Sleep and Immunomodulatory Responses to Systemic Lipopolysaccharide in Mice Selectively Expressing Interleukin-1 Receptor 1 on Neurons or Astrocytes

Ashley M. Ingiosi[1,2,3] and Mark R. Opp[3,4]

Sleep-wake behavior is altered in response to immune challenge. Although the precise mechanisms that govern sickness-induced changes in sleep are not fully understood, interleukin-1β (IL-1) is one mediator of these responses. To better understand mechanisms underlying sleep and inflammatory responses to immune challenge, we used two transgenic mouse strains that express IL-1 receptor 1 (IL1R1) only in the central nervous system and selectively on neurons or astrocytes. Electroencephalographic recordings from transgenic and wild-type mice reveal that systemic challenge with lipopolysaccharide (LPS) fragments sleep, suppresses rapid eye movement sleep (REMS), increases non-REMS (NREMS), diminishes NREM delta power, and induces fever in all genotypes. However, the magnitude of REMS suppression is greater in mice expressing IL1R1 on astrocytes compared with mice in which IL1R1 is selectively expressed on neurons. Furthermore, there is a delayed increase in NREM delta power when IL1R1 is expressed on astrocytes. LPS-induced sleep fragmentation is reduced in mice expressing IL1R1 on neurons. Although LPS increases IL-1 and IL-6 in brain of all genotypes, this response is attenuated when IL1R1 is expressed selectively on neurons or on astrocytes. Collectively, these data suggest that in these transgenic mice under the conditions of this study it is neuronal IL1R1 that plays a greater role in LPS-induced suppression of REMS and NREM delta power, whereas astroglial IL1R1 is more important for sleep fragmentation after this immune challenge. Thus, aspects of central responses to LPS are modulated by IL1R1 in a cell type-specific manner.

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Key words: Glia, IL-1, IL1R1, LPS, cytokine, fever, brain, illness

Introduction

There are bidirectional links between sleep and the immune system such that sleep loss impairs immune function, and sleep-wake behavior is modified in response to immune challenge [reviewed by (Besedovsky et al., 2012; Krueger, 2008; Imeri and Opp, 2009)]. Although mechanisms underlying these interactions are not completely understood, the proinflammatory cytokine interleukin-1β (IL-1) is one immunomodulator that mediates sleep and immune interactions [reviewed by (Jewett and Krueger, 2012; Kapsimalis et al., 2005; Krueger et al., 2001; Zielinski and Krueger, 2011)].

Systemic immune challenge triggers acute host defense responses that upregulate IL-1 in the brain and elicits clinical signs of illness (Bluthé et al., 2000; Davis et al., 2015; Granger et al., 2013; Van Dam et al., 1992, 1995; Zielinski et al., 2013). Indeed, symptoms of sickness, such as altered sleep, anorexia, weight loss, social withdrawal, and fever are induced by central administration of IL-1 [reviewed (Dantzer and Kelley, 2007; McCusker and Kelley, 2013; Opp, 2005)]. Brain regions associated with the regulation of sleep-wake behavior, feeding, social behavior, and thermoregulation are immunoreactive for IL-1 and IL-1 receptor 1 [IL1R1; (Breder

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et al., 1988; Konsman and Dantzer, 2001)]. IL-1 is involved in sleep regulation [reviewed (Krueger, 2008; Imeri and Opp, 2009)]; IL-1 increases non-rapid eye movement sleep (NREMS) by acting on defined sleep circuitry (Imeri and Opp, 2009), and suppresses rapid eye movement sleep [REMS; (Fang et al., 1998; Imeri et al., 2004; Ingiosi et al., 2015; Olivadoti and Opp, 2008; Opp et al., 1991)] by mechanisms that remain largely unknown. Furthermore, inflammatory-induced alterations in sleep are attenuated when IL-1 is inhibited (Baracchi and Opp, 2008; Fang et al., 1998; Opp and Krueger, 1991). IL-1 exerts its effects via IL1R1 which is widely and constitutively expressed in the brain and is present on neurons and astrocytes (Farina et al., 2007; Smith et al., 2009).

Although neurons produce cytokines (Bartfai and Schultzberg, 1993; Breder et al., 1988; Ingiosi et al., 2015; Tsakiri et al., 2008), much of their production and release is managed by glial cells, including astrocytes and microglia (Baumann et al., 1993; Choi et al., 2014; Dong and Benveniste, 2001; Mason et al., 2001; Qiu and Li, 2015). However, unlike astrocytes, microglia have negligible expression of IL1R1 (Ban et al., 1993; Pinteaux et al., 2002). As such, our studies of glial responses to IL-1 focus on astrocytes. Astrocytes respond rapidly to inflammation and produce sleep regulatory substances in response to immune challenge (Farina et al., 2007; Halassa et al., 2007; Ingiosi et al., 2013, 2015; Pinteaux et al., 2002; Sugama et al., 2011). Astrocytes also modulate physiological and pathophysiological sleep (Frank, 2013; Halassa et al., 2009; Ingiosi et al., 2015; Nadjar et al., 2013). However, the extent to which neuronal-glial interactions influence sleep and other physiological processes during immune challenge remains largely unknown.

Using transgenic mice that express IL1R1 only in the central nervous system (CNS) and selectively on neurons or on astrocytes, we previously showed that neuronal IL1R1 and astroglial IL1R1 differentially mediate sleep and inflammatory responses to sleep deprivation and central administration of IL-1 (Ingiosi et al., 2015). The objective of this current study was to determine cellular contributions of IL1R1 to altered sleep after systemic immune challenge. To accomplish this goal, these transgenic mice expressing IL1R1 selectively on neurons or on astrocytes were given a peripheral immune challenge in the form of a bolus injection of lipopolysaccharide (LPS). Our results reveal that IL1R1 differentially modulates distinct facets of sleep after LPS depending on whether it is expressed on neurons or on astrocytes.

