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Full-length Article

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Microglial homeostasis disruption modulates non-rapid eye movement sleep duration and neuronal activity in adult female mice

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The raw data supporting the findings of this study are available from the corresponding authors upon reasonable request.

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Abstract

Sleep is a natural physiological state, tightly regulated through several neuroanatomical and neurochemical systems, which is essential to maintain physical and mental health. Recent studies revealed that the functions of microglia, the resident immune cells of the brain, differ along the sleep-wake cycle. Inflammatory cytokines, such as interleukin-1β and tumor necrosis factor-α, mainly produced by microglia in the brain, are also well-known to promote sleep. However, the contributing role of microglia on sleep regulation remains largely elusive, even more so in females. Given the higher prevalence of various sleep disorders in women, we aimed to determine the role of microglia in regulating the sleep-wake cycle specifically in female mice. Microglia were depleted in adult female mice with inhibitors of the colony-stimulating factor 1 receptor (CSF1R) (PLX3397 or PLX5622), which is required for microglial population maintenance. This led to a 65-73% reduction of the microglial population, as confirmed by immunofluorescence staining against IBA1 (marker of microglia/macrophages) and TMEM119 (microglia-specific marker) in the reticular nucleus of the thalamus and primary motor cortex. The spontaneous sleep-wake cycle was evaluated at steady-state, during microglial homeostasis disruption and after complete microglial repopulation, upon cessation of treatment with the inhibitors of CSF1R, using electroencephalography (EEG) and electromyography (EMG). We found that microglia-depleted female mice spent more time in non-rapid eye movement (NREM) sleep and had an increased number of NREM sleep episodes, which was partially restored after microglial total repopulation. To determine whether microglia could regulate sleep locally by modulating synaptic transmission, we used patch clamp to record spontaneous activity of pyramidal neurons in the primary motor cortex, which showed an increase of excitatory synaptic transmission during the dark phase. These changes in neuronal activity were modulated by microglial depletion in a phase-dependent manner.

Altogether, our results indicate that microglia are involved in the sleep regulation of female mice, further strengthening their potential implication in the development and/or progression of sleep disorders. Furthermore, our findings indicate that microglial repopulation can contribute to normalizing sleep alterations caused by their partial depletion.

Keywords

Microglia, sleep, EEG, microglia depletion, CSF1R, NREM, synaptic plasticity

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1. Introduction

Sleep is a physiological state highly conserved across species (Cirelli and Tononi, 2008; Vorster and Born, 2015). While sleep is essential to maintain physical and mental health, its exact function remains unclear (Frank and Heller, 2019). Notably, it is proposed that sleep may be involved in the replenishment of the brain's energy stores (Scharf et al., 2008), clearance of metabolic waste (Xie et al., 2013), repair of neuronal DNA damage (Zada et al., 2019), downscaling of weak synapses (Tononi and Cirelli, 2020, 2003) and strengthening of neuronal connections established during learning and memory consolidation (Klinzing et al., 2019; Zhou et al., 2020). Moreover, sleep can be modulated by diverse sleep-regulatory substances, including neurotransmitters (e.g., acetylcholine, dopamine, noradrenaline) and cytokines (e.g., interleukin [IL]-1β and tumor necrosis factor [TNF]α) (Eban-Rothschild et al., 2018). Sleep is also tightly regulated through the body's circadian rhythm—the endogenous circadian clock normally lasting 24 hours—*via* multiple dedicated neuronal networks (Eban-Rothschild et al., 2018). In addition, local sleep occurs throughout the brain constantly, resulting in small areas that can be asleep while the rest of the brain is awake (Krueger et al., 2019). The mechanisms of local sleep are not restricted to specific neuronal networks and involve various molecular factors, including those aforementioned, together with other cell types such as (D'Ambrosio et al., 2019; Krueger et al., 2019). Indeed, there is now increasing evidence that glial cells, especially astrocytes, play a role in sleep regulation and functions undertaken during sleep (Bojarskaite et al., 2020; Haydon, 2017; Ingiosi et al., 2020). Astrocytes can regulate sleep drive (Halassa et al., 2009), clear toxic substances through the glymphatic system (Xie et

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al., 2013) and synchronize the neuronal activity during sleep (Fellin et al., 2012). Less is known about the involvement of microglia, the resident innate immune cells of the central nervous system, but key roles have been hypothesized (Frank, 2019; Garofalo et al., 2020; Nadjar et al., 2017).

Indeed, recent work demonstrates that microglia present morphological and functional variations along the circadian rhythm and the sleep-wake cycle (Garofalo et al., 2020). Microglia in male mice show diurnal rhythms in their morphology, displaying more ramified processes during the dark phase compared to the light phase within the primary somatosensory cortex (Hayashi, 2013). Microglial expression of cathepsin S, a lysosomal cysteine protease, and the purinergic $P2Y_{12}$ receptor, which both have circadian rhythms, are necessary for these morphological changes (Hayashi, 2013; Hayashi et al., 2013). It is hypothesized that these microglia-specific molecules could contribute to the diurnal variation of neuronal activities in the cerebral cortex (Nakanishi et al., 2021). Cortical and hippocampal microglia additionally possess an intrinsic molecular clock. Isolated cortical microglia from adult male mice and hippocampal microglia from adult male rats vary their expression of four main clock genes, Period1 (*Per1*), *Per2*, *Rev-erbα (Nr1d1)*, and Brain and muscle ARNT-like 1 (*Bmal1*), along the circadian cycle (Fonken et al., 2015; Hayashi et al., 2013).

Although these findings indicate that the sleep-wake cycle has diverse effects on microglia, only a few microglia-mediated processes have been hypothesized to participate in sleep regulation. A recent study demonstrates that microglia can modulate stable wakefulness during the dark phase. Specifically, microglia in adult mice regulate neuronal activity in

the anterior thalamic reticular nucleus (TRN) *via* ceramide signaling (H. Liu et al., 2021). It was also showed by Rowe *et al.* that microglial depletion induced by PLX5622, a colony-stimulating factor 1 receptor (CSF1R) inhibitor, could lead to an increased percentage of sleep time during the light period in male mice in the first week following complete microglial depletion (Rowe et al., 2022). Moreover, a study by our group shows that microglia can modulate sleep duration through the fractalkine signaling pathway in male mice (Corsi et al., 2021). Microglia could also contribute to sleep regulation by inducing or sustaining sleep through their production of cytokines such as IL-1β and TNFα, which peak midway through the light-sleep phase in male rats (Fonken et al., 2015). These cytokines regulate non-rapid eye movement (NREM) sleep duration and intensity by modulating neuronal activity in various brain regions (Krueger, 2008). It is also hypothesized that pro-inflammatory cytokines may exert their somnogenic effects by promoting microglial attraction to synapses (Karrer et al., 2015; Nadjar et al., 2017). Microglial production of TNFα is also involved in the phosphorylation of sleep-promoting kinases and numerous synaptic proteins (Pinto et al., 2022). Additionally, microglia may participate in the consolidation of learning and memory occurring during sleep by promoting the formation and elimination of dendritic spines (W. Li et al., 2017; Zhou et al., 2020) or by contributing to synaptic homeostasis, a downscaling process hypothesized to reduce the strength of weaker synapses during sleep (Tononi and Cirelli, 2020, 2003). Indeed, microglia can monitor local neuronal activity by detecting extracellular adenosine triphosphate (ATP) levels, and regulate synaptic activity notably through their phagocytosis of synapses and release of trophic factors (Wake et al., 2019; York et al., 2018). A recent study showed that, within the prefrontal cortex of male rats, microglia

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phagocytose more synaptic elements (both pre- and post-synaptic) that were opsonized (i.e., synapses with 'eat-me' signals) during sleep, thus possibly contributing to the memory consolidation process taking place during this period (Choudhury et al., 2020). This increase in phagocytosis could be influenced by norepinephrine and glucocorticoids, which are increased respectively in the brain and circulation at the onset of the wakefulness period (Choudhury et al., 2020).

While microglial functions across the sleep-wake states have been investigated in males (Corsi et al., 2021), no studies have specifically addressed this in females despite numerous sexual dimorphisms reported in microglial properties (Rahimian et al., 2019; Villa et al., 2019, 2018). Sleep features were also shown to be sexually dimorphic (e.g., sleep latency, NREM sleep duration), in both rodents and humans (Mallampalli and Carter, 2014). Similarly, there are sex differences in sleep-related pathological conditions, where women have a higher prevalence of sleep disorders, such as circadian rhythm sleep disorders, insomnia and restless leg syndrome, and are more affected by their comorbidities (Mallampalli and Carter, 2014; Shaib and Attarian, 2017). We thus aimed to determine the role of microglia in the regulation of sleep-wake cycle specifically in female mice. We partially depleted microglia in adult female mice using either PLX3397 by gavage or PLX5622 in the diet. Both drugs are inhibitors of CSF1R, a factor essential for microglial proliferation and survival (Elmore et al., 2014). Partial microglial depletion was confirmed in two regions involved in sleep regulation, the thalamic reticular nucleus (TRN) and primary motor cortex (MOp) (Durkin and Aton, 2019; Krone et al., 2021), by double immunofluorescence staining against the macrophage and microglia marker ionized calcium-binding adapter molecule 1 (IBA1) (Ito et al., 1998) and the resident microglia-

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specific marker transmembrane protein 119 (TMEM119) (Bennett et al., 2016). To evaluate the importance of microglia in regulating different stages of the sleep-wake cycle, neuronal activity was also recorded by electroencephalography (EEG) coupled with electromyography (EMG) before and after partial microglial depletion, during the period where microglia homeostasis is disrupted. For each animal, the time spent in each sleep-wake stage, as well as the power of cortical slow wave oscillations were measured to assess sleep pressure, an indicator of sleep quality. To test for a causal role of microglia, we determined if microglial repopulation normalized the sleep-wake activity by recording EEG/EMG activity 4 weeks after the end of PLX3397, since microglia can repopulate the central nervous system following cessation of CSF1R inhibitor administration (Elmore et al., 2014). We then investigated the effect of microglial depletion, induced using PLX5622, on the excitatory synaptic transmission of MOp pyramidal neurons to determine if microglia could regulate sleep through their modulation of neuronal activity.

2. Methods

2.1 Animals

All experiments were approved and performed under the guidelines of the EU Directive 2010/63/EU, carried out according to the Quality Reference System of INRAE and approved by the French ethical committee for care and use of animals (Direction Départementale de la Protection des Animaux, approval ID: A33-063-920, #18662), and by the Italian Ministry of Health (Italian D. Leg. 26/2014). Young adult C57BL/6J female mice (2-4 months of age) were housed under a 12:12 hr light-dark cycle (light onset at 7a.m.) with *ad libitum* access to food and water. Animals were generated from in house

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breeding, except for those used for electrophysiology experiments which were C57BL/6 female mice obtain from the European Mouse Mutant Archive (EMMA, Monterotondo, Italy).

2.2 Surgical procedures

Adult female mice were implanted with headmounts (Pinnacle Technologies, Lawrence, Kansas, USA) to record their EEG and EMG activities. They were anaesthetized with isoflurane (induction at 4% and sustained at 1-2%) and received a subcutaneous (s.c.) injection of buprenorphine (0.05 mg/kg). For EEG recordings, 4 stainless steel screws were implanted along the sagittal suture, two anterior and two posterior to Bregma. For EMG recordings, electrodes were inserted into the neck muscles. The implant was secured to the skull using dental acrylic (Decoeur et al., 2020; Nadjar et al., 2013). After surgery, mice were given one s.c. injection of carprofen (5 mg/kg). One week post-surgery, mice were connected to the recording device for 3 days of habituation and their EEG/EMG data were recorded on the fourth day over a period of 24 hrs.

