Diurnal Variation of Heart Rate, Locomotor Activity, and Body Temperature in Interleukin-1α/β Doubly Deficient Mice

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Abstract: This study was investigated the roles of interleukin-1 (IL-1) on diurnal rhythms of heart rate (HR), locomotor activity (LA), and body temperature (BT). For this purpose, HR, LA, and BT were recorded from conscious and unrestrained IL-1α/β doubly deficient (KO) and normal C57BL/6J mice using a telemetry system. These parameters were continuously recorded from just after to 2 weeks after transmitter implantation, because we thought that the surgical stress-induced IL-1 might affect the biobehavioral activities of the animals. At 1 day after implantation, HR and LA in IL-1α/β KO mice were higher than those in C57BL/6J mice. While BT in IL-1α/β KO mice was lower than that in C57BL/6J mice. Moreover, diurnal rhythmicity in these parameters after implantation in IL-1α/β KO mice appeared earlier than in C57BL/6J mice. At 2 weeks after implantation, there were no significant differences in the light- and dark-phase values of each parameter between IL-1α/β KO and C57BL/6J mice, however, IL-1α/β KO mice showed clear ultradian rhythmicity. It is thought that a phenotypical difference in biobehavioral activities between IL-1α/β KO and C57BL/6J mice may reflect IL-1 induced febrile and behavioral responses. These results suggest that IL-1 may play important physiological and pathophysiological roles on biobehavioral activities.

Key words: body temperature, circadian rhythm, heart rate, Interleukin-1, knockout mice, radiotelemetry, sleep

Introduction

Interleukin-1 (IL-1) is a proinflammatory cytokine with multiple cellular and systemic effects such as fever, sickness syndrome, activation of the hypothalamic-pituitary-adrenal axis, and induction of cytokines mediating the acute phase response [4]. IL-1 consists of two molecular species, IL-1α and IL-1β. Although IL-1α and IL-1β are agonists to the IL-1 type 1 receptor, biological activities induced by these molecules are not completely overlapping. To study the roles of IL-1 in febrile responses, IL-1β knockout [1]

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and gene deficient [9] mice were bred. However, the febrile responses to lipopolysaccharide (LPS) were different among these mice. We have recently bred IL-1α/β doubly deficient (KO) mice [7], because there is a possibility that IL-1α compensates for the IL-1β deficiency, and we thought that the IL-1α/β KO mice were essential for investigating the roles of IL-1 in febrile and neuro-immuno-endocrine responses.

Many investigators have made efforts to elucidate both the peripheral and central roles of IL-1. There is considerable evidence that IL-1 expression is upregulated during pathology [3, 5, 15, 20]. However, the physiological functions of IL-1 are limited, although IL-1 can be detected in a variety of physiological compartments such as plasma and milk [2, 14]. Recently, it is thought that central IL-1 is important in sleep regulation, because IL-1 mRNA expressions in hypothalamus, hippocampus, and cortex have diurnal variations [19], and hypothalamic IL-1 mRNA is affected by sleep deprivation [13]. If endogenous IL-1 is inhibited using antibodies, soluble receptors, or IL-1 receptor antagonist, spontaneous non-rapid eye movement sleep (NREMS) is inhibited [10, 11]. However, several physiologic processes such as the circadian rhythm, exercise, and infection affect both sleep regulation and thermoregulation. Therefore, it is important to obtain long-term recordings of these parameters to know the mechanisms underlying IL-1 mediated changes in biobehavioral activities. A telemetric monitoring system, that can simultaneously record heart rate (HR), locomotor activity (LA), and body temperature (BT) from conscious and unrestrained animals, has been developed, and this system can provide specific information concerning the diurnal variation of biobehavioral activities in mice.

We hypothesized that the biobehavioral activities including the sleep-awake cycle in IL-1α/β KO mice were different from those in strain controls. Therefore, the purpose of this study was to investigate the biobehavioral activities in IL-1α/β KO mice. For this purpose, HR, LA, and BT were continuously recorded from just after 2 weeks after implantation of a small telemetric transmitter in these mice to investigate the effect of the surgical stress-induced acute phase response and the steady state response of IL-1.

