

Physiology and pathophysiology of sleep

(Beta version of working text for students: Short overview of the sleep problematic. Text contains figures from many other authors).

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Sleep definition and functions

How can we define sleep?

Many authors have defined sleep from different perspectives (philosophical, biological, electrophysiological etc...). Also, different animals present very variable forms of sleep. Humans sleep exhibits complicated choreography of the activity in comparison to a simple regular inactivity in a worm with simple neural system. In general, sleep might be defined as a: "**Natural state of inactivity and temporarily reduced response to the external environment, accompanied by a reversible loss of consciousness.**"

So, for which functions is sleep responsible?

Despite the general awareness of the necessity of sleep for life and a rich knowledge about particular sleep functions, we still lack a unifying theory about sleep function. Sleep is absolutely essential for the life of the most living organisms. Usually, sleep takes place in a regular intervals and is homeostatically regulated, what means that amount and intensity of sleep is controlled, and lack of sleep for some time leads to the compensatory increase in a consecutive sleep periods. However, persistent longer sleep deprivation can lead to the **weight loss**, disruption of **thermoregulation** and **immune system** and consequently to the death. So, sleep has a general restorative effect for the whole body and **preserving energy**. And really, several studies showed activation of genes relating to the energy saving, e.g. expression of genes responsible for the glycogen production during sleep. Sleep is very important for **regeneration of tissues**, **development of the nervous system**, **clearance of the toxic substances** from the brain. One of the most important physiological functions of the sleep is its necessity for many **cognitive processes**, mainly for **memory consolidation**. Some of these functions will be discussed in detail below in the text.

How does a sleep structure look in humans?

Sleep structure in humans

Nathaniel Kleithman and Eugen Aserinski (1953) used EEG recordings (EEG was discovered few years ago in that time) for a monitoring sleep in volunteers. Nathaniel Kleithman made every night recording of signal on a paper tape long several hundred meters and then he manually analyzed it and it is also known that he widely used his ten years old son as an experimental object. Finally both experimenters discovered fundamental EEG patterns of electrical activity during sleep. Interestingly, during very deep sleep epochs EEG waves started to be slower with bigger amplitudes and consecutively those epochs were interrupted with EEG activity challenges reminiscent awake activity. In that period subjects have very often dreams, didn't moved most of the parts of their body, except eye bowls. For the paradox, that activity of the brain looked like awake, but whole body, except eye bowls, was that phase called as paradoxical **or Rapid Eye Movement (REM)**. Sleep phase with gradually deeping level of sleep (e.g. it is harder to awake person from that sleep...) were then called as a **Non-Rapid Eye Movement (NREM)**.



Nathaniel Kleithman and Eugen Aserinski (1953)

Let's describe REM and NREM slightly more in detail.

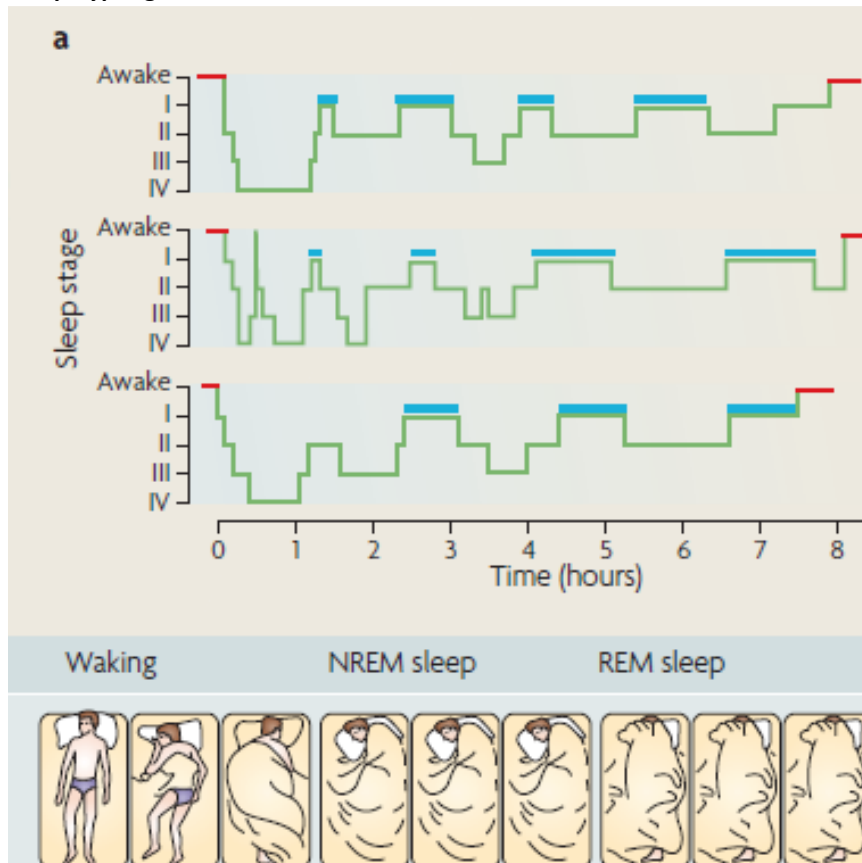
REM sleep characteristics:

Evolutionary occurrence of REM / NonREM sleep is known in mammals and birds. Evolutionary younger REM sleep, sometimes called also as a **rhombencephalic**¹ is characterized by a desynchronized EEG activity, accompanied by the occurrence of a low voltage activity, saw tooth waves, and mix of a theta, beta activity (see figures below). It was mentioned that REM sleep is accompanied by a rapid eye movements, drop of antigravity tone muscles, head muscles and chins. Paradoxically, during a REM is present a rapid and irregular eye ball movement. Both

¹ Note that older brain structure is responsible for the evolutionary younger type of sleep. As you see later, for the evolutionary older NREM sleep are responsible younger brain areas.

phases REM X NREM alternate periodically, in the first half of sleep is greater occurrence of NREM, in the second half dominates REM (see figure below). REM sleep is characterized by the presence of dreams usually with strong emotional content. REM episodes are accompanied by other physiological changes. Autonomous system during REM sleep is very unstable.

Sleep hypnogram in humans



Alternation of NREM – REM sleep. Green line means different depth of the NREM sleep, red line is awake state, blue line is REM sleep. Note proportion of REM-NREM per night, note behavior of the subject during different epochs.

NREM sleep characteristics:

NREM is characterized by a synchronized slow wave EEG activity with choreography of the activity typical for each NREM sleep stages (See schema below).

Stage 1: typically phase theta (4 – 7 Hz).

Stage 2: occurrence of sleep spindles and K - complexes.

Stage 3: predominate sleep spindles, occurrence of slow waves (0,5 – 4 Hz).

Stage 4: dominates high voltage delta activity.

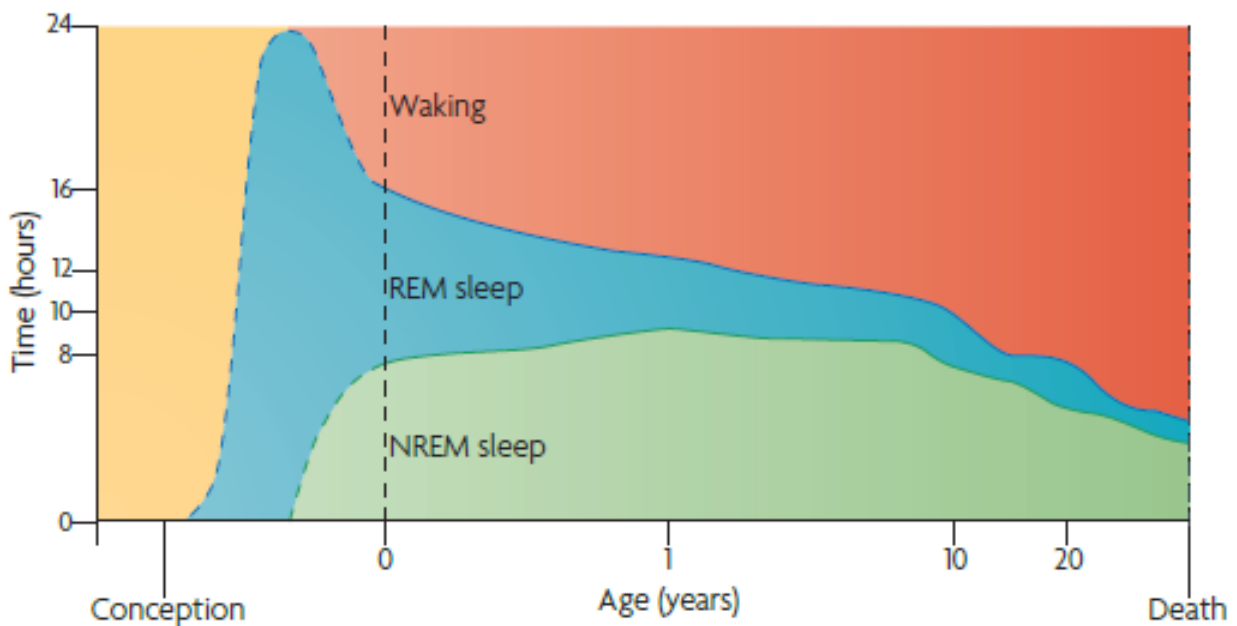
For NREM sleep is characteristics predominance of the parasympathetic system. NREM sleep is also important for memory consolidation.