Materials and Methods

Animals

Transgenic (Tg) mice used in these studies were engineered on a background lacking endogenous IL1R1, as previously described (Ingiosi et al., 2015). Briefly, IL1R1 was expressed on neurons by using the neuron specific enolase promoter [NSE; (Forss-Petter et al., 1990)], or on astrocytes using the human glial fibrillary acidic protein promoter [GFAP; (Brenner et al., 1994)]. Therefore, experimental mice (Tg(IL1R1)$Il1r1^{-/-}$) express IL1R1 only in the CNS and selectively on neurons or on astrocytes. Mice expressing IL1R1 specifically on neurons are called Tg NSE-IL1R1, whereas mice with astroglial localized expression of IL1R1 are referred to as Tg GFAP-IL1R1. Non-transgenic progeny expressing endogenous IL1R1 ($Il1r1^{+/+}$) were used as wild-type (WT) controls. Cell-type IL1R1 receptor expression specificity was previously reported (Ingiosi et al., 2015). The IL1R1 transgene is overexpressed relative to WT mice on neurons and astrocytes in some brain regions but not in others (Ingiosi et al., 2015). However, basal IL-1 protein concentrations in brains of Tg NSE-IL1R1 and Tg GFAP-IL1R1 mice do not differ from those of WT animals (Ingiosi et al., 2015).

Adult male WT, Tg NSE-IL1R1, and Tg GFAP-IL1R1 mice (20–30 g, 8–12 weeks at time of surgery) were obtained from our breeding colony and individually housed in standard cages on a 12:12 h light:dark cycle at 29 ± 1°C, an ambient temperature that is within the murine thermoneutral zone (Gordon and White, 1985; Rudaya et al., 2005). Food and water were available *ad libitum*. All procedures involving the 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.

Surgical Procedures

Mice used for electroencephalographic (EEG) recordings were 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; Ingiosi et al., 2015). 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 on the incision. Penicillin (1,200,000 IU/kg; SC) and topical triple antibiotic treatment of the surgical site were provided to minimize risk of infection. Additional buprenorphine (0.03 mg/kg; SC) was administered 24 h postsurgery. Animals were allowed at least 7 days recovery before initiation of experimental protocols.

Data Acquisition and Determination of Sleep-Wake Behavior

Mice were connected to the recording system using a counterbalanced, flexible tether which allowed for unrestricted cage navigation. EEG and brain temperature signals were conditioned and recorded, and general cage activity was monitored by an overhead infrared sensor (BioBserve, GmbH, Bonn, Germany) as previously described (Baracchi and Opp, 2008; Ingiosi et al., 2015). Digitized EEG signals were filtered using Chebyschev filters with third-order coefficients into delta (0.5–4.5 Hz) and theta (6.0–9.0 Hz) frequency bands and stored as binary files for subsequent analysis.

Arousal state was assigned with 10-s resolution based on visual inspection of the EEG waveform, theta-to-delta EEG frequency ratio, brain temperature, and cage activity using custom software (ICELUS, M. Opp, University of Washington) written in LabView for Windows (National Instruments) and previously published criteria (Opp, 1998; Opp and Krueger, 1994). Epochs containing artifacts were tagged and excluded from subsequent spectral analyses. The EEG was subjected to fast Fourier transformation to produce power spectra between 0.5 and 30.0 Hz in 0.5 Hz bins as previously described (Baracchi and Opp, 2008; Ingiosi et al., 2015). Power within the delta bands was normalized among animals by summing total NREM power across all frequency bins from 0.5 to 30.0 Hz for the 12 h dark and light periods. Values within individual 0.5 Hz bins were then expressed as a percentage of total power. Additionally, hourly NREM delta power values obtained after experimental manipulation (LPS injection) were expressed as relative change from the average values obtained during the 12 h dark and light periods after control manipulations (vehicle injection).

Quantification of Cytokine Protein Concentrations

Protein was extracted from tissue and quantified as previously described (Datta and Opp, 2008; Granger et al., 2013; Ingiosi et al., 2015; Sutton and Opp, 2015). Frozen brain tissue was thawed in ice cold lysis buffer from the Bio-Plex Cell Lysis Kit (171-304012; Bio-Rad Laboratories, Inc., Hercules, CA) containing 500 mM phenylmethanesulfonyl fluoride (P7626PMSF; Sigma-Aldrich, St. Louis, MO) and 1% protease inhibitor (S8830, Sigma-Aldrich). Tissue was homogenized using a sonic dismembrator. Homogenates were agitated for 40 min on ice and subsequently centrifuged at 8,000*g* for 20 min at 4°C. The supernatant was removed, aliquoted, and stored at −80°C until further processing. The total protein concentration of each sample was determined using the bicinchoninic acid (BCA) protein assay kit (23225, Thermo Scientific, Rockford, IL).

IL-1 and IL-6 concentrations were quantified using a bead-based assay and Luminex xMAP® technology (Luminex Corporation, Austin, TX) as previously described (Ingiosi et al., 2015; Sutton and Opp, 2015). Custom beads sets were developed using IL-1β (DY401) and IL-6 (DY406; R&D Systems, Inc., Minneapolis, MN) DuoSet antibodies and the xMAP Antibody Coupling Kit (40-50016, Luminex Corporation). Diluents were used as appropriate for the sample type: cell lysis buffer (171-304012, Bio-Rad Laboratories, Inc.) for protein extracts and 5% normal donkey serum (017-000-121, Jackson ImmunoResearch, West Grove, PA) in phosphate buffered saline for plasma samples. IL-1 and IL-6 recombinant proteins were used to generate a 7-point standard curve (27–20,000 pg/mL). Samples were loaded in duplicate, and IL-1 and IL-6 protein was detected by sequential 30 min incubations with the respective DuoSet detection antibodies and streptavidin-phycoerythrin (S866; Life Technologies, Carlsbad, CA). Plates were read on a Bio-Plex 200 system (Bio-Rad Laboratories, Inc.) and data analyzed using Bio-Plex Manager 4.1 software with five-parameter logistic regression (5PL) curve fitting. Observed concentrations obtained from brain tissue were adjusted for sample protein concentrations and are expressed as pg/mL/μg of protein loaded whereas those obtained from plasma samples are reported as pg/mL.

