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Sleep promotes T-cell migration towards CCL19 via growth hormone and prolactin signaling in humans

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ABSTRACT

Sleep strongly supports the formation of adaptive immunity, e.g., after vaccination. However, the underlying mechanisms remain largely obscure. Here we show in healthy humans that sleep compared to nocturnal wakefulness specifically promotes the migration of various T-cell subsets towards the chemokine CCL19, which is essential for lymph-node homing and, thus, for the initiation and maintenance of adaptive immune responses. Migration towards the inflammatory chemokine CCL5 remained unaffected. Incubating the cells with plasma from sleeping participants likewise increased CCL19-directed migration, an effect that was dependent on growth hormone and prolactin signaling. These findings show that sleep selectively promotes the lymph node homing potential of T cells by increasing hormonal release, and thus reveal a causal mechanism underlying the supporting effect of sleep on adaptive immunity in humans.

1. Introduction

Sleep is a profound regulator of adaptive immunity (Besedovsky et al., 2019; Irwin, 2019). This has been shown e.g. in the context of vaccination: in experimental studies in humans, sleep after vaccination doubled the antigen-specific antibody and T-cell response compared with staying awake at night (Lange et al., 2011; Lange et al., 2003). Such findings are corroborated by observational studies of larger populations demonstrating a close association between short habitual sleep duration and reduced responses to vaccinations (Prather et al., 2012), and by a recent *meta*-analysis showing that objectively-assessed short sleep duration was consistently associated with a reduced immune response to anti-viral vaccination across studies (Spiegel et al., 2023). The underlying mechanisms of this effect of sleep on the formation of adaptive immunity are not well understood. Based on the robust finding that sleep, compared with nocturnal wakefulness, acutely reduces the number of T cells in blood (Besedovsky et al., 2016; Born et al., 1997), it has been suggested that sleep fosters the extravasation of T cells and their subsequent redistribution to lymph nodes (LN), where adaptive immune responses are initiated (Butcher and Picker, 1996; von Andrian and Mackay, 2000). Given that a higher number of T cells recruited to LN is associated with a stronger adaptive immune response (Pulendran and Ahmed, 2006; Soderberg et al., 2005), a promoting effect of sleep on T-cell migration to LN could be a mechanism underlying the potentiation of vaccination responses by sleep. Animal studies have shown a reduced lymphocyte number in LN (Ruiz et al., 2017) and a decrease in the mRNA expression levels of genes important for immune cell recruitment to LN in sleep-deprived mice (Tune et al., 2020). Specifically, Tune et al. (2020) found that sleep deprivation reduced mRNA levels of the selectin CD62L, which is highly expressed on naïve and central memory T cells and enables their rolling along the endothelial walls (Raffler et al., 2005), the chemokine C-C motif chemokine ligand 19 (CCL19), which is expressed in high endothelial venules (HEVs) in LN, and of its receptor C-C motif chemokine receptor 7 (CCR7) expressed on naïve and central memory T cells (Stein and Nombela-Arrieta, 2005). However, so far evidence that sleep indeed fosters lymphocyte migration towards LN in humans is lacking.

In the present study, we therefore investigated in healthy humans whether sleep as well as the associated endocrine milieu increase the migration of various T-cell subpopulations towards the LN-homing

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chemokine CCL19. To test whether a potential effect of sleep on T-cell migration is specific to CCL19 or whether sleep rather increases reactivity towards chemokines in general, we also tested the effect on the migration towards another chemokine. For this purpose, we selected CCL5 (also known as RANTES), because it is a main regulator of T-cell recruitment to inflammatory sites (Appay and Rowland-Jones, 2001) and thus serves a very different function than CCL19. We show that sleep compared to nocturnal wakefulness indeed selectively increases T-cell migration towards CCL19 but not towards CCL5 in healthy males and females. We also demonstrate that this effect of sleep can be mimicked *in vitro* by incubating T cells with plasma from sleeping participants. This effect was dependent on growth hormone (GH) and prolactin (PRL), two

