Ambulatory assessment of human circadian phase and related sleep disorders from heart rate variability and other non-invasive physiological measurements
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Document Version
Publisher's PDF, also known as Version of record

Publication date:
2017

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

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ALTERED TEMPORAL INDICES OF HEART RATE VARIABILITY IN SLEEP ONSET INSOMNIA

Gil EA, Aubert XL, Penzel T, Beersma DGM. (submitted), *Altered temporal indices of heart rate variability in sleep onset insomnia.*
6.1 Abstract
Heart rate variability features have been used in various scientific and medical fields for diagnostic, monitoring, and research purposes. As a measure of autonomic nervous system activity, these signals are relevant in the sleep and chronobiology domains. Heart rate variability has been assessed in various sleep disorders and healthy subjects, however a thorough analysis of heart rate variability features in sleep onset insomnia patients has not yet been provided. In this study, we assessed temporal and spectral heart rate variability features derived from 24-hour electrocardiograms of 14 sleep onset insomnia patients. A previously unreported pattern was found in the temporal heart rate variability. While in healthy sleepers the standard deviation of inter-beat intervals peaks shortly before waking up, our data shows that in sleep onset insomnia patients it peaks on average shortly before sleep onset (Fisher’s Exact Test, p = 0.0063). This increase in the standard deviation of heart beats could be indicative of abnormal physiological or psychological processes related to the occurrence of sleep onset insomnia.

6.2 Introduction
Heart rate variability (HRV) encompasses a wide array of signals, both in the temporal and spectral domain, which have clinical relevance in many fields such as cardiology, psychology, sleep medicine, and chronobiology. The significance of the various HRV features has been well established in some fields, while it is still being developed and explored in others. In sleep medicine and chronobiology, HRV features have been used to understand autonomic changes during the sleep/wake transition and sleep cycles [1–3], the effects of different sleep disorders[4], to monitor treatments and therapies [5], and to estimate circadian phase [6]. Nevertheless, the depth and breadth of possible HRV analyses still allows for new findings and interpretations.

Most HRV signals have been shown to follow a circadian pattern [7,8]. HRV signals are used as measures of the autonomic nervous system [9] which is known to be influenced by the circadian system. The autonomic nervous system is further divided into the sympathetic and the parasympathetic (vagal) nervous systems. The balance between these two systems varies throughout the day, with the parasympathetic system being predominant during the night and vice versa [9]. The modulation seen in HRV features has been shown to be caused by the endogenous circadian system, both in the presence and absence of sleep [7,8,10].

In addition, HRV signals have been shown to be related to the sleep/wake cycle [2,11] and sleep architecture [3]. Although the circadian clock has a predominant effect on some HRV features, others are primarily affected by sleep. This has been assessed in several studies, with the findings being inconsistent. Carrington et al. found that the modulation in heart rate and sympathetic activation was mostly due to the circadian clock influence, while the nighttime increase in parasympathetic
activity was primarily caused by sleep [11]. However, Viola et al. found that heart rate and heart rate variability are more influenced by sleep than by the circadian clock [2]. HRV varies with the sleep stages and sleep stage transitions [2,3], making it possible to assess sleep quality through HRV measures. Furthermore, differences in the dynamics of HRV features during sleep can often be seen when comparing healthy sleepers to people with sleep disorders [12].

A recent review of the literature has found that the evidence for HRV impairment in insomnia patients is in fact conflicting [13]. For example, studies have found an increase in sympathetic activity in primary insomnia patients [14] or reduced parasympathetic activity in insomnia patients with reduced sleep duration [15], while others have not found a significant difference [16–18]. One way of assessing sympathovagal activation is through heart rate variability measures [9]. Reduced parasympathetic activity can be reflected in a decrease in the high frequency power of the HRV [15]. Furthermore, it has been shown that different types of insomnia present different characteristics when assessing the electroencephalogram (EEG), particularly during the sleep onset period of sleep onset insomnia patients and sleep maintenance insomnia patients [19]. Therefore, it is possible that the HRV characteristics are also different among the various types of insomnia. Sleep onset insomnia (SOI) is characterized by a difficulty in initiating sleep. The cause of this disorder might be attributed to a misalignment of the circadian clock or other (external) factors [20]. Studies incorporating 24-hour HRV recordings of insomnia patients are needed in order to better understand the dynamics of the complex HRV signals in the context of insomnia [13]. In this study, we have aimed at characterizing various temporal and spectral heart rate variability features over 24 hour periods as a way of finding physiological differences which could allude to the cause of the condition.

