Sleep changes induced by lipopolysaccharide in the rat are influenced by age

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Sleep changes induced by lipopolysaccharide in the rat are influenced by age. Am J Physiol Regulatory Integrative Comp Physiol 280: R398–R403, 2001.—In mammals, aging is associated with immune senescence. To examine whether the sleep changes occurring during immune challenge are affected by age, we assessed sleep alterations induced by the administration of lipopolysaccharide (LPS) in young and middle-aged rats. During vehicle, the middle-aged rats exhibited less pre-rapid eye movement sleep (pre-REMS) as well as REMS, due to a smaller number and shorter duration of REMS episodes, than young rats. LPS elevated body temperature, increased non-REMS, and suppressed both pre-REMS and REMS in the young as well as in the middle-aged rats. However, in the young animals, LPS significantly enhanced slow-wave activity in the electroencephalogram (EEG) within non-REMS, reflecting an increase in sleep intensity. In contrast, LPS attenuated EEG power in most frequency bands in the older animals. This finding indicates age-related changes in the modulation of sleep by LPS.

EEG spectral analysis; aging; sickness behavior; and consistent changes in sleep-wake behavior, consisting of a promotion of non-rapid eye movement sleep (non-REMS), an enhancement of slow-wave activity (SWA) in the electroencephalogram (EEG) within non-REMS, and an inhibition of REMS. Animal studies indicate that these effects are mediated by cytokines, particularly IL-1 and TNF-α (reviewed in Ref. 16). IL-1β and TNF-α also evoke fever, increase non-REMS, enhance SWA, and decrease REMS, whereas antagonists of their activity block these effects (4, 17, 23, 28, 36). It is generally assumed that the promotion of non-REMS and enhancement of SWA in the EEG within non-REMS support host defense (reviewed in Ref. 16), e.g., rabbits reacting with a robust increase in non-REMS time and SWA within non-REMS during immune challenge have a higher chance of survival than those responding with a decrease in both sleep parameters (40). Additionally, prolonged sleep deprivation in rats has been shown to prominently attenuate host defense against indigenous and pathogenic microorganisms, resulting in increased mortality (3). The objective of the present experiment is to investigate whether the sleep response to LPS in the rat is affected by age.

