Published in final edited form as:
Sleep. ; 43(2): . doi:10.1093/sleep/zsz222.

Short-term sleep deprivation in mice induces B cell migration to the brain compartment

Ben Korin[1,2], **Shimrit Avraham**[3], **Hilla Azulay-Debby**[1,2], **Dorit Farfara**[1,2], **Fahed Hakim**[5,6], **Asya Rolls**[1,2,4]

[1]Department of Neuroscience, Rappaport Faculty of Medicine, Technion - Israel Institute of Technology, Haifa, Israel

[2]Department of Immunology, Rappaport Faculty of Medicine, Technion - Israel Institute of Technology, Haifa, Israel

[3]Department of Cell Biology and Cancer Science, Rappaport Faculty of Medicine, Technion - Israel Institute of Technology, Haifa, Israel

[4]Technion Integrated Cancer Center (TICC), Technion - Israel Institute of Technology, Haifa, Israel

[5]Pediatric Pulmonary Unit, Rambam Health Care Campus, Haifa, Israel

[6]Cancer Research Center, EMMS Hospital, Nazareth, Israel

Abstract

Increasing evidence highlight the involvement of immune cells in brain activity and its dysfunction. The brain's immune compartment is a dynamic ensemble of cells that can fluctuate even in naïve animals. However, the dynamics and factors that can affect the composition of immune cells in the naïve brain are largely unknown. Here we examined whether acute sleep deprivation can affect the brain's immune compartment (parenchyma, meninges and choroid plexus). Using high-dimensional mass cytometry analysis we broadly characterized the effects of short-term sleep deprivation on the immune composition in the mouse brain. We found that after 6 hours of sleep deprivation there was a significant increase in the abundance of B cells in the brain compartment. This effect can be accounted for, at least in part, by the elevated expression of the migration-related receptor, CXCR5, on B cells and its ligand, *cxcl13*, in the meninges following sleep deprivation. Thus, our study reveals that short-term sleep deprivation affects the brain's immune compartment, offering a new insight into how sleep disorders can affect brain function and potentially contribute to neurodegeneration and neuroinflammation.

Correspondence to: Asya Rolls.

Corresponding author: Asya Rolls; Efron 1, Haifa, Israel; rolls.asya@gmail.com.

Disclosure statement
Financial Disclosure: none.
Non-financial Disclosure: none.

Author contributions
B.K. designed and carried out the experiments, analyzed and interpreted the results and wrote the manuscript; S.A. contributed to the execution and design of experiments, interpretation of results and the manuscript; H.A-D. carried out the qPCR analyses and contributed to the manuscript; D.F. performed the immunofluorescence staining and acquisition of images; F.H. contributed to the interpretation of results; A.R. contributed to the experimental design, interpretation of results, and revised the manuscript.

Europe PMC Funders Author Manuscripts

Keywords

Neuroimmunology; sleep deprivation; immunology; immune cells; B cells; brain compartment; CXCR5; *cxcl13*

Introduction

Many neurodegenerative conditions such as Alzheimer's disease (AD)[1,2], multiple sclerosis (MS)[3,4], and Parkinson's disease (PD)[5] are accompanied by disordered sleep. These same conditions are also often characterized by immune dysfunction in the brain and in the periphery[6–14]. However, the nature of the relationship between sleep, immunity and neurodegeneration remains unknown. Moreover, it is still unclear whether sleep has an effect on the brain's immune compartment under normal conditions. Uncovering such a connection is important since increasing evidence reveals the central role played by immune cells in brain plasticity[15], memory[16,17], cognition[18] and emotional processes[19,20], all of which are also affected by sleep, and especially by sleep deprivation.

Previous studies have demonstrated that sleep affects peripheral immunity[21–24]. For example, circadian fluctuations and sleep were suggested to induce the migration of lymphocytes between the blood and the lymphatic system[25,26], and sleep deprivation has been shown to impair immunological memory formation following hepatitis B vaccination[27,28]. Notably, the relationship between sleep and immunity is bi-directional, as made evident by sickness behavior and specific induced immune fluctuations, when immune activity alters sleep architecture[29–31]. For example, when centrally administered, interleukin (IL-1) was shown to increase non-rapid eye movement sleep[32,33]. This cytokine has been shown to affect neuronal activity in the wake-regulating centers of the hypothalamic preoptic area/basal forebrain[34], and consequently increase sleep duration. Additionally, sleep restriction has been associated with increased secretion of proinflammatory cytokines[35], such as tumor necrosis factor α (TNF-α) in the blood plasma[36], and neurodegeneration and neuroinflammation are commonly characterized by sleep disturbances[2,37,38]. More specifically, microglia, the brain's resident immune cell populations, has been proposed as a key player in the pathophysiology of both sleep disorders and neurodegeneration[39–42]. Thus, understanding how sleep affects the "brain's immune compartment" may uncover novel sleep-regulatory mechanisms.

The "brain's immune compartment" includes the composition of immune cells in the brain parenchyma, its surrounding tissues, the choroid plexus and meninges, and the perivascular spaces. It is characterized by resident myeloid cells and peripheral cells, which enter the brain compartment from the blood. These hematopoietic myeloid and lymphoid populations are mostly located in the meninges and choroid plexus[43]. Under pathological conditions, these cells can also be detected within the brain parenchyma[44–47]. In recent years, our understanding of the resident and infiltrating immune populations in the brain compartment has been expanded considerably. The emergence of high-dimensional cell characterization techniques such as mass cytometry (CyTOF) and single-cell RNA sequencing, revealed several largely uncharacterized immune populations in the naïve brain[43,48–50]. Especially

interesting among these populations are B cells, which comprise ~10% of the brain's blood-derived immune populations. Even in peripheral tissues, new evidence highlights the effects of B cells as antigen-presenting cells and cytokine-producing entities, in addition to their well-characterized role in antibody production[51]. Similarly, new roles for B cells within the brain compartment are constantly being revealed; these cells were shown to play a role in brain myelination processes during development[52]. Following stroke, analysis of postmortem tissue suggested that B cells reaching the damaged area are associated with the subsequent development of dementia[53]. These findings are supported by studies conducted in mice where pharmacologic ablation of B-lymphocytes using an anti-CD20 antibody prevented the appearance of delayed cognitive deficits following stroke[53]. Other studies identified regulatory B cells as immune modulators in stroke[54], in autoimmune conditions and under various stressors [55]. For example, B cells were found within MS plaques[9,56,57] and targeting B cells was shown to attenuate clinical and pathological symptoms of experimental autoimmune encephalomyelitis (EAE)[58–61]. Psychological stress has been suggested as a modulator of B cell numbers, via glucocorticoids that induce apoptosis of pre-B-cells[62]. Collectively, these findings contribute to the emerging perspective of the brain as a privileged, yet vibrant immune environment, and has led us to characterize immune cells in the brain compartment following acute sleep deprivation. Nevertheless, the specific role of B cells in the brain's immune compartment is still largely unknown.

Methods

Mice

Adult (8-10 weeks of age; 20-25 gr) male C57BL/6 mice were used in all experiments. Mice were maintained under Specific-Pathogen-Free (SPF) conditions; four mice were housed in each cage maintained on a 12:12 light cycle (lights on at 07:00) 24±1°C, humidity 30–70%. All experiments were performed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals. All procedures and protocols were approved by the Technion Administrative Panel of Laboratory Animal Care.

Sleep deprivation

Mice were left to sleep, or sleep-deprived for 6 hours, starting at light onset (ZT0). Sleep deprivation was maintained by gentle handling as shown previously[96], to minimize stress responses.

Brain single-cell dissociation

Mice were fully anesthetized (ketamine 80 mg/kg, xylazine 15-20 mg/kg) and perfused with at least 20 ml ice-cold $PBS^{-/-}$ (without Mg^{+2} and Ca^{+2}; Sigma-Aldrich) through the left ventricle of the heart. Brain tissue was dissociated as previously described[43,71,97], with several modifications. Briefly, brains were dissected and placed in $PBS^{-/-}$ on ice. Brain tissue was cut to small pieces, transferred to 7 ml of RPMI-1640 (Sigma-Aldrich) dissociation solution, with collagenase D (0.4 mg/ml; Sigma-Aldrich), DNase I (0.2 mg/ml; Sigma-Aldrich), 1% fetal bovine serum (FBS; Biological Industries), and 1 mM HEPES (Sigma-Aldrich), and mechanically dissociated with a dounce homogenizer. Following incubation (30 min, 37°C. shaking 200 rpm), immune cells were isolated by centrifugation

(30 min at 500 g, 18°C) using a 30% Percoll (Sigma-Aldrich, GE Healthcare Bio-Sciences) gradient, comprised of 3 ml stock isotonic Percoll (SIP; 10% X10 $PBS^{-/-}$ diluted in 90% Percoll) and 7 ml of the RPMI-1640 dissociation solution containing the cells. The top myelin layer and supernatant were removed, and the cell pellet was washed once with 10 ml of $PBS^{-/-}$ (7 min at 500 g, 4°C), then re-suspended in 1 ml of staining buffer ($PBS^{-/-}$, containing 1% bovine serum albumin and 0.05% sodium azide).

Blood extraction

Prior to perfusion, the right atrium of the heart was cut and blood was collected into EDTA coated sterile test tubes, lysed with 10 ml red blood cell lysis buffer for 15 min (BD Biosciences, NJ, US), and then washed twice with staining buffer.

