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Selective contributions of neuronal and astroglial interleukin-1 receptor 1 to the regulation of sleep

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ABSTRACT

Interactions between sleep and immune function are bidirectional. Although the mechanisms that govern these interactions are not fully elucidated, the pro-inflammatory cytokine, interleukin-1β (IL-1), is a known regulator of sleep and mediator of immune responses. To further clarify the underlying substrates of sleep and immune interactions, we engineered two transgenic mouse lines that express interleukin-1 receptor 1 (IL1R1) only in the central nervous system (CNS) and selectively on neurons (NSE-IL1R1) or astrocytes (GFAP-IL1R1). During spontaneous sleep, compared to wild type (WT) animals, NSE-IL1R1 and GFAP-IL1R1 mice have more rapid eye movement sleep (REMS) that is characterized by reduced theta power in the electroencephalogram (EEG) spectra. The non-REM sleep (NREMS) EEG of each of the IL1R1 transgenic mouse strains also is characterized by enhanced power in the delta frequency band. In response to 6 h of sleep deprivation, sleep of both IL1R1 transgenic mouse strains is more consolidated than that of WT animals. Additionally, the NREMS EEG of NSE-IL1R1 mice contains less delta power after sleep deprivation, suggesting astroglial IL1R1 activity may modulate sleep homeostasis. Intracerebroventricular injection of IL-1 fails to alter sleep or brain temperature of NSE-IL1R1 or GFAP-IL1R1 mice. These data suggest that selective IL1R1 expression on neurons or on astrocytes is not sufficient for centrally-administered IL-1 to induce sleep or fever. Lack of sleep and febrile responses to IL-1 in these IL1R1 transgenic mouse strains may be due to their inability to produce IL-6 in brain. Overall, these studies demonstrate, through the use of novel transgenic mice, that IL1R1 on neurons and astrocytes differentially mediates aspects of sleep under physiological conditions and in response to central IL-1 administration.

1. Introduction

The reciprocal interactions between sleep and immune function are extensively studied. We now know that insufficient sleep alters immune function and immune activation alters sleep [reviewed by (Imeri and Opp, 2009; Krueger et al., 2011; Besedovsky et al., 2012)]. Interleukin-1β (IL-1) is a well-characterized pro-inflammatory cytokine that plays critical roles in host defense and mediates, in part, sleep and immune responses. Although IL-1 is commonly associated with pathological states, this cytokine also is involved in the regulation of physiological sleep [reviewed (Imeri and Opp, 2009; Krueger et al., 2011)].

Sleep is composed of two distinct phases that differ from each other and from wakefulness in electroencephalogram (EEG) characteristics, autonomic tone, and other physiological processes. Pre-clinical sleep studies generally categorize these sleep phases as non-rapid eye movement sleep (NREMS) and rapid eye movement sleep (REMS). Laboratory rodents, including mice, are polyphasic sleepers, with sleep distributed during the light and the dark periods of the light:dark cycle. However, the majority of laboratory rodent sleep occurs during the light period. It is during the sleep period that IL-1 concentrations peak, whereas waking hours coincide with the diurnal nadir of IL-1 (Moldofsky et al., 1986; Lue et al., 1987; Taishi et al., 1998). IL-1 also exerts effects on known sleep circuitry (Breder et al., 1988; Alam et al., 2004; Brambilla et al., 2007; Brambilla et al., 2010). Central administration of IL-1 increases NREMS and suppresses REMS (Opp et al., 1991; Olivadoti and Opp, 2008). IL-1-induced alterations of NREMS and REMS are attenuated by pharmacological and genetic

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manipulations of IL-1 activity (Opp and Krueger, 1991; Fang et al., 1998; Imeri and Opp, 2009). IL-1 binds to IL-1 receptor 1 (IL1R1) present on neurons and astrocytes (Smith et al., 2009), and these cells also produce IL-1 (Bartfai and Schultzberg, 1993; Baumann et al., 1993; Dong and Benveniste, 2001). Indeed, mice lacking IL1R1 spend less time in NREMS under baseline conditions, and administration of IL-1 does not alter sleep in these animals (Fang et al., 1998).

Much of our current understanding of sleep and immune function is based on neuron-centric studies. However, recent research implicates astrocytes as critical, active mediators of central nervous system (CNS) processes. Astrocytes are the most abundant glial cell type in the brain, are responsible for homeostasis of neuronal function, express receptors for immunomodulators, respond rapidly to inflammation, and have a newly demonstrated role in sleep-wake processes (Aschner, 1998; Farina et al., 2007; Halassa et al., 2009; Frank, 2011; Ingiosi et al., 2013). These observations suggest astrocytes are well-positioned to contribute to brain immunity and to participate in the regulation of sleep. However, *in vivo* investigations of astroglial contributions to CNS-mediated processes and behaviors are generally lacking. Consequently, much remains to be elucidated regarding the role of astrocytes in the regulation of sleep.

To further our understanding of cellular contributions to sleep and immune interactions in the brain, we engineered two transgenic mouse lines that express IL1R1 only in the CNS and selectively on neurons or astrocytes. Although these mice offer a unique opportunity to dissect neuronal and astroglial roles in variety of processes, behaviors, and pathologies, this study focuses on sleep.

2. Methods

2.1. Engineering of transgenic mice

All protocols for creating transgenic mice were approved by the University of Michigan Committee on Use and Care of Animals and the University of Washington Institutional Animal Care and Use Committee. Full length *Il1r1* cDNA was generated by RT-PCR (primers listed in Table 1) from a total RNA sample extracted from mouse liver using Trizol reagent. The NSE-IL1R1 transgene was constructed by excising the rat neuron-specific enolase (NSE) promoter [described by (Forss-Petter et al., 1990; Mucke et al., 1994; Race et al., 1995; Kearney et al., 2001); from Dr. Miriam Meisler, University of Michigan, Ann Arbor, MI] using SalI and HindIII digests and ligating with the pBluescriptII SK vector (−). *Il1r1* cDNA was inserted into the NSE-containing vector at the EcoRV site. The transgene fragment for microinjection was excised with SalI and SacII digests.

The GFAP-IL1R1 transgene was built in the pGfa2-cLac plasmid containing the promoter for human glial fibrillary acidic protein (GFAP) [described by (Brenner et al., 1994); from Dr. Michael Brenner, University of Alabama, Birmingham, AL]. The *lacZ* gene was excised via digestion with BamHI. BglII ends were added to *Il1r1* cDNA that was subsequently cloned into the BamHI site of the pGfa2 plasmid. The transgene fragment for microinjection was excised with BglII digest.

Two independent strains were generated for each transgene. Two NSE-IL1R1 lines and one GFAP-IL1R1 line were generated by the Transgenic Animal Model Core at the University of Michigan via pronuclear injection of the transgenes into (C57BL/6 X SJL)F2 oocytes. The second GFAP-IL1R1 strain was generated by the Preclinical Research and Transgenic Services core at the University of Washington via pronuclear injection of the transgene into (B6C3 X C57BL/6) oocytes. Tg(NSE-IL1R1) mice were identified via PCR of tail snip DNA which produced a 433 bp product. PCR of Tg(GFAP-IL1R1) DNA yielded a 489 bp product. Primer pairs (Table 1) were complementary to the promoters and *Il1r1* cDNA sequences. Initial phenotyping results were the same for both founder lines for each transgene indicating that the observed phenotypes were caused by the transgene itself and not by the integration sites. Results presented in this study are from only one founder line per transgene.

Transgenic founders were bred with mice null for *Il1r1* and *Tnfr1* genes (B6;129S-Tnfrsf1a^{tm1Imx} Il1r1^{tm1Imx}/J, stock #003244, The Jackson Laboratory, Bar Harbor, ME). *Il1r1*$^{-/-}$*Tnfr1*$^{-/-}$ mice were used for breeding because transgenic TNFR1 counterparts were simultaneously engineered with the transgenic IL1R1 mice. To generate transgenic IL1R1 strains null for the endogenous *Il1r1* gene and wild type for the endogenous *Tnfr1* gene, transgenic progeny of the founders were crossed with non-transgenic littermates (Tg(IL1R1)*Il1r1*$^{+/-}$*Tnfr1*$^{+/-}$ × *Il1r1*$^{+/-}$*Tnfr1*$^{+/-}$). Non-transgenic progeny carrying endogenous *Il1r1* and *Tnfr1* (*Il1r1*$^{+/+}$*Tnfr1*$^{+/+}$) were used as wild type (WT) controls. Mice were genotyped for wild type and mutant alleles of *Il1r1* and *Tnfr1* using custom primers (Table 1).

To determine cell-type specificity for the IL1R1 transgene, mice were lightly anesthetized, euthanized via bilateral pneumothorax, and transcardially perfused with 10% buffered formalin. Brains were paraffin embedded and cut sagittally on a sliding microtome in 10 μM sections. Identification of IL1R1 in NSE-IL1R1 brain tissue was achieved via *in situ* hybridization using a digoxigenin (DIG)-labeled probe generated from murine IL1R1 cDNA using the AmpliScribe T7-Flash Transcription Kit (ASB71110, Epicentre, Madison, WI). After *in situ* hybridization, tissue was blocked in 10% normal donkey serum (017-000-121, Jackson ImmunoResearch, Inc., West Grove, PA) and incubated with anti-DIG made in sheep (11333089001, Roche Nutley, NJ) for 1 h. DIG was then visualized via 1 h incubation with Alexa Fluor 488 donkey anti-sheep (A-11015) secondary antibody. Neurons were identified by sequential 1 h incubations with the primary antibody mouse anti-NeuN (MAB377, EMD Millipore, Temecula, CA) and the secondary antibody Alexa Fluor 555 donkey anti-mouse (A-31570). All antibody solutions were made in 1% normal donkey serum at a concentration of 1:500. Secondary antibodies were purchased from Life Technologies (Carlsbad, CA).

