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Effects of intracerebroventricular administration of interleukin (IL)-1 on sleep and body temperature of IL-6-deficient mice

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Abstract

Cytokines in brain contribute to the regulation of physiological processes and complex behavior, including sleep. The cytokines that have been most extensively studied with respect to sleep are interleukin (IL)-1 β , tumor necrosis factor (TNF)- α , and IL-6. Administration of these cytokines into laboratory animals, or in some cases into healthy human volunteers, increases the amount of time spent in non-rapid eye movement (NREM) sleep. Although antagonizing the IL-1 or TNF systems reduces the amount of time laboratory animals spend in NREM sleep, interactions among these three cytokine systems as they pertain to the regulation of physiological NREM sleep are not well understood. To further elucidate mechanisms in brain by which IL-1 β , TNF α , and/or IL-6 contribute to NREM sleep regulation, we injected recombinant murine IL-1 β (muIL-1 β) into C57BL/6J mice and into IL-6-deficient mice (IL-6 knockout, KO). IL-6 KO (B6.129S6-*Il6*^{tm1Kopf}; n = 13) and C57BL/6J mice (n = 14) were implanted with telemeters to record the electroencephalogram (EEG) and core body temperature, as well as with indwelling guide cannulae targeted to one of the lateral ventricles. After recovery and habituation, mice were injected intracerebroventricularly (ICV) just prior to dark onset on different days with either 0.5 μ l vehicle (pyrogen-free saline; PFS) or with 0.5 μ l PFS containing one of four doses of muIL-1 β (2.5 ng, 5 ng, 10 ng, 50 ng). No mouse received more than two doses of muIL-1 β , and administration of muIL-1 β doses was counter-balanced to eliminate potential order effects. Sleep-wake behavior was determined for 24 h after injections. ICV administration of muIL-1 β increased in NREM sleep of both mouse strains in a dose-related fashion, but the maximal increase was of greater magnitude in C57BL/6J mice. muIL-1 β induced fever in C57BL/6J mice but not in IL-6 KO mice. Collectively, these data demonstrate IL-6 is necessary for IL-1 to induce fever, but IL-6 is not necessary for IL-1 to alter NREM sleep.

Keywords

Cytokine; fever; rodent; knockout; neuroimmunology; CNS

Introduction

There is ample evidence that cytokines such as interleukin (IL)-1 and tumor necrosis factor (TNF) are involved in the regulation of non-rapid eye movement (NREM) sleep. With respect

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to IL-1, there are diurnal rhythms to IL-1 message and protein that are in phase with major sleep periods (Moldofsky et al., 1986; Lue et al., 1987; Taishi et al., 1998). Administration of IL-1 into laboratory animals increases the amount of time spent in NREM sleep, whereas antagonizing the IL-1 system with receptor antagonists, soluble receptors or antibodies reduces NREM sleep [reviewed (Krueger et al., 2001; Opp and Toth, 2003; Opp, 2005)]. Mice lacking functional IL-1 type I receptors spend less time in NREM sleep than genetically intact mice (Fang et al., 1998; Baracchi and Opp, 2008). IL-1 decreases discharge rates of wake-related neurons and increases the discharge rates of a subpopulation of sleep-related neurons in the preoptic area and basal forebrain (Alam et al., 2004), brain regions implicated in the regulation of NREM sleep. IL-1 microinjected into the dorsal raphe nucleus increases NREM sleep of rats, and *in vitro* IL-1 inhibits firing rates of dorsal raphe serotonergic neurons (Manfridi et al., 2003). Collectively these and other data indicate a role for IL-1 in regulating spontaneous NREM sleep, in healthy animals not subjected to immune challenge [interested readers are referred to comprehensive reviews (Krueger et al., 2001; Opp and Toth, 2003; Opp, 2005)].

Cytokine systems in brain, as in the peripheral immune system, are complex, with multiple overlapping actions attributed to several cytokines. For example, many of the biologic responses that are initiated by IL-1 are mediated downstream by TNF and/or IL-6. Such interactions among cytokines make it difficult to ascribe biologic actions to a single cytokine. There is accumulating evidence that IL-6 may mediate, in part, alterations in sleep during some pathologic conditions associated with excessive daytime sleepiness. ICV administration of IL-6 into rats increases NREM sleep (Hogan et al., 2003) and intravenous administration of IL-6 into human volunteers increases slow-wave sleep (Späth-Schwalbe et al., 1998). IL-6 is elevated in patients suffering from insomnia, narcolepsy and sleep apnea (Vgontzas et al., 1997; Vgontzas et al., 1999; Vgontzas et al., 2002; Okun et al., 2004; Burgos et al., 2006), suggesting IL-6 may play a role in the excessive daytime sleepiness and fatigue associated with these sleep disorders. Although NREM sleep of mice lacking IL-6 is normal (Morrow and Opp, 2005b), these mice respond to immune challenge in the form of bacterial lipopolysaccharide (LPS) with increases in NREM sleep that are about 16 % – 50 % of the LPS-induced increases in NREM sleep of C57BL/6J control mice, depending on the timing of administration (Morrow and Opp, 2005a). The observation that LPS-induced increases in NREM sleep of IL-6 KO mice are less than those of control animals suggests that IL-6 is necessary for the complete manifestation of LPS challenge on sleep-wake behavior.

In addition to sleep and other complex behavior, IL-1 and IL-6 have been implicated in the initiation of febrile responses to immune challenge [e.g., (LeMay et al., 1990; Rothwell et al., 1991; Kozak et al., 1998)]. Of importance to this present study, low doses of LPS administered intraperitoneally (IP) do not induce fever in IL-6 KO mice (Kozak et al., 1998; Zetterstrom et al., 1998; Morrow and Opp, 2005a). Similarly, IL-6 KO mice do not fever in response to IP administration of IL-1 (Chai et al., 1996). Collectively, these data indicate a role for IL-6 in the production of fever that is downstream of IL-1 and/or TNF, at least in response to some types of immune challenge [reviewed (Leon, 2002)]. The aim of the present study was to further elucidate the relationships between IL-1 and IL-6 as they pertain to the regulation of sleep and body temperature. Although sleep and body temperature may be strongly influenced by peripheral processes, they are first and foremost processes that are regulated by the brain. Unlike the previously-cited studies, our focus in this study was on the brain. To that end, we injected C57BL/6J mice and mice lacking a functional IL-6 gene (IL-6 knockout, KO) intracerebroventricularly (ICV) with recombinant murine (μ) IL-1 β to test the hypothesis that sleep and temperature responses of IL-6 KO mice to central administration of IL-1 would differ from those of genetically-intact mice. We now report that C57BL/6J mice and IL-6 KO mice respond to IL-1 with increases in NREM sleep, although the maximal increase in NREM sleep of IL-6 KO mice is less than that of C57BL/6J mice. Furthermore, under the conditions of this study, IL-1 induces fever in C57BL/6J mice but not in IL-6 KO mice. These data suggest

that IL-6 in brain plays a greater role in temperature responses to IL-1 than it does in the alterations in sleep induced by this challenge.

