Brain Research Bulletin 137 (2018) 166–177

Contents lists available at ScienceDirect

Brain Research Bulletin

journal homepage: www.elsevier.com/locate/brainresbull

Research report

Sleep deprivation decreases neuronal excitability and responsiveness in rats both *in vivo* and *ex vivo*

Sándor Borbély*, Ildikó Világi, Zsófia Haraszti, Örs Szalontai, Tünde Hajnik, Attila Tóth, László Détári

Department of Physiology and Neurobiology, Eötvös Loránd University, Budapest, Hungary

ARTICLE INFO

Keywords:
Evoked potentials
Excitability
Microelectrophysiology
Sleep deprivation
Spontaneous epileptiform activity

ABSTRACT

Sleep deprivation has severe consequences for higher nervous functions. Its effects on neuronal excitability may be one of the most important factors underlying functional deterioration caused by sleep loss. In the present work, excitability changes were studied using two complementary *in vivo* and *ex vivo* models. Auditory evoked potentials were recorded from freely-moving animals *in vivo*. Amplitude of evoked responses showed a near-continuous decrease during deprivation. Prevention of sleep also reduced synaptic efficacy *ex vivo*, measured from brain slices derived from rats that underwent sleep deprivation. While seizure susceptibility was not affected significantly by sleep deprivation in these preparations, the pattern of spontaneous seizure activity was altered. If seizures developed, they lasted longer and tended to contain more spikes in slices obtained from sleep-deprived than from control rats. Current-source density analysis revealed that location and sequence of activation of local cortical networks recruited by seizures did not change by sleep deprivation. Moderate differences seen in the amplitude of individual sinks and sources might be explained by smaller net transmembrane currents as a consequence of decreased excitability. These findings contradict the widely accepted conception of synaptic homeostasis suggesting gradual increase of excitability during wakefulness. Our results also indicate that decreased neuronal excitability caused by sleep deprivation is preserved in slices prepared from rats immediately after deprivation. This observation might mean new opportunities to explore the effects of sleep deprivation in *ex vivo* preparations that allow a wider range of experimental manipulations and more sophisticated methods of analysis than *in vivo* preparations.

1. Introduction

Sleep must have some essential homeostatic functions, as long-term sleep deprivation leads to severe pathological changes affecting psychomotor, cognitive and behavioral functions (Aldabal and Bahammam, 2011). These pathological changes show that sleep is needed to provide a proper stage for some restorative processes to take place. Proportionally with the length of wakefulness, a drive for sleep ("sleep pressure") builds up that is relieved when environmental conditions allow sleep to occur. Accordingly, sleep pressure is high at the beginning of sleep and decreases afterwards. During sleep deprivation, "sleep pressure" accumulates. When sleep deprivation is over and sleep becomes possible, rebound or recovery sleep is initiated. Duration and intensity of sleep show a clear rebound effect (Franken et al., 1991). Rebound can be characterized by intensive slow wave activity (Borbely et al., 1981, 1984; Trachsel et al., 1989). The magnitude of slow wave activity depends on the duration of prior wakefulness (Tobler and Borbely, 1990), and may reflect restorative processes in direct connection with the function of sleep.

Sleep deprivation results in a variety of changes that were examined at the level of genes (Cirelli, 2002), transmitter receptor expression (Longordo et al., 2009), neurotransmitter release (Dash et al., 2009), neuronal networks (Krueger et al., 2013) and even behavior of the whole organism (Vollert et al., 2011).

Excitability refers to the capacity of single neurons as well as neuronal networks to generate action potentials as output measures. On the input side, excitability determined by the number and distribution of ion channels and receptors that contribute the electrical properties and depolarization potential of the neuron (Daoudal and Debanne, 2003). Effects of prolonged wakefulness or sleep deprivation on neuronal excitability are extensively studied, but these experiments have yielded contradicting results.

Abbreviations: ACSF, artificial cerebrospinal fluid; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; CSD, current-source density; EP, evoked potential; EFP, evoked field potential; FP, field potential; I - O, input – output; NMDA, N-methyl-D-aspartate; T, threshold

* Corresponding author at: Department of Physiology and Neurobiology, Eötvös Loránd University, Pázmány Péter sétány 1/C, H-1117, Budapest, Hungary.
E-mail address: sandor.borbely@ttk.elte.hu (S. Borbély).

https://doi.org/10.1016/j.brainresbull.2017.12.003
Received 13 September 2017; Received in revised form 5 December 2017; Accepted 8 December 2017
Available online 11 December 2017
0361-9230/

Several studies have shown increased excitability, in agreement with the more than 50 years old hypothesis claiming that permanent sleep deprivation increases the probability of nearly all types of epilepsy (Gastaut and Tassinari, 1966; Badawy et al., 2006) via various mechanisms, like lowering the threshold (T) to induce convulsions by electroshocks (Cohen and Dement, 1965), or the threshold for kindling (Shouse, 1988a). It is important to note that the above mentioned older clinical studies were later criticized because of their methodological shortcomings (Malow, 2004). The conception of increased excitability was supported by data from not only the disordered, but from the healthy brain as well, as amplitude of the early component of the auditory event-related potential has been shown to increase after whole-night total sleep deprivation in humans (Bortoletto et al., 2011). In rats, increased excitability during SD was demonstrated by increased firing of cortical neurons (Vyazovskiy et al., 2009).