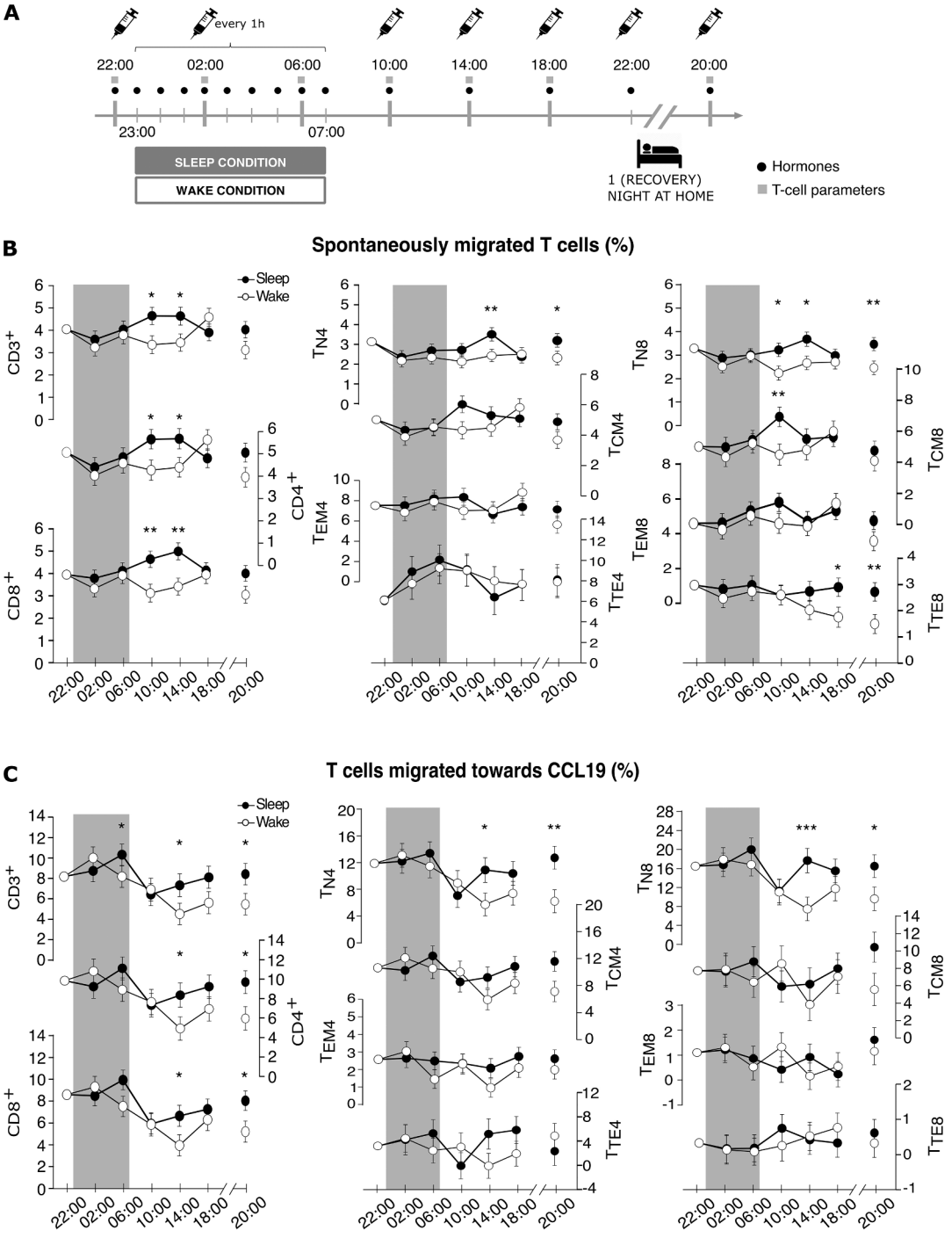

Fig. 1. Sleep increases the spontaneous and CCL19-directed migration of different T-cell subsets. (A) Healthy participants spent two 24-hour sessions in the sleep laboratory with repeated blood sampling for the determination of T-cell parameters (gray squares) and hormones (black circles). In the Sleep condition, participants had regular sleep at night while in the Wake condition, they spent the 24-hour period awake while staying in bed during the night-time period. They spent the subsequent night at home before returning the next day to the laboratory for a further blood sampling at 20:00 h. (B) Estimated marginal means ± SEM of the percentage of migrated $CD3^+$, $CD4^+$, and $CD8^+$ T cells and their T_N, T_{CM}, T_{EM}, and T_{TE} $CD4^+$ and $CD8^+$ subsets in the absence of any exogenous chemokine (i.e., spontaneous migration) or (C) in the presence of the chemokine CCL19 during a regular sleep-wake cycle (filled circles) and during 24 h of continuous wakefulness (open circles). Gray area indicates time in bed. n = 14 (7 males, 7 females). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ for pairwise comparisons between conditions. See Table 1 for overall results of the linear mixed models analyses.

hormones that support adaptive immune functions and are released in high amounts during sleep.

2. Materials and methods

2.1. Participants and study design

Fourteen healthy participants, 7 women and 7 men (mean ± SD age: 23.9 ± 3.37 years), took part in this study. None of the participants had a medical history of any chronic disease or mental disorder, including depression, anxiety, and sleep disorders as assessed by self-report. All participants reported having regular sleep/wake patterns (i.e., usual sleep between 23:00 h ± 1 h and 07:00 h ± 1 h). Acute illness was excluded by physical examination and routine laboratory investigation. Participants did not show any abnormalities in the polysomnographic analysis suggestive of a sleep disorder, did not take any medications at the time of the experiment (except oral contraceptives in females), and were nonsmokers. Further inclusion criteria were age between 18 and 30 years, a body-mass index between 20–25 kg/m^2, no shift work or intercontinental flights within the last 6 weeks, no alcohol or drug dependence, and no blood or plasma donation within the last 4 weeks. All of these measures were collected by self-report. On the days of the experiment, participants refrained from drinking alcohol and avoided having large physical or emotional effort. In addition, they refrained from drinking caffeinated beverages and eating chocolate after 12:00 h. All participants gave written informed consent prior to participation in accordance with the Declaration of Helsinki. The study was approved by the Ethics Committee of the University of Tübingen.

The sample size was calculated using a power analysis based on a previous study investigating the effects of a sleep manipulation on circulating T-cell numbers (Besedovsky et al., 2016). The number of circulating T cells is closely related to T-cell migration, which was the primary outcome of interest in the present study. The above-mentioned study found effect sizes of $\eta_p^2 = 0.171$ and 0.228 for CD4$^+$ and CD8$^+$ T cells, respectively, for the ANOVA interaction effect Condition (sleep vs. wake) x Time point. Using a significance criterion of $\alpha = 0.05$ and a power of 0.8, a sample size of $n = 12$ is sufficient to detect such effects.

The study was designed according to a randomized, within-subjects cross-over study, in which the participants spent two 24-hour experimental sessions in the sleep laboratory. In both conditions, participants arrived at the laboratory at 20:30 h for the preparation of blood sampling and polysomnographic recordings, and stayed until 22:30 h the next day. In the Sleep condition, they were allowed to sleep for an eight-hour period starting at 23:00 h (lights off ± 15 min), whereas in the Wake condition, they spent the 24-hour session continuously awake and stayed in bed in a half-supine position during night time (Fig. 1A). During this time, they were allowed to watch TV, listen to music, and talk to the experimenter at dim room light (i.e., < 6 lx measured at the eye level, to minimize effects on melatonin secretion). Both experimental sessions were separated by at least four weeks and the order of conditions was balanced across participants. In order to control for the influence of the menstrual hormones, both conditions took place in the same phase of the menstrual cycle in the female participants. Previous to the beginning of the experiments, the participants spent one adaptation night in the laboratory to become accustomed to the experimental setting. During the week prior to the experimental night and until the last blood sampling, they filled in a sleep diary and wore an actigraph (MotionWatch 8, ©CamNtech Ltd, Cambridgeshire, UK) in order to ensure regular sleep.

