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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

used. For each mouse, the (i) absolute time spent in a specific stage during succeeding two hour intervals and (ii) the percentage of sleep time spent in a specific sleep stage during succeeding 2-h intervals was determined. Additionally, duration and number of sleep episodes, SWS, pre-REM and REM sleep episodes were calculated for succeeding 2-h intervals.

Additionally, the EEG pattern during each sleep/wake state was characterized using Fast Fourier Transformation. Epochs containing EEG artifacts were excluded from spectral analyses. For SWS average power was computed for the slow-wave activity (SWA, 0.5–4.0 Hz), slow oscillation (0.5–2.0 Hz) and delta (2.0–4.0 Hz) frequency bands. For REM sleep and pre-REM sleep average power for the theta band (4.0–10.0 Hz) was calculated.

Sleep spindles were detected using an algorithm described in detail by Eschenko et al. (2006). Briefly, EEG signals were filtered between 12 and 15 Hz, the root mean square (rms) of the filtered signals was calculated, and the episodes where the rms signal was 1.5 SD above the mean for periods longer than 0.5 s were scored as spindle events.

2.5. Statistical analyses

Differences in sleep architecture, as well as in EEG power spectra among genotypes were analyzed using Analysis of Variance (ANOVA) including the group factor 'genotype' (the three mice strains of interest) and, when appropriate, the repeated-measures factor 'time' (representing the succeeding 2-h intervals). The Greenhouse–Geisser correction of degrees of freedom was applied where appropriate. We used two-sampled Student's *t*-tests to specify significant ANOVA main and interaction effects. Note, post hoc comparisons were only performed when ANOVA revealed significance for a test of interest. Because the ANOVA was considered the primary statistical tests, we did not introduce any correction for multiple comparison (e.g., Bonferroni) for the post-tests. A $p < 0.05$ (two-tailed) was considered significant. For statistical analysis, the SPSS 21.0 software (IBM, Armonk, USA) was used.

3. Results

3.1. 24-h sleep-wake architecture

Fig. 1 summarizes the time spent awake, in SWS, pre-REM and REM sleep for succeeding 2-h intervals across the 24-h cycle, separately for the three mouse strains. As expected in a nocturnal species, mice were mostly awake during the dark period of the day and much less awake during the light period. PEPCK mice, with peripheral blockade of IL-6 trans-signaling, showed a distinct increase in the time spent awake, which focused on the second half of the light period ($F(22,297) = 2.07$, $p = 0.004$, for genotype × time interaction, see Fig. 1A for post hoc pairwise comparisons between GFAP and WT mice). Consistent with this increased time spent awake, PEPCK mice spent less time in all sleep stages, especially towards the end of the light period. Thus PEPCK mice showed less SWS ($F(16.589,223.954) = 1.78$, $p = 0.033$, for genotype × time), less time in pre-REM sleep ($F(13.369,180.479) = 3.13$, $p < 0.001$, for genotype × time, ($F(2,27) = 6.02$, $p = 0.007$, for genotype main effect) and less time in REM sleep ($F(22,297) = 2.86$, $p < 0.001$ ($F(11,297) = 39.28$, $p < 0.001$, for genotype × time; ($F(2,27) = 7.15$, $p = 0.003$, for genotype main effect) than the WT control mice (see Fig. 1B–D, for respective post hoc pairwise comparisons). Sleep in GFAP mice, with CNS blockade of IL-6 trans-signaling, did not differ from sleep in WT mice except for a slight decrease in time in REM sleep ($F(1,17) = 7.20$, $p = 0.016$).

The decreased sleep time during the late light period in PEPCK mice was associated with a decreased number of episodes in SWS, pre-REM sleep and REM sleep ($F(10,135) = 2.62$, $p = 0.006$, $F(10,135) = 3.65$, $p < 0.001$, and $F(10,135) = 1.99$, $p = 0.039$, respectively, for genotype × time). The duration of episodes were not changed in the PEPCK mice (all *p*'s > 0.3).

Fig. 2 shows the percentages (of sleep time) spent in the different sleep stages during the 24-h cycle for the three strains). The proportion of SWS within sleep period was increased in PEPCK mice compared to the other two mice strains ($F(2,26) = 5.52$, $p = 0.010$), with this effect more pronounced during the late light phase ($F(12.995,168.931) = 1.56$, $p = 0.1$, for genotype × time, see Fig. 2A, for respective post hoc pairwise comparisons. By contrast proportions of pre-REM sleep and REM sleep were decreased in PEPCK mice ($F(2,26) = 3.96$, $p = 0.031$ and $F(2,26) = 4.80$, $p = 0.017$, for respective main effects of genotype. Post-hoc pairwise comparisons (Fig. 2B and C) revealed this effect to be particularly robust during the late light period, although respective genotype × time ANOVA interaction effects failed to reach significance (*p*'s > 0.15).