Experiment 1 Protocol: Determination of LPS-Induced Alterations in Sleep, Brain Temperature, and Clinical Symptoms of Illness

After recovery from surgery and habituation to the recording apparatus, WT ($n = 7$), Tg NSE-IL1R1 ($n = 7$), and Tg GFAP-IL1R1 ($n = 7$) mice received an intraperitoneal (i.p.) injection of vehicle [0.2 mL, pyrogen-free saline (PFS)], and recordings were obtained from undisturbed animals for 48 h. All mice then received 0.4 mg/kg LPS (*Escherichia coli*, serotype 0111:B4; Sigma-Aldrich), the dose and serotype of LPS that we have used in previous studies (Morrow and Opp, 2005; Olivadoti et al., 2011; Opp and Toth, 1998; Ringgold et al., 2013). All injections were given 15 min before dark onset (DO). Measures of body weight, food consumption, and water intake were obtained daily immediately before DO injections.

Experiment 2 Protocol: LPS-Induced Cytokine Expression

Two groups of mice were used to determine the impact of LPS on cytokine protein concentrations. The first group [WT ($n = 6$), Tg NSE-IL1R1 ($n = 5$), Tg GFAP-IL1R1 ($n = 5$)] received a single i.p. injection of vehicle (PFS; 0.2 mL). The second group [WT ($n = 7$), Tg NSE-IL1R1 ($n = 7$), Tg GFAP-IL1R1 ($n = 7$)] was injected with 0.4 mg/kg LPS i.p. Injections were given at light onset. All mice were anesthetized 4 h postinjection, after which blood was obtained via orbital bleed, mice decapitated, brains rapidly extracted, and meninges removed (Datta and Opp, 2008; Ingiosi et al., 2015). The hypothalamus, hippocampus, and brainstem were dissected while the brain was on an ice-cold surface and snap frozen in liquid nitrogen. Blood was centrifuged for 20 min at 3,000*g* at 4°C and plasma collected. All samples were stored at −80°C until subsequent protein extraction and/or analysis.

Statistical Analyses

Statistical analyses were performed using SPSS for Windows (IBM Corporation, Armonk, NY). Data are presented a 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 tests.

Within strain comparisons for percentage of time in REMS and NREMS, normalized NREM delta power, sleep state transitions, and brain temperature were performed using a general linear model for repeated measures with time (hours) as the repeated measure and manipulation (PFS *vs*. LPS) as the between-subjects factor. LPS effects on 12 h dark and light period normalized NREM delta power and brain temperature means for each strain were analyzed with one-way analysis of variance (ANOVA) using manipulation (PFS *vs*. LPS) as the independent factor.

Between strain comparisons for calculated difference scores of LPS effects were performed in 12 h time blocks using one-way ANOVA with strain (WT, Tg NSE-IL1R1, Tg GFAP-IL1R1) as the independent variable. If statistically significant strain effects were revealed, post-hoc comparisons by Tukey's HSD test were used to determine differences among mouse strains.

Within and between strain comparisons for body weight, food consumption, water intake, and cytokine protein concentrations were made with two-way ANOVAs using a within-in subjects factor of manipulation (PFS *vs*. LPS) and between-subjects factor of strain (WT, Tg NSE-IL1R1, Tg GFAP-IL1R1).

Results

Experiment 1: Determination of LPS-Induced Alterations in Sleep, Brain Temperature, and Clinical Symptoms of Illness

Sleep-wake behavior and brain temperature. Intraperitoneal administration of LPS altered sleep and brain temperature (Tbr) of WT, Tg NSE-IL1R1, and Tg GFAP-IL1R1 mice in a manner similar to responses previously reported (Jhaveri et al., 2006; Morrow and Opp, 2005; Olivadoti et al., 2011; Taishi et al., 2012; Zielinski et al., 2013). Specifically, LPS suppressed REMS, increased NREMS, and fragmented sleep of mice of all genotypes (Fig. 1). LPS induced a hypothermic response immediately after administration, although the LPS-induced reduction in Tbr did not achieve statistical significance in Tg NSE-IL1R1 mice. Febrile responses to LPS were apparent during the subsequent light period in all genotypes (Fig. 1).

The influence of neuronal and astroglial IL1R1 on LPS-induced alterations of NREM EEG spectra were evaluated as a measure of sleep intensity [e.g. (Borbély, 1982)]. Examination of hourly values demonstrated that LPS diminished NREM delta power for 18 h postinjection in WT mice, which then was followed by a significant increase (Fig. 1). LPS also reduced hourly NREM delta power in Tg NSE-IL1R1 mice, but not in Tg GFAP-IL1R1 mice. However, analysis of 12 h mean values revealed that LPS reduced NREM delta power of WT and Tg NSE-IL1R1 mice during the dark period, and increased this measure during the light period only in Tg GFAP-IL1R1 mice (Fig. 1).

Genotype differences in response to LPS were directly compared by calculating difference scores. Values obtained after injection of PFS (control) were subtracted from values obtained after LPS administration and then analyzed. There were no genotype differences observed in LPS-induced alterations of NREMS or brain temperature (Fig. 2). However, REMS suppression after LPS in Tg GFAP-IL1R1 mice was of greater magnitude and of longer duration than in Tg NSE-IL1R1 animals (Figs. 1 and 2). Additionally, NREM delta power in Tg GFAP-IL1R1 mice was enhanced during the subsequent light period after LPS administration as compared with either WT or Tg NSE-IL1R1 mice. Finally, sleep of Tg NSE-IL1R1 mice during the dark period was less fragmented by LPS challenge than in WT or Tg GFAP-IL1R1 mice as evidenced by fewer arousal state transitions.