Blood for assessment of T-cell numbers, T-cell migration, and hormone concentration was sampled via an intravenous forearm catheter that was connected to a long thin tube that enabled blood collection from an adjacent room without disturbing the participant's sleep. We processed the blood immediately after sampling. To prevent clotting, we infused approximately 800 mL of saline solution during the experimental period.

For lymphocyte phenotyping and assessment of T-cell migration, blood was sampled every 4 h from 22:00 h (baseline) until 18:00 h of the next day. In addition to these time points, we collected blood every hour from 22:00 h until 7:00 h during the experimental night and at 22:00 h on the following day for measurement of hormone concentration. During the time in between blood sampling, the participants stayed in the laboratory with the experimenter to control that all of them engaged in comparable activities and they had regular walks outside. The participants received meals at 08:00 h, 12:00 h, and 18:15 h.

After the last blood sampling at 22:00 h, the participants left the laboratory and had a regular night of sleep at home. The next day, they engaged in their usual activities and came back to the laboratory for an extra blood sampling at 20:00 h to assess whether any effect of the sleep manipulation persisted after recovery sleep.

2.2. Polysomnography and sleep EEG analyses

Sleep was determined from polysomnographic recordings from electrodes attached at positions C3 and C4 (according to the international 10–20 system, referenced to electrodes attached to the mastoids) as well as bipolar electrooculographic, electromyographic, and electrocardiographic electrodes. Sleep stages were determined off-line for subsequent 30-s recording epochs following standard criteria (Rechtschaffen, 1968).

2.3. Measurement of T-cell subpopulations

Using a "lyse no-wash" flow cytometry procedure, we determined the absolute counts of total CD3$^+$ T cells, CD4$^+$ T cells, CD8$^+$ T cells as well as their naïve (CD45RA$^+$CD62L$^+$), central memory (CD45RA$^-$CD62L$^+$), effector memory (CD45RA$^-$CD62L$^-$), and terminally differentiated effector (CD45RA$^+$CD62L$^-$) subsets (see Supplementary Fig. S1 for the specific gating strategy). We immunostained 90 µl of whole blood with anti-CD3/BV510, anti-CD4/BV421, anti-CD8/PerCP, anti-CD45RA/AF700, and anti-CD62L/FITC (Catalogue numbers: 317332, 357424, 980916, 304120, 304838; all from BioLegend, San Diego, CA, US), in Trucount^{TM} tubes (BD Biosciences, San Jose, CA, US). After 15 min of incubation at room temperature in the dark, we added 900 µl of FACS lysing solution (BD Biosciences) and incubated the cells for another 15 min. We acquired at least 10,000 CD3$^+$ cells on a FACSCalibur using DIVA Software (BD Biosciences) and calculated the absolute number of cells per µl using the following formula: cells/µl = (acquired cell events in the respective gate) x (number of beads per tube)/((acquired bead events) x (sample volume [µl])).

2.4. Transwell chemotaxis assay

We performed chemotaxis assays using 5-µm pore-sized polycarbonate membrane Transwell® inserts (Corning Incorporated, Tewksbury, MA, US). Briefly, we added 200 µl of a 1:4 dilution of the participant's fresh whole blood and RPMI 1640 (Gibco, Life Technologies Corporation, Carlsbad, CA, US) to the upper chamber. In the lower chamber, we added RPMI with Recombinant Human CCL19 (400 ng/ml; 582104, BioLegend), Recombinant Human CCL5 (200 ng/ml; 580204, BioLegend), or RPMI alone (for assessment of spontaneous migration), in a total volume of 600 µl. After 4 h of incubation at 37 °C and 5 % CO_2, we collected 100 µl of the liquid from both, the upper and lower chambers, and immunostained them with anti-CD3/BV510, anti-CD4/BV421, anti-CD8/PerCP, anti-CD45RA/AF700, and anti-CD62L/FITC (Catalogue numbers: 317332, 357424, 980916, 304120, 304838; all from BioLegend). We counted the number of cells that had migrated to the lower chamber as well as the number of cells in the upper chamber with a BD LSRFortessa flow cytometer, and calculated a migration index: [number of migrated cells / number of non-migrated cells] x 100. Targeted migration towards CCL19 and CCL5 was calculated by subtracting their migration index from the RPMI control. Besides CCL19, also CCL21

binds to the LN-homing receptor CCR7. However, we focused here on CCL19, because it seems to be more specifically involved in T-cell migration to LN and has a higher affinity for CCR7 than CCL21 (Lo et al., 2003; Yoshida et al., 1998), which was also confirmed by our pilot experiments, in which we observed a stronger migration of T cells towards CCL19 than CCL21.

2.5. Hormones assays

Blood for assessment of GH, PRL, and cortisol concentrations was centrifuged immediately and then stored at −80 °C until assay. The hormones were measured using commercial assays (Siemens Healthcare Diagnostics Inc., Tarrytown, NY, US). The lower limit of detection was 0.05 μg/l for GH, 0.3 μg/l for PRL, and 5.5 nmol/l for cortisol.

2.6. In vitro experiments

We incubated blood, sampled at 18:00 h, from seven additional healthy participants with sleep-like concentrations of GH (10 μg/l; PHH0014, Gibco, Life Technologies Corporation, Carlsbad, CA, US), and PRL (20 μg/l; SRP9000, Sigma-Aldrich, Saint Louis, MO, US), or with phosphate buffered saline (PBS) (as a "no hormone" control condition) for 2 h at 37 °C and 5 % CO_2. We then performed a Transwell® chemotaxis assay using the same procedure as for the *in vivo* experiment. The indicated sleep-like concentrations refer to peak concentrations of the hormones during sleep as derived from previous studies (Dimitrov et al., 2004; Lange et al., 2006).