6.3 MATERIALS AND METHODS

6.3.1 Participants
Twenty diagnosed sleep onset insomnia patients participated in an 8 day long study which combined ambulatory and in-clinic recordings. The patients were either referred to us by our collaborating physicians at the Charité University Hospital or were recruited from an existing patient database at the Advanced Sleep Research institute in Berlin. Patients were diagnosed with sleep onset insomnia when having a sleep onset latency longer than 30 minutes. The patients were further examined and screened by a medical sleep specialist. Exclusion criteria included psychiatric or cardiac disorders, sleep apnea, restless legs syndrome, travel across time-zones and shift work in the previous two months, and use of sleep medication or other medication that could influence sleep. Participants meeting the inclusion criteria were explained the study protocol by the sleep physician and signed an informed consent form in accordance to the Declaration of Helsinki.
From all the SOI patients that took part in this study, 14 SOI patients were used in the present analysis due to problems with the equipment or data quality. The characteristics of the subset of participants included are shown in Table 6.1.

**Table 6.1 Subject characteristics and questionnaire outcomes.**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>SOI (Mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>11 female/3 male</td>
</tr>
<tr>
<td>Age</td>
<td>44.60 ± 12.44</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.43 ± 2.45</td>
</tr>
<tr>
<td>Subjective SOL (min)</td>
<td>43.57 ± 26.13</td>
</tr>
<tr>
<td>MEQ</td>
<td>57.21 ± 9.73</td>
</tr>
<tr>
<td>PSQI</td>
<td>10.07 ± 3.08</td>
</tr>
<tr>
<td>MSFsc (MCTQ)</td>
<td>02:45 ± 00:48</td>
</tr>
</tbody>
</table>

Subjective sleep onset latency (SOL) was obtained via questionnaires. The Morningness-Eveningness Questionnaire (MEQ) aims at determining the intrinsic chronotype of the subjects [21]. The corrected midsleep on free days (MSFsc) obtained from the Munich Chronotype Questionnaire (MCTQ) is indicative of behavioral chronotype while also considering the effects of different sleep timing on work days versus free days [22]. The Pittsburgh Sleep Quality Index (PSQI) is a measure of sleep quality, where scores above 5 are considered indicative of poor sleep [23].

**6.3.2 Protocol**

Participants wore an Actiwatch Spectrum (Philips Respironics, Pittsburgh, USA) which measured activity levels and light exposure for 7 days to monitor sleep timing. On the seventh day, participants came to the sleep clinic in the afternoon and were equipped with an ambulatory Holter ECG monitor (CardioMed CM3000, Getemed, Teltow, Germany). Beginning 5 hours before their habitual bedtime determined by questionnaires and actigraphy recordings, participants were placed in constant low intensity lighting conditions where they remained for the rest of the evening. The participants were connected to a PSG system (Embla N7000, Broomfield, USA) and monitored over one night. The following morning, the PSG electrodes were removed and the participants were allowed to go about their usual routine. The participants continued to wear the Holter ECG monitor during this time. In the evening, participants returned to the clinic, the Holter monitor was removed and the Actiwatch Spectrum was returned. Holter ECG recordings were ensured to be at least 26 hours in duration. The study protocol received Institutional Review Board approval by the ethics committee at the Charité University Hospital.

**6.3.3 Heart rate variability**

Ambulatory 1-lead ECG recordings at 256Hz from all participants were collected. An R-peak detection algorithm was used to extract the RR intervals, defined as the time
between R-peaks on a standard ECG. The detected peaks were inspected and artefacts were corrected. Spectral and temporal heart rate variability (HRV) features were extracted in 5 minute windows from all RR interval streams. The spectral HRV features of interest were the low frequency power (LF, 0.04-0.15 Hz), high frequency power (HF, 0.15-0.4 Hz) and the LF/HF ratio. The temporal HRV features extracted were the standard deviation of normal beats (SDNN), the root mean square of successive differences (RMSSD), and the proportion of normal beats that differ by more than 50 milliseconds divided by the total number of normal beats (pNN50). The maximum and minimum values for each feature were determined, and these were used to calculate the amplitude of the waveform (difference between maximum and minimum), as well as the temporal organization of each signal.

6.3.4 Sleep measures
Sleep statistics were determined from the PSG recordings, including sleep onset time, sleep onset latency, sleep duration, and time of midsleep. In addition, all participants completed sleep diaries and questionnaires. The questionnaires used were the Morningness-Eveningness Questionnaire (MEQ) [21], Munich Chronotype Questionnaire (MCTQ) [22], Pittsburgh Sleep Quality Index (PSQI) [23] and the Sleep Timing Questionnaire (STQ) [24].