3.2. EEG power spectra during wake and specific sleep stages, spindles

Fig. 3 shows average power spectra during wake, SWS, pre-REM sleep and REM sleep for the three genotypes. All genotypes showed the typical increase in theta power (4.0–10.0 Hz) during wakefulness, pre-REM sleep, and REM sleep (in comparison with SWS), and the typical increase in slow wave activity (SWA, 0.5–4.0 Hz) during SWS and pre-REM sleep. Notably, GFAP mice (with blocked CNS IL-6 trans-signaling) displayed distinctly higher theta power basically throughout the whole recording period. This increase was very prominent during the stages characterized by high theta activity, i.e., wakefulness, pre-REM sleep and REM sleep compared to the other two strains ($F(2,26) = 4.21$, $p = 0.026$, $F(2,26) = 4.76$, $p = 0.017$, and $F(2,26) = 4.30$, $p = 0.024$, for respective main effect of genotype, see Fig. 3 for respective pairwise comparisons), but also reached significance in an additional analysis performed on SWS ($F(2,26) = 4.22$, $p = 0.026$). No significant differences between strains were found for SWA (0.5–4.0 Hz) or the slow oscillation (0.5–2.0 Hz) and delta (2.0–4.0 Hz) sub-frequency bands (all *p*'s > 0.2).

Analysis of spindles during SWS revealed a slight increase in spindle density (number of spindles per minute SWS) from the beginning to the end of the light period, followed by a drop right after the lights were turned off. However, these dynamics were not significantly altered in the two transgenic strains (*p*'s > 0.3). Also average spindle density was comparable for the three strains (WT: 1.87 ± 0.07/min, PEPCK: 1.99 ± 0.13/min, GFAP: 1.92 ± 0.09/min, $p > 0.6$).

3.3. Sleep deprivation

Sleep deprivation by gentle handling decreased time asleep during the 6-h interval by 74.95 ± 1.08%, compared with the corresponding time interval in undisturbed conditions, 24 h earlier. Recovery sleep as well as differences in recovery sleep between the strains concentrated on the 6-h interval following sleep deprivation (Fig. 4). WT mice recovered primarily SWS. Accordingly, these mice showed a significant increase in SWS ($p = 0.040$) accompanied by an increase in slow wave activity during SWS ($p = 0.029$) in the 6-h interval following sleep deprivation, compared with the corresponding 6-h interval in undisturbed conditions, 24 h before, whereas time in REM sleep was unchanged and time in pre-REM even decreased ($p = 0.004$) during this interval. The rebound in SWS was likewise seen in GFAP mice ($p = 0.050$) but, not in PEPCK mice ($p = 0.333$), and both mutants did not exhibit any rebound in slow wave activity during SWS ($p > 0.271$). The respective genotype × time interactions failed to reach significance