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**Materials and Methods**

**Animals**

Seven male adult IL-1α/β KO and C57BL/6J mice (3 to 5 months old; weight 23.0 to 30.0 g) were studied. IL-1α/β KO mice were bred at the Laboratory Animal Research Center, Institute of Medical Science, The University of Tokyo, and C57BL/6J mice are a strain that was used in the breeding of IL-1α/β KO mice. Mice were kept under specific pathogen-free conditions in an environmentally controlled clean room. All equipment and supplies, including cages, water bottles, wooden chips, and food pellets, were sterilized. The experiments were carried out according to institutional ethical guidelines for animal experiments, which meet the generally accepted international criteria for human treatment, sparing animals needless pain and suffering, and the experiments conducted were of scientific benefit to mankind.

**Implanting the transmitter**

A telemetric transmitter for electrocardiogram (ECG) and BT (TA10ETA-P20, Data Sciences, St. Paul, MN, USA) was implanted under pentobarbital sodium anesthesia (40 mg/kg, i.p.), as described previously [8]. Although the weight of this transmitter was 3.9 g, the transmitter did not affect locomotor activity in mice [8]. The paired wire electrodes in a precordial bipolar lead (Apex-Base lead) were placed at the cervical subcutaneous region over the trapezius and the skin was closed by suture. This transmitter also detected LA together with ECG and BT signals. Although animals are usually given postoperative analgesia and antibiotic medication, any medication was not carried out in this study to investigate the effect of the surgical stress-induced acute phase response of IL-1.

**Data recording**

The mice housed in individual cage were placed on a signal receiving board (CTR-86, Data Sciences, St. Paul, MN, USA) in a light-proof chamber (Sanyo, MIR-252, Tokyo, Japan). In the chamber, a light-dark cycle (LD 12:12; light-on at 8:00) was maintained under constant temperature (24 ± 1 C), and food and water were supplied ad libitum. The signals of HR, BT, and LA were continuously recorded every 5 min by a Data Quest analyzing system (Data Sciences, St Paul, MN, USA) for 2 weeks.
Data analysis

The hourly values for each mouse in each period were taken and then summarized for groups to get a mean ± SEM. Average waveforms were calculated for each mouse using smoothed data obtained by a 30 min moving average. Ultradian rhythmicity was evaluated by chi-squared periodogram with 95.0% confidence levels [17]. The Qp values and confidence values (i.e., oblique line) indicate significant periodicity.

Statistical analysis

All values are expressed as mean ± SEM. An analysis of variance (ANOVA) was used to compare the values in IL-1α/β KO and C57BL/6J mice. A paired Student’s t-test was used to compare the mean light and dark values. A value of P<0.05 was considered significant.

Results

Typical recordings and light- and dark-phase values of HR, BT and LA during 10 days after implantation of the transmitter in both mice are shown in Fig. 1 and 2. It took more than a few days and a week for a signifi-
Fig. 2. Changes in hourly averaged values for 10 days of heart rate, body temperature, and locomotor activity in C57BL/6J (left) and IL-1α/β KO mice (right). Values are mean ± SEM. * P<0.05 dark vs light.

cant diurnal rhythmicity to appear in HR, BT, and LA after implantation in IL-1α/β KO mice and C57BL/6J mice, respectively. The rhythmicity in IL-1α/β KO mice appeared earlier than in C57BL/6J mice, and light- and dark-phase differences in each parameter in IL-1α/β KO mice were larger than those in C57BL/6J mice.

As shown in Figs. 3 and 4, diurnal rhythmicity observed at 2 weeks after implantation did not exist at 1 day after implantation in any parameter in C57BL/6J mice. But the diurnal pattern of each parameter in IL-1α/β KO mice was almost the same between 1 day and 2 weeks after implantation. In both mice, the diurnal pattern of average waveforms for HR and BT resembled that for LA (Fig. 4). There were clear bimodal peaks early and late in the dark-phase in C57BL/6J mice, however, there were three peaks in the dark-phase in IL-1α/β KO mice. Furthermore, chi-squared periodogram analysis clearly showed consistency with ultradian rhythmicity of LA in IL-1α/β KO mice (Fig. 5).