Experimental Procedures

Substances

Recombinant murine IL-1 β (muIL-1 β) was purchased from R & D Systems (Minneapolis, MN) and reconstituted in pyrogen-free saline (PFS; Abbott Laboratories, North Chicago, IL). Aliquots were stored at -80°C until use, when they were thawed and brought to an appropriate concentration. Aliquots containing IL-1 were not subjected to repeated freeze-thaw cycles. PFS was used as vehicle in these studies.

Animals

Breeding pairs of B6.129S2-*Il6^{tm1Kopf}* (IL-6 KO) mice were purchased from the Jackson Laboratory (Bar Harbor, ME). A breeding colony was established under the oversight of the Unit for Laboratory Animal Medicine at the University of Michigan. Adult male IL-6 KO mice (22 – 28 g) were used in these studies. Originally generated on a 129S6 background (Kopf et al., 1994), this IL-6 KO strain has been backcrossed with C57BL/6J mice for 11 generations with seven generations of sibling mating. Therefore, the IL-6 KO mice have been bred to homogeneity and the genetic background of this strain is considered to be identical to that of C57BL/6J at all unlinked loci. Adult male C57BL/6J mice (25 – 28 g) were purchased from Jackson Laboratory and used as controls. Animals were individually housed in standard cages (18 $\times$ 28 $\times$ 12 cm) without running wheels on a 12:12 h light:dark cycle at an ambient temperature of $29 \pm 1^{\circ}\text{C}$, a temperature within the thermoneutral zone of mice (Gordon and White, 1985; Rudaya et al., 2005). All mice received rodent laboratory chow (Lab Diet 5001, PMI Nutrition International, Brentwood, MO) and drinking water ad libitum. All procedures involving the use of animals were approved by the University of Michigan Committee on Care and Use of Animals 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

Telemeters (model # ETA10-F20, Data Sciences International, St. Paul, MN) to record the electroencephalogram (EEG) and core body temperature were surgically implanted in the peritoneal cavity under isoflurane anesthesia (4% induction, 2% maintenance) using sterile technique, as previously described (Tang and Sanford, 2002; Morrow and Opp, 2005b). Transmitter leads were passed subcutaneously to the base of the skull, where they were attached to stainless steel screws that served as EEG recording electrodes. Mice recovered from anesthesia on a heating pad at 37°C until ambulatory. Analgesia was provided by administration of ibuprofen (0.2 mg/ml) in the drinking water beginning 24 h before surgery and continuing for 48 h after surgery (Hayes et al., 2000), and by subcutaneous administration of 0.05 mg/kg buprenex at the time of surgery. A broad-spectrum antibiotic (imipenem, 25 mg/kg subcutaneous) was given immediately after surgery to minimize risk of infection.

A second surgical procedure was used to implant chronic intracerebroventricular (ICV) guide cannulae. This surgical procedure was done 21 to 28 days after implantation of the biotelemeters. Briefly, mice were anesthetized with isoflurane and positioned in a stereotaxic instrument with a mouse adapter and an anesthesia mask (David Kopf Instruments, Tujunga, CA). Using sterile technique, a burr hole was drilled in the skull and the guide cannula was positioned at stereotaxic coordinates of AP: -0.5 mm , relative to bregma; lateral: 0.8 mm ; dorsal-ventral: -2.5 mm . Cannula placement in the lateral ventricle was determined during implantation by a pressure drop in the level of saline in a length of silastic tubing connected

to the cannula. The cannula was then cemented in place with dental acrylic. Post-surgical care included application of a topical analgesic (4% lidocaine) to the scalp incision, a subcutaneous injection of 0.05 mg/kg Buprenex, and a single subcutaneous injection of 25 mg/kg imipenem. After recovery, the patency of the cannula was tested by injection of 250 ng of angiotensin II in 0.5 μ l PFS. Angiotensin II induces a drinking response by stimulating pre-optic structures (Epstein et al., 1970; Denton et al., 1990; Weisinger et al., 1999; Skott, 2003); only data from mice with positive drinking responses were included in subsequent analyses. At least seven days were allowed for recovery after the implantation of the ICV guide cannula before experimental protocols were begun.

Experimental Protocols

C57BL/6J (n = 14) and IL-6 KO (n = 13) mice were injected ICV with vehicle (pyrogen-free saline; PFS), and on separate days with vehicle containing 2.5 ng, 5 ng, 10 ng or 50 ng of muIL-1 β (n = 6 – 7 per dose). All injections consisted of a volume of 0.5 μ l. No mouse received more than two doses of muIL-1 β , and administration of muIL-1 β doses was counter-balanced to eliminate potential order effects. Injections were given 15-min prior to dark onset. Recordings began at dark onset and continued for 24 h. A minimum of 48 h separated injections of muIL-1 β .

Data Acquisition

Signals from telemeters were fed to a DSI analog converter (ART Analog-8 CM), where each EEG and temperature channel was converted to voltage using a transmitter-specific calibration factor provided by DSI. The output from the DSI analog converter was captured by an A/D board (model PCI-3033E, National Instruments, Austin, TX), which re-digitized the data at 128 Hz with 16-bit precision. The temperature voltages were converted to engineering units ($^{\circ}$ C) by regression using calibration coefficients specific for each transmitter. Gross body activity was detected by an infrared sensor (BioBserv, GmbH, Bonn, Germany) placed above the shoebox. Movements detected by the infrared sensor were converted to a voltage output, which was captured by the National Instruments A/D board, digitized and integrated into 1-s bins. All signals (EEG, body temperature, integrated activity) were stored as binary files until further processing.

