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The short- and long-term proteomic effects of sleep deprivation on the cortical and thalamic synapses

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ABSTRACT

Acute total sleep deprivation (SD) impairs memory consolidation, attention, working memory and perception. Structural, electrophysiological and molecular experimental approaches provided evidences for the involvement of sleep in synaptic functions. Despite the wide scientific interest on the effects of sleep on the synapse, there is a lack of systematic investigation of sleep-related changes in the synaptic proteome. We isolated parietal cortical and thalamic synaptosomes of rats after 8 h of total SD by gentle handling and 16 h after the end of deprivation to investigate the short- and longer-term effects of SD on the synaptic proteome, respectively. The SD efficiency was verified by electrophysiology. Protein abundance alterations of the synaptosomes were analyzed by fluorescent two-dimensional differential gel electrophoresis and by tandem mass spectrometry. As several altered proteins were found to be involved in synaptic strength regulation, our data can support the synaptic homeostasis hypothesis function of sleep and highlight the long-term influence of SD after the recovery sleep period, mostly on cortical synapses. Furthermore, the large-scale and brain area-specific protein network change in the synapses may support both ideas of sleep-related synaptogenesis and molecular maintenance and reorganization in normal rat brain.

1. Introduction

The idea that sleep contributes to maintain normal brain functions as memory formation and behavior has been developed several decades ago (Bloch et al., 1977, 1979; Blissitt, 2001; Dang-Vu et al., 2006). It is supported by the facts that the majority of brain disorders are accompanied by sleep disturbances (Reynolds et al., 1988; Vitiello et al., 1990, 1991; Starkstein et al., 1991; Wiegand et al., 1991; Donnet et al., 1992; Baker and Richdale, 2015; Murphy and Peterson, 2015) and sleep deprivation causes memory impairment (Youngblood et al., 1997; Ishikawa et al., 2006), perception (Goel et al., 2005; Lei et al., 2015) and mood (Short and Louca, 2015) deficits.

Recently, two dominant ideas are formed concerning the general function of sleep, focusing mainly on the synapses. The synaptic homeostasis hypothesis (Tononi and Cirelli, 2003, 2006) suggests that sleep is necessary to decrease the enhanced synaptic strength that gradually develops during wakefulness to maintain the optimal balance between flexibility and rigidity in synapses which is crucial for normal brain function. This hypothesis predicted and partly proved the weakening of synaptic connection strength during sleep particularly in the cerebral cortex (Watson and Buzsáki, 2015). The synaptic homeostasis hypothesis emphasizes that the continuous molecular adjustment of the synapses during the active period makes them rigid through a "saturation" of the synaptic strength, therefore, decreases the ability of learning novel information. Thus, sleep reduces synaptic strength in general (Tononi

Abbreviations: 2D-DIGE, two-dimensional differential gel electrophoresis; EEG, electroencephalogram; EMG, electromyogram; FFT, Fast Fourier transform; MS/MS, tandem mass spectrometry; LTP, long-term potentiation; RS, recovery sleep; RS, experiment 2: the brains of rats were removed 16 h after the end of deprivation; SD, sleep deprivation; SD, experiment 1: the brains of rats were removed after 8 h of total sleep deprivation performed by gentle handling.

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and Cirelli, 2014). Another theory assumes the formation of novel dendritic spines, and in turn, synapses during slow-wave sleep, suggesting long-term memory inscription via development of novel synapses during sleep (Yang et al., 2014). The hypothesis of enhanced synaptogenesis during sleep points out that long-term memory trace consolidation is an important function of sleep and it is based on the genesis of novel dendritic spines and synapses (Matsuzaki et al., 2004) to change the neuronal connectome (Chow et al., 2013; Picchioni et al., 2013). While the synaptic homeostasis theory attempts to explain the restoration of "printability" of synapses, the intensive learning-induced, sleep-dependent synaptogenesis hypothesis suggests a mechanism which underlies the consolidation of long-term memory traces into the neuronal connectome. Both ideas are based on the fact that long-term memory traces require the ability of generating novel synapses and fundamental maintenance of flexibility in the protein composition of existing synapses (Trachtenberg et al., 2002; Klann and Sweatt, 2008).

Synaptogenesis and adjustment of synaptic strength are the results of molecular changes in synapses due to *de novo* protein synthesis (Martin et al., 2000) and/or incorporation of trafficking proteins into the synapse (Rumpel et al., 2005) during the memory consolidation process. As a model of synaptic plasticity induced by strong stimuli, long-term potentiation (LTP) is a good tool for studying molecular changes in synapses (Abraham and Otani, 1991; Sweatt, 1999). A fast imprinting into the synapse mediated by e.g., short-term kinase activity and protein trafficking is the major mechanism of early-phase LTP, lasting from a few seconds up to several hours after stimulation onset (Huang, 1998). The synaptogenesis and synaptic size increase were shown in the late phase of LTP (Tominaga-Yoshino et al., 2008). Most importantly, LTP is affected by sleep and sleep deprivation (McDermott et al., 2003; Blanco et al., 2015). These results further strengthen the idea that some sort of molecular maintenance and reorganization in synapses in conjunction with memory consolidation and recovery of learning capabilities are major functions of sleep.

