Interaction of Brain and Heart Activity during Sleep

Master Thesis

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Interaction of Brain and Heart Activity during Sleep

Master Thesis

In partial fulfilment of the requirements for the Master of Science ETH
in Health Sciences and Technology (MSc ETH HST)

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Abstract

Sleep is essential for human life and plays an important role in restorative processes for brain and cardiovascular health. Heart activity is predominantly regulated by the interaction of the parasympathetic and sympathetic branch of the autonomic nervous system. Dysbalance of parasympathetic and sympathetic activity has been associated with increased risks for cardiovascular diseases, for which aging represents the major risk factor. Since aging is also related to decreased slow wave sleep (SWS) and sleep quality, discovering associations in the underlying processes of nocturnal cardiac autonomic regulation in elderly may be important to find objectives for improvements of cardiovascular health and to ultimately promote healthy aging. Therefore, we measured in-home polysomnography in seven healthy elderly, aged 63 - 69 years (two females, five males) in combination with heart rate, heart rate variability (HRV) and blood pulse wave (BPW). Analysis of 26 nights revealed parasympathetic activity, as inferred from HRV, to be significantly decreased during rapid eye movement sleep (REM), whereas sympathetic activity, as inferred from BPW, was increased during REM compared to non-REM sleep (NREM) in the first part of the night. During the second part of the night, cardiac autonomic regulation changed to decreased sympathetic activity in NREM stage 2 and REM, whereas parasympathetic activity inconsistently changed. Unexpectedly, the sympathetic activity in the deepest stage of sleep in the second part was higher than during REM. Additionally, increased slow wave activity was associated with increased sympathetic modulation, whereas low frequency slow waves were related to higher parasympathetic influence on the heart. Hence, our research suggests an important role of low frequency slow waves for body energy conservation, which is indicated by parasympathetic predominance. Moreover our findings demonstrate a significant change in cardiovascular autonomic regulation during the second part of the night in elderly, which might be associated with the increased risks of cardiovascular diseases in aging.

Chapter 1

Introduction

Sleep plays an essential role in human life. During this stage of unconsciousness, various restorative brain and body processes occur to maintain general health and well-being[1–3]. Consequences of sleep deficits range from increased sleepiness or decreased performance to higher risks for inflammation, obesity and diabetes, which are all risk factors for cardiovascular disease, that are for instance stroke, hypertension and coronary heart diseases. Moreover, sleep deficiency is related to decreased cardiovascular health and mortality[1,4–8]. According to the World Health Organisation cardiovascular diseases are the leading cause of death in the world[9] for which age represents the major risk[10]. Since population in the world is getting older[11], more insight into nocturnal processes that regulate the cardiovascular restorative functions to ultimately promote healthy aging are needed.

1.1 Sleep

Sleep can be monitored by electroencephalography (EEG), which is recording changes in electrical activity produced by large neuron populations in the brain. In addition, eye activity can be tracked by electrooculography (EOG) and chin muscle activity by electromyography (EMG). EEG, EOG and EMG are commonly referred to as polysomnography (PSG). The American Academy of Sleep Medicine[12] provides guidelines for the categorisation of different sleep stages according to the underlying frequency bands of the neuronal activity and additional PSG information. Primarily rapid eye movement sleep (REM) and non-REM sleep (NREM) are distinguished, which usually alternate throughout the night, with REM getting longer towards the morning. Awake brain activity may consist of alpha waves ranging from 8 - 13 Hz, a usually high chin EMG activity and a combination of eye blinks, reading eye and rapid eye movements. REM is characterised by rapid eye movements, brain activity similar to wakefulness and lowest muscle tone, whereas NREM is further divided into three stages: Stage 1 (N1), stage 2 (N2) and stage 3 (N3). N1 contains low-amplitude mixed-frequency theta waves, predominantly ranging between 4 - 8 Hz with N1 characteristic slow eye movements. N2 is indicated by either sleep spindles, which are bursts of neural oscillatory activity in the 11 - 16 Hz frequency range lasting longer than 0.5 seconds, or K-complexes with a prominent high voltage, sharp negative peak followed by a positive component. N3, in which more than 20% of an 20 seconds epoch consists of slow waves, represents further deepening of sleep. Therefore, slow waves, which are defined as 0.5 - 2 Hz waves with a minimum peak-to-peak amplitude of 75 μV, are related to the depth of sleep. N3 is often referred to as deep sleep or slow-wave sleep (SWS) and represents the sleep stage with highest synchronisation of neurons[2,3,7,12–14]. Slow wave activity (SWA) represents the power spectral density

in the frequency range of 0.75 - 4.5 Hz, which shows cyclic patterns during sleep, with increased activity during NREM and low activity during REM episodes[15].

1.2 Cardiac autonomic regulation and its measurements

Besides the prominent change in brain activity, many additional changes in body functions are taking place during sleep. For instance heart rate and blood pressure decrease with NREM, but are similar to wakefulness during REM[3, 16, 17]. These cardiovascular functions are prevalently regulated by the sympathetic and parasympathetic branch of the autonomic nervous system (ANS), particularly during sleep[18]. The parasympathetic nervous system (PNS) and the sympathetic nervous system (SNS) are both continuously active to regulate body organs through changes in their tone and thus, a change in sympathovagal balance[2]. This regulation needs to be flexible for quick adaptation to changing environments and hence, bringing the body systems to a favourable state for a specific situation[19]. The SNS can provide extra activation to the body in stress situations which may result in a fight-or-flight response whereas the PNS is prevalently known for its rest and digest functions and its favourable role for energy conservation[2, 20]. Increased SNS activity has been associated with mental stress[21], which is a common risk factor for cardiovascular disease. Furthermore, stress frequently coincides with decreased PNS activity, which has been related to higher risks for all-cause mortality. Sympathetic overactivity results in higher energy demand of the body, which may cause premature aging and increased morbidity and might be a target to improve health and to limit risks and progression for cardiovascular disease[2, 22–24].

Measurement of ANS activity in humans is challenging, since many methods rely on testing cardiovascular reflexes that induce changes in ANS activity or are highly invasive like microneurography, which directly records action potentials in nerves to measure SNS activity[25, 26]. Heart rate variability (HRV) provides a non-invasive measurement of autonomic activity and tone[27–29], due to the influence of heart activity by both branches of the ANS[2, 30, 31]. Without any activity of SNS and PNS, the intrinsic heart rate generated by autorhythmic cells in the sinoatrial node is around 100 beats per minutes (bpm)[32]. PNS activity quickly (< 1 s) slows down the heart rate, the heart's strength of contraction and rate of conduction through the release of acetylcholine. On the other hand, SNS activity slowly (> 5 s) increases the heart rate, the heart's strength and rate of conduction through release of norepinephrine[2, 18, 27, 30, 31, 33]. This regulation of the heart will be from now on referred to as cardiac autonomic regulation and results in a variability of the time between consecutive heart beats, which is referred to as HRV. HRV is strongly related to heart rate[34], which simply represents how many times the heart beats each minute. With a slower heart rate, there is more time between consecutive heart beats and thus, more time for variability[19]. HRV has been proposed to reflect the cardiovascular autonomic regulation with the complex interaction between the brain and cardiovascular system[35]. Depressed HRV has been associated with aging, higher risk of all-cause mortality and cardiac events, lower self-reported health and increased mortality after acute myocardial infarction[36–40]. Additionally, HRV has been related to cognitive performance and emotional processing relying on prefrontal cortex and the body's ability to adapt to changes in environment[20, 24, 35, 41, 42]. Furthermore, HRV has been proposed to be a marker of healthy aging[43].