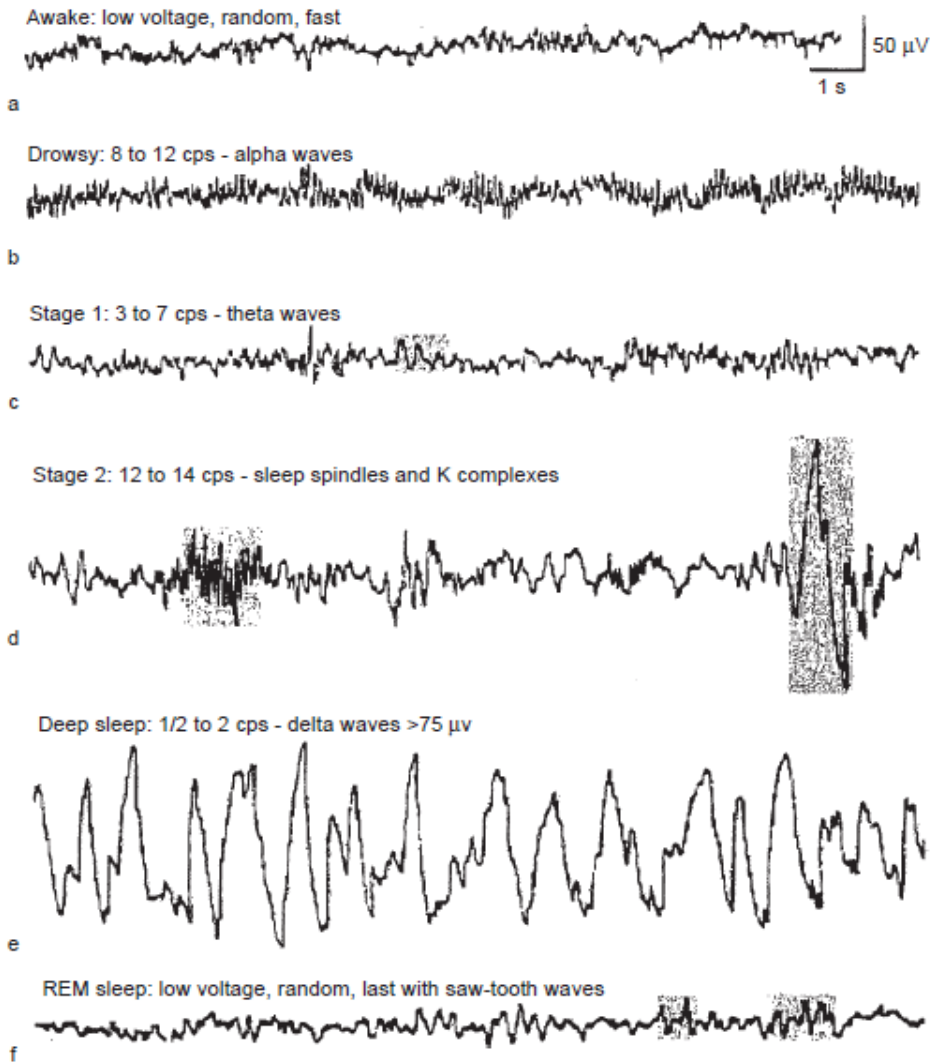
Length of sleep, proportion of REM and NREM sleep stages changes across human life. During sleep aging, the ratio of Non-REM / REM increases. In the figure below high proportion of the REM stage on the very beginning of life is obvious (e.g. 18 hours of sleep in newborns and 50 % of REM sleep). In old people is overall sleep shorter as well as proportion of REM episode.

Control question:

Q.1.: When in evolution, do you think, animals and humans can have REM sleep?

Q.2.: After birth, REM sleep is represented in 50% of sleep. Why do you think such a big proportion of REM in that period is so important?





Sleep EEG patterns across different phases.

Control question:

Q.3.: Why do you think are waves in deeper NREM sleep bigger in comparison to REM sleep or an Awake state?

Which brain structures are responsible for a wake/sleep and NREM/REM periods?

Case of epidemy „Encephalitis Lethargica“

In the last century Austrian doctor Constantin Von Economo evaluated patients with acute inflammation occurred in epidemic form from 1915 – 1924. Some patients suffered by strong

somnolence sleep. Those of patients was extensively slept (more than 20 hours per day) and very often fallen to coma. Those patients had an affliction of a middle brain and posterior hypothalamus. However small part of patients suffered from insomnia with the affection of the diencephalon and midbrain boundaries, the front brain and the anterior hypothalamus. Discovery of brain areas responsible for sleep/awake cycles initiated research of sleep responsible brain areas.

The following studies in monkeys (1939) and rats (1946) confirmed the importance of **hypothalamus** and **forebrain** for waking and sleeping. Another important discovery was the discovery of the Reticular Activated System (RAS) in the brain stem (Morruzi and Magoun, 1940). These authors have shown that RAS stimulation in the brain stem causes states of similar activity (desynchronized EEG, small amplitudes etc.) in anesthetized cats. Moreover, also people with damage of these structures have very often coma.