During acquisition, the EEG was digitally filtered using Chebyshev filters with 3rd order coefficients into delta (0.5 to 4.5 Hz) and theta (6.0 to 9.0 Hz) frequency bands. These filtered EEG signals were integrated over 1-s periods, and stored as part of the binary file structure. Arousal state designations were made with 10-s resolution on the basis of visual inspection of the recordings using custom software (ICELUS, M. Opp, University of Michigan) written in LabView for Windows (National Instruments). Determination of arousal state, made on the basis of EEG, body movements, and integrated delta and theta frequency values, was classified as wakefulness, NREM sleep, or REM sleep. Briefly, wakefulness was defined on the basis of a low amplitude, mixed frequency (delta $\cong$ theta) EEG accompanied by body movements. Increases in body temperature during wakefulness are associated with activity. NREMS was identified by an increased absolute EEG amplitude, integrated values for the delta frequency band greater than those for theta, and lack of body movements. Body temperature declines upon entry into NREMS until it reaches a regulated asymptote. REMS was characterized by a low amplitude EEG, with integrated values for the delta frequency band less than those for the theta frequency band. Epochs containing either movement artifacts or electrical noise were tagged and excluded from subsequent spectral analyses. The raw, non-integrated EEG signals were also processed offline using fast Fourier transformations (FFT) to yield power spectra between 0.5 and 20 Hz in 0.5 Hz frequency bins. These spectra were computed from the five consecutive 2-s EEG segments comprising the 10-s epoch. These five spectra were averaged to produce one spectrum for the epoch, which was matched to state to provide state-specific

power spectra. Because delta power during NREM sleep is now accepted as a measure of depth or intensity of sleep (Borbély, 1982; Borbely and Achermann, 1999), we focused our spectral analyses on IL-1-induced changes in this spectral frequency band (0.5 – 4.5 Hz).

Measures of sleep consolidation / fragmentation were based upon determination of the number of transitions from one arousal state to the next. These determinations were made for each 10 s epoch, irrespective of the arousal state designation. For example, a series of 10-s epochs designated as W,W,N,N,N,W,N,R would be determined to include four (4) state transitions. This approach provides a measure of sleep consolidation / fragmentation that is not based on arbitrary criteria for sleep architecture parameters.

Statistical Analyses

Statistical analyses were performed using SPSS for Windows. Two types of analyses were conducted. To determine the effects of ICV administration of muIL-1 β on sleep of C57BL/6J mice and IL-6 KO mice, analyses were constrained to within strain and comparisons were made by evaluating data in 4 h time blocks using one-way ANOVA. In these analyses, manipulation (vehicle, muIL-1 β dose) was the fixed effect (independent variable), and the amount of time spent in vigilance states, core body temperature, transitions from one state to another, and delta power during NREM sleep were the dependent variables. An alpha level of $p \leq 0.05$ was accepted for all statistical tests as indicating significant departures from control values.

To determine if there were dose-related responses to muIL-1 β , difference scores were calculated for each parameter by subtracting control values from experimental values. Analyses using one-way ANOVA were constrained to within strain and comparisons were made by evaluating average difference scores across the 12 h time blocks that comprised the dark period or the light period. In these analyses, muIL-1 β dose was the fixed effect (independent variable), and the difference scores for amount of time spent in vigilance states, core body temperature, transitions from one state to another, and delta power during NREM sleep were the dependent variables. An alpha level of $p \leq 0.05$ was accepted as indicating significant departures from control values. If one-way ANOVA revealed statistically significant differences across doses, post-hoc pair-wise comparisons by the method of Scheffé were used to determine values from which doses differed statistically from the others.

Potential strain differences in responses to muIL-1 β were evaluated across 4 h time blocks using the difference scores and one-way ANOVA in which strain (C57BL/6J, IL-6 KO) was the independent variable and the difference scores for time spent in vigilance states, core body temperature, transitions from one state to another, and delta power during NREM sleep were the dependent variables. An alpha level of $p \leq 0.05$ was accepted for all statistical tests as indicating significant departures from control values.

Results

Baseline measures of body temperature and sleep-wake behavior

C57BL/6J mice injected ICV with vehicle exhibited normal diurnal rhythms of body temperature and sleep-wake behavior (Figure 1, Table 1). Average body temperatures were higher during the 12 h dark period than during the 12 h light period, with a 1.4 ± 0.04 °C amplitude difference between the dark and light periods. C57BL/6J mice spent more time in wakefulness and less time in NREM and REM sleep during the dark period (Figure 1, Table 1). Conversely, during the light period, C57BL/6J mice spent more time in NREM sleep than in wakefulness (Figure 1, Table 1). Average body temperature and the amount of time spent in vigilance states by C57BL/6J mice injected ICV with vehicle in this study are essentially

identical to values previously obtained in our laboratory from C57BL/6J mice that did not receive any injections (Morrow and Opp, 2005b), or from C57BL/6J mice that received intraperitoneal injections of vehicle (Morrow and Opp, 2005a). Furthermore, the duration vigilance states in this study by the C57BL/6J mice from which vehicle control recordings were obtained are within the range of baseline values reported in the literature for this mouse strain (Table 2). Collectively, these data indicate that recordings obtained from C57BL/6J mice via telemetry are comparable to those obtained using cable tethers, and that ICV injections of vehicle do not alter normal sleep-wake behavior and body temperature rhythms of these mice.

IL-6 KO mice injected ICV with vehicle exhibited normal diurnal rhythms of body temperature and sleep-wake behavior (Figure 2, Table 1). Average body temperatures were higher during the 12 h dark period than during the 12 h light period, with peak-to-peak amplitude of 2.1 ± 0.7 °C and an average 12 h light:dark difference of 1.4 ± 0.1 °C (Table 1). IL-6 KO mice exhibited more wakefulness and less NREM and REM sleep during the dark period, whereas more NREM and REM sleep and less wakefulness was observed during the light period (Table 1). Average body temperature and duration of vigilance states for IL-6 KO mice injected ICV with vehicle in this present study were similar to values previously obtained in our laboratory from this mouse strain when either not subjected to injections (Morrow and Opp, 2005b), or when injected intraperitoneally with vehicle (Morrow and Opp, 2005a). As such, ICV injection of vehicle did not alter the normal diurnal variation of body temperature or vigilance states in IL-6 KO mice.