HRV analysis is usually performed in the time-domain or the frequency-domain[24, 27, 41]. Time domain methods involve statistical analysis across a time period of e.g. the time between two normal heartbeats, which were initiated by depolarisation of the sinoatrial node. This period is called NN interval or inter-beat interval. Additionally, analysis about the differences in consecutive inter-beat intervals can be analysed as well. The time period for calculations of time-domain measurements are varying, however, five minutes was

suggested by the Task Force[27]. The most prominent time-domain measurement are the root-mean square of successive differences of successive NN intervals (RMSSD) or the standard deviation of NN intervals (SDNN)[24, 27, 41]. RMSSD is believed to reflect vagal (PNS) activity[20, 41], whereas SDNN reflects overall variability in the time period[27, 44]. Frequency-domain measurements show the power distribution across the different frequency bands and can provide further information about the underlying regulations of the heart. Mainly three frequency bands are of interest for the analysis of short-term HRV: the high frequency (HF) band for frequencies of 0.15-0.4 Hz, the low frequency (LF) band for frequencies of 0.04 – 0.1 Hz and the very low frequency (VLF) for frequencies below 0.04 Hz[27]. In addition to the absolute power in frequency bands, LF and HF may be calculated in units normalized to the sum of LF and HF power, called LFnu and HFnu, whereas LFnu is exactly predictable from HFnu and vice versa[45].

Changes in volume and frequency of respiration can change HRV and primarily affect the HF band[24] and therefore represent vagal modulation[24, 27, 28, 46]. During one respiratory cycle, the heart rate changes inasmuch as during inhalation, heart rate increases since the cardio-respiratory centre inhibits vagal outflow, whereas during exhalation, heart rate decreases since vagal outflow is no longer inhibited[47]. This alteration in heart rate, which is called respiratory sinus arrhythmia (RSA), is generally not observable during daytime, but clearly detectable while resting or sleeping[48]. Additionally, baroreceptors are signalling changes in arterial pressure to the brain, which stimulate the ANS to bring arterial pressure back to a normal level. If baroreceptors detect excessive arterial pressure, SNS is more inhibited whereas PNS gets more activated, which results in decreasing heart rate, decreased strength of contraction and vasodilatation of the veins to reduce arterial pressure. This regulation of the heart rate contributes to the changes in especially the LF band[2, 41].

The blood pulse wave (BPW) is a novel stress measurement and was introduced by Biovotion AG (Zürich, Switzerland), which can be used in addition to HRV[49]. Blood is ejected into the arteries when the heart contracts and travels in form of a pressure pulse through the body. The difference between the higher systolic pressure and the lower diastolic pressure is called pulse pressure. Since SNS activation constricts veins and arteries and thereby increases its resistance, blood pressure gets elevated[2]. Pulse wave velocity has been demonstrated to predict future cardiovascular event and all-cause mortality[50]. Blood pressure was shown to be related to blood pulse amplitude during sleep and can be well estimated by including waveform morphologies in combination with time information about the pressure wave[51–53]. Biovotion used the shape, rhythmicity and speed of the photoplethysmographic (PPG) derived blood pressure wave for the BPW. High rhythmicity is related to low HRV and results in increased BPW. Furthermore, arterial stiffness results in increased blood pressure which is indicated by elevated BPW as well. Overall, BPW should be more robust than HRV[49], nevertheless, the specifics about BPW have not been published yet. Despite the lack of knowledge about BPW, it could provide novel insights into cardiac autonomic regulation during sleep with being an indicator for SNS activity.

1.3 Interaction of sleep and cardiac autonomic regulation

Context matters while assessing HRV measurements. Thereby position, activity and movements, noise and emotions can influence the outcome[24, 54]. Sleep and especially deep sleep is as a condition with almost no external disturbing events. Therefore, sleep may be a stationary state for optimal ANS function assessment[54–57]. NREM has been demonstrated to be accompanied with higher PNS and lower SNS activity. Additionally, PNS activity was increasing through the course of the night. During REM, vagal activity is decreased and SNS activity reaches high levels, which may be even higher than during wakeful-

ness[16, 58–63]. Moreover, increased sympathetic activity during sleep might be related to the increased frequency of cardiovascular adverse events in early morning hours after awakening[16, 64]. Overall, differences in LF power were observed to be higher (>140%) compared to HF power differences (>25%) during whole nights recordings, which may indicate higher variation in SNS activity than PNS activity throughout the night[55, 61]. The cyclic pattern of SWA was inversely coupled with LFnu and positively correlated with HF power[61, 65, 66]. In addition, RMSSD was shown to correlate well with nocturnal HF power and therefore PNS activity, but not with EEG mean frequency[67]. However, SDNN was shown to be higher in REM than in NREM, even though REM has been established to be sympathetically dominated illustrated by lower levels of HFnu and higher levels of LFnu[68].

The relationship between heart and brain activity is bidirectional. Not only changes in ANS activity can alter sleep regulation, but also disturbances in sleep influence the autonomic cardiac regulation. Thus, outflow from the heart affects the brain as well as the outflow from the brain affects the heart[20, 69]. Resulting from healthy aging, the elderly tend to have less SWS compared to younger adults, more arousals and longer periods of wakefulness after sleep onset[14, 70]. Additionally, time-domain HRV measures decrease throughout the course of the life as well as overall nocturnal HFnu, whereas nocturnal LFnu was increased compared to younger subjects. Those findings associated aging with decreased PNS activity and increased SNS activity [27, 37, 68, 71], which is additionally found in stress conditions[21, 63]. Since the length of SWS was demonstrated to be positively correlated with HFnu[68] and therefore PNS activity, SWS may be the leading process for the cardiovascular restorative functions during sleep. However, the underlying fundamental body processes resulting in lack of SWS and decrease in parasympathetic activity during sleep are still unknown. Despite this important relationship, no one, to the best of our knowledge, has studied the interaction between sleep, cardiac autonomic regulation and aging in subjects older than 60 years and across multiple nights. Moreover, previous work focussing on the relation of cardiac autonomic regulation and sleep has been mainly limited to correlations of SWA without considering slow wave specific parameters within NREM. Therefore, the aim of our research was to investigate sleep stage dependent differences in the cardiovascular parameters of heart rate, HRV and BPW in elderly across multiple nights per subject. Furthermore, since NREM and SWS have been indicated to be the most restful state for the body[15, 72], we aimed to assess correlations within NREM of specific slow wave and sleep parameters (e.g. amplitude, slope, frequency, number of slow waves or spindle power) with heart rate, HRV and BPW, which are indicating cardiac autonomic regulation, across multiple nights, to account for intra-individual differences.

Chapter 2

Materials and Methods

2.1 Subjects

This study was conducted as part of the wearable enhancement of sleep in aging (WESA) study using a mobile EEG recording device for in-home recordings. The study was approved by the Kantonale Ethikkommission Zürich (BASEC 2017-01436) and Swissmedic (2017-MD-0027). The EEG recording device records PSG and uses it for enhancing slow waves by targeted auditory stimulation. Participants were recruited through word-of-mouth advertisements, flyers and with the help of a centre for aging (Zentrum Dynamischen Alterns) and the elderly university (Seniorenuniverität Zürich). Subjects were between 60 - and 84 years old, German-speaking, with good general health status and living in a stable home situation in Switzerland. Women needed to take hormonal contraception if the menstrual cycle had not been absent for more than a year previous to the study start. Furthermore, we applied the following exclusion criteria: pregnant women, drug or alcohol abuse, intake of sleep altering medication, presence or history of diagnosed psychiatric or neurologic disorder, lesion of central nervous system, diagnosed internal disease (including cardiovascular disease), presence of sleep disorders, shift-work or participants living in situations which require several awakenings during the night, travelling more than two time zones in the month before intervention starts, skin disorders in face region, nicotine or cannabis use, high caffeine consumption or cognitive inability to follow study procedures.

2.2 Study design

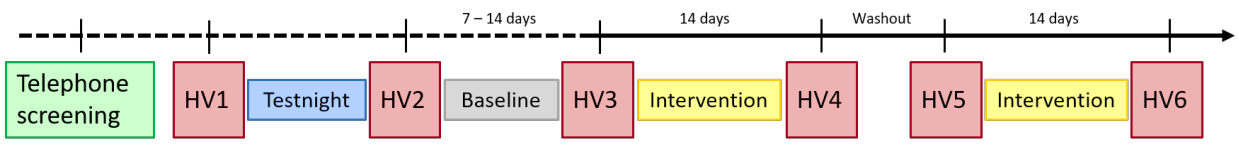

Figure 2.1: Study procedure of wearable enhancement of sleep in aging study. HV stands for homevisits. Intervention periods are either in sham or verum condition.