2.3 EEG/EMG experiments

2.3.1 Experimental design

Light phase onset was designated as Zeitgeber time (ZT) 0 (7 a.m.) and dark phase onset as ZT12 (7 p.m.). After the first recording, mice were unplugged from the system and received either PLX3397 (100 mg/kg, MedChemExpress, cat# HY-16749) (n = 6-8 mice) or the vehicle solution (n = 5-6 mice) at an equivalent volume once a day at 8 a.m. (ZT1) for 21 days by oral gavage by the same experimenter to reduce the stress generated by the procedure. The vehicle solution, in which the PLX3397 was dissolved, consisted of 45%

(v/v) polyethylene glycol 300, 5% (v/v) dimethyl sulfoxide and 5% (v/v) Tween20 in double-distilled water. After the substance administration period, mice were reconnected to the system for 48 hrs of habituation followed by 24 hrs of recording, during a period of microglial homeostasis disruption induced by the partial depletion and repopulation, as microglial repopulation was shown to start within a few days following cessation of the PLX3397 administration (Elmore et al., 2015; Li et al., 2021; O'Neil et al., 2018). We thus refer to this timepoint as microglial homeostasis disruption. Mice were then unplugged and returned to their home cages for 4 weeks to allow total microglial repopulation. After the 4 weeks, mice were reconnected to the system for 3 days of habituation followed by 24 hrs of recording. They were then perfused for immunofluorescence studies aimed to assess the percentage of microglial repopulation in regions of interest (Fig. 1). Mice were excluded from the experiments if they presented two or more signs of sickness behavior, i.e., loss of more than 20% of their weight, decreased locomotion, hunched posture, piloerection, and seizures. They were also excluded if the wound around the implant did not heal properly. Only one mice was removed following these criteria.

2.3.2 Data recording and analyses

EEG and EMG signals were recorded using the 8400 recording system from Pinnacle Technology Inc. at a sampling rate of 400 Hz. The EEG/EMG signals were analyzed semi-automatically as described before (Decoeur et al., 2020; Nadjar et al., 2013) in four-second periods by a blinded experimenter using the SleepSign software (KISSEI COMTEC CO., LTD, Matsumoto, Japan). We defined 3 vigilant states based on the level of EEG and EMG activity: wakefulness (Wake) and 2 sleep stages, non-rapid eye movement (NREM) and rapid eye movement (REM). After stage scoring, data were analyzed as a percentage of

total recording time over 12 or 24 hrs. Vigilant states bouts' duration and number were also quantified. Spectral analyses were performed using a Fast Fourier Transform (Hanning Window) and a spectrogram for each vigilance state was obtained. Quantitative measurement of sleep pressure was obtained using the amplitude of delta oscillations (between 0.5 and 4 Hz, also named slow-wave activity) and were restricted to the NREM activity over the light phase (Decoeur et al., 2020; Halassa et al., 2009; Nadjar et al., 2013). Sleep pressure, which builds up during the Wake phase and is gradually eliminated by NREM sleep, can be used as an indicator of the sleep quality (Borbély et al., 2016). Epochs containing movement artifacts were excluded from spectral analyses, but included in the state quantification.

2.4 Microglial density

2.4.1 Tissue preparation

To characterize microglial depletion, we used a different cohort of mice that received by oral gavage either PLX3397 (medChemExpress, cat# HY-16749) or vehicle (45% (v/v) polyethylene glycol 300, 5% (v/v) dimethyl sulfoxide and 5% (v/v) Tween20 in double-distilled water). To characterize total microglial repopulation, we used mice that underwent EEG/EMG recording, to reduce the total number of mice used in this study. Mice were anesthetized with a mixture of pentobarbital and lidocaine (300 mg/kg and 30 mg/kg, respectively) and blood was transcardially flushed with ice-cold phosphate-buffered saline [50mM] (PBS) followed by 4% paraformaldehyde (PFA; Sigma-Aldrich) between 8 a.m. and 10 a.m. (ZT1 and ZT3). Brains were post-fixed in 4% (w/v) PFA overnight at 4°C, cryoprotected in 30% (w/v) sucrose during 48 hrs at 4°C, rapidly frozen with isopentane and stored at -80°C until sectioning. 40 µm-thick coronal sections were cut with a cryostat

(Leica Biosystems), collected and stored in cryoprotectant solution (20% (v/v) glycerol and 30% (v/v) ethylene glycol in PBS) at -20°C until use.

2.4.2 Immunofluorescence

Immunofluorescence staining against IBA1 and TMEM119 was performed as previously described (Bordeleau et al., 2020; González Ibanez et al., 2019). Sections containing the TRN and the MOp (Bregma -0.58 mm to -1.58 mm) were selected based on the Paxinos and Franklin Stereotaxic Atlas, 4th edition (Paxinos and Franklin, 2012). These regions were chosen considering their involvement in the regulation of sleep, including the generation of sleep rhythm (TRN) and slow-wave activity during NREM sleep (MOp) (Durkin and Aton, 2019; Krone et al., 2021).

Free-floating sections were first incubated in citrate buffer [0.1M] at 70°C for 40 min to retrieve antigens. After cooling down, the sections were placed in 0.1% $NaBH_4$ solution for 30 min and washed with PBS. They were incubated in a blocking solution of 10% donkey serum with 0.5% (v/v) gelatin and 0.1% (v/v) Triton X-100 in PBS [PBS(T)] for 1 hr at room temperature (RT). Sections were then incubated with mouse anti-IBA1 (1:150, cat# MABN92, EMD Millipore) and rabbit anti-TMEM119 (1:300, cat# ab209064, Abcam) primary antibodies in blocking buffer at 4°C overnight. The next day, sections were rinsed in PBS(T), then incubated with blocking buffer containing donkey anti-mouse Alexa Fluor ® 555-conjugated (1:300, cat# A31570, Invitrogen) and donkey anti-rabbit Alexa Fluor ® 647-conjugated (1:300, cat# A31573, Invitrogen) secondary antibodies for 90 min at RT. Sections were washed in PBS, counter-stained with DAPI (4',6-diamidino-

2-phénylindole; 1:20000, Thermo-scientific), mounted on slides, and coverslipped with an anti-fading medium (Fluoromount-G, SouthernBiotech).

2.4.3 Imaging and analysis

For imaging, we used 6 brain sections per mice from 3 to 4 mice per experimental group. The sections, containing the TRN and the MOp (Bregma -0.58 mm to -1.58 mm), were imaged in a single plane at 20x magnification using an Axio Imager M2 epifluorescence microscope (Zeiss, Oberkochen, Germany) equipped with an ORCA-Flash 4.0 digital camera (Hamamatsu, Japan). The slide scanning workflow from MBF Bioscience was used to create the mosaics.

To prevent bias, the analysis of microglial density and distribution was conducted in all the captured images and was performed blinded to the experimental conditions with ImageJ software (National Institutes of Health) as previously described (Tremblay et al., 2012). Microglial density and spacing were calculated, respectively, using the "analyze particles" and "nearest neighbour distance" plugins. Microglial density was defined as the total number of IBA1 positive (+)/TMEM119+ cells divided by the total area (microglia/mm^2) for each region of interest in each animal. Spacing index was calculated as the square of the average nearest neighbour distance multiplied by microglia density per region of interest per animal. The total counts of IBA1+/TMEM119+ (microglia) and IBA1+/TMEM119 negative (-) (possible infiltrating macrophages) cells were compiled per region of interest per animal, using the analyze particles plugin as previously described to account for potential peripheral myeloid cell infiltration (González Ibanez et al., 2019). It is however possible that some IBA1+/TMEM119- observed are not infiltrating cells, but microglia that downregulated their TMEM119 expression in response to the homeostasis

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perturbation caused by CSF1R-induced microglial depletion (Ruan and Elyaman, 2022) which warrants further investigation.

2.5 Electrophysiology

For electrophysiology recordings, microglia were depleted by administering the Plexxikon CSF1R inhibitor PLX5622, which was kindly provided by Plexxikon inc. (Berkeley, USA), and formulated in standard chow AIN-76A by Research Diets (1200 ppm added to AIN-76A chow, Research Diets). PLX5622 also acts through CSF1R as PLX3397 and has a similar structure to PLX3397, with structural alterations on the two pyridine moieties, which allow for a higher specificity (Spangenberg et al., 2019). PLX5622 was used instead of PLX3397 for the electrophysiology experiments as it was the CSF1R inhibitor accessible to Dr. Limatola's group. C57BL/6 female mice received the inhibitor starting 7 days before the experiment onset until the end of the experiment (Fig. 1C). An age-matched control group received a control diet for the same duration (AIN-76A, Research Diets).

2.5.1 Slice preparation

Mice were euthanized at either ZT4 or ZT16. These two time points were chosen for being away from the daily changes in ambient light (ZT0 and ZT12), thus better representing animal behavior during the two phases (light and dark). To carry out all the electrophysiological recordings during working hours, mice that were euthanized at ZT16 received a light-dark cycle inversion (light on 7 p.m.) starting at least 14 days prior to euthanasia, which allowed for all (ZT4 or ZT16) slices to be harvested at 11 a.m. (Corsi et al., 2021). Mice were anesthetized with halothane then decapitated and their whole brains were rapidly extracted and immersed in ice-cold gassed (95% O_2, 5% CO_2), sucrose-based

artificial cerebrospinal fluid (ACSF) containing (in mM) 87 NaCl, 75 Sucrose, 2 KCl, 7 $MgCl_2$, 0.5 $CaCl_2$, 25 $NaHCO_3$, 1.2 NaH_2PO_4 and 10 glucose, pH 7.4, 300–305 mOsm, then sectioned (ThermoScientific HM 650 V) in coronal slices (300 μm). Following sectioning, slices were stored in oxigenated artificial cerebrospinal fluid (ACSF; NaCl 125 mM, KCl 4 mM, $CaCl_2$ 2.5 mM, $MgSO_4$ 1.5 mM, NaH_2PO4 1 mM, $NaHCO_3$ 26 mM, glucose 10 mM; 295-300 mOsm), and were allowed to recover for at least 1 hr after their preparation.

2.5.2 Patch-clamp recordings

Whole-cell patch clamp recordings were performed on MOp pyramidal neurons (layer 2/3) at RT using a Multiclamp 700B amplifier (Molecular Devices, USA) (n = 7-14 cells, in N = 2-4 mice per experimental group). The ACSF was perfused at a rate of approximately 2 ml/min using a gravity-driven perfusion system. Glass electrodes (3-4 mΩ) were pulled with a vertical puller (PC-10, Narishige) and pipettes were filled with 140 mM Cs-methanesulfonate, 10 mM Hepes, 0.5 mM EGTA, 2 mM Mg-ATP, 0.3 mM Na_3-GTP, 2mM $MgCl_2$, 2 mM QX314Br (295-300 mOsm, pH 7.2). Cell capacitance was constantly monitored and the experiments where access resistance changed more than 20% were discarded. Spontaneous excitatory post-synaptic currents (sEPSCs) were recorded by holding the cell -65 mV. Signals were acquired with DigiData-1440A, sampled at 10 kHz and low-pass filtered at 2 kHz using pCLAMP-v10 software (Molecular Devices, USA).

Analysis of sEPSCs was performed offline using MiniAnalysis software (Mini Analysis, Synaptosoft Fort Lee, NJ, USA) with the threshold for detection set at 5 pA. Cumulative probability curves were constructed by considering 100 consecutive events for each cell.

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2.6 Statistical analyses

Statistical analyses were performed with Prism software (GraphPad, version 9). Normality was verified using a Shapiro-Wilk test. For normally distributed datasets, a Grubbs' test (two-tailed, $\alpha = 0.05$) was used to identify outliers that were removed from the datasets prior to performing parametric statistical analysis. Overall, 4 outliers were removed: one in Duration Wake episode – Baseline PLX3397 group, one in Percentage of Wake time over 24h – Baseline vehicle animal, one in Percentage of REM sleep over 12h – PLX3397 Repopulation dark phase, and one in Sleep pressure PLX3397 – Baseline ZT 1-2 group. Data were analyzed using a two-way ANOVA for microglial density (n = 3-4 animals per group) or a mixed-effect model for the sleep analysis (n = 5-8 animals per group), followed by Tukey *post-hoc* test when applicable. The cumulative distribution analyses were analysed with the Kolmogorov–Smirnov two-sample test (KS) using the MiniAnalysis software (Synaptosoft Fort Lee, NJ, USA). Mean differences were considered statically significant when $p < 0.05$. All reported values are mean ± standard error of the mean (SEM).