Responses to muIL-1 β

C57BL/6J mice—ICV administration of muIL-1 β induced fever, increased NREM sleep and reduced REM sleep and wakefulness (Figure 1, Table 1). The two lowest doses of muIL-1 β used in this study (2.5, 5.0 ng) had little effect on body temperature. Increases in body temperature became apparent 2 – 3 h after injection of 10 ng or 50 ng of muIL-1 β (Figure 1). The fever induced by 10 ng muIL-1 β had a peak magnitude of 1.14 ± 0.45 °C and lasted until postinjection hour 8 (Figure 1). Body temperatures remained elevated during the subsequent light period (postinjection hours 13 – 24) after 10 ng muIL-1 β . However, the magnitude of increase in body temperature during postinjection hours 13 – 24 was less than 0.5 °C (Table 1), and as such is not considered a fever. The fever induced by 50 ng muIL-1 β was 1.36 ± 0.07 °C in magnitude and lasted about 8 h (Figure 1). Body temperature remained elevated during the subsequent light period after this dose of muIL-1 β as well, with average body temperatures 0.7 ± 0.15 °C greater than during the same period after vehicle injections (Figure 1, Table 1).

Relative to values obtained after ICV administration of vehicle, NREM sleep of C57BL/6J mice increased after 3 of the 4 doses of muIL-1 β tested (Figure 1). The increase in NREM sleep after muIL-1 β lasted from 4 – 12 h, depending on dose (Figure 1). The greatest increase in NREM sleep occurred after administration of 50 ng muIL-1 β ; during the initial 12 h postinjection the amount of time spent in NREM sleep almost doubled (Figure 1, Table 1). The majority of increased NREM sleep after this dose occurred during postinjection hours 1 – 8 (Figure 1). Increases in NREM sleep after muIL-1 β were mirrored by reductions in wakefulness (Figure 1, Table 1). Under these conditions, REM sleep was not dramatically altered, although the two highest doses (10 ng, 50 ng) modestly suppressed REM sleep for several hours (Figure 1).

Sleep of C57BL/6J mice was fragmented by muIL-1 β , as evidenced by an increase in transitions from one arousal state to another (Table 1). The two highest doses of muIL-1 β tested in this study, 10 ng and 50 ng, increased the number of transitions from one arousal state to another relative to values obtained after vehicle administration by about 190% and by about 250%, respectively. The increase in number of transitions was limited to the first 12 h postinjection (Table 1). Delta power during NREM sleep was decreased after muIL-1 β relative to values

obtained after vehicle administration. The reduction in delta power during NREM sleep was greatest after the 10 ng and 50 ng doses (Table 1).

The muIL-1 β -induced changes in sleep-wake behavior and body temperature of C57BL/6J mice were dose dependent (Figure 3). The amount of NREM sleep of C57BL/6J mice during the 12 h after ICV administration of 50 ng muIL-1 β was statistically greater than that observed after the 2.5 ng, 5 ng or 10 ng doses. Concomitant with increased NREM sleep were dose-related reductions in wakefulness. The muIL-1 β -induced reduction in wakefulness after 50 ng differed significantly from the reduction induced by ICV administration of 5 ng (Figure 3). The effects of muIL-1 β on REM sleep of C57BL/6J mice were less consistent; 10 ng muIL-1 β reduced REM sleep to a greater extent than after any of the other doses used in this study (Figure 3). The fever induced by 10 ng and 50 ng muIL-1 β was of greater magnitude than that induced by the 2.5 ng and 5 ng doses (Figure 3).

IL-6 KO mice—ICV administration of muIL-1 β increased NREM sleep, suppressed wakefulness and (at the highest dose) REM sleep without inducing fever (Figure 2, Table 1). The 2.5 ng and 5 ng doses of muIL-1 β did not affect body temperature (Figure 2, Table 1). After administration of 10 ng or 50 ng muIL-1 β , there were transient hypothermic responses that were followed by periods during which modest increases in body temperature were apparent (Figure 2). These maximal increases in body temperature of IL-6 KO mice were limited to 0.57 ± 0.23 °C and 0.51 ± 0.25 °C during postinjection hour 4 after administration of 10 ng or 50 ng muIL-1 β , respectively. Body temperatures of IL-6 KO mice remained elevated during the subsequent light period, 16 – 24 hours postinjection (Figure 2, Table 1). The 12 hour average elevations in body temperature during postinjection hours 16 – 24 amounted to $0.31 \pm .03$ °C after 10 ng muIL-1 β and 0.42 ± 0.03 °C after 50 ng muIL-1 β .

NREM sleep increased after each of the doses of muIL-1 β tested in this study (Figure 2, Table 1). These increases in NREM sleep were accompanied by reductions in wakefulness (Figure 2, Table 1). IL-1 effects on NREM sleep and wakefulness persisted for 4 – 12 h, depending on dose. REM sleep was suppressed during postinjection hours 4 – 8 after the 50 ng dose of muIL-1 β , which was followed by a statistically significant increase in REM sleep during postinjection hours 9 – 12 (Figure 2). As in C57BL/6J mice, sleep of IL-6 KO mice was fragmented after administration of muIL-1 β , as evidenced by more transitions from one behavioral state to another (Table 1). In contrast to C57BL/6J mice, sleep of IL-6 KO mice remained fragmented into the subsequent dark period after 3 of the 4 doses of muIL-1 β used in this study. Delta power during NREM sleep was not greatly affected by muIL-1 β in IL-6 KO mice (Table 1).

The effects ICV administration of muIL-1 β body temperatures of IL-6 KO mice did not differ across doses tested (Figure 3). There were however, dose-related increases in NREM sleep, with the amount of time spent in this vigilance state during the 12 h after injection being greater after the 50 ng dose than after the 2.5 ng dose (Figure 3). There were no dose-related effects on REM sleep, and the reductions in wakefulness were less consistent (Figure 3).

