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Research report

# Circadian modulation of sequence learning under high and low sleep pressure conditions

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**Abstract**

Humans are able to learn complex sequences even without conscious awareness. We have studied the repercussions of circadian phase and sleep pressure on the ability to learn structured sequences using a serial reaction time task (SRT). Sixteen young healthy volunteers were studied in a 40-h "constant posture protocol" under high sleep pressure (i.e. sleep deprivation) and low sleep pressure conditions (i.e. sleep satiation attained by multiple naps). Here we show that learning of different sequence structures improved after multiple naps, in particular after naps that followed the circadian peak of rapid-eye-movement (REM) sleep. This situation following sleep contrasted with the lack of learning without sleep. We have evidenced that the observed amelioration of learning new sequences came about by memorizing short sub-fragments ("chunks") of the sequence train. However, SRT performance did not deteriorate under high sleep pressure, despite the high level of sleepiness. Our data indicate that sequence learning is modulated by circadian phase, and the neurophysiological medium required for this type of learning is related to sleep.


*Keywords:* Sequence structure; Skill acquisition; Serial reaction time; Implicit learning; Melatonin; Rapid-eye-movement sleep; Non-rapid-eye-movement sleep; Fragment-based learning

## 1. Introduction

One of the proposed functions of sleep for which there is a considerable amount of evidence is memory consolidation [1]. According to this view, sleep is a period of reduced sensory input during which the brain rehearses or replays events or newly learned procedural tasks. Recent reports show that sleep plays a role in visual discrimination task performance [2,3], and that sleep deprivation hours after training can interfere with memory consolidation [4]. Despite the growing evidence of data supporting sleep's role in memory consolidation the connection between, in particular rapid-eye-movement sleep (REM) sleep and learning, are still debated (for reviews see [5–7]). On the other hand, there is general consensus that sleep is important to cognitive function. Performance in explicit learning tasks such as list learning and probed recall memory tests show a clear deterioration in subjects deprived from sleeping [8,9]. Whether such performance decrements are related to learning and whether they can be separated from learning effects is still an unresolved issue.

In this study, we aimed at distinguishing between performance and learning effects by using a sequence learning task. Performance on this task is simply measured by reaction times, i.e. how long it takes for a subject to react to each element of a sequentially structured visual sequence of events in the context of a serial reaction time (SRT) task [10]. Unbeknownst to the subjects, the sequence of successive stimuli follow a repetitive pattern (i.e. a fixed structured sequence) for some blocks of trials. The difference in reaction time speed between the sequenced trials with a repetitive pattern and the trials with a pseudo-random pattern was considered as the learning effect on this task (i.e. acquiring specific procedural knowledge). Therefore, whenever a subject takes this SRT task, his or her performance level and the learning effect can be quantitatively assessed.

It is not known whether these two aspects (i.e. performance and learning) of the SRT task depend on changes in sleep pressure and the phase of the endogenous circadian pacemaker. To answer this question, we have used a

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40-h protocol where circadian phase and sleep pressure were changed simultaneously. Differential sleep pressure conditions were achieved by depriving the subjects of sleep during a 40-h protocol (high sleep pressure condition) or by interspersing naps throughout the 40-h protocol sleep pressure (low sleep pressure condition).

We hypothesized that performance on the SRT task deteriorates under the high sleep pressure condition, with no discrimination between structured sequences and random sequences (i.e. no learning). In contrast, under low sleep pressure conditions performance on the SRT task is postulated to show no decrements and learning of sequences can occur. If learning on the SRT task depends on sleep per se we furthermore hypothesized a temporal relationship between the time course of sleep structure (e.g. REM sleep) and the sequence learning curve in the nap protocol.

## 2. Methods

### 2.1. Study volunteers

Eight male and eight female participants (age range: 20–31 years, mean = 25.1; S.D. = 3.4) were non-smokers, free from medical, psychiatric, and sleep disorders. They abstained from caffeine and alcohol for 1 week before study begin. Drug free status was verified through urinary toxicologic analysis. Subjects were asked to keep a regular sleep–wake schedule (bedtimes and waketimes within ±30 min of self-selected target time) prior to their admission to the laboratory, verified with a wrist actigraph (Cambridge Neurotechnologies®, UK) and sleep diaries. The range of bedtimes was from 22:50 p.m. to 01:00 a.m. and the range of waketimes was 06:50–09:12 a.m. during the baseline week prior to study begin. The participants were neither extreme morning nor evening types (defined by scores <12 or >23 on the Torsvall–Åkerstedt morning–evening-type questionnaire [11]). Female participants were studied during their follicular phase. All participants gave written informed consent. The protocol, screening questionnaires and consent form were approved by the local Ethical Committee.

### 2.2. Protocol

The entire study comprised two parts (5 days each) with an off-protocol episode of 2–4 weeks in between. Following two scheduled days in the laboratory (<8 lx and sleep at habitual times), participants underwent a 40-h sleep deprivation protocol (high sleep pressure condition) in constant routine conditions (for details on Section 2 see [9]) or a 40-h constant posture nap protocol (low sleep pressure conditions, alternating cycle of 150 min of wakefulness and 75 min of sleep) in a balanced crossover design (Fig. 1), starting at habitual waketime (lights on, Fig. 1) after, and