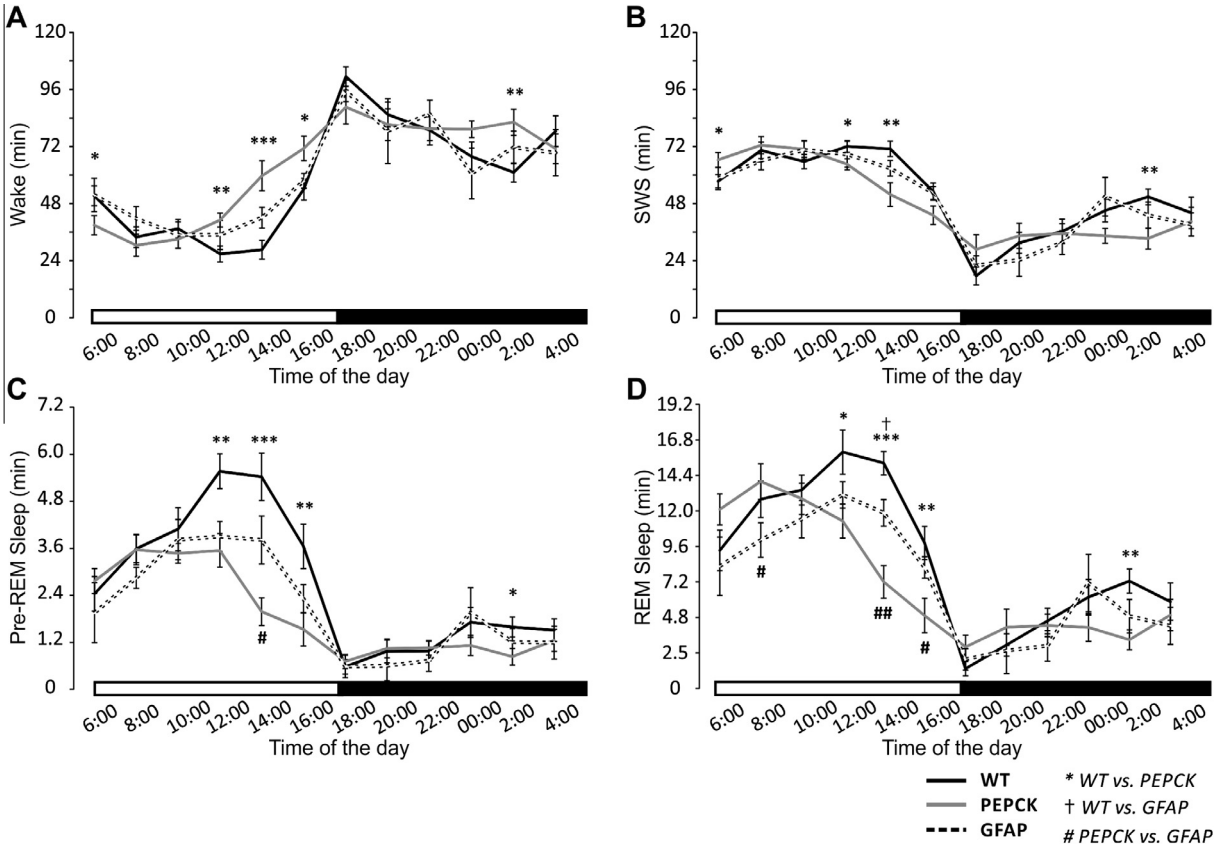

Fig. 1. Sleep during the 24-h cycle. Time (in min) spent (A) awake, (B) in SWS; (C) pre-REM sleep; and (D) REM sleep for succeeding 2-h intervals during light (empty bar on *x* axis) and dark period (black bar). Recordings started at 6:00 am. Wild-type (WT) mice – black lines, PEPCK mice – gray lines, GFAP mice – dashed line. Means ± SEM are shown. Significant differences (obtained from post hoc Student's *t*-tests, uncorrected for multiple comparisons) are indicated between WT and PEPCK mice by *, between WT and GFAP mice by †, between PEPCK and GFAP mice by # (single, double, triple symbols – $p < 0.05$, $p < 0.01$, $p < 0.001$, respectively).

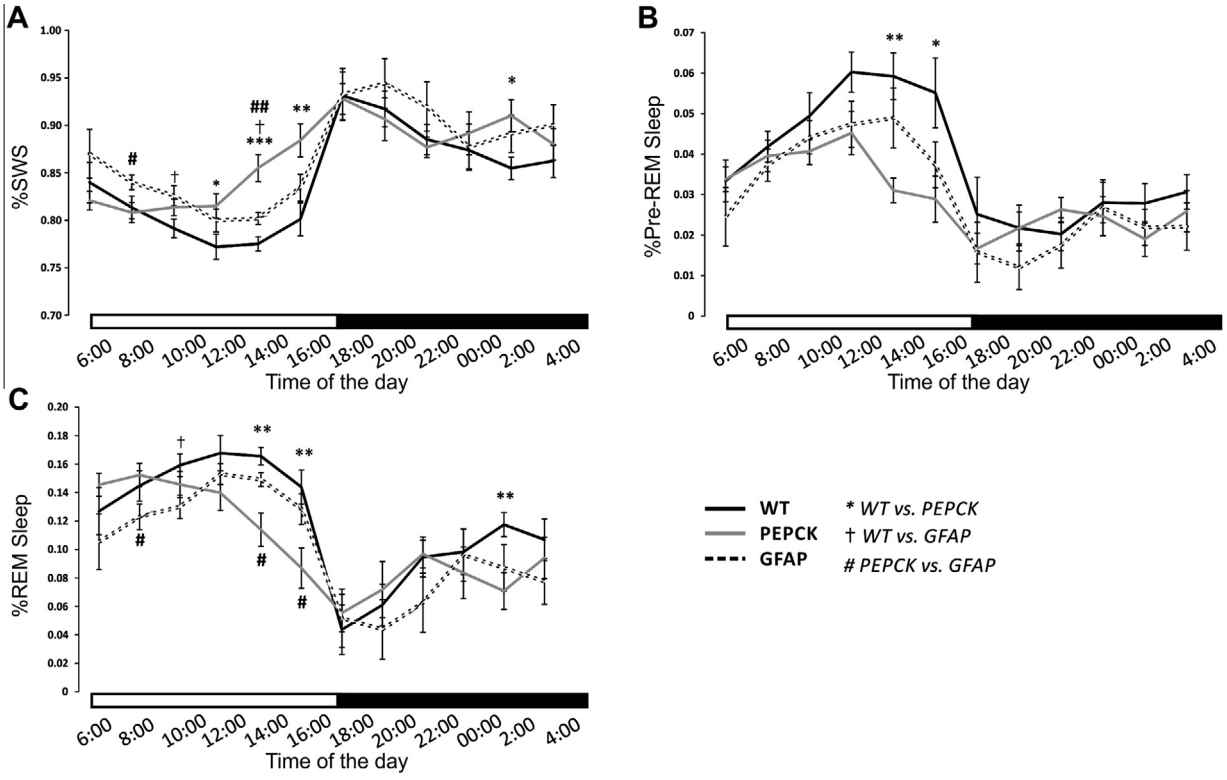

Fig. 2. Percentages (of time in sleep) of SWS (A), pre-REM sleep (B), and REM sleep (C) for succeeding 2-h intervals during the 24-h cycle in WT mice (black lines), PEPCK mice (gray lines), and GFAP mice (dashed lines). Significant (obtained from post hoc Student's *t*-tests, uncorrected for multiple comparisons) differences are marked as in Fig. 1.