6.4 RESULTS
Sleep statistics were derived from the one night PSG recording. Although it has been reported that one night is not consistently representative of characteristics of a patient’s habitual sleep pattern [25,26], such as sleep duration, we found no statistically significant difference when compared to actigraphy recordings over the preceding week. The sleep statistics extracted from the PSG are shown in Table 6.2.

<table>
<thead>
<tr>
<th>Sleep Statistic (hh:mm)</th>
<th>SOI (Mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bed-in time</td>
<td>22:38 ± 00:38</td>
</tr>
<tr>
<td>Sleep onset time</td>
<td>23:05 ± 00:20</td>
</tr>
<tr>
<td>Sleep duration</td>
<td>07:29 ± 00:24</td>
</tr>
<tr>
<td>Midsleep time</td>
<td>02:45 ± 00:26</td>
</tr>
<tr>
<td>Sleep onset latency</td>
<td>00:27 ± 00:20</td>
</tr>
</tbody>
</table>

The manually scored hypnograms of the SOI patient population were further analyzed in terms of sleep architecture and distribution of wake, S1, S2, slow wave sleep (SWS) defined as sleep in stages S3 and S4, and rapid eye movement (REM) sleep. The percentage of each of the sleep stages was assessed in the first and second half of the night. In addition, REM sleep latency and SWS latency were calculated. The percent wake is calculated with respect to the wake after sleep onset.
REM sleep latency was found to be 108.32 ± 46.81 minutes (mean ± SD) and SWS latency was 45.86 ± 27.06 minutes (mean ± SD). Patients presented an average of 3.8 REM cycles per night. In healthy normal young adults, wakefulness should account for approximately 5% of the night, S1 for 2% to 5%, S2 for 45% to 55%, SWS for 15% to 25%, and REM for 20% to 25% [27]. The SOI patients in our study presented higher than normal percent wakefulness and S1 sleep, yet lower than normal REM sleep. S2 and SWS sleep were within the healthy ranges.

The RR intervals of the sleep onset insomnia patients were aligned at the median sleep onset (median = 22:55; interquartile range = 00:29) and the average curve was plotted. The RR intervals increased during the evening and continued to increase at a slower rate during the night after sleep onset. During the night, the average heart rate was 59 beats per minute. Figure 6.1 shows the RR intervals over 24 hours.

Spectral (HF, LF, LF/HF ratio) and temporal (SDNN, RMSSD, pNN50) HRV features were calculated for each participant over 24 hours. Although these recordings took place over one night at the sleep clinic, it has been reported that one night is enough
when assessing HRV during sleep [25]. These signals were median filtered and aligned at the sleep onset of each participant. A two harmonic fitting was applied with periods corresponding to 24 and 12 hours. The average ± SEM of all participants is plotted in Figure 6.2 and Figure 6.3, where the vertical line represents the sleep onset time from all participants.

Figure 6.2 Spectral HRV features from 24 hour ECG recordings from sleep onset insomnia patients. The vertical line shows the sleep onset time. Error bars represent the standard errors of the measurements.
Figure 6.3 Temporal HRV features from 24 hour ECG recordings from sleep onset insomnia patients. The vertical line shows the sleep onset time. Error bars represent the standard errors of the measurements.

The SDNN showed an unexpected pattern, particularly in regards to the location of the SDNN maximum with respect to the overall 24 hour recording. It has been reported in studies on healthy sleepers that the SDNN reaches a peak in the early morning [2,8,28,29]. Table 6.4 shows a summary of studies which have reported the SDNN pattern of healthy subjects.

Table 6.4 Summary of studies reporting the timing of the SDNN maximum in healthy subjects.

<table>
<thead>
<tr>
<th>Study</th>
<th>Reported Time of SDNN max</th>
<th>Population</th>
<th>Mean Age</th>
<th>Setting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viola et al. 2002</td>
<td>0533hr ± 17 min</td>
<td>7 healthy males</td>
<td>Range 21 to 28</td>
<td>Lab</td>
</tr>
<tr>
<td>Vandewalle et al. 2007</td>
<td>0659hr (95% CI, 0525-0832)</td>
<td>8 healthy males</td>
<td>24.4 ± 4.4</td>
<td>Lab</td>
</tr>
<tr>
<td>Bonnemeier et al. 2003</td>
<td>Wake up time, 0700hr (approx. from SDNN plot)</td>
<td>166 healthy (81f/85m)</td>
<td>42 ± 15 (range 20-70)</td>
<td>Ambulatory</td>
</tr>
<tr>
<td>Yoshizaki et al. 2013</td>
<td>0700hr (approx. from SDNN plot)</td>
<td>27 healthy females</td>
<td>40 (range 25-53)</td>
<td>Ambulatory</td>
</tr>
</tbody>
</table>
For a different study, we collected ambulatory heart rate data from healthy sleepers over a period of 32 hours [6]. Although the data were collected from a different age group than the current SOI data, the findings reported in literature were confirmed by our healthy sleeper data. The mean SDNN from the sleep onset insomnia patients was plotted together with our healthy sleepers’ data to demonstrate the significant difference in temporal organization of the signals. The mean of the signals has been removed to emphasize the temporal differences and the data are plotted in Figure 6.4.