2.7. Plasma experiments

Blood sampled at 18:00 h from three healthy donors was incubated with plasma that had been collected at 02:00 h from seven participants of the *in vivo* experiment and was kept frozen at −80 °C until the day of the experiment. On the day of the experiment, freshly sampled whole blood from the donor participants was incubated with plasma collected in the sleep condition, plasma collected in the wake condition, plasma collected in the sleep condition + Human Growth Hormone Receptor Antibody (1 ug/ml; AF1210), and plasma collected in the sleep condition + Human Prolactin Receptor Antibody (400 ng/ml; MAB1167; both from R&D Systems, Minneapolis, MN, US). The blood was incubated with the plasma mixtures in a 1:4 dilution for 2 h at 37 °C and 5 % CO_2. Afterwards, a Transwell® chemotaxis assay was performed using the same procedure as for the *in vivo* and *in vitro* experiments. Experiments were performed in duplicates.

2.8. Statistical analysis

We performed all statistical analyses using IBM SPSS Statistics, version 26 (IBM Corp., Armonk, N.Y., US). We applied linear mixed models to assess the influence of sleep on T-cell numbers and migration, with "Condition" (wake vs. sleep), "Time" (22:00 h, 02:00 h, 06:00 h, 10:00 h, 14:00 h, 18:00 h, and 20:00 h of the subsequent day), and "Condition x Time" as fixed effects, and "participant" as random effect. The baseline measurement (at 22:00 h) was entered as a covariate. To assess the effect of sleep on hormone concentration, we performed comparable linear mixed models with "Condition" (wake vs. sleep), "Time" (22:00 h, 02:00 h, 06:00 h, 10:00 h, 14:00 h, 18:00 h, 22:00 h, and 20:00 h of the subsequent day), and "Condition x Time" as fixed effects, "Participant" as random effect, and with the baseline measure as a covariate. We also run a model dividing the time points according to Early (22:00 h-03:00 h) and Late night (04:00 h-07:00 h) to study the acute effect of sleep on hormonal levels. For the *in vitro* and plasma experiments analyses, paired-samples t tests were applied to analyze differences between conditions. A p value < 0.05 was considered statistically significant. Data was tested for normality with the Kolmogorov-Smirnov test.

3. Results

3.1. Sleep selectively increases the migration of T cells towards the LN-homing chemokine CCL19

Sleep compared to continuous wakefulness selectively promoted the migratory potential of various T-cell subpopulations. Specifically, sleep increased the spontaneous migration of total $CD3^+$, $CD4^+$, and $CD8^+$ T cells, with a significant difference during the day after the experimental night at 10:00 h and at 14:00 h (Fig. 1B, see Table 1 for results of linear mixed models analyses). Within the eight subpopulations measured (i.e., $CD4^+$ and $CD8^+$ naïve (T_{N4}, T_{N8}), central memory (T_{CM4}, T_{CM8}), effector memory (T_{EM4}, T_{EM8}), and terminally differentiated effector (T_{TE4}, T_{TE8}) T cells), sleep increased the spontaneous migration of T_{N4}, T_{N8}, T_{CM8}, and T_{TE8} (Fig. 1B, Table 1). Importantly, for T_{N4}, T_{N8}, and T_{TE8}, the differences between conditions in spontaneous migration were still present even after a night with (recovery) sleep at home.

On top of the promotion of spontaneous migration, sleep increased the directed migration of total $CD3^+$, $CD4^+$, and $CD8^+$ T cells, as well as T_{N4} and T_{N8} towards the chemokine CCL19 (Fig. 1C, Table 1), which is essential for homing of T cells to LN (Ebert et al., 2005). Again, these differences between conditions were still present even after a full night of (recovery) sleep. A similar effect was evident in T_{CM4} and T_{CM8} subsets, although it did not reach significance. As expected, sleep did not affect the CCL19-directed migration of T_{EM} and T_{TE}, which do not express CCR7, the receptor of CCL19 (Mahnke et al., 2013). Importantly, sleep also did not influence, in any of the subsets studied, the migration towards the chemokine CCL5 (Table 1, Supplementary Fig. S2), which promotes lymphocyte migration to sites of inflammation (Appay and Rowland-Jones, 2001).

As already shown in previous studies (Besedovsky et al., 2016; Born et al., 1997), sleep reduced the number of circulating $CD3^+$, $CD4^+$, and $CD8^+$ T cells, as well as the counts of all $CD4^+$ and $CD8^+$ T-cell subsets during the night except for the T_{TE4} population (Supplementary Fig. S3, Supplementary Table S1).

3.2. GH and PRL in sleep-like concentrations promote CCL19-directed migration

Hormonal changes during sleep have been suggested as potential mediators of the effects of sleep on peripheral immune functions (Besedovsky et al., 2019; Rolls et al., 2015). GH and PRL are two immune-modulating hormones that are among the hormones most strongly regulated by sleep (Spiegel et al., 1994; Takahashi et al., 1968). As expected (e.g., refs (Besedovsky et al., 2012; Lange et al., 2010)), levels of both hormones were increased in the Sleep condition compared to the Wake condition, while cortisol levels remained unchanged (Supplementary Fig. S4). In subsequent *in vitro* experiments, we therefore examined effects of GH and PRL in sleep-like concentrations on T-cell migration as potential endocrine mediators of the observed sleep effect. We focused on T_N and T_{CM} subsets, which recirculate through LN and showed robust migration towards CCL19 (Fig. 1C). Following a short incubation period of 2 h, GH specifically increased the migration of total $CD3^+$ and $CD4^+$ T cells as well as T_{N4} and T_{N8} towards CCL19 compared to a PBS control (Fig. 2A and B), without affecting the spontaneous migration (Supplementary Fig. S5).