Until the eighties of the last century, the role of RAS was considered to be a functionally homogeneous cluster of neurons with no different functions. However, recently, the role of individual cores is gradually being elucidated.

(Short) Overview of most important mediators of wake-up circuits

Cholinergic innervation:

pedunculo-pontine nuclei, laterodorsal tegmental nuclei.

These nuclei are responsible for the activity during waking and also for the initial triggering of the REM sleep („**REM – ON**“). Those nuclei are situated in basal forebrain.

Monoaminergic innervation (catecholamines, serotonin, histamine):

Those nuclei are situated in the ventrolateral medulla and locus coeruleus (Noradrenalin), and they seem to be a key structure for a keeping an awake state.

Dopaminergic innervation: periaqueductal grey matter

Serotonergic innervation: ncl. Raphe – keeping an awake state and „turn off“ REM sleep („**REM – OFF**“).

Histaminergic: tuberomammilar nuclei.

Hypocretin (orexin) innervations

These neurons are situated in the lateral hypothalamus and are important for **active awareness** because they stimulate monoaminergic neurons. In NREM sleep their activity is consequently attenuated, in the end of REM is their activity again going up.

Why do we sleep and wake up again?

Regulatory circuits of sleep/wakefulness

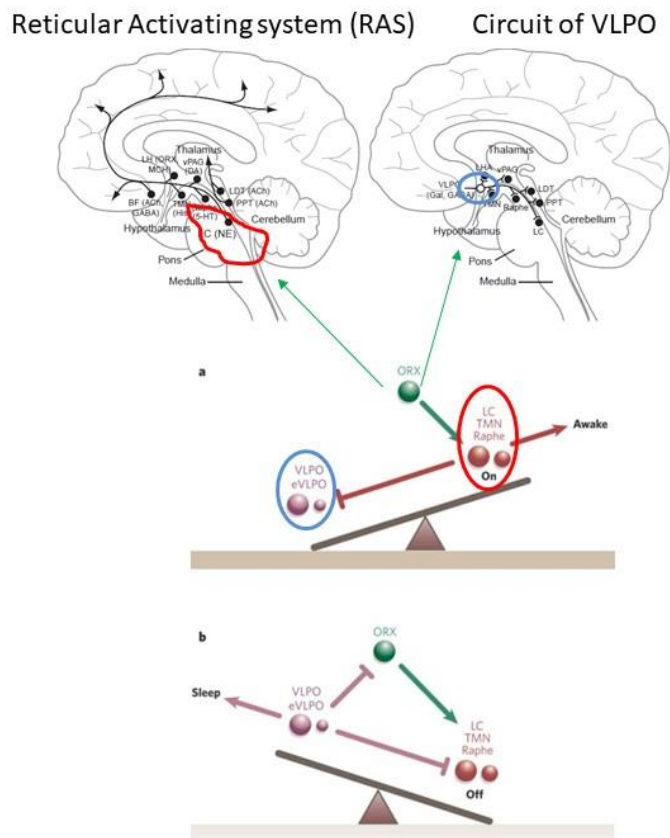
In 1990s were discovered an important role of the **Ventrolateral preoptic nuclei (VLPO)**. These nuclei inhibit circuits for activation awareness states (e.g. hypocretin neurons, see above) and

are active during sleep. For the inhibition of other neurons these nuclei are using inhibitory mediators (galanin, **GABA**). Their lesions cause **insomnia** or fragmented sleep.

Flip-flop model of sleep

Alternation of sleep and wakefulness (flip-flop sleep model, see below) is ensured by a change in the balance between neurons **RAS** a **VLPO**. This model ensures a **smooth** transition between sleep and wakefulness, instead of unexpected episodes of sleep / wakefulness (which are obvious in some pathological states, see below).

Delaying VLPO, for example in an elderly, leads to the **fragmentation** of sleep. Same effect is possible to see after the experimental destruction (studies in rodents shown that animals can initiate sleep, but they had a problem to maintain ongoing sleep). Also, patients with **narcolepsy** have disturbed this regulatory circuit because of the (**hypocretin / orexin system**).



Some chemical substances, called **somnogens**, are capable to disinhibit neurons in **VLPO**, and these neurons are inhibiting **hypocretin / orexin** neurons. This inhibition is then after some time suppressed by the feedback loop through inactivation of monoamines and inhibition of **hypocretin / orexin** is disrupted.

Which somnogens do we know?

Adenosine is one of the most known somnogen with specific receptors in several brain areas (e.g. forebrain, cortex, hippocampus). In awake state its level is increasing and consecutively during sleep is again dropping down. Astrocytes are biggest sources of the extracellular adenosine and concentrations there is regulated by means of kinases. Administrations of the adenosine agonists promotes NREM sleep. Inhibition of those receptors by antagonist **caffeine** supports an awake state.