![Figure 6.4 Difference in the timing of the SDNN peak in sleep onset insomnia patients. The circles and dark fit correspond to the sleep onset insomnia patients, while the triangles and gray fit correspond to the healthy sleepers. The traces have been shifted and aligned at the sleep onset, represented by the vertical line. Error bars show standard errors of the measurements.](image)

As can be seen, the average SDNN curve for the sleep onset insomnia patients reached its maximum before sleep onset, on average at 22:30, while the healthy sleepers had a maximum SDNN value around wake up time at approximately 06:00.

### 6.5 Discussion
Sleep onset insomnia patients were characterized in terms of sleep architecture and circadian heart rate variability. These characteristics were compared to reported values in literature for healthy sleepers.

#### 6.5.1 Sleep architecture
As expected, the first half of the night presented more SWS than REM sleep, while the opposite was true for the second half of the night. The REM sleep latency of 108.32 minutes was within the normal range of 90-110 minutes [27]. Age is known to be an important factor influencing the percentage of each sleep stage over a given sleep period [27]. The percentage of SWS and REM sleep as well as REM latency are known to decrease with age, while the percentage of S1, S2 and WASO increase with age [30]. Keeping these effects in mind, the hypnograms of our sample of SOI patients were in-line with what would be expected from healthy sleepers, with the
exception of a prolonged sleep onset latency. This is consistent with earlier comparisons between sleep onset insomnia patients and healthy sleepers [31].

6.5.2 Heart rate variability

The pattern of RR intervals shows that the reduction in heart rate that occurs at the transition from wake to sleep becomes less pronounced. This is different than what would commonly be expected in healthy sleepers, where the RR intervals would show a steady or accelerated increase. This difference has been previously reported by Spiegelhalder et al. [15].

Circadian rhythmicity was observed in all HRV features as shown in Figures 2 and Figure 6.3 with harmonic fittings of 24 and 12 hours. Large inter-subject variability was found in the means of most HRV features as seen from the large SEM values. This might be due to the variability in the severity of the sleep onset insomnia in each of the patients. In the spectral domain, the HF and LF components of the HRV both reached a maximum during the night, while the LF/HF ratio presented its minimum. In the temporal domain, the SDNN, RMSSD, and pNN50 all showed a maximum during the night. Nevertheless, the temporal distribution of maximum and minimum of the SDNN feature did not match what has been presented in literature for other patient and healthy subject populations.

6.5.3 SDNN maximum

The SDNN of our sample of sleep onset insomnia patients reached a maximum approximately at sleep onset time. All reports of HRV features in healthy subjects have found that the SDNN peak occurred at the end of the sleep interval or around wake up time. As shown in Table 6.4, these findings include studies done in healthy sleepers both in laboratory [2,8] and in ambulatory settings [28,29] including both young and older subject populations comprising both male and female participants. In one study, Bonnemeier et al. presented data from 166 healthy subjects (81f/85m) with an age range of 20 to 70 years, and divided their analysis per decade. They showed that the SDNN amplitude decreased with age but the timing remained consistent, with the maximum occurring around wake up time [28].

As an indication of the discriminative value of the SDNN feature for our patient and subject populations, 10 of the 14 sleep onset insomnia patients (71.4%) and only 2 of the 14 healthy participants (14.3%) showed an SDNN maximum within 2 hours of sleep onset (Fisher’s Exact Test, p = 0.0063).

The SDNN is used as a global index of heart rate variability and is said to reflect all cyclic components responsible for the variability of the heart rate in the recording period. This feature is not commonly used in sleep monitoring or assessment, therefore its significance in the sleep domain is not well understood. Viola et al. assessed the effects of sleep and the circadian system on several HRV features over 24 hours by shifting the subjects’ sleep episode by 8 hours. This study found that the only feature which presented a clear circadian modulation, independent from the
occurrence or lack of sleep, was the SDNN [2]. Building upon this finding, one cannot exclude the possibility of circadian misalignment causing a shifted SDNN peak. Assuming that the remaining HRV features are mostly sleep dependent, their apparent alignment corresponds to the sleep/wake cycle and not the endogenous circadian clock. Abnormalities in the timing of the circadian system with respect to the patient’s sleep schedules could come forth as a shifted SDNN curve. It has been shown that the correlations between the dim light melatonin onset (DLMO), accepted as the gold standard measure of circadian phase, and sleep timing features decrease in sleep onset insomnia patients compared to healthy sleepers [32,33]. This could be indicative of a misalignment between the endogenous circadian signal and the sleep/wake cycle.