Mass cytometry

For mass cytometry analyses, we pooled six mouse brains for each sample. Pooled cells ($3\text{-}4{\cdot}10^6$) from each tissue were incubated with TruStain fcX™ (anti-mouse CD16/32, clone 93; 1:100) for FC blocking, and rhodium-103 (1:2000; Rh; Fluidigm) for live/dead discrimination (20 min, 4°C). Samples were washed twice (5 min, 500 g, 4°C) with staining buffer and then treated for palladium barcoding[98] according to the manufactures' instructions (Cell-ID 20-Plex Pd Barcoding Kit; Fluidigm). Briefly, each sample was incubated in X1 fix buffer (10 min, room temperature; RT), then washed twice with X1 perm buffer, and incubated with a palladium barcode for 30 min, RT. Following barcode incubation, samples were washed twice (5 min, 500 g, 4°C), pooled to a single sample, and stained with the metal-conjugated antibody mix (1 hr, 4°C; a complete list of antibodies is provided in Supplementary Table 1). The pooled sample was washed twice (5 min, 500 g) and fixed in 1.6% freshly made PFA in $PBS^{-/-}$ (1 hr, RT; Sigma-Aldrich), then incubated with iridium-191/193 (1:2000, 20 min, RT; Ir; Fluidigm) for live/dead discrimination, washed in ultrapure H_2O (5 min, 500 g) and analyzed on a CyTOF I machine (Fluidigm). Antibodies were conjugated in-house using the MAXPAR reagent (Fluidigm), and the optimal concentration for staining was determined by titration. Internal metal isotope bead standards were added for sample normalization by Matlab as shown previously[99], to account for the decline in mean marker intensity over time. Acquired data were uploaded to Cytobank[100] for data processing, exclusion of dead cells and normalization beads, and analysis. Immune population in were defined as (Figure 1, Figure S1): resident myeloid cells ($CD45^{low}CD44^{neg}CX3CR1^{+}CD11b^{+}$), monocytes ($CD45^{high}CD11b^{+}Ly6C^{+}$), granulocytes ($CD45^{high}CD11b^{+}Gr\text{-}1^{+}$), DCs ($CD45^{high}MHC\text{-}II^{+}CD11c^{+}$), CD4 T cells ($CD45^{high}TCR\text{-}\beta^{+}CD4^{+}$), CD8 T cells ($CD45^{high}TCR\text{-}\beta^{+}CD8^{+}$), B cells ($CD45^{high}MHC\text{-}II^{+}CD19^{+}$), NK cells ($CD45^{high}TCR\text{-}\beta^{neg}NK1.1^{+}CD49b^{+}$).

Flow cytometry

Cells were stained with the following antibodies: Pacific Blue or PE/Cy7-conjugated anti-CD19 (6D5, 115523, 115520, Biolegend), Alexa Fluor 488 or Pacific Blue-conjugated anti-MHC-II (M5/114.15.2, 107616,107620, Biolegend), PE or Alexa Fluor 700-conjugated anti-CD45 (30-F11, 103106, 103128, Biolegend), Alexa Fluor 488-conjugated anti-CD40 (HM40-3, 102910, Biolegend), APC-conjugated anti-IgM (1B4B1, 1140-11, Southern Biotech), APC-conjugated anti-IgG (X56, 550874, BD Bioscience), Alexa

Fluor 647-conjugated anti-TLR-2 (QA16A01, 153008, Biolegend), APC-conjugated anti-CX3CR1 (SA011F11, 149008, Biolegend), PE-conjugated anti-CD54 (YN1/1.7.4, 116108, Biolegend), PerCP or Brilliant Violet 605-conjugated anti-CD62L (MEL-14, 104430, 104438, Biolegend), PE or Brilliant Violet 510-conjugated anti-CD44 (IM7, 103008, 103043, Biolegend), PE-conjugated anti-CXCR4 (L276F12, 146506, Biolegend), PE-conjugated anti-CXCR5. Zombie NIR™ Fixable Viability (423106, Biolegend) staining was performed for live/dead discrimination according to the manufacturer's instructions. In each sample, $1{\cdot}10^6$ cells were incubated (30 min, 4°C) with the antibody mixture in staining buffer (total volume 50 µl, in a 96 well U-shaped plate), then washed twice with staining buffer. Samples were re-suspended in 200 µl of 1% PFA in staining buffer, and analyzed by flow cytometry using CytoFLEX S flow cytometer (Beckman Coulter).

Flow cytometry data analysis

Flow cytometry analysis was performed using FlowJo 10.1r5 software (Tree Star). Pre-gating for double discrimination of cells was performed in each analysis, followed by the selection of live cells. Marker expression level was analyzed by median fluorescence intensity (MFI).

Immunofluorescence

Following perfusion, mice were decapitated, and the skin was removed from the skull. The skull and the brain compartment within it were transferred to 10 ml of 4% PFA in PBS (48 hours, 4°C). Tissues were then transferred to 10 ml of 0.5 M EDTA in $PBS^{-/-}$ for 3-5 days, at room temperature. Following cryoprotection in 30% sucrose in $PBS^{-/-}$ (48 hours, 4°C), tissues were frozen in dry ice. Cryosections of the tissues were sliced at 10 µm thickness and mounted on super-frost slides (Fisherbrand). Tissues were stained with purified anti-CD19 (1:100; 6D5, 115502, Biolegend) and Alexa Fluor 488 AffiniPure Donkey Anti-Rat IgG (1:100; AB_2340684, Jackson), and with PE-conjugated anti-CD45 (1:250; 30-F11, 103106, Biolegend). All images were taken at 40X magnification using an Axio imager M2 microscope (Carl Zeiss Inc. US).

Corticosterone ELISA

Mouse blood was collected in EDTA coated tubes and centrifuged for 10 min at 300×g. Plasma was collected and stored at -80°C until analyzed. Corticosterone levels in plasma were analyzed using Corticosterone ELISA kit (Enzo Life Sciences, US) according to the manufacturer's instructions.

Quantitative PCR (qPCR) analysis

Brain parenchyma, meninges and choroid plexus were dissected from mice as previously shown[101]. We have included all meningeal compartments (the dural/arachnoid meninges and the leptomeninges) in our analyses. Tissues were lysed in TRI-Reagent (Sigma) and stored at −80°C overnight. Total RNA was isolated with TRI-Reagent according to the manufacturer's protocol. The concentration and purity of RNA samples were determined using Take3 Trio Micro-Volume Plate (BioTek, USA). Total RNA (0.1 µg) was reverse transcribed (RT) with the High-Capacity cDNA Reverse Transcription Kit

(Applied Biosystems). Quantitative Real-time PCR analysis was performed on an Applied Biosystems StepOnePlus Real Time PCR System (Foster City, CA) in two independent experiments in duplicates using the Fast SYBR Green Master Mix (Applied Biosystems). Appropriate no-RT and nontemplate controls were included in each 96-well PCR reaction, and dissociation analysis was performed at the end of each run to confirm the specificity of the reaction. Real-time PCR efficiencies were determined for all sets of primers used. The cycle conditions for real-time PCR were 95°C for 20 s, followed by 40 cycles of 95°C for 3 s, 60°C for 30 s and a melt curve stage (95°C for 15 s, 60°C for 1 min and 95°C for 15 s). Relative quantification of gene expression was performed according to the ΔΔ-CT method using StepOne Software 2.3 (Applied Biosystems). The following primers were used: GAPDH forward: 5′-TGAAGCAGGCATCTGAGGG-3′, reverse: 5′-CGAAGGTGGAAGAGTGGGAG-3′; CXCL12 forward: 5′-TGCATCAGTGACGGTAAACCA-3′, reverse: 5′-TCTTCAGCCGTGCAACAATC-3′; CXCL13 forward: 5′-GGCCACGGTATTCTGGAAGC-3′, reverse: 5′-GGGCGTAACTTGAATCCGATCTA-3′.

Statistics

For each mass cytometry experiment, pools of six mouse brains were analyzed as a single sample. Two tailed Student's *t-test* was used for the analysis of differences between sleep-control and sleep-deprived mice. When indicated, multiple comparisons correction was performed using Holm-Sidak method; multiple comparisons using one-way ANOVA were performed with Fisher's LSD test. qPCR data were normalized according to the mean relative expression of the control group and analyzed using One column t-test. Statistics were calculated using GraphPad Prism7. Possible outliers were identified using GraphPad Prism7 ROUT algorithm, based on false discovery rate (FDR) with Q = 5%. One outlier was detected in Figure S5 (indicated in the figure legend) and included in the analysis (no change in the statistical outcome of not significant). Results are reported as mean ± standard error of the mean (sem). Each replicated experiment is a biological repeat, indicated in the text as "representative data of X experiments" so that the presented figure includes the data of one of these repeats. Randomization and blinding were not applied in this study. No statistical methods were used to pre-determine sample sizes, but our sample sizes are similar to those reported in previous publications[43,102]. Data distribution was assumed to be normal, but this was not formally tested.