PRL also promoted T-cell migration towards CCL19, with even broader effects than those of GH, including effects on total $CD3^+$, $CD4^+$, and $CD8^+$ T cells, T_{N4}, T_{N8}, T_{CM4}, and T_{CM8} (Fig. 2A and B). Like GH, PRL did not affect the spontaneous migration of the cells (Supplementary Fig. S5).

3.3. Incubation of T cells with plasma from sleeping participants promotes T-cell migration dependent on GH and PRL signaling

To verify that the effects of sleep on T-cell migration observed in the

Table 1
Results of linear mixed models analyses for spontaneous, CCL19-directed, and CCL5-directed migration of various T-cell subsets in the *in vivo* sleep experiment.

	Spontaneous migration			**CCL19-directed migration**			**CCL5-directed migration**		
	Condition main effect	Time main effect	Condition x Time interaction	Condition main effect	Time main effect	Condition x Time interaction	Condition main effect	Time main effect	Condition x Time interaction
$CD3^+$	**P = 0.010**	P > 0.1	P = 0.082	**P = 0.004**	**P = 0.001**	P > 0.1	P > 0.1	**P = 0.041**	P > 0.1
$CD4^+$	**P = 0.013**	P > 0.1	P = 0.068	**P = 0.014**	**P = 0.012**	P > 0.1	P > 0.1	**P = 0.046**	P > 0.1
T_{N4}	**P = 0.005**	P > 0.1	P > 0.1	**P = 0.015**	**P = 0.006**	P = 0.062	P > 0.1	P = 0.053	P > 0.1
T_{CM4}	P = 0.060	P = 0.067	P > 0.1	P = 0.066	P = 0.052	P > 0.1	P > 0.1	P > 0.1	P > 0.1
T_{EM4}	P > 0.1	P = 0.087	P > 0.1	P > 0.1	P > 0.1	P > 0.1	P > 0.1	P = 0.081	P > 0.1
T_{TE4}	P > 0.1	P > 0.1	P > 0.1	P > 0.1	P > 0.1	P > 0.1	P > 0.1	P > 0.1	P > 0.1
$CD8^+$	**P < 0.001**	P > 0.1	P > 0.1	**P = 0.015**	**P < 0.001**	P > 0.1	P > 0.1	**P = 0.018**	P > 0.1
T_{N8}	**P < 0.001**	P > 0.1	P > 0.1	**P = 0.004**	**P = 0.005**	P > 0.1	P > 0.1	**P = 0.017**	P > 0.1
T_{CM8}	**P = 0.047**	P > 0.1	P > 0.1	P > 0.1	P > 0.1	P > 0.1	P > 0.1	P > 0.1	P > 0.1
T_{EM8}	P > 0.1	P = 0.065	P > 0.1	P > 0.1	P > 0.1	P > 0.1	P > 0.1	P > 0.1	P > 0.1
T_{TE8}	**P = 0.002**	P > 0.1	P > 0.1	P > 0.1	P > 0.1	P > 0.1	P > 0.1	**P < 0.001**	P > 0.1

P < 0.05 are shown in bold.