Differences between strains—There were strain differences with respect to the impact of IL-1 on NREMS (Figure 3). The increase in NREM sleep after the 5 ng muIL-1 β doses was statistically greater in IL-6 KO mice than in C57BL/6J mice, whereas after 50 ng muIL-1 β NREM sleep of C57BL/6J mice increased to a greater extent than did that of IL-6 KO mice (Figure 3). Strain differences in responsiveness to muIL-1 β were less consistent with respect to REM sleep or wakefulness, with REM sleep differing only after the 10 ng dose and wakefulness differing only after the 5 ng dose (Figure 3). The impact of muIL-1 β on body temperature of C57BL/6J and IL-6 KO mice differed significantly after the 10 ng and 50 ng

doses during the initial 12 h postinjection (dark period; Figure 3), but not during postinjection hours 13 – 24 (subsequent light period).

Discussion

There are two major findings of this study: 1) IL-6 is not necessary for central IL-1 to increase NREM sleep of mice, and 2) IL-6 is necessary for central IL-1 to induce fever in mice. To our knowledge, the effects of central (ICV) administration of IL-1 on sleep of mice have not previously been reported.

Sleep of genetically intact C57BL/6J mice is altered in response to ICV administration of IL-1. This present study demonstrates that central administration of muIL-1 β increases NREM sleep, reduces wakefulness, and at higher doses suppresses REM sleep. These results generally agree with previously published studies of the effects of intraperitoneal (IP) injection of human recombinant (hu) or murine recombinant (mu) IL-1 β on sleep of mice (Fang et al., 1998; Toth and Opp, 2001). Fang and colleagues (Fang et al., 1998) report that IP administration of 0.1 μ g or 0.4 μ g muIL-1 β into C7BL6x129sv mice increases NREM sleep, reduces wakefulness and suppresses REM sleep. Under conditions of that study (Fang et al., 1998), maximal effects were observed after the 0.4 μ g muIL-1 β , and these lasted about six hours. We previously demonstrated (Toth and Opp, 2001) that IP administration of 0.4 μ g huIL-1 β into inbred C57BL/6J mice transiently increases NREM sleep and reduces wakefulness without altering REM sleep. Although comparisons of effects of muIL-1 β administered ICV in our present study with those after IP administration of hu- or muIL-1 β in previous studies must be made with care, all reports indicate IL-1 β administered into genetically intact mice (C57BL/6J or C57BL/6x129) increases the amount of time spent in NREM sleep and reduces wakefulness.

IL-1, IL-6, and TNF are the cytokines that have been the most extensively studied with respect to sleep-wake behavior [reviewed (Krueger et al., 2001; Opp, 2005)]. Nevertheless, interactions among these three cytokine systems as they pertain to the regulation of sleep are not well understood. Cytokine networks are complex, and are characterized by overlapping biologic activities and redundancy of function (Vitkovic et al., 2000). As such, it is often difficult to ascribe a specific effect to a single cytokine. Our present data demonstrate that central administration of low doses of muIL-1 β increases NREM sleep of mice lacking IL-6 to the same extent as in genetically intact C57BL/6J mice. These data suggest that IL-6 is not an essential mediator of IL-1-induced alterations in NREM sleep. There are numerous mechanisms by which IL-1 can increase NREM sleep, including direct actions on sleep-active neurons of the preoptic area of the hypothalamus (Alam et al., 2004; Baker et al., 2005) and on arousal-promoting serotonergic neurons of the dorsal raphe (Brambilla et al., 2007). Endogenous IL-6 exerts negative feedback control on TNF, and IL-6 KO mice exhibit an approximately threefold higher increase in serum TNF after immune challenge with LPS than do control mice (Fattori et al., 1994; Kozak et al., 1998). TNF is also involved in the regulation of NREM sleep and increases NREM sleep of mice (Fang et al., 1997). Elevated TNF in response to IL-1 is another possible mechanism by which NREM sleep of IL-6 KO mice could be increased after low doses of IL-1. In fact, the finding that 5 ng muIL-1 β increased NREM sleep of IL-6 KO mice to a greater extent than in C57BL/6J mice may result from an IL-1-induced increase in TNF. Experiments to test this hypothesis have not been conducted.

IL-1 also induces the synthesis and release of transmitters, peptides, and hormones that are involved in the regulation or modulation of NREM sleep, including nitric oxide, adenosine, and growth hormone releasing hormone, to name but a few [reviewed (Obál, Jr. and Krueger, 2003)]. Therefore, observations that NREM sleep of IL-6 KO mice increases after low doses of muIL-1 β administered ICV indicates that these effects are mediated by IL-1-specific actions and/or actions of other systems that are stimulated by IL-1. However, the highest dose of

muIL-1 β used in this study increases NREM sleep of C57BL/6J mice to a greater extent than in IL-6 KO mice. These data suggest that as the dose of IL-1 increases, the IL-6 system plays some role in mediating IL-1 effects on NREM sleep. Additional studies are necessary to determine the extent of interactions between IL-1 and IL-6 as they pertain to the regulation and/or modulation of NREM sleep.

Previously published reports of the effects of IP administration of IL-1 on sleep of genetically-intact mice differ somewhat in the precise manner in which sleep is altered by this challenge. Fang and colleagues (Fang et al., 1998) demonstrate muIL-1 β -induced alterations in NREM sleep of mice that persist for up to six hours following IP administration, whereas Toth and Opp (Toth and Opp, 2001) report huIL-1 β -induced increases in NREM sleep that last for two hours after the same dose. There are several factors that could account for the differences in time course of responses to IL-1 β reported in previous studies. Toth and Opp (Toth and Opp, 2001) used inbred C57BL/6J mice maintained at 22 °C, whereas Fang et al., (Fang et al., 1998) used mixed background C57BL/6x129sv mice housed at 30 °C. It has been known for many years that different genetic backgrounds may influence the expression of complex behavior of mice, including sleep (Friedmann, 1974; Valatx and Bugat, 1974; Daszuta et al., 1983; Roussel et al., 1984; Tafti et al., 1997; Toth and Williams, 1999; Huber et al., 2000). However, data from our present study suggest that the ambient temperature at which mice are housed may also be a critical determinant of precise alterations in sleep induced by IL-1 β . The ambient temperature at which mice are housed impacts spontaneous sleep; C57BL/6J mice housed at ambient temperatures of 30 – 34 °C spend more time in NREM sleep than do mice housed at 25 – 26 °C (Roussel et al., 1984; Jhaveri et al., 2007). Data from our present study and those of others suggest the impact of IL-1 β on sleep of mice may also depend on the temperature at which the animals are housed; ICV administration of muIL-1 β into inbred C57BL/6J mice maintained at 29 °C (this study) alters sleep in a manner similar to that observed when mixed background C57BL/6x129sv mice maintained at 30 °C are injected IP with muIL-1. (Fang et al., 1998). Similarities in these data suggest that differences previously reported (Fang et al., 1998; Toth and Opp, 2001) in responses of mice to IP administration of the same dose of IL-1 β may be due to the ambient temperature at which the animals were housed rather than the genetic background of the strains used or the route of administration.