Results

Sleep is a homeostatic process that affects various physiological mechanisms and has long-term effects on endocrine and metabolic pathways[63–66]. Thus, manipulations that examine the effects of sleep deprivation inevitably include many contributing factors (e.g., the secretion of stress hormones) that can account for some of the observed changes in physiological and mental processes[67–69]. Although these accompanying changes are in fact endogenous aspects of sleep deprivation, in this study, to minimize the impact of these alternate processes, we chose to focus on short-term sleep deprivation (SSD). We sleep-deprived mice by gentle handling, which is considered to have minimal effect on stress. Control mice were allowed to sleep at the same time (6 hours Zeitgeber time (ZT) 0 to

ZT6). We broadly characterized the complete immunological brain compartment, including the parenchyma, the choroid plexus, and the meninges of mice in both experimental groups (Figure 1A). We used mass cytometry as an initial screening tool to identify possible phenotypic changes in the resident myeloid cells and in blood-derived immune cell populations. To distinguish between resident and blood-derived cells, we used their expression of the extracellular markers CD45 and CD44. Resident myeloid cells were identified by their relatively low or absent expression of these markers ($CD45^{low}$ $CD44^{neg}$), along with expression of CX3CR1 and CD11b (see Figure S1 for gating strategy). Blood-derived cells were defined by their high levels of CD45 ($CD45^{high}$). For further identification of each cell population, we used additional cell surface markers in our analyses (Figure S1). We characterized resident myeloid cells of sleep-control and sleep-deprived mice using viSNE analysis[70]. We used viSNE analysis to describe broad changes in the resident myeloid cell population while preserving the fine details of the single-cell level. However, our analysis did not reveal changes in the proportions of the various resident myeloid cell subsets (Figure 1B) or in the expression of specific cell markers between the groups (Figure 1C, Figure S2A). Even with a more targeted approach, utilizing flow cytometry, which has higher sensitivity compared to mass cytometry and used as an additional validation method, we could detect only limited changes in resident myeloid cells. Most notably, we found a significant decrease in CX3CR1 expression, yet the effect size of this change was relatively small (sleep-control: 1±0.02; SSD: 0.91±0.01; n=4, Student's t-test, P<0.003; Figure S2B). Thus, we concluded that the resident myeloid cells were largely unaffected by our short-term sleep deprivation.

Next, we examined the blood-derived immune cell populations in the brain compartment of sleep-control and sleep-deprived mice: monocytes, granulocytes, dendritic cells (DCs), CD8 T cells, CD4 T cells, B cells, and natural killer (NK) cells from total blood-derived cells ($CD45^{high}$; Figure 1D). While we could not detect any changes in the phenotype (Figure S3) or overall number of blood-derived cells between the groups (Figure S4), there was a significant increase in the relative abundance of B cells in the tissue derived from sleep-deprived mice ($CD45^{high}MHC\text{-}II^{+}CD19^{+}$; sleep-control: 9.92±0.56%, SSD: 13.43±0.54%; n=6, multiple t-tests, corrected P<0.002; Figure 1D). We validated this effect and its specificity to B cells using flow cytometry (sleep-control: 9.48±0.39%, SSD: 14.8±0.94%; n=9, Student's t-test, P<0.001; Figure 1E; Figure S5). While B cell proportion increased following sleep deprivation, there was no specific population whose levels were decreased following sleep deprivation (Figure 1D; Figure 1E; Figure S5). Such overall decrease in the proportion of all other cell types, further highlights the specificity of the effect on B cells. Since B cells are the most abundant population in the mouse blood[43], blood contamination in the brain may affect our analyses. We have previously described the identification of blood contamination levels in the brain following perfusion[43,71]. Using this technique, we have showed that our potential error from blood contamination is approximately 1% for all blood-borne cells in the brain compartment, and 0.25% for B cells[43]. Given the limited contamination rate, the described effect observed following sleep deprivation, cannot be accounted by contamination from the blood.

To uncover whether the detected difference in B cell abundance was due to a sleep-induced decrease of B cell numbers or a sleep deprivation induced increase, we compared the

proportion of B cells in the sleep-control and sleep-deprived group to the baseline condition, analyzed at ZT0 (control; Figure 2A). There was no difference in the abundance of B cells in the sleep group compared to baseline conditions (ZT0), while in the sleep deprivation group, there was an increase in the percentage of B cells in the brain compartment (control: 9.36±0.84%; sleep: 9.3±0.93%; SSD: 12.62±1.32%; n=9, one-way ANOVA followed by Fisher's LSD, P<0.04; Figure 2A). Thus, sleep on its own had no effect on the number of B cells, but sleep deprivation increased their abundance in the brain compartment. Moreover, this suggests that the effect on B cells was not dependent on circadian mechanisms as both sleep-control and sleep-deprived mice were analyzed at the same time point.

Next, we wanted to address the possibility that the observed increase in B cells abundance was induced by the gentle handling procedure rather than the sleep manipulation. Although gentle handling is considered to be mild intervention that does not induce a significant stress response (validated by corticosterone measurement in our experimental mice; Figure S6), we repeated gentle handling during waking hours (ZT12-ZT18). Under these conditions, we did not observe any effect on B cell percentage (Figure 2B). Moreover, some level of stress is an integral part of sleep deprivation even if it is not made evident by corticosterone levels. Thus, we evaluated the direct effects of stress induced by damp bedding on B cell abundance in the brain. However, when we exposed mice to 6 hours of stress using damp bedding[72], there was no increase of B cells in the brain compartment. Rather, mice in the stress group exhibited a significant reduction in B cells in the brain compartment (control: 9.88±0.8%, damp bedding: 6.27±0.77%; n=5, Student's t-test, P<0.012; Figure 2C), further highlighting the unique effect induced by sleep deprivation. Circadian timing had no significant effect on the proportions of other cell types in the brain compartment (Figure S6B).

Blood-derived immune cells in the brain compartment enter from the blood and under naïve conditions are mainly located in the border areas, the choroid plexus and meninges[43]. To determine whether sleep deprivation affected the spatial localization of B cells, we performed an immunofluorescence analysis of the brains in the sleep-control and sleep-deprived groups. Sleep deprivation did not induce B cell infiltration to the parenchyma, and all were located only in the border tissues of brains of both groups (Figure S7). It is important to note that given the relatively low abundance of B cells under naïve conditions (i.e., no central nervous system inflammation), these results are mainly qualitative. It was designed to evaluate cell location rather than a numeric comparison in cell number, since quantitative immunofluorescence analysis, with such a low number of cells, has limited power.

To determine whether the cellular profile of B cells was affected by the sleep deprivation, we characterized cell surface markers indicative of specific cell subsets and B cell activation (Figure S8). We found a mild increase in IgG expression (sleep-control: 1±0.08; SSD: 1.21±0.07; n=20; Student's t-test, P<0.043; Figure S8B), yet there was no significant change in the proportion of IgM^+ or IgG^+ B cells, or in the relative expression level of IgM on B cells in the brain (Figure S8A, S8B). We additionally found an increase in CD40 expression (sleep-control: 1±0.04; SSD: 1.16±0.04; n=20, Student's t-test, P<0.004; Figure S8E), a costimulatory membrane protein required for the generation of germinal centers, isotype switching, and sustained antibody production[73]. Nonetheless, we could not identify

a comprehensive change in B cell profile following sleep deprivation (Figure S8F) and the major effect was evidently in the number of these cells. Despite our relatively broad analysis, further studies may provide additional insight on the brain's B cell population, while extending the characterization of B cell markers, as shown by single-cell RNA sequencing of B cells in the periphery[74–76].

The size of a specific immune population can be affected by changes in cell proliferation, death or cell migration. Given the short duration of our manipulation (6 hours), we excluded the possibility of cell proliferation. Flow cytometry analysis using Zombie staining (live/dead discrimination) demonstrated that there was no change in cell viability (Figure S9), suggesting that sleep deprivation induced infiltration and B cell homing towards the brain compartment.

Homing of immune cells to different tissues is regulated by cytokines, chemokines, and their receptors. Therefore, we used flow cytometry to identify changes in migration-related receptors on B cells extracted from the brain compartment or blood of mice in the sleep-control and sleep-deprived groups (Figure 3). CX3CR1 and CD54 on B cells were not affected in the brain compartment, but we detected an increase in their expression on B cells in the blood (CX3CR1: sleep-control:1±0.02, SSD: 1.3±0.07, n=11, Student's t-test, P<0.001; CD54: sleep-control:1±0.03, SSD: 1.67±0.09, n=11, Student's t-test, P<0.001). CD62L was elevated in the brain compartment (sleep-control:1±0.05; 1.37±0.09, n=11, Student's t-test, P<0.003) but not in the blood. CD44 expression decreased in the brain, yet increased in the blood (brain: sleep-control:1±0.0.02, SSD: 0.85±0.04, n=12, Student's t-test, P<0.002; blood: sleep-control:1±0.11, SSD: 2.51±0.25, n=10, Student's t-test, P<0.001). By contrast, CXCR4 and CXCR5 were elevated on B cells derived from both the brain compartment and blood of sleep-deprived mice compared to sleep-controls (CXCR4: brain: sleep-control:1±0.08, SSD: 1.85±0.11, n=12, Student's t-test, P<0.001; blood: sleep-control:1±0.03, SSD: 2.07±0.14, n=9, Student's t-test, P<0.001; CXCR5: brain: sleep-control:1±0.12, SSD: 1.77±0.14, n=11, Student's t-test, P<0.001; blood: sleep-control:1±0.01, SSD: 2.2±0.12, n=11, Student's t-test, P<0.001; Figure 3). The interaction between CXCR4 and its ligand, CXCL12 (also known as stromal cell derived factor 1; SDF-1), regulates the trafficking of all lymphocytes[26]. This chemokine receptor has been shown to be affected by various neuronal and hormonal signals, such as catecholamines and glucocorticoids[77]. It has been recently described as a major regulator of circadian-dependent immune cell trafficking, controlling the migration of multiple immune cell types between various tissues[26]. On the other hand, the interaction between CXCR5 and its ligand, CXCL13 (also known as B cell–attracting chemokine 1; BCA-1), is more specific to B cells[78–80] (ImmGen). Based on Allen Brain Atlas, within the brain, *cxcl13* is mainly expressed in the meninges[81].

