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Peripheral and central blockade of interleukin-6 trans-signaling differentially affects sleep architecture

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ABSTRACT

The immune system is known to essentially contribute to the regulation of sleep. Whereas research in this regard focused on the pro-inflammatory cytokines interleukin-1 and tumor necrosis factor, the role of interleukin-6 (IL-6) in sleep regulation has been less intensely studied, probably due to the so far seemingly ambiguous results. Yet, this picture might simply reflect that the effects of IL-6 are conveyed via two different pathways (with possibly different actions), i.e., in addition to the 'classical' signaling pathway via the membrane bound IL-6 receptor (IL-6R), IL-6 stimulates cells through the alternative 'trans-signaling' pathway via the soluble IL-6R. Here, we concentrated on the contributions of the trans-signaling pathway to sleep regulation. To characterize this contribution, we compared the effect of blocking IL-6 trans-signaling (by the soluble gp130Fc fusion protein) in the brain versus body periphery. Thus, we compared sleep in transgenic mice expressing the soluble gp130Fc protein only in the brain (GFAP mice) or in the body periphery (PEPCK mice), and in wild type mice (WT) during a 24-h period of undisturbed conditions and during 18 h following a 6-h period of sleep deprivation. Compared with WT mice, PEPCK mice displayed less sleep, particularly during the late light phase, and this was accompanied by decreases in slow wave sleep (SWS) and rapid eye movement (REM) sleep. Following sleep deprivation PEPCK mice primarily recovered REM sleep rather than SWS. GFAP mice showed a slight decrease in REM sleep in combination with a profound and persistent increase in EEG theta activity. In conclusion, peripheral and central nervous IL-6 trans-signaling differentially influences brain activity. Peripheral IL-6 trans-signaling appears to more profoundly contribute to sleep regulation, mainly by supporting SWS.

1. Introduction

The immune system profoundly influences the pattern of sleep, not only in many pathological conditions but even under normal physiological conditions (Krueger, 2008; Imeri and Opp, 2009; Lange et al., 2010; Besedovsky et al., 2012). This influence is thought to be conveyed via the release of cytokines from immune cells in the body periphery or in the brain itself. Whereas studies have so far focused on the role of the pro-inflammatory cytokines interleukin-1 and tumor necrosis factor, which appear to regulate sleep via an action on slow wave sleep (SWS) promoting mechanisms (Takahashi et al., 1999; Clinton et al., 2011; Jewett and Krueger, 2012; Schmidt et al., 2015), possible contributions of IL-6 signaling to sleep regulation received less attention, which might be partly ascribed to the seemingly inconclusive pattern of result from these studies. Thus, in humans, an association was reported between impaired sleep and elevated IL-6 and cortisol levels (Vgontzas et al., 2003; Burgos et al., 2006; Riemann et al., 2009). IL-6 enhanced non-rapid eye movement (NonREM) sleep in rats (Hogan et al., 2003), and enhanced slow wave activity during SWS in humans (Benedict et al., 2009), suggesting IL-6 signaling to favor SWS-related processes. However, IL-6 knock-out mice spent more time in REM sleep than control mice (Morrow and Opp, 2005a). Additionally, these mice showed a slower

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recovery of sleep after a 6-h period of sleep deprivation. In other experiments no effects of IL-6 on sleep were observed in rabbits (Opp et al., 1989), and antagonizing IL-6 activity by neutralizing antibodies in rats also did not affect sleep (Hogan et al., 2003).

The heterogeneity of IL-6 effects on sleep observed in previous studies could be at least partially due to the fact that IL-6 can act on cells through two different signaling pathways, *classical signaling* and *trans-signaling*. In classic signaling, IL-6 binds to a membrane-bound receptor (mbIL-6R). Thereafter, the IL-6/mbIL-6R complex interacts with the trans-membrane protein gp130, inducing its dimerization and downstream signaling via the JAK/STAT pathway (Heinrich et al., 2003; Rose-John, 2012). Only cells, which express mbIL-6R are able to respond to IL-6 via classic signaling. In trans-signaling, IL-6 binds to a soluble form of the receptor (sIL-6R), present in the extracellular space. This complex of IL-6/sIL-6R can stimulate gp130 expressing cells, including those that lack membrane-bound IL-6R (Rose-John, 2012). Of note, the latter cells are completely unresponsive to IL-6 alone (Rose-John, 2012). Whereas membrane-bound IL-6R is mostly expressed by hepatocytes and some leukocytes, gp130 is expressed by virtually all cells in the body including different types of glia cells (März et al., 1999) and neurons (März et al., 1998). Accordingly, IL-6 trans-signaling has been demonstrated to be of particular importance in the central nervous system (CNS) (Campbell et al., 2014).