IL1R1 in GFAP-IL1R1 brain sections was detected with sequential 1 h incubations with the primary antibody rabbit anti-IL1R1 (sc-25775, Santa Cruz Biotechnology, Inc., Dallas, TX) and secondary antibody Alexa Fluor 555 donkey anti-rabbit (A-31572, Life Technologies). Astrocytes were identified via sequential treatment with the primary antibody anti-GFAP made in chicken (GFAP,

Table 1
List of primers used in these studies.

Gene	Primers (5′–3′)	Use
Il1r1	F:ATGGAGAATATGAAAGTGCTACTGG R:CTAGCCGAGTGGTAAGTGTGTT	RT-PCR
Tg(NSE-IL1R1)	F:GGCAAGGGAGAACCCCTTCTA R:AATCTCCAGCGACAGCAGAGG	PCR
Tg(GFAP-IL1R1)	F:AGAGCCAGAGCAGGTTGGAGA R:TGGGGGTCTTGCTGTCATTCT	PCR
IL1R1 (wild type)	F:GGTTTGAATGTTGGGGTTTG R:CACCACCACCTGGCTACTTT	PCR
IL1R1 (mutant)	F:TCTGGACGAAGAGCATCAGGG R:CAAGCAGGCATCGCCATG	PCR
TNFR1 (wild type)	F:GGATTGTCACGGTGCCGTTGAAG R:TGACAAGGACACGGTGTGTGGC	PCR
TNFR1 (mutant)	F:TGCTGATGGGGATACATCCATC R:CCGGTGGATGTGGAATGTGTG	PCR

Aves Labs, Inc., Tigard, OR) and the secondary antibody Alexa Fluor 488 donkey anti-chicken (703-545-155, Jackson ImmunoResearch, Inc.). All antibody solutions were made in 1% normal donkey serum at a concentration of 1:500.

Experimental animals express IL1R1 only in the CNS and selectively on neurons or astrocytes. Those mice with selective neuronal expression of IL1R1 are referred to as NSE-IL1R1, and mice expressing IL1R1 selectively on astrocytes are called GFAP-IL1R1. Fig. 1 panels A–C show co-localization of IL1R1 expression with neuronal staining in NSE-IL1R1 mouse brain, and panels D–F demonstrate that IL1R1 expression specifically co-localizes with astrocytes in GFAP-IL1R1 mouse brain.

We used qRT-PCR to determine relative expression and regional distribution of the IL1R1 transgene. Tissue samples were obtained from adult male mice of each of four strains: NSE-IL1R1, GFAP-IL1R1, WT, IL1R1$^{-/-}$ (knockout). Mice were euthanized with isoflurane, perfused, brains removed, and tissues/brain regions of interest dissected. The olfactory bulb, frontal cortex, hippocampus, hypothalamus, cerebellum, and brainstem were collected from brains and $n = 5$ tissue samples per genotype were analyzed for NSE-IL1R1, GFAP-IL1R1, and WT mice. Samples from $n = 1$–2 IL1R1$^{-/-}$ (KO) mice were analyzed for these brain regions. Peripheral tissues, heart, lung, liver, spleen, and colon, were analyzed from $n = 2$ mice of each genotype, except IL1R1$^{-/-}$ (KO) mice, from which $n = 1$ sample per tissue was analyzed. All samples were stored at −80 °C until assay. Total RNA from brain tissue was isolated with RNeasy Lipid Tissue Mini Kit (74804, QIAGEN Inc., Valencia, CA), whereas total RNA from peripheral tissues was isolated with RNeasy Mini Kit (74104, QIAGEN Inc.). All RNA samples were treated with DNase (79254, QIAGEN Inc.) according to the manufacturer's protocol. RNA quantity and quality were determined using A260/A280 readings by NanoDrop (Thermo Scientific, Rockford, IL). Reverse transcription (RT) was performed using a High Capacity cDNA Reverse Transcription Kit (4368814, Applied Biosystems, Inc, Foster City, CA) following the manufacturer's protocol. Controls included "no template" and "no RT". Real-time PCR was performed on an ABI 7300 Real Time PCR system (Applied Biosystems, Inc.) using TaqMan Gene Expression Assays (Applied Biosystems, Inc.) according to the manufacturer instructions. The probe used was IL1R1 (4351372, Mm01226960_g1, Applied Biosystems, Inc.). Ribosomal 18s RNA (18s rRNA; 4331182, Mm03928990_g1, Applied Biosystems, Inc.) was used as a reference gene. Gene expression was calculated relative to 18s rRNA and WT by the $2^{-\Delta\Delta Ct}$ method using the 7300 System SDS software.

IL1R1 was not detected in any brain region obtained from IL1R1$^{-/-}$ (KO) mice (Table 2). IL1R1 transgene expression was normalized by setting WT values equal to 1.0 and calculating the fold difference. The IL1R1 transgene was overexpressed (greater than 2-fold difference) relative to WT in olfactory bulb and hypothalamus of both transgenic mouse strains, and in cortex and brainstem of GFAP-IL1R1 mice. Relative to expression in WT mice, IL1R1 transgene expression was detected in some peripheral tissues of the transgenic mouse strains, but at negligible levels (data not shown).

2.2. Animals

During the studies, adult male WT, NSE-IL1R1, and GFAP-IL1R1 mice (20–30 g, 8–12 weeks at time of surgery) from our breeding colony were individually housed in standard cages on a 12:12 h light:dark cycle at 29 ± 1 °C, an ambient temperature within the murine thermoneutral zone (Gordon, 1985; Rudaya et al., 2005). Food and water were available *ad libitum*. All procedures involving the experimental use of animals were approved by the University of Washington Institutional Animal Care and Use Committee in accordance with the US Department of Agriculture Animal Welfare Act and the National Institutes of Health policy on Humane Care and the Use of Laboratory Animals.

2.3. Surgical procedures

Electroencephalographic (EEG) recordings were obtained from mice stereotaxically instrumented under isoflurane anesthesia with three stainless steel screws (MPX-0080-02P-C, Small Parts Inc., Logansport, IN) and a calibrated 10 kΩ thermistor (AB6E3-GC16KA103L, Thermometrics, Northridge, CA) as previously described (Baracchi and Opp, 2008).

Mice receiving intracerebroventricular (i.c.v.) injections were also provided a chronic guide cannula (C315GS, Plastics One Inc., Roanoke, VA) placed into the right lateral ventricle (AP: −0.5 mm, relative to Bregma; lateral: 1.25 mm relative to the central suture; DV: −2.0 mm relative to the dura). Patency of the cannula was assessed after recovery from surgery by central injection of 250 ng angiotensin II (H-1750, Bachem Americas, Inc., Torrance, CA) in 0.5 µl pyrogen-free saline (PFS). If the cannula is patent and ventricular circulation unobstructed, angiotensin II stimulates a drinking response upon activation of pre-optic structures (Epstein et al., 1970; Skott, 2003). Only mice with positive drinking responses were included in experimental analyses.

Analgesia was provided at the time of surgery by subcutaneous (SC) injection of buprenorphine (0.05 mg/kg) as well as topical application of 4% lidocaine at the incision site. Penicillin (1,200,000 IU/kg; SC) and topical triple antibiotic treatment of the surgical site was also provided to minimize risk of infection. Additional buprenorphine (0.03 mg/kg; SC) was administered

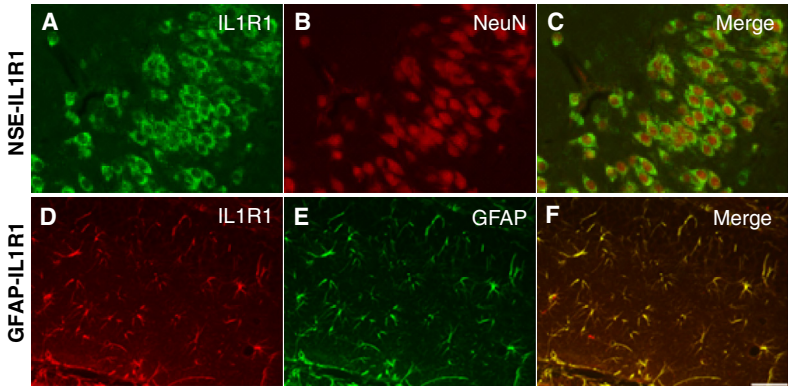

Fig. 1. The interleukin-1 receptor 1 (IL1R1) is selectively expressed on neurons or astrocytes. *In situ* hybridization for IL1R1 (green; panel A), immunofluorescent neuronal staining (NeuN; red; panel B), and merged image (panel C) indicate that IL1R1 is expressed on neurons of NSE-IL1R1 mice. Immunofluorescent detection of IL1R1 (red; panel D), and glial fibrillary acid protein (GFAP; green; panel E) shows that IL1R1 co-localizes with astrocytes in GFAP-IL1R1 mice (merged image; panel F). All images were acquired from hippocampus of an NSE-IL1R1 mouse and a GFAP-IL1R1 mouse. Images are 40×; scale bar = 50 µM.

Table 2
IL1R1 transgene expression in selected brain regions.

Brain Region	IL1R1$^{-/-}$	IL1R1$^{+/+}$	Tg(NSE-IL1R1)	Tg(GFAP-IL1R1)
Olfactory bulb	ND	1.0 ± 0.2	6.7 ± 1.4	4.9 ± 1.5
Frontal cortex	ND	1.0 ± 0.2	1.6 ± 0.5	2.3 ± 0.4
Hippocampus	ND	1.0 ± 0.1	0.5 ± 0.1	0.6 ± 0.1
Hypothalamus	ND	1.0 ± 0.2	3.8 ± 0.4	5.9 ± 0.7
Cerebellum	ND	1.0 ± 0.2	2.0 ± 0.8	0.7 ± 0.2
Brainstem	ND	1.0 ± 0.1	2.0 ± 0.3	3.0 ± 0.4

Values are the mean ± sdev fold differences in IL1R1 transgene expression relative to WT expression (normalized to a value of 1.0). Sample sizes: $n = 5$ for each strain, except IL1R1$^{-/-}$ (knock out), from which $n = 1$–2 per region; ND: not detectable; IL1R1$^{+/+}$ = wild type.