Impact of LPS on symptoms of clinical illness. LPS effects on body weight, food consumption, and water intake were used as measures of clinical illness. During control conditions (i.e. after PFS administration), there were no genotype differences in body weight or 24 h food consumption, but Tg GFAP-IL1R1 mice drank less during the 24-h postinjection period than did WT or Tg NSE-IL1R1 mice (Fig. 3). Injection of LPS reduced body weight and 24 h food consumption and water intake in all mouse strains, but the LPS-induced decrease in water intake was attenuated in Tg NSE-IL1R1 mice compared with WT and Tg GFAP-IL1R1 animals. Calculated difference scores revealed these responses to be of the same magnitude for all genotypes with the exception that the reduction in water intake by Tg NSE-IL1R1 mice was less than that of WT mice (Fig. 3).

Experiment 2: LPS-Induced Cytokine Expression

IL-1 and IL-6 are produced in response to LPS as part of the cytokine cascade (Amiot et al., 1997; Zetterstrom et al., 1998). There were no differences among genotypes in IL-1 or IL-6 concentrations after injection of vehicle (PFS), although IL-6 was not detected in plasma from Tg mice (Fig. 4). LPS increased IL-1 and IL-6 protein concentrations in brain tissue samples from hypothalamus, hippocampus, and brainstem, and in plasma, in all genotypes (Fig. 4) with one exception; the trend for increased IL-1 in hypothalamus of Tg NSE-IL1R1 did not achieve statistical significance. LPS-induced increases in IL-1 and IL-6 were attenuated in all brain regions assayed from Tg NSE-IL1R1 and Tg GFAP-IL1R1 mice with the exception of hypothalamic IL-1.

Discussion

IL-1 is a critical mediator of many of the changes that occur in complex behavior and CNS processes during immune challenge. IL-1 receptors (IL1R) are widely distributed throughout the CNS and are expressed on multiple cell types, including neurons and astrocytes (Farina et al., 2007; Smith et al., 2009). Importantly within the context of this study, IL-1 is involved in the regulation of normal, physiological sleep and in the alterations of sleep that occur during immune activation (Krueger et al., 2001; Imeri and Opp, 2009). Although much is known about IL-1 and sleep regulation, comparatively little is understood of the CNS cell types by which IL-1 exerts these effects. Using these same transgenic mouse lines that express IL1R1 only in the CNS and selectively on neurons or on astrocytes, we recently reported that IL1R1 on astrocytes modulates the homeostatic response to sleep deprivation (Ingiosi et al., 2015). The aim of this present study was to determine the relative contributions of neuronal and astroglial IL1R1 to changes in sleep during systemic immune challenge with LPS. Although LPS upregulates