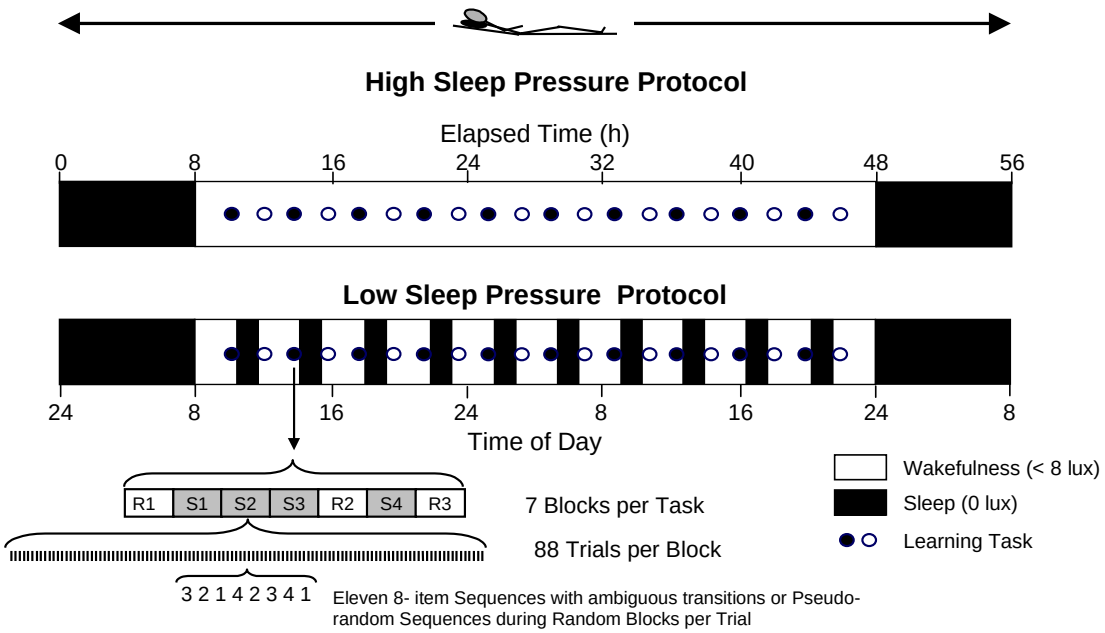


Fig. 1. Overview of the SD- and nap protocol. Subjects were scheduled to 8-h sleep episodes and 16 h of wakefulness according to their habitual bedtimes (24:00−08:00 h in the present subject, black bars). Subjects remained in a constant semi-recumbent posture during scheduled wakefulness and supine during scheduled sleep. The light levels during scheduled wakefulness were <8 lx, typically between 3 and 5 lx. During scheduled sleep/nap episodes subjects were in complete darkness (0 lx). Upper panel: SD protocol (high sleep pressure): 40 h of extended wakefulness starting at habitual waketime. Lower panel: nap protocol (low sleep pressure): 40 h of an alternating regimen of 150 min of scheduled wakefulness and 75 min of scheduled sleep, starting at habitual waketime on day 2. Filled (first presentation) and open circles (second presentation) indicate the timing of the SRT tasks. A single SRT task consisted of seven blocks, (three random and four sequenced blocks), which itself comprised 88 trials (11 eight-item sequences or 11 pseudo-random sequences).

followed by, an 8-h night centered at the midpoint of the subject's habitual sleep episode. During both protocols body posture remained controlled (semi-recumbent during scheduled wakefulness and fully recumbent during scheduled sleep). Here we only report data from the first part of the study (inter-subject comparison).

### 2.3. Assessment of sequence learning in the SRT task

None of the participants had ever performed a SRT task before. They lay semi-recumbent in bed facing a Macintosh ibook computer screen displaying four permanent position markers (circles). They were asked to react as quickly and accurately as possible to the appearance of a stimulus within one of the markers (circles) by pressing the spatially corresponding key. No feedback was given on their performance. Each stimulus followed by fixed 225-ms response-to-stimulus interval, until seven blocks of 88 trials had been completed. The duration of each SRT task was 5–8 min. Unknown to the participants, the sequential structure of the stimulus material was manipulated: blocks 1, 5, and 7 (Ran 1–3) were pseudo-random stimuli (i.e. without repetition of a predefined sequence), blocks 2–4 (Seq 1–4) and 6 presented a fixed eight-item sequence. Stimuli were generated based on a deterministic finite-state grammar (for an example see Fig. 2) that defines legal transitions between successive trials [12,13]. All sequences applied in the experiment can be classified as ambiguous (all stimuli positions (1–4) appear multiple times in the sequence while no subsequent stimulus can be unambiguously predicted from the previous one [14]. Furthermore, none of the sequences comprised "trivial" transitions such as "1-2-3." In total, participants were exposed to 10 different, but formally equivalent, sequences (i.e. only ambiguous sequences). Each sequence was applied twice in the experiment (Presentation 1: before each nap in the LSP (low sleep pressure) or corresponding wake episode in the HSP (high sleep pressure) and Presentation 2: after each nap in the LSP or corresponding wake episode in the HSP, see Fig. 1). Sequence learning occurs when the repeating sequence of target locations elicits shorter reaction times than does a random sequence of target locations. Therefore, the primary measure of sequence learning is the reaction time difference between responses occurring to sequentially determined events versus pseudo-randomly occurring events (Seq−Ran). Such reaction time differences can be inflated by increases in overall reaction times [15]. Realizing this problem and according to [15], we have expressed sequence learning as the proportion of the reaction time difference over the baseline reaction time ([Seq−Ran]/Ran). For sake of clarity, most of the statistics are based on these transformed reaction times.

