Sleep and Hibernation
### Same phylogenetic order, different sleep times

<table>
<thead>
<tr>
<th>Animal</th>
<th>Total Sleep</th>
<th>REM Sleep</th>
</tr>
</thead>
<tbody>
<tr>
<td>Golden Mantled Ground Squirrel</td>
<td>15.9 Hours</td>
<td>3.0 Hours</td>
</tr>
<tr>
<td>Degu</td>
<td>7.7 Hours</td>
<td>0.9 Hours</td>
</tr>
<tr>
<td>Cat</td>
<td>12.5 Hours</td>
<td>3.2 Hours</td>
</tr>
<tr>
<td>Genet</td>
<td>6.3 Hours</td>
<td>1.3 Hours</td>
</tr>
<tr>
<td>Owl monkey</td>
<td>17.0 Hours</td>
<td>1.9 Hours</td>
</tr>
<tr>
<td>Man</td>
<td>8.0 Hours</td>
<td>1.9 Hours</td>
</tr>
</tbody>
</table>

### Different phylogenetic order, similar sleep times

<table>
<thead>
<tr>
<th>Animal</th>
<th>Total Sleep</th>
<th>REM Sleep</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guinea Pig</td>
<td>9.4 Hours</td>
<td>0.8 Hours</td>
</tr>
<tr>
<td>Baboon</td>
<td>9.4 Hours</td>
<td>1.0 Hours</td>
</tr>
<tr>
<td>Goat</td>
<td>5.3 Hours</td>
<td>0.6 Hours</td>
</tr>
<tr>
<td>Eastern Tree Hyrax</td>
<td>5.3 Hours</td>
<td>0.5 Hours</td>
</tr>
<tr>
<td>Eastern American Mole</td>
<td>8.4 Hours</td>
<td>2.1 Hours</td>
</tr>
<tr>
<td>Man</td>
<td>8.0 Hours</td>
<td>1.9 Hours</td>
</tr>
</tbody>
</table>

*Current Biology*
A typical night’s sleep
• Why do we sleep?
  – Sleep is essential for survival
    • Fatal Familial Insomnia
    • Animal studies of sleep deprivation
  – What essential function does sleep serve?
    • Human studies:
      – Not physical or physiological well-being
      – Cognitive-sleep deprive, get hallucinations, impaired perceptions, and decreased concentration
      – Missed sleep is ‘made up’, especially Stage 4 and REM sleep
      – Blood flow and cerebral metabolism decrease in stage 4 sleep, so is sleep a way to get brain rest?
• Why do we sleep?
  – Animal Studies:
    • Sleep deprive without increasing exercise/stress factors: Yolked-control rotating platform expt.
    • Experimental animals were 87% sleep deprived, controls ~30%
      – Sleep deprivation led to sickness, cessation of grooming fur, weak, uncoordinated movement, inability to regulate body temp, increased eating, but weight loss due to increased metabolic rate, and death
      – Cause of death undetermined. NOT stress hormones, inflammation, changes to brain or internal organs, or decreased caloric intake (when fed high calorie meals)
• Why do we sleep?
  – More clues...
    • No correlation between sleep and physical exercise
    • Positive correlation between daytime brain activity and nighttime delta wave activity in human subjects
      – Somatosensory cortex--stimulate hand, see increase in delta activity in contralateral SS cortex

  – Function of REM sleep
    • Increased pressure to get ‘rebound sleep’ when deprived, most of which is REM sleep.
    • Suggests it is homeostatically regulated.
    • Highest proportion of REM sleep during development
      – Does brain development lead to REM sleep?
      – Does REM sleep promote brain development?*
• Why do we sleep?
  – REM sleep in adults--2 possibilities
    1. Learn new things, need to strengthen synapses, make new synapses, changes in AMPA and NMDA receptors occur during sleep
    2. Need to rid the brain of all the extraneous information you ‘learned’ during the day; sleep gets rid of irrelevantly strengthened synapses
      – Called depotentiation; allows you to learn new things the next day
      – If sleep deprived (maintain potentiated state) animals had a harder time achieving LTP
Asleep
Protein synthesis

Ribosome

RNA

For example, EF2, IF4A11 (F, M, H, S)

Lipid metabolism

Cholesterol

For example, cholesterol synthesis enzymes (F, M, S)

Awake
Energy metabolism

For example, mitochondrial genes and glucose transporters (F, M, H, S)

Stress response

For example, heat shock proteins and BiP (F, M, H, S)

Synaptic depression

Synaptic potentiation
Figure 4 | The major functional categories of genes with increased expression in the rat brain after several hours of wakefulness or after several hours of sleep.
Sleep is a homeostatic process

(a) Many hours of wakefulness → Sleep-promoting chemical accumulates → Sleep

Sleep-promoting chemical is destroyed during sleep

(b) Many hours of wakefulness → Wakefulness-promoting chemical is depicted → Wakefulness

Depletion of chemical leads to loss of wakefulness

Sleep is no longer inhibited

Wakefulness-promoting chemical is produced during sleep
Norepinephrine
(a) Inhibited
Sleep-promoting region in VLPA