The effect of ambient temperature on thermoregulatory responses of mice to immune challenge has also been demonstrated. For example, C57BL/6J mice maintained within their thermoneutral zone at an ambient temperature of 31 °C exhibit a different time course of lipopolysaccharide (LPS)-induced alterations in body temperature than when housed at temperatures below thermoneutrality (Rudaya et al., 2005). C57BL/6J mice infected with influenza virus become hypothermic when housed at 22 °C or 26 °C, but maintain normothermia when housed at an ambient temperature of 30 °C (Jhaveri et al., 2007).

We further contribute to the literature of IL-1 effects on sleep of mice by demonstrating dose-related responses to ICV administration. Our previous study determined the impact on sleep of mice of a single dose of huIL-1 β administered IP (Toth and Opp, 2001), whereas Fang et al., (Fang et al., 1998) demonstrated dose-related responses to IP administration of muIL-1 β . Our results indicate that increasing doses of muIL-1 β administered ICV induce larger increases in NREM sleep. Furthermore, low doses of muIL-1 β administered ICV do not suppress REM sleep, whereas REM sleep of C57BL/6J mice is suppressed as the dose of muIL-1 β increases. These responses of mice to ICV administration of muIL-1 β are generally similar to those of rats injected ICV with huIL-1 β (Opp et al., 1991; Opp and Krueger, 1991; Imeri et al., 1993; Imeri et al., 1999; Imeri et al., 2004). In rats however, there appears to be a difference in time course and magnitude of responses to IL-1 depending on the species-specificity of the IL-1 injected. Rat recombinant IL-1 β injected ICV into rats (Baker et al., 2005) increases NREM sleep and induces fever with a slower time course than does huIL-1 β (Opp et al., 1991; Opp

and Imeri, 2001). To our knowledge, studies have not been conducted that directly compare effects of huIL-1 β vs. muIL-1 β on sleep or thermoregulation of mice.

Previous studies demonstrate a role for IL-6 in the production of fever in response to some types of immune challenge. For example, Chai and colleagues report that IP or ICV administration of IL-1 does not induce fever in IL-6 KO mice (Chai et al., 1996). Similarly, IL-6 KO mice do not fever in response to intravenous administration of IL-1 α (Kagiyada et al., 2004). In addition, IP administration of low doses of LPS does not induce fever in IL-6 KO mice (Chai et al., 1996; Kozak et al., 1998; Zetterstrom et al., 1998; Morrow and Opp, 2005a). However, IL-6 KO mice develop fever after ICV administration of recombinant human IL-6 (Chai et al., 1996). Collectively, these data indicate a role for IL-6 in the production of fever that is downstream of IL-1 and/or TNF, at least in response to some types of immune challenge [reviewed (Leon, 2002)]. Data from our present study generally corroborate these previous findings; central administration of muIL-1 β into IL-6 KO mice induces modest, yet statistically significant elevations in body temperature. The changes in body temperature of IL-6 KO mice after ICV administration of muIL-1 β are significantly reduced with respect to the magnitude and duration of those observed in genetically intact C57BL/6J mice. We have yet to conduct definitive studies to determine whether the modest increases in body temperature of IL-6 KO mice after 10 ng or 50 ng of ICV IL-1 β are merely a shift in the diurnal rhythm of body temperature due to actions of muIL-1 β on the suprachiasmatic nucleus. The recent demonstration that IL-1 increases transcriptional activity of nuclear factor- κ B in suprachiasmatic nuclei astrocytes in culture (Leone et al., 2006) indicates a mechanism by which cytokines may alter the timing of behavior and/or physiologic processes. Nevertheless, the changes in body temperature of IL-6 KO mice during the first 12 h after ICV administration of 10 ng or 50 ng muIL-1 β in our present study are similar to those reported by Chai et al., (Chai et al., 1996) after ICV administration of 100 ng muIL-1 β , which in their study did not deviate statistically from controls and was not considered a fever.

Previous studies from our laboratory demonstrate that IL-6 KO mice respond to administration of LPS at dark onset with a hypothermic response (Morrow and Opp, 2005a). Bolus LPS administration is used as one model that mimics some facets of sepsis, yet administration of LPS does not constitute infection. Cecal ligation and puncture (CLP) is used as a model of sepsis because it results in polymicrobial infection that develops over a period of hours to days. Control mice become hypothermic during the early stages of sepsis induced by CLP, and subsequently develop fever. In response to CLP, IL-6 KO mice initially become hypothermic, but do not exhibit subsequent fever (Leon et al., 1998). Similarly, administration of turpentine results in fever in control animals, but not in IL-6 KO mice (Kozak et al., 1998). Therefore, changes in body temperature of IL-6 KO mice after immune challenge depend on the nature of that challenge: body temperature of IL-6 KO mice is not dramatically altered after administration of turpentine or in response to ICV administration of muIL-1 β , yet hypothermia develops after IP LPS or CLP and IL-6 KO mice fever after central administration of IL-6. Collectively, these data demonstrate that physiological regulation of body temperature is intact in IL-6 KO mice and that IL-6 plays an important role in fever, but not all, thermoregulatory responses to immune challenge.

Finally, although sleep and thermoregulation are tightly coupled [reviewed (Krueger and Takahashi, 1997)], our data contribute to a growing literature demonstrating that these processes may be uncoupled under some conditions. In response to ICV administration of 2.5 ng of muIL-1 β , NREM sleep of C57BL/6J mice increases without changes in body temperature. As the dose of muIL-1 β is increased, there are greater increases in NREM sleep and a strong febrile response ensues. NREM sleep of IL-6 KO mice increases in response to each of the doses of muIL-1 β tested in this study, although there are only modest changes in body temperature.