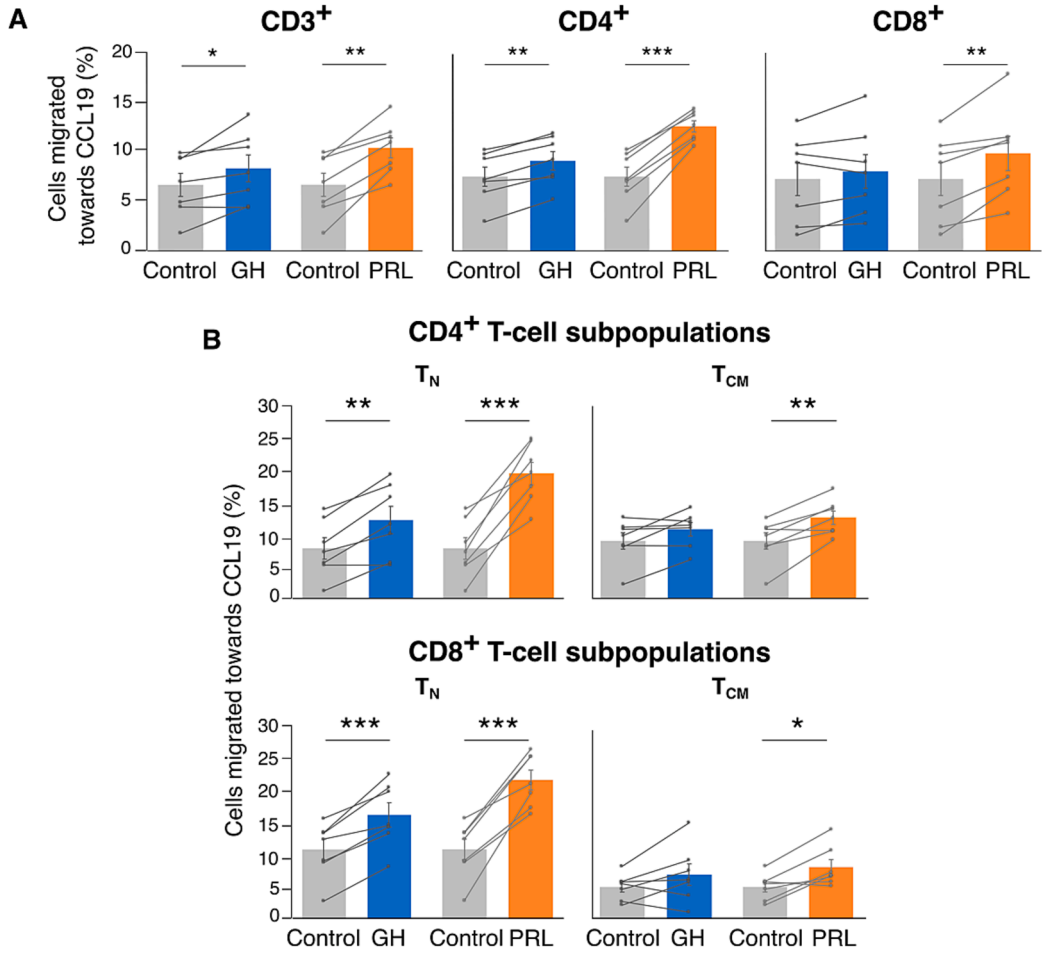

Fig. 2. Growth hormone (GH) and prolactin (PRL) increase CCL19-directed T-cell migration *in vitro*. (A) Means ± SEM of the percentage of migrated $CD3^+$, $CD4^+$, and $CD8^+$ T cells and (B) their T_N and T_{CM} $CD4^+$ and $CD8^+$ subsets in the presence of the chemokine CCL19 (CCL19-directed migration) after incubating the cells with GH (blue), PRL (orange), or a PBS control (gray). n = 7. *p < 0.05, **p < 0.01, ***p < 0.001 for pairwise comparisons between conditions (paired t tests). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

in vivo sleep manipulation experiments were indeed mediated by endocrine factors, and specifically by GH and PRL, we incubated whole blood from healthy donors for 2 h with plasma that had been previously collected at 2:00 h during the Sleep and the Wake conditions of the *in vivo* sleep experiments. The 2:00 h time point was selected a priori, because at this time, levels of GH and PRL are both close to their maximum during sleep (Dimitrov et al., 2015; Van Cauter and Plat, 1996). Whole blood from the donor participants was freshly collected at 18:00 h, when endogenous concentrations of GH and PRL are low. Incubation with the plasma derived from the Sleep condition specifically increased CCL19-directed migration of total $CD3^+$, $CD4^+$, and $CD8^+$ T cells (Fig. 3A) as well as of all subsets of interest (i.e., T_{N4}, T_{N8}, T_{CM4}, and T_{CM8}) (Fig. 3B), without affecting their spontaneous migration (Supplementary Fig. S6). This effect was largely dependent on GH and PRL signaling, as adding antagonists of these hormones to the "sleep plasma" significantly decreased the migration towards CCL19 compared to