Neuronal excitability and cortical bistability appearing as an alternation between active (UP) and silent (DOWN) states have been shown to increase during prolonged wakefulness (Vyazovskiy et al., 2013). Cortical neurons responded to stimulation faster, stronger and with increased synchrony compared to the control stage (Vyazovskiy et al., 2013). Although neuronal responsiveness to incoming stimuli increased, behavioral performance was reduced because of the increased bistability. Cortical neurons were more responsive, when they were in the silent state, while neurons in the active state had a stronger tendency to enter the silent state after a short period of increased activity induced by the stimulation. Inter-trial variability of evoked potentials (EPs) was also increased during sleep deprivation (Vyazovskiy et al., 2013). It was suggested that not only neuronal responsiveness, but also intracortical connectivity can become stronger as a consequence of sleep deprivation (Huber et al., 2013).

On the other hand, some studies found decreased cortical excitability after sleep deprivation. In experiments in humans, amplitude of the P1 component of the visual event-related potentials diminished while performing a psychomotor vigilance task during sleep deprivation (Hoedlmoser et al., 2011). In rat hippocampal slices, sleep deprivation impaired long-term potentiation (LTP). Compared to the controls, the amplitude of population spikes was smaller following sleep deprivation tested thirty minutes after tetanic stimulation (Campbell et al., 2002; Kreutzmann et al., 2015).

To reconcile the contradicting results (increased vs. decreased excitability), a third group of investigators suggested that sleep deprivation changes the balance between excitatory and inhibitory neurotransmitter functions. These changes might allow both increased and decreased excitability to occur depending on the actual conditions, e.g. on the length of sleep deprivation and on the level of stress accompanying sleep deprivation (Civardi et al., 2001).

Although there is no agreement regarding the direction of changes in neuronal excitability during sleep deprivation, its negative functional consequences has been repeatedly demonstrated using various experimental paradigms both in rodents (Hagewoud et al., 2011; Ishikawa et al., 2014) and humans (Van Dongen et al., 2003; Mander et al., 2011). It can be hypothesized, that excitability changes are caused by complex processes that cannot be simply characterized by the direction of these changes.

We performed parallel *in vivo* and brain slice studies to test excitability changes after sleep deprivation. We recorded acoustic EPs in freely-moving, unanesthetized rats, while we also examined sleep deprivation effects ex vivo, in brain slices prepared immediately after sleep deprivation. We assumed that sleep deprivation might cause long-lasting changes in neuronal excitability in the cortex that would only be relieved during a rebound sleep that also depends on subcortical networks. Thus, we supposed that effects of sleep deprivation on the cortical networks themselves can be studied ex vivo as in brain slices subcortical afferents are missing. In the complementary in vivo model, sleep deprivation-evoked changes can be examined in the cortex when intact cortical-subcortical networks are available.

2. Experimental procedures

Experiments were carried out in accordance with the Hungarian Act of Animal Care and Experimentation (1998, XXVIII) and with the directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. Experimental protocols were approved by the regional ethical committee (license number XIV-I-001/519-4/2012 for in vivo experiments, XIV-I-001/516-4/2012 for ex vivo experiments). Efforts were made to minimize the number of animals used.

2.1. *In vivo electrophysiological recordings*

2.1.1. *Surgery and animal housing*

Experiments were performed on 6 male Wistar rats (Toxicoop, Budapest) weighing between 380 and 420 g at the time of the surgery. The animals were anesthetized with ketamine-xylazine (ketamine: 80 mg/kg, xylazine: 10 mg/kg, i.p.) then placed in a stereotaxic instrument (David-Kopf) using blunt ear bars to protect the middle ear from injury.

To record EEG activity and EPs stainless steel screws (1.1 mm, Fine Science Tools) were inserted into the skull above the primary somatosensory cortex (Br-2.0 L3.0) and occipital cortex (vertex; Br −4.5; L 2.0) on both sides. Potentials were recorded between the screws located ipsilaterally in case of both hemispheres. An additional screw implanted into the bone over the cerebellum served grounding purposes. Coordinates are based on the stereotaxic atlas of Paxinos and Watson (1998). To monitor EMG activity, a pair of Teflon-insulated stainless steel wires (250 μm) was inserted into the neck musculature close to the caudal surface of the skull. All leads were soldered to a female connector fixed to the bone with dental acrylic. Following surgery, the animals were kept warm, and painkillers (50 mg/kg metamizole, i.p.) were administered for 3 days.

After surgery, animals were kept in a windowless, quiet room at the basement of the building in individual Plexiglas cylinders with an internal diameter of 280 mm. The cylinders were covered by Plexiglas lids accommodating also the speakers. Room temperature was kept at 21 °C, food and water were available *ad libitum*. Light was controlled in a LD12:12 cycle, with light on between 8:00 to 20:00.

Rats were connected to the recording apparatus three days after surgery to ensure their adjustment. A zigzag flat cable with a rubber string in the middle connected to a swivel (Litton) was used as recording cable to provide free movements for the rats. The first stage of the amplifier was built into the male connector at the end of the cable to decrease artefacts.

2.1.2. *Electrophysiological recording and acoustic stimulation*

Experiments started after one week of recovery period with a baseline recording of spontaneous sleep-wake activity and auditory EPs for 24 h. The next day, sleep deprivation was performed by gentle handing during the first 6 h of the light period, followed by an 18-h recovery period. Only one sleep deprivation session was performed in each rat.

Auditory click stimuli with 500 μs duration were delivered every 10 s by a piezoelectric speaker attached to the top of the cage, approximately 30 cm high above the animal. Stimulus intensity was set empirically, to ensure that no startle response or any twitch of the auricles was induced by the click. The intensity was assessed to be about 60 dB also depending on the position of the animal.

Signals were amplified (500x) and filtered (0,3 Hz − 3 kHz) (Elsoft BT), then digitalized at a 16-bit resolution by an analog-to-digital (A/D) converter card (Labview, National Instruments, Austin, TX, USA). The A/D sampling frequency was set to 8192 Hz (a power of 2) to facilitate Fast Fourier analysis.