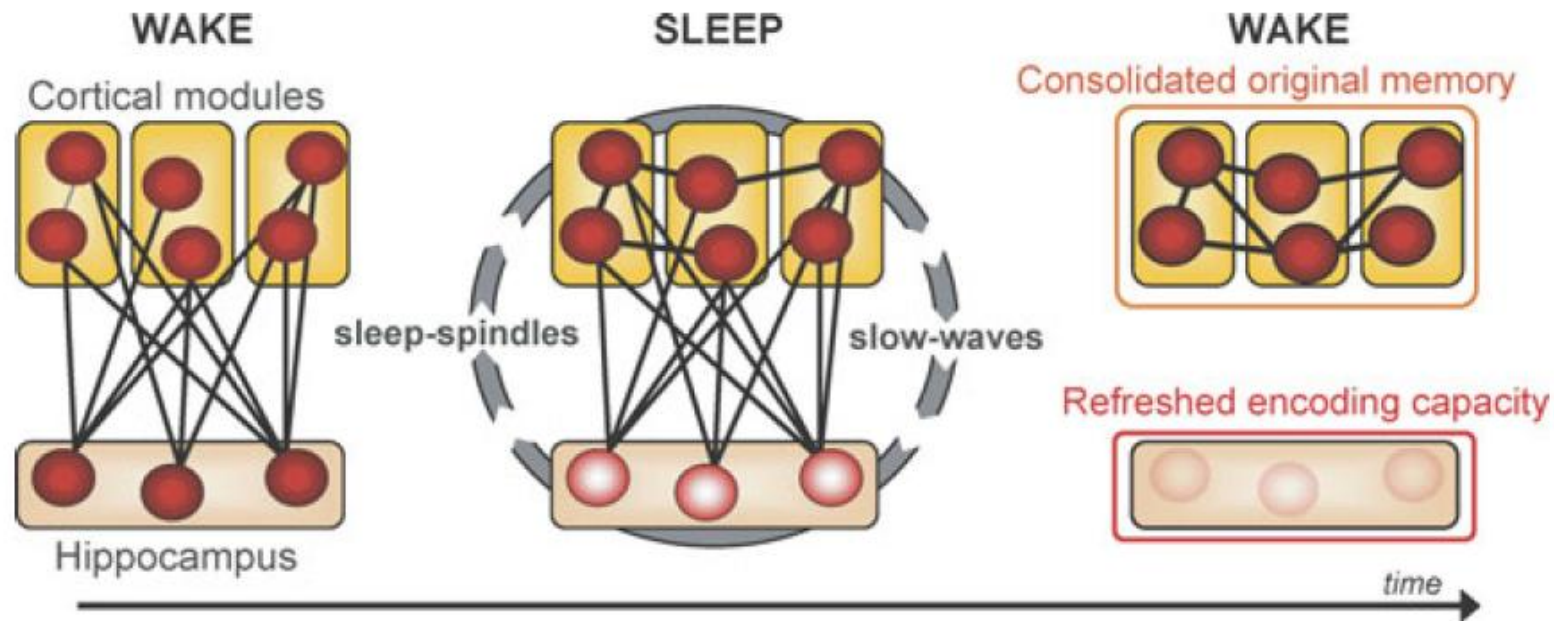
Activation of synapses causes influx of  $Ca^{++}$  which starts internal cascade.

This leads to actin remodeling and shuttling of new channels; growing synapse.

These changes are consolidated.

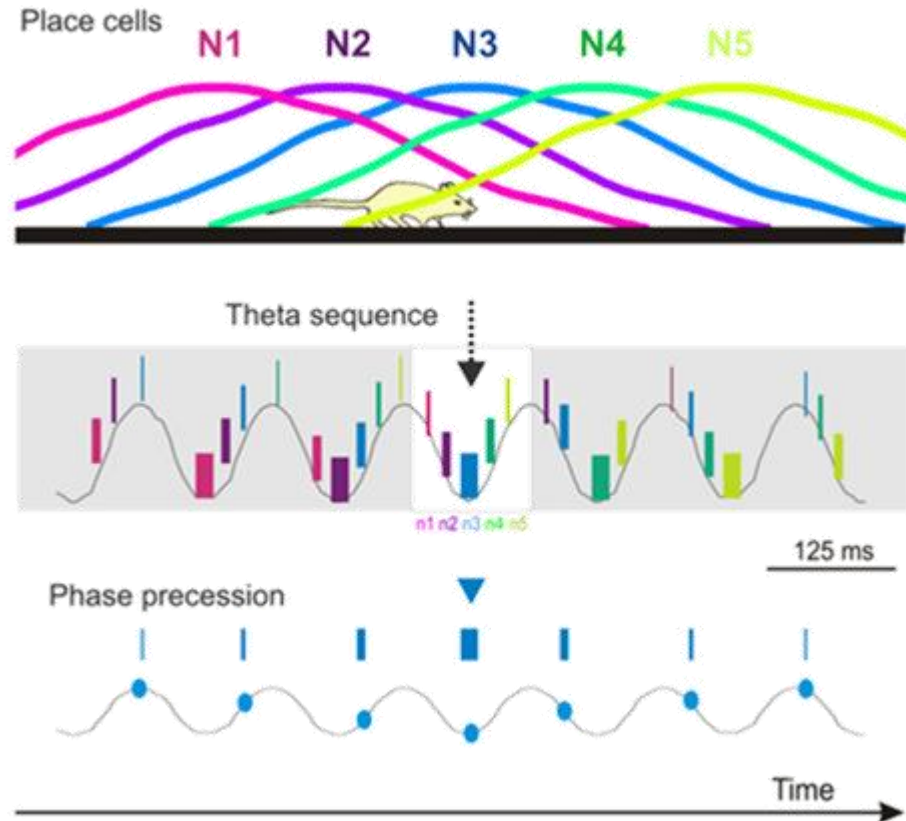
SWS causes global downscaling of synapses.

# Consolidation of Memories Hypothesis



# Experiences are replayed during sleep

Sequences of place cell activation in HPC as the animal navigates through a 1D run:



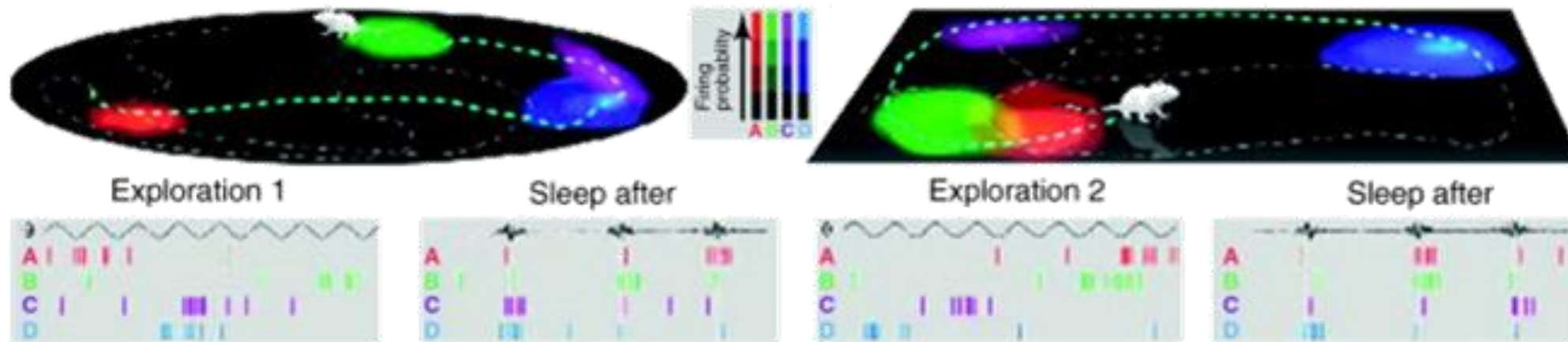
These sequences are replayed during sleep, particularly during SWR during NREM:

Replay 



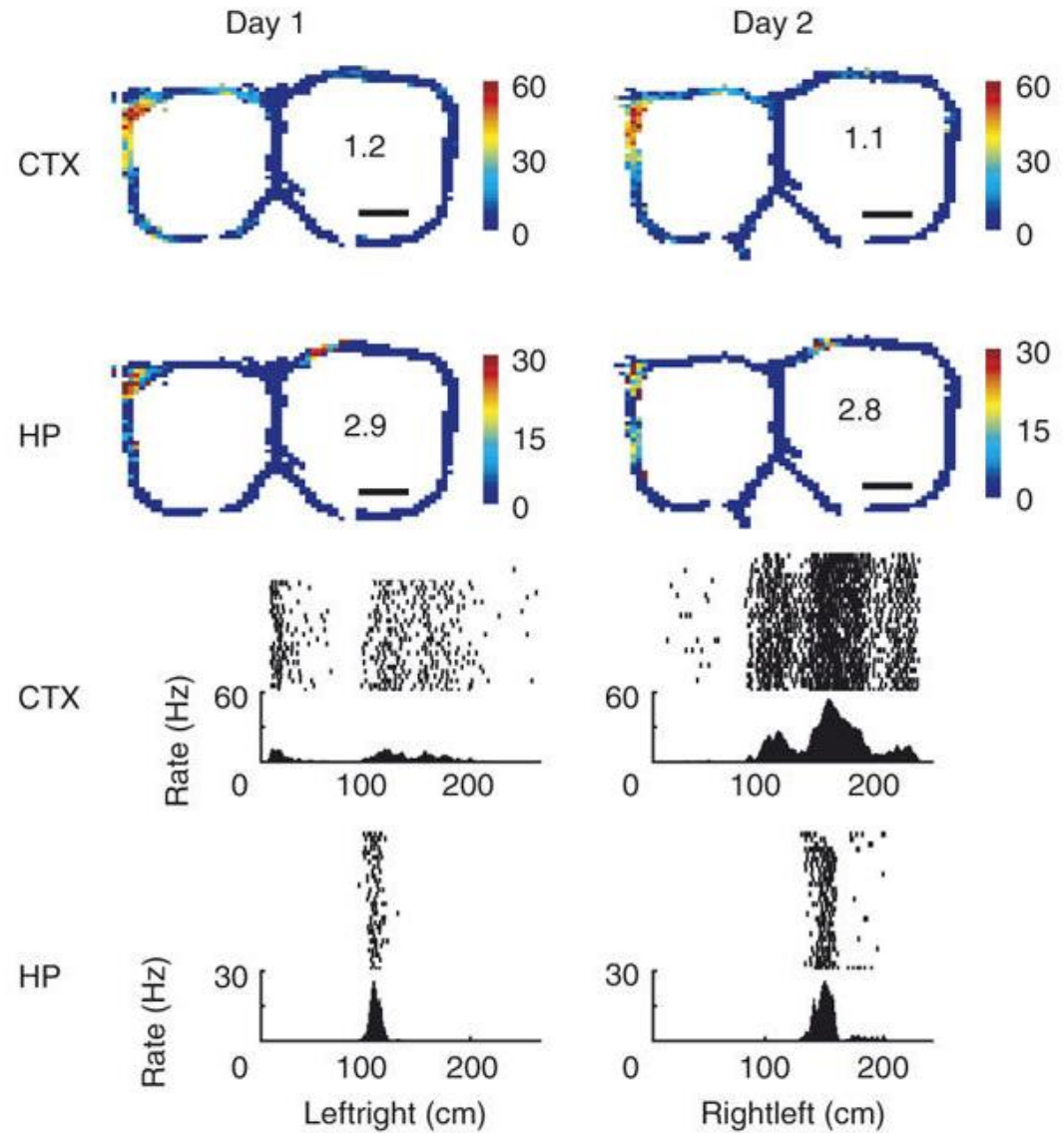
# Experiences are replayed during sleep

Sequences are also seen in two-dimensional environments:



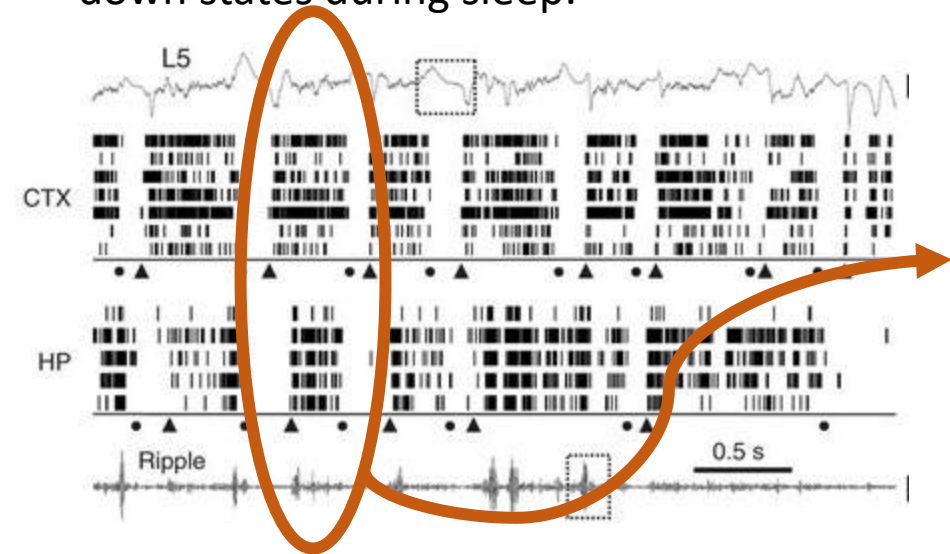
# Replay events are also seen in cortex

Neurons in visual cortex have spatially localized responses. These are likely due to selectivity of neuron for the specific environment.

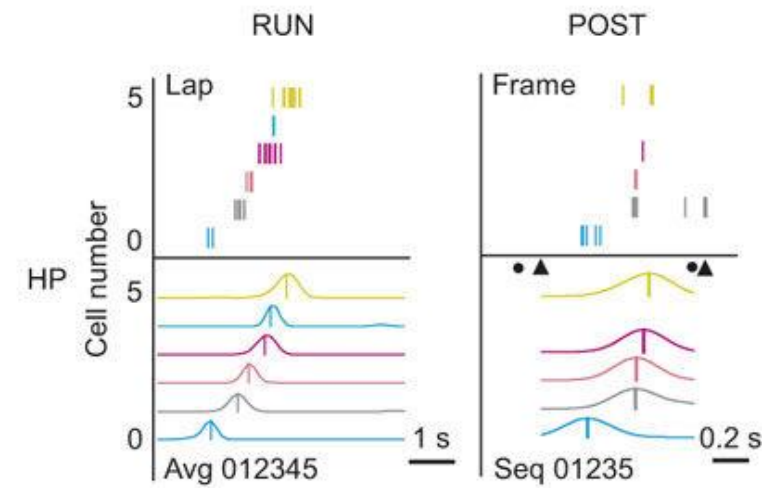
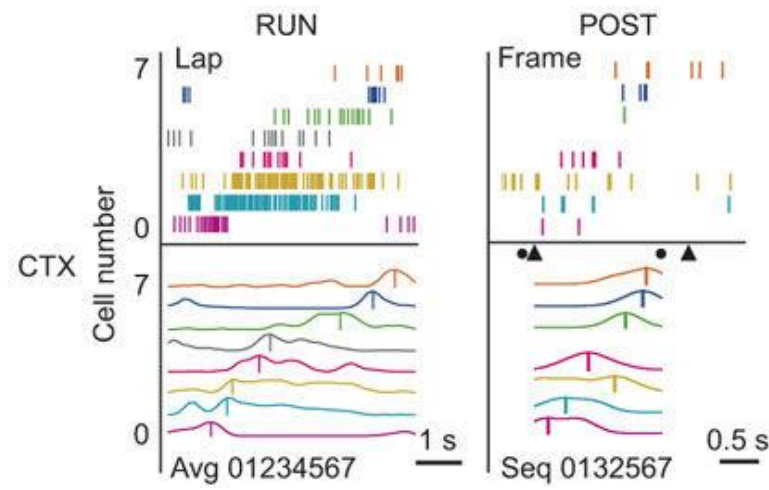


# Replay events are also seen in cortex

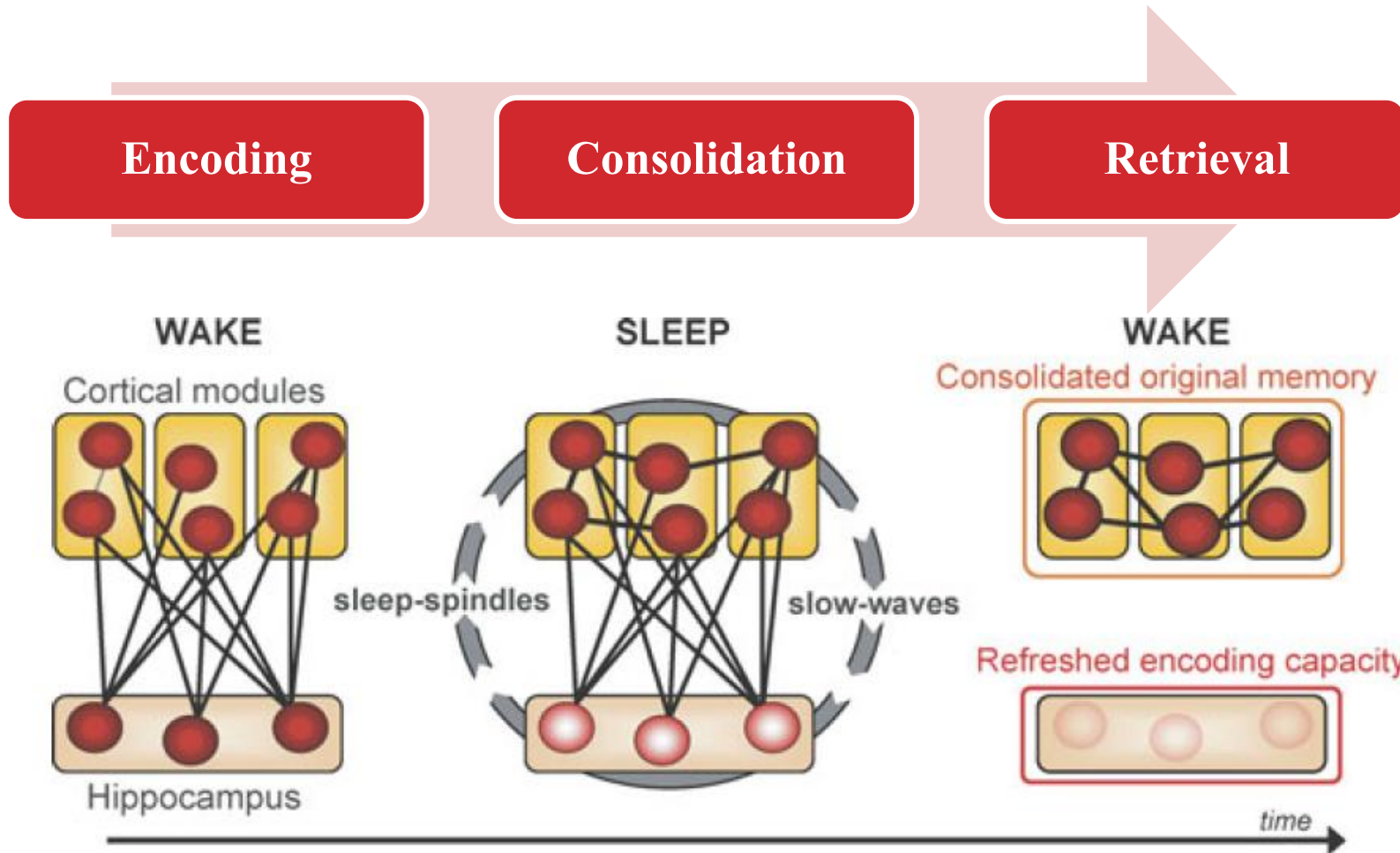
Activity alternates between up and down states during sleep:



Neurons in both HPC and visual cortex show replay events during the UP states of NREM sleep:



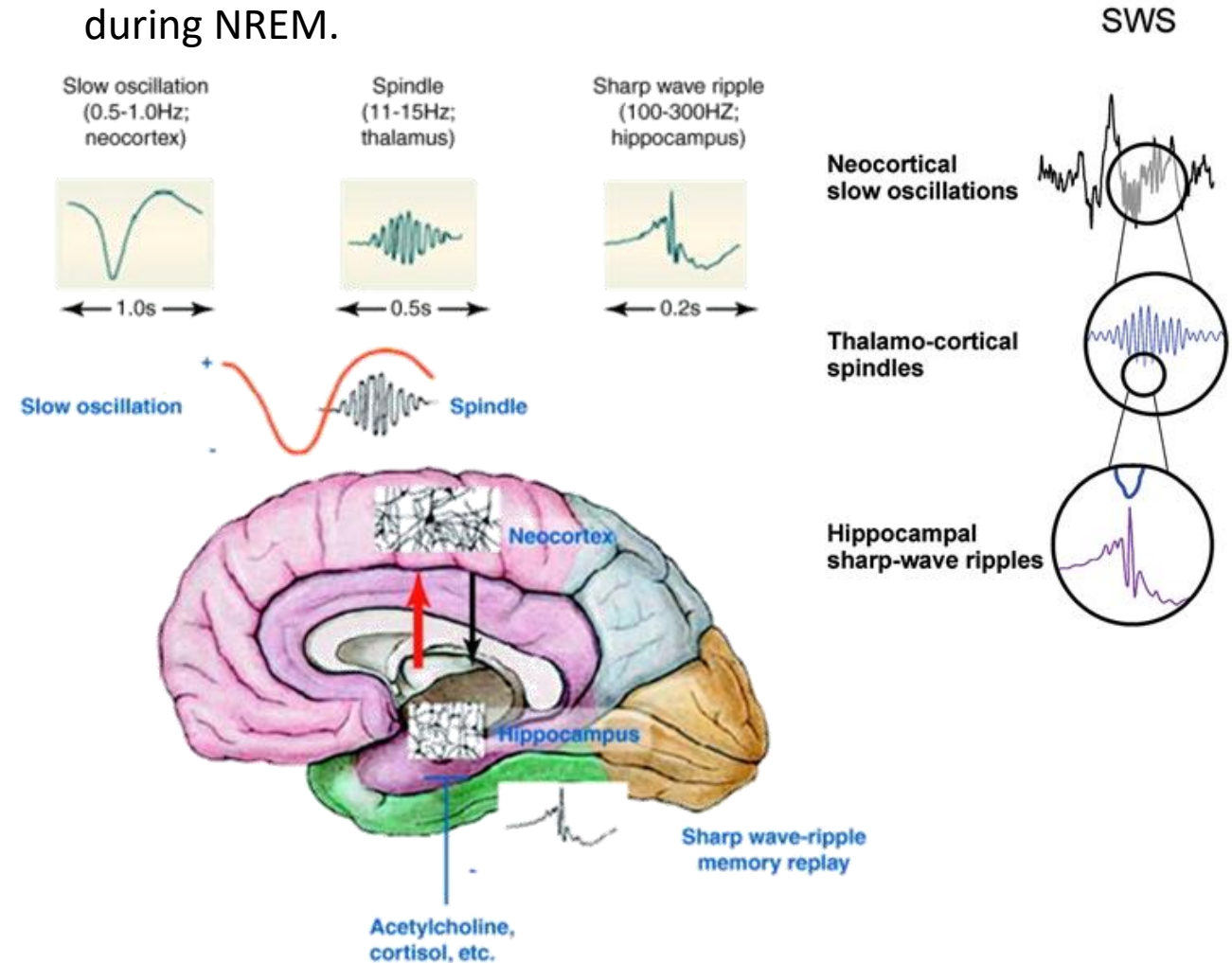
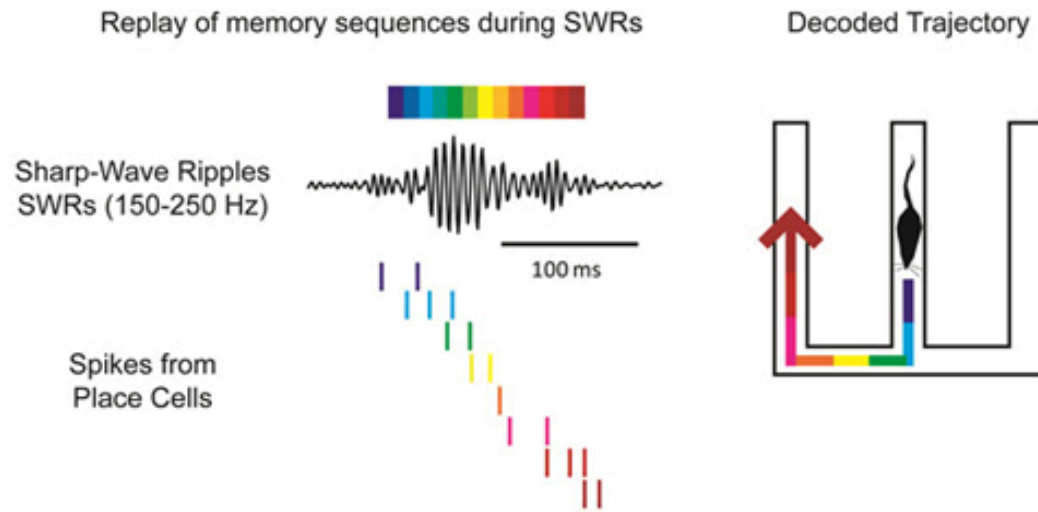
# Consolidation Hypothesis



# Replay occurs during sharp-wave ripple events during NREM

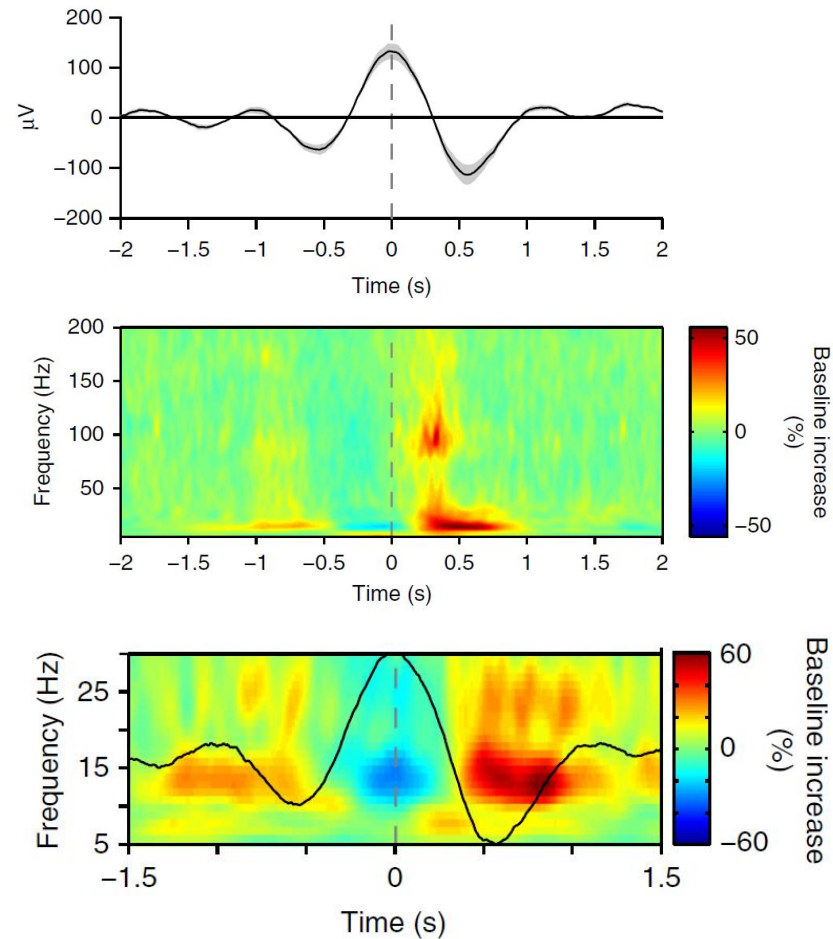
Replay events happen during sharp-wave ripples during non-REM sleep.

Sharp-wave ripples in HPC and spindle events in neocortex are correlated through slow oscillations during NREM.



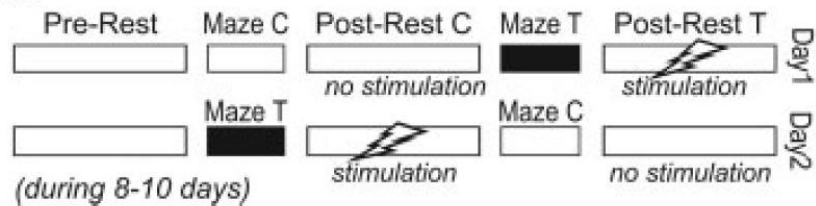
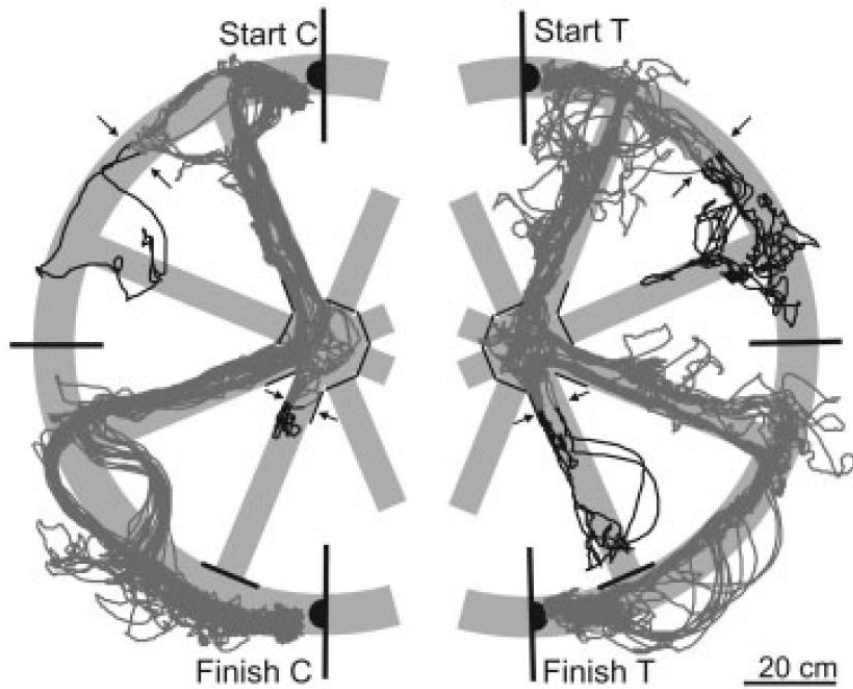
# Nesting of oscillations may facilitate communication between HPC and CTX during NREM

Align on the very slow oscillation ( $\sim 1$  Hz) in human recordings and you see organization of SWRs and spindles during sleep:

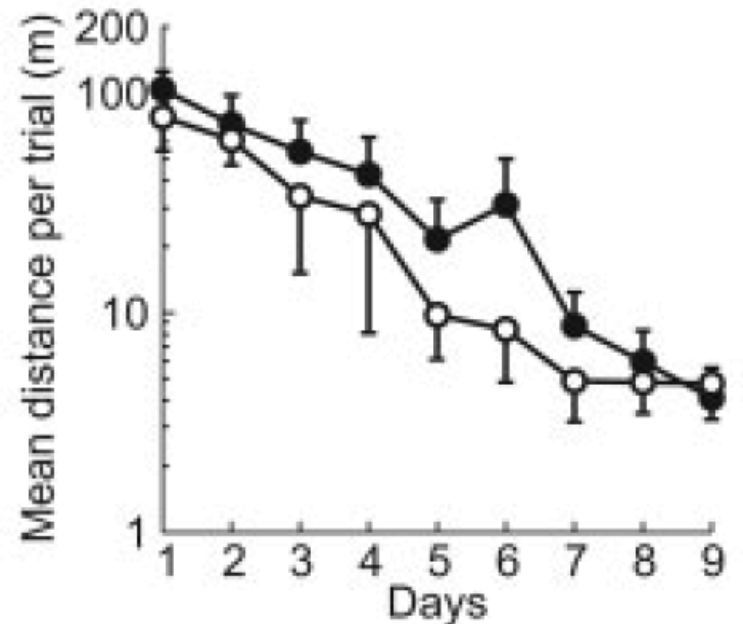
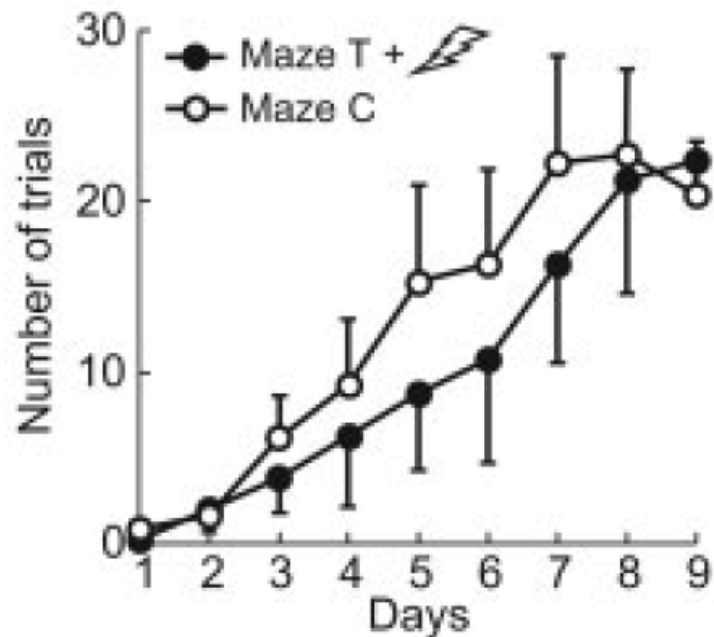


# Disrupting SWRs impairs consolidation

Rat learned two different mazes. On the test (T) maze, stimulation was delivered during ripple events during NREM sleep:

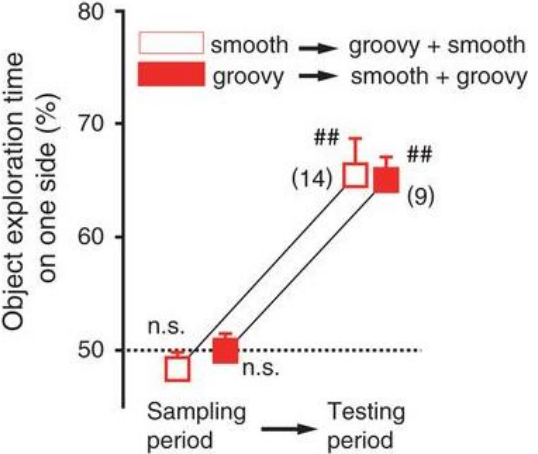
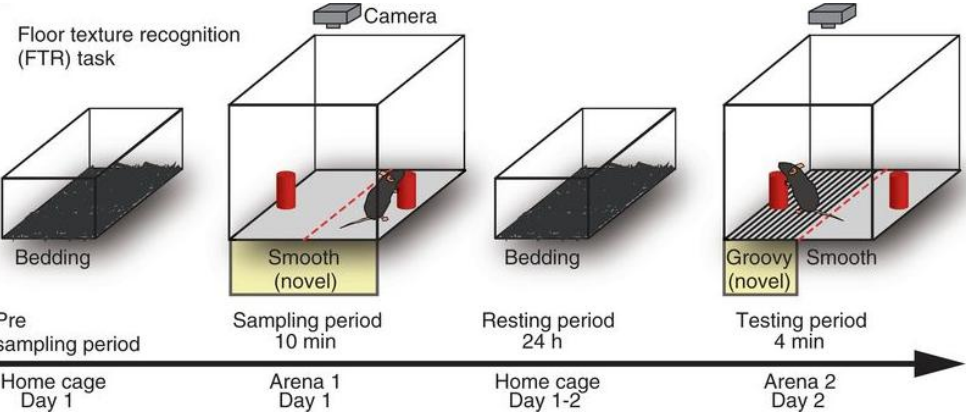


Stimulation during sleep reduced the ability of the rat's ability to learn the maze:

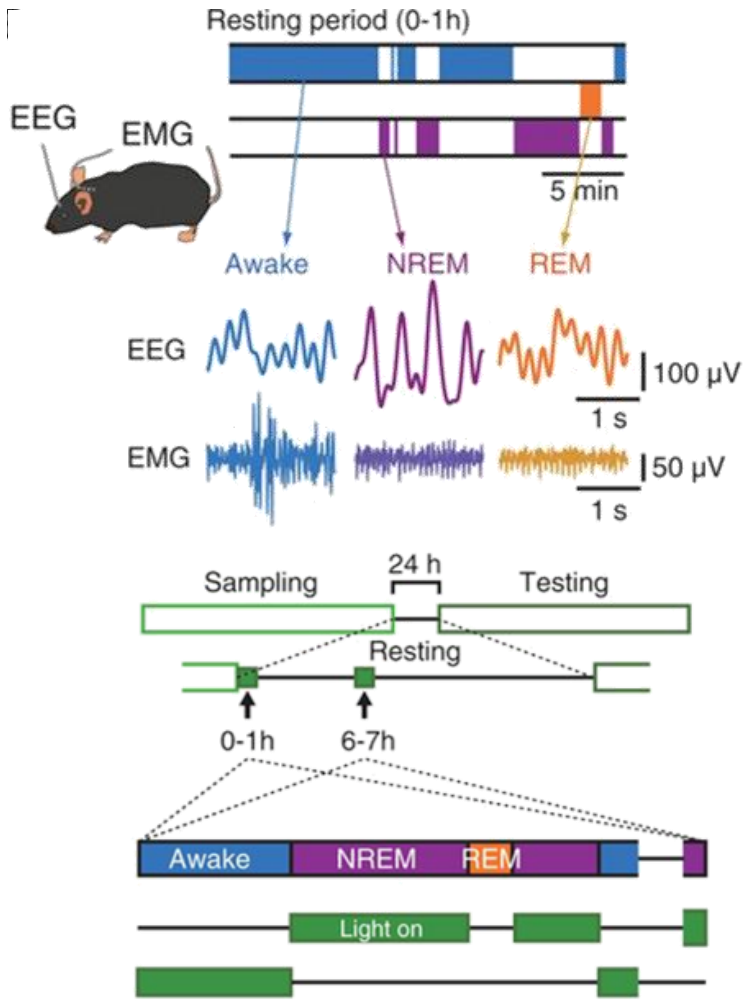
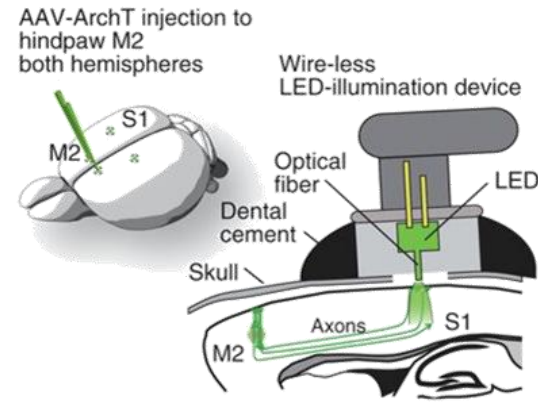


# Consolidation requires top-down cortical input

Using novel object exploration as a test of memory:



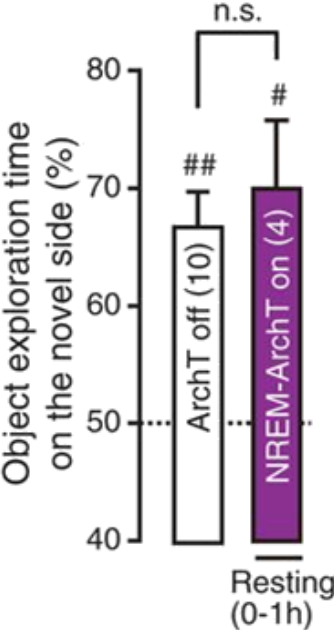
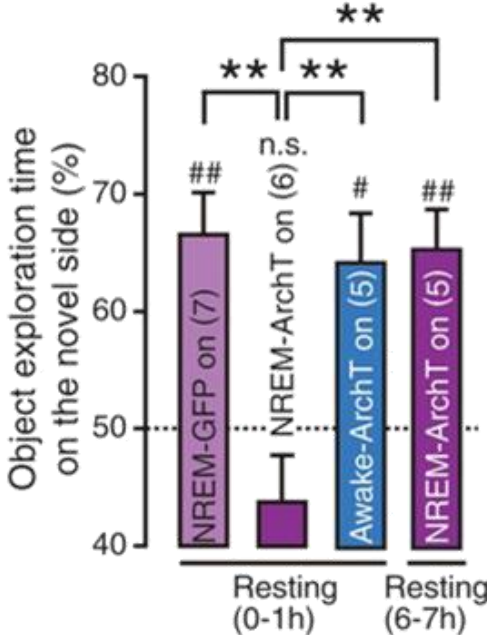
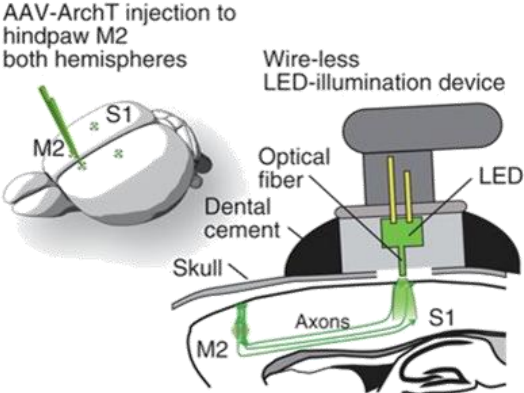
Targeted optogenetic inhibition of M2->S1 projections during NREM sleep.