3. Results

3.1 Oral gavage with PLX3397 partially depletes microglia

To confirm microglial depletion induced by PLX3397, we quantified microglial density in the TRN and the MOp, two regions involved in sleep regulation (Durkin and Aton, 2019; Krone et al., 2021). We performed a double immunofluorescence staining against IBA1 (labels all myeloid cells, including microglia) and TMEM119 (specific to resident microglia under normal physiological conditions) (Bennett et al., 2016). In the TRN,

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microglial population was reduced by 65% (timepoint*treatment $F_{(1,10)} = 85.5$, $p < 0.0001$; vehicle *vs* PLX3397, $p < 0.0001$) (Fig. 2A, C; Supp. Table 1). In the MOp, there was a 73% microglial depletion (timepoint*treatment $F_{(1,10)} = 46.6$, $p < 0.0001$; vehicle *vs* PLX3397, $p < 0.0001$) (Fig. 2B, G; Supp. Table 1). Accordingly, microglial nearest neighbour distance was increased in the TRN (timepoint*treatment $F_{(1,10)} = 61.2$, $p < 0.0001$; vehicle *vs* PLX3397, $p < 0.0001$) and the MOp (timepoint*treatment $F_{(1,10)} = 14.9$, $p = 0.00315$; vehicle *vs* PLX3397, $p = 0.00349$) (Fig. 2E, I; Supp. Table 1) following PLX3397 oral gavage. Microglial spacing index was reduced following partial depletion in the MOp (timepoint*treatment $F_{(1,10)} = 7.98$, $p = 0.0180$; vehicle *vs* PLX3397, $p = 0.0150$), but was relatively unchanged in the TRN (treatment*timepoint $F_{(1,10)} = 2.37$, $p = 0.155$) (Fig. 2I, J; Supp. Table 1). Moreover, the presence of IBA1+/TMEM119- cells was increased after partial microglial depletion in the MOp (timepoint*treatment $F_{(1,10)} = 17.4$, $p = 0.00192$; vehicle *vs* PLX3397, $p = 0.00192$) but not in the TRN (treatment*timepoint $F_{(1,10)} = 1.03$, $p = 0.3351$) (Fig. 2D, H; Supp. Table 1). At the end of PLX3397 treatment, we confirmed the microglial repopulation of mice that underwent EEG/EMG recording, i.e., 4 weeks after the end of the oral gavage coinciding with the last EEG/EMG recording. After repopulation, microglial density was not statistically different between female mice that received the vehicle and those who received PLX3397 in both examined regions (Fig. 2C,G; Supp. Table 1). Microglial nearest neighbour distance (Fig. 2E, I; Supp. Table 1) and myeloid cell infiltration were also restored following repopulation (Fig. 2D, H; Supp. Table 1).

While we only characterized microglial depletion and repopulation in the TRN and Mop as they are involved in sleep regulation (Durkin and Aton, 2019; Krone et al., 2021), that doesn't rule out microglial depletion in other central nervous system (CNS) regions.

3.2 Microglial homeostasis disruption increases the amount of NREM sleep

After confirming the partial depletion of microglia in the MOp and TRN, we evaluated the time spent in the different vigilance states across 24 hrs (Fig. 3; Supp. Tables 2 and 3). Following microglial homeostasis disruption, mice had a significant decrease in the percentage of time in Wake ($F_{(1.62,8.10)} = 7.91$, $p = 0.0151$; Baseline *vs* PLX3397, $p = 0.0152$), which was restored by total microglial repopulation (Baseline *vs* Repopulation, $p = 0.648$; PLX3397 *vs* Repopulation, $p = 0.0397$) (Fig. 3A; Supp. Table 2). Mice with microglial homeostasis disruption also showed an increase of the percentage of time spent in NREM sleep ($F_{(1.25,6.27)} = 7.86$, $p = 0.0260$; Baseline *vs* PLX3397, $p = 0.0135$), which was similarly normalized after microglial repopulation (Fig. 3E; Supp. Table 2). No significant difference was found for the relative time spent in REM sleep ($F_{(1.46,12.4)} = 1.29$, $p = 0.297$) (Fig. 3I; Supp. Table 2).

Analysis of the distribution of vigilance states across the light and dark phases did not identify any significant differences between groups (Fig. 3B, F, J; Supp. Table 3), indicating that the Wake (day phase*timepoint $F(_{1.24,3.72}) = 1.99$, $p = 0.245$) and NREM (day phase*timepoint $F(_{1.22,3.67}) = 2.23$, $p = 0.222$) sleep changes were evenly spread across the recording period. Quantification of the number and duration of the different state episodes over a 24 hrs period identified changes after microglial homeostasis disruption. Indeed, mice displayed a significant increase in their number of NREM episodes ($F_{(1.71,8.58)}$

= 5.63, p = 0.0306; Baseline *vs* PLX3397, p = 0.0201), but their average NREM episode duration was not affected ($F_{(0.93,4.65)}$ = 3.57, p = 0.122) (Fig. 3G-H; Supp. Table 2). Wake and REM number of episodes (Wake $F_{(1.46,12.4)}$ = 1.43, p = 0.269; REM $F_{(1.75,14.9)}$ = 1.16, p = 0.334) and duration (Wake $F_{(1.13,9.05)}$ = 1.95, p = 0.197; REM $F_{(1.05,5.26)}$ = 0.753, p = 0.430) were also not affected (Fig. 3C, D, K, L; Supp. Table 2). Total microglial repopulation presented a partial restoration of the number of NREM episodes, which was not statistically significant from Baseline or PLX3397 timepoints (PLX3397 *vs* Repopulation, p = 0.107; Baseline *vs* Repopulation, p = 0.945) (Fig. 3G; Supp. Table 2).

All of these parameters (percent time in vigilance states, hourly analysis of vigilance states, number and duration of episodes) were also measured in mice which received the vehicle, where no significant difference was observed, thus confirming that the oral gavage procedure did not affect sleep duration (Supp. Fig. 1; Supp. Table 2 and 3).

3.3 Microglial homeostasis disruption does not affect the power of delta oscillations

A spectral analysis of EEG signals was performed to evaluate the outcomes on sleep quality after microglial homeostasis disruption and microglial repopulation. The amplitude of oscillations was not changed in any of the vigilant states across the 24-hr recording period (ZT*timepoint Wake $F_{(1.55,7.72)}$ = 0.233, p = 0.744; NREM $F_{(1.60,7.98)}$ = 0.483, p = 0.594; REM $F_{(1.25,6.21)}$ = 0.211, p = 0.714) (Fig. 4A-C; Supp. Table 4). During the light phase, we also looked at sleep pressure, which is known to positively correlate with the amplitude of delta oscillations (Borbély et al., 2016). No change was observed after microglial homeostasis disruption and microglial repopulation (ZT*Timepoint $F_{(2.50,10.4)}$ = 3.23, p = 0.074) (Fig. 4D; Supp. Table 5). In vehicle treated animals, none of these parameters were

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impacted either (Supp. Fig. 2; Supp. Table 4 and 5), indicating the oral gavage procedure did not affect sleep oscillations.

3.4 Microglia modulate excitatory synaptic transmission in a phase-dependent manner

Several studies show light-dark changes in the expression and phosphorylation levels of AMPA receptors in different brain regions, as well as ultrastructural synaptic modifications, which support synaptic scaling across the wake/sleep cycle (de Vivo et al., 2017; Liu et al., 2010; Maret et al., 2011; Vyazovskiy et al., 2008; Yang et al., 2014). To determine whether microglia could modulate sleep through a modulation of synaptic transmission, we quantified the sEPSCs from pyramidal neurons in motor cortex slices harvested at ZT4 (light phase) and ZT16 (dark phase) from control or mice depleted of microglia using PLX5622.

The cumulative probability curve of the sEPSC amplitude was significantly shifted to the right at ZT16 compared to ZT4 in both the control group (KS, p = 1.92 x 10^{-9}) and the PLX5622 group (KS, p = 0.0007) (Fig. 5B), indicating increased sEPSC amplitude. This is in line with a net synaptic potentiation during Wake as reported in cortical and hippocampal regions (Corsi et al., 2021; Vyazovskiy et al., 2008). In addition, PLX5622 treatment shifted to the right the amplitude cumulative probability curve at ZT4 compared to the control animals, indicating an increased amplitude of sEPSC during the light phase with PLX5622 (KS, p = 0.0008), but no effect was observed at ZT16. Moreover, the inter-event intervals (IEIs) cumulative distributions were similar in the ZT4 and ZT16 control groups but shifted towards the left in the mice treated with PLX5622 at both Zeitgeber times (ZT4: KS, p = 0.0004; ZT16: KS, p = 0.0150), reflecting increased sEPSCs frequency

after microglial depletion (Fig. 5C). These results indicate that microglial depletion affects excitatory post-synaptic transmission in the MOp at pre and post-synaptic levels, with some differences observed at the two Zeitgeber times considered. These findings suggest a microglial contribution to synaptic strength regulation in the motor cortex across the light-dark cycle in females.

4. Discussion

In this study, we investigated the role of microglia in the regulation of sleep and wake phases throughout the 12:12 light-dark cycle, upon microglial homeostasis disruption with CSF1R inhibitors and following their repopulation, in female mice. The partial depletion achieved by gavage with PLX3397 that we obtained is consistent with other studies showing that not all microglia are depleted by the treatment with CSFR1 inhibitors (Elmore et al., 2014; M. Li et al., 2017). However, the percentage of depletion we observed is lower than what was previously reported in other studies using a lower dose of PLX3397 through oral gavage (40 mg/kg) (M. Li et al., 2017; Yang et al., 2018). Another team also found a lower depletion rate (around 50%) in the nucleus accumbens of Swiss male mice (da Silva et al., 2021). These discrepancies between studies could be due to depletion efficacy variability between species, sexes and brain regions. The depletion variability between brain regions could be related to the diversity of states within the microglial population resulting in their different functional responses across brain regions (Tan et al., 2020). We observed a different percentage of depletion in the two investigated regions (65% *vs* 73%), which is line with the work of Berve *et al.* who also observed regional differences in microglial depletion (Berve et al., 2020). Interestingly, we also observed in the MOp region

that 19.8% of the cells remaining did not express TMEM119, generally considered a specific marker of resident microglia during normal physiological conditions (Bennett et al., 2016). This could indicate that partial depletion induced infiltration of peripheral myeloid cells in the MOp. Peripheral myeloid cells have been shown to simultaneously repopulate the CNS in combination with the surviving resident microglia following diphtheria induced depletion in *Cx3cr1*$^{CreER/+}$*R26*$^{DTR/+}$ mice which express the diphtheria toxin in myeloid cells (Lund et al., 2018; Zhou et al., 2022). Another possibility is that the remaining IBA1+/TMEM119- cells might be microglia that downregulated their expression of TMEM119 to levels that are too low to be detected by traditional immunofluorescence in the MOp. Indeed, microglial TMEM119 can be downregulated in inflammatory conditions (Cao et al., 2021; Vankriekelsvenne et al., 2022; Young et al., 2021). This is in line with previous studies which showed the remaining microglia following depletion had reduced homeostatic signature genes, including *Tmem119* (Hohsfield et al., 2021; Najafi et al., 2018; Zhan et al., 2020). This downregulation could have affected our microglial density results, potentially indicating an higher depletion rate than what was achieved, which warrants further investigation. This microglial subset could have a low or negative expression of CSF1R, display different properties, as well as influence the changes in sleep-wake cycle we observed. In a mouse model of neuronal ceroid lipofuscinosis disease treated with PLX3397, microglia with a higher expression level of CSF1R and TMEM119 were correlated with a more efficient depletion (Berve et al., 2020).