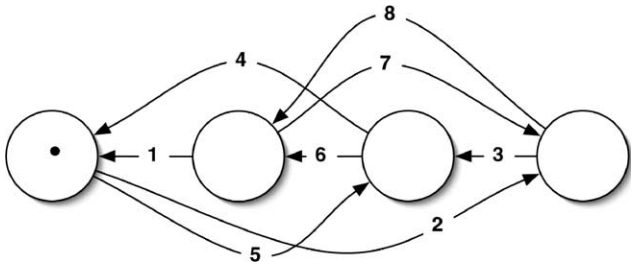


Fig. 2. Stimuli were generated based on a deterministic finite-state grammar that defines legal transitions between successive trials. All sequences applied in the experiment can be classified as ambiguous (all stimuli positions (1–4) appear multiple times in the sequence while no subsequent stimulus can be unambiguously predicted from the previous one. None of the sequences comprised "trivial" transitions such as "1-2-3").

All other measures of the protocol have been detailed in [16]. Briefly, subjective sleepiness was assessed every 30 min using the Karolinska Sleepiness Scale (KSS) [17], at the same time as collection of saliva samples for melatonin assay [18]. A reaction time task was used to evaluate sustained attention (Psychomotor Vigilance Performance, PVT) [19]. Sleep EEG recording (Vitaport-3 digital recorder, TEMEC Instruments B.V., Kerkrade, The Netherlands) and analyses as in [16]. EEG data derived from $C3-(A2+A1)$ were scored according to standard criteria [20].

### 2.4. Circadian phase assessment

The circadian phase was estimated from salivary melatonin data. Saliva was collected at ∼30-min intervals during scheduled wakefulness. Saliva samples were assayed for melatonin using a direct double-antibody radio-immunoassay validated by gas chromatography-mass spectroscopy with an analytical least detectable dose of 0.15 pg/ml and a functional least detectable dose of 0.65 pg/ml (Bühlmann Laboratories, Allschwil, Switzerland [18]). Comparative analysis has shown that melatonin phase is a more reliable and accurate measure of circadian phase than the core body temperature rhythm [21,22]. For each subject, the upward and downward crossing times of the 24-h mean melatonin concentration (between hours 5 and 29 of the 40-h LSP and HSP protocol) was calculated in addition to the timing of the midpoint between (for details see [23]).

### 2.5. Statistics

The statistical packages SAS® (SAS® Institute Inc., Cary, North Carolina, USA, Version 6.12) and Statistica® (StatSoft Inc. 2000, STATISTICA for Windows, Tulsa, OK, USA) were used. Analyses of variance for repeated measures (rANOVA) with the repeated factors "random versus structured sequences," "Presentation 1 versus Presentation 2," "time," and the nominal factor "condition" (high versus low sleep pressure) were performed for collapsed random blocks (mean of Ran 1–3) and collapsed sequenced blocks (mean of Seq 3 and Seq 4). The standardized experimental conditions of our study (very stringent subject selection, constant routine conditions, etc.) allowed a very precise measurement of performance on the SRT task. In fact, the mean standard deviation per subject (intra-subject S.D.) was only 40.5 ms. Since all participants showed error rates lower than 5%, error

data were not analyzed. However, reaction times during an error (i.e. wrong key press) were discarded from the analysis. The time course of subjective sleepiness ratings, PVT performance, salivary melatonin levels during the nap protocol were analyzed with one-way rANOVAs with factor "time interval". Before entering the one-way rANOVA, subjective sleepiness ratings, PVT performance, and salivary melatonin levels were binned into 3.75-h intervals. All *P*-values derived from rANOVAs were based on Huynh–Feldt's corrected degrees of freedom, but the original degrees of freedom are reported. For post hoc comparisons the Duncan's multiple range test—or if the data did not meet the criteria for normal distribution the Kolmogorov–Smirnov two-sample test or the Wilcoxon matched paired test was used.

## 3. Results

In a first step, we compared performance and learning on the SRT task at the beginning and the end of the 40-h sleep deprivation protocol (HSP condition) and the 40-h nap protocol (LSP condition; Fig. 3), respectively. There was a significant improvement in SRT performance at the end of the LSP condition compared to the HSP condition, but only for the sequenced trials, particularly Seq 3 and Seq 4 ($P < 0.05$ Kolmogorov–Smirnov two-sample test, Fig. 3 right hand panel). Additionally, learning to discriminate sequenced from random trials ([Seq−Ran]/Ran) was significantly better at the end of the LSP condition compared to the HSP condition (Fig. 3 bottom panel, $P < 0.05$, Kolmogorov–Smirnov two-sample test). At the beginning of the two protocols, there was no significant difference between the HSP and LSP condition for either measure, indicating similar initial conditions for both groups (Fig. 3 left-hand side panel, $P > 0.05$ for all types of sequences, Kolmogorov–Smirnov two-sample test).

To investigate the temporal evolution of SRT performance and learning, collapsed random and sequenced blocks as well as the ratio ([Seq−Ran]/Ran) were calculated for each task and plotted against time elapsed into the protocol (i.e.