MATERIALS AND METHODS

Seven young male Wistar rats (Charles River Laboratories, Sulzfeld, Germany), ~3 mo old and weighing 250–350 g, and seven middle-aged male Wistar rats, ~14 mo old and weighing 750–900 g, were prepared for EEG and electromyogram (EMG) recordings. Under deep halothane (Hoechst, Frankfurt am Main, Germany) anesthesia, they were implanted with four stainless steel screws to record EEG (frontal cortex: 3.9 mm anterior and ±2 mm lateral to bregma; occipital cortex: 6.4 mm posterior and ±4 mm lateral to bregma) and with two stainless steel screws to record neck-muscle EMG. The EEG signal was derived from a frontal electrode and the contralateral occipital electrode. Animals were housed individually in a sound-attenuated Faraday room under a 12:12-h light-dark schedule (lights on at 7:30 AM; 50–120 lx) at an ambient temperature of 20 ± 1°C with food and water available ad libitum. After 2 wk of recovery, the rats were connected to recording cables for adaptation. During the following 5 days, body temperature (Tb) was measured every 30 min; Tb was recorded during sleep measurements to confirm that the animal was asleep. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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measured 6, 9, and 24 h after light onset every day. On day 6, the animals were injected intraperitoneally with vehicle (pyrogen-free saline), and on day 7 they were injected with 100 μg/kg LPS (Salmonella abortus equi, Sigma, Deisenhofen, Germany) immediately after light onset. Tc was measured 6, 9, and 24 h after light onset, and EEG and EMG were continuously recorded during the first 12-h postinjection. EEG and EMG signals were amplified and filtered (EEG: high pass 0.3 Hz and low pass 29 Hz, 48 dB/octave; EMG: high pass 16 Hz and low pass 3,000 Hz, 6 dB/octave). Both the EEG and the rectified and integrated EMG were digitized with a sampling rate of 64 Hz. The EEG signal was subjected to an online fast Fourier transform routine, and for each 2-s epoch, an EEG power spectrum was computed for the frequencies between 0.5 and 25 Hz (0.5-Hz bins in the 0.5- to 4.5-Hz frequency range and 1-Hz bins for the higher frequencies). Power spectra were averaged over 10-s epochs. Recordings were analyzed offline by visual scoring of the raw EEG and the rectified and integrated EMG displayed on-screen in 10-s epochs, distinguishing wakefulness, non-REMS, REMS, and pre-REMS (for scoring criteria, see Ref. 21). Pre-REMS, also called the intermediate stage (7), precedes REMS and is characterized by high-amplitude spindles on a background of theta (6–9 Hz) activity. For each recording period, the latency to non-REMS and REMS (arbitrarily defined as the 20th epoch of non-REMS and the 3rd epoch of REMS) and the number and average duration of the non-REMS (including pre-REMS) and REMS episodes were computed. Time in each vigilance state and average EEG power densities within non-REMS (excluding pre-REMS) were determined per 2-h interval. For standardization, the EEG values were expressed as percentage of the average power density within non-REMS in the same frequency band during the corresponding 12-h vehicle period and were then log transformed. To analyze the effects of LPS within each and between the two age groups, a two- or three-factor repeated-measures ANOVA (Greenhouse Geisser correction) was performed with age (young vs. middle-aged rats) as between-subjects factor and treatment (vehicle vs. LPS) and time (6 × 2-h intervals for sleep variables and 3 time points for Tc) as within-subjects factor. Where appropriate, the ANOVAs were followed by two-sided, paired or unpaired t-tests.

RESULTS

Tc. Analysis of Tc yielded a significant effect of treatment (F1,12 = 20.1, P = 0.0007). Irrespective of age, LPS increased Tc. Pairwise comparisons over both age groups revealed significant increases at 9 and 24 h after LPS administration (Fig. 1).

Vigilance states. For wakefulness and non-REMS, ANOVA found a significant treatment effect (see Fig. 1 legend for results of ANOVA). Independent of age, LPS increased both wakefulness and non-REMS. Pairwise comparisons showed that during the first 2-h interval, wakefulness was significantly increased and non-REMS decreased (Fig. 1). The increase in total non-REMS was mainly due to changes during the second half of the recording period. Pre-REMS was significantly affected by age and by treatment. The middle-aged rats exhibited less pre-REMS than the young rats during both the vehicle and the LPS condition. Yet, LPS powerfully suppressed this state throughout the recording period to a similar extent in young and middle-aged rats. For REMS, a significant effect of age was found. Compared with the young rats, the middle-aged rats had less REMS during both conditions. Furthermore, a significant effect of treatment and of age × treatment × time emerged, reflecting that LPS-evoked changes evolved differently between the groups. In the young rats, LPS significantly decreased REMS during the first four 2-h intervals, whereas in the middle-aged...
rats, LPS almost completely abolished REMS throughout the recording period.

Sleep architecture. ANOVA did not yield significant effects of LPS on the latency to non-REMS nor on the duration of non-REMS episodes (Table 1), but it revealed a significant treatment effect ($F_{1,12} = 7.1, P = 0.02$) for the number of non-REMS episodes. LPS increased the number of non-REMS episodes to a similar degree in the young and middle-aged rats. Although the latency to REMS was not affected by age or treatment, significant age and treatment effects were observed for the number of REMS episodes ($F_{1,12} = 17.7, P < 0.001$ and $F_{1,12} = 37.6, P < 0.0001$). Irrespective of treatment, middle-aged rats had less REMS episodes than young rats. LPS reduced the number of REMS episodes in both age groups. Analysis of the duration of REMS episodes revealed a significant effect of age ($F_{1,12} = 15.3, P = 0.002$), which was due to the fact that the middle-aged rats exhibited shorter REMS episodes than the young rats during both vehicle and LPS.