24 h post-surgery. Animals were allowed at least seven days recovery prior to initiation of experimental protocols.

2.4. Data acquisition

Ten days post-surgery, mice were connected to the recording system by a lightweight, flexible tether that allowed unrestricted movement. The EEG and brain temperature were recorded, and general cage activity monitored using an infrared sensor (BioBserve, GmbH, Bonn, Germany), as previously described (Baracchi and Opp, 2008). Digitized signals were stored as binary files until subsequent analysis.

Arousal state was determined by visual inspection of the EEG waveform, calculated EEG theta-to-delta frequency ratio, brain temperature, and cage activity with 10 s resolution using custom software (ICELUS, M. Opp, University of Washington) written in LabView for Windows (National Instruments, Austin, Texas) based on previously published criteria (Opp and Krueger, 1994a; Opp, 1998). The EEG was subjected to fast Fourier transformation (FFT) to produce power spectra between 0.5 and 30 Hz in 0.5 Hz bins as previously described (Baracchi and Opp, 2008). Spectral bins within the delta (0.5–4.5 Hz) and theta (6.0–9.0 Hz) bands were normalized to the total state-specific power (delta in NREMS; theta in REMS) summed across all frequency bins from 0.5 to 30 Hz for the 12 h light and dark periods and expressed as a percentage of total power. Hourly NREM delta power data from the light and dark periods were normalized to 12 h light and dark averages, respectively, derived from control conditions. EEG epochs containing artifacts were excluded from spectral analyses.

2.5. Experiment 1: determination of sleep and brain temperature phenotypes and responses to sleep deprivation

After recovery from surgery and habituation to the recording environment, 48 h of undisturbed baseline recordings beginning at light onset (LO) were obtained from WT ($n = 7$), NSE-IL1R1 ($n = 8$), and GFAP-IL1R1 ($n = 7$) mice. Animals were then subjected to 6 h sleep deprivation via gentle handling beginning at LO. Following sleep deprivation, animals were undisturbed and recordings continued for 18 h.

2.6. Experiment 2: effects of central administration of IL-1 on sleep and brain temperature

Separate groups of mice were used to determine responses to central administration of IL-1. After recovery from surgery and adaptation to the recording apparatus, WT ($n = 4$), NSE-IL1R1 ($n = 5$), and GFAP-IL1R1 ($n = 5$) mice were injected i.c.v. in a counterbalanced schedule with vehicle (PFS, pyrogen-free saline; 0.5 μl) and 50 ng recombinant mouse IL-1β (401-ML-005/CF, R&D Systems Minneapolis, MN). All injections were given 15 min prior to dark onset (DO) and recordings obtained for 48 h.

2.7. Experiment 3: IL-1-induced cytokine protein in brain

Two groups of mice were used to determine effects of IL-1 administration on cytokine protein concentrations in brain. The first group [WT ($n = 6$), NSE-IL1R1 ($n = 7$), GFAP-IL1R1 ($n = 6$)] received a single i.c.v. injection of vehicle (PFS; 0.5 μl). Mice in the second group [WT ($n = 6$), NSE-IL1R1 ($n = 7$), GFAP-IL1R1 ($n = 7$)] were injected with 50 ng IL-1β in 0.5 μl PFS. All injections were given at LO. Mice were lightly anesthetized and sacrificed via decapitation 4 h post-injection. Brains were extracted, and the hypothalamus, hippocampus, and brainstem dissected. Samples were snap frozen in liquid nitrogen and stored at −80 °C until subsequent protein extraction.

2.7.1. Cytokine protein quantification

Protein was extracted from thawed frozen tissues as previously described (Datta and Opp, 2008; Granger et al., 2013). The protein concentration of each sample was determined using the bicinchoninic acid (BCA) protein assay (kit 23225, Thermo Scientific, Rockford, IL).

IL-1, IL-6, and tumor necrosis factor α (TNF) protein concentrations were quantified using a bead-based assay and Luminex xMAP technology (Luminex Corporation, Austin, TX) as previously described (Sutton and Opp, 2015). Custom beads were developed with IL-1β (DY401), IL-6 (DY406), and TNF DuoSets (DY410; R&D Systems). Diluents were generated as appropriate for the sample type: cell lysis buffer (171-304012, Bio-Rad) for protein extracts and 0.1% BSA in phosphate buffered saline (PBS) for cell culture supernatant. Observed concentrations were obtained from the Bio-Plex software for all sample types and reported as pg/ml for culture supernatant samples. Observed concentrations obtained from brain tissue were adjusted for sample protein concentrations determined from the BCA assay and final values expressed as pg/mL/μg of protein loaded.

2.8. Experiment 4: IL-1-induced cytokine production in vitro

2.8.1. Neuronal culture

Whole brains from NSE-IL1R1 P0–P2 pups were harvested to generate neuronal cultures. Neurons were plated on poly-L-lysine coated coverslips at a density of 80,000 cells/cm^2 in 24-well plates in NbActiv4 media (Nb4-500, BrainBits, LLC, Springfield, IL) containing 1% penicillin–streptomycin–glutamine (10378-016, Life Technologies). Cultures were incubated at 37 °C with 5% CO_2 and received a 50% media change every 3–4 days. Neuronal cultures were ready for experimental use 12–14 days later.

2.8.2. Astroglial culture

Astroglial cultures were obtained from whole brains of P0–P2 GFAP-IL1R1 pups and cultured according to a protocol adapted from Schildge et al. (2013). Astrocytes were plated at 10–15 × 10^6 cells on poly-L-lysine coated T75 flask in high glucose Dulbecco's Modified Eagle Medium (DMEM; 11965-092, Life Technologies) containing 10% heat inactivated fetal bovine serum (10082-147, Life Technologies) and 1% penicillin–streptomycin–glutamine. Once confluent, cultures were astrocyte enriched via agitation on an orbital shaker. Astrocytes then were re-plated in two poly-L-lysine coated T75 flasks. Approximately 14 days post-split when cells were confluent, cells were trypsinized and evenly distributed to poly-L-lysine-coated 24-well plates. Cultures were incubated at 37 °C with 5% CO_2. Cells were ready for experimental use 24–48 h post-plating once confluent.

2.8.3. IL-1 stimulation of cell cultures

Mature neuronal and astroglial cultures were subjected to three treatment conditions: untreated, vehicle (0.1% BSA in PFS), or IL-1

(neuronal: 50 ng/well; astroglial: 10 ng/well). After 24 h, cell culture supernatant was aliquoted and stored at −80 °C until cytokine analysis. IL-6 and TNF protein was quantified via bead-based assay using Luminex xMAP technology as described in Section 2.7.1. IL-1 in supernatant was not quantified as the assay cannot differentiate between the recombinant murine IL-1 used to stimulate the cultures and *de novo* IL-1 produced by the murine cells in culture.

2.9. Statistical analysis

Statistical analyses were performed using SPSS for Windows (IBM Corporation, Armonk, NY). Data are presented as means ± standard error of the mean (SEM) or means ± standard deviation (SDEV), as appropriate. An alpha level of $p < 0.05$ was accepted as indicating significant departures from control conditions or strain differences for all statistical comparisons.

Between strain comparisons for undisturbed sleep-wake parameters, brain temperature, cage activity, EEG spectral analyses, and responses to sleep deprivation were performed in 12 h and 6 h time blocks using one-way analysis of variance (ANOVA) with strain (WT, NSE-IL1R1, GFAP-IL1R1) as the independent variable. One-way ANOVA was also used to determine IL-1-stimulated *in vitro* cytokine protein concentrations with treatment (untreated, vehicle, IL-1) as the independent variable. If statistically significant strain or treatment effects were revealed, post hoc comparisons by Tukey's HSD test were used to determine differences among conditions.

Within strain comparisons for percentage of time in REMS and NREMS, normalized NREM delta power, and brain temperature values were performed via a general linear model for repeated measures using time (hours) as the repeated measure and manipulation (undisturbed baseline vs. 6 h sleep deprivation; PFS vs. IL-1) as the between-subjects factor.

Between strain comparisons of *in vivo* cytokine protein concentrations were made using a mixed-effects ANOVA designating manipulation (PFS vs. IL-1) and strain (WT, NSE-IL1R1, or GFAP-IL1R1) as between-subjects factor. Post-hoc comparisons by Tukey's HSD test were used to determine differences among mouse strains where appropriate.