In our study, we show that microglial homeostasis disruption induced by PLX3397 treatment increases the time spent in NREM sleep during the dark phase, and accordingly,

the number of NREM episodes in female mice. Intriguingly, REM sleep was not affected. We performed the recording on day 3 after termination of the PLX3397 administration, where some level of repopulation most likely had already started. The depletion in our experiments was only partial, and the rate of the repopulation was previously found to differ based on the initial rate of depletion (Li et al., 2021; Mendes et al., 2021; Najafi et al., 2018; O'Neil et al., 2018; Zhan et al., 2019). These studies observed varying rates of total microglial repopulation, between 3 and 14 days. However, in the case that it affected the results, this could indicate that we would have observed a stronger effect on the first day following the withdrawal of the PLX3397. Rowe *et al*, as well as our group recently obtained similar changes regarding the sleep duration in male mice using the same method (Corsi et al., 2021; Rowe et al., 2022). Similar findings were also recently observed in male and female mice by Liu *et al.*, showing a reduction of stable wakefulness due to an increase in the transitions between wakefulness and NREM sleep using a transgenic model, through ceramide signaling. In this model, the diphtheria toxin receptor was conditionally expressed in microglia under the CX3CR1 promoter (H. Liu et al., 2021). However, Gentry *et al.* found no sleep architecture changes in male and female mice that were depleted in microglia following PLX5622 administration through chow. While not significant, they did find a potentially slightly increased sleep duration (~25 min) (Gentry et al., 2022). A study by Barahona *et al.* also investigated whether microglia could regulate sleep through their modulation of canonical cycling genes, but mice receiving PLX5622 in their chow showed no differences in the diurnal expression patterns of these genes (Barahona et al., 2022). Another pathway through which microglia could be involved in sleep regulation is through the production of cytokines such as IL-1β and TNFα, which are known to be sleep-

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regulatory substances (Clinton et al., 2011). Accordingly, microglia-derived TNFα was shown to modulate cortical protein phosphorylation during the sleep period, including sleep-promoting kinases and synaptic proteins (Pinto et al., 2022).

We then investigated sleep pressure, which was not changed by microglial homeostasis disruption, suggesting that NREM sleep is still efficiently reducing it thoughout the light phase. It would be interesting to see in the long term if this increase in NREM sleep duration could lead to behavioral deficits and sleep disorders. Valuable information could be obtained through the investigation of sleep spindles, which are generated from the TRN and occur throughout NREM sleep (Fernandez and Lüthi, 2020). Indeed, it was shown that these waves play an important role in memory consolidation (Rasch and Born, 2013), a process in which microglia are also involved (Nguyen et al., 2020).

Microglial repopulation did restore the NREM sleep duration, but only partially restored the number of NREM episodes, thus resulting in a fragmentation of NREM sleep. This result could indicate that the new microglial population does not accomplish exactly the same roles as the original one, which is intriguing considering that repopulating microglia have a transcriptomic state profile resembling the microglial homeostatic state profile (Elmore et al., 2015). Microglial repopulation originates from the proliferation of the residual pool of resident microglia (Huang et al., 2018; Weber et al., 2019), and from nestin-positive progenitor cells (Elmore et al., 2014), with a potential contribution from macrophages form the periphery (Lund et al., 2018; Zhou et al., 2022). This difference could also be due to others cells being affected by the repopulation. For instance, the expression of glial fibrillary acidic protein, an astrocyte marker, was increased in male mouse cerebral cortex following microglial depletion and repopulation using PLX3397

(Najafi et al., 2018), and astrocytes are well-known to be involved in the regulation of sleep (Garofalo et al., 2020). It is also possible that the partial depletion permantly affected neuronal circuits.

We then looked into synaptic transmission in MOp pyramidal neurons to determine if microglia could regulate sleep by modulating synaptic transmission. We observed a shift to the right of the amplitude cumulative curve of sEPSCs during the dark phase, but the cumulative curves for the IEIs, which are inversely correlated to frequency and rely on pre-synaptic modifications, were not different. This finding possibly indicates underlying post-synaptic modifications across the daily cycle. This is in line with what we observed in males (Corsi et al., 2021) and with other studies showing an increase in synaptic strength during wakefulness (de Vivo et al., 2017; Diering et al., 2017; Liu et al., 2010; Vyazovskiy et al., 2008). These results are also in agreement with the synaptic homeostasis hypothesis which proposes an overall synaptic downscaling during sleep following synaptic strengthening during wakefulness (Tononi and Cirelli, 2020, 2003). However, other groups have instead demonstrated the opposite finding, i.e., that sleep promotes spine formation (W. Li et al., 2017; Yang et al., 2014) and sleep deprivation leads to synaptic loss (Havekes et al., 2016; Raven et al., 2019). These different results could be due to region, sex and/or the sleep deprivation model used (Havekes and Aton, 2020). Moreover, our results show that microglial depletion by PLX5622 increased both the amplitude and the frequency of sEPSC during the light phase, but only the sEPSC frequency during the dark phase. This result could indicate that the reduced presence of microglia compromises the synaptic downscaling process occurring during sleep, thus resulting in an increased synaptic strength in the sleep phase. These results highlight the role of microglia in modulating

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neuronal activity among the motor cortex through the light-dark cycle. The motor cortex is part of the thalamocortical loop, which is involved in the generation of oscillations in NREM sleep (Durkin and Aton, 2019). Thus, a change in synaptic transmission in this region could have resulted in a modification of NREM sleep. It is however possible that there are other regions involved in the changes we found, such as the hypothalamus, from which arise some of the sleep regulatory substances (i.e., orexin, melanin) (Ono and Yamanaka, 2017). Contrary to the current results in female mice, in our previous study in male mice, microglial depletion resulted in the reduction of both amplitude and frequency of sEPSC during the dark phase, and abolished the differences between light phase in the control mice (Corsi et al., 2021). These differences could be due to the measurements being done within different regions, i.e. the motor cortex *versus* the hippocampus. Recent studies have shown that microglial depletion (Yegla et al., 2021) decreases synaptic transmission in the hippocampus in young and aged male rats (Yegla et al., 2021) and impairs glutamatergic synaptic transmission in adult male mice (Basilico et al., 2022), while it increases excitatory and inhibitory synaptic connections in the visual cortex of adult male and female mice (Y.-J. Liu et al., 2021). It could also be caused by differences in the microglial depletion efficacy, which can differ between males and females when using CSF1R inhibitors in rodents (Berve et al., 2020; Easley-Neal et al., 2019; Sharon et al., 2022).

In future experiments, it would be important to look at other CNS cells with which microglia interact, such as astrocytes and oligodendrocytes (Kalafatakis and Karagogeos, 2021; Matejuk and Ransohoff, 2020). It is possible that microglial depletion could have affected their functions, hence indirectly contributing to the results we observed. It would

also be relevant to investigate how the synaptic transmission changes caused by the microglial depletion could be affected by sleep deprivation. Indeed, it was recently shown in male and female mice depleted of microglia using PLX5622 that while the homeostatic sleep rebound following acute sleep deprivation was not altered, the mice presented memory deficits when exposed to a fear conditioning and acute sleep deprivation paradigm (Gentry et al., 2022).

It is important to take into consideration some limitations of our study. CSF1R inhibitors were shown to affect peripheral immune cells, notably by eliminating circulating CX3CR1+, CD115+, F4/80+, and myeloid-epithelial-reproductive tyrosine kinase (MerTK)+ blood cells in mice (Lei et al., 2020; Šimončičová et al., 2022). This could have an impact on the sleep changes we have detected, considering that the peripheral immune system is involved in the regulation of sleep (Besedovsky et al., 2012; Irwin and Opp, 2017). Moreover, two different CSF1R inhibitors (PLX3397 and PLX5622) were used in this study. While both act through CSF1R, they have different specificity (Spangenberg et al., 2019). However, we do observe the same impact on the sleep in females as we did in males in our previous study (Corsi et al., 2021), which suggest that both inhibitors have the same effect on sleep and could be interchangeable in this context. Another limitation to take into consideration is the use of a different anesthetic for the electrophysiology experiments. Microglial morphology and motility, and thus their interactions with neurons, can be impacted by anesthetics. Isoflurane and fentanyl increased microglial process ramification and dynamic parenchymal surveillance in the visual and somatosensory cortices of male and female mice (Liu et al., 2019; Stowell et al., 2019), while ketamine/xylazine and sodium pentobarbital instead reduced motility in the somatosensory

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cortex of male mice (Hristovska et al., 2020). It is thus possible this could have affected our results.

5. Conclusion

In the current study, our results demonstrate that microglia participate in the regulation of sleep/wake activity, likely through the modulation of synaptic transmission in female mice. Future studies will be needed to better characterize these specific microglia-synapse interactions and the underlying molecular mechanisms. Altogether, our findings indicate that microglia play an important role in the regulation of sleep in female mice, and could potentially be involved in the development or progression of sleep disorders in women.

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References

Barahona, R.A., Morabito, S., Swarup, V., Green, K.N., 2022. Cortical diurnal rhythms remain intact with microglial depletion. Sci Rep 12, 114. https://doi.org/10.1038/s41598-021-04079-w

Basilico, B., Ferrucci, L., Ratano, P., Golia, M.T., Grimaldi, A., Rosito, M., Ferretti, V., Reverte, I., Sanchini, C., Marrone, M.C., Giubettini, M., De Turris, V., Salerno, D., Garofalo, S., St-Pierre, M.-K., Carrier, M., Renzi, M., Pagani, F., Modi, B., Raspa, M., Scavizzi, F., Gross, C.T., Marinelli, S., Tremblay, M.-È., Caprioli, D., Maggi, L., Limatola, C., Di Angelantonio, S., Ragozzino, D., 2022. Microglia control glutamatergic synapses in the adult mouse hippocampus. Glia 70, 173–195. https://doi.org/10.1002/glia.24101

Bennett, M.L., Bennett, F.C., Liddelow, S.A., Ajami, B., Zamanian, J.L., Fernhoff, N.B., Mulinyawe, S.B., Bohlen, C.J., Adil, A., Tucker, A., Weissman, I.L., Chang, E.F., Li, G., Grant, G.A., Hayden Gephart, M.G., Barres, B.A., 2016. New tools for studying microglia in the mouse and human CNS. PNAS 113, E1738–E1746.