An abnormal cardiovascular regulatory system could attribute to the difference in the occurrence of the SDNN peak. Maintaining the notion that the endogenous circadian system is primarily responsible for the modulation of the various temporal and spectral HRV features as a reflection of the autonomous nervous system, one would expect that an abnormal circadian system would influence all HRV features. As that is not the case, one possible explanation is an abnormal cardiovascular regulatory system.

Studies have shown that stress results in an increase in heart rate and a decrease in SDNN [34,35]. The feelings of stress or anxiety that are often experienced by insomnia patients prior to bedtime did not seem to be present in this study. It is possible that these patients have learned over the years to apply relaxation techniques in order to more easily achieve sleep onset. However, then one would expect that in addition to the evening peak, the major peak seen in healthy participants around 06:00 would also be present in the SOI data.

A related psychological state which has been correlated with a decreased SDNN is major depression [36]. Major depression is associated with a reduction in deep sleep, prolonged REM sleep, and a reduced REM sleep latency [37]. As can be seen in Table 6.3, the sleep architecture of the SOI patients did not present the characteristics of patients suffering from depression.

Variations in heart rate are also known to be caused by hormones, such as cortisol. The cortisol awakening response (CAR) can affect heart rate and it has been shown that an attenuated CAR is associated with an increase in SDNN [38]. A related process which is controlled by the circadian system is hypothalamo-pituitary-adrenal (HPA) activity. Nevertheless, the CAR and the circadian rise in HPA activity are thought to be two distinct processes [38,39]. The cortisol rhythm and in particular the CAR, are very complex signals which can be influenced by numerous physiological processes. An overview of these processes is presented by Clow et al. [38]. Cortisol profiles for healthy and insomnia patients generally show the same timing structure, with some differences in amplitude [32,40]. However, these studies pertain to primary insomnia and not specifically to sleep onset insomnia,
which could yield different results. Furthermore, considering the gender
distribution, studies have shown that women entering menopause commonly suffer
from disturbed sleep and this could be due to hormonal changes [41]. Menopause
is associated with higher levels of cortisol during sleep, as well as an increased
susceptibility to nocturnal rises in cortisol as a result of stress [42]. Although
menopausal stage was not investigated, the average age in our female population
of 44.6 could be associated with perimenopause, and could therefore present some
early menopausal sleep and hormonal characteristics.

Further research is required into the processes which could influence the
modulation of the SDNN feature in terms of sleep and circadian rhythms. The results
we presented seem to indicate an interaction between the SDNN and sleep,
particularly with the sleep-wake and wake-sleep transitions. A controlled study
involving circadian markers, cardio-regulatory measurements, relevant hormone
concentrations, as well as psychological measures of depression and other mental
states, could shed light into the processes involved in the abnormal temporal
organization of the SDNN feature in sleep onset insomnia patients. The main
limitation of our study is the lack of a control group of healthy volunteers matched
on gender and age. This limitation has been addressed by doing a comprehensive
evaluation of the literature and referring to published results as an indication of
what would be expected in such a cohort. The consistency of the literature across
age groups, gender, and protocols justifies the comparison between our healthy and
insomnia populations. It suggests that the differences observed in the SDNN feature
are caused by factors related to the insomnia diagnosis.

6.6 CONCLUSION
Heart rate variability analyses of 24-hour electrocardiograms from sleep onset
insomnia patients show an altered SDNN profile, with its maximum occurring shortly
before sleep onset when compared to healthy cases which present the SDNN
maximum shortly before wake-up. In the same manner as hypnograms of sleep
onset insomnia patients are similar to healthy sleepers and, therefore, different
than other insomnia patients, sympathovagal activity as assessed by heart rate
variability measures could also present different characteristics within the various
types of insomnia. Physiological changes taking place directly before sleep onset
might be of greater importance in understanding the mechanisms associated with
the occurrence of sleep onset insomnia, as opposed to nycthemeral processes which
affect broader sleep disorder classifications.

6.7 ACKNOWLEDGEMENTS
This work was supported by the EU Marie Curie Network iCareNet under grant
number 264738.
6.8 References