An overview of the study procedure is illustrated in Figure 2.1. During the screening phase of the WESA study, participants completed a telephone screening to check for inclusion and exclusion criteria as well

(A) SleepLoop Device (B) Headband (C) Everion

Figure 2.2: Illustration about the used devices. (A) shows the SleepLoop device for sleep recordings and stimulation of slow waves. (B) illustrates the headband used in the study to connect seven electrodes with the SleepLoop device. The cardiovascular parameters and additional physiological parameters were recorded with the Everion, which is shown in (C).

as to explain study duties. Afterwards, a testnight was scheduled. Participants signed informed consent and completed questionnaires, cognitive tasks, physiological measurements during the homevisit (HV) 1 and HV2 at each participant's home. Most importantly, we introduced all the devices used throughout the study, in particular the SleepLoop device, a smartphone for questionnaires and vigilance assessment and a wearable monitor Everion (Biovotion AG, Zürich, Switzerland), which is worn around the upper arm. We trained the application of the headband, which is shown in Figure 2.2b, in order to assure correct appliance in the evenings. Additionally, participants completed an audiometry to check for their hearing abilities. After the testnight, we checked for uncertainties and comfort with the device and conducted a lungfunction assessment. If subjects passed the testnight, the intervention phase started after 7 to 14 days of a baseline period, which consisted of only cardiovascular recordings with the Everion and daily questionnaires. Sleep was recorded, in addition to the tasks from the baseline, during two intervention periods of 14 consecutive days with a wash-out phase of 14 days in between. One intervention period was randomly assigned to auditory stimulation (verum) and the other one to no auditory stimulation (sham). However, only the nights in the sham condition were used for this research. Participants and the team involved in hvs were blinded about the conditions.

2.3 Biosignal recordings

2.3.1 EEG

The self-developed SleepLoop device (Mobile Health Systems Lab, Zurich, Switzerland), which is shown in Figure 2.2a, was used in combination with a self developed headband illustrated in Figure 2.2b. Auditory stimulation was provided through headphones (SleepPhones, AcousticSheep LLC, Erie PA, USA), which were embedded in the headband. Seven electrodes (Ambu BlueSensor N, Ballerup, Denmark) were located on each participant's face and connected with the SleepLoop device through connection cables (camed GmbH, Baden-Dättwil, Switzerland). EEG is measured with one electrode located at Fpz, two are used for eye activity, two for reference and ground behind the ears and two for EMG on the chin.

The polysomnographic recordings were sampled with 250 Hz and pre-processed using the EEGLAB toolbox for MATLAB (R2017b, Mathworks Inc., Natick, MA, United States). Recordings were band-pass filtered from from 0.5 to 35 Hz with `pop_eegfiltnew`. Afterwards, the data was down sampled to 128 Hz using `pop_resample` and 20 seconds power spectral density was calculated. We scored four nights

for every subject according to standard criteria[12]. Additionally, automated MATLAB scripts for slow wave detection were used to calculate different slow wave parameters (peak amplitudes, slope, frequency, number) for each 20 seconds epoch. Slow waves were defined if the frequency was below 4.5 Hz and either the negative-positive or positive-negative amplitude was higher than 50 μV. Power spectral density in the frequency range of 0.5 - 4.5 Hz was calculated for SWS, whereas sigma represented the power in the 11 - 16 Hz spindle band. Outliers were detected using the generalized extreme studentized deviate test for outliers, and linearly interpolated using the function `filloutliers`. Outliers at the 15 epochs in the beginning or the end were interpolated using nearest method.

2.3.2 Cardiovascular parameters

Measurements of the Everion, which is illustrated in Figure 2.2c, included heart rate, skin temperature, accelerometer data, steps, respiration rate, inter-beat-intervals, BPW, HRV, skin blood perfusion, galvanic skin response and energy expenditure. For further analysis, we selected heart rate, HRV and BPW as cardiovascular parameters and the accelerometer data in addition. All data was sampled with 51.2 Hz except accelerometer data with 50 Hz, and were downsampled and stored with 1 Hz. Biovotion calculated HRV using RMSSD of the inter-beat intervals detected by PPG with a five minutes sliding window. Inter-beat intervals prone to artefacts were not used for RMSSD calculations. RMSSD will be from now on referred to as HRV unless otherwise stated. Every measurement of the Everion included a related quality in which quality above 50 indicates compliance with medical grade data quality[49]. Therefore, only data coincide with higher data quality than 50 were used for analysis and statistics.

2.3.3 Synchronisation of cardiovascular parameters with EEG recordings

Due to the lack of a precise timestamp in the EEG data recorded by the SleepLoop device, the polysomnographic recordings needed to be synchronised with the Everion recordings for further analysis. Thus, start time of EEG recordings had to be determined. Since actigraphy is a well-established tool for sleep assessment[73, 74], the accelerometer signal of the Everion recording was used for alignment with EEG spectral power in the frequency range of 26.25 – 32.0 Hz, which was suggested to detect muscle artefacts in EEG[75].

The entire Everion accelerometer recording over the span of the 14 days in sham condition was split into 14 single nights, each ranging from 20:00 to 10:00. Afterwards, the nights were shortened if participants took off the Everion for more than 10 minutes in this timespan. To detect the time spent in bed, the following labelling procedure was used, which is illustrated in Figure 1 in the Appendix. First, each night was labelled with activity *on* or activity *off*, if threshold of the minimum accelerometer value of 0.8743 a.u. plus 0.4 a.u. was reached. The 0.4 a.u. was selected, since it fitted best for detection of activities during sleep. Then, periods in which activity was *on* for less than three seconds were set to *off*. Afterwards, activity *off* periods for less than five minutes were turned to *on*. Lastly, shorter *on* sections than five minutes were turned *off* again. Every *on* or *off* period was either classified as wake, rest, sleep or arousal, depending on the previous and later period. Finally, subjective information about the participant's bed-time, provided by a next morning questionnaire, was also considered for the classification. Bedtime was defined as the first timestamp in the first period identified as sleep and sleep offset as the last timestamp during the last sleep period.

To align EEG and Everion signals, accelerometer data was first averaged into 20 seconds segments by calculating the mean for every segment. Afterwards, the sleep offset time was synchronised with last point of the EEG recordings, in which the spectral power in frequency range of 26.25 – 32.0 Hz were below the

92nd percentile value. Afterwards, both recordings were modified by assigning each data point either *0* if its value was below the 92nd percentile, or *1*, if it was larger or equal to the 92nd percentile. The final start of recording time was determined using `finddelay` of the Signal Processing Toolbox with a maximal lag of 60 minutes. `finddelay` uses cross-correlation to identify the maximum correlation within the defined lag. The synchronised recordings were visually inspected for correctness. One illustrative example of a synchronised night is shown in Figure 2.3.

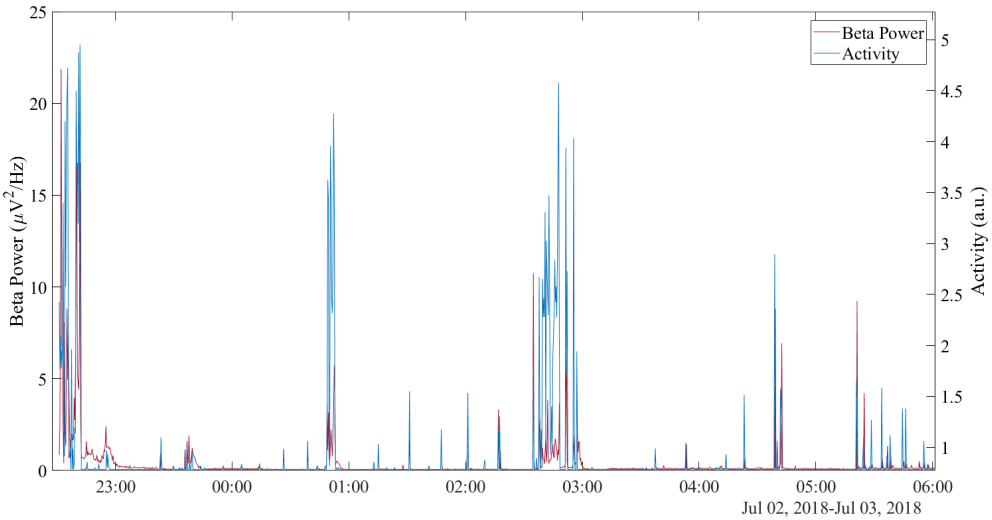

Figure 2.3: Illustrative example of synchronisation of beta power (26.25 – 32.0 Hz) of electroencephalography and activity signal of Everion for one subject across one night.