Berve, K., West, B.L., Martini, R., Groh, J., 2020. Sex- and region-biased depletion of microglia/macrophages attenuates CLN1 disease in mice. Journal of Neuroinflammation 17, 323. https://doi.org/10.1186/s12974-020-01996-x

Besedovsky, L., Lange, T., Born, J., 2012. Sleep and immune function. Pflugers Arch 463, 121–137. https://doi.org/10.1007/s00424-011-1044-0

Bojarskaite, L., Bjørnstad, D.M., Pettersen, K.H., Cunen, C., Hermansen, G.H., Åbjørsbråten, K.S., Chambers, A.R., Sprengel, R., Vervaeke, K., Tang, W., Enger, R., Nagelhus, E.A., 2020. Astrocytic Ca2+ signaling is reduced during sleep and is involved in the regulation of slow wave sleep. Nat Commun 11, 3240. https://doi.org/10.1038/s41467-020-17062-2

Borbély, A.A., Daan, S., Wirz-Justice, A., Deboer, T., 2016. The two-process model of sleep regulation: a reappraisal. J Sleep Res 25, 131–143. https://doi.org/10.1111/jsr.12371

Bordeleau, M., Lacabanne, C., Fernández de Cossío, L., Vernoux, N., Savage, J.C., González-Ibáñez, F., Tremblay, M.-È., 2020. Microglial and peripheral immune priming is partially sexually dimorphic in adolescent mouse offspring exposed to maternal high-fat diet. J Neuroinflammation 17, 264. https://doi.org/10.1186/s12974-020-01914-1

Cao, Z., Harvey, S.S., Chiang, T., Foltz, A.G., Lee, A.G., Cheng, M.Y., Steinberg, G.K., 2021. Unique Subtype of Microglia in Degenerative Thalamus After Cortical Stroke. Stroke 52, 687–698. https://doi.org/10.1161/STROKEAHA.120.032402

Choudhury, M.E., Miyanishi, K., Takeda, H., Islam, A., Matsuoka, N., Kubo, M., Matsumoto, S., Kunieda, T., Nomoto, M., Yano, H., Tanaka, J., 2020. Phagocytic elimination of synapses by microglia during sleep. Glia 68, 44–59. https://doi.org/10.1002/glia.23698

Cirelli, C., Tononi, G., 2008. Is Sleep Essential? PLoS Biol 6.

https://doi.org/10.1371/journal.pbio.0060216

Clinton, J.M., Davis, C.J., Zielinski, M.R., Jewett, K.A., Krueger, J.M., 2011. Biochemical Regulation of Sleep and Sleep Biomarkers. J Clin Sleep Med 7, S38–S42. https://doi.org/10.5664/JCSM.1360

Corsi, G., Picard, K., di Castro, M.A., Garofalo, S., Tucci, F., Chece, G., Del Percio, C., Golia, M.T., Raspa, M., Scavizzi, F., Decoeur, F., Lauro, C., Rigamonti, M., Iannello, F., Ragozzino, D.A., Russo, E., Bernardini, G., Nadjar, A., Tremblay, M.E., Babiloni, C., Maggi, L., Limatola, C., 2021. Microglia modulate hippocampal synaptic transmission and sleep duration along the light/dark cycle. Glia. https://doi.org/10.1002/glia.24090

da Silva, M.C.M., Gomes, G.F., de Barros Fernandes, H., da Silva, A.M., Teixeira, A.L., Moreira, F.A., de Miranda, A.S., de Oliveira, A.C.P., 2021. Inhibition of CSF1R, a receptor involved in microglia viability, alters behavioral and molecular changes induced by cocaine. Sci Rep 11, 15989. https://doi.org/10.1038/s41598-021-95059-7

D’Ambrosio, S., Castelnovo, A., Guglielmi, O., Nobili, L., Sarasso, S., Garbarino, S., 2019. Sleepiness as a Local Phenomenon. Front. Neurosci. 13. https://doi.org/10.3389/fnins.2019.01086

de Vivo, L., Bellesi, M., Marshall, W., Bushong, E.A., Ellisman, M.H., Tononi, G., Cirelli, C., 2017. Ultrastructural Evidence for Synaptic Scaling Across the Wake/sleep Cycle. Science 355, 507–510. https://doi.org/10.1126/science.aah5982

Decoeur, F., Benmamar-Badel, A., Leyrolle, Q., Persillet, M., Layé, S., Nadjar, A., 2020. Dietary N-3 PUFA deficiency affects sleep-wake activity in basal condition and in response to an inflammatory challenge in mice. Brain Behav Immun 85, 162–169. https://doi.org/10.1016/j.bbi.2019.05.016

Diering, G.H., Nirujogi, R.S., Roth, R.H., Worley, P.F., Pandey, A., Huganir, R.L., 2017. Homer1a drives homeostatic scaling-down of excitatory synapses during sleep. Science 355, 511–515. https://doi.org/10.1126/science.aai8355

Durkin, J.M., Aton, S.J., 2019. How Sleep Shapes Thalamocortical Circuit Function in the Visual System. Annu Rev Vis Sci 5, 295–315. https://doi.org/10.1146/annurev-vision-091718-014715

Easley-Neal, C., Foreman, O., Sharma, N., Zarrin, A.A., Weimer, R.M., 2019. CSF1R Ligands IL-34 and CSF1 Are Differentially Required for Microglia Development and Maintenance in White and Gray Matter Brain Regions. Frontiers in Immunology 10.

Eban-Rothschild, A., Appelbaum, L., Lecea, L. de, 2018. Neuronal Mechanisms for Sleep/Wake Regulation and Modulatory Drive. Neuropsychopharmacology 43, 937. https://doi.org/10.1038/npp.2017.294

Elmore, M.R.P., Lee, R.J., West, B.L., Green, K.N., 2015. Characterizing newly repopulated microglia in the adult mouse: impacts on animal behavior, cell morphology, and neuroinflammation. PLoS One 10, e0122912. https://doi.org/10.1371/journal.pone.0122912

Elmore, M.R.P., Najafi, A.R., Koike, M.A., Dagher, N.N., Spangenberg, E.E., Rice, R.A.,

Kitazawa, M., Matusow, B., Nguyen, H., West, B.L., Green, K.N., 2014. CSF1 receptor signaling is necessary for microglia viability, which unmasks a cell that rapidly repopulates the microglia-depleted adult brain. Neuron 82, 380–397. https://doi.org/10.1016/j.neuron.2014.02.040

Fellin, T., Ellenbogen, J., De Pittà, M., Ben-Jacob, E., Halassa, M., 2012. Astrocyte regulation of sleep circuits: experimental and modeling perspectives. Frontiers in Computational Neuroscience 6.

Fernandez, L.M.J., Lüthi, A., 2020. Sleep Spindles: Mechanisms and Functions. Physiological Reviews 100, 805–868. https://doi.org/10.1152/physrev.00042.2018

Fonken, L.K., Frank, M.G., Kitt, M.M., Barrientos, R.M., Watkins, L.R., Maier, S.F., 2015. Microglia inflammatory responses are controlled by an intrinsic circadian clock. Brain Behav Immun 45, 171–179. https://doi.org/10.1016/j.bbi.2014.11.009

Frank, M.G., 2019. The Role of Glia in Sleep Regulation and Function. Handb Exp Pharmacol 253, 83–96. https://doi.org/10.1007/164_2017_87

Frank, M.G., Heller, H.C., 2019. The Function(s) of Sleep. Handb Exp Pharmacol 253, 3–34. https://doi.org/10.1007/164_2018_140

Garofalo, S., Picard, K., Limatola, C., Nadjar, A., Pascual, O., Tremblay, M.-È., 2020. Role of Glia in the Regulation of Sleep in Health and Disease, in: Comprehensive Physiology. American Cancer Society, pp. 687–712. https://doi.org/10.1002/cphy.c190022

Gentry, N.W., McMahon, T., Yamazaki, M., Webb, J., Arnold, T.D., Rosi, S., Ptáček, L.J., Fu, Y.-H., 2022. Microglia are involved in the protection of memories formed during sleep deprivation. Neurobiology of Sleep and Circadian Rhythms 12, 100073. https://doi.org/10.1016/j.nbscr.2021.100073

González Ibanez, F., Picard, K., Bordelau, M., Sharma, K., Bisht, K., Tremblay, M.-È., 2019. Immunofluorescence Staining Using IBA1 and TMEM119 for Microglial Density, Morphology and Peripheral Myeloid Cell Infiltration Analysis in Mouse Brain. J Vis Exp. https://doi.org/10.3791/60510

Halassa, M.M., Florian, C., Fellin, T., Munoz, J.R., Lee, S.-Y., Abel, T., Haydon, P.G., Frank, M.G., 2009. Astrocytic modulation of sleep homeostasis and cognitive consequences of sleep loss. Neuron 61, 213–219. https://doi.org/10.1016/j.neuron.2008.11.024

Havekes, R., Aton, S.J., 2020. Impacts of Sleep Loss versus Waking Experience on Brain Plasticity: Parallel or Orthogonal? Trends Neurosci 43, 385–393. https://doi.org/10.1016/j.tins.2020.03.010

Havekes, R., Park, A.J., Tudor, J.C., Luczak, V.G., Hansen, R.T., Ferri, S.L., Bruinenberg, V.M., Poplawski, S.G., Day, J.P., Aton, S.J., Radwańska, K., Meerlo, P., Houslay, M.D., Baillie, G.S., Abel, T., 2016. Sleep deprivation causes memory deficits by negatively impacting neuronal connectivity in hippocampal area CA1. eLife 5, e13424. https://doi.org/10.7554/eLife.13424

Hayashi, Y., 2013. Diurnal Spatial Rearrangement of Microglial Processes through the

Journal Pre-proofs

Rhythmic Expression of P2Y12 Receptors. J Neurol Disord 01. https://doi.org/10.4172/2329-6895.1000120

Hayashi, Y., Koyanagi, S., Kusunose, N., Okada, R., Wu, Z., Tozaki-Saitoh, H., Ukai, K., Kohsaka, S., Inoue, K., Ohdo, S., Nakanishi, H., 2013. The intrinsic microglial molecular clock controls synaptic strength via the circadian expression of cathepsin S. Sci Rep 3. https://doi.org/10.1038/srep02744

Haydon, P.G., 2017. Astrocytes and the modulation of sleep. Curr Opin Neurobiol 44, 28–33. https://doi.org/10.1016/j.conb.2017.02.008

Hohsfield, L.A., Najafi, A.R., Ghorbanian, Y., Soni, N., Crapser, J., Figueroa Velez, D.X., Jiang, S., Royer, S.E., Kim, S.J., Henningfield, C.M., Anderson, A., Gandhi, S.P., Mortazavi, A., Inlay, M.A., Green, K.N., 2021. Subventricular zone/white matter microglia reconstitute the empty adult microglial niche in a dynamic wave. eLife 10, e66738. https://doi.org/10.7554/eLife.66738

Hristovska, I., Verdonk, F., Comte, J.-C., Tsai, E.S., Desestret, V., Honnorat, J., Chrétien, F., Pascual, O., 2020. Ketamine/xylazine and barbiturates modulate microglial morphology and motility differently in a mouse model. PLOS ONE 15, e0236594. https://doi.org/10.1371/journal.pone.0236594

Huang, Y., Xu, Z., Xiong, S., Sun, F., Qin, G., Hu, G., Wang, J., Zhao, L., Liang, Y.-X., Wu, T., Lu, Z., Humayun, M.S., So, K.-F., Pan, Y., Li, N., Yuan, T.-F., Rao, Y., Peng, B., 2018. Repopulated microglia are solely derived from the proliferation of residual microglia after acute depletion. Nat Neurosci 21, 530–540. https://doi.org/10.1038/s41593-018-0090-8

Ingiosi, A.M., Hayworth, C.R., Harvey, D.O., Singletary, K.G., Rempe, M.J., Wisor, J.P., Frank, M.G., 2020. A Role for Astroglial Calcium in Mammalian Sleep and Sleep Regulation. Current Biology 30, 4373-4383.e7. https://doi.org/10.1016/j.cub.2020.08.052

Irwin, M.R., Opp, M.R., 2017. Sleep Health: Reciprocal Regulation of Sleep and Innate Immunity. Neuropsychopharmacol 42, 129–155. https://doi.org/10.1038/npp.2016.148

Ito, D., Imai, Y., Ohsawa, K., Nakajima, K., Fukuuchi, Y., Kohsaka, S., 1998. Microglia-specific localisation of a novel calcium binding protein, Iba1. Brain Res Mol Brain Res 57, 1–9. https://doi.org/10.1016/s0169-328x(98)00040-0

Kalafatakis, I., Karagogeos, D., 2021. Oligodendrocytes and Microglia: Key Players in Myelin Development, Damage and Repair. Biomolecules 11, 1058. https://doi.org/10.3390/biom11071058

Karrer, M., Lopez, M.A., Meier, D., Mikhail, C., Ogunshola, O.O., Müller, A.F., Strauss, L., Tafti, M., Fontana, A., 2015. Cytokine-induced sleep: Neurons respond to TNF with production of chemokines and increased expression of Homer1a in vitro. Brain, Behavior, and Immunity, Sleep, Brain, Behavior, and Immunity 47, 186–192. https://doi.org/10.1016/j.bbi.2014.11.008

Klinzing, J.G., Niethard, N., Born, J., 2019. Mechanisms of systems memory consolidation during sleep. Nat Neurosci 22, 1598–1610. https://doi.org/10.1038/s41593-019-0467-3

Journal Pre-proofs

Krone, L.B., Yamagata, T., Blanco-Duque, C., Guillaumin, M.C.C., Kahn, M.C., van der Vinne, V., McKillop, L.E., Tam, S.K.E., Peirson, S.N., Akerman, C.J., Hoerder-Suabedissen, A., Molnár, Z., Vyazovskiy, V.V., 2021. A role for the cortex in sleep–wake regulation. Nat Neurosci 24, 1210–1215. https://doi.org/10.1038/s41593-021-00894-6

Krueger, J.M., 2008. The role of cytokines in sleep regulation. Curr Pharm Des 14, 3408–3416. https://doi.org/10.2174/138161208786549281

Krueger, J.M., Nguyen, J.T., Dykstra-Aiello, C.J., Taishi, P., 2019. Local sleep. Sleep Med Rev 43, 14–21. https://doi.org/10.1016/j.smrv.2018.10.001

Lei, F., Cui, N., Zhou, C., Chodosh, J., Vavvas, D.G., Paschalis, E.I., 2020. CSF1R inhibition by a small-molecule inhibitor is not microglia specific; affecting hematopoiesis and the function of macrophages. PNAS 117, 23336–23338.