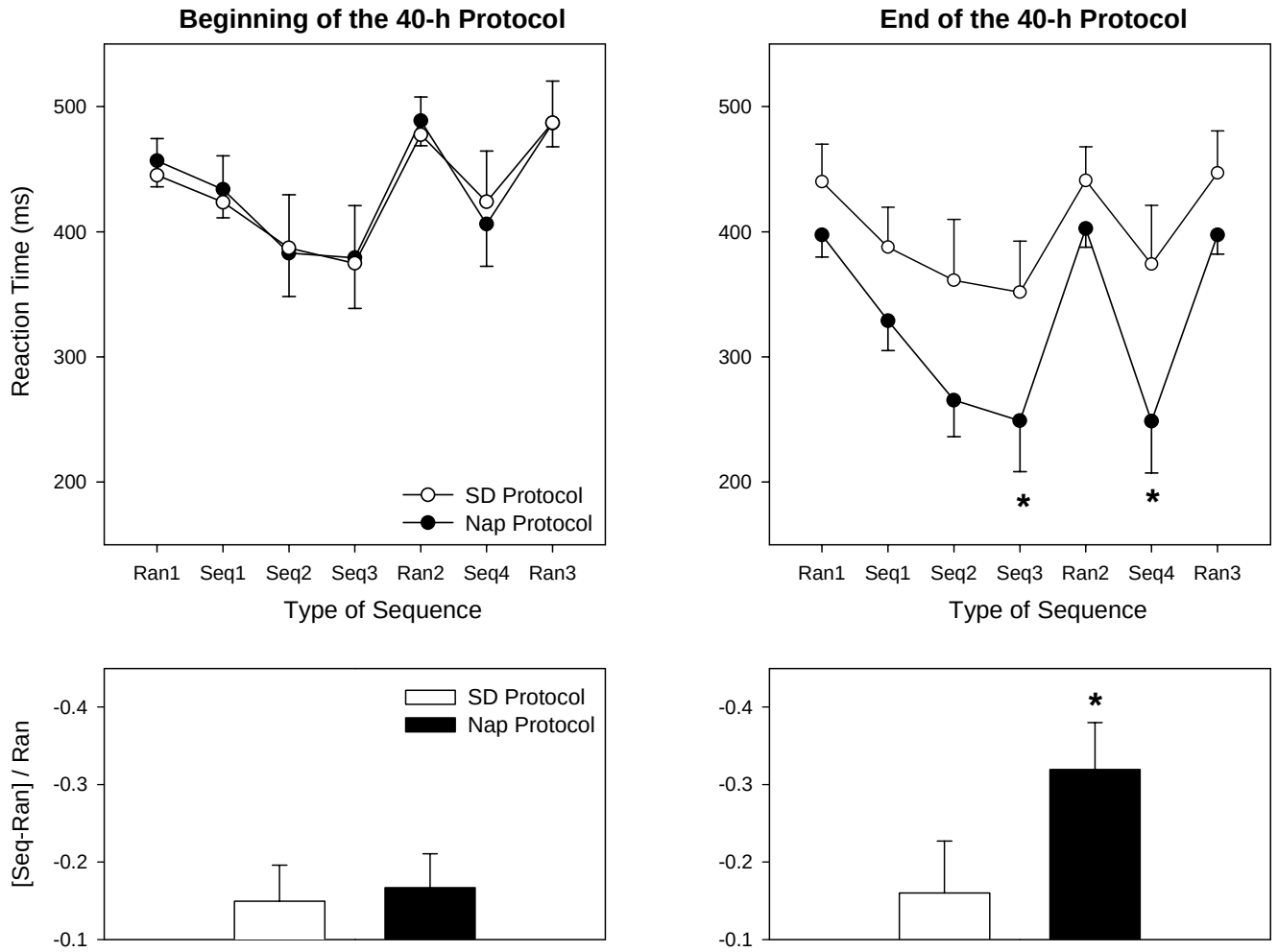


Fig. 3. Upper panels: average median reaction time during the SRT task at the beginning and at the end of the SD- and nap-protocol ($n = 8$; ±S.E.M.). Ran 1–3: random blocks, Seq 1–4: blocks containing sequenced structures. Lower panels: learning to discriminate sequenced from random trials ([Seq−Ran]/Ran) at the beginning and end of the SD- and nap-protocol. For statistics see text.