In summary, our results demonstrate that mice respond to ICV administration of muIL-1 β with increases in NREM sleep and reductions in REM sleep and wakefulness. These effects on sleep-wake behavior are generally similar to those previously reported to occur after IP administration of hu- or muIL-1 β into mice (Fang et al., 1998; Toth and Opp, 2001). Our results add to the literature of sleep-immune interactions by demonstrating dose-dependent effects of ICV administration of muIL-1 β on NREM sleep and body temperature of C57BL/6J mice. Furthermore, we demonstrate that IL-6 is not necessary for low doses of IL-1 to increase NREM sleep, although IL-6 contributes, in part, to increases in NREM sleep induced by higher doses of IL-1.

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Comprehensive List of Abbreviations

ANOVA, analyses of variance
 EEG, electroencephalogram
 FFT, fast Fourier transform
 IL-1/IL-1 β , interleukin-1
 IL-6, interleukin-6
 ICV, intracerebroventricular
 KO, knockout
 LPS, lipopolysaccharide
 NREM, non-rapid eye movement
 ng, nanogram
 REM, rapid eye movement
 PFS, pyrogen-free saline
 TNF α , tumor necrosis factor
 μ g, microgram

Figure 1.

Effects of murine recombinant interleukin-1 β (IL-1) on core body temperature (Tcore), wakefulness, non-rapid eye movement (NREM) sleep, and rapid eye movement (REM) sleep of C57BL/6J mice. Symbols are means $\pm$ SEM for 10-min (Tcore) or hourly (wakefulness, NREM, REM) values after intracerebroventricular administration of vehicle (pyrogen-free saline, open symbols, thin lines) or IL-1 (closed symbols, thick lines). Sample sizes are $n = 7$ for each dose of IL-1. The black bar on the x-axis denotes the dark period of the light:dark cycle. Asterisks and horizontal lines with asterisks identify time blocks during which values differed statistically between conditions ($p < 0.05$).

Figure 2.

Effects of murine recombinant interleukin-1 β (IL-1) on core body temperature (Tcore), wakefulness, non-rapid eye movement (NREM) sleep, and rapid eye movement (REM) sleep of IL-6-deficient (knock out) mice. Symbols are means $\pm$ SEM for 10-min (Tcore) or hourly (wakefulness, NREM, REM) values after intracerebroventricular administration of vehicle (pyrogen-free saline, open symbols, thin lines) or IL-1 (closed symbols, thick lines). Sample sizes are: 2.5 ng IL-1, $n = 7$; 5 ng IL-1, $n = 6$; 10 ng IL-1, $n = 6$; 50 ng IL-1, $n = 7$. The black bar on the x-axis denotes the dark period of the light:dark cycle. Asterisks and horizontal lines with asterisks identify time blocks during which values differed statistically between conditions ($p < 0.05$).

Figure 3.

Comparison between mouse strains of the effects of four doses of murine recombinant interleukin-1 (IL-1) on core body temperature (T_{core}), wakefulness (WAKE), non-rapid eye movement (NREM) sleep, and rapid eye movement (REM) sleep. Values are the mean $\pm$ SEM 12 h averages for the period immediately following intracerebroventricular (ICV) administration of IL-1, ie., during the dark period of the light:dark cycle. These values are expressed as differences from values obtained after ICV injection of vehicle (pyrogen-free saline), which is represented by the zero line. Statistically significant differences between mouse strains within the same dose of IL-1 are depicted by asterisks (*). Statistically significant differences among doses within the same mouse strain are depicted by the letters a, b, c, or d.

These letters refer to IL-1 doses as follows: a = 2.5 ng, b = 5 ng, c = 10 ng, d = 50 ng. open bars: C57BL/6J mice; filled bars: IL-6 KO mice. An alpha value of $p \leq 0.05$ was accepted as indicating statistical significance.

Table 1

Effects of intracerebroventricular administration of recombinant murine interleukin-1 β (muIL-1 β) on sleep-wake behavior, core body temperature, delta power during non-rapid eye movement sleep, and sleep consolidation of mice. muIL-1 β was administered just prior to dark onset and recordings continued for 24 h.