The present study aimed at dissecting the contributions of IL-6 trans-signaling in the body periphery and in the CNS on sleep regulation. In this vein, it complements and extends previous experiments (May et al., 2009), in which we stimulated IL-6 trans-signaling by an introcerebroventricular infusion of Hyper-IL-6 (a fusion protein of human IL-6 and human soluble IL-6 receptor, Fischer et al., 1997) in rats. In that study, stimulation of the IL-6 trans-signaling pathway increased REM sleep and decreased power of the EEG theta activity during REM sleep. In the present study, we selectively blocked IL-6 trans-signaling in the CNS or in the body periphery. To this end two different types of transgenic mice were used, which expressed a soluble and dimerized form of gp130 (sgp130Fc) – a fusion protein that selectively inhibits IL-6 trans-signaling, and leaves classic signaling via the membrane-bound IL-6R intact (Jostock et al., 2001; Rabe et al., 2008; Braun et al., 2013; Campbell et al., 2014). In one transgenic line (PEPCK mice) IL-6 trans-signaling was blocked in the periphery whereas in the other line (GFAP mice) it was blocked in the CNS. The two groups of mice were compared to age-matched C57Bl/6J wild type (WT) mice. We compared the sleep architecture and EEG between the three groups during 24 h in undisturbed conditions, and during 18 h of recovery from a 6-h period of sleep deprivation. We hypothesized that central blockade of IL-6 trans-signaling induced effects opposite to those seen after central nervous administration of Hyper-IL-6 in a previous study (May et al., 2009), i.e., a decreasing rather than increasing effect on REM sleep, whereas peripheral blocking of IL-6 trans-signaling was suspected to suppress promoting effects on sleep and SWS, which were associated with IL-6 activity in previous studies.

2. Material and methods

2.1. Animals

Mice of three different genotypes were used. The first line of transgenic mice expressed sgp130Fc as a transgene from a liver promoter PEPCK (PEPCK group, Rabe et al., 2008); therefore, sgp130Fc was present in the blood and peripheral body fluids. The second line of transgenic mice expressed sgp130Fc as a transgene from the astrocyte specific GFAP promoter (GFAP group, Campbell et al., 2014), producing high levels of the protein in the CNS. Eleven PEPCK-sgp130Fc mice with C57BL/6J background, eight GFAP-sgp130Fc mice with C57BL/6J background, and eleven wild type C57BL/6J mice (aged between 8 and 12 weeks) were used. The transgenic mice were generated at one of the coauthors lab (S.R.-J.), and the genotypes were verified by PCR analysis of tail and hear DNA. The transgenic mice do not exhibit any apparent behavioral alteration. Animals were housed and experiments were performed at controlled temperature (20 ± 2 °C) and humidity (55 ± 10%), and a controlled 12 h/12 h light/dark cycle with light onset at 6 a.m. Water and food were available *ad libitum*. All experimental procedures were performed in accordance with the European animal protection laws and policies (Directive 86/609, 1986, European Community) and were approved by the Baden-Württemberg state authority (MPV 1/12).

2.2. Surgery

The animals were anesthetized with intraperitoneal injection of fentanyl (0.05 mg/kg of body weight), midazolam (5.00 mg/kg), and medetomidin (0.50 mg/kg). They were placed into a stereotaxic frame and were supplemented with isoflurane anesthesia (0.5%) as necessary. The scalp was removed and 4 holes were drilled into the skull. Four EEG screw electrodes were implanted: one frontal electrode (AP: +1.5 mm, L: +1.0 mm, relative to Bregma), two parietal (AP: −2.0 mm, L: ±2.5 mm), and one occipital reference electrode (AP: −10.0 mm, L: 0 mm). Two stainless steel wire electrodes were implanted bilaterally in the neck muscles for EMG recordings. The electrodes were fixed to the skull with cold polymerizing dental resin and the wound was sutured. At the end of the surgery, an anesthesia antidote (naloxone 1.2 mg/kg, flumazenil 0.5 mg/kg, and atipamezole 2.5 mg/kg) was applied subcutaneously. The animals were given analgesics (carprofen 0.05 mg/kg) for 3 days following the surgery. At least seven days were allowed for recovery.

2.3. Experimental protocol and EEG/EMG recordings

The recordings took place in a quadratic recording box (30 × 30 cm, 40 cm high) made of dark gray PVC. Mice were habituated to the recording box for two days. After habituation, the EEG and EMG were recorded continuously for 48 h. During the first 24 h the mice were left undisturbed. The second day started with a 6-h period of sleep deprivation, followed by an 18-h recovery period. Sleep deprivation was achieved by gentle handling; if the animal displayed a sleeping posture and the EEG confirmed signs of sleep the mouse was aroused by tapping on the box, gently shaking the box or, if necessary, disturbing the nest. Note, because gentle handling starts with confirmation of EEG signs of sleep and may not immediately arouse the animal, the procedure does not completely abandon sleep. During recordings, the electrodes were connected through a swiveling commutator to an amplifier (Model 15A54, Grass Technologies, USA). EEG and EMG signals were amplified, filtered (EEG: 0.01–300 Hz; EMG: 30–300 Hz), and sampled at a rate of 1017 Hz.

2.4. Assessment of sleep-wake architecture

Sleep stages, i.e., slow wave sleep (SWS), pre-rapid eye movement (pre-REM) sleep, and rapid eye movement (REM) sleep, and wakefulness were scored off-line by visual inspection using 10-s epochs according to standard criteria (Neckelmann et al., 1994). Pre-REM is scored specifically in rodents and normally occurs at the transition into REM sleep. It is mainly characterized by a progressive decrease in EEG slow wave activity and EMG activity, and a concurrent increase in EEG theta activity. For sleep scoring, the Sleep-Sign for Animal software (Kissei Comtec, Japan) was

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