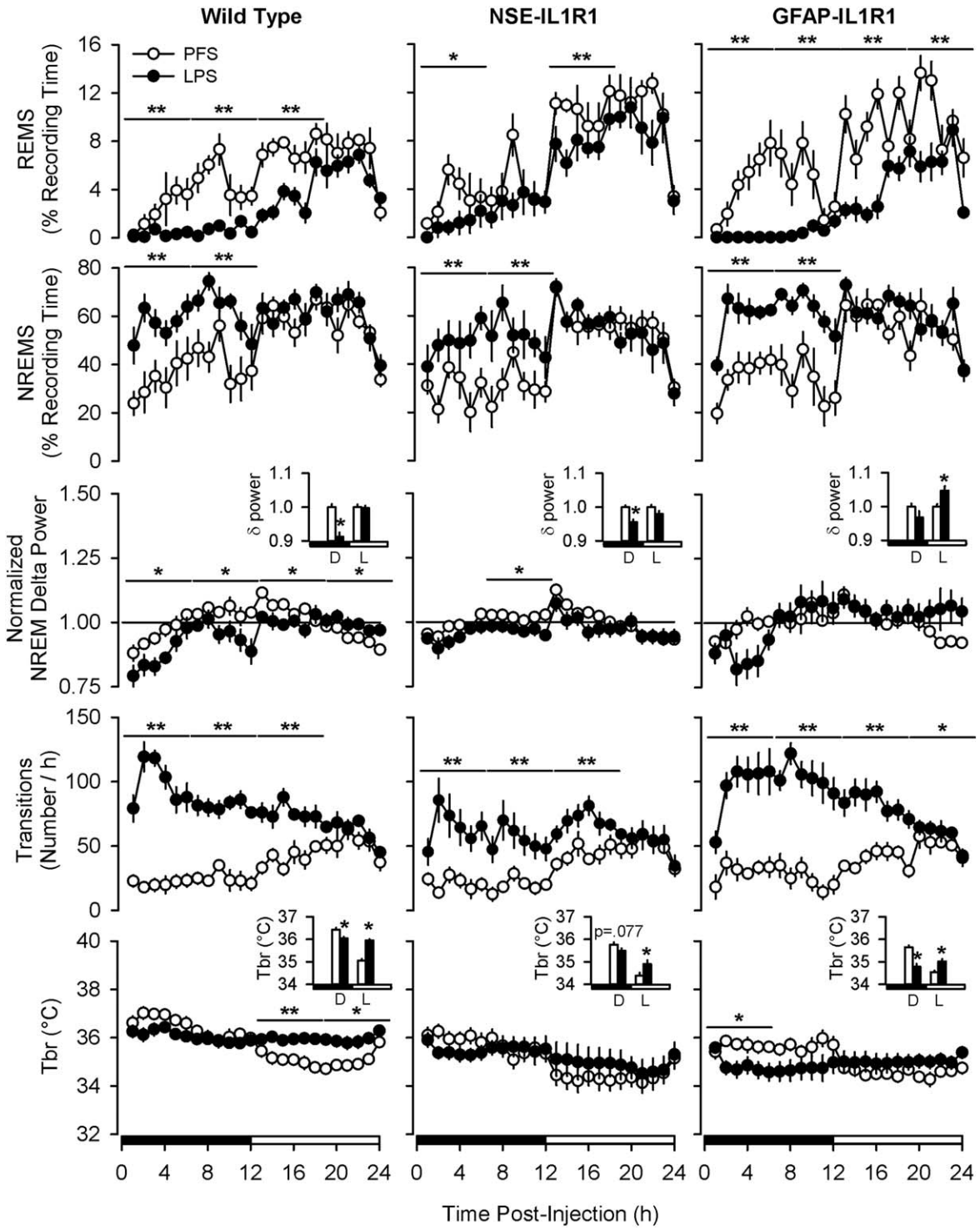

FIGURE 1: Selective expression of interleukin-1 receptor 1 on neurons or on astrocytes is sufficient for sleep and temperature responses to lipopolysaccharide (LPS). The percentage of recording time spent in rapid eye movement sleep (REMS) and non-REMS (NREMS), normalized NREM delta power, number of sleep state transitions, and brain temperature (Tbr) were determine from wild type, Tg NSE-IL1R1, and Tg GFAP-IL1R1 mice after intraperitoneal administration of vehicle (pyrogen-free saline, PFS; open symbols) or 0.4 mg/kg LPS (filled symbols). Values are means ± SEM. Hourly data for electroencephalographic (EEG) NREM delta power were normalized relative to average 12 h control (PFS) values for the dark and light periods, respectively. Graphical insets for NREM delta power (δ power) and Tbr depict 12 h mean comparisons between PFS (open bars) and LPS (filled bars) effects for the dark (D) and light (L) periods. The filled and open bars on the *x*-axis indicate dark and light periods of the light–dark cycle, respectively. *$P < 0.05$ *vs.* corresponding control (PFS) values. **$P < 0.01$ *vs.* corresponding control (PFS) values. $n = 7$ mice per genotype.

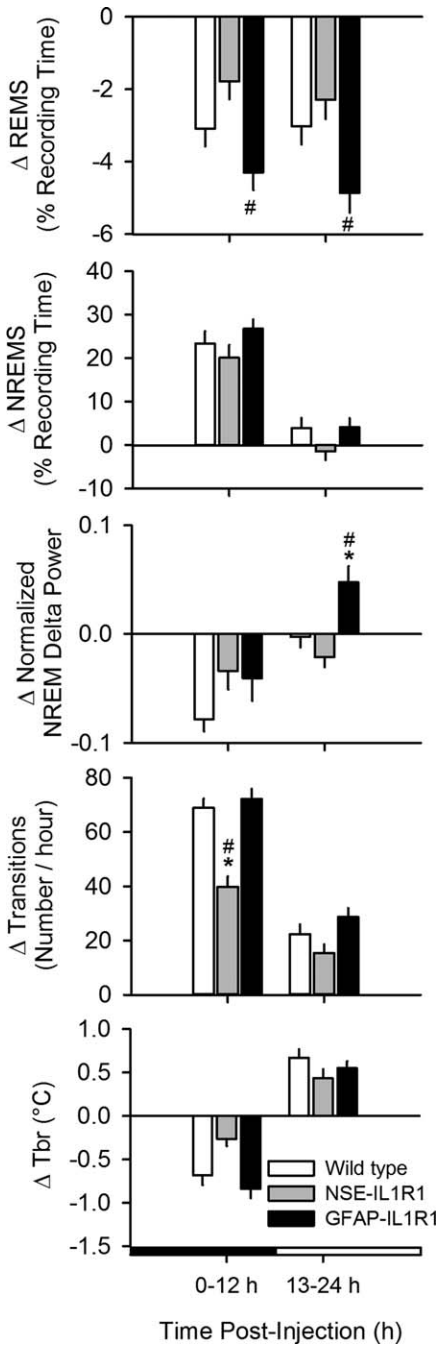

FIGURE 2: In response to lipopolysaccharide (LPS), rapid eye movement sleep (REMS) is suppressed to a greater extent when interleukin-1 receptor 1 (IL1R1) expression is localized to astrocytes, but sleep is more consolidated when IL1R1 is expressed selectively on neurons. Effects of intraperitoneal injection of 0.4 mg/kg LPS are shown as differences from vehicle (pyrogen-free saline), represented as the "zero" line. Percentage of recording time spent in REMS and non-REMS (NREMS), normalized electroencephalographic (EEG) delta power during NREMS, number of sleep state transitions, and brain temperature (Tbr) are plotted in 12 h time blocks for wild type, Tg NSE-IL1R1, and Tg GFAP-IL1R1 mice. Values are means ± SEM. Filled and open bars on the X-axis indicate dark and light portions of the light-dark cycle, respectively. *$P < 0.05$ *vs.* wild-type mice. #$P < 0.05$ *vs.* Tg IL1R1 mice. $n = 7$ per genotype.

expression of several inflammatory mediators, genotype difference in responses to LPS in this study are attributed to cell-type expression of IL1R1 on neurons or on astrocytes, in spite of the fact that other immunomodulatory systems are intact in these transgenic mice. Our new results demonstrate that neuronal and astroglial IL1R1 differentially modulate distinct facets of sleep responses to LPS challenge: neuronal IL1R1 modulates LPS-induced changes in REMS and NREM delta power, whereas astroglial IL1R1 plays a role in LPS-induced sleep fragmentation.

