Tumor necrosis factor ligand and receptor deficiency affects sleep in mice

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INTRODUCTION

Tumor necrosis factor (TNF) is a proinflammatory cytokine involved in host defense and pathogenesis of various diseases.1 There is extensive literature available suggesting that TNF is also involved in sleep. Thus, administration of TNF increases non-rapid eye movement (NREM) sleep.2,3 Moreover, inhibition of endogenous TNF inhibits spontaneous sleep4 and attenuates the increase in sleep after sleep deprivation (SD).5

Two cell surface receptors for TNF, TNFR1 and TNFR2 (55 kDa and 75 kDa in size, respectively), have been characterised.6 They mediate distinct actions, but cannot be differentiated by pharmacological means.7 Lymphotoxin (LT)-α binds to the same receptors as TNF, and administration of TNF and LT-α leads to similar responses.8,9 To enable functional dissection, TNF receptors deficient mice were generated. It was shown that TNFR1 knockout (R1KO) mice sleep less during the light period, indicating that TNF affects sleep via TNFR1.3

An alternative approach is removal of the ligands, TNF and LT-α.8,9 Our aim was to investigate the role of TNF receptors in sleep and sleep regulation in these mice (Ligand KO). On the basis of the results with R1KO mice we expected that the Ligand KO mice would sleep less as well. Sleep and sleep regulation of the Ligand KO was compared with that of wild-type control mice (WT) and R1KO and TNFR2 knockout (R2KO) mice. The R1KO is not the same construct as the one used by Fang et al.7

MATERIALS AND METHODS

Animals

Adult mice (16 weeks) of the four genotypes were used (WT n=12; Ligand KO n=9; R1KO n=8; R2KO n=8). The KO mice were generated on 129/SV background and backcrossed for 6 (Ligand KO) or 10 generations (R1KO and R2KO) with C57Bl/6. C57Bl/6 mice were used as WT controls. The animals were maintained on a 12:12 h light:dark schedule, individually kept in sound-attenuated chambers.
Food and water were available ad libitum. The mice were implanted with EEG and EMG electrodes as previously described. At least three weeks were allowed for recovery.

**Experimental protocol and analysis**

A 24-h baseline recording starting at lights on preceded the 6-h SD. Recordings continued during the SD and the following 18 h. SD was performed by gentle handling. The mice were never disturbed during feeding and drinking. The EEG and EMG signals were amplified and filtered as previously described. EEG power spectra were computed for consecutive 4-s epochs by a FFT routine. EMG signals were integrated over 4-s. Vigilance states (waking, NREM sleep and REM sleep) were determined as described previously. Epochs containing EEG artifacts were marked and excluded from the spectral analysis. Duration and frequency of vigilance state episodes were determined according to criteria previously described.

Overall effects were analyzed by two-way ANOVA, with factors ‘time’ and ‘condition’. Differences between the four genotypes were analyzed by two-way ANOVA with factors ‘genotype’ and ‘time’. Contrasts were tested by post-hoc two-tailed t-tests.

**RESULTS**

**Vigilance states**

Ligand KO and R2KO had 15% less REM sleep during the baseline light period compared to WT (Table 1). There were no differences in the amount of sleep between R1KO and WT. The decreased REM sleep was caused by a reduction in REM sleep episode frequency, which was not compensated by an increase in REM sleep episode duration (Table 1). Also NREM sleep episode frequency was reduced in Ligand KO and R2KO, but this was compensated by an increase in duration (data not shown). During the dark period REM sleep episode frequency was reduced in all KO mice, which was compensated by an increase in episode duration reaching similar durations as in the light period (Table 1). SD elicited similar responses in the vigilance states in all genotypes.

**EEG power spectra**

After SD all mice showed a similar increase in SWA (mean EEG power density 0.75-4.0 Hz) in NREM sleep (not shown). However, distinct differences were found within the SWA band. The increase in Ligand KO was mainly in the range of 2.75-4.0 Hz, while in WT it was most prominent between 0.75-2.5 Hz (Figure 1). During the recovery light period the 2.75-4.0 Hz range remained above BL in Ligand KO and R2KO and above WT in Ligand KO. In most intervals of the dark period power density in this frequency range was above WT levels in Ligand KO and R2KO (data not shown).

**DISCUSSION**

No difference was found in the amount of NREM sleep between the genotypes, contrasting previous data where a 20% reduction in NREM sleep was found in R1KO. Since this reduction was attributed to the deficiency of the TNF R1 receptor, we expected that the Ligand KO and our R1KO would sleep less as well. It is unlikely that in our mice compensation occurs via alternative mechanisms, since we obtained similar results in the three genotypes. The present results support the notion that the previous reduction in NREM sleep is caused by differences in genetic background between R1KO and WT.
EEG POWER DENSITY IN NREM SLEEP (W/Hz)

FIGURE 1. Time course of EEG power density in non-rapid eye movement (NREM) sleep in the light period after sleep deprivation in three genotypes. Curves connect means of relative power density for the first and second 3-h interval of recovery expressed relative to the first and second 3-h interval of the light period of baseline, respectively (>100%). Lines above the abscissa indicate frequencies, which differ significantly from baseline (p<0.05, two-tailed paired t-test after significant ANOVA factor ‘day’) or WT (p<0.05, two-tailed t-test, after significant ANOVA factor ‘genotype’).

The clear reduction in REM sleep in Ligand KO and R2KO contrasted the lack of difference in NREM sleep. This reduction was mainly due to a lower number of REM sleep episodes. Also in the dark period the KO mice showed a reduction in REM sleep episode frequency, but this was accompanied by an increase in episode duration. The absence of TNF or TNF receptors seems to reduce the probability of initiating REM sleep. The effects on REM sleep in the KO mice are similar to the effects of administration of a serotonin (5-HT) 1A receptor agonist in the pons, which reduced REM sleep episode frequency without changing duration. Since TNF is known to increase 5-HT uptake, removal of TNF or its receptor may increase 5-HT levels in several brain areas, including the medulla oblongata/pons. The present data suggest that TNF influences REM sleep by changing 5-HT levels through both TNF receptors.

Different frequencies within SWA were enhanced after SD between the genotypes. The slow portion of SWA, increased in WT, R1KO, and R2KO, corresponds to the frequency of slow cortical oscillations (<1 Hz) and thalamic clock-like oscillations (1-4 Hz), typical for sleep. The fast SWA band, increased in WT, R2KO and the Ligand KO, corresponds to intrinsic 3-4 Hz activity of cortical neurons. The latter is the only known slow-wave oscillation, which is induced by depolarization of the cell membrane. Our results suggest that the increase in SWA after SD in the Ligand KO is caused by an excitatory input to the cortex, which is enhanced above the normal input occurring during the dark period, when fast SWA is increased as well (data not shown).

In conclusion, the deficiency of one of two TNF receptors or the Ligand does not affect the amount of NREM sleep in mice. However, it reduces REM sleep in a similar way as after administration of 5-HT. The increase in SWA after SD is shifted in Ligand KO and R2KO in the direction of fast slow-waves. In vitro these fast slow-waves are induced by an excitatory input. The data suggest that, in Ligand KO and R2KO, SD causes an excitatory input above the level normally found during the dark period.

REFERENCES