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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.

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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.

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

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,

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