# Consolidation requires top-down cortical input

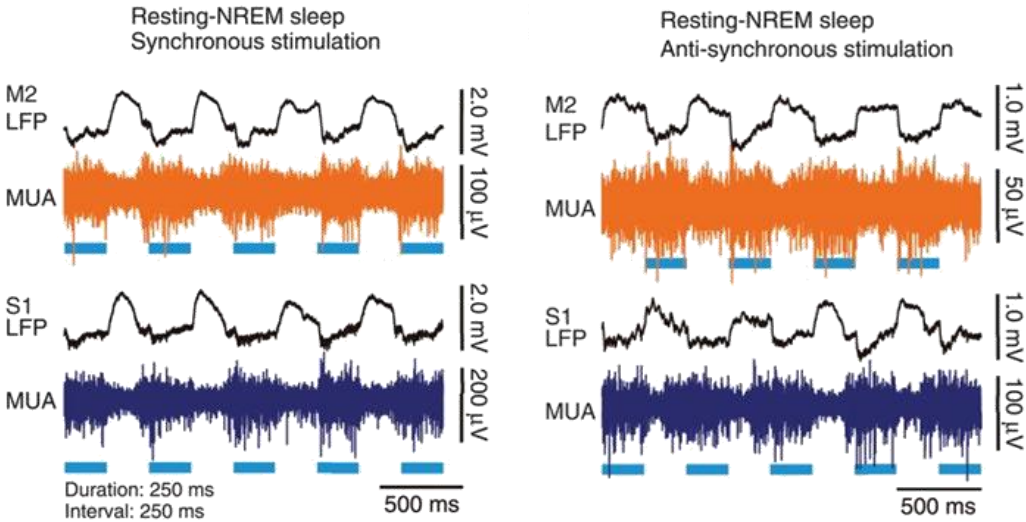
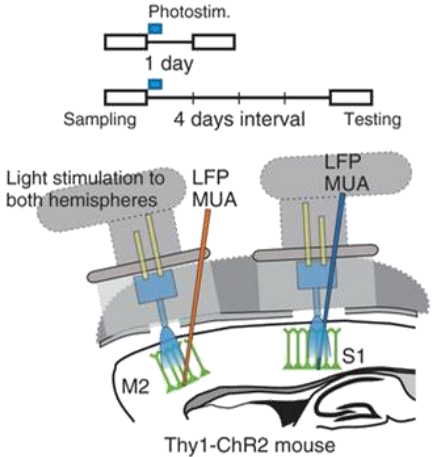
Inhibition of M2->S1 projections reduces the response to novel stimulus

Inhibition of S1->M2 projections does not change response to novel stimulus

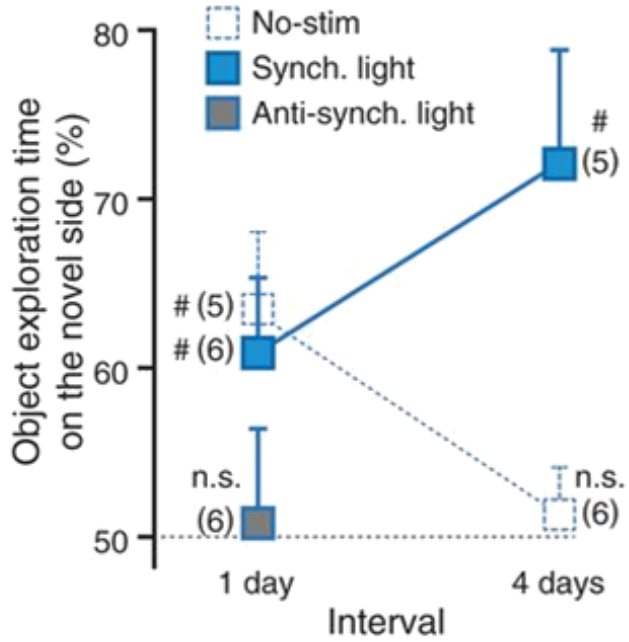


# Consolidation requires top-down cortical input

## Stimulation of S1 and M2 during NREM was either synchronous or asynchronous

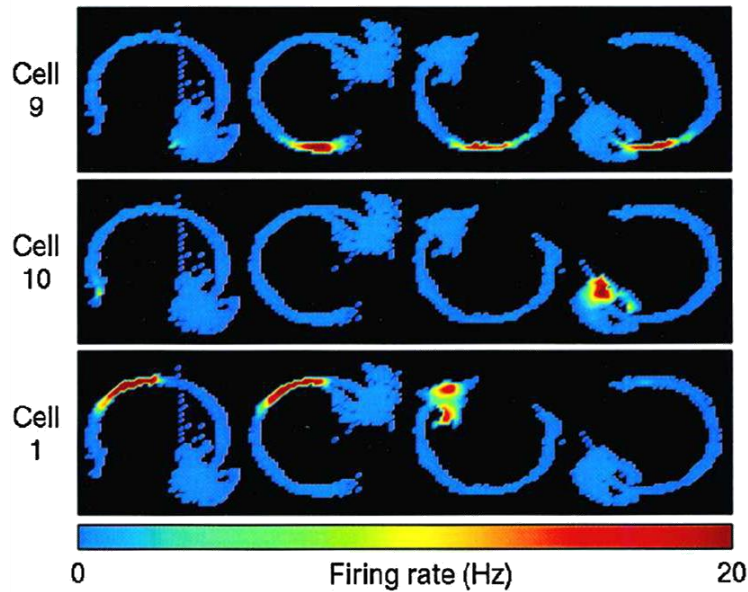
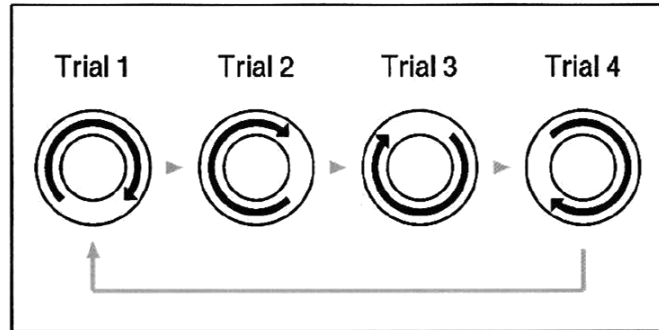


## Synchronous stimulation enhances memory relative to no stimulation and asynchronous stimulation

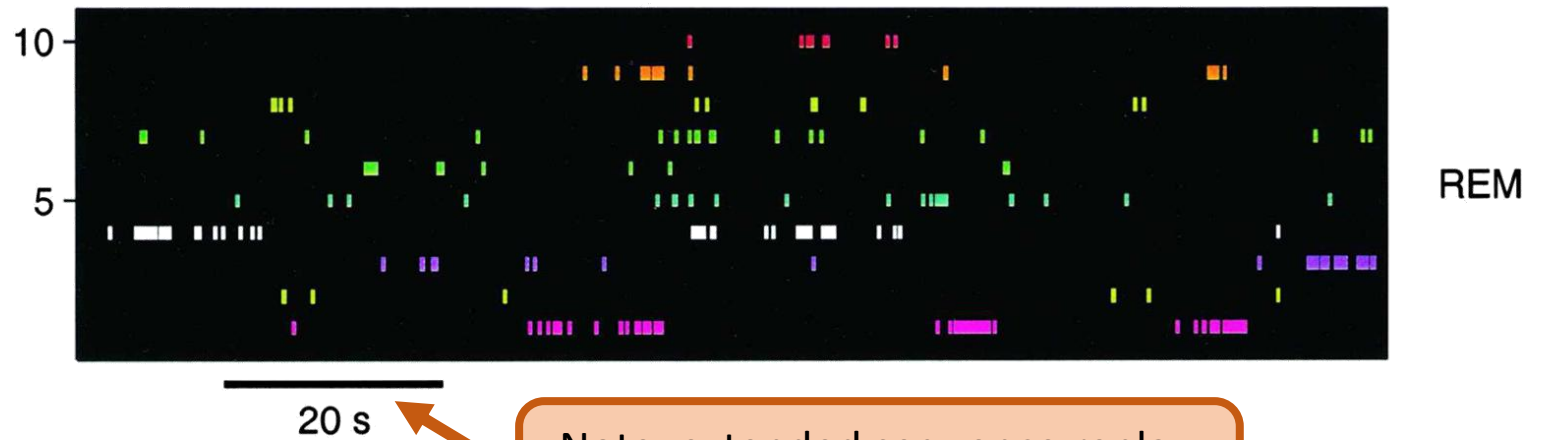
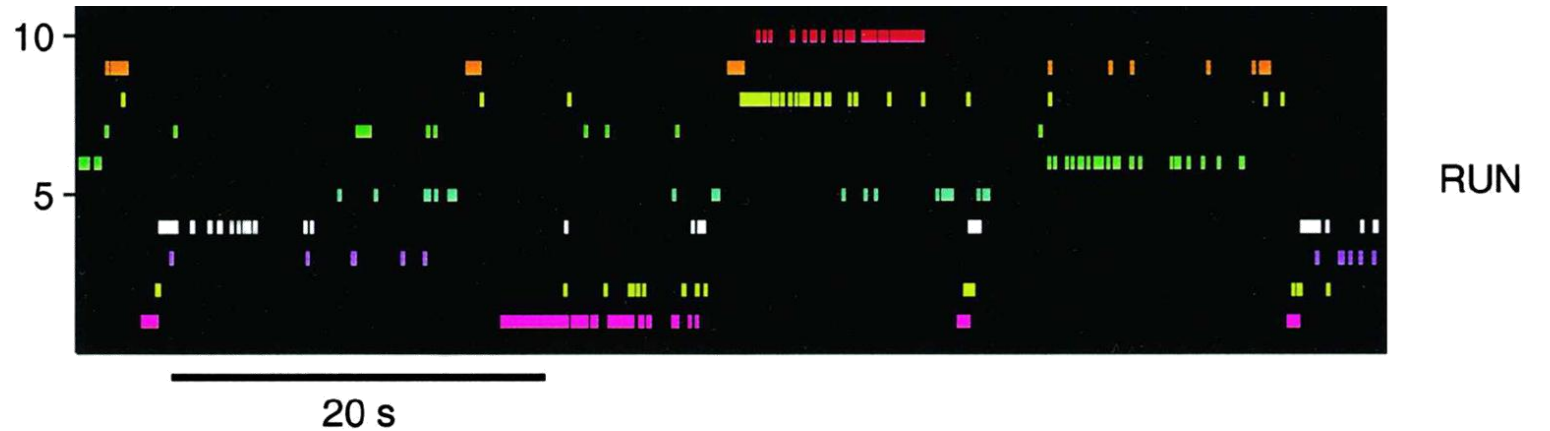