There are several molecular changes in sleep and sleep deprivation uncovered by measuring mRNA level changes (Cirelli and Tononi, 1998; Cirelli et al., 2006; Terao et al., 2003a, 2003b; Mackiewicz et al., 2007; Jones et al., 2008; Vecsey et al., 2012) and also some protein level alterations have been revealed (Basheer et al., 2005; Pawlyk et al., 2007; Poirrier et al., 2008). However, focused high-throughput examination of the synaptic proteome is still lacking. The synaptic proteome contains more than 1,000 known proteins, but this number can be higher, since the available literature provides very different numbers of synaptic proteins probably due to the methodological heterogeneity in the field of proteomics (see http://www.synprot.de) (Pielot et al., 2012). Moreover, the majority of the synaptic proteins are also crucial in other cellular compartments of the neurons. Synapses are supplied by proteins from the local protein synthesis (Martin et al., 2000) and also by selection of proteins from axonal and dendritic protein trafficking systems (Vallee and Bloom, 1991). Interestingly, a general increase in the brain tissue protein synthesis has been revealed during sleep (Ramm and Smith, 1990; Nakanishi et al., 1997) but the data are not specific for the synaptic proteome.

The changes specific to the synaptic protein network underlying the sleep-related adjustment of synaptic strength are poorly understood. In this study, we performed a parietal cortical and thalamic synaptosome proteomic study of rats. The parietal cortex receives inputs from the thalamus which is necessary for genesis of synchronous sleep-related activity in the cortex as extensively studied by Steriade and others (for review, see Steriade and Llinás, 1988). It is also known that the detrimental effects of sleep deprivation are particularly pronounced in the thalamus and parietal cortex (among other cortical structures) (Chee and Choo, 2004; Chee et al., 2006). Therefore, the proteomics study was conducted on samples of thalamo-cortical cross-linked areas of the brain highly sensitive to sleep deprivation. Surprisingly, sleep deprivation inversely affects the activation-state of thalamus and the parietal cortex (Tomasi et al., 2009), emphasizing the importance of separately assessing molecular changes in these brain areas. The proteomic changes were characterized in both brain areas after 8 h of total sleep deprivation (SD) and 16 h after the end of deprivation, when recovery sleep (RS) of sleep deprived animals took place. This experimental design enabled monitoring the effects of SD and RS on the synaptic proteome in relevant brain areas.

2. Materials and methods

2.1. Animals

Adult male Sprague-Dawley rats (4 months old, weighing 350–400 g; Charles River Laboratories, Hungary) were used ($n = 24$ for proteomic experiment, $n = 6$ for electron microscopy and sleep deprivation validation). Animals were housed under standard laboratory conditions (lights on at 9:00 AM, lights off at 9:00 PM), with free access to water and food. The care and treatment of all animals were in conform to Council Directive 86/609/EEC, the Hungarian Act of Animal Care and Experimentation (1998, XXVIII), and local regulations for the care and use of animals in research. All efforts were taken to minimize the animals' pain and suffering and to reduce the number of animals used.

2.2. Experimental paradigm

Sleep deprivation started at 9:00 AM (lights on) and lasted 8 h long until 5:00 PM. In the SD experiment, brains of sleep deprived ($n = 6$) and control ($n = 6$) rats were removed right after the ending of the deprivation. In the RS experiment, brains of sleep deprived ($n = 6$) and undisturbed, control ($n = 6$) rats were removed 16 h after finishing the deprivation, at 9:00 AM, on the next day. For the experimental paradigm, see Fig. 1.

2.3. Sleep deprivation procedure and estimation of its effectivity by EEG and EMG

Sleep deprivation was carried out using the gentle handling method which is the least stressful method of total sleep deprivation (Ledoux et al., 1996; Rechtschaffen et al., 1999; Fenzl et al., 2007).

For electroencephalogram (EEG) recordings, rats were implanted with stainless steel screw electrodes (0.8 mm o.d.) and with teflon-coated, stainless steel multiwire muscle electrodes for electromyogram (EMG) recordings. Animals were anesthetized with 1% (v/v) isoflurane. The screw electrodes were implanted into the skull, bilaterally above the occipital, parietal and frontal cortices. Ground and reference electrodes were placed above the cerebellar cortex. The electrodes were fixed on the skull using dentacrylate cement, and were soldered to ten-pin sockets. Sleep deprivation and polygraphic recordings were performed after one week recovery period.

EEG and EMG were recorded by a Grass Model 8B (Grass Instrument Company) electroencephalograph attached to a CED 1401 mkII data capture and analysis device, using Spike 2 software (Cambridge Electronic Design Limited). The bandwidth of the EEG recording was 0.5–70 Hz and 5–300 Hz for the EMG recording. Signals were digitalized at 500 Hz sampling rate for EEG and at 900 Hz for EMG. Power density analysis was performed using Fast Fourier transform (FFT size 8192, Hanning window) in Spike 2. Somnograms were produced by sleep scoring that was made in 30 s epochs by a script provided by Cambridge Electronic Design Limited for the Spike 2 software ("RatSleepAuto" script; Costa-Miserachs et al., 2003).

2.4. Synaptosome preparation

Synaptosome isolation was performed immediately after the brain removal. Quickly removed brains were placed into ice-cold artificial cerebrospinal fluid and brain structures were dissected on a dry ice-cooled plate. Subsequently, parietal cortices and thalami were removed. From