As previously reported for rats (Imeri et al., 2006; Kapas et al., 1998; Lancel et al., 1995, 1997) and mice (Morrow and Opp, 2005; Nadjar et al., 2013; Olivadoti et al., 2011; Szentirmai and Krueger, 2014; Taishi et al., 2012; Toth, 2001; Zielinski et al., 2013), LPS administration into mice in this study decreases REMS, an effect observed in each of the genotypes used. Mechanisms by which LPS suppresses REMS are not well understood, but IL-1 administration into rodents suppresses REMS (Baker et al., 2005; Fang et al., 1998; Imeri et al., 1999, 2004; Manfridi et al., 2003; Olivadoti and Opp, 2008; Opp and Imeri, 2001; Opp and Toth, 1998; Opp et al., 1991). Our new data, and those previously published (Datta and Opp, 2008; Henry et al., 2009; Sehgal et al., 2011; Wu et al., 2012), demonstrate that LPS upregulates IL-1 in brain, suggesting that LPS-induced suppression of REMS may be mediated, in part, by downstream actions of IL-1. The LPS-induced increase in IL-1 protein in brain is attenuated in the transgenic mice used in this study irrespective of whether IL1R1 is selectively expressed on neurons or on astrocytes. As such, although attenuated relative to that of WT mice, the LPS-induced increase of IL-1 in brain is sufficient to suppress REMS. Interestingly, the extent to which REMS is suppressed in these transgenic mice differs when IL1R1 is selectively expressed on neurons or on astrocytes, even when IL-1 concentrations in brain are equivalent. These observations suggest that cell type-specific mechanisms downstream of IL1R1 activation play a role in this response to LPS challenge.

Accessory proteins are required for IL-1 signal transduction. Upon ligand binding, the IL-1 receptor accessory protein (IL1RAcP) binds to the IL-1/IL1R1 complex and the signaling cascade is initiated (Greenfeder et al., 1995; Wesche et al., 1997). An alternatively spliced IL1RAcP isoform appears to be brain specific and is thus termed the brain-dominant IL1RAcP [IL1RAcPb; (Smith et al., 2009)]. IL1RAcPb is present primarily in neurons, but *not* astrocytes (Smith et al., 2009; Huang et al., 2011). The IL1RAcPb binds to the IL-1R/IL1RAcP complex and alters the subsequent signaling response such that it acts as an *intracellular* inhibitor of IL-1 actions. Recent work demonstrates that IL1RAcPb knockout mice have greater REMS suppression in

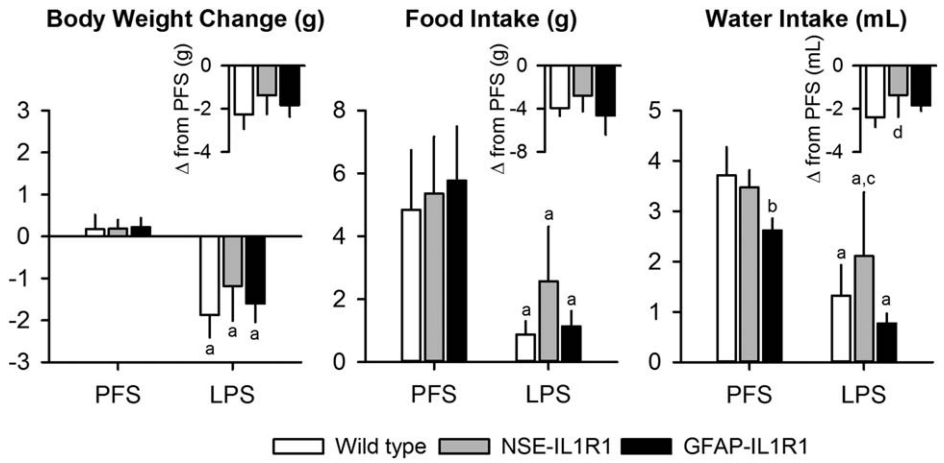

FIGURE 3: Lipopolysaccharide (LPS)-induced reduction in water intake is attenuated in transgenic mice that express interleukin-1 receptor 1 selectively on neurons. Intraperitoneal administration of 0.4 mg/kg LPS decreases body weight, food consumption, and water intake of wild type, Tg NSE-IL1R1, and Tg GFAP-IL1R1 mice compared with injection with vehicle (pyrogen-free saline, PFS). Graphical insets depict differences from control (PFS). Values are means ± SDEV collected 24 h postinjection. a = $P < 0.05$ *vs.* control (PFS) values. b = $P < 0.05$ *vs.* wild-type and Tg NSE-IL1R1 control (PFS) values. c = $P < 0.05$ *vs.* wild-type and Tg GFAP-IL1R1 LPS values. d = $P < 0.05$ *vs.* wild type. $n = 7$ per genotype.

response to LPS than do wild-type mice (Taishi et al., 2012). Our new data obtained from transgenic mice expressing IL1R1 selectively on neurons or on astrocytes are in agreement with these previous observations of LPS-induced REMS suppression in IL1RAcPb knockout mice. Because IL1RAcPb is a neuron-specific inhibitory mechanism for IL-1 actions, and because the magnitude of LPS-induced REMS suppression is the same in transgenic mice expressing IL1R1 selectively on neurons as in WT mice, the collective results of these two studies indicate that IL1R1 on neurons contributes to "normal" LPS-induced REMS suppression. That the magnitude of LPS-induced REMS suppression is greater in transgenic mice expressing IL1R1 selectively on astrocytes is likely due to the fact that there are no IL-1 receptors on neurons in