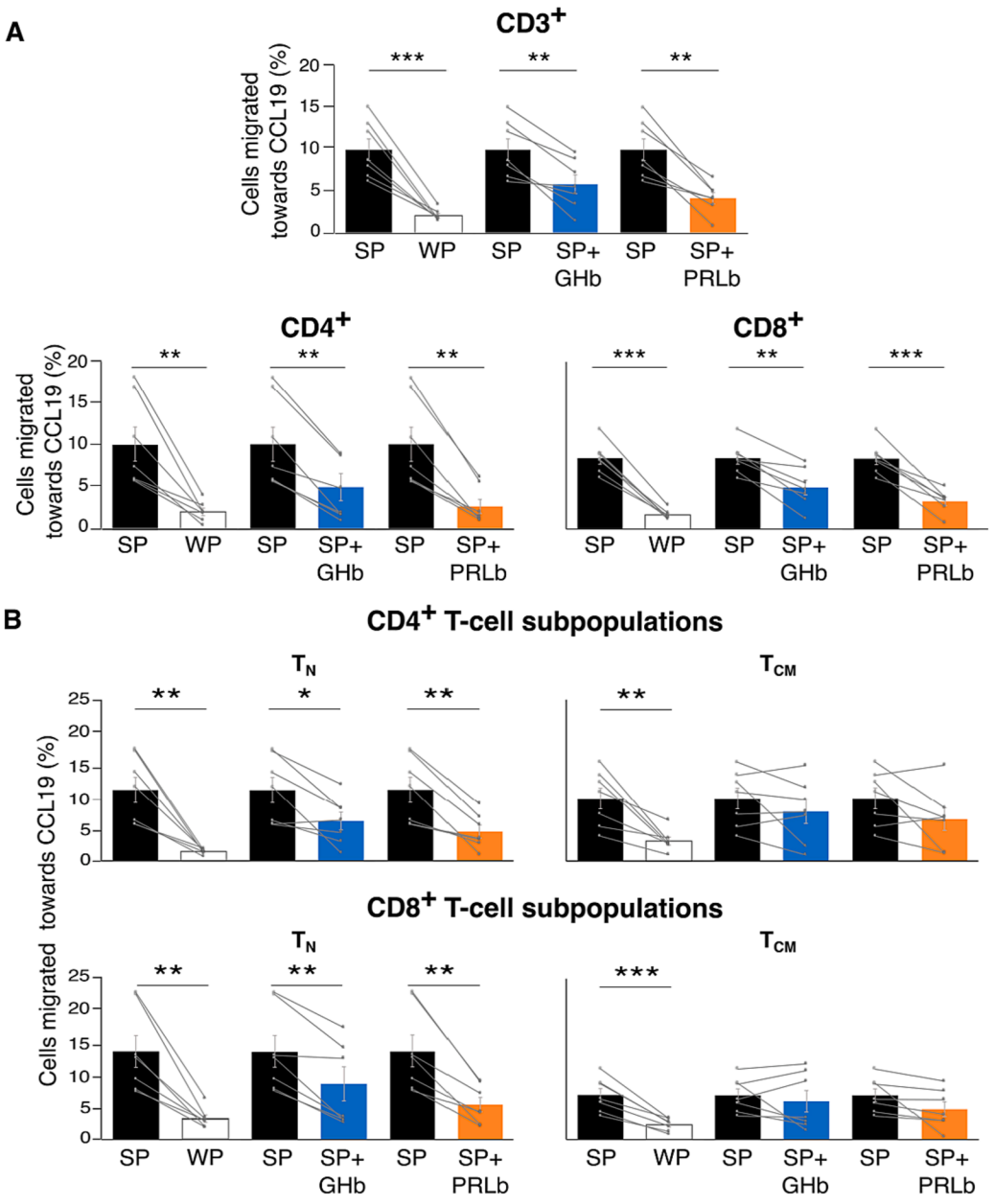

Fig. 3. Incubation of T cells with plasma from sleeping vs. awake participants increases CCL19-directed T-cell migration. (A) Means ± SEM of the percentage of migrated $CD3^+$, $CD4^+$, and $CD8^+$ T cells and (B) their T_N and T_{CM} $CD4^+$ and $CD8^+$ subsets in the presence of the chemokine CCL19 after incubation with plasma from sleeping participants ("sleep plasma", SP, black), from awake participants ("wake plasma", WP, white), sleep plasma plus a growth hormone receptor blocker (SP + GHb; blue), or sleep plasma plus a prolactin receptor blocker (SP + PRLb; orange). n = 7. *p < 0.05, **p < 0.01, ***p < 0.001 for pairwise comparisons between conditions (paired t tests). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

incubation with the sleep plasma without antagonists in total $CD3^+$, $CD4^+$, $CD8^+$ T cells (Fig. 3A), T_{N4}, and T_{N8} (Fig. 3B).

4. Discussion

We show here that sleep, compared to 24-hours of wakefulness, selectively promotes the migration of various T-cell subsets towards the LN-homing chemokine CCL19. This effect could be mimicked by incubating T cells with plasma from sleeping participants. In addition, blocking GH and PRL in the sleep plasma dampened the effect, whereas incubation of T cells with GH and PRL in sleep-like concentrations *in vitro* mimicked the *in vivo* effect of sleep on T-cell migration. Together, these findings suggest a GH- and PRL-driven increase in the migratory potential of T cells towards LN as a potential mechanism underlying the potent effect of sleep on adaptive immune functions.

The effects of the *in vivo* sleep manipulation on CCL19-directed migration were shown for total $CD3^+$, $CD4^+$, and $CD8^+$ T cells as well as for their T_N subsets. Similar effects in T_{CM4} and T_{CM8} did not reach significance, possibly due to the lower expression of CCR7, the receptor of CCL19, in these subsets compared to T_N subsets (Besedovsky et al., 2014). As expected, the migration towards CCL19 remained unaffected by the sleep manipulation in T_{EM} and T_{TE}, which are indeed T-cell subsets not readily migrating to LN (Mahnke et al., 2013). Our findings are in line with previous experiments in mice, showing that sleep deprivation reduces lymphocyte numbers in LN (Ruiz et al., 2017) and decreases the expression of genes important for immune cell recruitment to LN (i.e., *CCL19*, *CCR7*, *CD62L*) (Tune et al., 2020).

In contrast to CCL19-directed migration, the migration towards CCL5

remained unaffected by sleep in all the subsets studied. While CCL19 regulates migration to and within LN, CCL5 attracts T cells to sites of inflammation (Appay and Rowland-Jones, 2001). Our findings thus suggest that sleep specifically promotes the directed migration of T cells to LN and does not increase the reactivity towards chemokines in general. While we did not find an effect of sleep on the migration towards CCL5, there appeared to be a 24-hour rhythm in the migration of T cells towards this chemokine, with a steady decline during the night and an increase in the afternoon. Further studies are needed to specifically investigate whether there is indeed an endogenous circadian rhythm in CCL5-directed T-cell migration and what the physiological function of such a rhythm may be.

Hormonal changes hallmarking sleep have been considered a candidate mechanism for mediating the effects of sleep on peripheral immune functions (Besedovsky et al., 2019; Rolls et al., 2015). Specifically, GH and PRL are released in a strongly sleep-dependent fashion, as shown in previous studies (e.g., refs (Besedovsky et al., 2012; Lange et al., 2010)) and confirmed in the present study. These hormones act as pro-inflammatory signals that promote immune cell activation, proliferation, differentiation, and the production of pro-inflammatory cytokines (Haus, 2007; Kelley et al., 2007), and, thus, eventually aid the initiation of adaptive immune responses. In our *in vitro* experiments, we could show that GH and PRL additionally promote CCL19-directed migration. Previous studies in animals have shown that GH-transgenic mice display higher amounts of lymphocytes in LN and enhanced lymphocyte migration compared to wild-type animals (Smaniotto et al., 2010). Furthermore, injection of GH into the thymus of mice has been shown to promote the trafficking of naïve $CD4^+$ T cells to LN (Smaniotto et al., 2004). Our findings significantly advance these animal experiments by demonstrating that physiological, i.e., sleep-like, concentrations of GH have fast, promoting effects on the LN homing potential of human T cells and their T_N subsets.

So far, an effect of PRL on LN homing was unrecognized, except of one animal study showing that mice treated with PRL for 28 days display an increase in $CD4^+$ and $CD8^+$ T-cell numbers in LN (Dill and Walker, 2017). Our findings thus unravel an acute effect of physiological PRL levels on the LN-homing potential of T cells, pointing to a new role of PRL in acutely promoting adaptive immune responses in humans.

Our plasma experiments demonstrate that the promoting effects of sleep on CCL19-directed T-cell migration can be mimicked *ex vivo* by a short-term incubation of T cells with plasma collected from sleeping participants and that this effect is largely dependent on GH and PRL signaling. Together, our series of experiments suggest that the effects of sleep on T-cell migration towards CCL19 were mediated by these hormones. These findings pave the way for future studies aiming to investigate the molecular mechanisms underlying the effects of GH and PRL on T-cell migration and whether their effects are additive or even synergistic. A common and principal signaling pathway of GH and PRL receptor activation is the JAK/STAT pathway (Chilton and Hewetson, 2005), which could thus be a potential downstream molecular mediator. Because the effects of sleep on chemokine-dependent migration were specific to CCL19, a likely molecular target mediating this effect is CCR7, the receptor for CCL19 expressed on T cells. However, further studies are necessary to specifically address the questions whether and how GH and PRL signaling affects the expression and responsivity of CCR7 and other potential molecular targets.