# Replay during REM is slower and more extended

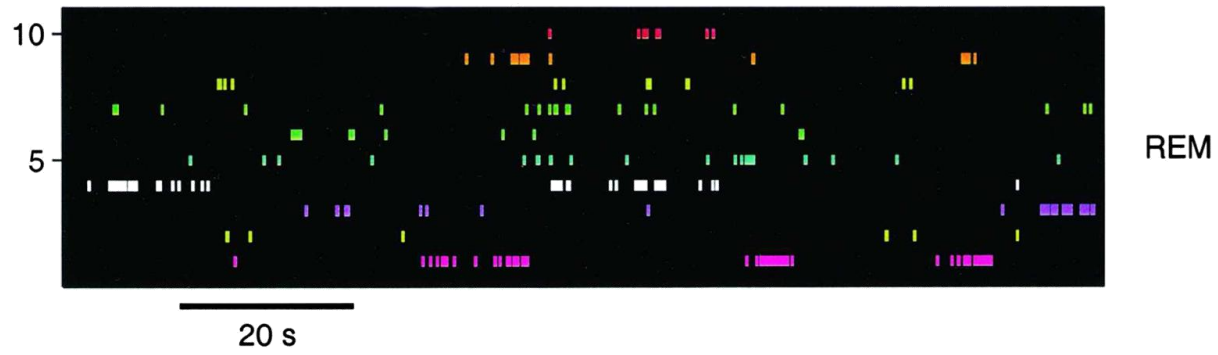
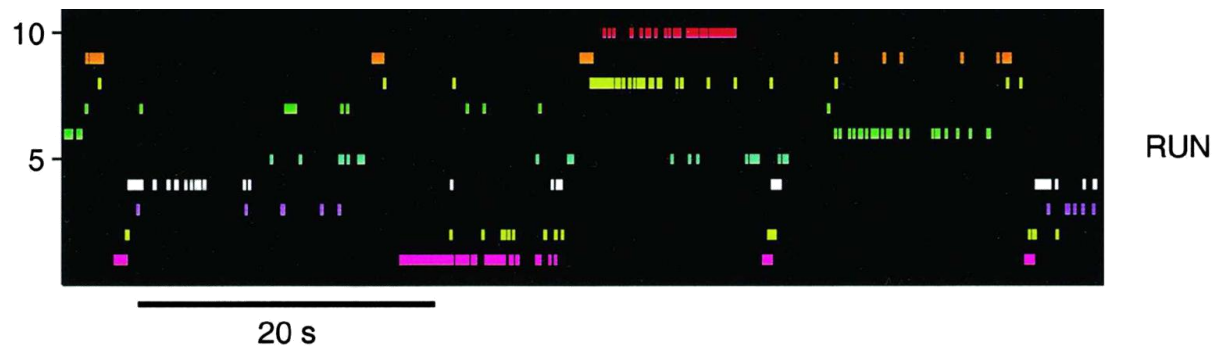
Rats repeatedly ran a circular track, alternating between directions:



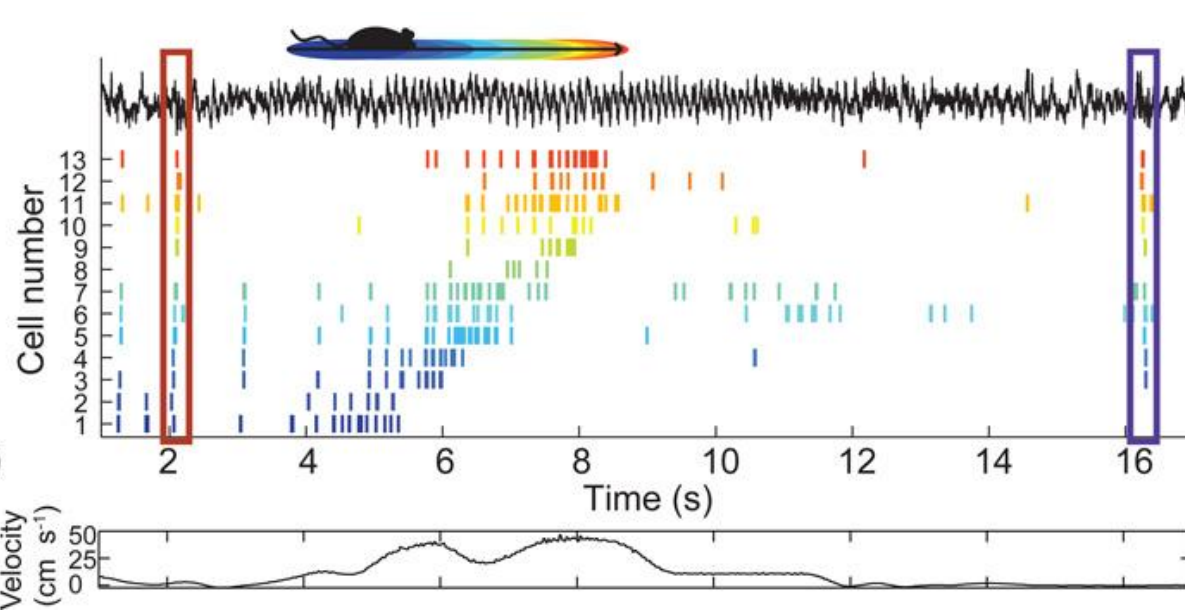
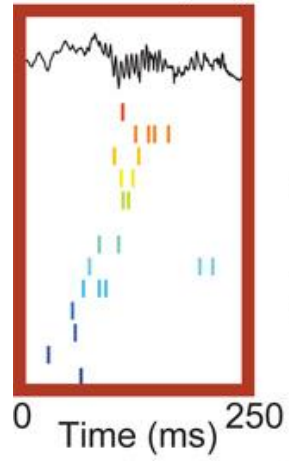
Neural sequences are similar across RUN and REM:



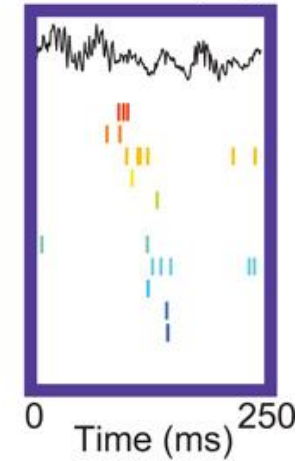
Note: extended sequence replay, but at about 1.6x compression