The Everion recordings were averaged again into 20 second periods matching the EEG epochs. Only epochs with more than 50% compliance with the medical grade data quality (> 50) were used to calculate the mean. Data points associated with inferior data quality and outliers detected by a moving median with a window width of five minutes were interpolated using the piecewise cubic hermite interpolating polynomial method.

2.4 Statistics

Statistical analysis was performed using R (3.5.2, R Core Team, Vienna, Austria). The residuals of the models were inspected for normal distribution, that is why SWA and the sigma band were log-transformed to approximate normal-distribution. Since we collected many datapoints, we analysed all scored nights with different dimension reductions, to test the effects for robustness. The different dimensions of these datasets are shown in Table 2.1. A median-night dataset consisted out of the calculated median of every sleep stage of each night and participant. Thus, one measurement for each sleep stage of every night was included in this dataset. For the epoch matched dataset, the same number of epochs for all sleep stages within one night were included. Therefore, the shortest sleep stage was selected and the identical number of epochs were randomly chosen from the other sleep stages in this night. This procedure was performed for every night of each subject. We calculated linear mixed effect models for all datasets using lme4 package of R[76] for heart rate, HRV, and BPW as dependent factors and the sleep stages as independent factor, with subject being entered as random factor (to account for repeated measures). Chi square test was used for determining fixed-effects terms. Posthoc p-values were obtained with Satterthwaite's method using the R package lmerTest. To compare the first and the second part of the night, the cardiovascular parameters

were entered as dependent factors, subject was entered as random factor and stage and part of the night as independent variables with interactions.

Inter-participant and intra-participant correlations during NREM (N2 and N3) between cardiovascular parameters and amplitude, frequency, number and slope of slow waves as well as the sigma band and SWA were calculated using the package rmcorr[77]. Since the HRV was calculated based on a five minutes window, the mean of heart rate, BPW and NREM parameters were calculated for five minutes windows as well. All data of five minutes epochs containing amplitudes or frequency equal zero were not considered for the analysis of all scored nights. The variability of the NREM characteristics represents the standard deviation within this five minutes window and with inclusion of epochs containing amplitude or frequency equal zero. We considered $p < 0.05$ as significant.

Table 2.1: Dimensions of analysed datasets

	Complete dataset	**Epoch-matched dataset**	**Median-night dataset**
N3	3'632	2'460	26
N2	14'546	2'460	26
REM	6'730	2'460	26
Wake	4'701	2'460	26
Total Observations	29'609	9'840	104

Table represents the number of observations in the sleep stages rapid-eye movement sleep (REM), non-REM (NREM) stage 2 (N2), NREM stage 3 (N3) and in wake, used for analysis of the three datasets. The complete dataset used all data for every sleep stage. In the epoch-matched dataset, the same number of epochs for all sleep stages within one night were included. Therefore, the shortest sleep stage was selected and the identical number of epochs were randomly chosen from the other sleep stages in this night. This procedure was performed for every night of each subject. The calculated median of every sleep stage of each night and participant was used for the median-night dataset.

Chapter 3

Results

3.1 Datasets used

Seven healthy elderly (two women, five men) aged 66.17 $\pm$ 2.57 years were included in this analysis. Out of the possible 98 (14*7) SHAM nights, a total of 15 nights were not complete due to bad quality of EEG recordings (1), bad quality of Everion recordings (1), missing EEG recordings (4) or missing Everion recordings (9). The Everion recordings were missing due to synchronisation problems between the coupled iPhone and the Biovotion cloud. The synchronisation of the EEG and the cardiovascular parameters of the Everion was successful for all complete datasets. One illustrative example of a synchronised night is shown in Figure 2.3. For each subject, four nights were scored. However, for one participant only two out of the four nights could be used due to missing Everion recordings. Therefore, 26 scored nights of the seven participants were used for data analysis. The sleep architecture of the scored nights is shown in Table 3.1.

Table 3.1: Sleep variables of subjects derived from visual scoring

	Time ± SD (min)	**Time ± SD (% Bedtime)**
Bedtime	443.13 ± 38.53	
Sleep period time	418.50 ± 38.31	94.44 ± 8.65
Total sleep time	382.28 ± 38.32	86.27 ± 8.65
Latency to N1	20.96 ± 17.80	4.73 ± 4.02
WASO	36.21 ± 12.44	8.17 ± 2.81
N1	61.94 ± 17.34	13.98 ± 3.91
N2	187.44 ± 30.39	42.30 ± 6.86
N3	46.63 ± 41.88	10.52 ± 9.45
REM	86.28 ± 28.52	19.47 ± 6.44
Sleep efficiency		91.31 ± 2.91

Data is presented as mean ± standard deviation of seven subjects and 26 nights in total (four nights each for six subjects and two nights for one). Total Bedtime: Time of wearing device. Sleep period time: Total sleep time + wake after sleep onset (WASO). Total sleep time: Time in non-rapid eye movement sleep (NREM) stage 1 (N1), NREM stage 2 (N2), NREM stage 3 (N3) and rapid eye movement sleep (REM). WASO: wake after sleep onset. N1 latency: time until first occurence of N1. Sleep efficiency = $\frac{Totalsleeptime*100}{totalbedtime}$ [13]

3.2 Cardiovascular parameters in different sleep stages

In order to investigate the changes of the cardiovascular parameters depending on the underlying sleep stage, we used linear mixed effect models for the complete dataset of the 26 scored sham nights. The progression of the SWA, heart rate, HRV, BPW and the sleep stages throughout the course of one night is illustrated in Figure 3.1. Additionally, nocturnal changes of heart rate, HRV and BPW with their corresponding sleep stage for an illustrative night are shown in Figure 3.2. The changes in the cardiovascular parameters indicated a slightly sine-wave like oscillation within NREM-REM cycles, which is e.g. best seen in the heart rate. Another example of one night is illustrated in Figure 3 in the Appendix, in which an opposite cyclic trend of BPW and heart rate depending on the underlying sleep stage is seen. Additionally, sleep stage specific differences are identifiable. Mean heart rate, HRV and BPW of every subject in the corresponding sleep stages N3, N2, REM and wake are shown in Figure 3.3. The black dots indicate the participant-corrected estimates for each sleep stage calculated with the linear mixed effect model. Analysis revealed that the heart rate is significantly dependent on sleep stage ($p < 0.001$) with the lowest heart rate of 59.88 bpm is found in N2. Heart rate in N3 is 1.08 bpm ($p < 0.001$) higher than N2 and 0.31 bpm higher than REM ($p = 0.01$). The highest heart rate of 66.27 bpm occurred during wake and was 5.32 bpm higher than N3 ($p < 0.001$) and 5.64 bpm higher than REM ($p < 0.001$).