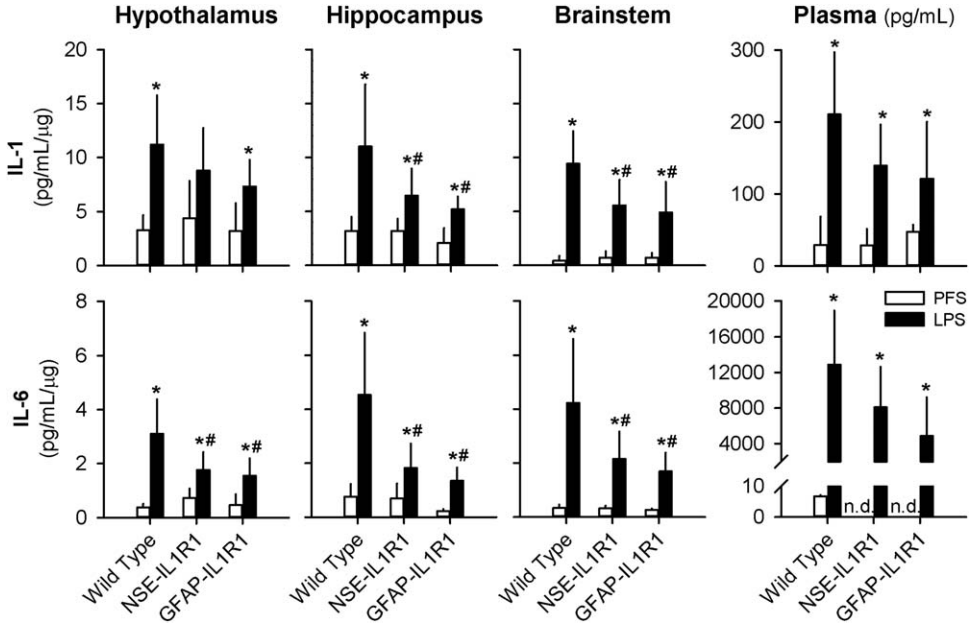

FIGURE 4: Lipopolysaccharide (LPS)-induced increases in interleukin-1 (IL-1) and IL-6 in brain are attenuated in transgenic IL-1 receptor 1 (IL1R1) mice. IL-1 and IL-6 protein was quantified from hypothalamus, hippocampus, brainstem, and plasma samples harvested from wild type, Tg NSE-IL1R1, and Tg GFAP-IL1R1 mice 4 h post-intraperitoneal injection of vehicle (pyrogen-free saline, PFS) or 0.4 mg/kg LPS. Values are means ± SDEV. *$P < 0.05$ *vs.* control (PFS) values. #$P < 0.05$ *vs.* LPS values obtained from wild-type mice. n.d. = not detected. $n = 5$–7 samples per genotype/condition.

these animals, and thus intracellular inhibitory mechanisms for IL-1, such as the IL1RAcPb, do not exert their effects. Definitive experiments to test this hypothesis remain to be conducted.

Although NREMS is elevated to the same extent in wild-type and IL1R1 transgenic mice, LPS-induced alterations in the NREM EEG spectra differ among strains in a time-dependent manner. The immediate effects do not differ among genotypes; NREM delta power is suppressed during the dark period following LPS administration to the same extent in each of the three mouse strains used in this study. During the subsequent light period, however, 12 h after LPS injection, NREM delta power increases in transgenic mice expressing IL1R1 on astrocytes, but not in transgenic mice expressing IL1R1 on neurons or in wild-type animals. NREM delta power is considered an index of sleep quality and intensity (Borbély, 1982). Therefore, despite increased time spent in NREMS during the dark period, these data suggest that sleep after LPS administration may be less "restorative" during this period of the light:dark cycle. These findings also suggest that for transgenic mice expressing IL1R1 on astrocytes, NREM sleep after LPS is deeper and perhaps more "restorative." Importantly, because NREM delta power after LPS is the same in wild-type and in transgenic mice expressing IL1R1 selectively on neurons, these observations during the light period suggest that neuronal IL1R1 contributes to, or drives, LPS-induced reductions in NREM delta power that would otherwise be elevated by actions of astroglial IL1R1.

The mechanisms that govern spectral characteristics of the NREM EEG have yet to be fully identified. However, astrocyte-derived adenosine may mediate, in part, LPS-induced increases in NREM delta power. Nadjar et al. administered LPS at light onset and demonstrated that the increase in NREM delta power is attenuated when astroglial gliotransmission of adenosine is inhibited (Nadjar et al., 2013). We previously reported that diurnal timing of LPS challenge is a determinant of EEG and behavioral outcomes (Morrow and Opp, 2005). Despite the differences in the protocols used, our current study also implicates astrocytes (via IL1R1) as modulators of LPS-induced increases in NREM delta power. Indeed, IL-1 treatment of hippocampal slices drives adenosine release (Sperlagh et al., 2004; Zhu et al., 2006) that is partly dependent on intracellular calcium signaling (Zhu et al., 2006), a key intracellular signaling mechanism in astrocytes [reviewed by (Frank, 2013)]. Collectively, the data suggest that LPS may induce astroglial IL1R1-modulated gliotransmission of adenosine to increase NREM delta power. Our data also indicate that although astroglial IL1R1 actions increase NREM delta power during the subsequent light period after LPS administration, neuronal IL1R1 modulates the LPS-induced reduction in NREM delta power during the initial post-LPS injection period, as observed in wild-type mice and in previous studies that administered LPS at dark onset (Kapas et al., 1998; Morrow and Opp, 2005; Szentirmai and Krueger, 2014; Taishi et al., 2012; Zielinski et al., 2013).

An additional measure of sleep quality and efficiency is sleep state consolidation [reviewed by (Imeri and Opp, 2009)]. LPS increases the number of arousal state transitions in all mouse strains we used, a response generally indicative of poor sleep. However, sleep of transgenic mice expressing IL1R1 selectively on neurons is less fragmented/more consolidated after LPS than that of wild-type mice or transgenic mice expressing IL1R1 on astrocytes. Our present study, and those of others (Arai et al., 2004; Ban et al., 1992; Bluthé et al., 2000; Datta and Opp, 2008; Konsman et al., 2008; Zielinski et al., 2013), demonstrate that LPS upregulates IL-1 in brain and that intracerebroventricular administration of IL-1 fragments sleep (Olivadoti and Opp, 2008; Opp et al., 1991). Collectively, these data suggest that IL1R1 activation on astrocytes may modulate sleep state maintenance and/or fragmentation after LPS. However, although mechanisms are not known, IL1R1 on astrocytes normally plays a role in LPS-induced sleep fragmentation because in their absence sleep of Tg mice is less fragmented/more consolidated. One previously discussed possibility for neuronal IL1R1 consolidation of LPS-induced sleep fragmentation is disinhibition of negative regulatory actions of the IL1RAcPb, which is specific to neurons. It is not currently known to what extent, if at all, the negative regulatory elements of the IL-1 system (e.g. AcPb, IL-1 receptor antagonist, non-signaling IL1R2) or its secondary mediators (e.g. anti-inflammatory cytokines) are affected in these transgenic IL1R1 mice. However, without IL1R1-derived inputs from non-neuronal cells, it is feasible that negative regulation of IL1R1 by IL1RAcPb in transgenic mice expressing IL1R1 selectively on neurons may manifest as more consolidated sleep.