3. Results

3.1. Experiment 1: determination of sleep and brain temperature phenotypes and responses to sleep deprivation

3.1.1. Spontaneous sleep and temperature

Undisturbed baseline recordings were obtained from WT and transgenic mice to determine neuronal- and astroglial-specific contributions of IL1R1 to sleep-wake behavior and thermoregulation. There were no differences among strains in the percentage of

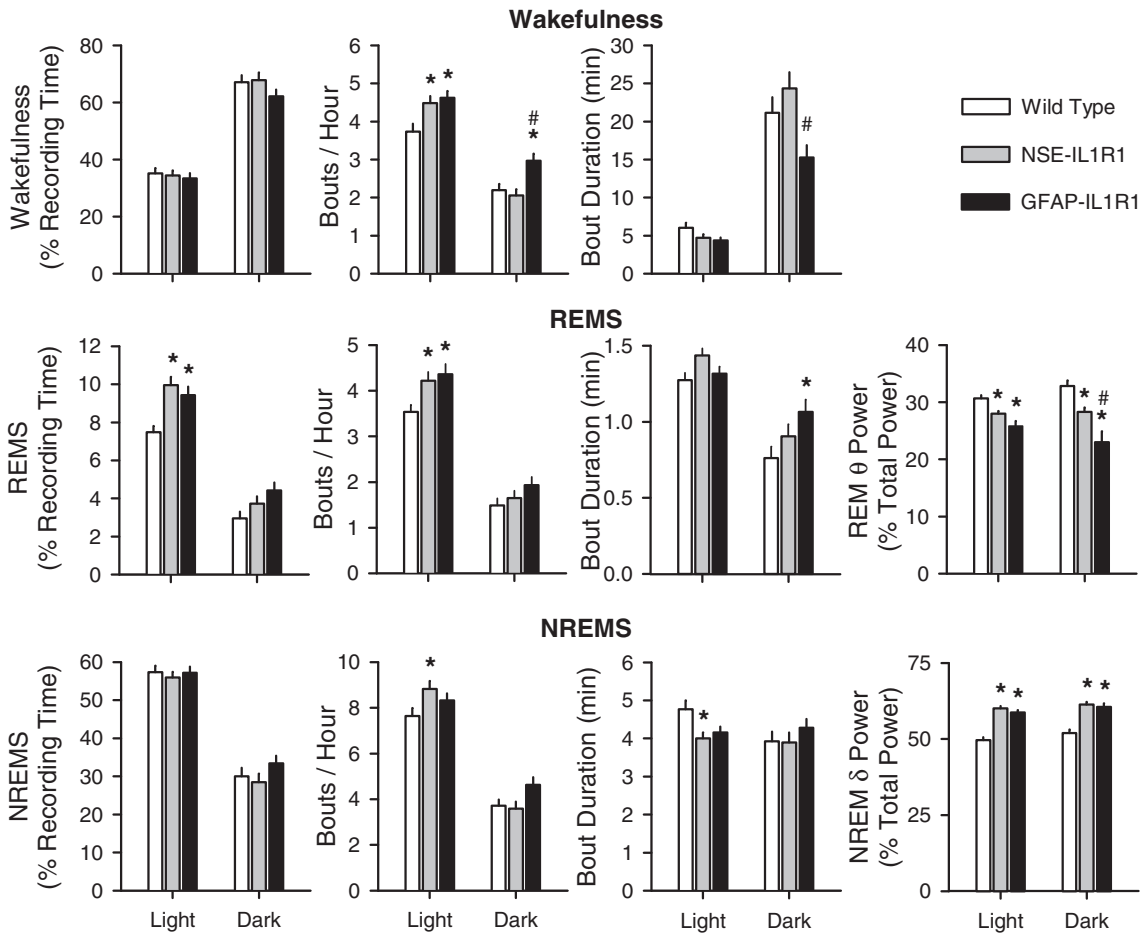

Fig. 2. Selective expression of IL1R1 on neurons or astrocytes alters sleep and the electroencephalogram (EEG) of transgenic mice. Percentage of time spent in non-rapid eye movement sleep (NREMS), rapid eye movement sleep (REMS) and wakefulness, as well as the number of state-specific bouts and their duration were determined from undisturbed wild type mice, transgenic NSE-IL1R1 mice, and transgenic GFAP-IL1R1 mice. Values are means ± SEM obtained during the light period or dark period. Power in the EEG theta (θ; 6–9 Hz) and delta (δ; 0.5–4.5 Hz) frequency bands are presented as percent of total power. * = $p < 0.05$ vs. values obtained from wild type mice; # = $p < 0.05$ vs. values obtained from NSE-IL1R1 mice.

recording time spent in wakefulness or NREMS during the 24 h baseline recording period (Fig. 2). However, NSE-IL1R1 and GFAP-IL1R1 mice had more wake bouts during the light period, and GFAP-IL1R1 mice also had more wake bouts during the dark period. Wake bouts during the dark period were shorter in GFAP-IL1R1 mice than in NSE-IL1R1 animals. NSE-IL1R1 mice had more bouts of NREMS during the light period that were of shorter duration than those of WT mice. Both IL1R1 transgenic lines spent more time in REMS during the light period in comparison to WT counterparts. This increase in REMS is due, in part, to more REM bouts. However, during the dark period, GFAP-IL1R1 mice had longer REM bouts than did WT mice.

Artifact-free normalized EEG spectra obtained during NREM and REM sleep differed among strains (Figs. 2 and 3). NREM delta power (0.5–4.5 Hz) was greater in NSE-IL1R1 and GFAP-IL1R1 mice than in WT mice. The REMS EEG of each transgenic strain contained less theta power (6.0–9.0 Hz) than that of WT mice, and REMS theta power was reduced in GFAP-IL1R1 mice compared to NSE-IL1R1 animals.

Transgenic NSE-IL1R1 and GFAP-IL1R1 mice had lower brain temperatures than did WT mice (Fig. 4). These brain temperature differences among strains were apparent during the light period and the dark period. Strain differences in brain temperature cannot be attributed to differences in activity as generalized home-cage activity did not differ among strains (Fig. 4).

3.1.2. Responses to sleep deprivation

Sleep of WT and transgenic mice was restricted to determine the extent to which neuronal and astroglial IL1R1 mediate responses to insufficient sleep. Sleep deprivation induces IL-1 (Mackiewicz et al., 1996; Taishi et al., 1998; Opp and Krueger, 1994a,b). To that end, WT and transgenic mice were sleep deprived for 6 h via gentle handling, which essentially abolished REMS and NREMS during this 6 h period (Fig. 5).

Within strain analyses revealed that NREMS and REMS increased in all strains during the recovery period after sleep deprivation ended (Fig. 5). Sleep deprivation induced a statistically significant REMS rebound in the dark period for all strains. WT mice and each of the IL1R1 transgenic mouse strains also had transient increases in NREMS during the first 6 h of recovery. This modest increase in NREMS was accompanied by increased NREM delta power in all strains for the 6 h period immediately post-deprivation (Fig. 5). NSE-IL1R1 mice also exhibited a greater amount of time spent in NREMS in the early portion of the dark period.

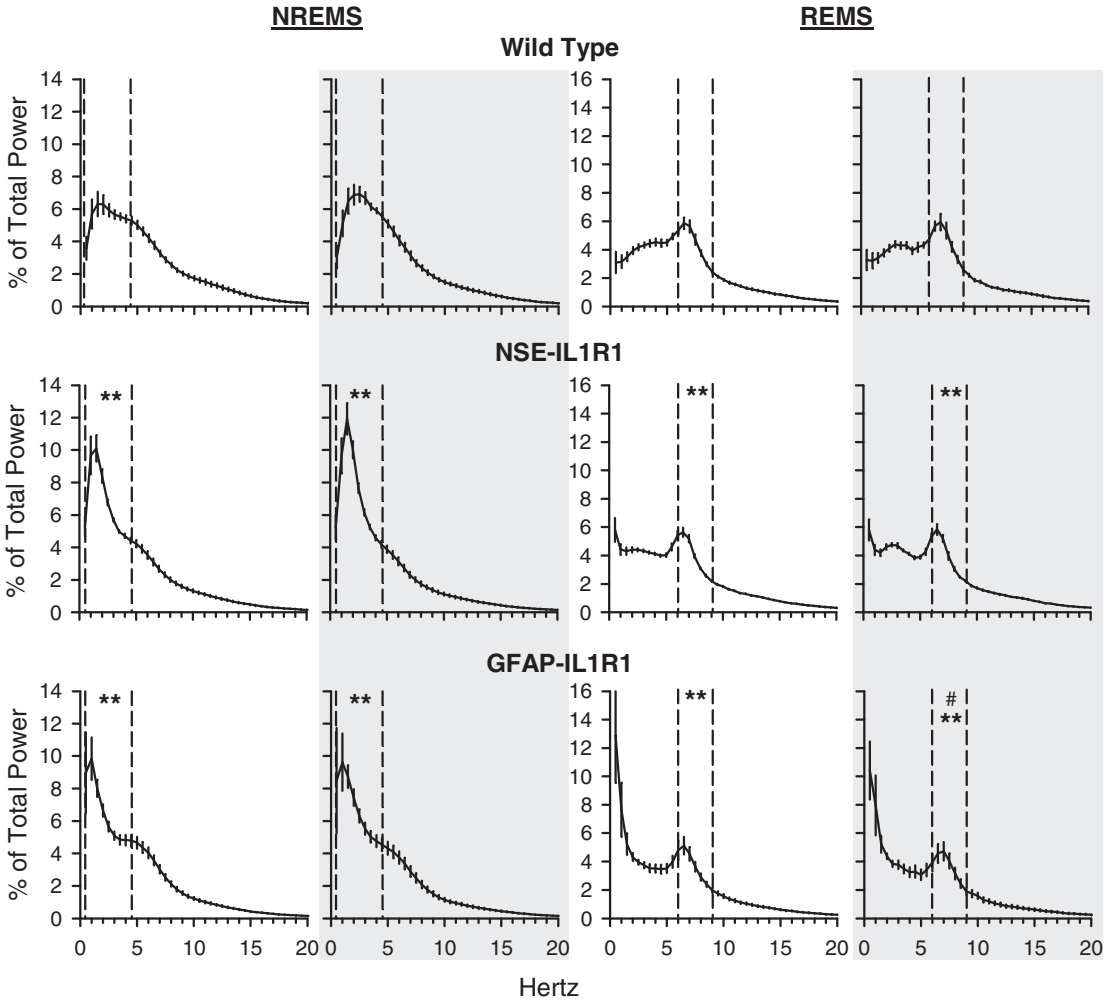

Fig. 3. Selective expression of the interleukin-1 receptor 1 (IL1R1) on neurons or astrocytes alters spectral characteristics of the electroencephalogram (EEG). Artifact-free, state-specific epochs of the EEG were obtained from wild type mice, transgenic NSE-IL1R1 mice, and transgenic GFAP-IL1R1 mice during undisturbed baseline recordings. Spectral analyses were performed on frequencies 0.5–30.0 Hz, but data depicted are limited to 0.5–20.0 Hz. Spectra were normalized as a percentage of total power across all frequencies for specific sleep states within the 12 h light or dark periods. Values are plotted as means ± SEM for each frequency bin. Statistical analyses were conducted on the delta (0.5–4.5 Hz) frequency band for NREMS and the theta (6.0–9.0 Hz) frequency band for REMS. Shaded areas indicate spectra derived from the 12 h dark period. ** = $p < 0.01$ vs. values obtained from wild type mice. # = $p < 0.05$ vs. values obtained from transgenic NSE-IL1R1 mice.

