Impairment of the Sleep Response to Influenza Infection in Mice with a Defective GHRH-receptor.

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Introduction: Sleepiness is a common experience during infectious diseases. Viral infections induce many cytokines, including interleukin-1β (IL-1β), which has somnogenic activity. Previous results suggest that IL-1β stimulates growth hormone-releasing hormone (GHRH), a peptide also implicated in promotion of non-rapid eye movement (NREMS). Recently via quantitative trait loci analysis, the GHRH-receptor (GHRH-R) was identified as a candidate gene responsible for NREMS responses of BALB/c and C57BL/6 mice to influenza challenge(1). To test the hypothesis that the GHRH-R is important for the excess NREMS induced by influenza we used the dwarf C57BL/6 (lit/lit) mouse which has a point mutation in the GHRH-R gene resulting in the loss of receptor function (2).

Methods: Mice (control C57BL/6, n=11: dwarf lit/lit, n=10) were implanted with electrodes over the frontal and parietal cortices for EEG recordings and with EMG electrodes in the dorsal neck muscles. Animals were individually housed in Plexiglas cages on a 12:12-h light-dark cycle at an ambient temperature of 30 °C in sound-attenuated chambers. After 4-5 days of habituation, spontaneous sleep-wake activity was recorded for 48 h. Data from these two days were averaged and used as baseline values. Mice were then intranasally infected with A/PR/8/34 (H1N1) influenza virus (2.5×10^6 $TCID_{50}$ in 50μl) at light onset. The EEG and EMG signals were collected by computers and states of vigilance were visually determined in consecutive 10-s intervals. The percent time spent in wakefulness, NREMS and REMS were calculated for each recording hour. Changes in NREMS and REMS were compared by means of two-way ANOVA for each day between groups.

Results: Lit/lit mice had significantly less NREMS and REMS than controls on the baseline day. In control mice influenza virus induced a long-lasting increase in NREMS and suppression of REMS, while lit/lit mice showed a progressive decrease in NREMS (figure) with a slight suppression in REMS. The sleep responses were statistically different. The lit/lit mice exhibited pathological EEG slow wave and spike activity after infection, an abnormality, that occurred only prior to death in normal mice. By day 4, 7 of 10 lit/lit mice died, whereas the mortality of the controls was 4/11.

Conclusion: Results suggest that the GHRH-R is important for influenza-induced sleep responses and, perhaps, for survival.

Supported by NIH HD36520

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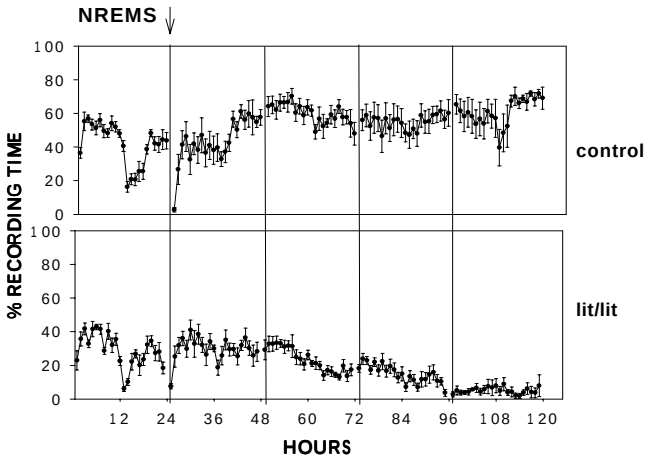

After PR8 challenge (arrow), NREMS in lit/lit mice decreases. Each data point represents percentage of NREM sleep, ± SEM.