! But keep in mind, if you blockade your adenosine receptors with caffeine, your adenosine is still cumulated and once caffeine is cleaved from the receptor, tiredness is stronger because the effect of higher level of adenosine!

Tumor necrotic factor alpha (TNF – α)

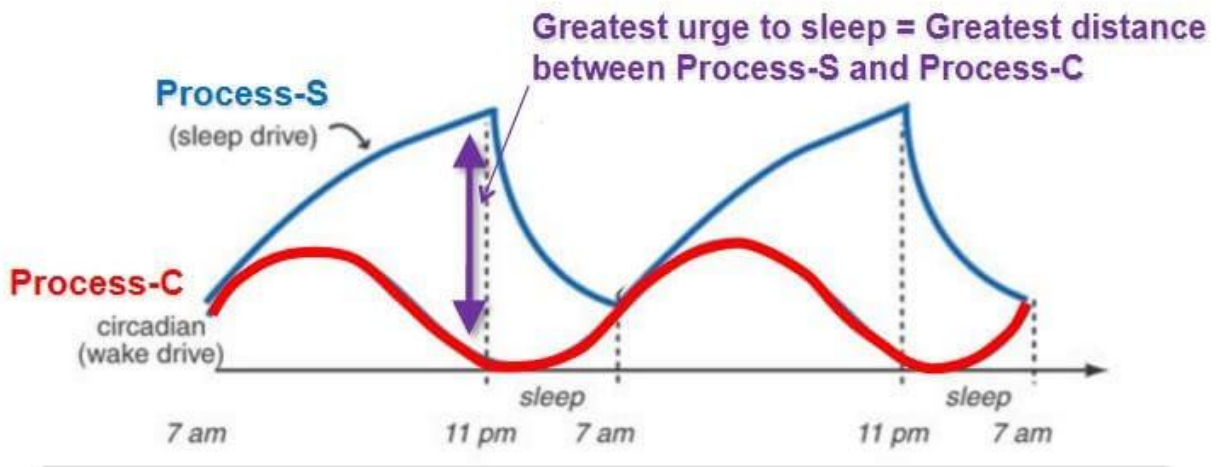
Level of **TNF – α** correlates with NREM sleep and it is believed that it promotes this sleep episode. Experimental administration of **TNF – α** extends length of sleep (approximately 3 μ g of **TNF – α** for 90 minutes). Biological effect for sleep is **VLPO**.

Have you known, that an experimental transfer of cerebrospinal fluid from the sleep deprived subject causes the recipient's extended sleep?

Higher level of **TNF – α** increases level of delta EEG activity (0.5 – 4 Hz). It is also known, that during sleep deprivation is also increasing concentration of **TNF – α** , and some specific groups of patients (with sleep apnea, chronic fatigue, chronic insomnia, heart attack) have also increased level of **TNF – α** .

Two Factor Theory of sleep

Is a model which is trying to explain relationship between circadian oscillatory tendency to be awake – Process C, and tendency to sleep – Process S. Best time to go sleep is at time when is the maximum difference between Processes C and S (see fig. below, purple arrow).



You can imagine process S as an effect of adenosine to VLPO for gradual tendency to fall asleep during a time. Process C is a complex circadian rhythm regulating many physiological functions and also level of wakefulness.

Control question:

Q.4.: When is better to go sleep after a whole night party? Immediately in the morning or rather later during the afternoon?

REM/ NREM sleep control circuits

Ventrolateral preoptic area and **medial preoptic nucleus** contain neurons specific for NREM sleep. This effect is ensured by the rich innervation of inhibitory GABA neurons into the neural network for waking, especially in the hypothalamus and brain stem. Basal forebrain contains subgroup of GABA neurons which inhibit cortex.

REM Sleep Control Circuits

Initiation of REM sleep is provided by **cholinergic neurons pedunculopontine a laterodorsal tegmental nuclei**. **Counterweight is monoaminergic activity** which has inhibitory effect to REM sleep (LC, nucleus raphe, ventrolateral periaqueductal grey). **Sublaterodorsal nucleus** ensure loss of the muscle tone during REM phase.

Control question:

Q.5.: Do you know which drug is blocking REM sleep?

*Have you known that an experimental lesion of **Sublaterodorsal nucleus** in cats caused a behavioral disorder during REM sleep? The animals moved exactly according to the content of their dreams (absence of muscle atony).*

Similar symptoms can be observed in patients with cerebral degeneration (idiopathic, but also with narcolepsy or Parkinson's disease, Lewy dementia, etc.).