In our *in vivo* sleep-manipulation experiment, sleep did not only promote CCL19-directed migration but also spontaneous migration (i.e., migration of the cells in the absence of a chemokine). Of note, in contrast to CCL19-directed migration, spontaneous migration was not affected by the incubation of T cells with GH or PRL, or with plasma from sleeping participants. This finding indicates that factors mediating the effects of sleep on spontaneous T-cell migration are either not present in the plasma or that their actions take longer than 2 h (the duration of the incubation with the hormones or plasma) to emerge. Future studies will be necessary to identify the mediators and the physiological significance of the effect of sleep on spontaneous T-cell migration.

Another important finding is that the differences between the Sleep and the Wake conditions with regard to both CCL19-directed and spontaneous T-cell migration were still evident after a full night of (recovery) sleep. This demonstrates that the effects of sleep loss on cell migration are quite persistent and take more than one night of sleep to normalize. These findings are consistent with other studies, in which different immune parameters (mainly numbers of various leukocyte subsets and cytokine production) were investigated and which show that a single recovery night is often not sufficient to normalize the changes in immune parameters following short-term experimental sleep deprivation (Besedovsky et al., 2019).

As already shown in previous studies (Besedovsky et al., 2016; Born et al., 1997), the reducing effect of sleep on the number of circulating T cells emerged already early during the night (i.e., at 2:00 h). At a first glance, this seems to be discrepant with the effects of sleep on the T-cell migratory potential, which appeared to emerge with some delay. However, this discrepancy in the timing likely reflects the fact that sleep, by acutely promoting the extravasation of T cells during the night, leaves only those T cells in the circulation (and for our analyses) that have a lower migration potential. This effect tends to obscure the actual strength of the effect of sleep versus wakefulness on the migration potential at night. This explanation is corroborated by the above-mentioned finding that incubation of T cells (collected at 18:00 h) with plasma collected as early as 2:00 h at night from sleeping participants did in fact promote T-cell migration.

The main aim of our study was to shed light onto the mechanisms underlying the effects of sleep on adaptive immunity and, specifically, the response to vaccination. We compared a 24-hour sleep-wake cycle to 24 h of continuous wakefulness, because this is the experimental manipulation employed most often in previous experimental sleep studies investigating effects on vaccination (Benedict et al., 2012; Lange et al., 2011; Lange et al., 2003). The advantage of this study design compared to more prolonged forms of sleep deprivation or restriction is that it does not activate the stress axes to a significant extent (evidenced, e.g., by a lack of or only small changes in cortisol levels, as also shown in the present study). This design is therefore useful to investigate the *active* role of sleep for the immune system compared with a condition without sleep, as it minimizes potential detrimental effects of prolonged sleep deprivation (Besedovsky et al., 2019). However, this type of sleep deprivation is not necessarily comparable to the more chronic forms of sleep deficiency occurring in large parts of the general population. Future studies will therefore be necessary to investigate how more prolonged and partial sleep deprivation affects T-cell migration. Previous studies specifically deepening sleep (compared to unstimulated natural sleep) have shown that sleep actively reduces circulating T-cell numbers and increases GH and PRL levels (Besedovsky et al., 2022a; Besedovsky et al., 2017). Further studies using such designs would be helpful to support our conclusion that the observed effects on T-cell migration are also actively mediated by sleep *per se* and are not a consequence of the deprivation of sleep.

A recent *meta*-analysis about the association between insufficient sleep and antibody responses to vaccination found important sex differences, with the association between sleep duration and vaccination responses being significant only in males but not in females (Spiegel et al., 2023). These authors speculate that the lack of effect in females might be explained by the larger variability in sex hormone levels, which were not controlled in the studies included in the *meta*-analysis. In our study, both conditions took place in the same phase of the menstrual cycle in females to reduce such variability. However, our study was not powered to specifically assess sex differences and, therefore, cannot address the question whether sleep differentially affects T-cell migration in females and males. Given previous findings showing prominent sex differences in the effects of sleep not only on vaccination responses but also on other aspects of immune function (Besedovsky et al., 2022b; Irwin et al., 2010; Prather et al., 2013), future studies should be

designed to specifically examine sex differences in the effects of sleep on immune cell migration.

Although the hypothesis that sleep promotes T-cell migration to LN has been around already for more than 20 years (Born et al., 1997), evidence for this notion in humans was lacking so far. In combination, our series of experiments demonstrate that sleep selectively promotes the LN homing potential of T cells by increasing GH and PRL levels, thus revealing a fundamental mechanism underlying the supporting effect of sleep on adaptive immunity. Our findings uncovering acute effects of GH and PRL in physiological concentrations on CCL19-directed T-cell migration also have potential clinical implications. Thus, GH and PRL could be considered as new adjuvants to promote immune responses following vaccination, especially in aged people, who typically display reduced levels of these hormones (Van den Berghe et al., 1998) together with impairments in sleep (Gulia and Kumar, 2018; Li et al., 2018) and in vaccine-driven immune responses (Hainz et al., 2005; Murasko et al., 2002).

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CRediT authorship contribution statement

Estefanía Martínez-Albert: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis. **Nicolas D. Lutz:** Writing – review & editing, Investigation, Formal analysis. **Robert Hübener:** Writing – review & editing, Investigation. **Stoyan Dimitrov:** Writing – review & editing, Methodology. **Tanja Lange:** Writing – review & editing, Conceptualization. **Jan Born:** Writing – review & editing, Conceptualization. **Luciana Besedovsky:** Writing – original draft, Supervision, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbi.2024.02.021.

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