Light- and dark-phase values of HR, BT, and LA in both mice are summarized in Table 1. At 1 day after implantation, HR and LA in IL-1α/β KO mice tended to be higher than those in C57BL/6J mice. Especially, the dark-phase value of HR in IL-1α/β KO mice was significantly higher than that in C57BL/6J mice, while
light-phase and overall values of BT in IL-1α/β KO mice were significantly lower than those in C57BL/6J mice. At 2 weeks after implantation, there was no significant difference in the light- and dark-phase values of each parameter between IL-1α/β KO and C57BL/6J mice.

Discussion

This study has demonstrated the rhythmicity of HR, BT, and LA in conscious and unrestrained IL-1α/β KO and C57BL/6J mice. The recovery process from transmitter implantation was totally different between IL-1α/β KO and C57BL/6J mice. At 1 day after implantation, HR and LA in IL-1α/β KO mice were higher than those in C57BL/6J mice. While BT in IL-1α/β KO mice was lower than that in C57BL/6J mice. Although there was no significant difference in the light- and dark-phase values of each parameter in IL-1α/β KO and C57BL/6J mice at 2 weeks after implantation, diurnal rhythmicity of HR, BT, and LA were different between IL-1α/β KO and C57BL/6J mice. These re-
C57BL  IL-1 α / β KO

![Graphs showing heart rate, body temperature, and locomotor activity over time for C57BL and IL-1 α / β KO mice.](image)

Fig. 4. Group mean waveforms of heart rate, body temperature, and locomotor activity at 2 weeks after implantation in C57BL/6J (left) and IL-1α/β KO mice (right). Dark phase is indicated by shaded bar along the abscissa. Data show group mean ± SEM.

Results suggest that IL-1 may play important physiological and pathophysiological roles on biobehavioral activities.

Although a telemetry system is useful for a long-term recording of biobehavioral parameters from conscious and unrestrained animals, it is usually necessary to wait for animals to recover from the surgical stress of transmitter implantation. Earlier studies showed that it took 3 days to recover food and water intake patterns from surgical stress [12]. Moreover, anorexia is a well-known phenomenon in which IL-1 plays a central role [16, 18]. We thought that surgical stress-induced IL-1 might affect the biobehavioral activities in animals. Therefore, HR, BT, and LA were continuously recorded from just after to 2 weeks after implantation of a small telemetric transmitter in IL-1α/β KO and C57BL/6J mice to investigate effects of IL-1 on their biobehavioral parameters.

One of the major findings of the present study is a phenotypical difference in febrile and locomotive responses to surgical stress between IL-1α/β KO and C57BL/6J mice. BT in IL-1α/β KO mice was lower.
Fig. 5. Representative chi-squared periodogram of locomotor activity at 2 weeks after implan- 
tation in C57BL/6J (left) and IL-1α/β KO mice (right). Oblique solid lines indicate a significant 
level (P<0.05). Qp values above the line indicate statistically significant periodicity (P<0.05). 
Ultrasound rhythmicity is observed only in IL-1α/ 
β KO mice.

Table 1. Light- and dark-phase values of heart rate, body tem-
perature, and locomotor activity in C57BL/6J and IL-
1α/β KO mice