Li, M., Li, Z., Ren, H., Jin, W.-N., Wood, K., Liu, Q., Sheth, K.N., Shi, F.-D., 2017. Colony stimulating factor 1 receptor inhibition eliminates microglia and attenuates brain injury after intracerebral hemorrhage. J Cereb Blood Flow Metab 37, 2383–2395. https://doi.org/10.1177/0271678X16666551

Li, Q., Shen, C., Liu, Z., Ma, Y., Wang, J., Dong, H., Zhang, X., Wang, Z., Yu, M., Ci, L., Sun, R., Shen, R., Fei, J., Huang, F., 2021. Partial depletion and repopulation of microglia have different effects in the acute MPTP mouse model of Parkinson’s disease. Cell Proliferation 54, e13094. https://doi.org/10.1111/cpr.13094

Li, W., Ma, L., Yang, G., Gan, W., 2017. REM sleep selectively prunes and maintains new synapses in development and learning. Nat Neurosci 20, 427–437. https://doi.org/10.1038/nn.4479

Liu, H., Wang, X., Chen, Lu, Chen, Liang, Tsirka, S.E., Ge, S., Xiong, Q., 2021. Microglia modulate stable wakefulness via the thalamic reticular nucleus in mice. Nat Commun 12, 4646. https://doi.org/10.1038/s41467-021-24915-x

Liu, Y.-J., Spangenberg, E.E., Tang, B., Holmes, T.C., Green, K.N., Xu, X., 2021. Microglia Elimination Increases Neural Circuit Connectivity and Activity in Adult Mouse Cortex. J. Neurosci. 41, 1274–1287. https://doi.org/10.1523/JNEUROSCI.2140-20.2020

Liu, Y.U., Ying, Y., Li, Y., Eyo, U.B., Chen, T., Zheng, J., Umpierre, A.D., Zhu, J., Bosco, D.B., Dong, H., Wu, L.-J., 2019. Neuronal network activity controls microglial process surveillance in awake mice via norepinephrine signaling. Nature Neuroscience 22, 1771–1781. https://doi.org/10.1038/s41593-019-0511-3

Liu, Z.-W., Faraguna, U., Cirelli, C., Tononi, G., Gao, X.-B., 2010. Direct Evidence for Wake-Related Increases and Sleep-Related Decreases in Synaptic Strength in Rodent Cortex. J. Neurosci. 30, 8671–8675. https://doi.org/10.1523/JNEUROSCI.1409-10.2010

Lund, H., Pieber, M., Parsa, R., Han, J., Grommisch, D., Ewing, E., Kular, L., Needhamsen, M., Espinosa, A., Nilsson, E., Överby, A.K., Butovsky, O., Jagodic, M., Zhang, X.-M., Harris, R.A., 2018. Competitive repopulation of an empty microglial niche yields functionally distinct subsets of microglia-like cells. Nat Commun 9, 4845. https://doi.org/10.1038/s41467-018-07295-7

Journal Pre-proofs

Mallampalli, M.P., Carter, C.L., 2014. Exploring Sex and Gender Differences in Sleep Health: A Society for Women's Health Research Report. J Womens Health (Larchmt) 23, 553–562. https://doi.org/10.1089/jwh.2014.4816

Maret, S., Faraguna, U., Nelson, A.B., Cirelli, C., Tononi, G., 2011. Sleep and wake modulate spine turnover in the adolescent mouse cortex. Nat Neurosci 14, 1418–1420. https://doi.org/10.1038/nn.2934

Matejuk, A., Ransohoff, R.M., 2020. Crosstalk Between Astrocytes and Microglia: An Overview. Frontiers in Immunology 11.

Mendes, M.S., Le, L., Atlas, J., Brehm, Z., Ladron-de-Guevara, A., Matei, E., Lamantia, C., McCall, M.N., Majewska, A.K., 2021. The role of P2Y12 in the kinetics of microglial self-renewal and maturation in the adult visual cortex in vivo. eLife 10, e61173. https://doi.org/10.7554/eLife.61173

Nadjar, A., Blutstein, T., Aubert, A., Laye, S., Haydon, P.G., 2013. Astrocyte-derived adenosine modulates increased sleep pressure during inflammatory response. Glia 61, 724–731. https://doi.org/10.1002/glia.22465

Nadjar, A., Wigren, H.-K.M., Tremblay, M.-E., 2017. Roles of Microglial Phagocytosis and Inflammatory Mediators in the Pathophysiology of Sleep Disorders. Front Cell Neurosci 11. https://doi.org/10.3389/fncel.2017.00250

Najafi, A.R., Crapser, J., Jiang, S., Ng, W., Mortazavi, A., West, B.L., Green, K.N., 2018. A limited capacity for microglial repopulation in the adult brain. Glia 66, 2385–2396. https://doi.org/10.1002/glia.23477

Nakanishi, H., Ni, J., Nonaka, S., Hayashi, Y., 2021. Microglial circadian clock regulation of microglial structural complexity, dendritic spine density and inflammatory response. Neurochemistry International 142, 104905. https://doi.org/10.1016/j.neuint.2020.104905

Nguyen, P.T., Dorman, L.C., Pan, S., Vainchtein, I.D., Han, R.T., Nakao-Inoue, H., Taloma, S.E., Barron, J.J., Molofsky, A.B., Kheirbek, M.A., Molofsky, A.V., 2020. Microglial Remodeling of the Extracellular Matrix Promotes Synapse Plasticity. Cell 182, 388-403.e15. https://doi.org/10.1016/j.cell.2020.05.050

O'Neil, S.M., Witcher, K.G., McKim, D.B., Godbout, J.P., 2018. Forced turnover of aged microglia induces an intermediate phenotype but does not rebalance CNS environmental cues driving priming to immune challenge. Acta Neuropathol Commun 6, 129. https://doi.org/10.1186/s40478-018-0636-8

Ono, D., Yamanaka, A., 2017. Hypothalamic regulation of the sleep/wake cycle. Neuroscience Research, Cutting-edge Approaches to Unwrapping the Mysteries of Sleep 118, 74–81. https://doi.org/10.1016/j.neures.2017.03.013

Paxinos, G., Franklin, K.B.J., 2012. Paxinos and Franklin's the Mouse Brain in Stereotaxic Coordinates, 4e édition. ed. Academic Press Inc, Amsterdam.

Pinto, M.J., Cottin, L., Dingli, F., Laigle, V., Ribeiro, L.F., Triller, A., Henderson, F.J., Loew, D., Fabre, V., Bessis, A., 2022. Microglial TNFα orchestrates brain phosphorylation during the sleep period and controls homeostatic sleep.

Journal Pre-proofs

https://doi.org/10.1101/2022.03.24.485623

Rahimian, R., Cordeau, P., Kriz, J., 2019. Brain Response to Injuries: When Microglia Go Sexist. Neuroscience, Microglia-Neuron interactions in health and disease - novel perspectives for translational research 405, 14–23. https://doi.org/10.1016/j.neuroscience.2018.02.048

Rasch, B., Born, J., 2013. About sleep’s role in memory. Physiol Rev 93, 681–766. https://doi.org/10.1152/physrev.00032.2012

Raven, F., Meerlo, P., Van der Zee, E.A., Abel, T., Havekes, R., 2019. A brief period of sleep deprivation causes spine loss in the dentate gyrus of mice. Neurobiol Learn Mem 160, 83–90. https://doi.org/10.1016/j.nlm.2018.03.018

Rowe, R.K., Green, T.R.F., Giordano, K.R., Ortiz, J.B., Murphy, S.M., Opp, M.R., 2022. Microglia Are Necessary to Regulate Sleep after an Immune Challenge. Biology 11, 1241. https://doi.org/10.3390/biology11081241

Scharf, M.T., Naidoo, N., Zimmerman, J.E., Pack, A.I., 2008. The energy hypothesis of sleep revisited. Progress in Neurobiology 86, 264–280. https://doi.org/10.1016/j.pneurobio.2008.08.003

Shaib, F., Attarian, H., 2017. Chapter 40 - Sex and Gender Differences in Sleep Disorders: An Overview, in: Legato, M.J. (Ed.), Principles of Gender-Specific Medicine (Third Edition). Academic Press, San Diego, pp. 585–601. https://doi.org/10.1016/B978-0-12-803506-1.00046-2

Sharon, A., Erez, H., Spira, M.E., 2022. Significant Sex Differences in the Efficacy of the CSF1R Inhibitor-PLX5622 on Rat Brain Microglia Elimination. Pharmaceuticals 15, 569. https://doi.org/10.3390/ph15050569

Šimončičová, E., Andrade, E.G. de, Vecchiarelli, H.A., Awogbindin, I.O., Delage, C.I., Tremblay, M.-È., 2022. Present and future of microglial pharmacology. Trends in Pharmacological Sciences 0. https://doi.org/10.1016/j.tips.2021.11.006

Spangenberg, E., Severson, P.L., Hohsfield, L.A., Crapser, J., Zhang, J., Burton, E.A., Zhang, Y., Spevak, W., Lin, J., Phan, N.Y., Habets, G., Rymar, A., Tsang, G., Walters, J., Nespi, M., Singh, P., Broome, S., Ibrahim, P., Zhang, C., Bollag, G., West, B.L., Green, K.N., 2019. Sustained microglial depletion with CSF1R inhibitor impairs parenchymal plaque development in an Alzheimer’s disease model. Nat Commun 10, 3758. https://doi.org/10.1038/s41467-019-11674-z

Stowell, R.D., Sipe, G.O., Dawes, R.P., Batchelor, H.N., Lordy, K.A., Whitelaw, B.S., Stoessel, M.B., Bidlack, J.M., Brown, E., Sur, M., Majewska, A.K., 2019. Noradrenergic signaling in the wakeful state inhibits microglial surveillance and synaptic plasticity in the mouse visual cortex. Nature Neuroscience 22, 1782–1792. https://doi.org/10.1038/s41593-019-0514-0

Tan, Y.-L., Yuan, Y., Tian, L., 2020. Microglial regional heterogeneity and its role in the brain. Mol Psychiatry 25, 351–367. https://doi.org/10.1038/s41380-019-0609-8

Tononi, G., Cirelli, C., 2020. Sleep and synaptic down-selection. Eur J Neurosci 51, 413–

421. https://doi.org/10.1111/ejn.14335

Tononi, G., Cirelli, C., 2003. Sleep and synaptic homeostasis: a hypothesis. Brain Research Bulletin 62, 143–150. https://doi.org/10.1016/j.brainresbull.2003.09.004