To determine whether the expression of these chemokines in the brain compartment was affected by sleep deprivation, we analyzed their mRNA levels in the meninges, choroid plexus and brain parenchyma. Given that by 6 hours we had already observed an increase in B cell abundance, we analyzed the mRNA levels of the ligands after 2 hours of sleep or sleep deprivation (ZT2). *cxcl12* levels decreased in all isolated sites of the brain (meninges, choroid plexus and brain parenchyma; Figure 4A). However, *cxcl13* levels were

elevated only in the meninges of sleep-deprived mice compared to sleep-controls (SSD/sleep expression ratio: 1.33±0.08, n=12, One sample t-test, P<0.002; Figure 4B). Thus, sleep deprivation induced the increase in CXCR5 on B cells and the elevated expression of *cxcl13* in the meninges, which formulates preferential conditions for B cell trafficking to the brain compartment.

Discussion

The involvement of B cells and other immune cells in brain function, repair or neurodegeneration has become increasingly apparent. However, the factors and the conditions regulating their infiltration and surveillance in the naïve brain, are not fully elucidated. Our study introduces short-term sleep deprivation as such condition. This effect on immune cell trafficking is in line with previous reports that sleep affects the distribution of immune cells between peripheral tissues[25]. We suggest that the interaction between CXCR5 and its ligand *cxcl13* mediated this increase in B cell abundance in the brain compartment following sleep deprivation. However, further studies are required to identify the factors that promote these changes.

In this study, we focused on the immune effects of short term sleep deprivation, which is a common affliction in modern society. Our approach has enabled us to evaluate the immediate effects of sleep deprivation independently from other factors that are associated with longer or chronic sleep deprivation (e.g., stress, metabolic changes). Chronic and prolonged sleep restrictions can alter the homeostatic state of the organism and thus, some factors commonly associated with sleep deprivation may not be apparent [82–84]. For example, in some cases no change or even a decrease in plasma corticosterone levels were previously described even though acute sleep deprivation is accompanied by a prominent stress response [85–88]. Thus the transition from the acute sleep restricted phase to the chronic one is likely to be evident in the immune compartment of the brain. As many neurodegenerative conditions are accompanied by chronic sleep disruptions [37,89,90], it would be especially interesting to evaluate the immune outcomes of sleep manipulations in specific models of neurodegeneration. Moreover, sleep deprivation can potentially impact various autoimmune conditions in the central nervous system, specifically MS, in which the involvement of B cells has become increasingly evident[12,56,57,91,92]. Under these conditions, future studies should address how chronic sleep deprivation affects the brain immune compartment of these illnesses. Moreover, the brain is a very complex organ, that holds numerous unique niches, further research may also reveal changes in the spatial localization of the infiltrating immune cells in these various conditions. Such studies will become possible with the emergence of novel analysis techniques allowing us to decipher the immune phenotypes in different brain sites[93–95]. Thus, our study, demonstrating that acute sleep deprivation affects the immune compartment in the brain, mainly introduces new questions and potentially, an opportunity for intervention.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Prof. Ami Aronheim, Prof. Doron Melamed, Dr. Slava Berger, Maya Schiller and Mariam Amer for their support and helpful discussions, and Shelley Schwarzbaum for editing the paper. We thank Tania Dubovic, Dr. Amir Grau and the Biomedical Core Facility at the Technion Faculty of Medicine for technical support and comments. This work benefited from data assembled by the ImmGene consortium. This study was supported by the Israeli Ministry of Science and Technology, the Adelis Foundation and Prince Center for Neurodegenerative Diseases, A.R. is an International Howard Hughes Medical Institute (HHMI) - Wellcome Trust researcher.

References

1. Mander BA, Winer JR, Jagust WJ, Walker MP. Sleep: A Novel Mechanistic Pathway, Biomarker, and Treatment Target in the Pathology of Alzheimer's Disease? Trends Neurosci. 2016; 39 (8) 552–566. DOI: 10.1016/j.tins.2016.05.002 [PubMed: 27325209]
2. Peter-Derex L, Yammine P, Bastuji H, Croisile B. Sleep and Alzheimer's disease. Sleep Med Rev. 2015; 19: 29–38. DOI: 10.1016/j.smrv.2014.03.007 [PubMed: 24846773]
3. Sahraian MA, Rezaali S, Hosseiny M, Doosti R, Tajik A, Moghadasi AN. Sleep Disorder as a Triggering Factor for Relapse in Multiple Sclerosis. Eur Neurol. 2017; 77 (5-6) 258–261. [PubMed: 28359058]
4. Brass SD, Duquette P, Proulx-Therrien J, Auerbach S. Sleep disorders in patients with multiple sclerosis. Sleep Med Rev. 2010; 14 (2) 121–129. [PubMed: 19879170]
5. Yeh N-C, Tien K-J, Yang C-M, Wang J-J, Weng S-F. Increased Risk of Parkinson's Disease in Patients With Obstructive Sleep Apnea. Medicine (Baltimore). 2016; 95 (2) doi: 10.1097/MD.0000000000002293 [PubMed: 26765405]
6. Lai KSP, Liu CS, Rau A, et al. Peripheral inflammatory markers in Alzheimer's disease: a systematic review and meta-analysis of 175 studies. J Neurol Neurosurg Psychiatry. 2017; 88 (10) 876–882. [PubMed: 28794151]
7. Akiyama H, Barger S, Barnum S, et al. Inflammation and Alzheimer's disease. Neurobiol Aging. 2000; 21 (3) 383–421. DOI: 10.1016/s0197-4580(00)00124-x [PubMed: 10858586]
8. Heppner FL, Ransohoff RM, Becher B. Immune attack: the role of inflammation in Alzheimer disease. Nat Rev Neurosci. 2015; 16 (6) 358–372. [PubMed: 25991443]
9. Dendrou CA, Fugger L, Friese MA. Immunopathology of multiple sclerosis. Nat Rev Immunol. 2015; 15 (9) 545–558. [PubMed: 26250739]
10. McGeer PL, McGeer EG. Inflammation and neurodegeneration in Parkinson's disease. Parkinsonism Relat Disord. 2004; 10: S3–S7. [PubMed: 15109580]
11. Mosley RL, Hutter-Saunders JA, Stone DK, Gendelman HE. Inflammation and adaptive immunity in Parkinson's disease. Cold Spring Harb Perspect Med. 2012; 2 (1) Accessed April 3, 2014 doi: 10.1101/cshperspect.a009381 [PubMed: 22315722]
12. Pikor NB, Prat A, Bar-Or A, Gommerman JL. Meningeal Tertiary Lymphoid Tissues and Multiple Sclerosis: A Gathering Place for Diverse Types of Immune Cells during CNS Autoimmunity. Front Immunol. 2016; 6 doi: 10.3389/fimmu.2015.00657 [PubMed: 26793195]
13. Mesquita SD, Louveau A, Vaccari A, et al. Functional aspects of meningeal lymphatics in ageing and Alzheimer's disease. Nature. 2018; 560 (7717) 185. doi: 10.1038/s41586-018-0368-8 [PubMed: 30046111]
14. Mundt S, Mrdjen D, Utz SG, Greter M, Schreiner B, Becher B. Conventional DCs sample and present myelin antigens in the healthy CNS and allow parenchymal T cell entry to initiate neuroinflammation. Sci Immunol. 2019; 4 (31) eaau8380 [PubMed: 30679199]
15. Ziv Y, Ron N, Butovsky O, et al. Immune cells contribute to the maintenance of neurogenesis and spatial learning abilities in adulthood. Nat Neurosci. 2006; 9 (2) 268–275. [PubMed: 16415867]
16. Baruch K, Deczkowska A, David E, et al. Aging-induced type I interferon response at the choroid plexus negatively affects brain function. Science. 2014; 346 (6205) 89–93. DOI: 10.1126/science.1252945 [PubMed: 25147279]
17. Schmidt E-M, Linz B, Diekelmann S, Besedovsky L, Lange T, Born J. Effects of an interleukin-1 receptor antagonist on human sleep, sleep-associated memory consolidation, and blood monocytes. Brain Behav Immun. 2015; 47: 178–185. [PubMed: 25535859]