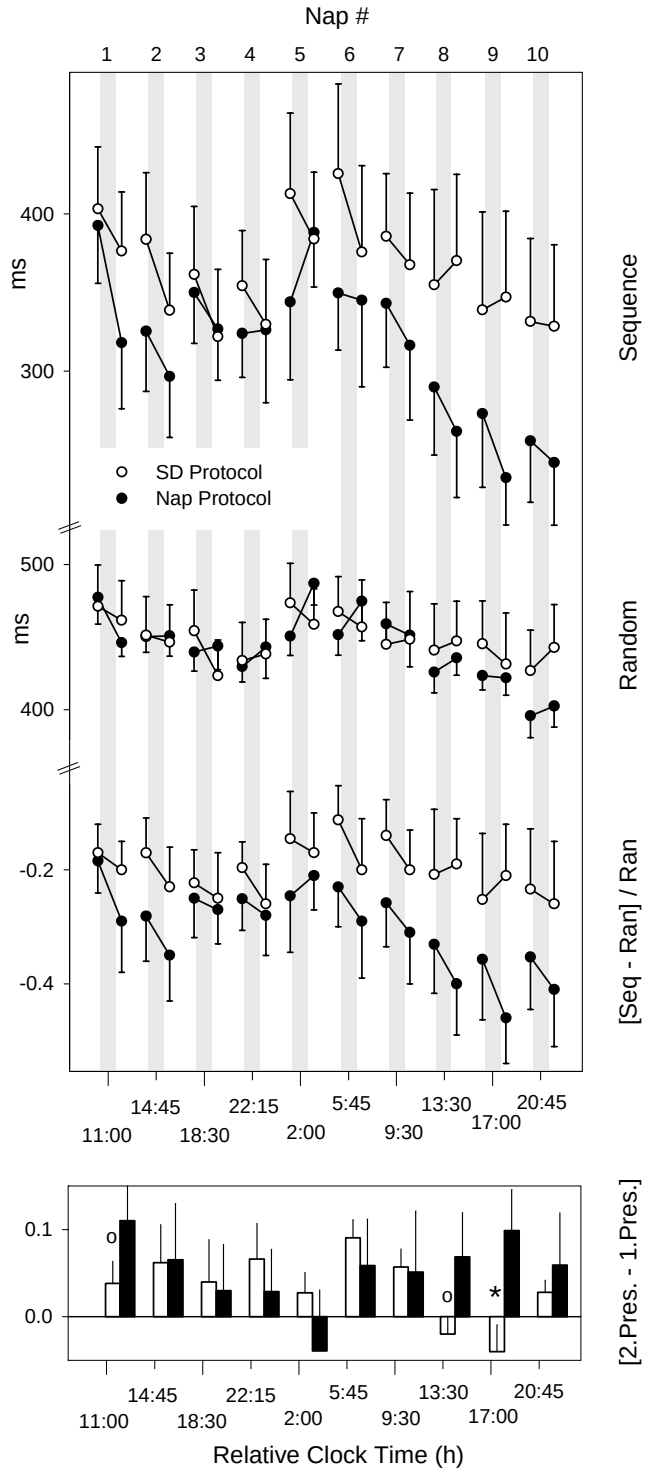


Fig. 4. Average median reaction time (ms) during random and sequenced trials and learning of discriminating sequenced from random trials ([Seq−Ran]/Ran; $n = 8$; ±S.E.M.) during in the first presentation and second presentation as they occurred relative to the timing of the naps (gray shaded areas) in the SD- and nap protocol. Lower panel: difference in learning to discriminate sequenced from random trials ([Seq−Ran]/Ran) between the second and first presentation. Filled circles indicate the low sleep pressure condition (nap protocol), open circles the high sleep pressure condition (SD protocol). Time of day is expressed in hours.

Table 1
Three-way repeated measure ANOVA for learning of discriminating sequenced from random trials ([Seq−Ran]/Ran)

| Factor | d.f. | $F$ | $P$ |
|---|---|---|---|
| Condition (high vs. low sleep pressure) | 1, 14 | 0.5 | 0.5 |
| Elapsed time (time into protocol) | 9, 126 | 7.3 | <0.0001 |
| Elapsed time × condition | | 1.2 | 0.1 |
| Presentation (first vs. second) | 1, 14 | 10.9 | 0.005 |
| Presentation × condition | | 0.1 | 0.7 |
| Presentation × elapsed time | 9, 126 | 2.2 | 0.02 |
| Presentation × elapsed time × condition | | 2.1 | 0.03 |

Results of three-way ANOVA for repeated measures with the factors condition (high- vs. low sleep pressure), elapsed time into the protocol, d.f.: degrees of freedom, $F$: $F$-value, $P$: Huyn–Feldth corrected $P$-values.

relative clock time; Fig. 4). Furthermore, the difference between the first and second presentation (i.e. over-nap change in the LSP condition) was calculated and plotted against elapsed time into protocol (bottom panel of Fig. 4). A three-way rANOVA for the ratio ([Seq−Ran]/Ran) with the factors: condition (LSP versus HSP), elapsed time into the protocol, and presentation (first versus second) was performed (Table 1). Both factors, elapsed time into protocol and presentation yielded significance, as well as the interaction presentation × elapsed time, and the three-way interaction of the above mentioned factors. Limiting the rANOVA to the second day of the study protocols yielded a significant interaction between condition and presentation ($F_{1,14} = 4.6$, $P < 0.05$). A two-way rANOVA with the factors elapsed time into the protocol and condition for the difference between the first and second presentation yielded a significant interaction of these two factors ($F_{9,126} = 2.2$; $P < 0.03$, Fig. 4 bottom panel). Post hoc comparisons between the LSP and HSP-condition, revealed a significant more improvement in SRT learning after naps 1, 8, and 9 in the LSP condition ($P$ at least <0.05).

In a next step, we aligned the data for each individual subject according to his or her endogenous ciracadian phase calculated by the melatonin midrange crossing time (see Section 2). Post hoc analyses yielded a significant correlation between the at home assessed habitual bedtime and the melatonin midpoint measured in the laboratory ($r = 0.61$; $P < 0.007$). Therefore, time courses of SRT performance and learning (Fig. 5) plotted against circadian phase did not substantially differ from the ones plotted against elapsed time into the protocol (Fig. 4). A two-way ANOVA with the factors condition (LSP versus HSP) and circadian phase for the first and second presentation separately, revealed a significant interaction condition × circadian phase for the second presentation ($F_{8,112} = 2.5$; $P < 0.02$) whereas the factor circadian phase was significant for both the first and second presentation ($P$ at least <0.002). Post hoc analyses for the second presentation yielded a significant improvement in sequence learning at the end of the LSP protocol but no significant change at the end of the HSP protocol ($P$ at least <0.02 for comparisons for the last two time points versus the preceding time points, Duncan's multiple range test).