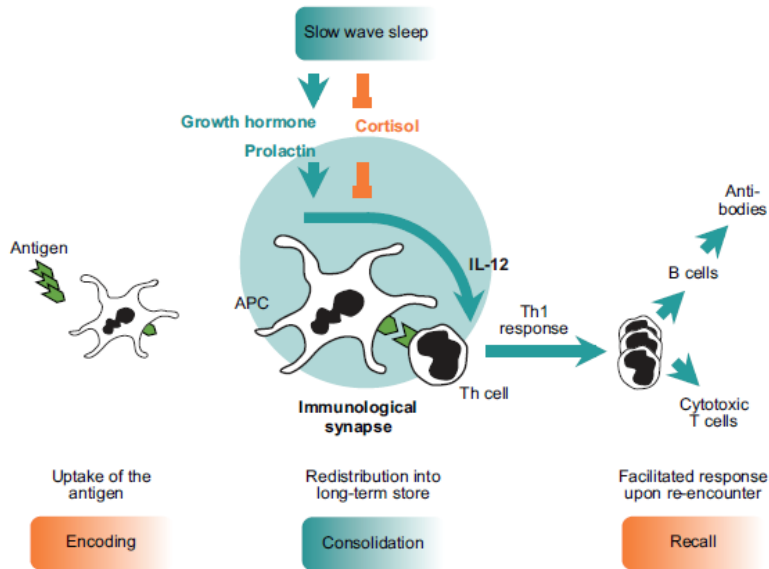
Sleep and dynamics of hormonal regulation

Some hormones show strict circadian rhythmicity regardless of the sleep cycle (e.g. Cortisol, TSH). Other hormones like **growth hormone** (50-70% of his total production is in the first half of sleep) and **prolactin** are dependent on the presence of sleep or to specific its phases. Sleep deprivation leads to an increase of Cortisol, TSH, T3 (T4 is unchanged), and decrease in Prolactin. Another example is **leptin**, a hormone important for appetite regulation. Level of the **leptin** increases during sleep and decrease during awake state. Sleep deprivation level decrease leads to a higher appetite and caloric intake.

Sleep and memory of the immune system

Beneficial role of rest and sleep for healing and recovery process is very well known across cultures. Once infection is established subjects are tired and sleep more extensively. On the other hand, long sleep deprivation disturbed strong immune response. Sleep supports antigen presenting cells (APC) and T – lymphocytes interactions, migration of T-lymphocytes into the lymphoid tissue. NREM sleep is important for production of proinflammatory factors which have somnogenous effect with a prolongation of deep NREM sleep.

Immune system represents a **long-term memory** for antigens of varying character = **adaptive immune response**. For instance, APC are in lymphatic organs interacting with naive T-cells and compose “immunological synapses”. Activation of T-lymphocytes leads to an increase of pro-inflammatory factors (e.g. Interleukins (IL) 12, 2, 10) what supports production antibodies and memory for antigens. During NREM sleep is lower level of cortisol with immunological inhibitory effect. Level of growth hormone and prolactin on the other hand go up, and both hormones support immunological memory (e.g. true the IL 12 production). It is also well known, that anti-inflammatory factors, like glucocorticoids, markedly shorten duration of NREM sleep.



Autonomous system and sleep

NREM Sleep prevails influence of parasympathetic, what **stabilizes** the cardiovascular system: It is obvious a low blood pressure (“dipping phenomenon”) and low heart rate. Blood pressure fluctuations are mainly compensated by baroreceptors. Absence of dipping phenomenon is a risk factor for a number of cardiovascular disorders and higher mortality. During NREM sleep is frequently significant sinus arrhythmia.

Rem sleep is on the other hand accompanied by an **instability** of the cardiovascular system with sympathetic and parasympathetic fluctuations. On the top of that, skeletal muscle hypotonia eliminates the auxiliary breathing muscles → **instability and decrease of ventilation** (especially during dreams). From that reason is REM critical period for development of sleep apnea.

And also, **sleep deprivation** leads to a **metabolic dysregulation** and **an increase of the tone of sympathetic** → higher risk of cardiovascular diseases, especially hypertension.

Patients with cardiovascular diseases have during REM sleep higher susceptibility to onset of seizures of angina pectoris or heart attack.

Selected diseases related to Sleep:

Narcolepsy

Known as a Daniela Merrick's Syndrome (DMS) is a chronic neurological disorder where the brain is unable to properly regulate the sleep and wake cycle. This disease has a genetical background associated with genes HLA DR2 and HLA DQB1 * 0602.

Experimentally, narcolepsy can be induced in rodents by the destruction of neurons producing hypocretin (orexin) mediators, which provide vigilance through a broad interaction with wake-up neurons. It is assumed that in patients with narcolepsy some autoimmune process destroys these neurons.

Narcolepsy symptoms: Typical is occurrence of unexpected sleep attacks. Very often so-called **cataplexy** of various degree = loss of muscle tone, which is triggered by strong emotions.