EEG power densities within non-REMS. ANOVA run on the normalized and log-transformed EEG power densities within non-REMS yielded a significant effect of age $\times$ treatment for all frequencies $\geq$8 Hz. During the 12-h recording period, LPS significantly enhanced low-frequency activity in the young rats, whereas it tended to attenuate the same activity in the middle-aged rats (Fig. 2). ANOVA revealed a significant effect of treatment for the frequencies between 8 and 15 Hz, which was due to the fact that LPS depressed EEG activity in the respective frequency bands in both groups of rats. For the same frequency bands, a significant age $\times$ treatment $\times$ time effect was found, reflecting age-related differences in the time course of the LPS-evoked alterations. In the young rats, a dramatic attenuation of power in the frequencies between 8 and 15 Hz occurred mainly during the last 2-h interval, whereas it was evident throughout the entire recording period in the middle-aged rats (Fig. 2). A significant age $\times$ treatment $\times$ time effect also emerged for most frequencies $\geq$19 Hz. LPS slightly augmented high-frequency EEG activity during the third and fourth 2-h interval in the young rats and markedly depressed it during the last 2-h interval in both the young and middle-aged rats.

**DISCUSSION**

The present study demonstrates for the first time that specific aspects of the acute phase sleep response change as a function of age in the rat. In the young rats, LPS induced fever, increased the total amount of non-REMS that was related to an increase in the number of non-REMS episodes, enhanced SWA in the EEG within non-REMS, persistently inhibited pre-REMS, and transiently decreased REMS. In middle-aged rats, LPS elicited comparable alterations in Tc and, except for a longer-lasting suppression of REMS, in sleep architecture, but generally it attenuated power densities in the non-REMS-specific EEG signals, including the delta frequency bands.

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Table 1. Sleep architecture

<table>
<thead>
<tr>
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<th>Young Rats</th>
<th>Middle-aged Rats</th>
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<tr>
<td></td>
<td>Veh</td>
<td>LPS</td>
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<tr>
<td>non-REMS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Latency, min</td>
<td>13.9 ± 8.02</td>
<td>23.8 ± 1.70</td>
</tr>
<tr>
<td>Frequency, min</td>
<td>142 ± 19.2</td>
<td>160 ± 17.3</td>
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<tr>
<td>Duration, min</td>
<td>2.66 ± 0.39</td>
<td>2.50 ± 0.37</td>
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<tr>
<td>REMS</td>
<td></td>
<td></td>
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<tr>
<td>Latency, min</td>
<td>29.6 ± 8.90</td>
<td>92.0 ± 155</td>
</tr>
<tr>
<td>Frequency, min</td>
<td>71.1 ± 21.0</td>
<td>26.0 ± 18.9</td>
</tr>
<tr>
<td>Duration, min</td>
<td>1.28 ± 0.25</td>
<td>1.36 ± 0.41</td>
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</tbody>
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Data are means ± SD ($n = 7$). Values in bold type indicate significant differences between vehicle (Veh) and lipopolysaccharide (LPS) over both age groups ($P < 0.05$; 2-sided, paired t-test). *Significant age differences in the Veh or LPS condition ($P < 0.05$; 2-sided, unpaired t-test). REMS, rapid eye movement sleep.