18. Kipnis J, Gadani S, Derecki NC. Pro-cognitive properties of T cells. Nat Rev Immunol. 2012; 12 (9) 663–669. DOI: 10.1038/nri3280 [PubMed: 22903149]
19. Herz J, Kipnis J. Bugs and Brain: How Infection Makes You Feel Blue. Immunity. 2016; 44 (4) 718–720. [PubMed: 27096312]
20. Dantzer R, O'Connor JC, Freund GG, Johnson RW, Kelley KW. From inflammation to sickness and depression: when the immune system subjugates the brain. Nat Rev Neurosci. 2008; 9 (1) 46–56. DOI: 10.1038/nrn2297 [PubMed: 18073775]
21. Marshall L, Born J. Brain-immune interactions in sleep. Int Rev Neurobiol. 2002; 52: 93–131. [PubMed: 12498102]
22. Lange T, Dimitrov S, Born J. Effects of sleep and circadian rhythm on the human immune system. Ann N Y Acad Sci. 2010; 1193 (1) 48–59. [PubMed: 20398008]
23. Besedovsky L, Ngo H-VV, Dimitrov S, Gassenmaier C, Lehmann R, Born J. Auditory closed-loop stimulation of EEG slow oscillations strengthens sleep and signs of its immune-supportive function. Nat Commun. 2017; 8 (1) 1984 doi: 10.1038/s41467-017-02170-3 [PubMed: 29215045]
24. Opp MR, Krueger JM. Sleep and Immunity: A Growing Field with Clinical Impact. Brain Behav Immun. 2015; 47: 1–3. DOI: 10.1016/j.bbi.2015.03.011 [PubMed: 25849976]
25. Besedovsky L, Lange T, Born J. Sleep and immune function. Pflüg Arch - Eur J Physiol. 2011; 463 (1) 121–137. DOI: 10.1007/s00424-011-1044-0 [PubMed: 22071480]
26. He W, Holtkamp S, Hergenhan SM, et al. Circadian Expression of Migratory Factors Establishes Lineage-Specific Signatures that Guide the Homing of Leukocyte Subsets to Tissues. Immunity. 2018; 49 (6) 1175–1190. e7 doi: 10.1016/j.immuni.2018.10.007 [PubMed: 30527911]
27. Lange T, Dimitrov S, Bollinger T, Diekelmann S, Born J. Sleep after vaccination boosts immunological memory. J Immunol Baltim Md. 2011; 187 (1) 283–290. [PubMed: 21632713]
28. Prather AA, Hall M, Fury JM, et al. Sleep and Antibody Response to Hepatitis B Vaccination. Sleep. 2012; 35 (8) 1063–1069. DOI: 10.5665/sleep.1990 [PubMed: 22851802]
29. Imeri L, Opp MR. How (and why) the immune system makes us sleep. Nat Rev Neurosci. 2009; 10 (3) 199–210. DOI: 10.1038/nrn2576 [PubMed: 19209176]
30. Besedovsky L, Lange T, Haack M. The Sleep-Immune Crosstalk in Health and Disease. Physiol Rev. 2019; 99 (3) 1325–1380. DOI: 10.1152/physrev.00010.2018 [PubMed: 30920354]
31. Krueger, JM, Clinton, JM, Winters, BD. , et al. Progress in Brain Research. Slow Brain Oscillations of Sleep, Resting State and Vigilance. Van Someren, EJW, Van Der Werf, YD, Roelfsema, PR, Mansvelder, HD, Lopes Da Silva, FH, editors. Vol. 193. Elsevier; 2011. 39–47.
32. Krueger JM, Walter J, Dinarello CA, Wolff SM, Chedid L. Sleep-promoting effects of endogenous pyrogen (interleukin-1). Am J Physiol. 1984; 246 (6 Pt 2) R994–999. [PubMed: 6611091]
33. Tobler I, Borbély AA, Schwyzer M, Fontana A. Interleukin-1 derived from astrocytes enhances slow wave activity in sleep EEG of the rat. Eur J Pharmacol. 1984; 104 (1-2) 191–192. [PubMed: 6333990]
34. Alam MN, McGinty D, Bashir T, et al. Interleukin-1beta modulates state-dependent discharge activity of preoptic area and basal forebrain neurons: role in sleep regulation. Eur J Neurosci. 2004; 20 (1) 207–216. [PubMed: 15245493]
35. Irwin MR. Why Sleep Is Important for Health: A Psychoneuroimmunology Perspective. Annu Rev Psychol. 2015; 66 (1) 143–172. DOI: 10.1146/annurev-psych-010213-115205 [PubMed: 25061767]
36. Vgontzas AN, Zoumakis E, Bixler EO, et al. Adverse effects of modest sleep restriction on sleepiness, performance, and inflammatory cytokines. J Clin Endocrinol Metab. 2004; 89 (5) 2119–2126. [PubMed: 15126529]
37. Musiek ES, Holtzman DM. Mechanisms linking circadian clocks, sleep, and neurodegeneration. Science. 2016; 354 (6315) 1004–1008. DOI: 10.1126/science.aah4968 [PubMed: 27885006]
38. Holth J, Patel T, Holtzman DM. Sleep in Alzheimer's Disease - Beyond Amyloid. Neurobiol Sleep Circadian Rhythms. 2017; 2: 4–14. DOI: 10.1016/j.nbscr.2016.08.002 [PubMed: 28217760]
39. Nadjar A, Wigren H-KM, Tremblay M-E. Roles of Microglial Phagocytosis and Inflammatory Mediators in the Pathophysiology of Sleep Disorders. Front Cell Neurosci. 2017; 11 doi: 10.3389/fncel.2017.00250 [PubMed: 28912686]

40. Yang Q, Wang Y, Feng J, Cao J, Chen B. Intermittent hypoxia from obstructive sleep apnea may cause neuronal impairment and dysfunction in central nervous system: the potential roles played by microglia. Neuropsychiatr Dis Treat. 2013; 9: 1077–1086. DOI: 10.2147/NDT.S49868 [PubMed: 23950649]
41. Zhao Q, Xie X, Fan Y, et al. Phenotypic dysregulation of microglial activation in young offspring rats with maternal sleep deprivation-induced cognitive impairment. Sci Rep. 2015; 5 9513 doi: 10.1038/srep09513 [PubMed: 25830666]
42. Wisor JP, Schmidt MA, Clegern WC. Evidence for Neuroinflammatory and Microglial Changes in the Cerebral Response to Sleep Loss. Sleep. 2011; 34 (3) 261–272. DOI: 10.1093/sleep/34.3.261 [PubMed: 21358843]
43. Korin B, Ben-Shaanan TL, Schiller M, et al. High-dimensional, single-cell characterization of the brain's immune compartment. Nat Neurosci. 2017; 20 (9) 1300–1309. [PubMed: 28758994]
44. Ferretti MT, Merlini M, Späni C, et al. T-cell brain infiltration and immature antigen-presenting cells in transgenic models of Alzheimer's disease-like cerebral amyloidosis. Brain Behav Immun. 2016; 54: 211–225. [PubMed: 26872418]
45. Heneka MT, Kummer MP, Latz E. Innate immune activation in neurodegenerative disease. Nat Rev Immunol. 2014; 14 (7) 463–477. [PubMed: 24962261]
46. Schwartz M, Shechter R. Systemic inflammatory cells fight off neurodegenerative disease. Nat Rev Neurol. 2010; 6 (7) 405–410. [PubMed: 20531383]
47. Keren-Shaul H, Spinrad A, Weiner A, et al. A Unique Microglia Type Associated with Restricting Development of Alzheimer's Disease. Cell. 2017; 169 (7) 1276–1290. e17 [PubMed: 28602351]
48. Zeisel A, Muñoz-Manchado AB, Codeluppi S, et al. Cell types in the mouse cortex and hippocampus revealed by single-cell RNA-seq. Science. 2015; 347 (6226) 1138–1142. [PubMed: 25700174]
49. Mrdjen D, Pavlovic A, Hartmann FJ, et al. High-Dimensional Single-Cell Mapping of Central Nervous System Immune Cells Reveals Distinct Myeloid Subsets in Health, Aging, and Disease. Immunity. 2018; 48 (2) 380–395. e6 [PubMed: 29426702]
50. Ofengeim D, Giagtzoglou N, Huh D, Zou C, Yuan J. Single-Cell RNA Sequencing: Unraveling the Brain One Cell at a Time. Trends Mol Med. 2017; 23 (6) 563–576. DOI: 10.1016/j.molmed.2017.04.006 [PubMed: 28501348]
51. Tarlinton D. B cells still front and centre in immunology. Nat Rev Immunol. 2019; January. 1 [PubMed: 30602730]
52. Tanabe S, Yamashita T. B-1a lymphocytes promote oligodendrogenesis during brain development. Nat Neurosci. 2018; 21 (4) 506–516. [PubMed: 29507409]
53. Doyle KP, Quach LN, Solé M, et al. B-Lymphocyte-Mediated Delayed Cognitive Impairment following Stroke. J Neurosci. 2015; 35 (5) 2133–2145. DOI: 10.1523/JNEUROSCI.4098-14.2015 [PubMed: 25653369]
54. Ren X, Akiyoshi K, Dziennis S, et al. Regulatory B Cells Limit CNS Inflammation and Neurologic Deficits in Murine Experimental Stroke. J Neurosci. 2011; 31 (23) 8556–8563. DOI: 10.1523/JNEUROSCI.1623-11.2011 [PubMed: 21653859]
55. Andersen BL, Goyal NG, Weiss DM, et al. Cells, cytokines, chemokines, and cancer stress: A biobehavioral study of patients with chronic lymphocytic leukemia. Cancer. 2018; 124 (15) 3240–3248. DOI: 10.1002/cncr.31538 [PubMed: 29757455]
56. Michel L, Touil H, Pikor NB, Gommerman JL, Prat A, Bar-Or A. B Cells in the Multiple Sclerosis Central Nervous System: Trafficking and Contribution to CNS-Compartmentalized Inflammation. Front Immunol. 2015; 6 doi: 10.3389/fimmu.2015.00636 [PubMed: 26732544]
57. Stern JNH, Yaari G, Heiden JAV, et al. B cells populating the multiple sclerosis brain mature in the draining cervical lymph nodes. Sci Transl Med. 2014; 6 (248) 248ra107-248ra107 doi: 10.1126/scitranslmed.3008879 [PubMed: 25100741]
58. Cross AH, Trotter JL, Lyons J-A. B cells and antibodies in CNS demyelinating disease. J Neuroimmunol. 2001; 112 (1) 1–14. [PubMed: 11108928]
59. Matsushita T, Yanaba K, Bouaziz J-D, Fujimoto M, Tedder TF. Regulatory B cells inhibit EAE initiation in mice while other B cells promote disease progression. J Clin Invest. 2008; 118 (10) 3420–3430. DOI: 10.1172/JCI36030 [PubMed: 18802481]