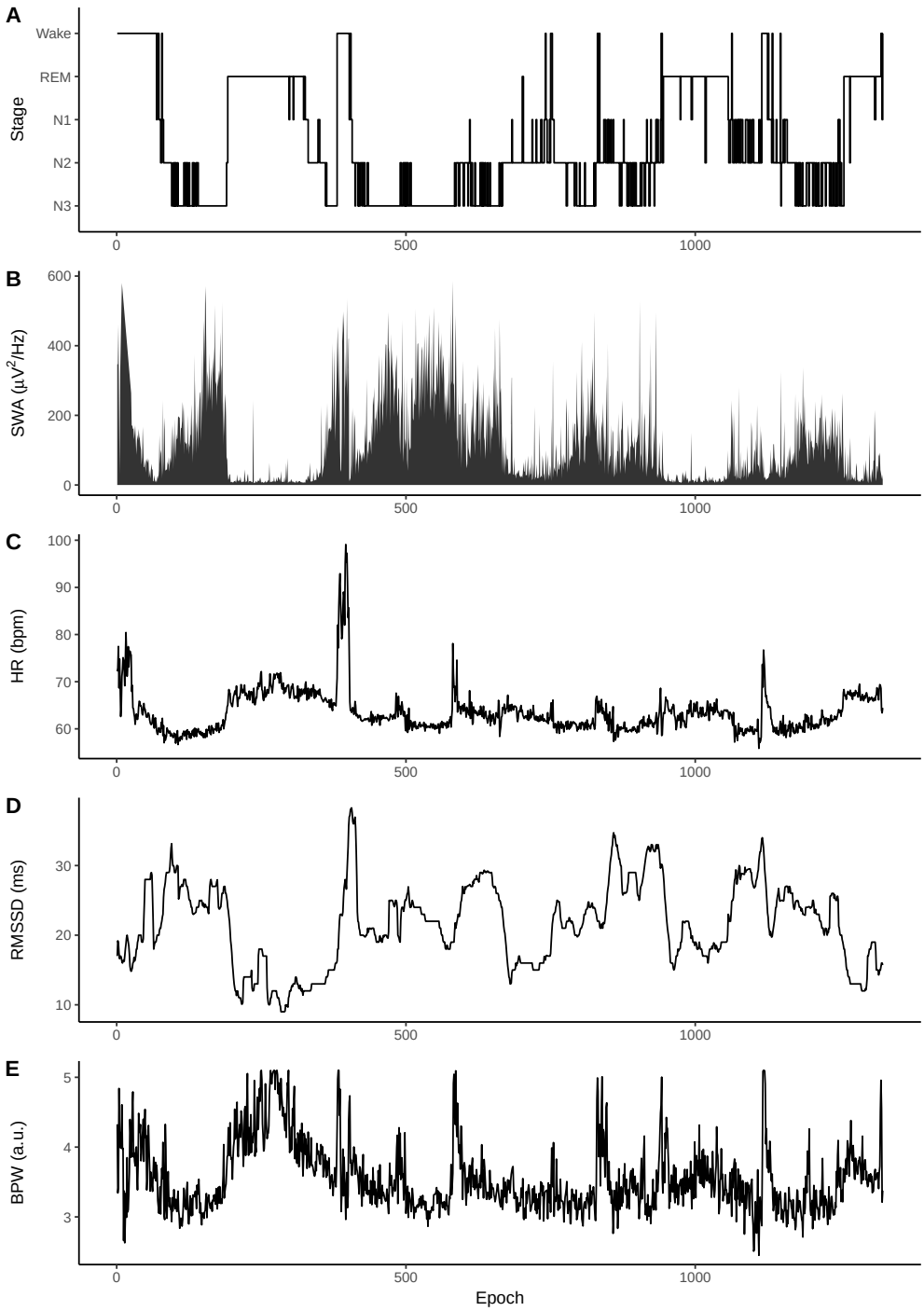

Figure 3.1: Recordings across one illustrative night. **A** shows hypnogram, which represents the sleep stage, **B** shows the slow wave activity (SWA), **C** the heart rate (HR), **D** heart rate variability (RMSSD) and **E** the blood pulse wave (BPW).

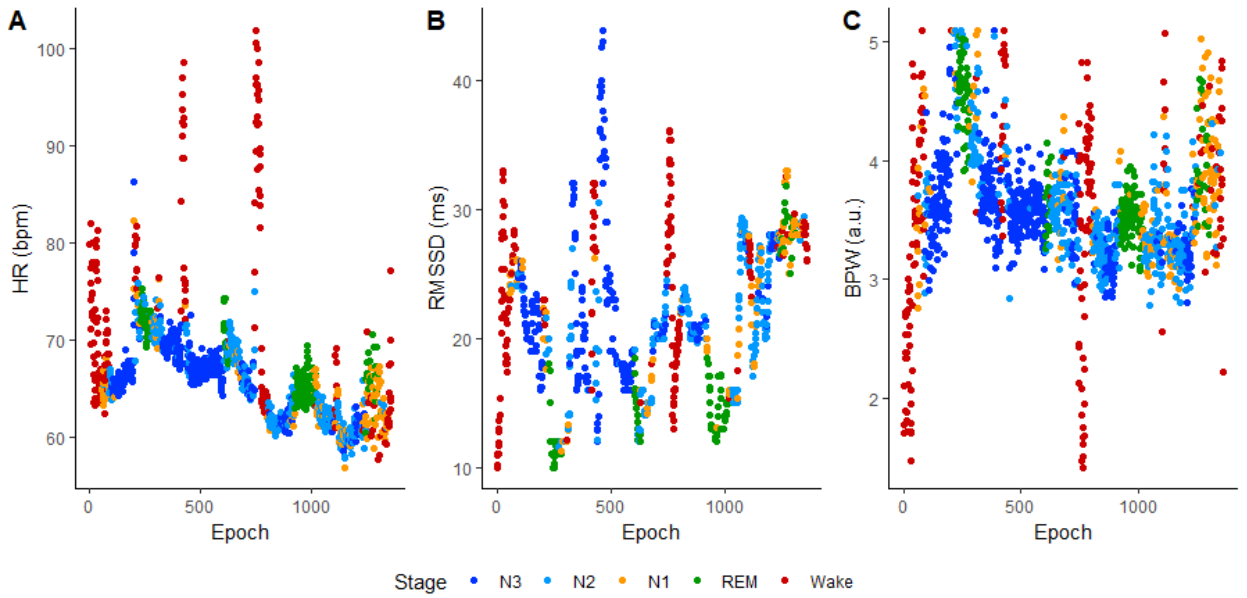

Figure 3.2: Cardiovascular parameters across one night of one subject with the coloured sleep stages representing non-rapid eye movement sleep (NREM) stage 3 (N3), NREM stage 2 (N2), NREM stage 1 (N1), rapid eye movement sleep (REM) and during wake. **A** illustrates nocturnal heart rate (HR) in which a overall decrease of HR throughout the night as well as a cyclic behaviour within NREM-REM alternations is identifiable. **B** shows heart rate variability (RMSSD) in which a cyclic behaviour is seen, which is interrupted by periods of wakefulness. **C** illustrates the blood pulse wave (BPW) in which predominantly periods of wakefulness interrupt the slightly decreasing BPW throughout the course of the night.

Sleep stage significantly predicted HRV ($p < 0.001$). HRV of N2 was the highest with 25.28 ms and no significant difference was found to N3. REM and wake HRV were both decreased by 3.28 ms and 0.78 ms, respectively, compared to N2 ($p < 0.001$ for both). Furthermore, HRV in N3 was 3.38 ms higher than REM ($p < 0.001$) and 0.87 ms higher than wake ($p < 0.001$). REM HRV of 22.00 ms was 2.51 ms lower than wake HRV ($p < 0.001$). BPW was highly dependent on sleep stage ($p < 0.001$) and was lowest during N2 with 2.76 a.u., N3 was 0.72% elevated (0.02 a.u., $p < 0.05$) compared to N2. REM BPW and wake BPW were the highest, with a BPW of 3.03 a.u. in wake, that is 9.78% higher than N2 (0.27 a.u., $p < 0.001$), 9.06% higher than N3 (0.25 a.u., $p < 0.005$) and 7.25% higher compared to REM (0.20 a.u., $p < 0.005$). A summary of the results is shown in Table 1 in the Appendix. Analysis of the reduced and median dataset showed comparable tendency of effects, but with less significance, and are illustrated in the Appendix in Table 3 and Table 4, respectively.