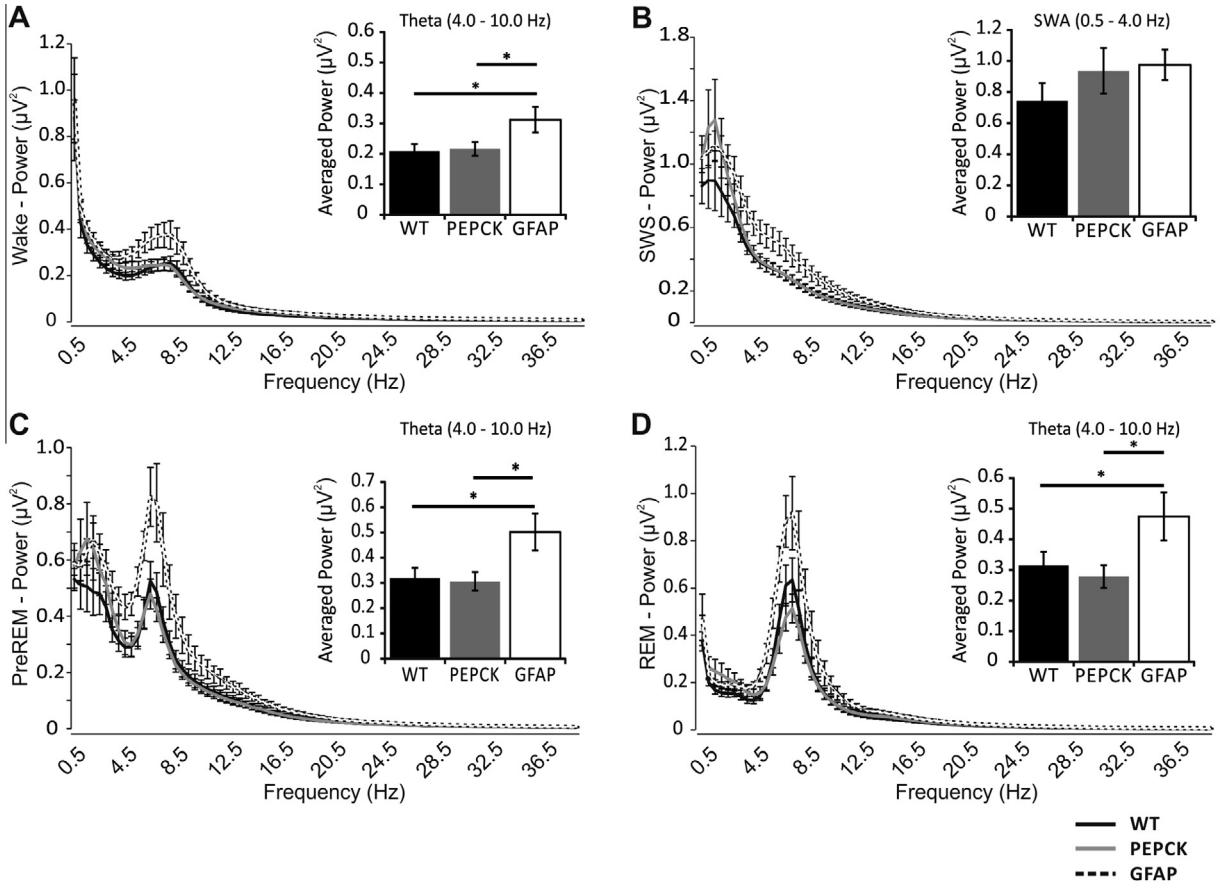

Fig. 3. EEG power spectra. Mean (±SEM) power spectra are shown separately for the four sleep/wake stages: (A) wake, (B) SWS, (C) pre-REM sleep, and (D) REM sleep, and separately for the three mouse strains (WT mice – black lines, PEPCK mice – gray lines, GFAP mice – dashed lines), across the whole 24-h cycle. Inserts show comparison of average power in selected frequency bands: theta power (4.0–10.0 Hz) during wake, pre-REM sleep, and REM sleep; slow wave activity (SWA; 0.5–4.0 Hz) during SWS. Significant differences (obtained from post hoc Student's *t*-tests, uncorrected for multiple comparisons) are indicated ($^{*}p \leqslant 0.05$).

(SWS rebound: $p > 0.130$, SWA rebound: $p > 0.316$). Interestingly, unlike WT mice, PEPCK mice showed a significant rebound of REM sleep during the 6-h recovery period ($p = 0.009$; $F(1,20) = 4.06$, $p = 0.058$ for genotype × time). Also, PEPCK mice, unlike WT mice, did not display any decrease in time in pre-REM during the recovery period ($p = 0.461$, $F(2,27) = 4.90$, $p = 0.015$, for genotype × time). Comparing the average time spent in the different sleep stages during the 6-h recovery period in particular confirmed the diverging sleep pattern in the PEPCK mice, which spent more time awake and less time in SWS and pre-REM sleep during this period ($F(2,27) = 7.15$, $p = 0.003$, ($F(2,27) = 7.23$, $p = 0.003$ and $F(2,27) = 6.74$, $p = 0.004$, respectively, for main effect of genotype, Fig. 4).