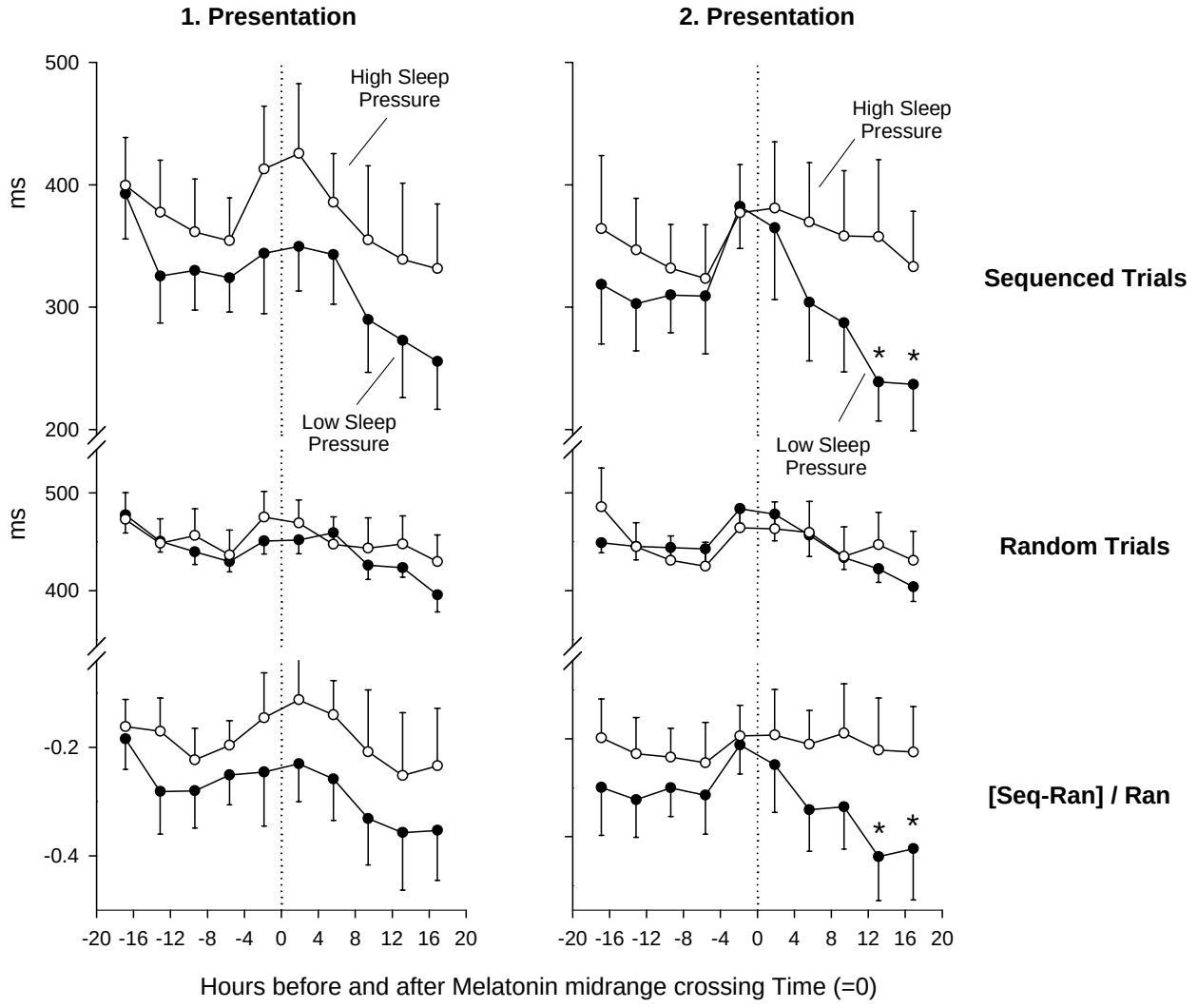


Fig. 5. Left-side panel: average median reaction time (ms) during random and sequenced trials and learning to discriminate sequenced from random trials ([Seq−Ran]/Ran; $n = 8$; ±S.E.M.) during the first presentation. Right-side panel: ditto for the second presentation. The data are expressed relative to each subject's melatonin midrange crossing time (0) in the evening. For statistics see text.

Data from the nap protocol (LSP) are presented in Fig. 6 to show the temporal relationships between salivary melatonin, subjective sleepiness, PVT, and the occurrence of REM- and slow-wave sleep with respect to the over-nap change in SRT learning and SRT performance during the first and second presentation. Maximum subjective sleepiness and slowest median reaction time were phase locked with the melatonin maximum, whereas the peak in REM sleep occurred later (∼10:00 a.m.). In none of these variables was an overall buildup or decrease correlated with elapsed time into the nap protocol. Daytime levels of subjective sleepiness, PVT performance, REM sleep, and melatonin did not change from day 1 to day 2 (see also [21]) indicating that we had attained conditions of low sleep pressure so as to effectively show mainly a circadian rhythm component. In contrast, sequence learning improved considerably with elapsed time into the nap protocol, particularly on day 2. Daytime levels of subjective sleepiness and PVT performance did not significantly differ between day 1 and day 2, so that the improvement in SRT performance is unlikely to be directly related. Since sleep structure (e.g. REM sleep) is modulated by circadian phase, any correlations between sequence learning and sleep structure needs to be done at a particular circadian phase. Therefore, we calculated the correlation between sleep structure per se and over-nap improvement in sequence learning for each nap separately. Only for REM sleep, and only for naps 1 and 2 were the correlations significant ($P$ at least $<0.05$; $r$ between 0.64 and 0.79, Pearson's product moment correlation) and for naps 8 and 9 a tendency was found ($P$ at least $<0.1$; $r$ between 0.4 and 0.49, Pearson's product moment correlation).