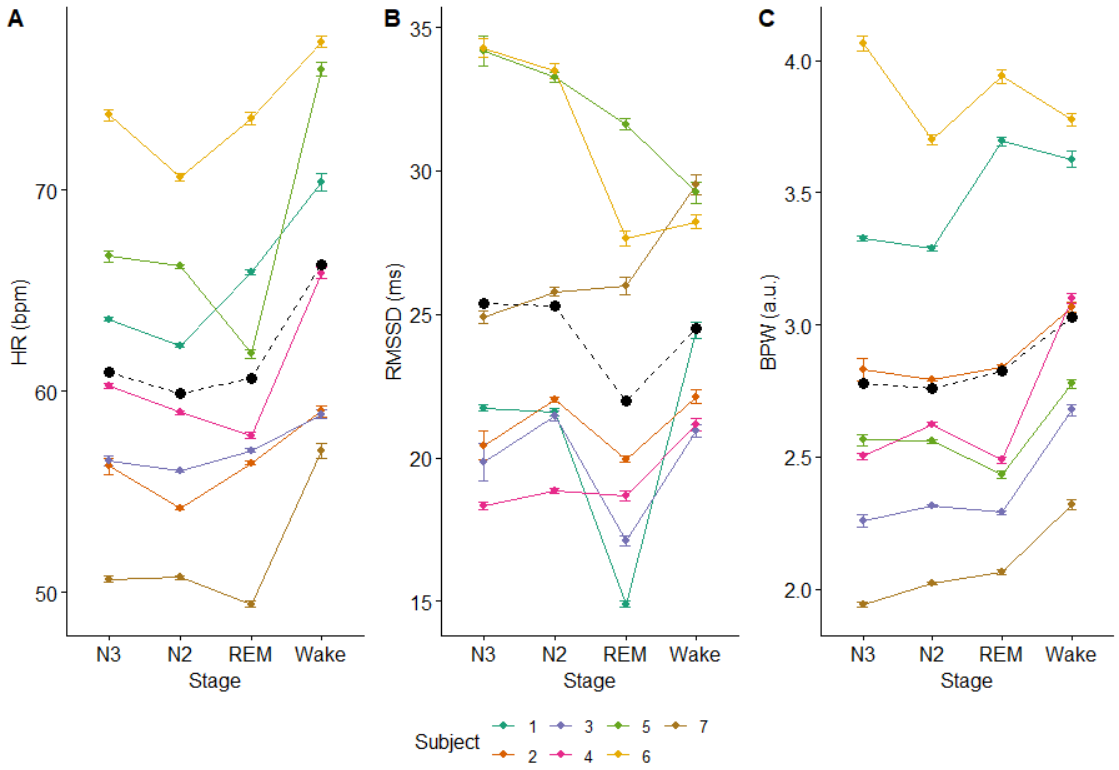

Figure 3.3: **A** shows mean heart rate (HR), **B** mean heart rate variability (RMSSD) and **C** blood pulse wave (BPW) in non-rapid eye movement sleep Stage 3 (N3), Stage 2 (N2), rapid eye movement sleep (REM) for every subject (n=7). Black dots indicate the for participant-corrected results of the linear mixed effect model of the complete dataset.

3.2.1 Effect of part of the night

Since NREM length is getting shorter towards the end of the night whereas REM episodes are getting longer, the distribution of the sleep stages changes throughout the course of the night. Therefore, we analysed both halves of the night separately, which will be from now on referred to as first and second part of the night. The linear mixed effect model revealed significant interactions of part of the night and sleep stage ($p < 0.001$). Therefore, we compared sleep stage specific fixed effects for both parts of the night individually, with the results illustrated in Table 3.2. Differences between the two parts of the cardiovascular parameters for each sleep stage are shown in Table 3.3. The heart rate in the second part of the night decreased significantly in every sleep stage, which was already indicated in Figure 3.1. REM and N3 heart rate did not differ in the second part. We found significantly higher HRV in N3 ($p < 0.05$) compared to N2 in the first part, whereas in the second part, HRV in N3 and N2 were not significantly different anymore. Additionally, BPW of N3 in part two was significantly higher compared to N2 and REM (+0.04 a.u., $p = 0.01$), whereas in part 1, BPW in N3 was significantly lower compared to N2 and REM (-0.14 a.u., $p < 0.001$). A summary of the findings of both parts is shown in Table 1 in the Appendix.

Table 3.2: Fixed effects of linear mixed effect model with N2 as reference

	HR (bpm)			RMSSD (ms)			BPW (a.u.)		
Predictors	Estimates	CI	P	Estimates	CI	P	Estimates	CI	P
First part									
N2 (Intercept)	61.20	56.47 –65.94		25.36	21.33 – 29.39		2.78	2.33 – 3.24	
N3	−0.16	−0.42 – 0.09		0.37	0.03 – 0.71	*	−0.07	−0.09 – −0.05	***
REM	1.35	1.10 – 1.59	***	−4.01	−4.34 – −3.68	***	0.07	0.05 – 0.09	***
Wake	5.73	5.49 – 5.97	***	−1.34	−1.66 – −1.02	***	0.16	0.14 – 0.18	***
Second part									
N2 (Intercept)	58.66	52.45 –64.87		25.12	20.81 – 29.42		2.75	2.30 – 3.20	
N3	1.12	0.72 – 1.53	***	0.31	−0.15 – 0.77		0.10	0.07 – 0.13	***
REM	0.72	0.51 – 0.94	***	−2.53	−2.77 – −2.28	***	0.06	0.04 – 0.07	***
Wake	7.01	6.72 – 7.30	***	−0.45	−0.78 – −0.12	*	0.43	0.41 – 0.46	***

Fixed effects (estimates) of linear mixed effect model of the first part of sleep with non-rapid eye movement sleep (NREM) stage 2 (N2) as reference, for all measurement points of each participant (n=7). NREM stage 3 (N3), REM and wakefulness values are shown with 95% confidence intervals (CI). Dependent variable is heart rate (HR), heart rate variability (RMSSD) and blood pulse wave (BPW) with subject as random factor and sleep stage as independent factor and 15'197 observations for the first part and 14'412 for the second part. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$

Table 3.3: Fixed effects of linear mixed effect model for part analysis

	HR (bpm)			RMSSD (ms)			BPW (a.u.)		
Predictors	Estimates	CI	P	Estimates	CI	P	Estimates	CI	P
N2 part 1 (In.)	61.01	55.57 – 66.45		25.40	21.35 –29.45		2.77	2.32 – 3.22	
N2 part 2	−2.21	−2.39 – −2.02	***	−0.23	−0.45 – 0.00	*	−0.02	−0.04 – −0.01	**
N3 Part 1 (In.)	61.21	55.77 – 66.66		25.31	21.25 –29.37		2.77	2.31 – 3.22	
N3 Part 2	−1.37	−1.80 – −0.94	***	0.25	−0.27 – 0.78		0.06	0.02 – 0.09	**
REM part 1 (In.)	62.63	57.19 – 68.07		21.13	17.07 –25.19		2.87	2.42 – 3.32	
REM part 2	−3.12	−3.40 – −2.84	***	1.38	1.03 – 1.72	***	−0.07	−0.09 – −0.04	***
Wake part 1 (In.)	66.50	61.05 – 71.94		24.34	20.28 –28.40		2.92	2.47 – 3.37	
Wake part 2	−0.59	−0.92 – −0.26	***	0.42	0.02 – 0.83	*	0.27	0.24 – 0.29	***

Fixed effects (estimates) of linear mixed effect model for part analysis of the night for the sleep stages non-rapid eye movement sleep (NREM) stage 2 (N2), NREM stage 3 (N3), rapid eye movement sleep (REM) and wake. Dependent variable is heart rate (HR), heart rate variability (RMSSD) and blood pulse wave (BPW) with sleep stage as independent factor and subject as random factor. Part 1 was taken as reference (In: Intercept) for comparisons to part 2. Results are shown with 95% confidence intervals (CI) of seven subjects. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$

3.3 Relationship of brain activity and cardiovascular parameters in NREM

In order to account for the individual fluctuations in sleep, that cannot be displayed by averaging each sleep stage, we investigated the relationship of within-subject and between-subject fluctuations in NREM oscillations with the cardiovascular parameters using repeated measures correlation analysis. In addition to SWA, we chose the slow waves parameters amplitude, frequency, number and slope since these are related to sleep depth. Information about the distribution of analysed NREM parameters can be found in the Appendix in Figure Figure 2. We found no significant relation ($p > 0.05$) between length of SWS or length of N2 with heart rate, HRV or BPW. However, within-participant correlation are positive, but non-significant, as illustrated in Figure 3.4d. The repeated measures correlation analysis revealed that increase in SWA is positively correlated with heart rate (see Figure 3.4b) and BPW ($r_{rm} = 0.09$, p = 0.002), although the relationship between HRV and SWA is non-significant. Increase in frequency of slow waves is positively correlated with heart rate and BPW and negatively correlated with HRV, which are illustrated in Figure 3.4. Additionally, increase in frequency variability is negatively correlated with HRV ($r_{rm} = -0.067$, $p < 0.005$). We found very weak positive correlations between amplitude of slow waves and heart rate ($r_{rm} = 0.09$, $p < 0.01$) and BPW ($r_{rm} = 0.06$, $p < 0.05$), however, amplitude and HRV are non-significantly related. Furthermore, increase in slope of slow waves is positively correlated with heart rate ($r_{rm} = 0.19$, $p < 0.001$) and BPW ($r_{rm} = 0.12$, $p < 0.001$), whereas slope of slow waves is slightly negatively correlated with HRV ($r_{rm} = -0.06$, $p < 0.05$). Number of slow waves are positively correlated with heart rate ($r_{rm} = 0.18$, $p < 0.001$) and BPW ($r_{rm} = 0.13$, $p < 0.001$), but analysis revealed no significant correlation between HRV and number of slow waves.