4. Discussion

We characterized the effects of peripheral and CNS blockade of IL-6 trans-signaling on sleep using two different transgenic mice strains, which express the selective IL-6 trans-signaling blocker sgp130Fc (Jostock et al., 2001) from the liver specific PEPCK and the astrocyte specific GFAP promoter. Our results show that blockade of IL-6 trans-signaling in the periphery and in the brain has distinct effects. Blocking IL-6 trans-signaling in the periphery (in PEPCK mice) suppressed sleep and thus increased the time animals spent awake, with this effect focusing on the second late half of the inactive (light) period. Concurrently, time in SWS, REM sleep and pre-REM sleep was diminished. Blocking IL-6 trans-signaling in the brain (in GFAP mice) slightly decreased REM sleep, in combination with a profound and persistent increase in EEG theta activity (4.0–10.0 Hz). The increase in theta activity was observed during virtually all wake and sleep states, and was most clearly seen in the states characterized by high theta activity, i.e., wakefulness, pre-REM sleep and REM sleep. Our data extend previous data in demonstrating an involvement of IL-6 trans-signaling in sleep regulation. We identify IL-6 trans-signaling in the periphery as a factor that contributes to the maintenance of sleep, probably via an activating influence on SWS generating mechanisms. By contrast, IL-6 trans-signaling in the CNS seems to mainly affect theta-generating networks, with this effect being independent of the brain state.

The mice models used in the present study to selectively block IL-6 trans-signaling in the CNS and periphery have been established in several previous studies (Rabe et al., 2008; Braun et al., 2013; Campbell et al., 2014). Measurements of soluble sgp130Fc levels in peripheral tissue (Rabe et al., 2008) and brain, confirmed highly increased peripheral sgp130Fc levels of 20–30 μg/ml in the PEPCK mice whereas they were below the detection threshold in brain tissue in these mice. Conversely, GFAP mice exhibited highly increased central sgp130Fc levels of 250–350 ng/ml in the perfused brain homogenates and no elevated sgp130Fc levels in the periphery. Moreover, the sgp130Fc protein was clearly detectable by Western blotting in the supernatant of primary astrocyte cultures from GFAP mice but not from WT mice (Campbell et al., 2014). It has been also shown that sgp130Fc does not cross the blood brain barrier under unchallenged conditions (Braun et al., 2013).