Tremblay, M.-È., Zettel, M.L., Ison, J.R., Allen, P.D., Majewska, A.K., 2012. Effects of aging and sensory loss on glial cells in mouse visual and auditory cortices. Glia 60, 541–558. https://doi.org/10.1002/glia.22287

Vankriekelsvenne, E., Chrzanowski, U., Manzhula, K., Greiner, T., Wree, A., Hawlitschka, A., Llovera, G., Zhan, J., Joost, S., Schmitz, C., Ponsaerts, P., Amor, S., Nutma, E., Kipp, M., Kaddatz, H., 2022. Transmembrane protein 119 is neither a specific nor a reliable marker for microglia. Glia 70, 1170–1190. https://doi.org/10.1002/glia.24164

Villa, A., Della Torre, S., Maggi, A., 2019. Sexual differentiation of microglia. Frontiers in Neuroendocrinology 52, 156–164. https://doi.org/10.1016/j.yfrne.2018.11.003

Villa, A., Gelosa, P., Castiglioni, L., Cimino, M., Rizzi, N., Pepe, G., Lolli, F., Marcello, E., Sironi, L., Vegeto, E., Maggi, A., 2018. Sex-Specific Features of Microglia from Adult Mice. Cell Reports 23, 3501–3511. https://doi.org/10.1016/j.celrep.2018.05.048

Vorster, A.P., Born, J., 2015. Sleep and memory in mammals, birds and invertebrates. Neuroscience & Biobehavioral Reviews, Brain, Memory and Development: The Imprint of Gabriel Horn 50, 103–119. https://doi.org/10.1016/j.neubiorev.2014.09.020

Vyazovskiy, V.V., Cirelli, C., Pfister-Genskow, M., Faraguna, U., Tononi, G., 2008. Molecular and electrophysiological evidence for net synaptic potentiation in wake and depression in sleep. Nat Neurosci 11, 200–208. https://doi.org/10.1038/nn2035

Wake, H., Horiuchi, H., Kato, D., Moorhouse, A.J., Nabekura, J., 2019. Physiological Implications of Microglia–Synapse Interactions, in: Garaschuk, O., Verkhratsky, A. (Eds.), Microglia: Methods and Protocols, Methods in Molecular Biology. Springer, New York, NY, pp. 69–80. https://doi.org/10.1007/978-1-4939-9658-2_6

Weber, M.D., McKim, D.B., Niraula, A., Witcher, K.G., Yin, W., Sobol, C.G., Wang, Y., Sawicki, C.M., Sheridan, J.F., Godbout, J.P., 2019. The Influence of Microglial Elimination and Repopulation on Stress Sensitization Induced by Repeated Social Defeat. Biological Psychiatry, The Biology of Stress-Induced Depression 85, 667–678. https://doi.org/10.1016/j.biopsych.2018.10.009

Xie, L., Kang, H., Xu, Q., Chen, M.J., Liao, Y., Thiyagarajan, M., O'Donnell, J., Christensen, D.J., Nicholson, C., Iliff, J.J., Takano, T., Deane, R., Nedergaard, M., 2013. Sleep drives metabolite clearance from the adult brain. Science 342, 373–377. https://doi.org/10.1126/science.1241224

Yang, G., Lai, C.S.W., Cichon, J., Ma, L., Li, W., Gan, W.-B., 2014. Sleep promotes branch-specific formation of dendritic spines after learning. Science 344, 1173–1178. https://doi.org/10.1126/science.1249098

Yang, X., Ren, H., Wood, K., Li, M., Qiu, S., Shi, F.-D., Ma, C., Liu, Q., 2018. Depletion of microglia augments the dopaminergic neurotoxicity of MPTP. FASEB J 32, 3336–3345. https://doi.org/10.1096/fj.201700833RR

Journal Pre-proofs

Yegla, B., Boles, J., Kumar, A., Foster, T.C., 2021. Partial microglial depletion is associated with impaired hippocampal synaptic and cognitive function in young and aged rats. Glia 69, 1494–1514. https://doi.org/10.1002/glia.23975

York, E.M., Bernier, L.-P., MacVicar, B.A., 2018. Microglial modulation of neuronal activity in the healthy brain. Developmental Neurobiology 78, 593–603. https://doi.org/10.1002/dneu.22571

Young, K.F., Gardner, R., Sariana, V., Whitman, S.A., Bartlett, M.J., Falk, T., Morrison, H.W., 2021. Can quantifying morphology and TMEM119 expression distinguish between microglia and infiltrating macrophages after ischemic stroke and reperfusion in male and female mice? J Neuroinflammation 18, 58. https://doi.org/10.1186/s12974-021-02105-2

Zada, D., Bronshtein, I., Lerer-Goldshtein, T., Garini, Y., Appelbaum, L., 2019. Sleep increases chromosome dynamics to enable reduction of accumulating DNA damage in single neurons. Nature Communications 10, 895. https://doi.org/10.1038/s41467-019-08806-w

Zhan, L., Fan, L., Kodama, L., Sohn, P.D., Wong, M.Y., Mousa, G.A., Zhou, Y., Li, Y., Gan, L., 2020. A MAC2-positive progenitor-like microglial population is resistant to CSF1R inhibition in adult mouse brain. eLife 9, e51796. https://doi.org/10.7554/eLife.51796

Zhan, L., Krabbe, G., Du, F., Jones, I., Reichert, M.C., Telpoukhovskaia, M., Kodama, L., Wang, C., Cho, S., Sayed, F., Li, Y., Le, D., Zhou, Y., Shen, Y., West, B., Gan, L., 2019. Proximal recolonization by self-renewing microglia re-establishes microglial homeostasis in the adult mouse brain. PLOS Biology 17, e3000134. https://doi.org/10.1371/journal.pbio.3000134

Zhou, K., Han, J., Lund, H., Boggavarapu, N.R., Lauschke, V.M., Goto, S., Cheng, H., Wang, Y., Tachi, A., Xie, C., Zhu, K., Sun, Y., Osman, A.M., Liang, D., Han, W., Gemzell-Danielsson, K., Betsholtz, C., Zhang, X.-M., Zhu, C., Enge, M., Joseph, B., Harris, R.A., Blomgren, K., 2022. An overlooked subset of Cx3cr1wt/wt microglia in the Cx3cr1CreER-Eyfp/wt mouse has a repopulation advantage over Cx3cr1CreER-Eyfp/wt microglia following microglial depletion. Journal of Neuroinflammation 19, 20. https://doi.org/10.1186/s12974-022-02381-6

Zhou, Y., Lai, C.S.W., Bai, Y., Li, W., Zhao, R., Yang, G., Frank, M.G., Gan, W.-B., 2020. REM sleep promotes experience-dependent dendritic spine elimination in the mouse cortex. Nat Commun 11. https://doi.org/10.1038/s41467-020-18592-5

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List of abbreviations

+: positive

-: negative

ACSF: artificial cerebrospinal fluid

ATP: adenosine triphosphate

a.u.: arbitrary units

Bmal1: brain and muscle ARNT-like 1

CD: cluster of differentiation

CNS: central nervous system

CSF1R: colony-stimulating factor 1 receptor

Cx3cr1: fracktalkine receptor

EEG: electroencephalography

EMG: electromyography

IBA1: ionized calcium-binding adapter molecule 1

Ic: internal capsule

IL: interleukin

IEIs: inter-event intervals

MerTK: myeloid-epithelial-reproductive tyrosine kinase

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MOp: primary motor cortex area

NND: nearest neighbour distance

NREM: non-rapid eye movement

PBS: phosphate-buffered saline

Per: period

PFA: paraformaldehyde

REM: rapid eye movement

RT: room temperature

s.c.: subcutaneous

sEPSCs: spontaneous excitatory post-synaptic currents

SEM: standard error of the mean

T: triton

TMEM119: transmembrane protein 119

TNF: tumor necrosis factor

TRN: thalamic reticular nucleus

ZT: Zeitgeber time

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Figures, Tables and additional files

Figure 1. Experimental time course. Adult female mice were first implanted with headmounts to record their EEG and EMG activities. One week post-surgery, mice were connected to the recording device for 3 days of habituation and their EEG/EMG data were recorded on the fourth day over a period of 24 hrs (Baseline). After the substance administration period, mice were reconnected to the system for 48 hrs of habituation followed by 24 hrs of recording (Microglial homeostasis disruption). Mice were then unplugged and returned to their home cages for 4 weeks to allow for microglial total repopulation. After 4 weeks, mice were reconnected to the system for 3 days of habituation followed by 24 hrs of recording (Repopulation). Mice were then perfused for immunofluorescence to assess the percentage of microglial repopulation. Two other cohorts were used to determine microglial density after partial microglial depletion (B) and for the patch-clamp experiments (C). EEG: electroencephalography, EMG: electromyography, ZT: Zeitgeber time.

Figure 2. Confirmation of microglial depletion and repopulation in the thalamic reticular nucleus and primary motor cortex. Representative epifluorescence pictures at a 20x magnification showing IBA1(red) and TMEM119 (green) stained microglia in the TRN (A) and MOp (B) of the 4 experimental groups. Scale bar is equivalent to 70 μm. White arrows: IBA1+/TMEM119- cells. PLX3397 treatment induced a 65% microglial depletion in the TRN (C), as well as an increase in IBA1+/TMEM119- cell density (D). Accordingly, microglial nearest neighbour distance (E) and spacing index (F) were reduced. PLX3397 treatment induced a 73% microglial depletion in the MOp (G), but did

not affect the IBA1+/TMEM119- cell density (H). Microglial nearest neighbour distance (I) was reduced following the administration of PLX3397, but the spacing index (J) was not affected. n = 3-4 mice/group. Data are expressed as mean ± standard error of the mean. Statistical significance was assessed by 2-way ANOVA followed by Tukey *post-hoc* analysis, where **p<0.01, ***p< 0.001, and ****p< 0.0001. a.u.: arbitrary units, TRN: reticular nucleus of the thalamus, Ic: internal capsule, NND: nearest neighbour distance.

Figure 3. Microglial homeostasis disruption alters the architecture of sleep/wake cycles over 24 hours. Percentage of time spent in Wake (A) was decreased after microglial homeostasis disruption following PLX3397 treatment and restored after microglial repopulation, but the percentage during light and dark phase (B), and the number and duration of episodes (C-D) were not affected. Accordingly, the percentage of time spent in NREM sleep (E) was increased with microglial homeostasis disruption, and was also restored after total microglial repopulation, but the percentage of time spent in NREM sleep during light and dark phase was not affected (F). The number of NREM episodes was increased by the microglial homeostasis disruption, but it was not entirely restored by the total repopulation (G). The duration of NREM episodes was unaltered (H). The REM sleep parameters (I-L) were not changed. n= 6-8 mice/group. Data are expressed as mean ± standard error of the mean. Statistical significance was assessed by a mixed-effects analysis followed by Tukey *post-hoc* analysis, where *p<0.05. NREM: non-rapid eye movement, REM: rapid-eye movement.

Figure 4. Microglial homeostasis disruption does not alter slow wave activity during NREM sleep. Normalized power of EEG traces over 24 hours during Wake (A), NREM (B) and REM (C) stages are not changed by microglial homeostasis disruption following

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PLX3397 treatment. The normalized power of delta oscillations (SWA) was also not changed (D). n = 6-8 mice/group. Data are expressed as mean ± standard error of the mean. Statistical significance was assessed by mixed-effects analysis. NREM: non-rapid eye movement, REM: rapid-eye movement.