Sleep spindles are prominent oscillations in NREM and therefore, we explored the relationship of the sigma band with the cardiovascular measurements. Increase in sigma band power is positively correlated with heart rate ($r_{rm} = 0.22$, $p < 0.001$) and BPW (see Figure 3.4f), but again, not significantly correlated with HRV. However, we found that increase in spindle band variability is negatively correlated with HRV ($r_{rm} = 0.058$, $p < 0.005$).

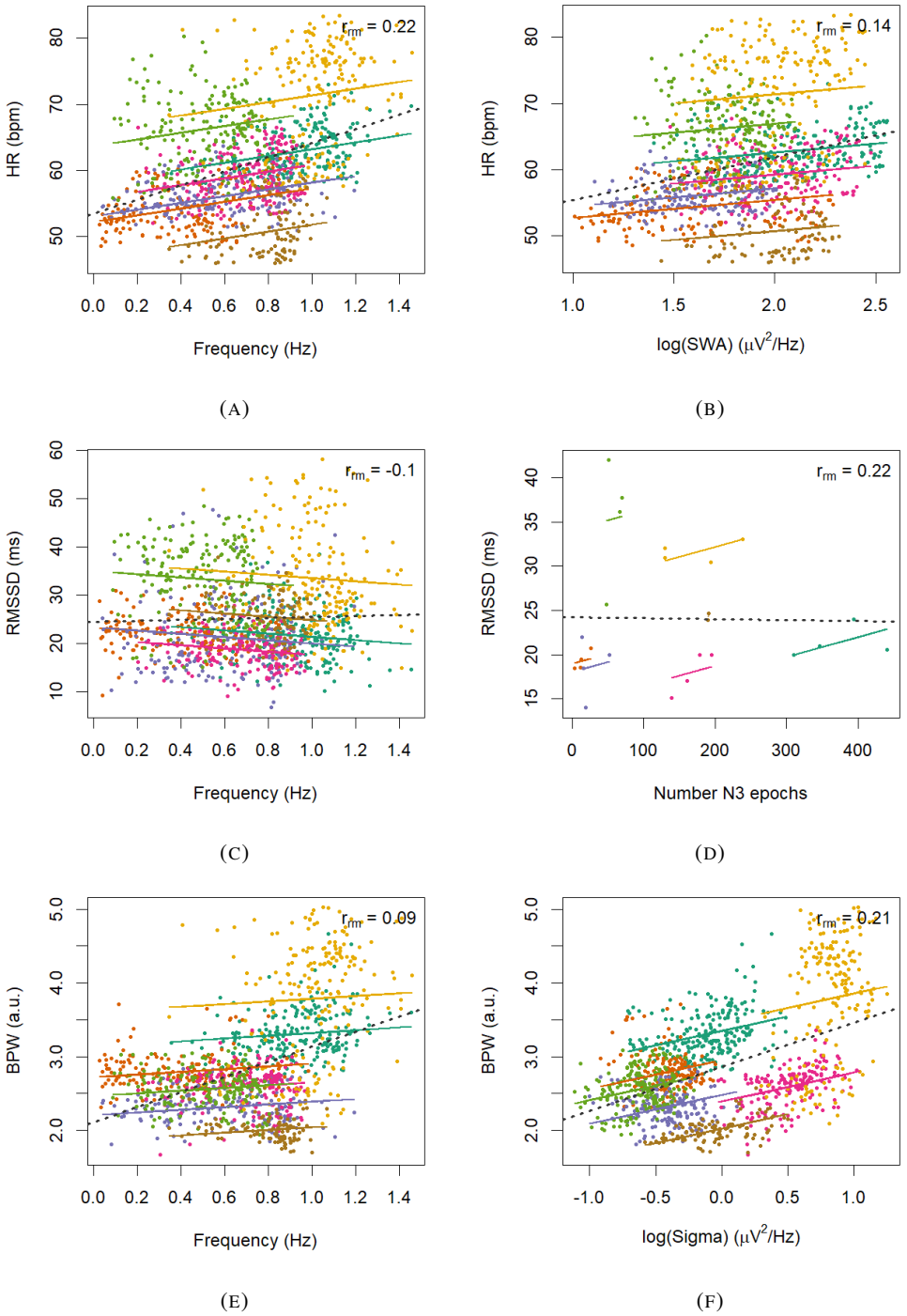

Figure 3.4: Repeated measures correlation for five-minutes average of non-rapid eye movement sleep (NREM) parameters and heart rate (HR), heart rate variability (RMSSD) and blood pulse wave (BPW). All repeated measures correlations were significant ($p < 0.001$) except (B) with $p = 0.34$. SWA is slow wave activity, N3 NREM stage 3. Each unique colour represents one participant, with intra-participant correlations shown by the coloured line. Overall repeated measures correlations are indicated by the black dotted line. All models have 1'206 degrees of freedom except (B), which has 18.

Chapter 4

Discussion

Our results demonstrate for the first time, that the sympathovagal balance in elderly in the second part of the night differs compared to the first part of the night. Furthermore, low frequency slow waves were associated with increased parasympathetic modulation of the heart, whereas increased SWA was related to increased sympathetic activity of the cardiac autonomic regulation.

4.1 Cardiac autonomic regulation across sleep stages

Based on our findings, ANS activity is changing as a result of the underlying sleep stage. We observed the highest heart rate during wake, followed by REM, which we expected, since sympathovagal balance is known to be shifted towards sympathetic predominance during those episodes. The lowest HRV was found in REM, which indicates the parasympathetic withdrawal[16, 58, 59, 61, 68] and increased BPW compared to NREM, which supports sympathetic predominance. By only assessing HRV, we would have concluded that PNS activity during wake periods is higher than during REM. However, BPW and heart rate were both significantly higher in wake episodes, indicating a shift towards parasympathetic withdrawal. Concurrent with the increase in heart rate in the beginning of an arousal, HRV was also increasing (see Figure 3.1). Since we only analysed RMSSD, which was calculated based on a five minutes window, arousals change HRV values for five more minutes after the end of the arousal. Therefore, wake values of HRV need to be interpreted with caution and in relation to the reported heart rate as suggested by Sacha[34]. The BPW observations support the tendency of higher sympathetic activity during REM and wake compared to NREM and appear to be less disturbed by arousals.

Unexpectedly, heart rate was found to be lowest during N2 in complete night recording. Reviewing literature, we expected heart rate to be lowest during N3, since the sympathetic activity was shown to be lower, the deeper the sleep is[16]. Additionally, BPW and HRV findings support that the lowest SNS activity and the highest PNS activity, respectively, are in N2, rather than in N3. Furthermore, we found heart rate in N3 to be even higher than during REM. Since literature reported a decrease in heart rate and increase in PNS activity throughout the course of the night[17, 63], analysing whole night recordings may not be sufficient to reveal all differences in cardiovascular parameters. We found significant decreases in heart rate in every sleep stage in the second part of the night compared to the first part. However, one study reported that heart rate increased during the first NREM episodes and decreased throughout the rest of the night thereafter[55], which we were able to identify in some nights (see e.g. the illustrative example in Figure 3.2). Since NREM

episodes are established to get shorter towards the morning, whereas length of REM episodes increase, the first increase in heart rate is influencing the longest NREM episode, in particular, since aging is known to diminish length of SWS[14, 70]. Therefore, relatively more epochs of SWS with the higher heart rate were used for the complete night analysis. However, since we found comparable tendencies for all the reduction levels of our datasets, however not with the same significance, the observed effect is indicated to be robust.