60. Fillatreau S, Sweenie CH, McGeachy MJ, Gray D, Anderton SM. B cells regulate autoimmunity by provision of IL-10. Nat Immunol. 2002; 3 (10) 944–950. [PubMed: 12244307]
61. Barr TA, Shen P, Brown S, et al. B cell depletion therapy ameliorates autoimmune disease through ablation of IL-6–producing B cells. J Exp Med. 2012; 209 (5) 1001–1010. DOI: 10.1084/jem.20111675 [PubMed: 22547654]
62. McGregor BA, Murphy KM, Albano DL, Ceballos RM. Stress, cortisol, and B lymphocytes: a novel approach to understanding academic stress and immune function. Stress. 2016; 19 (2) 185–191. DOI: 10.3109/10253890.2015.1127913 [PubMed: 26644211]
63. Sharma S, Kavuru M. Sleep and Metabolism: An Overview. Int J Endocrinol. 2010; 2010 doi: 10.1155/2010/270832 [PubMed: 20811596]
64. AlDabal L, BaHammam AS. Metabolic, endocrine, and immune consequences of sleep deprivation. Open Respir Med J. 2011; 5: 31. doi: 10.2174/1874306401105010031 [PubMed: 21754974]
65. Briançon-Marjollet A, Weiszenstein M, Henri M, Thomas A, Godin-Ribuot D, Polak J. The impact of sleep disorders on glucose metabolism: endocrine and molecular mechanisms. Diabetol Metab Syndr. 2015; 7 (1) 25. doi: 10.1186/s13098-015-0018-3 [PubMed: 25834642]
66. Schmid SM, Hallschmid M, Schultes B. The metabolic burden of sleep loss. Lancet Diabetes Endocrinol. 2015; 3 (1) 52–62. [PubMed: 24731536]
67. Kushida, CA. Sleep Deprivation: Basic Science, Physiology and Behavior. CRC Press; 2016.
68. Knutson KL, Spiegel K, Penev P, Van Cauter E. The metabolic consequences of sleep deprivation. Sleep Med Rev. 2007; 11 (3) 163–178. DOI: 10.1016/j.smrv.2007.01.002 [PubMed: 17442599]
69. Cirelli C, Faraguna U, Tononi G. Changes in brain gene expression after long-term sleep deprivation. J Neurochem. 2006; 98 (5) 1632–1645. [PubMed: 16923172]
70. Amir ED, Davis KL, Tadmor MD, et al. viSNE enables visualization of high dimensional single-cell data and reveals phenotypic heterogeneity of leukemia. Nat Biotechnol. 2013; 31 (6) 545–552. DOI: 10.1038/nbt.2594 [PubMed: 23685480]
71. Korin B, Dubovik T, Rolls A. Mass cytometry analysis of immune cells in the brain. Nat Protoc. 2018; 13 (2) 377. [PubMed: 29370157]
72. Jaggi AS, Bhatia N, Kumar N, Singh N, Anand P, Dhawan R. A review on animal models for screening potential anti-stress agents. Neurol Sci. 2011; 32 (6) 993–1005. [PubMed: 21927881]
73. Elgueta R, Benson MJ, de Vries VC, Wasiuk A, Guo Y, Noelle RJ. Molecular mechanism and function of CD40/CD40L engagement in the immune system. Immunol Rev. 2009; 229 (1) doi: 10.1111/j.1600-065X.2009.00782.x [PubMed: 19426221]
74. Andor N, Simonds EF, Czerwinski DK, et al. Single-cell RNA-Seq of follicular lymphoma reveals malignant B-cell types and coexpression of T-cell immune checkpoints. Blood. 2019; 133 (10) 1119–1129. DOI: 10.1182/blood-2018-08-862292 [PubMed: 30591526]
75. Nguyen A, Khoo WH, Moran I, Croucher PI, Phan TG. Single Cell RNA Sequencing of Rare Immune Cell Populations. Front Immunol. 2018; 9 doi: 10.3389/fimmu.2018.01553 [PubMed: 30022984]
76. Zhang L, Dong X, Lee M, Maslov AY, Wang T, Vijg J. Single-cell whole-genome sequencing reveals the functional landscape of somatic mutations in B lymphocytes across the human lifespan. Proc Natl Acad Sci. 2019; 116 (18) 9014–9019. DOI: 10.1073/pnas.1902510116 [PubMed: 30992375]
77. Ince LM, Weber J, Scheiermann C. Control of Leukocyte Trafficking by Stress-Associated Hormones. Front Immunol. 2019; 9 doi: 10.3389/fimmu.2018.03143 [PubMed: 30687335]
78. Heng TSP, Painter MW, The Immunological Genome Project Consortium. et al. The Immunological Genome Project: networks of gene expression in immune cells. Nat Immunol. 2008; 9: 1091–1094. [PubMed: 18800157]
79. Legler DF, Loetscher M, Roos RS, Clark-Lewis I, Baggiolini M, Moser B. B Cell–attracting Chemokine 1, a Human CXC Chemokine Expressed in Lymphoid Tissues, Selectively Attracts B Lymphocytes via BLR1/CXCR5. J Exp Med. 1998; 187 (4) 655–660. DOI: 10.1084/jem.187.4.655 [PubMed: 9463416]

80. Sáez de Guinoa J, Barrio L, Mellado M, Carrasco YR. CXCL13/CXCR5 signaling enhances BCR-triggered B-cell activation by shaping cell dynamics. Blood. 2011; 118 (6) 1560–1569. [PubMed: 21659539]
81. Lein ES, Hawrylycz MJ, Ao N, et al. Genome-wide atlas of gene expression in the adult mouse brain. Nature. 2007; 445 (7124) 168–176. [PubMed: 17151600]
82. Leemburg S, Vyazovskiy VV, Olcese U, Bassetti CL, Tononi G, Cirelli C. Sleep homeostasis in the rat is preserved during chronic sleep restriction. Proc Natl Acad Sci U S A. 2010; 107 (36) 15939–15944. DOI: 10.1073/pnas.1002570107 [PubMed: 20696898]
83. Everson CA. Functional consequences of sustained sleep deprivation in the rat. Behav Brain Res. 1995; 69 (1) 43–54. [PubMed: 7546317]
84. Everson CA, Wehr TA. Nutritional and metabolic adaptations to prolonged sleep deprivation in the rat. Am J Physiol-Regul Integr Comp Physiol. 1993; 264 (2) R376–R387. [PubMed: 8447493]
85. Everson CA, Crowley WR. Reductions in circulating anabolic hormones induced by sustained sleep deprivation in rats. Am J Physiol-Endocrinol Metab. 2004; 286 (6) E1060–E1070. [PubMed: 14871886]
86. Alexandre C, Latremoliere A, Ferreira A, et al. Decreased alertness due to sleep loss increases pain sensitivity in mice. Nat Med. 2017; 23 (6) 768–774. DOI: 10.1038/nm.4329 [PubMed: 28481358]
87. Everson CA, Reed HL. Pituitary and peripheral thyroid hormone responses to thyrotropin-releasing hormone during sustained sleep deprivation in freely moving rats. Endocrinology. 1995; 136 (4) 1426–1434. [PubMed: 7895653]
88. Salín-Pascual RJ, Ortega-Soto H, Huerto-Delgadillo L, Camacho-Arroyo I, Roldán-Roldán G, Tamarkin L. The effect of total sleep deprivation on plasma melatonin and cortisol in healthy human volunteers. Sleep. 1988; 11 (4) 362–369. [PubMed: 3206055]
89. Abbott SM, Videnovic A. Chronic sleep disturbance and neural injury: links to neurodegenerative disease. Nat Sci Sleep. 2016; 8: 55–61. DOI: 10.2147/NSS.S78947 [PubMed: 26869817]
90. Wulff K, Gatti S, Wettstein JG, Foster RG. Sleep and circadian rhythm disruption in psychiatric and neurodegenerative disease. Nat Rev Neurosci. 2010; 11 (8) 589–599. [PubMed: 20631712]
91. Milo R. Therapeutic strategies targeting B-cells in multiple sclerosis. Autoimmun Rev. 2016; 15 (7) 714–718. [PubMed: 26970489]
92. Wekerle H. B cells in multiple sclerosis. Autoimmunity. 2017; 50 (1) 57–60. [PubMed: 28166681]
93. Hove HV, Martens L, Scheyltjens I, et al. A single-cell atlas of mouse brain macrophages reveals unique transcriptional identities shaped by ontogeny and tissue environment. Nat Neurosci. 2019; 22 (6) 1021. [PubMed: 31061494]
94. Zhang Y, Chen K, Sloan SA, et al. An RNA-Sequencing Transcriptome and Splicing Database of Glia, Neurons, and Vascular Cells of the Cerebral Cortex. J Neurosci. 2014; 34 (36) 11929–11947. DOI: 10.1523/JNEUROSCI.1860-14.2014 [PubMed: 25186741]
95. McKenzie AT, Wang M, Hauberg ME, et al. Brain Cell Type Specific Gene Expression and Co-expression Network Architectures. Sci Rep. 2018; 8 (1) 8868 doi: 10.1038/s41598-018-27293-5 [PubMed: 29892006]
96. Rolls A, Pang WW, Ibarra I, et al. Sleep disruption impairs haematopoietic stem cell transplantation in mice. Nat Commun. 2015; 6 Accessed November 11, 2015 doi: 10.1038/ncomms9516 [PubMed: 26465715]
97. Kokiko-Cochran ON, Saber M, Puntambekar S, et al. Traumatic Brain Injury in hTau Model Mice: Enhanced Acute Macrophage Response and Altered Long-Term Recovery. J Neurotrauma. 2017; 35 (1) 73–84. DOI: 10.1089/neu.2017.5203 [PubMed: 28859549]
98. Zunder ER, Finck R, Behbehani GK, et al. Palladium-based mass tag cell barcoding with a doublet-filtering scheme and single-cell deconvolution algorithm. Nat Protoc. 2015; 10 (2) 316–333. DOI: 10.1038/nprot.2015.020 [PubMed: 25612231]
99. Finck R, Simonds EF, Jager A, et al. Normalization of mass cytometry data with bead standards. Cytometry A. 2013; 83A (5) 483–494. DOI: 10.1002/cyto.a.22271 [PubMed: 23512433]
100. Kotecha, N, Krutzik, PO, Irish, JM. Current Protocols in Cytometry. John Wiley & Sons, Inc; 2001. http://onlinelibrary.wiley.com/doi/10.1002/0471142956.cy1017s53/abstract [Accessed September 5, 2016]

101. Derecki N, Derecki N, Kipnis J. Mouse meninges isolation for FACS. Protoc Exch. 2014; September. doi: 10.1038/protex.2014.030

102. Guilliams M, Dutertre C-A, Scott CL, et al. Unsupervised High-Dimensional Analysis Aligns Dendritic Cells across Tissues and Species. Immunity. 2016; 45 (3) 669–684. DOI: 10.1016/j.immuni.2016.08.015 [PubMed: 27637149]

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

Statement of Significance

In this study, we show that even after a single and brief deprivation of sleep (6 hours), there is a significant increase in the B cell population in the mouse brain compartment. The recruitment of B cells is especially interesting given the emerging role of B cells in neurodegenerative and neuroinflammatory diseases, brain development and homeostasis. Furthermore, we provide a potential mechanism showing that sleep deprivation induces an increase in the expression levels of the homing receptor CXCR5 on B cells in the brain and in the blood, and a complementary increase in the CXCR5 ligand (*cxcl13*) in the brain.

