Project summary for Enough Sleep Project No 518189

Participants

Participant Name	Country
University of Helsinki	**Finland**
Karolinska Institutet	**Sweden**
University of Verona	**Italy**
Leiden University Medical Center	**Netherlands**
University of Zürich	**Switzerland**
National Public Health Institute	**Finland**
University of Milano	**Italy**
Nexstim	**Finland**

General objectives:

1) To spearhead the first integrated effort at understanding the different mechanisms of sleep regulation using a combined genetic, molecular, electrophysiological and systems approach.
2) To develop novel diagnostic tools (genetic, molecular and electrophysiological) for identifying disorders of sleep regulation and for characterizing their pathophysiology.
3) To apply such tools in the diagnosis and cure of disordered sleep, with special emphasis on insomnia and depression.

Specific objectives

1) Identify cortical mechanisms of sleep regulation
2) Identify sub-cortical mechanisms of sleep regulation
3) Identify humoral and genetic mechanisms of sleep regulation
4) Explore the role of glia in sleep regulation
5) Develop new diagnostic tools to specifically study sleep regulation in humans
6) Understand the mechanisms responsible for the alterations of sleep regulation in insomniac/depressed patients in order to envision new treatments

Summary of the results

The relationships of cortical activity and excitability and sleep/sleep homeostasis were examined using high density EEG and transcranial magnetic stimulation (TMS). The work was enabled by collaboration of a research group and a company that developed the necessary equipment and software that were suited not only for research purposes but also for patient work. The magnetic pulse used to stimulate the brain needs to be targeted with great accuracy; this has been improved in the present project. The equipment can be used e.g. in neurosurgery to accurately

localize the cortical motor areas. Software was developed to improve compatibility between the navigator data and data from ordinary hospital measurements (e.g. MRI).

An important advancement was to show that high sleep pressure is associated with an increased slope of slow waves, allowing the use of the slope as an indicator of sleep homeostasis. Interesting differences in the cortical excitability and slow wave activity (SWA) were found between normal subjects and bipolar patients. The amount of SWA was decreased in the frontal areas but increased in the occipital areas. In normal subjects cortical excitability decreased with sleep and increased with time spent in waking, but in patients this relationship was lost. However, during sleep deprivation the excitability changes normalized, suggesting that cortical excitability can still be affected also in patients by a strong stimulus, like sleep deprivation. This could possibly explain why sleep deprivation temporarily alleviates the symptoms of depression by increasing the excitability of frontal cortex.

New interesting results were found regarding memory and sleep. In mice sleep deprivation immediately following acquisition induced a memory deficit, when the experiment was performed during daytime (the rest period of mice), but had no effect when performed during night-time (the waking-period of mice). Moreover, voluntarily "sleep deprivation" induced by running wheels did not disturb learning either, raising the question whether sleep is really essential for learning.

In genetic studies (conducted on 1200 healthy sleepers) on the relations between plasticity-related genes and sleep duration one gene was found to be more related to both short (less than 6h) and long (more than 9 h) sleep than to normal sleep (7-8h). Serotoninergic genes were associated with depression and fatigue in women and one immediate early gene in depression in male. The results indicate sex-dependent and symptom-specific differences in the genetic background of depression. The differences may partially explain the large spectrum of depressive symptoms, and their systematic monitoring could potentially be used for diagnostic purposes

The roles of basal forebrain, suprachiasmatic nucleus (SCN) and thalamus were addressed with a series of experiments. It was shown that the basal forebrain cholinergic (BF) cells are essential for the production of recovery sleep (sleep that follows prolonged waking periods).

The SCN appears to be more susceptible to changes in sleep during early morning hours. Experiments using an interrupted sleep protocol showed that the intervention elicits changes in the neuronal activity of the SCN, increasing circadian signalling. This may indicate that recovery sleep is more effective at appropriate times of the day. Experiments with *trypanosoma brucei* showed that the SCN remains unaffected by this infection. Genomic experiments with circadian genes showed that in healthy sleepers five were associated with short sleep and four to long sleep.

Adenosine (ADE) and nitric oxide (NO) are important regulators of sleep and wakefulness. The waking-stimulating effect of caffeine is based on binding to adenosine receptors and prevention of adenosine-induced sleepiness. We showed that this effect is mediated through A2 receptors (as opposed to A1 receptors).

The relationships between immune system and sleep were addressed by measuring the expression of SOCS, regulators of cytokine activity, and iNOS induction as well as NO and microglia. We found that short term sleep deprivation did not induce SOCS expression, although SOCS genes are induced by cytokines, and IK-1b and TNFα are induced by sleep deprivation. In addition, microglia was activated by sleep deprivation. Inducible nitric oxide synthase (iNOS) was earlier shown to induce increase in NO concentration in the BF during sleep deprivation. In the present project the activation was further characterized and found to take place 30 min after

the initiation of sleep deprivation in the BF, but only after 6h sleep deprivation in the cortex. The source of NO, surprisingly, in the BF is neuronal – usually iNOS is expressed as response to immunological challenge in glia cells. We also showed that the effects of NO on recovery sleep are only partially mediated through the cGMP-pathway and that NO can either activate (low doses) or inhibit (high doses) BF neurons. The inhibition is partly dependent on increase in adenosine concentration.

Summary of the dissemination activities

Scientific activity:

Altogether 18 articles have so far been published and 8 have been submitted for publication. 10 manuscripts are under preparation and 23 citable abstracts have been presented in scientific meetings.

Dissemination for general public

There have been at least 86 presentations to the academic public and 50 presentations to the general public. These presentations include TV and radio programs, articles in health and other magazines, lectures in schools and health seminars, as well as a book addressed to the general public.

Exploitable products

Six items that will be in commercial use within years 2009-2011 either in research or clinical work were produced. Two of them carry patent and four software algorithms are protected by copyright.