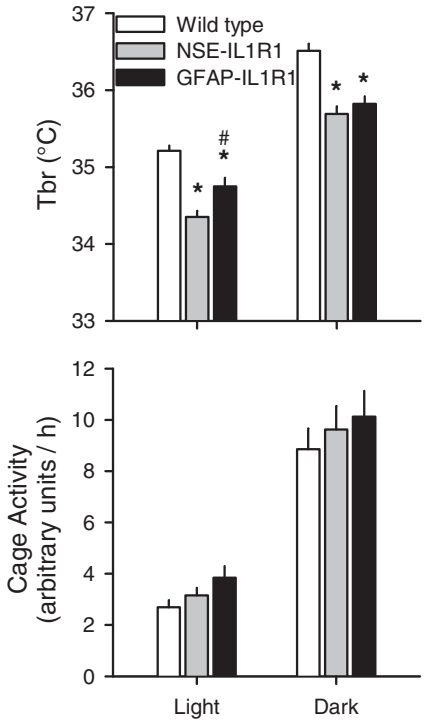

Fig. 4. Selective expression of interleukin-1 receptor 1 (IL1R1) on neurons or astrocytes reduces brain temperature without altering activity. Brain temperatures (Tbr) of transgenic NSE-IL1R1 mice and transgenic GFAP-IL1R1 mice are lower than those of wild type mice even though general cage activity does not differ among strains. Data were obtained during undisturbed baseline recordings and are presented as means ± SEM for the 12 h light and dark periods. * = $p < 0.05$ vs. values obtained from wild type mice. # = $p < 0.05$ vs. values obtained from NSE-IL1R1.

During the 6-h sleep deprivation period, brain temperatures of WT and NSE-IL1R1 mice, but not GFAP-IL1R1 mice, were significantly increased. Brain temperatures for all mouse strains were normal during the 18 h recovery period (Fig. 5).

Strain differences in response to sleep deprivation were determined by analyzing difference scores that were calculated by subtracting corresponding values obtained during undisturbed baseline period from the 18 h post-deprivation recovery period (Fig. 6). No strain differences were detected for the amount of REMS or NREMS acquired during the 18 h recovery period following sleep deprivation. NSE-IL1R1 mice had significantly less NREM delta power compared to WT during the first 12 h post-deprivation, suggesting less restorative sleep. However, sleep of NSE-IL1R1 and GFAP-IL1R1 mice was more consolidated than that of WT mice during the first 6 h of recovery as evidenced by fewer sleep state transitions.

3.2. Experiment 2: effects of central administration of IL-1 on sleep and brain temperature

Intracerebroventricular administration of IL-1 (50 ng) altered sleep and brain temperature of WT mice in a manner generally similar to that previously reported in mice (Olivadoti and Opp, 2008). IL-1 transiently increased NREMS, suppressed REMS and NREM delta power, and induced long-lasting fever in WT mice. In contrast, i.c.v. administration of the same IL-1 dose into the IL1R1 transgenic mouse strains did not alter brain temperature or any sleep-wake parameter quantified in this study (Fig. 7).

3.3. Experiment 3: IL-1-induced cytokine protein in brain

Interleukin-6 is produced subsequent to IL-1 in the cytokine cascade (Zetterstrom et al., 1998), and this proinflammatory cytokine mediates, in part, IL-1-induced fever and modulates pathophysiological sleep responses to IL-1 (Olivadoti and Opp, 2008). Similar to IL-1, plasma IL-6 in humans demonstrates diurnal oscillations with peaks at night (Bauer et al., 1994; Irwin, 2002). Furthermore, central administration of IL-6 alters NREMS of rats (Hogan et al., 2003). In this current study the cytokine response to IL-1 administration was determined 4 h post-injection by quantifying IL-1, IL-6, and TNF protein. I.c.v. administration of IL-1 increased *de novo* IL-1 in hypothalamus and brainstem and tended to increase IL-1 in hippocampus of WT mice (Fig. 8). IL-1 administration induced a significant increase in *de novo* IL-1 in hippocampus of NSE-IL1R1 mice and in hippocampus and brainstem of GFAP-IL1R1 mice. Central administration of IL-1 increased IL-6 in all brain regions assayed in WT mice, but not in either of the IL1R1 transgenic mouse strains. TNF was not detected in any brain region or condition used in this study (data not shown).

3.4. Experiment 4: IL-1-induced cytokine production in vitro

To determine the effect of IL-1 on IL-6 and TNF cytokine production from transgenic cells in isolation from *in vivo* conditions, *in vitro* responses of neurons and astrocytes to treatment with IL-1 were assessed from cultures of NSE-IL1R1 and GFAP-IL1R1 brains (Fig. 9). Stimulated IL-6 production by NSE-IL1R1 neurons and GFAP-IL1R1 astrocytes was apparent 24 h after adding IL-1 to the cultures. TNF was not detected in any condition (data not shown).

4. Discussion

4.1. Spontaneous behavior and brain temperature

There is an abundance of data demonstrating a role for IL-1 in the regulation of sleep [reviewed by (Krueger et al., 2011; Imeri and Opp, 2009)], yet specific contributions of neurons and astrocytes to sleep-wake behavior remain largely unknown. This study aimed to determine the relative contributions of neurons and astrocytes to IL-1 regulation of sleep. To this end, we engineered two transgenic mouse lines that express IL1R1 only in the CNS, and selectively on neurons (NSE-IL1R1) or astrocytes (GFAP-IL1R1). During periods when undisturbed mice are spontaneously behaving, transgenic NSE-IL1R1 and GFAP-IL1R1 mice have more REMS during the light period as compared to WT mice, and this REMS is characterized by less theta power in the EEG spectra. The EEG of each of the transgenic mouse strains is characterized by enhanced NREM delta power relative to WT animals. Although neuron- or astrocyte-specific expression of IL1R1 does not affect the amount of sleep after 6 h of sleep deprivation, the NREM EEG of NSE-IL1R1 mice contains less delta power, and sleep of both transgenic strains is more consolidated than that of WT animals. Finally, central administration of IL-1 does not alter sleep of either transgenic mouse strain, an effect that may be due to impaired *in vivo* production of IL-6.

Previous findings demonstrate that mice lacking IL1R1 exhibit less NREMS (Fang et al., 1998), and antagonizing IL-1 with the IL-1 receptor antagonist (Opp and Krueger, 1991), antibodies (Opp and Krueger, 1994a), or inhibiting its production (Imeri et al., 2006), reduces NREMS. Collectively, these and other data [reviewed (Imeri and Opp, 2009)] demonstrate a role for IL-1 in the regulation of spontaneous NREMS. In transgenic NSE-IL1R1 and GFAP-IL1R1 mice, the percentage of time spent in NREMS is not

altered compared to WT counterparts. Our new data suggest that selective IL1R1 expression on neurons or astrocytes is sufficient for normal NREMS to be manifest. Although the amount of time spent in NREMS is unchanged in transgenic animals, NREMS architecture during the light period is altered in NSE-IL1R1 mice, but not in GFAP-IL1R1 mice. Collectively, these results implicate a role for astroglial IL1R1 in at least some facets of the initiation and maintenance of NREMS.

Selective neuronal or astroglial expression of IL1R1 increases REMS during the light period, whereas mice lacking IL1R1 have normal REMS (Fang et al., 1998). These data suggest that neuronal and astroglial IL1R1 contribute to REMS regulation. Current knowledge regarding the brain regions and neurotransmitters involved in mediating effects of IL-1 on REMS is limited. Most studies demonstrate that IL-1 administration suppresses REMS (Opp et al., 1991; Opp and Toth, 1998; Fang et al., 1998; Olivadoti and Opp, 2008; Brambilla et al., 2010), an effect mediated, in part, by inhibition of brainstem cholinergic neurons (Brambilla et al., 2010).

Because IL-1 inhibits brainstem cholinergic neurons, the increased REMS phenotype of these transgenic mice may be due to altered IL1R1 modulation of cholinergic neurons. Data demonstrate that REMS is generated in the cholinergic brainstem, which contains REM-ON neurons in laterodorsal and pedunculopontine tegmental nuclei (LDT/PPT) [reviewed by (Brown et al., 2012)]. Microinjection of IL-1 into the LDT reduces REMS (Brambilla et al., 2010). Similarly, IL-1 inhibits firing rates of cholinergic LDT neurons *in vitro*. Attenuation of cholinergic activity appears to be a consequence of inhibiting evoked glutamatergic responses and, thus, excitatory drive in cholinergic LDT neurons (Brambilla et al., 2010). An essential function of astrocytes is to regulate extracellular glutamate. However, IL-1 suppresses glutamate uptake by astrocytes (Ye and Sontheimer, 1996; Hu et al., 2000). Consequently, there may be an excess of glutamate in the extracellular space of our IL1R1 transgenic mice. This putative overabundance of glutamate may, in turn, stimulate cholinergic REM-ON neurons of the LDT resulting in the initiation and enhancement of REMS. Additional experiments are necessary to determine the precise mechanisms by which neuronal and astroglial IL1R1 modulate REMS.

