In a previous report about the effects of sleep deprivation under dim-light conditions (Van Dongen et al. 1998) it was found that the circadian phase (time of body temperature minimum) was gradually delayed during three days of partial or total sleep deprivation. Although the subjects remained in a light- and temperature-controlled laboratory environment throughout the experiment and underwent a rigorous schedule of neurobehavioral test bouts, they were not subjected to a constant routine. Thus, the temperature measurements may have been influenced by masking factors such as physical activity and postural changes. In order to investigate this possibility, we assessed circadian phase delays for plasma melatonin and cortisol in the same experiment.

METHODS

Twenty-two subjects (all male, mean age 29.3 y, age range 22-42 y) were confined to an isolated laboratory with light not brighter than 50 lx (range 25-45 lx). On three subsequent baseline days (BL1, BL2, BL3), the subjects were allowed to sleep from 22:45 until 07:45 hours (BL1) and from 23:30 until 07:30 hours (BL2 and BL3). During the next three days (SD1, SD2, SD3) in the laboratory, they were either partially sleep deprived (PSD, 10 subjects) or totally sleep deprived (TSD, 12 subjects). During this 88-hour period of sleep deprivation, neurobehavioral tests were taken every 2 hours. In the PSD condition, naps were allowed from 14:45 until 16:45 hours and from 02:45 until 04:45 hours only. In a double-blind procedure starting at 05:30 hours of SD1, the subjects took a pill of caffeine (0.3 mg/kg) or placebo each hour (except when napping). As of yet, the authors are still blinded to this condition.
before the last baseline sleep (BL3). Blood plasma was analyzed for melatonin and cortisol secretion by means of radio-immuno-assays. For both hormones, data were available on 11 subjects.

Data analysis was restricted to the sleep deprivation period. Based on the notion that a circadian phase shift is mathematically equivalent to a (temporary) change in the circadian period (tau), circadian phase shifts in body temperature, plasma cortisol and plasma melatonin were determined per subject by assessing the circadian periods for these variables over the 88 hours of sleep deprivation, using the Lomb method of periodogram analysis (Lomb 1976, Scargle 1982). Any differential effect of the PSD versus the TSD condition on the circadian periods was tested by means of independent samples t-tests.

RESULTS

For body temperature, plasma melatonin and plasma cortisol, there were no statistically significant differences among the calculated circadian periods of the subjects in the PSD condition versus the TSD condition (P=0.24, 0.39, 0.50, respectively). Thus the data could be pooled. Table 1 shows the overall mean circadian periods (tau values) and the corresponding standard errors of the mean (s.e.m.). The table also shows the total circadian phase shifts over the 88 hours of sleep deprivation, derived from the (mathematically equivalent) mean circadian periods. One-sample t-tests revealed that the circadian periods of body temperature and plasma melatonin were significantly longer than 24 hours (P=0.002 for body temperature, P=0.012 for plasma melatonin), but this was not the case for plasma cortisol (P=0.31).

Table 1: Mean circadian periods (tau, in hours), standard errors of the mean (s.e.m., in hours), total circadian phase shifts (shift, in hours) and numbers of subjects analyzed (n) for body temperature, plasma melatonin, and plasma cortisol. The total circadian phase shifts (over the 88 hours of sleep deprivation) were derived from the (mathematically equivalent) mean circadian periods; positive values correspond to delays. The circadian periods of body temperature and plasma melatonin were significantly longer than 24 hours.

<table>
<thead>
<tr>
<th></th>
<th>tau (h)</th>
<th>s.e.m. (h)</th>
<th>shift (h)</th>
<th>n</th>
</tr>
</thead>
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<tr>
<td>Temperature</td>
<td>25.03</td>
<td>0.27</td>
<td>3.78</td>
<td>12</td>
</tr>
<tr>
<td>Melatonin</td>
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<td>0.14</td>
<td>1.32</td>
<td>11</td>
</tr>
<tr>
<td>Cortisol</td>
<td>23.93</td>
<td>0.13</td>
<td>-0.26</td>
<td>11</td>
</tr>
</tbody>
</table>
DISCUSSION

In the 88 hours of sleep deprivation in dim light, different circadian periods were found for body temperature, plasma melatonin and plasma cortisol. Thus, different circadian phase delays occurred in these variables. In order to understand this, at least two sources of variance should be considered. Firstly, at present the authors are still blinded to the distribution of the subjects across caffeine versus placebo intake. Secondly, there may be a natural, inter-individual variability in the susceptibility to the experimental conditions. These two factors may have had differential effects on the three mean circadian periods, because the groups of subjects for which these circadian periods were assessed only partially overlapped.

Humans have been shown to free-run in constant dim light, even when aware of the time of day (middleton et al 1996). Yet, it is remarkable that circadian periods longer than 24 hours were found at all in this experiment, i.e., that stable circadian phase positions could not always be maintained. In Syrian hamsters it has been shown that induced activity can entrain the endogenous circadian pacemaker (Reebs & Mrosovsky 1989). However, the human subjects in the present experiment apparently could not entrain to the rigorous schedule of neurobehavioral test bouts imposed upon them. It should be emphasized that the circadian periods shown in table 1 describe real properties of the gathered data (rather than being mathematical artifacts), as was ensured by applying the Lomb method of periodogram analysis (Van Dongen et al. 1997).

It is noteworthy that no significant differences were found between the circadian periods observed for subjects experiencing total sleep deprivation versus those allowed to nap. Given the constant dim light (less than 50 lx) in the laboratory, it is unlikely that differences in light exposure between the two groups due to the naps are a relevant factor. Thus, the results may suggest that the two 2-hour nap opportunities at 12-hour intervals did not entrain the subjects to a 24-hour day, unlike the single 4-hour anchor sleep opportunity at night in a study of Minors & Waterhouse (1983).

Although masking factors such as physical activity and postural changes may have influenced the apparent circadian rhythms of body temperature and plasma cortisol, no such effects have been reported in the literature for plasma melatonin. The mean circadian period of 24.36 h found for melatonin therefore seems to reflect a genuine phase delay in the endogenous circadian pacemaker across the 88 hours of sleep deprivation. The hourly caffeine intake may be involved in this finding, but caffeine has been reported to induce a circadian phase advance in rats (Redman & Rajaratnam 1998). Whether or not the present results therefore indicate that subjects were free-running remains to be established.
REFERENCES