In a further analysis of the sequence learning task itself, we assessed how many times repetitive elements within the eight-item sequences had been presented. Since there were only four discrete locations on the computer screen for the stimulus to move, together with a high number of SRT trials,

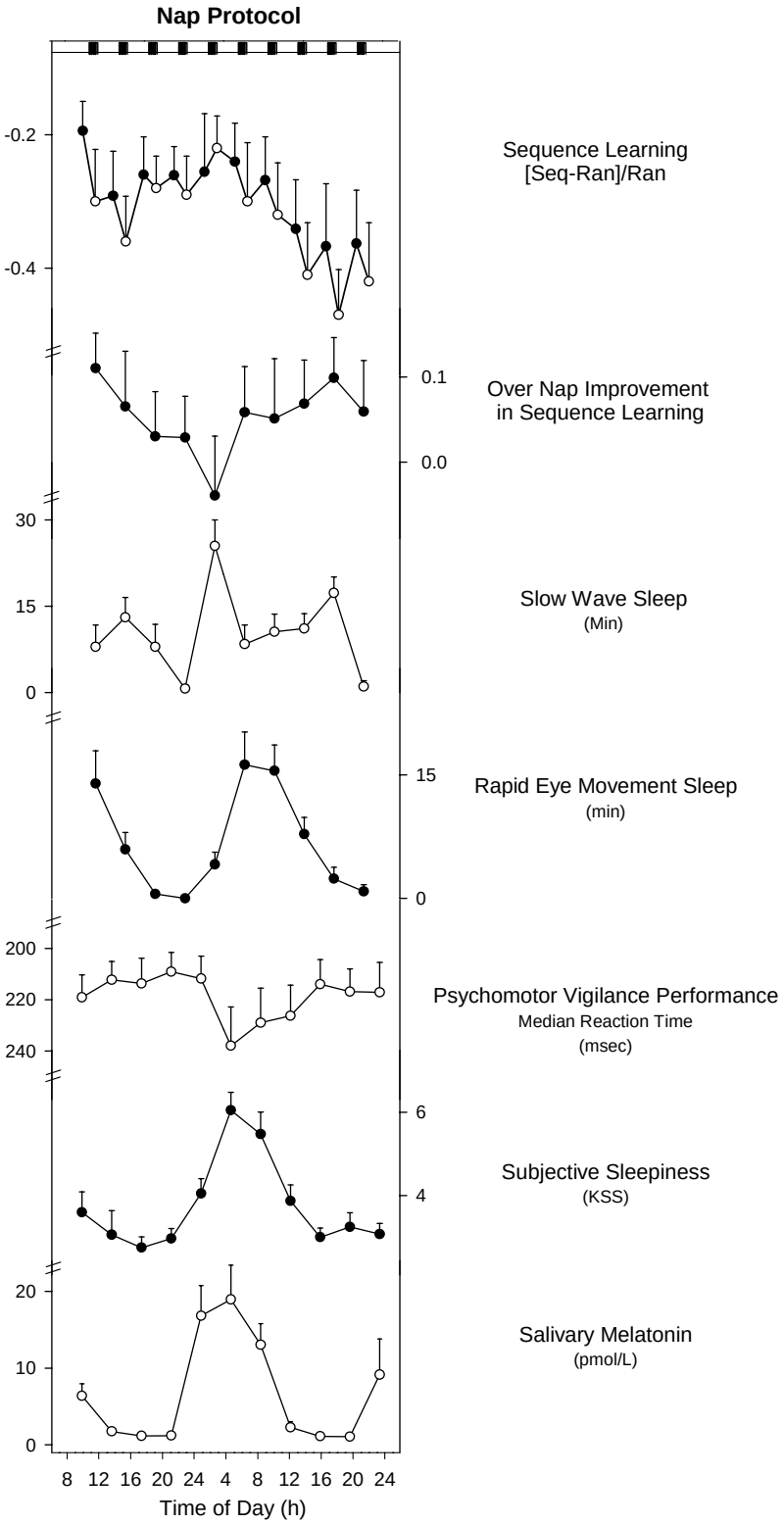


Fig. 6. Dynamics of sequence learning in the SRT task, the over-nap improvement in sequence learning, the amount of REM sleep and slow-wave sleep, Psychomotor Vigilance performance (PVT) (median reaction time), subjective sleepiness on the Karolinska Sleepiness Scale and endogenous melatonin across the low sleep pressure condition (nap protocol; mean values $\pm 1$ S.E.M.; $n = 8$). The top line indicates timing of the naps (black bars) and scheduled episodes of wakefulness (white bars). Alternating symbols in the SRT-panel represent reaction times during the first presentation (filled; before lights off of the nap) and during the second presentation of the sequence (open; after lights on). Data are plotted against the midpoint of the time intervals. Time of day represents the average clock time at which the time intervals occurred.

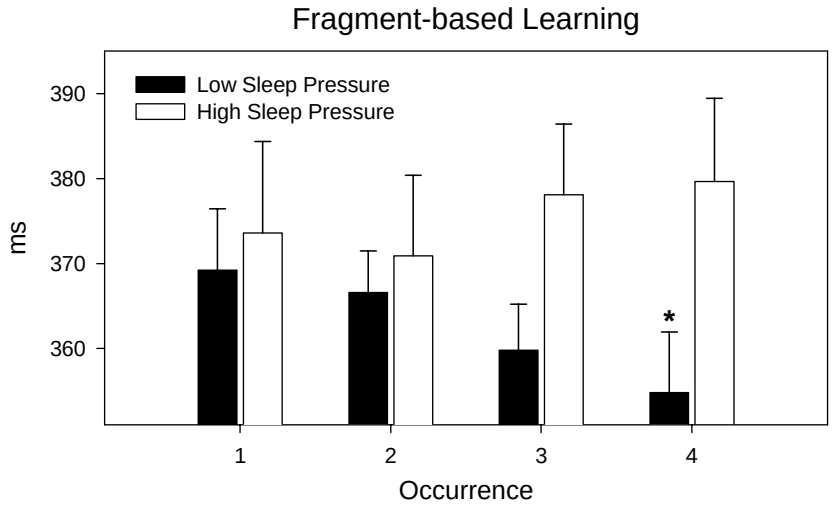


Fig. 7. Fragment-based learning during the low- and high sleep pressure protocol after its first, second, third and fourth occurrence. Reaction times become shorter when the fragments (average of triplets and quadruplets) are repeated (one to four times) during the LSP condition but not during the HSP condition. Data represent mean values of median reaction times across subjects. Asterisk indicates a significant difference between the fourth and first occurrence in the LSP protocol ($P < 0.05$; Duncan's multiple range test).