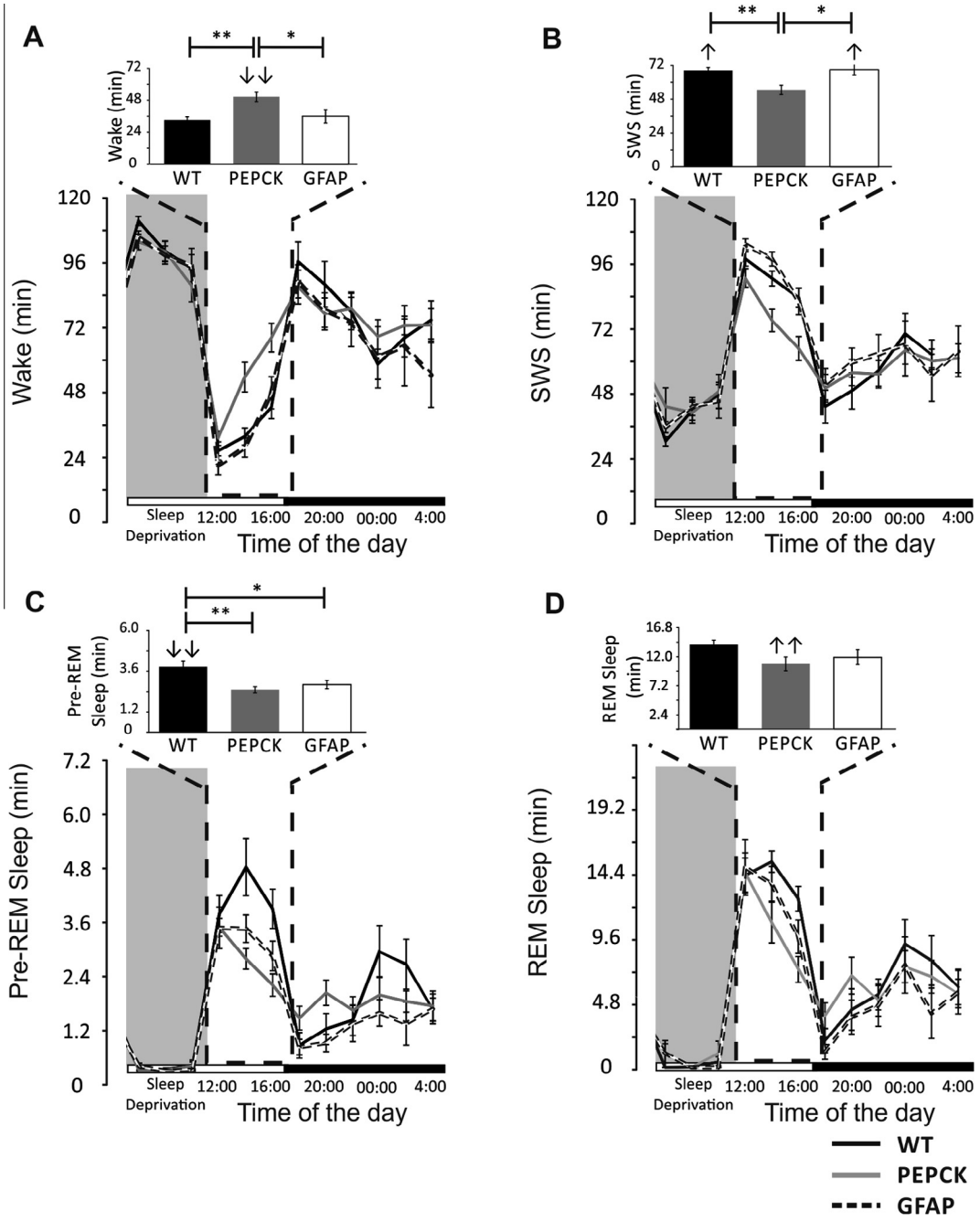

Fig. 4. Recovery sleep after sleep deprivation. Time (in min) spent (A) awake, (B) in SWS, (C) pre-REM sleep and (D) REM sleep during an 18-h interval following a 6-h interval of sleep deprivation (gray shaded area) during the early light period (empty bar on x axis). Wild-type (WT) mice – black lines, PEPCK mice – gray lines, GFAP mice – dashed lines. Means ± SEM are shown. Inserts show average time spent in the different stages in the 6-h interval immediately following sleep deprivation. Significant differences (obtained from post hoc Student's *t*-tests, uncorrected for multiple comparisons) are indicated: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. Arrows above the bar indicate significant decrease (↓) or increase (↑) with reference to the respective 6-h period in undisturbed sleep conditions, i.e., 24 h before (single, double symbols – $p < 0.05$, $p < 0.01$ respectively).

The most prominent effect of peripheral blockade of IL-6 trans-signaling (in the PEPCK mice) was the decrease in sleep time towards the late light period, with this decrease being accompanied by a reduction of number of episodes and time spent in each of the 3 sleep stages. As the percentage of SWS relative to the time spent in sleep, was enhanced during this period in the PEPCK mice it might be argued that the SWS process per se is preserved in these mice. However, probing sleep generating mechanisms by total sleep deprivation revealed clear signs of reduced SWS propensity in PEPCK mice. PEPCK mice not only exhibited reduced average time in SWS during the recovery period. They also failed to show a rebound in SWS or slow wave activity during this period when compared to their SWS levels during corresponding period of an undisturbed 24-h cycle. Different from WT mice, which primarily recovered SWS, PEPCK mice primarily recovered REM sleep during the 6-h period following sleep deprivation. The primary recovery of REM sleep is noteworthy, given that the sequential structure of sleep, with REM sleep always following SWS, is intact in PEPCK mice. Indeed, it speaks for an effect of peripheral IL-6 trans-signaling on mechanisms inducing sleep that in such conditions would promote, in the first place, SWS. Yet, the possibility cannot be entirely excluded that peripheral IL-6 trans-signaling

additionally directly suppresses REM sleep. This view would not only be consistent with the profound recovery of REM sleep observed in the PEPCK mice after total sleep deprivation but also with findings in IL-6 knock-out mice, which showed generally enhanced REM sleep (Morrow and Opp, 2005a).

Contrasting with the effects of peripheral blockade, CNS blockade of IL-6 trans-signaling in the GFAP mice did not alter sleep architecture, except for a slight decrease in REM sleep. The much more prominent change in GFAP mice was the persistent increase in theta activity. These changes strikingly complement the opposite effects observed after stimulating central IL-6 trans-signaling by intracerebroventricular administration of the designer cytokine Hyper-IL-6 (May et al., 2009). Interestingly, stimulation of classic signaling by the injection of IL-6 did not show this effect. Hyper-IL-6 reduced EEG theta activity, and this effect was accompanied by increased time in REM sleep. In combination, these results reveal theta-generating networks as a primary target of central nervous IL-6 trans-signaling. The theta increase in the EEG most likely reflects theta rhythm in the hippocampus as the major source of this rhythm in the rodent brain (Vanderwolf and Leung, 1983; Buzsáki, 2002). Considering the strong involvement of this rhythm in processing of spatial and episodic memory information (Whishaw and Vanderwolf, 1973; Buzsáki, 2005), future studies are indicated to examine the functionality of the increase in theta activity characterizing the GFAP mice.

While our results indicate that sleep-regulatory actions of IL-6 via the trans-signaling pathway mainly originate from the body periphery rather than CNS, we can only speculate about the mediating mechanisms and cells that convey influences of peripheral IL-6 trans-signaling to central nervous sleep-generating networks. The gp130 protein is expressed by virtually all cells in the body. Consequently, quite different pathways might be involved in this mediation process, including direct actions on afferent neurons of the vagus nerve (Goehler et al., 2000; Garcia-Oscos et al., 2015), on endocrine signals that cross the blood brain barrier (e.g., Späth-Schwalbe et al., 1996), or on cells of the blood–brain barrier regulating its permeability for other sleep-regulating signals (Brunello et al., 2000). Whatever the mediating mechanisms are, it is to note that the present alterations in sleep observed in PEPCK mice after peripheral blockade of IL-6 trans-signaling are well in line with major findings from previous studies examining influences on sleep following experimental manipulation of the IL-6 signal itself, rather than manipulating receptor pathways. Thus, IL-6 knock-out mice showed a delayed recovery of sleep after sleep deprivation (Morrow and Opp, 2005a), and when treated with lipopolysaccharide displayed diminished increases in SWS (Morrow and Opp, 2005b). In healthy men, administration of IL-6 enhanced SWS (Späth-Schwalbe et al. 1998; Benedict et al., 2009), and in pathological conditions, increased blood IL-6 concentrations were found to be associated with signs of sickness behavior including sleepiness (Vgontzas et al., 2005). Collectively these findings converge to the view that peripheral IL-6 via trans-signaling supports sleep by promoting sleep and SWS. The view is further corroborated by findings indicating that sleep is a condition substantially increasing circulating soluble IL-6 receptors and is, thus, associated with an upregulated trans-signaling in the body periphery (Dimitrov et al. 2006).