NREMS and REMS EEG spectral characteristics are also altered in IL1R1 transgenic mice. Although the amount of NREMS is normal in these transgenic animals, NSE-IL1R1 and GFAP-IL1R1 mice exhibit enhanced NREM delta power. NREM delta power is widely regarded as a marker of NREMS intensity or depth (Borbély,

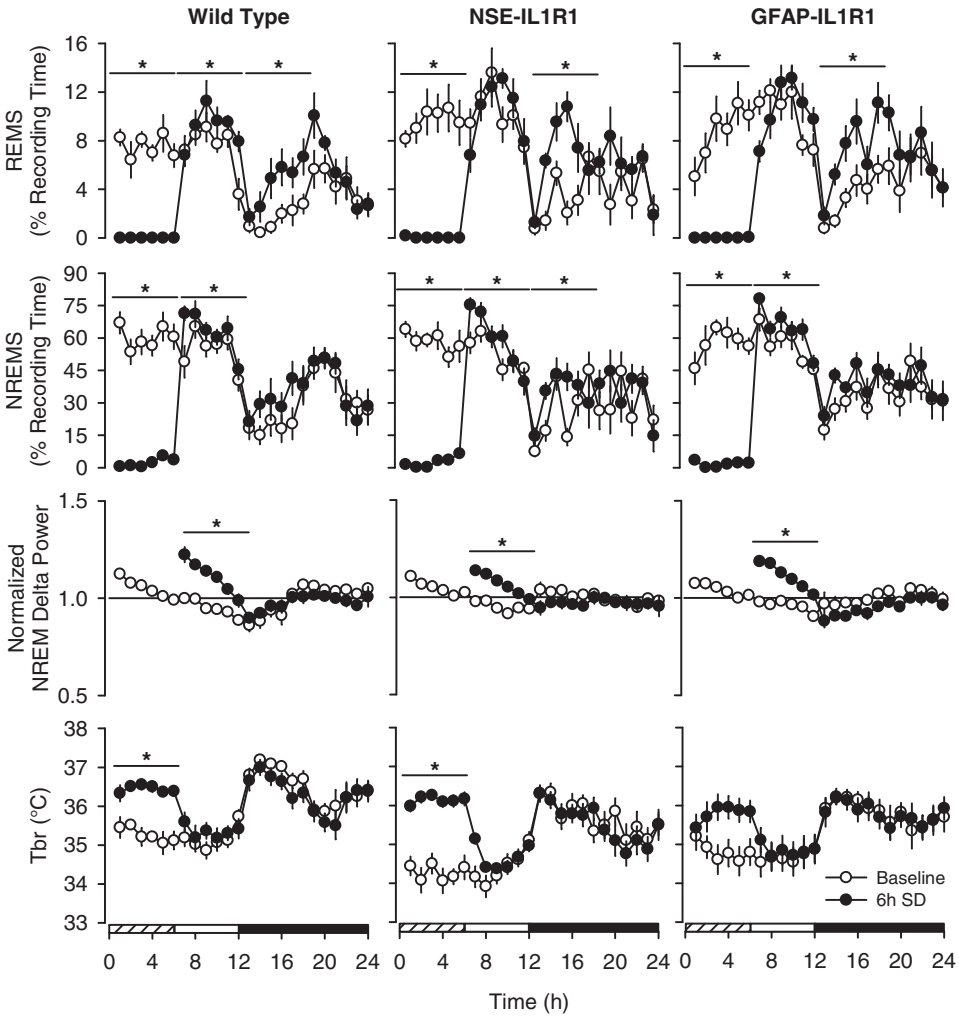

Fig. 5. Interleukin-1 receptor 1 expressed on neurons or on astrocytes is sufficient to mediate responses to sleep deprivation. The percentage of recording time spent in rapid eye movement sleep (REMS) and non-rapid eye movement sleep (NREMS), normalized electroencephalogram (EEG) NREM delta power, and brain temperatures (Tbr) were determined from mice during undisturbed baseline conditions (open symbols) and during and after 6 h sleep deprivation (SD; filled symbols). Values are means ± SEM from wild type mice, transgenic NSE-IL1R1 mice, and transgenic GFAP-IL1R1 mice. Hourly data for EEG delta power were normalized to 12 h light and dark undisturbed baseline averages. The open and filled bars on the *X*-axis indicate light and dark periods of the light–dark cycle, respectively. The cross-hatched portion of the *X*-axis denotes the 6 h sleep deprivation period. * = $p < 0.05$ vs. corresponding undisturbed baseline periods.

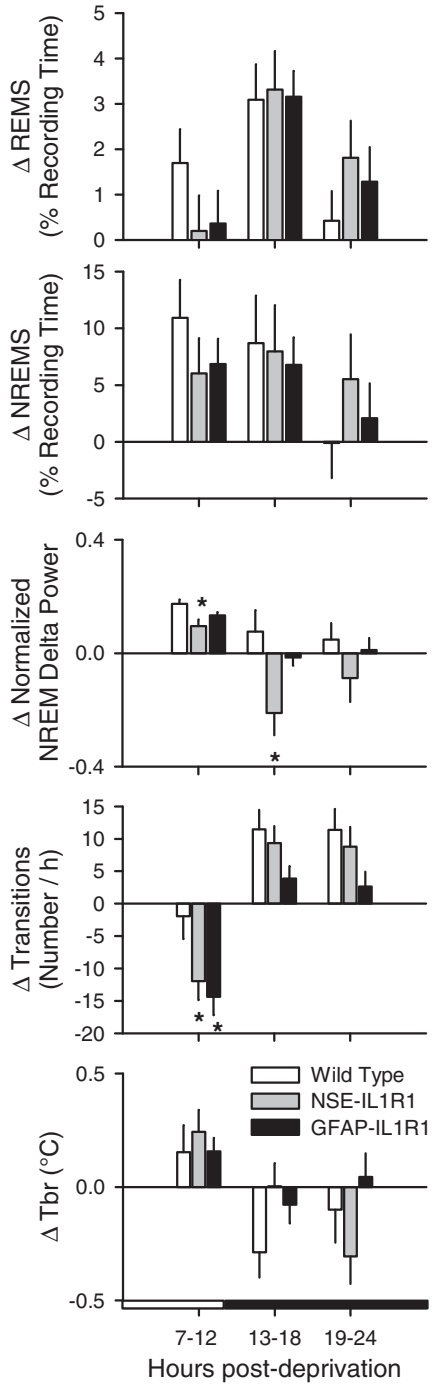

Fig. 6. Sleep is consolidated after sleep deprivation when interleukin-1 receptor 1 is selectively expressed on neurons or on astrocytes. Sleep of transgenic IL1R1 mice is more consolidated/less fragmented after sleep deprivation than is sleep of wild type mice. Normalized non-rapid eye movement sleep (NREM) electroencephalogram delta power is reduced in transgenic NSE-IL1R1 mice as compared to wild type mice. Difference scores were calculated by subtracting corresponding undisturbed baseline values from those obtained after sleep deprivation. Values for percentage of recording time spent in rapid eye movement sleep (REMS) and NREMS, normalized NREM delta power, number of sleep state transitions, and brain temperature (Tbr) are plotted in 6 h time blocks for the 18 h period following sleep deprivation. Values are means ± SEM. * = $p < 0.05$ vs. values obtained from wild type mice. Open and filled bars on the *X*-axis denote light and dark portions of the light–dark cycle, respectively.

1982). These results are consistent with previous studies that demonstrate dissociation of NREMS delta power from time spent in NREMS (Yasuda et al., 2005; Halassa et al., 2009; Davis et al., 2011). IL1R1 transgenic mice also have a reduced REMS theta peak as compared to WT mice. Therefore, increased REMS time and power in the EEG theta frequency band during REMS are inversely related in IL1R1 transgenic mice. The functional significance of this inverse relationship between REMS duration and theta power in the REMS EEG is not clear. Although NREM delta and REM theta power may not depend specifically on neuronal or astroglial IL1R1 expression, neuronal IL1R1 activity may contribute more prominently to REM theta power because power in the theta frequency band during the dark period is less in GFAP-IL1R1 mice than in WT or NSE-IL1R1 mice. Mechanisms underlying cytokine modulation of cortical EEG are not fully understood (Davis et al., 2011), but altered NREM delta and REM theta power in these transgenic mice may be due, in part, to actions on cholinergic neurons in the basal forebrain (BF). Indeed, inhibition of cholinergic BF neurons increases delta power and suppresses theta activity (Cape and Jones, 2000), and IL-1 suppresses discharge activity of wake-active BF neurons (Alam et al., 2004).

Brain temperatures of freely-behaving IL1R1 transgenic mice are lower than those of WT animals, an effect that does not appear to be neuron- or astrocyte-specific. It is not likely that reduced brain temperature in these IL1R1 transgenic mice is due to reduced activity as overall cage activity is similar in all mouse strains used in this study. Thermoregulatory centers reside in the preoptic area and anterior hypothalamus (Boulant, 2000) and are innervated by the brainstem LDT/PPT (Chiba and Murata, 1985). Stimulation of cholinergic neurons in these brainstem nuclei induces hypothermia (Imeri et al., 1995; Mallick and Joseph, 1997; Takahashi et al., 2001). Because transgenic mice have more REMS, which may be mediated, in part, by activation of LDT/PPT cholinergic neurons, it is possible that lower brain temperatures in transgenic mice result from enhanced stimulation of preoptic areas via LDT/PPT projections. Alternatively, the lower baseline temperatures of the transgenic animals might indicate a downward shift in the thermoregulatory setpoint of NSE-IL1R1 and GFAP-IL1R1 mice. No metabolic measures were obtained in this study, but increased time spent in REMS by transgenic mice is consistent with a change in thermoregulatory setpoint. Although further experimentation is necessary, a classic study in rats demonstrated that, independent of changes in NREMS, REMS is maximal when ambient temperature is within a narrow range of the thermoneutral zone (Szymusiak and Satinoff, 1981).