	NREM ² (Percent Recording Time)		REM ³ (Percent Recording Time)		WAKE ⁴ (Percent Recording Time)		Tcore ⁵ (° C)		NREM DELTA ⁶ (Arbitrary Units)		TRANSITIONS ⁷ (Number/Hour)	
	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light
C57BL/6J¹												
Vehicle (14)	31.0 $\pm$ 1.5	50.9 $\pm$ 1.3	3.0 $\pm$ 0.3	7.4 $\pm$ 0.3	66.0 $\pm$ 1.7	41.7 $\pm$ 1.5	37.2 $\pm$ 0.1	35.8 $\pm$ 0.1	1.6 $\pm$ 0.1	1.4 $\pm$ 0.1	22 $\pm$ 1	30 $\pm$ 1
2.5 ng (7)	39.7 $\pm$ 2.2 *	50.8 $\pm$ 1.8	3.1 $\pm$ 0.4	7.0 $\pm$ 0.5	57.3 $\pm$ 2.5 *	42.2 $\pm$ 2.1	37.2 $\pm$ 0.1	35.9 $\pm$ 0.1	1.2 $\pm$ 0.1	1.2 $\pm$ 0.1	29 $\pm$ 2 *	29 $\pm$ 1
5 ng (7)	36.8 $\pm$ 2.5	53.8 $\pm$ 2.3	3.2 $\pm$ 0.4	6.6 $\pm$ 0.5	60.0 $\pm$ 2.7	39.6 $\pm$ 2.6	37.2 $\pm$ 0.1	35.9 $\pm$ 0.1	1.7 $\pm$ 0.1	1.6 $\pm$ 0.1	27 $\pm$ 2	25 $\pm$ 2
10 ng (7)	43.3 $\pm$ 2.4 *	48.1 $\pm$ 1.6	3.2 $\pm$ 0.4	7.3 $\pm$ 0.5	53.5 $\pm$ 2.6	44.6 $\pm$ 1.9	37.6 $\pm$ 0.1 *	36.3 $\pm$ 0.0 *	1.4 $\pm$ 0.1 *	1.5 $\pm$ 0.1	41 $\pm$ 3 *	31 $\pm$ 1
50 ng (7)	57.6 $\pm$ 2.4 *	49.8 $\pm$ 1.7	1.8 $\pm$ 0.3	7.7 $\pm$ 0.5	40.6 $\pm$ 2.4 *	42.5 $\pm$ 2.1	37.9 $\pm$ 0.1 *	36.1 $\pm$ 0.1	0.9 $\pm$ 0.4 *	1.1 $\pm$ 0.1	54 $\pm$ 2 *	29 $\pm$ 10
IL-6 KO												
Vehicle (13)	28.7 $\pm$ 1.7	46.2 $\pm$ 1.8	2.8 $\pm$ 0.3	5.8 $\pm$ 0.3	68.4 $\pm$ 2.0	48.0 $\pm$ 2.0	37.1 $\pm$ 0.0	35.7 $\pm$ 0.1	1.0 $\pm$ 0.1	1.0 $\pm$ 0.0	20 $\pm$ 1	27 $\pm$ 2
2.5 ng (7)	40.0 $\pm$ 2.7 *	54.2 $\pm$ 1.9 *	2.3 $\pm$ 0.3	6.0 $\pm$ 0.4 *	57.7 $\pm$ 2.9 *	39.7 $\pm$ 2.1 *	37.1 $\pm$ 0.1	35.9 $\pm$ 0.1 *	1.0 $\pm$ 0.1	0.8 $\pm$ 0.1 *	25 $\pm$ 2 *	30 $\pm$ 1 *
5 ng (6)	39.8 $\pm$ 2.7 *	52.4 $\pm$ 1.7	2.6 $\pm$ 0.4	7.2 $\pm$ 0.5	57.6 $\pm$ 2.9 *	39.8 $\pm$ 1.7	37.2 $\pm$ 0.1	35.9 $\pm$ 0.1 *	0.9 $\pm$ 0.1	0.8 $\pm$ 0.1	30 $\pm$ 1 *	34 $\pm$ 2 *
10 ng (6)	44.9 $\pm$ 2.4 *	45.8 $\pm$ 2.3 *	3.2 $\pm$ 0.42	5.8 $\pm$ 0.6	51.9 $\pm$ 2.6 *	48.4 $\pm$ 2.6 *	37.1 $\pm$ 0.1	36.0 $\pm$ 0.1 *	0.9 $\pm$ 0.1	0.8 $\pm$ 0.1	36 $\pm$ 2 *	30 $\pm$ 2 *
50 ng (7)	53.0 $\pm$ 2.6 *	49.7 $\pm$ 4.6 *	3.0 $\pm$ 0.4	6.6 $\pm$ 0.4 *	44.0 $\pm$ 2.8 *	43.7 $\pm$ 1.9	37.1 $\pm$ 0.1	36.1 $\pm$ 0.1	0.9 $\pm$ 0.1	1.1 $\pm$ 0.1	47 $\pm$ 3 *	33 $\pm$ 12 *

Values are the mean $\pm$ SEM for 12 h periods of the light:dark cycle.

* Asterisks denote statistically significant departures from vehicle conditions ($p \leq 0.05$).

¹ Mouse strain and experimental conditions, with sample sizes in parentheses. Values are given for all animals after vehicle. However, statistical analyses were limited to within subjects and as such are based on sample sizes for muIL-1 β dose.

² non-rapid eye movement sleep;

³ rapid eye movement sleep;

⁴ wakefulness;

⁵ core body temperature;

⁶ delta power during NREM sleep;

⁷ number of transitions from one state of arousal to another.

Table 2

Published sleep parameters determined from C57BL/6 mice during baseline (undisturbed) conditions. Values obtained from this present study, in which mice were injected intracerebroventricularly with vehicle, are included to facilitate comparison.

Sleep Parameter and Reference	Light Period	Dark Period	Total 24-h
NREMS duration			
Franken P et al., (Franken et al., 1998)	49.3 ± 1.4	22.0 ± 3.4	35.6 ± 1.5
Huber R et al., (Huber et al., 2000)	54.4 ± 0.7	29.4 ± 1.6	41.9 ± 0.7
Veasey S et al., (Veasey et al., 2000)	56.9 ± 1.6	27.6 ± 1.8	42.3 ± 1.4
Toth L & M Opp, (Toth and Opp, 2001)	54.5 ± 1.4	32.0 ± 2.2	NR
* Tang X et al., (Tang and Sanford, 2002)	51.7	34.7	43.3
* Morrow J & M Opp, (Morrow and Opp, 2005b)	53.7 ± 1.4	32.4 ± 2.1	42.9 ± 2.8
* <i>This Study</i>	51.0 ± 1.3	31.0 ± 1.5	41.0 ± 1.1
REMS duration			
Franken	7.0 ± 0.3	2.7 ± 0.5	4.8 ± 0.3
Huber	9.7 ± 0.5	3.6 ± 0.4	6.6 ± 0.2
Veasey	5.4 ± 0.8	1.8 ± 0.3	3.6 ± 0.5
Toth	6.6 ± 2.1	2.5 ± 0.3	NR
Tang	5.5	3.4	4.4
Morrow	6.5 ± 0.4	3.2 ± 0.4	6.3 ± 0.5
<i>This Study</i>	7.4 ± 0.3	3.0 ± 0.3	5.0 ± 0.3
WAKE duration			
Franken	43.6 ± 1.4	75.3 ± 2.4	59.4 ± 1.8
Huber	35.9 ± 1.0	66.9 ± 1.9	51.4 ± 0.8
Veasey	37.6	70.1	53.8
Toth	38.8 ± 1.6	65.3 ± 2.4	NR
Tang	42.8	61.8	52.3
Morrow	40.3 ± 1.5	64.3 ± 2.3	52.5 ± 3.3
<i>This Study</i>	41.7 ± 1.5	66.0 ± 1.7	53.8 ± 1.3

Values are the mean ± SEM percent recording time, except for those from Tang et al., which were converted from minutes of recording time. C57BL/6 mice used in all studies except that of Huber et al. were purchased from the Jackson Laboratory, Bar Harbor, ME, ie., they are C57BL/6J mice.

* Studies that used telemetry to record the electroencephalogram. NR: not reported.