Our findings indicating stronger sleep-regulatory influences of peripheral than central IL-6 trans-signaling might surprise. Why should a peripheral cytokine signal be more potent than a central in promoting sleep? Possibly, this relates to the function of IL-6, signaling to the brain an increased need for sleep in conditions of acute infection, which mainly arise in the body periphery. Indeed, sleep-regulatory effects of IL-6 were also revealed to essentially depend on the presence or absence of an immune challenge (Morrow and Opp, 2005b; Campbell et al., 2014). Hence, the full characterization of the role of IL-6 in sleep regulation requires not only to directly compare effects of blocking trans-signaling and classical pathways, but should also comprise examinations of the two pathways in immunologically challenged and unchallenged conditions.

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References

Benedict, C., Scheller, J., Rose-John, S., Born, J., Marshall, L., 2009. Enhancing influence of intranasal interleukin-6 on slow-wave activity and memory consolidation during sleep. FASEB J. 23, 3629–3636.
Besedovsky, L., Lange, T., Born, J., 2012. Sleep and immune function. Eur. J. Physiol. 463, 121–137.
Braun, O., Dewitz, C., Möller-Hackbarth, K., Scheller, J., Schiffelholz, T., Baier, P.C., Rose-John, S., 2013. Effects of blockade of peripheral interleukin-6 trans-signaling on hippocampus-dependent and independent memory in mice. J. Interferon Cytokine Res. 33, 254–260.
Brunello, A.G., Weissenberger, J., Kappeler, A., Vallan, C., Peters, M., Rose-John, S., Weis, J., 2000. Astrocytic alterations in interleukin-6/soluble interleukin-6 receptor α double-transgenic mice. Am. J. Pathol. 157, 1485–1493.
Burgos, I., Richter, L., Klein, T., Fiebich, B., Feige, B., Lieb, K., Voderholzer, U., Riemann, 2006. Increased nocturnal interleukin-6 excretion in patients with primary insomnia: a pilot study. Brain Behav. Immun. 20, 246–253.
Buzsáki, G., 2002. Theta oscillations in the hippocampus. Neuron 33, 325–340.
Buzsáki, G., 2005. Theta rhythm of navigation: link between path integration and landmark navigation, episodic and semantic memory. Hippocampus 15, 827–840.
Campbell, I.L., Erta, M., Lim, S.L., Frausto, R., May, U., Rose-John, S., Scheller, J., Hidalgo, J., 2014. Trans-signaling is a dominant mechanism for the pathogenic actions of interleukin-6 in the brain. J. Neurosci. 34, 2503–2513.
Clinton, J.M., Davis, C.J., Zielinski, M.R., Jewett, K.A., Krueger, J.M., 2011. Biochemical regulation of sleep and sleep biomarkers. J. Clin. Sleep Med. 7, S38–S42.
Dimitrov, S., Lange, T., Benedict, C., Nowell, M.A., Jones, S.A., Scheller, J., Rose-John, S., Born, J., 2006. Sleep enhances IL-6 trans-signaling in humans. FASEB J. 20, 2174–2176.
Eschenko, O., Mölle, M., Born, J., Sara, S.J., 2006. Elevated sleep spindle density after learning or after retrieval in rats. J. Neurosci. 26, 12914–12920.
Fischer, M., Goldschmitt, J., Peschel, C., Brakenhoff, J.P., Kallen, K.J., Wollmer, A., Grötzinger, J., Rose-John, S., 1997. A bioactive designer cytokine for human hematopoietic progenitor cell expansion. Nat. Biotechnol. 15, 142–145.
Garcia-Oscos, F., Peña, D., Housini, M., Cheng, D., Lopez, D., Borland, M.S., Salgado, R., Salgado, H., D'Mello, S., Kilgard, M.P., Rose-John, S., Atzori, M., 2015. Vagal nerve stimulation block interleukin 6-dependent synaptic hyperexcitability induced by lipopolysaccharide-induced acute stress in the rodent prefrontal cortex. Brain Behav. Immun. 43, 149–158.
Goehler, L.E., Gaykema, R.P.A., Hansen, M.K., Anderson, K., Maier, S.F., Watkins, L.R., 2000. Vagal immune-to-brain communication: a visceral chemosensory pathway. Auton. Neurosci. 85, 49–59.
Heinrich, P.C., Behrmann, I., Haan, S., Hermanns, H.M., Müller-Newen, G., Schaper, F., 2003. Principles of interleukin (IL)-6-type cytokine signaling and its regulation. Biochem. J. 374, 1–20.
Hogan, D., Morrow, J.D., Smith, E.M., Opp, M.R., 2003. Interleukin-6 alters sleep of rats. J. Neuroimmunol. 137, 59–66.
Imeri, L., Opp, M.R., 2009. How (and why) the immune system make us sleep. Nat. Rev. Neurosci. 10, 199–210.
Jewett, K.A., Krueger, J.M., 2012. Humoral Sleep Regulation; interleukin-1 and tumor necrosis factor. Vitam. Horm. 89, 241–257.
Jostock, T., Müllberg, J., Özbek, S., Atreya, R., Blinn, G., Voltz, N., Fischer, M., Neurath, M.F., Rose-John, S., 2001. Soluble gp130 is the natural inhibitor of soluble interleukin-6 receptor transsignaling responses. Eur. J. Biochem. 268, 160–167.
Krueger, J.M., 2008. The role of cytokines in sleep regulation. Curr. Pharm. Des. 14, 3408–3416.
Lange, T., Dimitrov, S., Born, J., 2010. Effects of sleep and circadian rhythm on the human immune system. Ann. N.Y. Acad. Sci. 1193, 48–59.
März, P., Cheng, J.G., Gadient, R.A., Patterson, P., Stoyan, T., Otten, U., Rose-John, S., 1998. Sympathetic neurons can produce and respond to interleukin-6. Proc. Natl. Acad. Sci. U.S.A. 95, 3251–3256.
März, P., Heese, K., Dimitriades-Schmutz, B., Rose-John, S., Otten, U., 1999. Role of interleukin-6 and soluble IL-6 receptor in region-specific induction of astrocytic differentiation and neurotrophin expression. Glia 26, 191–200.