LPS effects on mouse brain temperature in this study are similar to those previously reported (Morrow and Opp, 2005), and no strain differences were detected. Pharmacologic or genetic inhibition of IL-1 or IL1R1 does not eliminate LPS-induced fever (Alheim et al., 1997; Leon, 1996; Lundkvist et al., 1999); our present results add to data indicating that LPS-induced temperature responses do not depend on the IL-1 system and/or selective neuronal or astroglial expression of IL1R1.

Also consistent with the literature, LPS induces weight loss and attenuates feeding and drinking behavior in each of the mouse strains we used. The magnitude of LPS effects on body weight and food consumption do not differ among wild-type and IL1R1 transgenic mouse strains. This result is not

entirely surprising as LPS induces weight loss and anorexia in IL1R1 knockout mice, effects that may be mediated more so in the periphery than centrally (Bluthé et al., 2000). The effect of LPS on water intake in transgenic mice expressing IL1R1 selectively on neurons is attenuated compared with wild-type mice, suggesting astroglial IL1R1 activity may play a role in drinking behavior after this immune challenge. Indeed, GFAP immunoreactivity, an astroglial marker, fluctuates in the supraoptic nucleus of the hypothalamus depending on hydration status (Hawrylak et al., 1998). Although the LPS-induced reduction in water intake of transgenic mice expressing IL1R1 on astrocytes does not differ from wild-type mice, these animals drink less water during control conditions compared with wild-type mice and transgenic mice with IL1R1 localized to neurons. These data indicate that IL1R1 on neurons plays a role in physiological drinking, whereas IL1R1 on astrocytes modulates water intake under pathological conditions.

We quantified cytokine protein concentrations in brain to determine the impact of cell-specific IL1R1 expression on the underlying innate immune responses to LPS as potential mediators of altered behavior. LPS upregulates IL-1 and IL-6 in brain and plasma of each mouse strain used in this study. This effect is, in most cases, less robust in brain tissue obtained from the IL1R1 transgenic mouse strains. Although the IL-6 system is intact in these mice, IL-1 mediates LPS-induced IL-6 expression (Kuida et al., 1995; Luheshi et al., 1996). Therefore, reduced IL-1 protein concentrations may account for the attenuated IL-6 response to LPS.

LPS binds to toll-like receptor 4, resulting in subsequent production of cytokines, including IL-1 and IL-6 (Amiot et al., 1997; Zetterstrom et al., 1998). Unlike in brain, there are no genotype differences in plasma IL-1 or IL-6 concentrations in response to LPS. Despite attenuated brain IL-1 and IL-6 protein concentrations, transgenic mice with cell-specific IL1R1 expression still manifest behavioral responses to peripheral LPS challenge. One interpretation of these data is that peripheral cytokine expression is driving the behavioral alterations to LPS, and central IL-1 and IL-6 may not be crucial mediators of these responses. However, this is not likely the case as blockade of central responses to peripheral challenge attenuates symptoms of sickness induced by peripheral LPS (Luheshi et al., 1996; Morrow and Opp, 2005; Imeri et al., 2006). LPS-induced increases in peripheral cytokines impact the central nervous system by several mechanisms, including activation of the vagus nerve, brain entry through circumventricular organs, and/or increased blood-brain barrier (BBB) transport or permeability [for review see (Dantzer et al., 1998a; Dantzer et al., 1998b; Banks and Erickson, 2010)]. It is unknown if IL1R1 is expressed on vagal nerve terminals in our transgenic mice. However, although peripheral administration of low doses alters centrally-mediated processes via vagal afferents (Kapas et al., 1998; Zielinski et al., 2013), higher doses of LPS like the one used in this study do not require vagal signaling to the central nervous system to alter sleep, temperature, feeding, or inflammatory responses (Hansen et al., 2000; Opp and Toth, 1998; Schwartz et al., 1997; Van Dam et al., 2000; Zielinski et al., 2013). As such, peripheral administration of LPS in this study likely induces central nervous system responses by different mechanisms, such as increasing BBB transport and/or permeability (Minami et al., 1998; Jaeger et al., 2009; Xaio et al., 2001), for example. Characteristics of BBB transport in these transgenic IL1R1 animals have not been determined. However, it should be noted that endothelial cells that comprise the BBB play an important role in the saturable transport and local production of cytokines (Banks, 2005; Skinner et al., 2009), and endothelial IL1R1 expression is lacking in our IL1R1 transgenic mice. Another possibility is that behavioral responses to LPS in transgenic mice may be modulated by compensatory actions of other cytokines, such as tumor necrosis factor-alpha (TNF-α), as is the case with IL1R1 knockout mice (Bluthé et al., 2000). The role of TNF in responses of these transgenic mice to LPS challenge remains to be explored.

Overall, our new data demonstrate the complexity of cell-specific IL1R1 mechanisms that underlie behavioral and immune responses to inflammatory challenge. To our knowledge, these are the first studies to investigate the role of neuronal- or astroglial-specific IL1R1 in meditating/modulating CNS-response to peripheral LPS challenge. Continued study of the role of non-neuronal cell types is essential to further our understanding of a variety of CNS-mediated processes and behaviors as they relate to sickness and disease.

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