“Such a condition can be a curse and suffering, let’s imagine a small kid which fall to cataplexy after the strong emotion of pleasure...”

Very often are present other symptoms as a **sleep paralysis** and **hypnagogic hallucinations**: Subject after awaking from REM sleep cannot moved and his reality is mixed with content of dream.

Very often is such a state confused with Schizophrenia.

Sleep is fragmented across into the whole day, but total time of sleep is normal.

REM Sleep Dissociation – subjects with narcolepsy can cross from a wake-up state "directly" to the REM Sleep, which is not physiological.

Sleep apnea syndrome

Breathing in sleep is interrupted several times per night for more than 10 s. According to etiology we distinguish two forms:

Obstructive (more frequently):

↓ oxygenation of the blood due to obstruction in the upper airways.

In most patients, the reduction of arterial blood is mainly in the REM phase (muscle atonia).

Upper pathways tend to collapse in horizontal position → it is necessary to increase the flow in the HDC → increased breathing → neurochemical changes at motoneuron level (serotonin,

noradrenalin, GABA) lead to decreased muscle tonus of m-genioglossus. Obstruction leads to snoring.

As a result of hypoxemia and hypocapnia, pulmonary hypertension, cor pulmonale, polycythemia may develop. Cardiovascular effects include hypertension, heart rhythm disturbances, myocardial infarction, and cerebrovascular disorders.

Central (rare):

Apneic Pauses in Non-REM Sleep Phases I and II. are caused by ↓CO₂ Breathing in these phases of sleep is triggered at values of PCO₂, which is not during awake breathing not inhibited, that's mean that it is problem of malfunction.

Insomnia

The impact of a socio-economic development of Western civilization strongly affects a quality of sleep especially in western civilization. From history, industrial revolution have changed previously two phase sleep structure (people before industry revolution slept in two 4 hours periods with 2 hours of brake), because middle class have started to work in a work shifts. Recently society is exposed to a noisy environment, artificial light, especially blue spectrum from a new technologies and high stress make sleep length inadequate. It is possible to classify particular causes of insomnia into the three groups:

Bad sleep hygiene

For high quality of sleep is an important to keep some basic rules, like sleep in quiet environment without light in a comfortable bed. Important might be also little colder temperature inside a bedroom. Some bad habits, like is drinking alcohol or coffee before sleep and eating heavy food can also deteriorate sleep quality. Mental strain before a sleep as well as long term stress is another reason leading to the sleep disturbance.

Disrupted circadian biorhythms

Shiftwork, traveling and jet-leg phenomenon, exposition to blue light in a new technologies is strongly affecting circadian rhythms and sleep quality. You can read more in detail in chapter which is devoted for this topic.

Symptoms of other diseases

Many types of diseases can affect quality of the sleep. Pain, cough, frequent urination, immobility, depression or anxiety. For some of those factors, such as cough, there may be not a single assignment to just one group.

Types of failures:

- Sleep initiation disorder (e.g. environmental impact, anxiety).
- Disorder of the sleep continuity (e.g. Sleep apnea, asthma, and dementia).
- Early awakening (e.g. seniority, depression).

Pseudoinsomnia

The patient is not aware that he sleeps more time than he estimates. His insomnia often overstates.

Hypersomnia

Excessive daytime sleepiness or excessive time of sleeping have many reasons. It might be accompanied by lower wakefulness and higher drowsiness.

Primary hypersomnia

Include states like narcolepsy and idiopathic or periodic hypersomnia's.

Secondary hypersomnia

As a symptom of sleep apnea syndrome, especially in obese people with pick syndrome. It is also frequent in children with restless leg syndrome and generally as a result of consequence of many night insomnias. Shift working people might have problem to have a full-fledged sleep, so they are tired and sleepy.

Parasomnias

Abnormal behavioral disorders during sleep. It is divided according to the NREM / REM phase.

NREM parasomnias

Somnambulism (night walker) – dissociated awakening in deep NREM sleep in the first cycle. The patient can perform various movements, but he is not aware of it.