Figure 5. Synaptic changes in sEPSCs over the light-dark cycle are affected by PLX5622 treatment. A) Representative traces of sEPSC currents recorded from neurons in motor cortex slices harvested at ZT4 (light phase) and ZT16 (dark phase) from control or PLX5622 treated mice. A significant rightward shift in the cumulative probability curve of the sEPSCs amplitude (B) was observed at ZT4, but not at ZT16 following PLX5622 treatment. The cumulative distribution of the inter-event intervals (IEIs) (C) was shifted to the left upon PLX5622 treatment at ZT4 and ZT16 (n = 7-14 cells, in N = 2-4 mice/group). Cumulative probability functions were compared with Kolmogorov-Smirnov test. Ctrl: control, sEPSCs: spontaneous excitatory post-synaptic currents, ZT: Zeitgeber time.

Figure 1

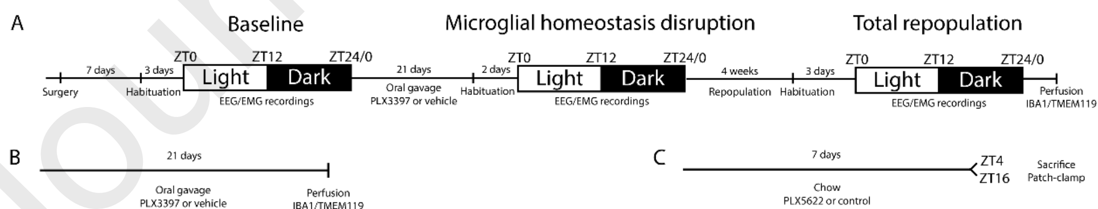

Figure 2

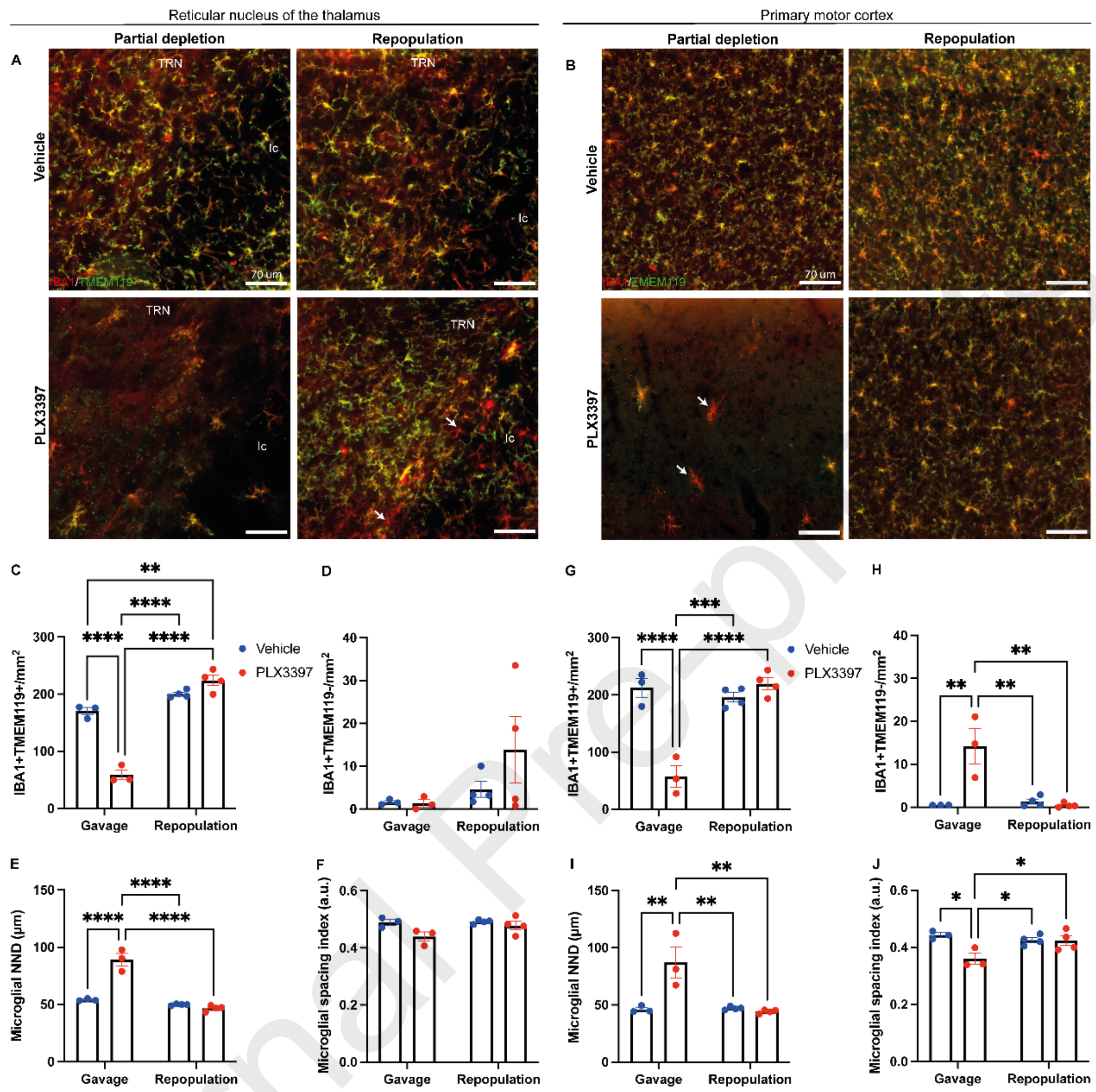

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Figure 3

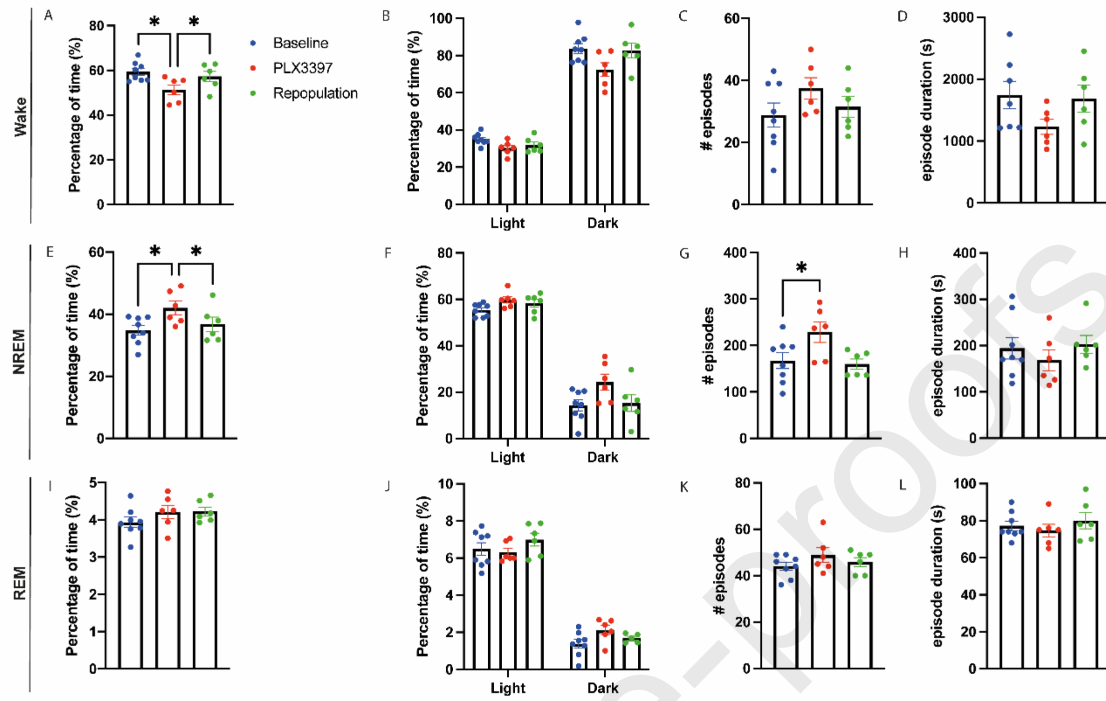

Figure 4

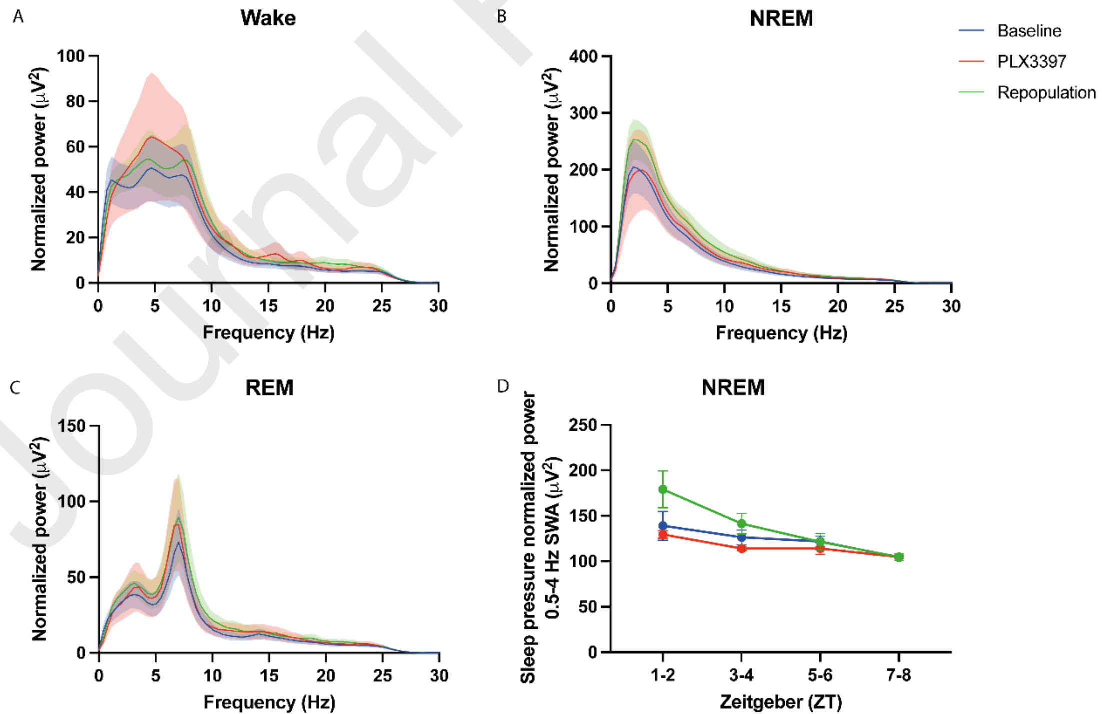

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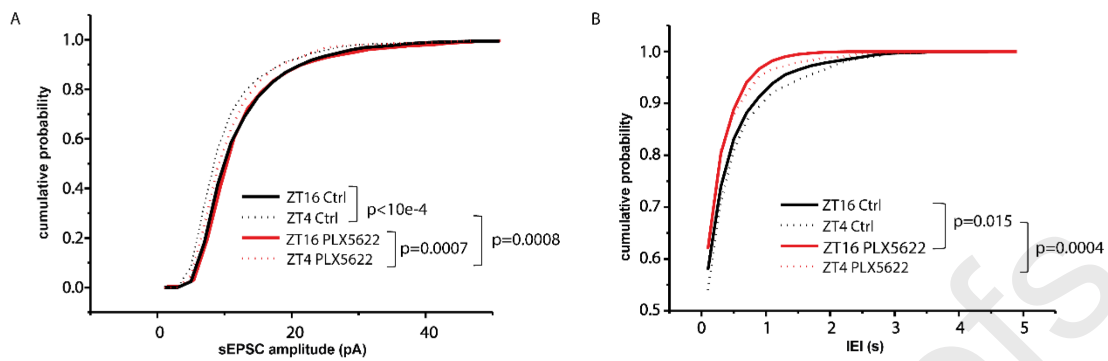
Figure 5



Highlights

- Microglial homeostasis disruption increases NREM sleep length and episodes number
- Microglial repopulation partially normalizes these outcomes on NREM sleep
- Microglia modulate excitatory synaptic transmission in a phase-dependent manner

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