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Fig. 2. Changes in electroencephalogram (EEG) power density within non-REMS during 2-h intervals after administration of LPS in young (c) and middle-aged (m) rats. Curves connect means ± SE ($n = 7$). For plotting purposes, the data are expressed as a percentage of the corresponding vehicle values. Bars below the graph refer to frequency bands for which post hoc analysis yielded significant differences between vehicle and LPS in each age group ($P < 0.05$, 2-sided, paired t-test).
In accordance with most earlier reports (9, 10, 34, 38), middle-aged rats displayed practically identical amounts of wakefulness and non-REMS during the vehicle light period as young rats, but they spent less time in REMS. The latter was due to fewer as well as shorter-lasting REMS episodes, which implicate an age-related disruption of REMS initiation and maintenance. Furthermore, the present study shows a concomitant reduction in pre-REMS. As this state constitutes the transition from non-REMS to REMS, this finding indicates that the age-related decrease in the number of REMS episodes may be a direct consequence of the decreased occurrence of pre-REMS.

The administration of microbial constituents that evoke an acute phase response, such as LPS, is a common strategy to examine the influence of an immune challenge on central nervous system function. The effects of LPS on sleep in the young rats are in accordance with previous findings in rodents (13, 15, 17, 29). LPS initially promoted wakefulness. This was followed by an increase in non-REMS, which was typically associated with many but relatively short-lasting non-REMS episodes, and an enhancement of SWA in the EEG within this state. Furthermore, LPS decreased REMS during the first 8 h, which was mainly caused by a reduction in the number of REMS episodes. Moreover, the present study reveals that LPS also robustly decreases pre-REMS. This observation implies that the decreased occurrence of REMS episodes during immune challenge may be partially due to the fragmentation of non-REMS, resulting in fewer entries in pre-REMS and, consequently, in REMS.

In agreement with an earlier investigation (5), LPS elicited similar increases in Tc in young and middle-aged rats. Furthermore, most LPS-induced changes in sleep architecture in the middle-aged and young rats were indistinguishable. As in the young animals, LPS increased non-REMS initiation and decreased both pre-REMS and REMS, the latter due to a reduction in the number of REMS episodes. However, in contrast to the young rats, REMS in the older rats was decreased over the entire recording period. The alterations in the EEG power density within non-REMS differed prominently between the age groups in that LPS did not evoke a decrease of non-REMS and SWA within non-REMS, a disruption of sleep continuity, and a reduction of REMS (reviewed in Refs. 6, 14, 37). Thus age-related alterations in the HPA axis may be, at least partially, responsible for the differences in the amount of pre-REMS and REMS during vehicle, the differences in the duration of REMS suppression, and the EEG responses to LPS between young and middle-aged rats.

Furthermore, changes in growth hormone-releasing hormone (GHRH) may be involved. The activity and efficacy of the GHRH system, including the hypothalamus and pituitary, decrease with advancing age (11, 20; reviewed in Refs. 16, 37). Exogenous GHRH is known to increase non-REMS and SWA within non-REMS and reduces REMS (14, 35, 41). Consequently, ACTH release from the pituitary during stress is higher in aged rats than in young rats (14). Intracerebroventricular injection of CRH has been shown to increase wakefulness at the expense of non-REMS and REMS in different rat strains and to reduce EEG power density in the delta range (2, 25). Accordingly, studies on humans have demonstrated that exogenous CRH and ACTH evoke a decrease of non-REMS and SWA within non-REMS, a disruption of sleep continuity, and a reduction of REMS (reviewed in Refs. 6, 14, 37). Thus age-related alterations in the HPA axis may be, at least partially, responsible for the differences in the amount of pre-REMS and REMS during vehicle, the differences in the duration of REMS suppression, and the EEG responses to LPS between young and middle-aged rats.

Perspectives

The present paper demonstrates that the changes in sleep architecture evoked by LPS are minimally affected by age, whereas the stimulation of the deep sleep-associated, slow-frequency components in the EEG within non-REMS declines as a function of age in
the rat. This observation indicates age-related changes in the sleep response to LPS and, possibly, also other immune challenges. Because the promotion of especially deep sleep is assumed to support host defense, the present findings may suggest an aging-associated reduction in the ability to recuperate from immune challenge during sleep. Further studies are needed to delineate whether this is the case and to determine the underlying neuroendocrine mechanisms.

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REFERENCES