the chance of getting the same fragments (i.e. subparts) of a sequence more than once was considerable. Triplet and quadruplet repeats (i.e. subparts of length 3 and 4) occurred sufficiently often to analyze their effects on reaction times. Fig. 7 shows that under the LSP condition, reaction time decreased after the second, third, and fourth occurrence of a triplet or quadruplet fragment, whereas under the HSP condition no such decrease was found (rANOVA, interaction term: condition × occurrence, $F_{3,27} = 3.1$; $P < 0.04$). Post hoc analysis revealed a significant drop in reaction time from occurrence 1 to 4 in the low sleep pressure condition ($P < 0.03$; Duncan's multiple range test).

## 4. Discussion

The present data demonstrate that performance and learning on a SRT task are modified by the phase position of the circadian pacemaker and the level of sleep pressure. Improvement in SRT performance occurred only in the condition when sleep was allowed to occur, and the learning of the same eight-item sequence was significantly better when a 75-min nap was scheduled in between the presentation and repetition of the sequence. The clear demonstration of greater learning during presentation and repetition in the latter part of the nap protocol was unrelated to sleep per se in the preceding nap. However, REM sleep correlated with the over-nap learning in some of the naps (1, 2, 7, and 8). Interestingly, these naps occurred all around the same circadian phase or time of day (10–16 h). This implies that at certain circadian phases, REM sleep may play a role in memory consolidation. Indeed, there is substantial evidence for REM "windows" in animal studies, where REM sleep episodes at certain times are the important times for memory consolidation to occur (for a review see [24]). Studies in humans also suggested the existence of a REM window at the end of the night of sleep [2,25]. Further evidence supporting the role of REM sleep in memory consolidation comes from a human functional imaging study, in which it has been shown that those cerebral brain areas which are involved in the execution of a SRT task similar to the one here, are reactivated during REM sleep [26]. In our study, however, we do not have evidence that the amount of REM sleep per se co-varies with the time course in sequence learning.

This study is the first to consider circadian phase under constant routine conditions and to manipulate the level of sleep pressure in order to estimate their involvement in performance and learning on a SRT task. The observed circadian modulation in SRT learning and performance during both the HSP- and LSP condition is in good accordance with earlier reports which have shown circadian modulation in more declarative memory tasks such as recall of word pairs or a cognitive throughput task [27]. The circadian-related decrease in SRT performance and learning during the habitual night across all types of sequences was associated with decreased subjective alertness and neurobehavioral performance at this time of day. In contrast to the tasks mentioned above, sequence learning can be implicit [28,29]. Some participants in our study became aware of the repetitive character of some sequences, although they could not explicitly describe the sequence. Therefore, sequence learning may have been to some extent explicit in our study. This is in accordance with the view of Cleremans and Jimenez [30], who have proposed that implicit and explicit learning of deterministic sequences can occur simultaneously.

How was it possible to improve on this task when the sequence structure changed in a pseudo-random manner? The detailed analysis indicates that memorization of

sequence-fragments (encoded as memory chunks) may have helped the participants to learn new sequences faster. Those fragments are repetitive in nature, because the succession of the chosen sequences was restricted to include only four spatially separated stimulus locations. Sequence-fragments were thus repeated frequently in the sense that they constituted subparts of the different sequences presented. During the HSP condition, the participants did not seem to be able to memorize such sequence chunks. When sleep-deprived, the continuous buildup of sleep pressure is reflected in a prominent overall increase of low-EEG-activity particularly in frontal brain areas [9,31]. The prefrontal cortex is a region of particular interest, as PET studies have revealed markedly reduced prefrontal cortical activity after sleep deprivation, together with decrements in neurobehavioral performance [32]. The prefrontal cortex also plays an important role in SRT performance [33] since its activity is increased during SRT learning [34–36], and prefrontal lesions impair implicit and explicit learning of sequences on visuomotor tasks [37]. If performance in sequence learning is based on the representation of different chunks, the prefrontal cortex may provide a powerful form of control over motor cortical areas that give rise to anticipatory responding [38]. Based on our waking EEG findings which show augmented frontal low-EEG activity during the HSP condition [16], we speculate that our study volunteers may have experienced “prefrontal tiredness” (i.e. reduced activity in the prefrontal cortex) during the HSP condition with a resulting loss of prefrontal control in SRT learning. In other words, in a sleep-deprived condition there is less ability to learn chunks and therefore virtually no improvement in sequence learning.

## 5. Conclusions

The controlled constant routine conditions revealed the role of circadian phase and sleep pressure in sequence learning. Interestingly, learning of new sequences was only possible under low sleep pressure conditions when sleep was allowed, and was positively correlated with the amount of REM sleep occurring during naps scheduled after the circadian peak. On the other hand, SRT performance did not deteriorate under high sleep pressure, despite the high level of sleepiness. This indicates that the neurophysiological medium required for this type of learning is related to sleep, but may also occur, to a much lesser extent, however, during episodes of quiet and relaxed wakefulness.

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