4.2. Responses to sleep deprivation

Responses to 6-h sleep deprivation at light onset of the mouse strains used in this study are generally similar to those previously reported for WT mice (Franken et al., 1991a, 1999; Huber et al., 2000; Morrow and Opp, 2005b; Baracchi and Opp, 2008). Following 6 h of sleep deprivation, there are REMS and NREMS rebounds, NREM delta power is enhanced, and sleep is consolidated. Previous studies demonstrate that the NREMS rebound after sleep deprivation of IL1R1 knockout mice is less than in WT animals (Schmidt and Wisor, 2012). Because REMS, NREMS, and wakefulness of IL1R1 transgenic mice do not differ from WT mice after 6 h sleep deprivation, these data suggest that neuronal or astroglial IL1R1 expression is sufficient for sleep responses during the post-deprivation recovery period.

One of the strain differences in response to sleep deprivation is that sleep of both IL1R1 transgenic mouse strains is more consolidated/less fragmented than that of WT mice during the first 6 h of the recovery period. Sleep consolidation after sleep deprivation generally indicates more efficient sleep, and as such, IL1R1 transgenic mice may recover from sleep loss more quickly than WT animals. Mechanisms by which selective IL1R1 expression on either neurons or astrocytes consolidates sleep/reduces sleep

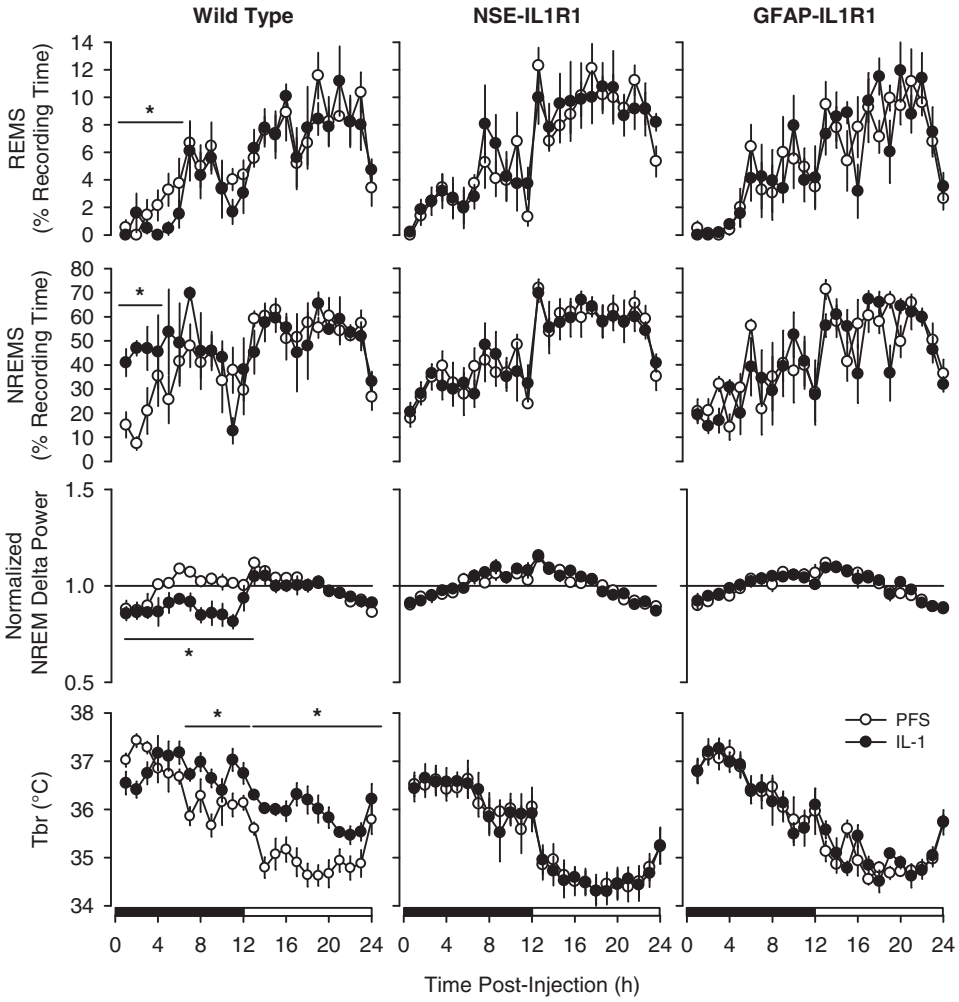

Fig. 7. Central administration of interleukin-1 (IL-1) does not alter sleep, the electroencephalogram (EEG), or brain temperature when IL1R1 is selectively expressed on neurons or on astrocytes. Intracerebroventricular (i.c.v.) injection of IL-1 does not alter the percentage of recording time spent in rapid eye movement sleep (REMS) or non-rapid eye movement sleep (NREMS), normalized EEG NREM delta power, or brain temperature (Tbr) in transgenic NSE-IL1R1 mice or in transgenic GFAP-IL1R1 mice. Values are mean ± SEM responses obtained after vehicle (pyrogen-free saline; PFS; open symbols) or 50 ng IL-1 (filled symbols). Hourly data for EEG delta power were normalized to 12 h dark and light averages obtained during undisturbed baseline recordings. The filled and open bars on the *X*-axis indicate dark and light periods of the light–dark cycle, respectively. * = $p < 0.05$ vs. values obtained after injection of vehicle.

fragmentation remain to be determined. Nevertheless, our new data demonstrate a role for neuronal and astroglial IL1R1 expression as mediators of this facet of responses to sleep deprivation.

Power in the EEG delta frequency band is generally considered to be an indicator of sleep intensity and/or quality (Borbély, 1982). Delta power during NREMS increases in proportion to the duration of prior wakefulness (Franken et al., 1991b, 1992), and as such this EEG parameter is also a marker of sleep pressure or sleep drive. After sleep deprivation, NREM delta power is reduced in transgenic NSE-IL1R1 mice as compared to WT mice, suggesting that NSE-IL1R1 mice are less affected by sleep loss under these conditions. Increased delta power after sleep deprivation is attenuated when gliotransmission by astrocytes is inhibited, an effect attributed to reduced astrocyte-derived adenosine (Halassa et al., 2009). IL-1 increases with prolonged time awake (Opp and Krueger, 1994b; Mackiewicz et al., 1996; Taishi et al., 1998), and IL-1 induces adenosine production (Sperlagh et al., 2004; Zhu et al., 2006). Additionally, IL-1 stimulation of astrocytes enhances effects of the adenosinergic precursor, adenosine triphosphate (Stella et al., 1997; Meme et al., 2004; Narcisse et al., 2005). Transgenic NSE-IL1R1 mice lack IL1R1 on astrocytes, which could result in lower extracellular concentrations of adenosine. Furthermore, selective neuronal IL1R1 activation *per se* may not stimulate as much adenosine release as activation of astroglial IL1R1. An alternate hypothesis is that neuron-derived adenosine may act via mechanisms that are distinct from astroglial-derived adenosine (Lovatt et al., 2012). Additional studies are necessary to determine precise mechanisms by which sleep deprivation-induced changes in EEG spectral characteristics are modulated/mediated by IL1R1 expression on specific cell types in brain.

4.3. In vivo and in vitro responses to IL-1

Wild type mice used in this study respond to central administration of IL-1 as previously reported for rodents, with suppressed REMS, increased NREMS, diminished NREM delta power, and a prolonged fever (Opp et al., 1991; Opp and Krueger, 1991; Olivadoti and Opp, 2008). Of interest, IL-1 has no effect on sleep or brain temperature of either IL1R1 transgenic mouse strain used in this study. These data indicate that IL1R1 expression specifically on neurons or on astrocytes is not sufficient for IL-1 to affect sleep or temperature regulation. Neurons and astrocytes in brain do not exist or function in isolation. *In vitro* studies show that some neuronal functions are dependent on astrocytes, and *vice versa*. Astrocytes mediate synaptic development and plasticity (Stevens, 2008), confer neuroprotection against immune effector cells