Mutual inhibition

Activated
Brain stem and forebrain arousal systems

ACh NE 5-HT Histamine
Alert waking state

(b) Activated
Sleep-promoting region in VLPA

Mutual inhibition

Inhibited
Brain stem and forebrain arousal systems

ACh NE 5-HT Histamine
Slow-wave sleep

Flip-flop is "on"

Flip-flop is "off"
Stabilizing the awake state

- **Inhibited**
  - Sleep-promoting region in VLPA

- **Mutual inhibition**

- **Activated**
  - Brain stem and forebrain arousal systems
  - ACh, NE, 5-HT, Histamine
  - Alert waking state

**Motivation to remain awake**
- Hypocretinergic neurons in the lateral hypothalamus
  - Activation holds flip-flop "on"
Stabilizing the sleep state

Prolonged metabolic activity of neurons in the brain

Accumulation of adenosine

Hypocretin neurons in LH

Inhibit wakefulness-promoting activity

Activated

Sleep-promoting region in VLPA

Inhibit waking mechanisms, switch flip-flop off

Neurons in basal forebrain that inhibit VLPA

Inhibits

Inhibition is removed; VLPA becomes activated

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Table 2. Conserved sleep mechanisms.

<table>
<thead>
<tr>
<th></th>
<th>Worms</th>
<th>Flies</th>
<th>Fish</th>
<th>Mammals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clock Genes</td>
<td>+1</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cyclic AMP</td>
<td>+</td>
<td>+</td>
<td>?</td>
<td>+</td>
</tr>
<tr>
<td>Cyclic GMP</td>
<td>+</td>
<td>+</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>EGF</td>
<td>+</td>
<td>+</td>
<td>?</td>
<td>+</td>
</tr>
<tr>
<td>GABA</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Adenosine</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Dopamine</td>
<td>?</td>
<td>+</td>
<td>?</td>
<td>+</td>
</tr>
<tr>
<td>Histamine</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Melatonin</td>
<td>?</td>
<td>?</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Hypocretin/orexin</td>
<td>?</td>
<td>?</td>
<td>+2</td>
<td>+</td>
</tr>
<tr>
<td>Potassium channels</td>
<td>?</td>
<td>+</td>
<td>?</td>
<td>+</td>
</tr>
</tbody>
</table>

1 Clock gene expression is associated with developmentally timed sleep.
2 The precise direction of hypocretin sleep regulation is under debate.

Allada and Siegel, 2008
Hibernation is not a long bout of sleep
Hibernation is characterized by very low body temperature.
Increased levels of the antioxidant ascorbic acid (vitamin C)
Takes a bigger injury to elicit pathology when hibernating
Immunosuppression during hibernation

- WBC ($10^3$/mm$^3$)
  - Euthermic: $T_b=36.6^\circ$C
  - Hibernating: $T_b=4.5^\circ$C
Mechanisms of HP control in hibernating chipmunks

• HP (140kD complex) is reduced in the blood during hibernation

• But concentrations are increased in the brain prior to hibernation
  – Transport into/by choroid plexus?
  – Probably not due to expression by brain

• Blocking brain HP complex prevents or shortens hibernation bouts

Kondo et al 2006
Levels of HP complex are regulated circannually under control of a circannual rhythm independently of Tb changes.

An association of HP20c (a complex of HP20, HP25, and HP27) with HP55 in the blood has previously been shown (Kondo and Kondo, 1992a). The HP complex in blood was compared to that in CSF using size exclusion chromatography. Although plasma HP20c coeluted with HP55 over several fractions, fractions of CSF containing HP20c did not overlap extensively with fractions containing HP55. Such a dissociation of the HP complex in CSF was supported by the results of immunoprecipitation using anti-HP27 antibody (Figure 5B). HP55 coimmunoprecipitated with HP20c from plasma, but was not present in immunoprecipitates of CSF. There were no changes in the molecular weights of HP20c components or HP55 between plasma and CSF (Figures 4B, inset, and 5). These findings suggest that some amount of the HP complex in the blood is transported into CSF in conjunction with dissociation of HP55 from the complex.