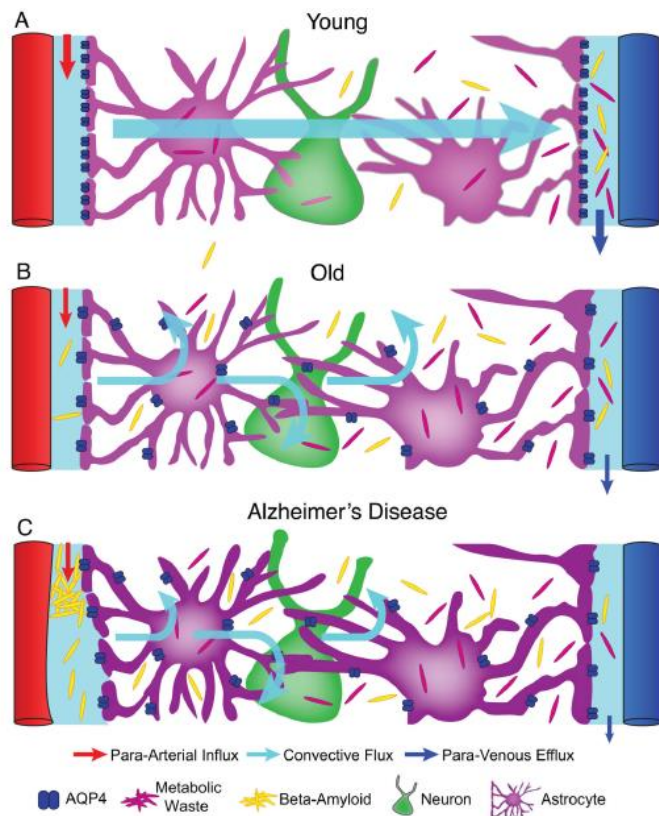
Pavor nocturnus the patient experiences great anxiety with strong vegetative symptoms, contact cannot be established with him, he does not remember anything in the morning.

REM parasomnias

Nightmare (horror dream) – In the second half of the night, the patient is awakened as a result of a dreadful dream, can be contacted with him, a dream remembers. Occurrence mainly in children, in adults rarely. Often in post-traumatic stress disorder.

Alzheimer disease and sleep

A recent study has shown significant increase of the beta amyloid level in sleep deprived people. Microglial clearance is in insufficient sleep and in the brain remains many waste products, including amyloid beta. This is a high-risk factor for the later development of Alzheimer's disease.



The microglia provide cleansing of metabolites from neurons and their synapses. It has been shown that this process is most intense while sleeping.

Pharmacological effect on sleep and awake

Ethanol - Supports GABAergic transfer of information. Excess alcohol leads to sleep disorders, especially insomnia in the second half of the night, when alcohol is already broken down and stimulated by the GABAergic system is transported by the Glutamatergic system .

It's just an illusion that alcohol will make better sleep!

Anesthetics and sedatives - effect at various sites of the neural network for sleep / wakefulness. Generally, GABAergic transmission of information is enhanced. Some anesthetics (like propofol) affect gap-junction connections. Benzodiazepines affect GABA - A receptors (suitable for sleeping patients with insomnia).

Antihistaminic – physiological histaminergic neurons inhibit NREM sleep and promote awakening. H receptor blockers block this effect.

Stimulancia – Caffeine blocks adenosine receptors (A1, A2). Amphetamine stimulates the effect of monoamines, especially Dopamine. Modafinil is a new stimulant (the effect is through Dopamine) used in therapy of narcolepsy. Significantly increases alertness in patients after stroke.

Literature for further reading:

The Neuroscience of Sleep. Edited by Robert Stickgold and Matthew Walker

Questions to repeat:

1. What is a main reason of death in subject with long time sleep deprivation?
2. What means REM and NREM sleep?
3. In which animals we can observe REM and NREM phases?
4. How is REM and NREM developed during human ontology?
5. In which Sleep phase is higher incidence of cardiovascular diseases, and why?
6. Which hormones are related to circadian rhythm and which to sleep phases?
7. Which brain center is responsible for Narcolepsy?
8. What is immunological memory?
9. Do you know some mechanism related to Alzheimer disease and sleep ?

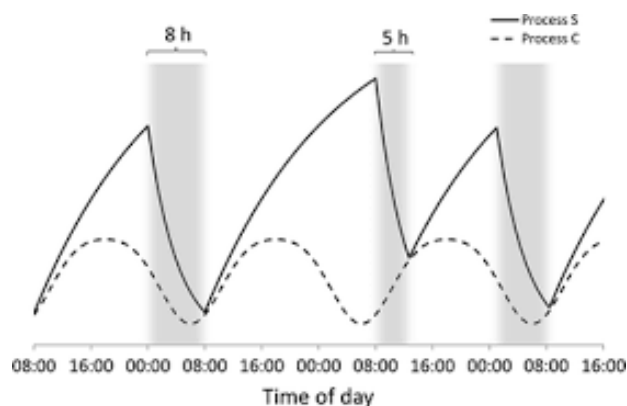
Answers for control questions:

Q1: Some people believe that REM sleep can developed it when muscle paralysis during sleep is not dangerous for the subject, e.g. in primates it was time when they didn't sleep on trees.

Q2: In newborns REM sleep works as a source of "internal" stimulation of the brain during long sleep, when there is a clear absence of external stimuli.

Q3: Because the synchrony of neurons is during NREM sleep higher, so general amplitude is bigger in contrast to awake state, when neuronal activity fires more "independently".

Q4: During afternoon, because the difference between C and S processes is bigger...



Q5:

Antidepressants with their influence *increase level of monoamines*, thereby completely suppressing REM sleep.