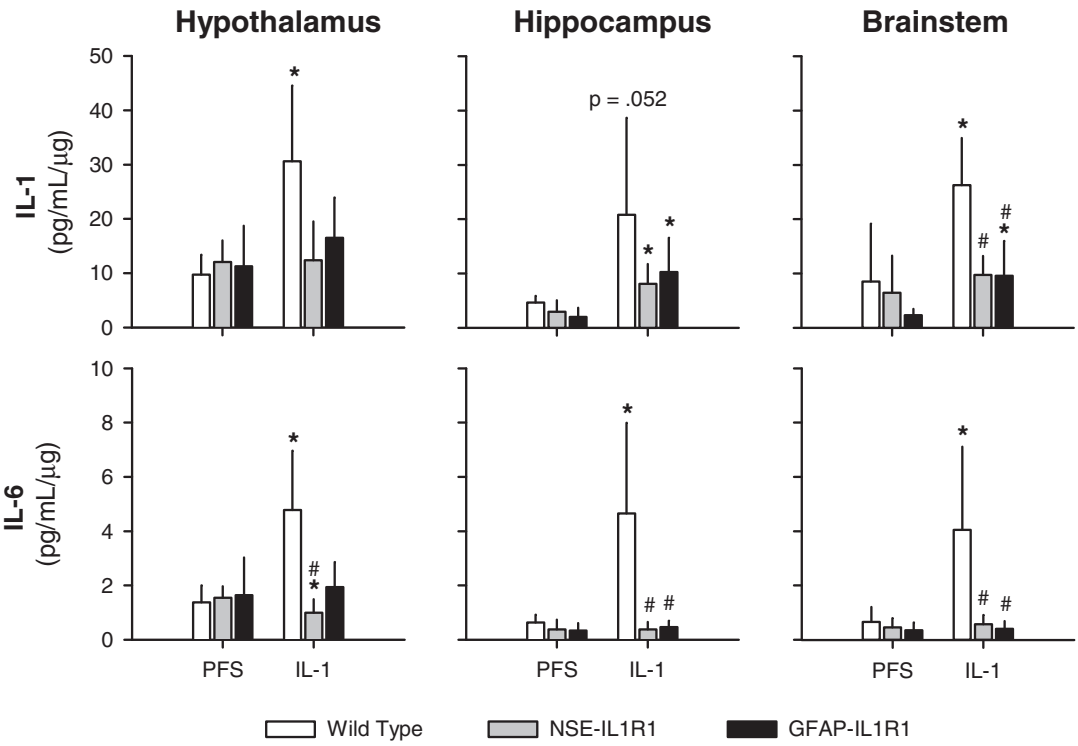

Fig. 8. Central administration of interleukin-1 (IL-1) increases IL-1, but not IL-6, in brain of transgenic IL1R1 mice. Intracerebroventricular (i.c.v.) administration of 50 ng IL-1 increases IL-1 in hippocampus of transgenic NSE-IL1R1 mice and transgenic GFAP-IL1R1 mice and brainstem of GFAP-IL1R1 mice. The hypothalamus, hippocampus, and brainstem of wild type, transgenic NSE-IL1R1 mice, and transgenic GFAP-IL1R1 mice were harvested 4 h post-i.c.v. injection with vehicle (PFS; pyrogen-free saline) or 50 ng IL-1. Values are means ± SDEV expressed as pg/mL/μg of protein loaded. * = $p < 0.05$ vs. PFS. # = $p < 0.05$ vs. wild type IL-1 values.

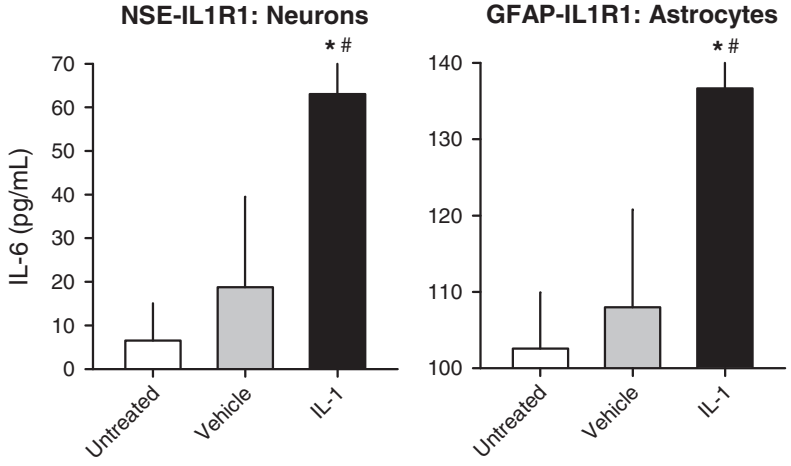

Fig. 9. Interleukin (IL)-6 increases *in vitro* when IL1R1 expressing neurons or astrocytes are stimulated with IL-1. Homogeneous transgenic NSE-IL1R1 neuronal cultures and transgenic GFAP-IL1R1 astroglial cultures were untreated, treated with vehicle, or stimulated with IL-1 (50 ng/well: neurons; 10 ng/well: astrocytes) for 24 h. Values are means ± SDEV. * = $p < 0.05$ vs. untreated conditions. # = $p < 0.05$ vs. vehicle.

(Darlington et al., 2008), and regulate hippocampal neurogenesis (Song et al., 2002). Likewise, neurons influence astroglial expression of glutamate transporters (Swanson et al., 1997) and mediate astroglial proliferation (Hatten, 1987). Astroglial-neuronal interactions can be modulated by secreted factors (Hayashi et al., 1988; Seil et al., 1992) or *via* direct cellular contact (Hatten, 1987). Our new data suggest that concurrent expression of IL1R1 on neurons and astrocytes may be necessary for sleep and brain temperature responses to centrally administered IL-1 to be fully manifest.

Potential mechanisms by which central administration of IL-1 alters sleep and brain temperature include downstream actions on cytokines and other inflammatory mediators [reviewed by (Allan et al., 2005)]. IL-1 stimulates its own production (Dinarello et al., 1987; Dinarello, 1988), and previous studies demonstrate the elimination half-life of exogenous IL-1 is approximately 40–60 min (Reimers et al., 1991; Loddick and Rothwell, 1996). Therefore, *in vivo* IL-1 detected 4 h post-injection in this study is *de novo* IL-1 produced in response exogenous IL-1 administration. Our data demonstrate that i.c.v injection of IL-1 induces *de novo* IL-1 production in all strains of mice we used. However, IL-1 is not increased in hypothalamic tissue obtained from IL1R1 transgenic mice, which may explain, at least in part, lack of sleep and temperature responses in these strains after central IL-1 administration.

In WT mice, i.c.v. IL-1 also increases IL-6 in brain, an effect previously reported (Romero et al., 1996; Tsakiri et al., 2008). IL-1 does not increase IL-6 in brain of either IL1R1 transgenic mouse strain. IL-6 is a known modulator of pathophysiological sleep and temperature (Morrow and Opp, 2005a; Olivadoti and Opp, 2008), and its production occurs subsequent to IL-1 in the cytokine cascade (Zetterstrom et al., 1998). We previously demonstrated that sleep and, more so, temperature responses to i.c.v. administration of IL-1 are attenuated in IL-6 knockout mice (Olivadoti and Opp, 2008). Collectively, previously published data and these new results suggest that IL-1-induced fever and, to some extent, changes in sleep require production of IL-6. Of note, NSE-IL1R1 neurons and GFAP-IL1R1 astrocytes in cell culture, which express IL1R1, are capable of producing IL-6 in response to IL-1 stimulation. IL-1 induces IL-6 release via signaling pathways that differ between neurons and astrocytes (Tsakiri et al., 2008). Because IL-1 stimulates IL-6 *in vitro* (this study), it is unlikely that both neuronal- and astroglial-specific downstream mechanisms of IL-1-induced IL-6 production are somehow disrupted in these IL1R1 transgenic mice. Therefore, lack of IL-1-induced IL-6 *in vivo* suggests that mediators upstream of these pathways are necessary. One potential mediator of *in vivo* IL-1-induced sleep and IL-6 production may be endothelial cells. Endothelial cells are an important substrate for IL-1 effects (Konsman et al., 2004; Thornton et al., 2010). Although little is known with respect to their contributions to sleep-wake behavior, febrile responses to IL-1 are attenuated in mice with inducible inhibition of IL1R1 on endothelial cells (Ching et al., 2007). Furthermore, endothelial-specific IL1R1 knockdown reduces IL-1-induced hypothalamic c-Fos expression (Ching et al., 2007). A recent study reports that IL1R1 selectively expressed on endothelial (and hematopoietic) cells rescues IL-1-induced COX-2 expression in brain compared to IL1R1 knockout mice (Liu et al., 2015). The transgenic mice used in our study lack IL1R1 on endothelial cells. Therefore, as with fever, expression of IL1R1 on endothelial cells may be necessary for IL-1-induced alterations in sleep. Although additional experiments are necessary, endothelial IL1R1 might be a critical initiator of behavioral responses to IL-1, whereas neuronal and astroglial IL1R1 may be necessary to potentiate such responses.

4.4. Methodological considerations

To our knowledge, these are the first *in vivo* studies to investigate the sufficiency of neuronal and astroglial-specific IL1R1 modulation of physiological sleep and IL-1-induced alterations in sleep. As with any model, there are strengths and limitations that must be recognized. Due to the approach used to express the transgene, IL1R1 is overexpressed relative to WT mice on neurons and astrocytes in some brain regions. However, baseline IL-1 and IL-6 protein concentrations in brains of NSE-IL1R1 and GFAP-IL1R1 mice do not differ from those of WT animals. Although IL1R1 expression in brain is constitutive and widespread (Farrar et al., 1987; Breder et al., 1988; Takao et al., 1990; Yabuuchi et al., 1994), to our knowledge, a comprehensive analysis of cell-specific IL1R1 expression by brain region has yet to be conducted. Therefore, it is unclear to what extent cell-specific IL1R1 expression patterns in our transgenic lines differ from WT mice. Additionally, the IL-1 system is self-regulated by a number of feed-forward and negative feedback regulatory components, including the non-signaling IL1R type II decoy receptor (McMahan et al., 1991), the endogenous IL-1 receptor antagonist (Hannum et al., 1990), and the IL-1 receptor brain-specific accessory protein (Taishi et al., 2012). The impact of selective neuronal- or astroglial expression of IL1R1 on these system components is currently unknown. IL-1 also induces a number of secondary mediators that exhibit inhibitory autocrine and paracrine effects on its activity. These factors include anti-inflammatory cytokines (Fiorentino et al., 1991; Pousset et al., 1999) and components of the hypothalamic–pituitary–adrenal (HPA) axis (Opp and Imeri, 2001; Knudsen et al., 1987; Lee et al., 1988). The impact of IL-1 on anti-inflammatory mediators and the HPA axis in these transgenic mice has not yet been investigated.

5. Conclusions

Overall, these studies demonstrate the complexity of IL1R1 and its cellular expression for sleep regulation and responses to exogenously administered IL-1. IL1R1 transgenic mice provide a unique tool to dissect interactions between neurons and astrocytes under a variety of conditions. Although other cell types, including microglia and endothelial cells, are important mediators of brain responses to immune challenge, understanding the molecular contributions of astrocytes to CNS-mediated processes and behaviors may provide new and important information that is relevant to multiple neuropathologies characterized by inflammation. Further, investigation of cell-specific contributions to physiological and pathophysiological CNS processes can aid in the development of targeted cellular therapies not only for chronic diseases, but potentially also for targeted treatment of individual symptoms associated with all inflammatory diseases.

Conflict of interest

The authors have no conflicts of interest to disclose.

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