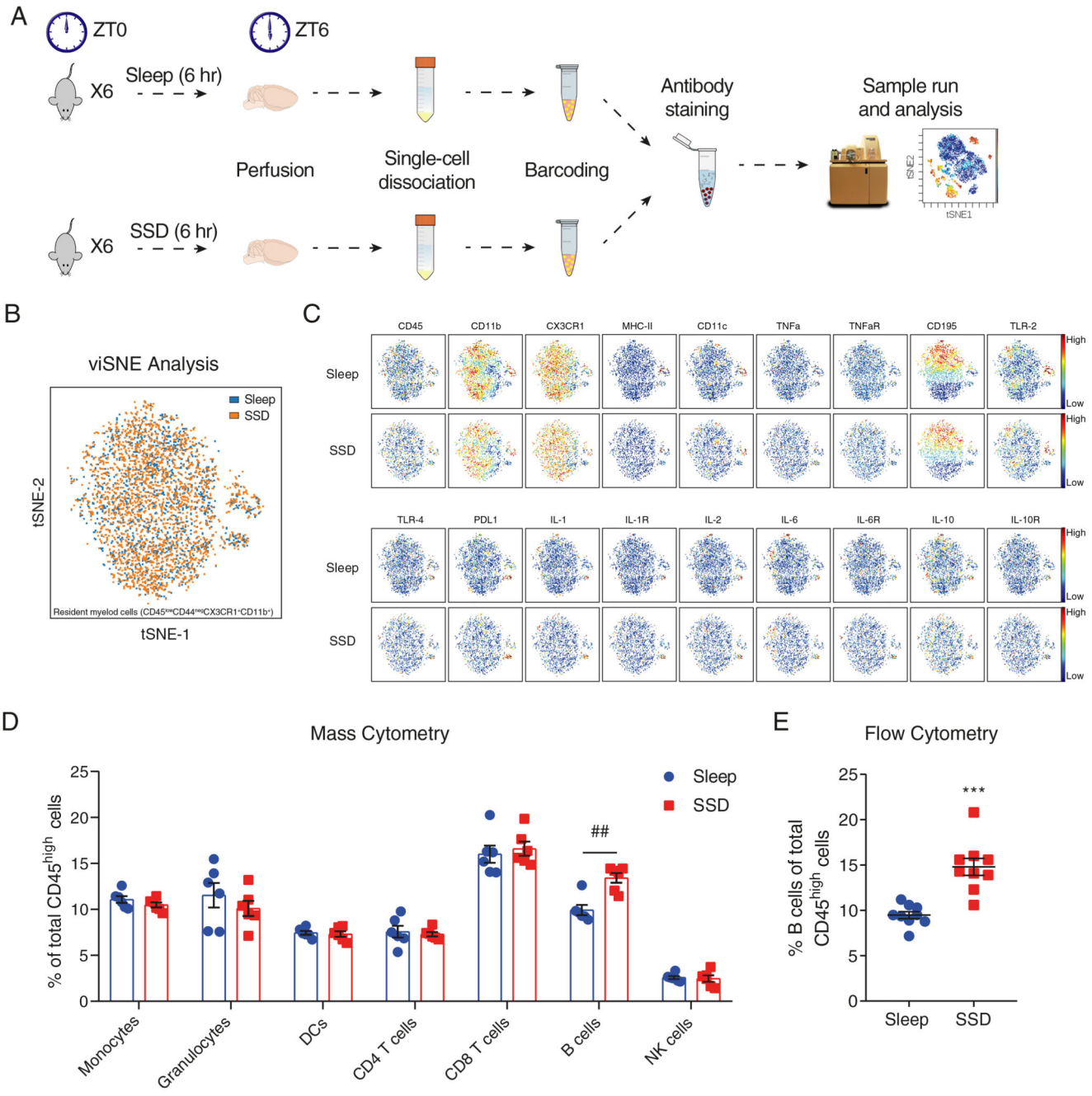

Figure 1. Characterization of immune cells in the brain compartment following short-term sleep deprivation.

(A) Schematic representation of the experimental procedure. Following 6 hours of sleep or sleep deprivation (ZT0 to ZT6), mice were sacrificed and perfused. Each mouse brain was dissociated to a single-cell suspension. Pools of single-cell suspensions from 6 mouse brains per group were barcoded, stained and analyzed using a mass cytometer. **(B)** viSNE analysis of sleep-control and sleep-deprived resident myeloid cells in an overlaid dot plot, and **(C)** in a color gradient plot for each marker. **(D)** The various blood-derived immune cell populations in the brain compartment, following 6 hours of sleep (sleep; left) or 6

Europe PMC Funders Author Manuscripts

hours of sleep deprivation (SSD; right), and the cell subset proportions of each group. Data are presented as mean±sem; multiple t-tests, n=6 pooled specimens of six mice in each sample, corrected *p-value* ##P<0.002; data of three experiments. **(E)** Flow cytometry analysis of the percentage of B cells ($CD45^{high/+}CD19^{+}MHC\text{-}II^{+}$) of total $CD45^{high}$ cells in the brain compartment, following 6 hours of sleep (sleep; blue circle) or sleep deprivation (SSD; red square). Data are presented as mean±sem; Student's t-test, n=9 individual mice; ***P<0.001; representative data of three experiments.

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

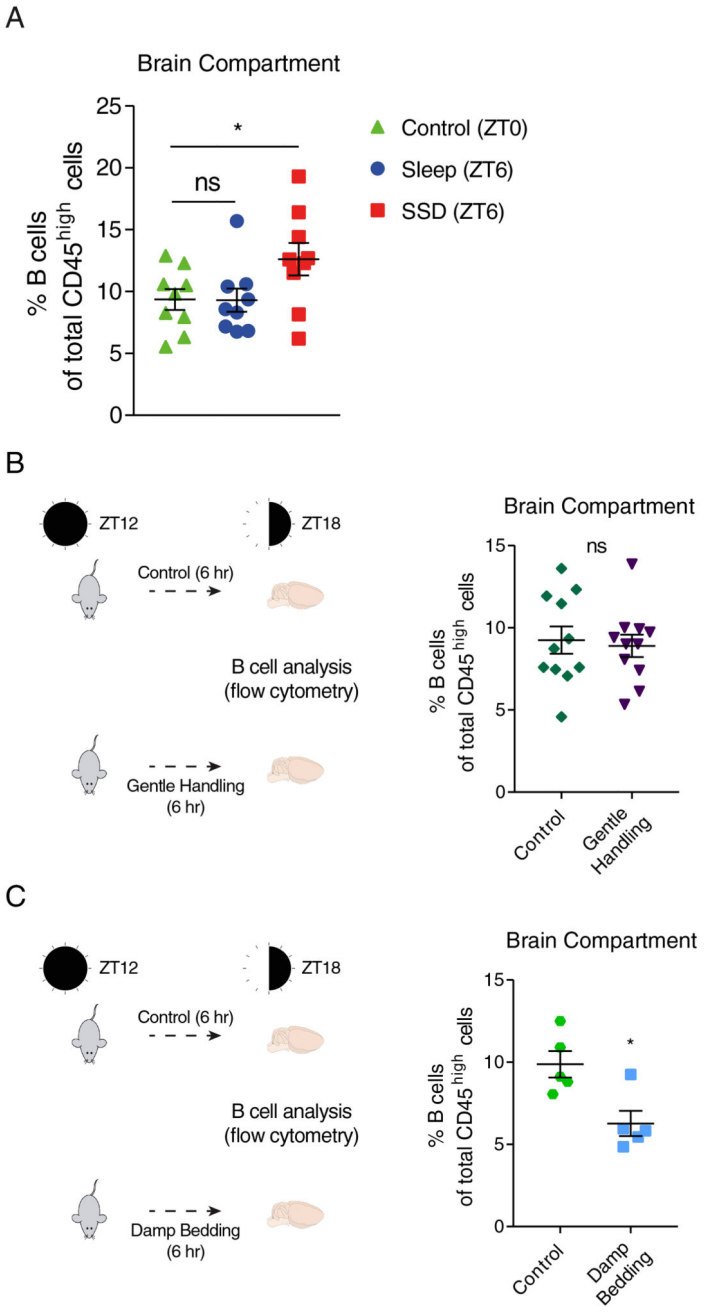

Figure 2. Comparison of B cell proportions in the brain compartment at baseline conditions (ZT0), and validation of the effects of gentle handling and stress during waking hours (ZT12-ZT18) on B cell proportion in the brain compartment.