Anti-HP20c Antibody Decreases Hibernation Time

The functional importance of HP20c in hibernation was examined in the brain. The circannual timing and duration of hibernation measured in the preceding hibernation season were found to be relatively constant in individuals (Figure 2A). In order to block HP20c activity in CSF, polyclonal anti-HP20c IgG, which immunoprecipitates HP20c in the blood and CSF and interferes with the interaction between HP20c and HP55 in the blood (Figure 6B, inset), or control IgG, was administered into the lateral ventricles of hibernating animals for 2 weeks (Figure 6A). For quantitative analysis of hibernation, the amount of time spent in low Tb (hibernation time) during the 2 weeks of antibody administration was compared to that during the 2 weeks prior to antibody treatment. The administration of preimmune IgG during the early, middle, and late stages of hibernation had only a slight effect on hibernation time, while that of anti-HP20c IgG markedly decreased hibernation time. When the anti-HP20c antibody was administered in the early and middle stages, the decreased hibernation time recovered to normal levels after finishing administration in all animals tested (n = 5 in each), while antibody administration in the late stage accelerated the termination of hibernation in 2 of 5 animals (Figures 6Aa–6Ac). The effect of the antibody, a decrease in hibernation time, was dramatic in early and late stages and somewhat less at the middle stage (Figure 6B). This may be because the largest amount of HP20c in CSF was seen in this middle stage (Figures 4A and 4B). The dose-inhibition relationship of anti-HP20c IgG on hibernation was examined in the latter half of the hibernation period (stages 3 to 4 in Figure 4B) where HP20c levels in CSF were relatively constant. Intraventricular administration of the IgG decreased the hibernation time in a dose-dependent manner (Figure 6C). The highest dose of IgG tested (0.045 mg/100 g body weight/day), where the hibernation time was shortened below 10% of control, was used in the experiments shown in Figures 6A and 6B. The antibody was reactive specifically to components of...
Epigenetic control of hibernation
Synaptic Remodeling

• Fewer synapses
  – Less maintenance energy
  – Less transmission energy
  – Reduced oxidants
  – Higher threshold to respond
Torpor-related loss of colabeled presynaptic and postsynaptic puncta

We investigated whether the loss of synaptic protein clusters in torpor affects synapse number, as defined by the colocalization of presynaptic and postsynaptic protein puncta. We analyzed two groups of animals: the first group was killed 6 days into a torpor bout at 5°C and the second group was killed 12 h after initiation of arousal to euthermic temperatures. Double labeling of slices from the four brain regions described above were performed with the presynaptic protein Piccolo and the postsynaptic protein PSD95.

Torpid animals exhibited a 50–65% decline in colabeled puncta relative to euthermic animals (p < 0.001 for all regions), as shown in Figure 4.

We tested whether this large decline of colabeled puncta could be accounted for merely by the torpor-related decrease in number of Piccolo and PSD95 puncta or whether colabeled puncta were targeted. The same analysis was repeated with the PSD95 image rotated by 90°, while the Piccolo image remained in its original orientation. This analysis ensured that presynaptic and postsynaptic puncta were not functionally related, enabling analysis of the torpor-related percentage decrease in randomly overlapping colabeled puncta. There was a 26% loss of randomly colabeled puncta, demonstrating that torpor results in a significant loss of synapses.

Total levels of synaptic protein immunofluorescence do not change with torpor

We analyzed whether the loss of area covered by synaptic protein clusters is attributable to an overall loss of immunofluorescence in slices from torpid animals. The measurement of area covered by immunofluorescent clusters was made after subtracting background levels of immunofluorescence, which left discrete hotspots of fluorescence that are thought to correspond to synapses. When we analyzed the intensity of immunofluorescence without subtracting background levels of immunofluorescence, we found that there were no differences between euthermic and hibernating animals (Table 1), indicating that overall levels of fluorescence, and thus the overall amount of these synaptic proteins, do not change between these groups. Together with the loss of area covered by discrete protein clusters during torpor, these data indicate that Piccolo, PSD95, and synaptophysin shift from a punctate, or synaptic, organization during euthermia to a more diffuse nonsynaptic pool of protein during torpor. The distribution of MAP2, a nonsynaptic protein, also changes markedly during torpor, as can be seen in Figure 1. MAP2 shifts from an organized and filamentous pattern in the euthermic animal (Fig. 1B) to a punctate and less organized pattern in the torpid animal (Fig. 1C). The change in distribution of these proteins likely results in the loss of high-intensity immunofluorescence during hibernation.
Torpor affects synapse number, as defined by the colocalization of presynaptic and postsynaptic protein puncta. We investigated whether the loss of synaptic protein clusters in hibernation.

We analyzed whether the loss of area covered by synaptic protein clusters is attributable to an overall loss of immunofluorescence, and thus the overall amount of these synaptic proteins, do not change between these groups. Together with the loss of area covered by synaptic protein clusters with torpor body temperature is shown for MAP2 and synaptophysin.

When we analyzed the intensity of immunofluorescence without background levels of immunofluorescence, which left discrete hotspots of fluorescence that are thought to correspond to synapses. There was a 26% loss of randomly overlapping colabeled puncta. There was a 50–65% decline in colabeled puncta in slices from torpid animals.

The proteins investigated include MAP2, Piccolo, PSD95, and synaptophysin. We tested whether this large decline of colabeled puncta could indicate that Piccolo, PSD95, and synaptophysin shift from a punctate, or synaptic, organization during euthermia to a more diffuse nonsynaptic pool of protein during torpor. The distribution of these proteins likely results in a change in distribution of MAP2, a nonsynaptic protein, also changes markedly during torpor, as can be seen in Figure 1. MAP2 shifts from an organized and filamentous pattern in the euthermic animal to a punctate and less organized pattern in the torpid animal (Fig. 1). The slopes of graphs from animals hibernating at 5 and 15°C, with arrows indicating the experimental groups, are shown in Figure 3.