May, U., Schiffelholz, T., Baier, P.C., Krueger, J.M., Rose-John, S., Scheller, J., 2009. IL-6-trans-signaling increases rapid-eye-movement sleep in rats. Eur. J. Pharmacol. 613, 141–145.

Morrow, J.D., Opp, M.R., 2005a. Sleep-wake behavior and responses of interleukin-6-deficient mice to sleep deprivation. Brain Behav. Immun. 19, 28–39.

Morrow, J.D., Opp, M.R., 2005b. Diurnal variation of lipopolysaccharide-induced alterations in sleep and body temperatures of interleukin-6-deficient mice. Brain Behav. Immun. 19, 40–51.

Neckelmann, D., Olsen, O.E., Fagerland, S., Ursin, R., 1994. The reliability and functional validity of visual and semiautomatic sleep/wake scoring in the Møll-Wistar rat. Sleep 17, 120–131.

Opp, M.R., Obal Jr., F., Cady, A.B., Johannsen, L., Krueger, J.M., 1989. Interleukin-6 is pyrogenic but not somnogenic. Physiol. Behav. 45, 1069–1072.

Rabe, B., Chalaris, A., May, U., Waetzig, G.H., Seegert, D., Williams, A.S., Jones, S.A., Rose-John, S., Scheller, J., 2008. Transgenic blockade of interleukin 6 transsignaling abrogates inflammation. Blood 111, 1021–1028.

Riemann, D., Kloepfer, C., Berger, M., 2009. Functional and structural brain alterations in insomnia: implications for pathophysiology. Eur. J. Neurosci. 29, 1754–1760.

Rose-John, S., 2012. IL-6 trans-signaling via the soluble IL-6 receptor: importance for the pro-inflammatory activities of IL-6. Int. J. Biol. Sci. 8, 1237–1247.

Schmidt, E.M., Linz, B., Diekelmann, S., Besedovsky, L., Lange, T., Born, J., 2015. Effects of an interleukin-1 receptor agonist on human sleep, sleep-associated memory consolidation, and blood monocytes. Brain Behav. Immun. (Epub ahead of print).

Späth-Schwalbe, E., Schrezenmeier, H., Bornstein, S., Burger, K., Porzsolt, F., Born, J., 1996. Endocrine effects of recombinant interleukin 6 in man. Neuroendocrinology 63, 237–243.

Späth-Schwalbe, E., Hansen, K., Schmidt, F., Schrezenmeier, H., Marshall, L., Burger, K., Fehm, H.L., Born, J., 1998. Acute effects of recombinant human interleukin-6 on endocrine and central nervous sleep functions in healthy men. J. Clin. Endocr. Metab. 83, 1573–1579.

Takahashi, S., Kapás, L., Fang, J., Krueger, J.M., 1999. Somnogenic relationships between tumor necrosis factor and interleukin-1. Am. J. Physiol. 276, R1132–R1140.

Vanderwolf, C.H., Leung, L.S., 1983. Hippocampal rhythmical slow activity: a brief history and effects of entorhinal lesions and phencyclidine. In: Seifert, W. (Ed.), The Neurobiology of the Hippocampus. Academic Press, London, pp. 275–302.

Vgontzas, A.N., Zoumakis, M., Bixler, E.O., Lin, H.M., Prolo, P., Vela-Bueno, A., Kales, A., Chrousos, G.P., 2003. Impaired nighttime sleep in healthy old *versus* young adults is associated with elevated plasma interleukin-6 and cortisol levels: physiologic and therapeutic implications. J. Clin. Endocr. Metab. 88, 2087–2095.

Vgontzas, A.N., Bixler, E.O., Lin, H.M., Prolo, P., Trakada, G., Chrousos, G.P., 2005. IL-6 and its circadian secretion in humans. NeuroImmunoModulation 12, 131–140.

Whishaw, I.Q., Vanderwolf, C.H., 1973. Hippocampal EEG and behavior: changes in amplitude and frequency of RSA (theta rhythm) associated with spontaneous and learned movement patterns in rats and cats. Behav. Biol. 8, 461–484.