Flow cytometry analysis. **(A)** The percentage of B cells ($CD45^{high/+}CD19^{+}MHC\text{-}II^{+}$) of total $CD45^{high}$ cells at ZT0 (control; green triangle), and following 6 hours of sleep (ZT6; blue circle) or sleep deprivation (SSD; ZT6; red square). Data are presented as mean±sem; one-way ANOVA followed by Fisher's LSD, n=9 individual mice; *P<0.05; data of two experiments. **(B)** Mice were housed normally or gently handled for 6 hours (ZT12-ZT18), and the percentage of B cells in the brain compartments of total blood-derived cells was

analyzed using flow cytometry. Data are presented as mean±sem; Student's t-test, n=11 individual mice; P>0.747; data of two experiments. **(C)** Mice were housed normally or exposed to damp bedding for 6 hours (ZT12-ZT18), and the percentage of B cells in the brain compartments of total blood-derived cells was analyzed using flow cytometry. Data are presented as mean±sem; Student's t-test, n=5 individual mice; *P<0.05. ns, not significant.

Europe PMC Funders Author Manuscripts

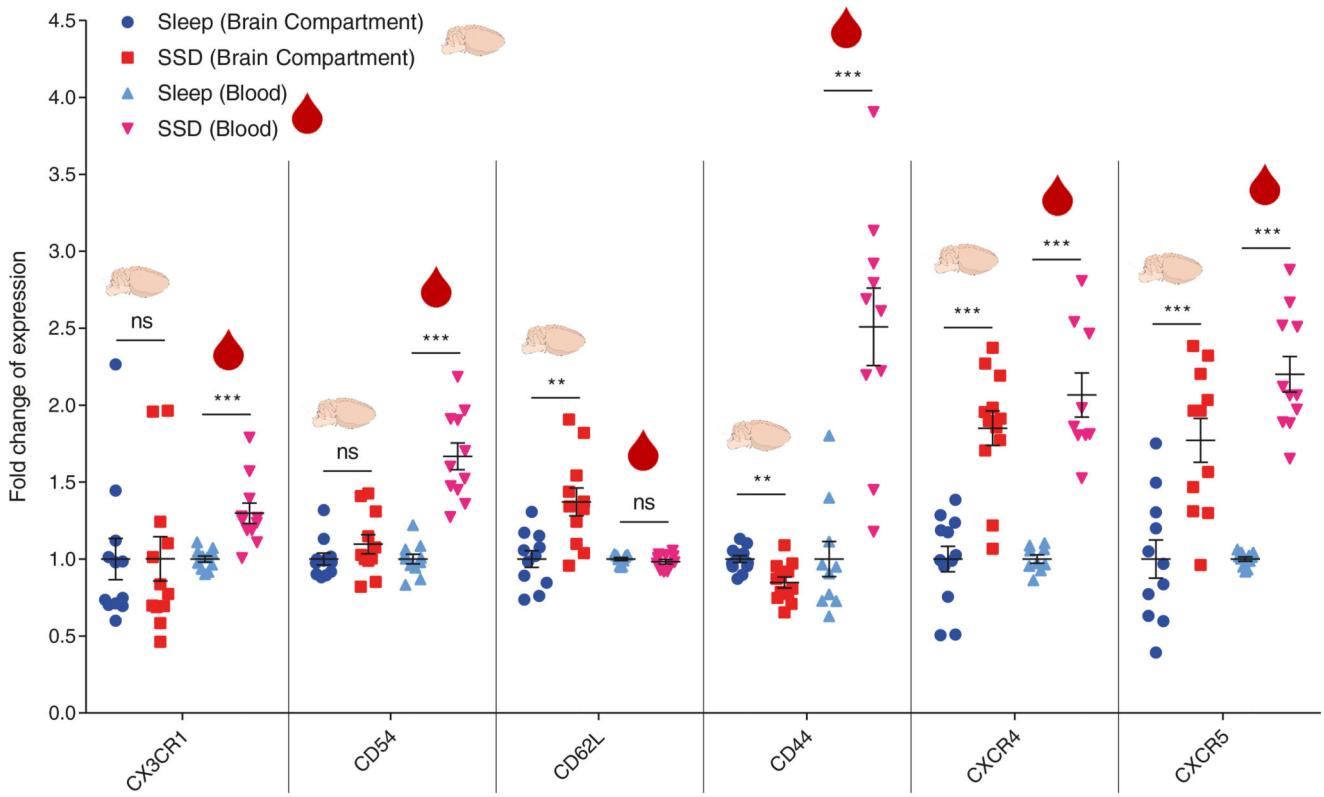

Figure 3. Characterization of a B cell migration phenotype in the brain compartment and blood following short-term sleep deprivation.

Flow cytometry analysis. Fold change of expression in migration-related markers (CX3CR1, CD54, CD62L, CD44, CXCR4, CXCR5), in B cells isolated from the brain compartment or in B cells in the blood following 6 hours of sleep (sleep; blue circle) or sleep deprivation (SSD; red square). Fold change of expression was calculated by dividing each value with the mean value of the sleep group for each experiment. Data are presented as mean±sem; Student's t-test, n=9-12 individual mice; **P<0.01; ***P<0.001; data of two experiments. ns, not significant.

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

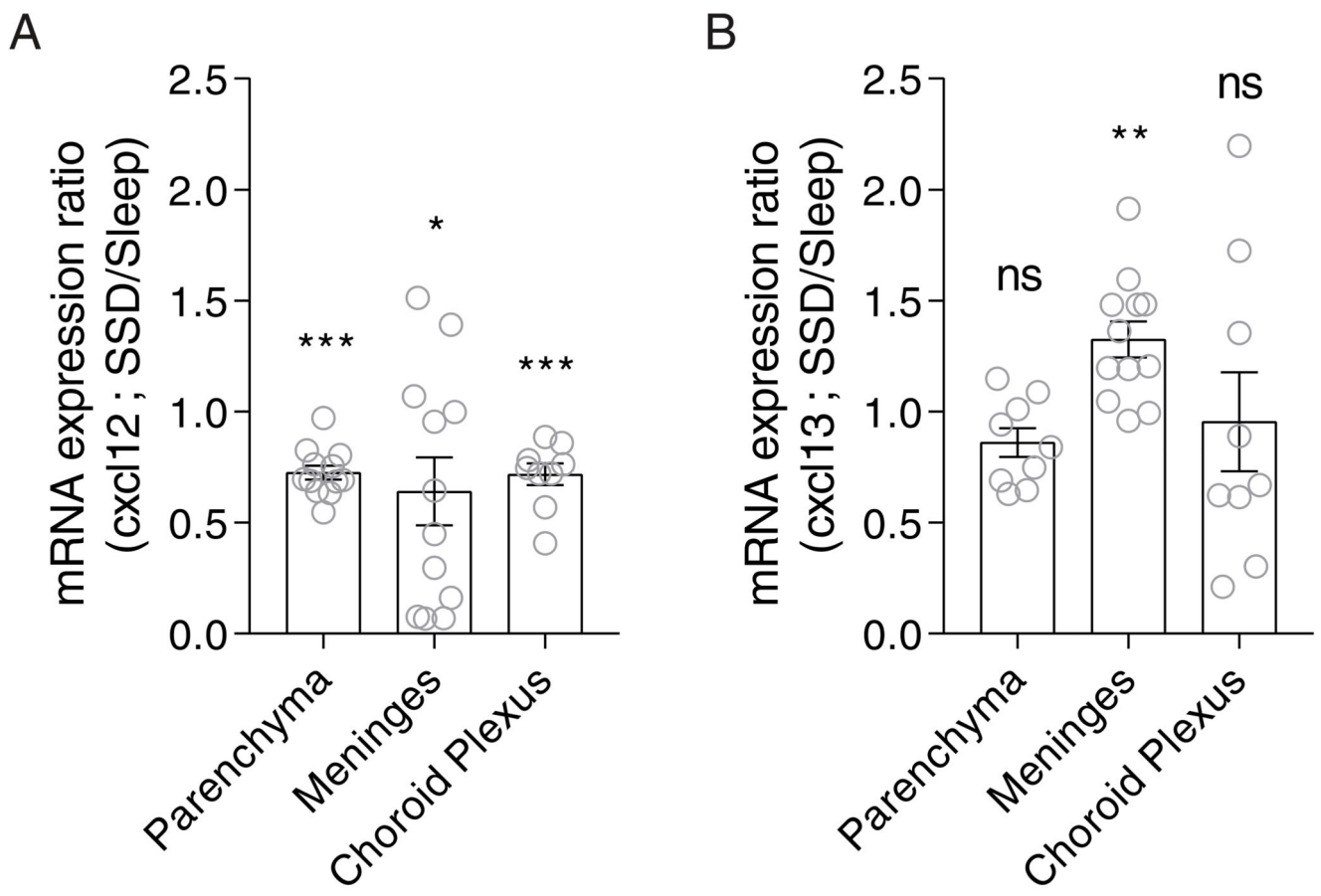

Figure 4. Relative mRNA expression of *cxcl12* and *cxcl13* in the meninges, choroid plexus (CP) and brain parenchyma.

qPCR analyses of (**A**) *cxcl12* and (**B**) *cxcl13* expression levels in the brain parenchyma, meninges, and choroid plexus following 2 hours of sleep deprivation. Relative mRNA expression was calculated by dividing each value in the sleep-deprived group with the mean value of the sleep group for each gene and tissue. Data are presented as mean±sem; One sample t-test, n=9-12 individual mice; *P<0.05; **P<0.01; ***P<0.001; data of three experiments. SSD, sleep-deprived; ns, not significant.