Forward replay

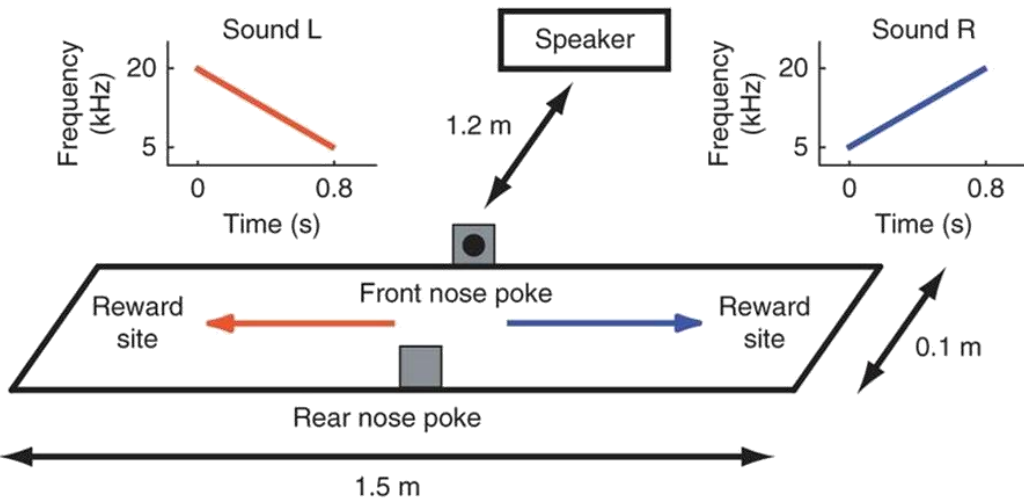


Reverse replay

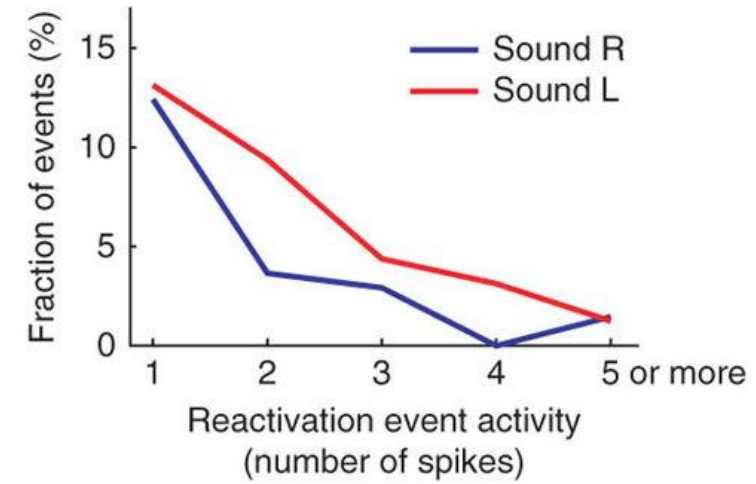
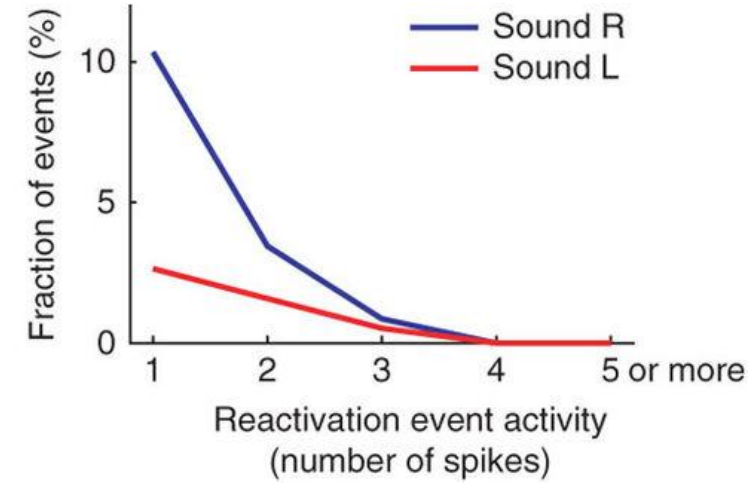
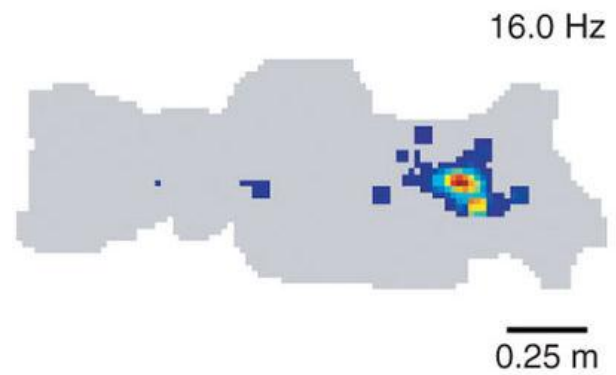


# Cue induced recall

Rats were trained to move to a specific port in response to a sound:

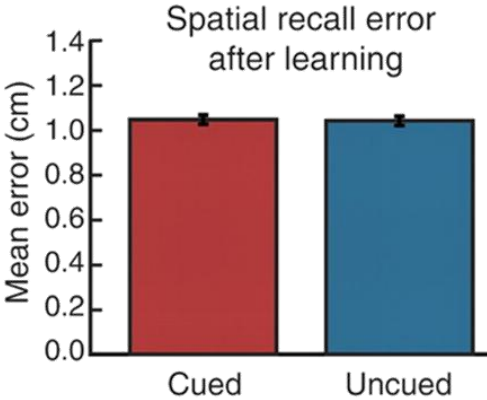
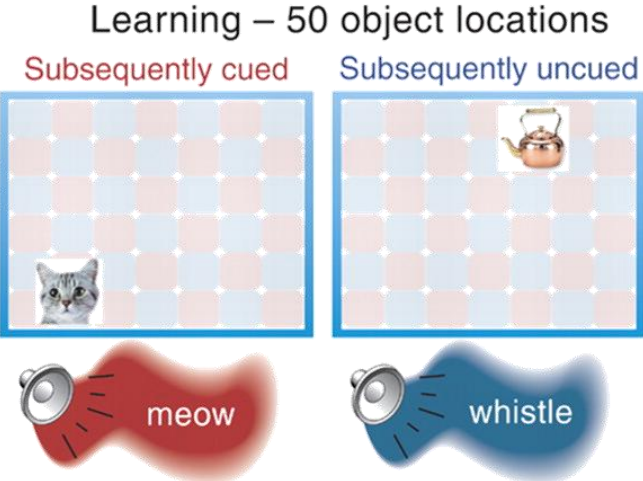


While sleeping, if a tone is played it induces a replay event towards the associated location:

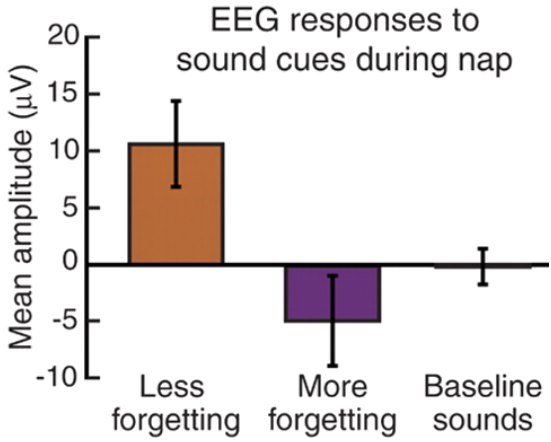
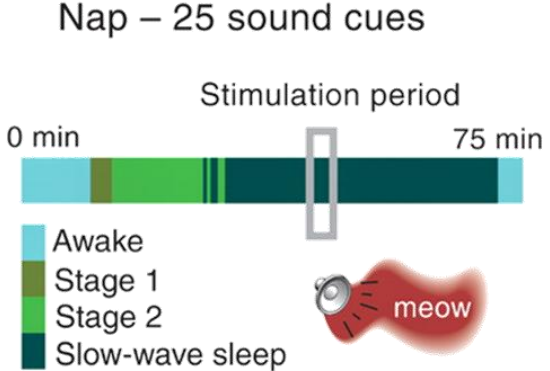


# Cue induced recall in humans

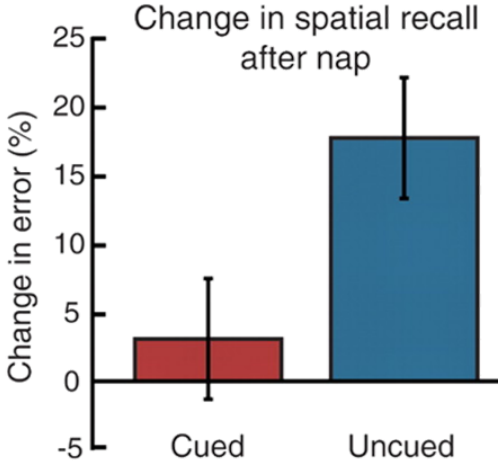
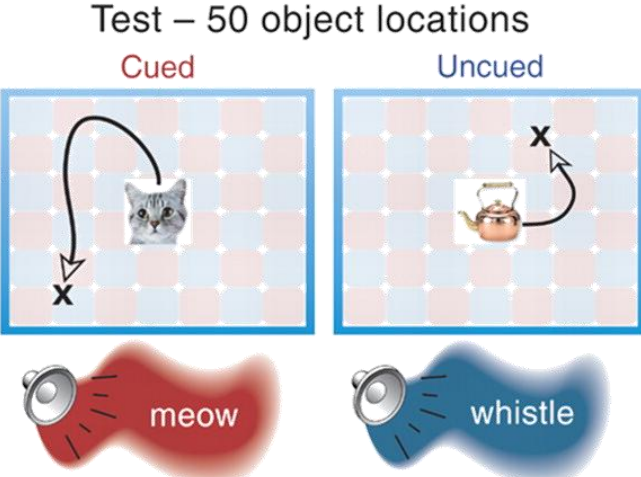
Subjects learned to associate specific stimuli with specific locations. During learning they were exposed to irrelevant associated sounds:



Subjects then took a nap and received auditory cues:

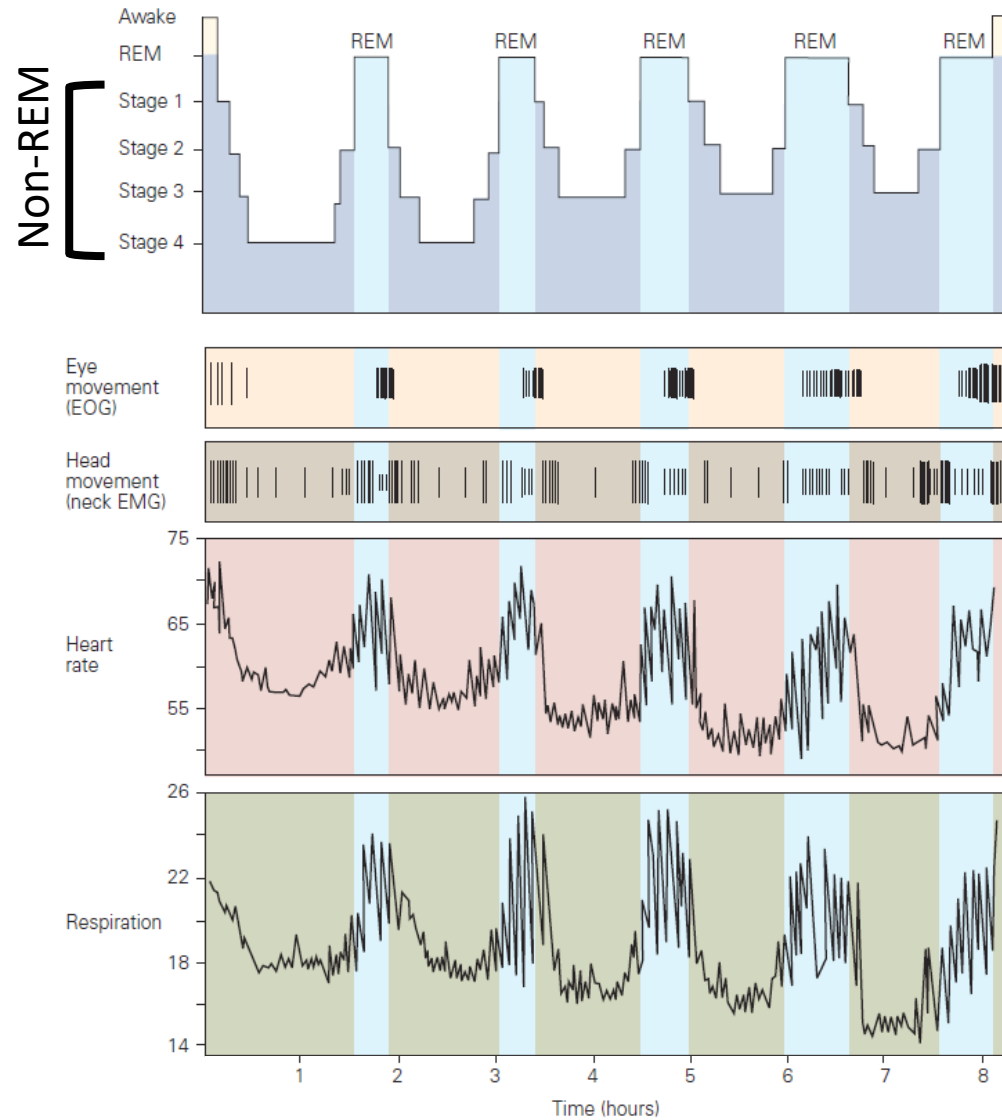
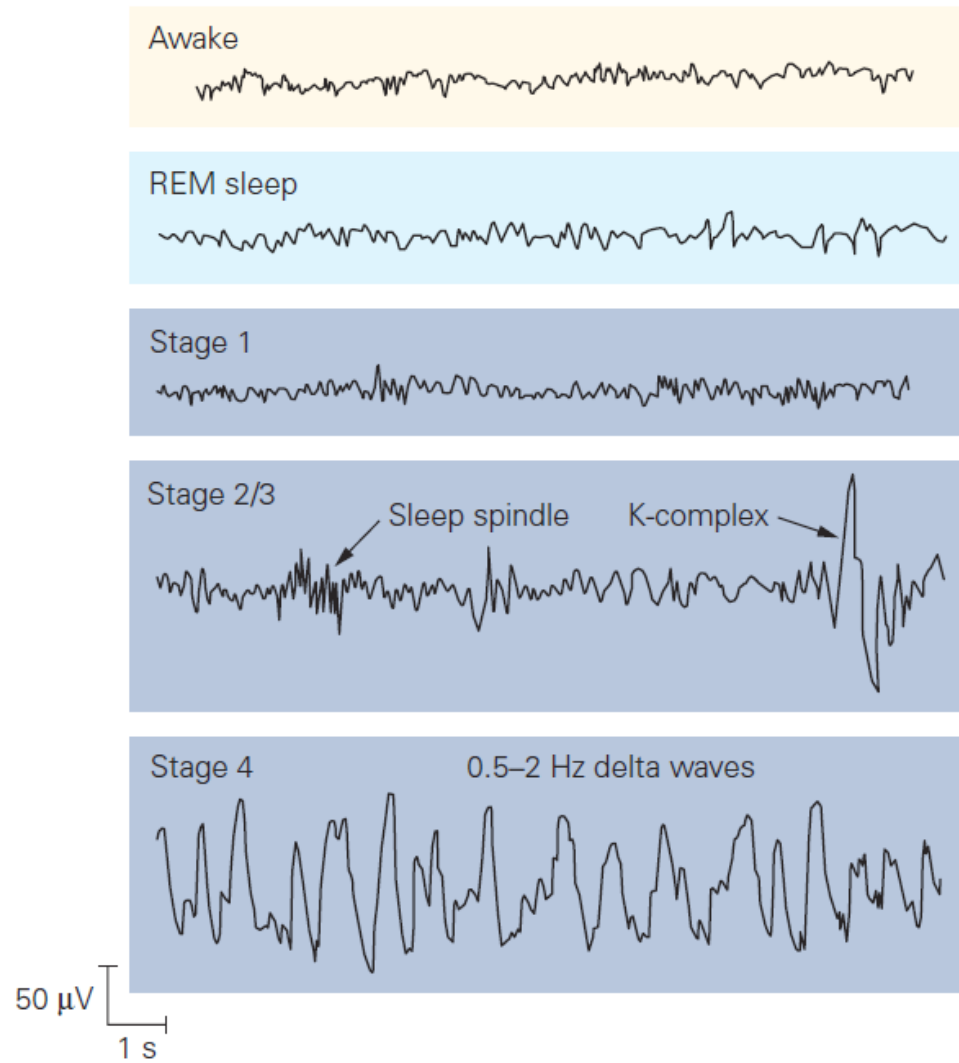


After sleep, all memories are more poorly localized, but the cued stimulus is significantly less impaired:



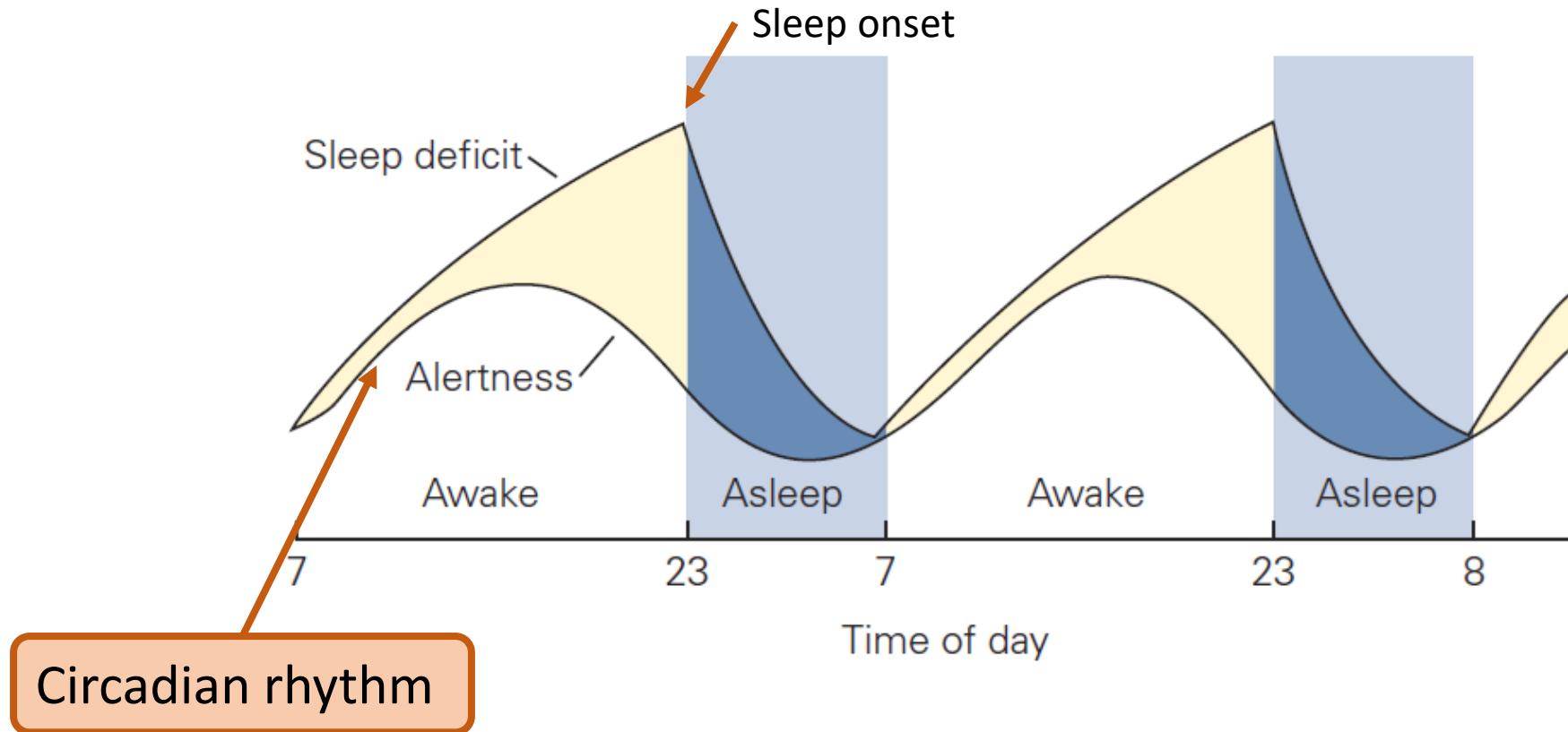
# Summary

# Summary of the Sleep Cycle

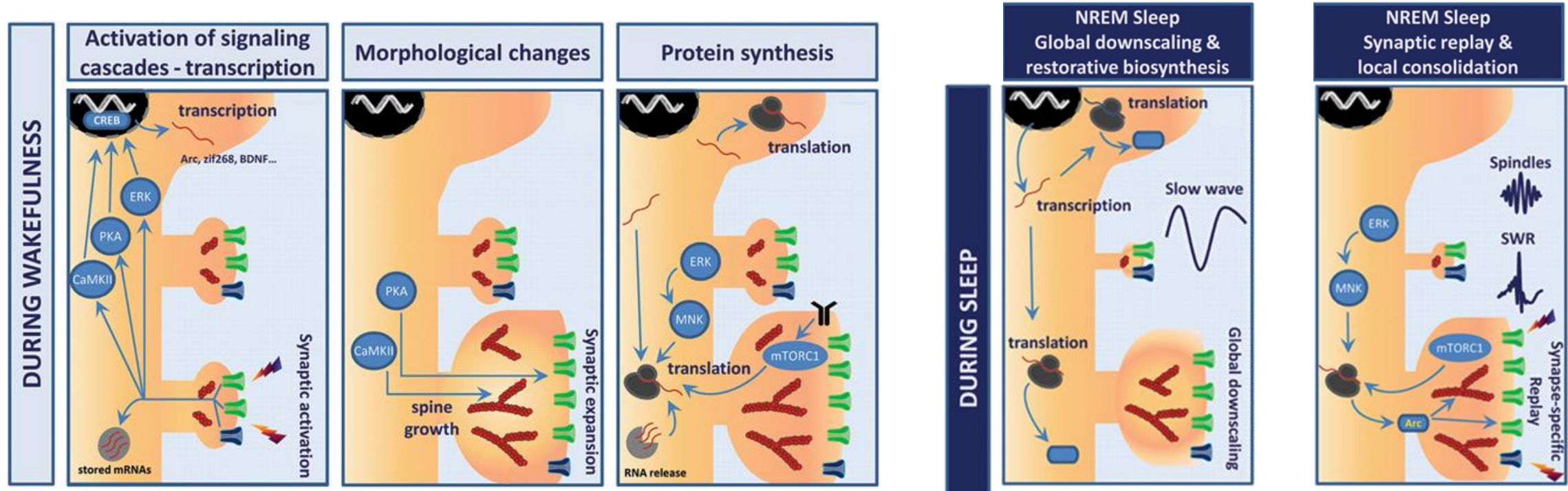


# Summary of Sleep Regulation

Sleepiness (or the 'drive for sleep') seems to depend on two factors: 1) time since last sleep and 2) time during the day. This has led to a two-process model being proposed for sleep initiation.



# Summary of Synaptic Homeostasis and Consolidation



Activation of synapses causes influx of  $Ca^{++}$  which starts internal cascade.

This leads to actin remodeling and shuttling of new channels; growing synapse.

These changes are consolidated.

SWS causes global downscaling of synapses.

Replay events would provide further consolidation of memories.