Acute sleep deprivation affects diurnal rhythmicity in granulocytes
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1. Introduction

Background
• Sleep deprivation and chronic partial sleep loss are often accompanied by disruption or desynchronisation of the circadian clock and have been associated with the development of severe pathologies, such as obesity, diabetes and hypertension.1-3
• There is accumulating evidence that sleep itself has a supportive role for the functioning of the immune system, while sleep loss is a risk factor for impaired immune function.4-5
• Under a normal sleep/wake schedule, diurnal rhythms have been identified in levels of circulating blood cell populations, with highest lymphocyte levels observed at night-time.6
• The effect of sleep deprivation on observed rhythmicity in circulating blood cell populations is currently controversial.6-10

Aims
• To provide an in-depth characterisation of diurnal rhythms in circulating levels of different blood cell populations
• To investigate the impact of a night of acute sleep deprivation on any observed rhythmicity in a wide range of circulating blood cells.

2. Methods

Laboratory study
• 11 young (mean age ± SD: 23.0 ± 5.8 years) healthy males participated
• Study protocol: Fixed sleep-wake schedule at home, adaptation night in the laboratory followed by an experimental period of 48 h, which included a nocturnal 8 h sleep schedule episode followed by 29 h of continuous wakefulness (Figure 1).
• During the 48 h experimental period, 3-hourly blood samples were taken for flow cytometry measurements.

Analysis
• Levels of circulating blood cell populations were quantitatively assessed by flow cytometry using a gating strategy (Figure 2).
• All blood samples were assayed within 24 h from the end of the laboratory session.
• Single cosinor analysis and non-linear curve fitting using a cosine function (lsf function of the stats package of R version 2.12.0) were employed to determine significant cosinor rhythmicity, peak time and amplitude of individual rhythms.11
• The effect of condition on peak time and amplitude was assessed by ANOVA. Paired T-test for each cell type and individual was performed to compare cell numbers on night 2 (N2) versus N3.
• Pearson's R squared estimation of r-scores between all pairs of independent cell types was computed for all individuals (SPSS).

3. Results

One subject had to be excluded from analysis due to constantly rising leucocyte levels, indicating an acute inflammation.

Individual rhythms and effect of sleep deprivation
• The number of subjects with significant rhythms in a given cell type was highest for the collapsed data (sleep plus sleep deprivation condition).
• Weakest rhythms were found in monocytes and CD8 m/e cells, strongest rhythms were present in CD4 naive cells.
• Paired T-test with data from all subjects revealed statistically significant differences in granulocyte levels when comparing the sleep and the sleep deprivation night (p = 0.0073).

Overall rhythmicity in blood cell subsets
• Significant diurnal rhythms were detected for all blood cell subsets investigated, independent of the experimental condition (Figure 3).
• Non-linear curve fitting confirmed single cosinor results concerning peak time and robustness of rhythms in blood cell subsets.
• The strongest effect of condition was found for granulocytes, with the biggest loss in both rhythmicity and amplitude observed during sleep deprivation (Figure 4).

Correlation of (independent cell types)
• Cell types from the lymphocyte lineage tended to show large and statistically significant correlations between each other (Figure 5).

Granulocytes showed lowest correlations with any other given cell type.

4. Discussion

• Results obtained with single cosinor analysis and non-linear curve fitting were in good agreement with each other concerning peak time and strength of rhythmicity, confirming previous reports.1-12
• Developmentally closely related cell populations (lymphocyte, especially cell subsets and T cells), also showed a strong correlation in diurnal rhythmic behaviour.
• The diurnal rhythm in granulocyte levels was the most strongly affected by acute sleep deprivation, with loss of rhythmicity and an overall increase in circulating levels. Granulocyte levels directly mirror the body's immediate stress response, thereby confirming and extending recent studies describing elevated granulocyte levels after sleep restriction.12

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References

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