<table>
<thead>
<tr>
<th></th>
<th>C57BL/6J</th>
<th>IL-1α/β KO</th>
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<tbody>
<tr>
<td>HR (bpm)</td>
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<tr>
<td>1 day</td>
<td>Overall</td>
<td>528 ± 12</td>
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<tr>
<td></td>
<td>Light</td>
<td>572 ± 10</td>
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<tr>
<td></td>
<td>Dark</td>
<td>594 ± 16</td>
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<tr>
<td>2 weeks</td>
<td>Overall</td>
<td>614 ± 17</td>
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<tr>
<td></td>
<td>Light</td>
<td>593 ± 19</td>
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<tr>
<td></td>
<td>Dark</td>
<td>634 ± 16*</td>
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<tr>
<td>BT (°C)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 day</td>
<td>Overall</td>
<td>35.0 ± 0.2</td>
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<tr>
<td></td>
<td>Light</td>
<td>34.8 ± 0.2</td>
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<tr>
<td></td>
<td>Dark</td>
<td>35.1 ± 0.3</td>
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<tr>
<td>2 weeks</td>
<td>Overall</td>
<td>35.1 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>Light</td>
<td>34.9 ± 0.3</td>
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<tr>
<td></td>
<td>Dark</td>
<td>35.4 ± 0.1*</td>
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<tr>
<td>LA</td>
<td></td>
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<tr>
<td>1 day</td>
<td>Overall</td>
<td>9.1 ± 1.3</td>
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<td></td>
<td>Light</td>
<td>7.5 ± 0.9</td>
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<tr>
<td></td>
<td>Dark</td>
<td>10.8 ± 2.0</td>
</tr>
<tr>
<td>2 weeks</td>
<td>Overall</td>
<td>19.2 ± 4.5</td>
</tr>
<tr>
<td></td>
<td>Light</td>
<td>12.9 ± 3.0</td>
</tr>
<tr>
<td></td>
<td>Dark</td>
<td>25.5 ± 6.5*</td>
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Values are mean ± SEM. *P<0.05 dark vs light; †P<0.05 C57BL vs IL-1 KO.

than in C57BL/6J mice. Moreover, diurnal variation of 
BT disappeared in C57BL/6J mice. These differences 
may reflect altered IL-1 production between IL-1α/β 
KO and C57BL/6J mice caused by the surgical stress.
Because IL-1 mediates febrile responses and affects 
behavioral activities, an elevated BT and a suppressed 
LA may be only observed in C57BL/6J mice. How-
ever, Alheim et al. [1] reported that IL-1β KO mice 
were hyperresponsive to LPS-induced fever develop-
ment. In contrast, Kozak et al. [9] reported that febrile 
response to LPS was reduced in the IL-1β gene defi-
cient mice. Although there is no clear explanation for 
this discrepancy, the mechanisms of thermoregulation 
to exogenous pyrogen may be altered in these KO mice.
Therefore, there is a possibility that IL-1α/β KO mice 
also have an altered metabolic response due to the level 
of heat generation. However, this is an unlikely possi-
bility because acute phase febrile responses to 
intrapertoneal injection of LPS or IL-1β in IL-1α/β 
KO mice were almost the same as those in C57BL/6J 
mice (unpublished observation).

Although there was no significant difference in the 
light- and dark-phase values of each parameter in IL-
1α/β KO and C57BL/6J mice at 2 weeks after implan-
tation, diurnal rhythmicity of HR, BT, and LA 
were different between IL-1α/β KO and C57BL/6J 
mice. There were clear bimodal peaks early and late in 
the dark-phase in C57BL/6J mice. This daily rhythmic-
ity was consistent with an earlier report on daily 
rhythms of mean arterial blood pressure and HR in 
C57BL/6J mice [12]. However, IL-1α/β KO mice 
showed ultrasound rhythmicity. It was reported that IL-
1 increased NREMS [10, 11]. Moreover, IL-1 type I 
receptor KO mice had slightly, but significantly, less
sleep during the dark-phase than their strain control mice [6]. Currently, we do not have any direct information for sleep, such as EEG or EMG recordings from IL-1α/β KO mice. It seems that IL-1α/β KO mice might have less NREMS and sleep shallowly the same as IL-1 type I receptor KO mice. If this is the case, then the sleep-awake cycle in IL-1α/β KO mice might be shorter than in C57BL/6J mice. In addition, LA in IL-1α/β KO mice expressed ultradian rhythmicity, therefore, our findings in IL-1α/β KO mice support the idea that endogenous IL-1 might be involved in physiological sleep regulation.

In conclusion, this study has demonstrated the rhythmicity of HR, BT, and LA in conscious and unrestrained IL-1α/β KO and C57BL/6J mice. The recovery process from surgical stress was totally different between IL-1α/β KO and C57BL/6J mice. Diurnal rhythmicity of HR, BT, and LA were different between IL-1α/β KO and C57BL/6J mice. Moreover, IL-1α/β KO mice showed ultradian rhythmicity. These results suggest that IL-1 may play important physiological and pathophysiological roles in biobehavioral activities.

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References