Cardiovascular parameters for part of the night differed compared to complete night. Surprisingly, heart rate in N3 in the first part of the night was slightly, but non-significant, lower than heart rate in N2, possibly due to the higher parasympathetic and lower sympathetic modulation. Additionally, the heart rate in N3 was significantly lower than during REM and therefore in contrast to the complete night analysis. Hence, the cardiac autonomic regulation in the first part of the night confirmed previous literature, with SWS being the most restorative state of sleep[16, 58, 59, 61, 68]. However, we found changes in sympathovagal balance of the underlying sleep stages in the second part of the night. First, sympathetic and parasympathetic activity were increased in wake episodes of the second part. The latter might be due to the more frequently occurring arousals towards the end of the night[14, 70] and therefore, the same limitations for RMSSD calculations as previously discussed apply. Arousals may also result in an increased sympathetic modulation. However, heart rate was decreased during wake, which might still indicate some degree of higher parasympathetic activity in the second part, which has been previously described[63]. Moreover, decreased heart rate in N2 and REM of the second part indicated a shift of the sympathovagal balance towards PNS predominance, which may be caused due to decreased BPW in both stages. However, absolute parasympathetic modulation increased in REM and decreased in N2. Thus, the decrease in heart rate of the second part in REM was 4.98% higher if compared to a decrease of 3.62% in N2. Paradoxically, we observed 2.17% increased sympathetic modulation in N3 in the second part, which resulted in significant higher sympathetic modulation compared with REM. Even though parasympathetic activity in N3 was still higher than in REM and heart rate in N3 decreased 2.24% compared to the first part, heart rate of both stages did not differ. Therefore, N2 seems to be the most favourable stage for energy conservation reflected by lowest sympathetic and highest parasympathetic activity in the second part[22]. Hence, our results of the second part suggest a shift in the nocturnal cardiac autonomic regulation in NREM and especially SWS when comparing elderly with younger, even though previous research suggested no difference in the underlying nocturnal cardiac autonomic regulation with aging[71].

This change in cardiac autonomic regulation, particularly in SWS, might be caused by decreasing length of SWS towards the end of the night[14, 15]. Moreover, SWS duration is decreasing with age[70] and therefore, NREM in elderly may often be on the border between N2 and N3. For N3, more than 20% of an epoch must consist of slow waves[12] and therefore, the differences in number of slow waves between N2 and N3 might not be that pronounced when compared to younger participants. Since analysis on a group level is not accounting for the inter-individual differences, the variability between the subjects cannot be demonstrated. In Figure 3.3, the differences in the cardiovascular parameters in the sleep stages as well as the approximate mean value across all the sleep stages are clearly seen. Furthermore, as illustrated in 2, the length of SWS was highly different across the subjects as well. The subject with longest SWS duration and one of the subjects with the second longest SWS duration did have slightly higher overall heart rate and BPW, compared to the others and were both women. Women not only tend to have more SWS[15], but also an increased mean heart rate and HF power compared to men[33, 78], even though aging was demonstrated to diminish the gender differences of heart rate and HRV in 24 hours recordings[37]. Thus, results for SWS may be influenced by gender imbalance and the high variability of SWS duration, which might be more pronounced in the second part of the night.

In summary, sleep stage and course of the night differentially affect cardiac autonomic regulation. Furthermore, inter-individual differences need to be considered in the analysis based on the group-level. Therefore, sleep stage, part of the night and the between-subject variability need to be carefully considered in future studies.

4.2 Relationship of brain activity and cardiovascular parameters in NREM

In contrast to previous findings[68], we found no significant correlation between length of SWS and HRV, which might be due to the low numbers of subjects and included nights. The positive correlations of heart rate and BPW, as well as the negative correlation of HRV with slow wave frequency may indicate a shift of the sympathovagal balance towards predominant PNS activity with low frequency slow waves compared to higher frequency slow waves. Thus, low frequency slow waves might be more restorative. However, amplitude and slope of slow waves were both positively correlated with heart rate and BPW, indicating higher SNS activity with increased amplitude, which is according to the likewise positive correlation with SWA. However, since SNS is known to be lowest in N3[16], in which additionally the highest SWA occurs[15], we expected heart rate and BPW to be negatively correlated with SWA. We found no correlations of HRV with SWA, although previous studies reported SWA to be correlated with HF power or LFnu[61, 65–67]. Nevertheless, the use of RMSSD might explain the lack of correlation. Since we found increased sympathetic modulation in N3 compared to N2 across the complete night, which was caused by the the second part, the correlation analysis indicates the previously discussed shift in cardiac autonomic regulation as well. Furthermore, previous findings suggested a time-lag of brain activity compared to cardiovascular parameters[54, 58, 61, 71]. However, since we did not account for a time-lag, the possible time-shifted correlation of SWA with HRV is not reflected in our results.

BPW and heart rate were both positively correlated with number of slow waves. However, the analysis of number of slow waves is ambiguous since there is a conflict with frequency. An epoch filled with low-frequency slow waves consists of less slow waves than an epoch filled with higher frequency slow waves and may not be distinguished from another epoch with the same number of higher frequency slow waves, although SWA is increased in the first epoch. Therefore, the weak positive correlation between heart rate and BPW with the number of slow waves needs to be interpreted in relation to the underlying frequency. The power spectral density of the spindle band correlated positively with heart rate and BPW, both illustrating higher heart rate and SNS activity with higher amount of sleep spindles. Although HRV did not correlate with the sigma band, HRV was slightly related with the variability of sigma power. Furthermore, we discovered very weak positive correlations between frequency variability and HRV. However, further research is needed to better understand possible interactions of the possible coupling of variability in the heart rhythm with variability in the brain rhythm.

Nevertheless, in general, all observed correlations were weak to very weak. Since we only analysed correlations within N2 and N3 sleep, differences in slow wave parameters and SWA may be dampened compared to correlation analysis across all sleep stages, even though Rothenberger et al. reported positive correlations of HF power and SWA within NREM in midlife women[65]. Additionally, the correlations in NREM across the complete night may be different from changes within one NREM-REM cycle, since the coupling of HF power and SWA was demonstrated to decrease from cycle to cycle and is even different within one cycle[65]. To conclude, the slow wave specific parameters and spindle band power differentially influence the cardiac autonomic regulation within NREM sleep.

4.3 Limitations

A major limitation of our research is the low numbers of participants, even though we used repeated measures correlations to account for the within-participant nightly differences. High inter-participant variations in heart rate, HRV and BPW as well as length of SWS may have influenced the results. Additionally, there was a gender bias, since only two out of seven participants were female. Heart rate and HRV has been previously established to significantly differ for gender[78] and therefore, higher number of participants would allow correcting for gender as well. Furthermore, we only used RMSSD for HRV. Although we found changes in the cardiovascular parameters, the underlying processes causing those changes remains unknown. Decreased HRV cannot only be caused by decreased PNS function or increased SNS activity, but can be due to decreased responsiveness of the heart or lack of reflex feedback to the central nervous system[18]. Therefore, time domain measures of HRV are not capable of representing the autonomic dynamics underlying the changes in HRV, RMSSD can only be used to estimate changes in PNS activity[19]. To better identify specific dynamic relationships of the interaction of sleep and cardiovascular autonomic regulation, frequency domain analysis need to be included in future research.

The required synchronisation of Everion recordings with the EEG prior to analysis is another important limitation. Hence, our results are not as accurate as if both recording devices were synchronised. However, since we averaged the NREM episodes to five minutes segments, effects of possible slight misalignments should not fundamentally influence the results. Different studies have previously demonstrated that changes in HRV preceded changes in brain activity by around 5-10 minutes[54,58,61,71], which might be ambiguous without perfect synchronisation. Thus, the lack of correlation between SWA and HRV might be since we did not account for the possible delay, which needs to be considered in future studies. Furthermore, since the validation of BPW and its possible correlation with stress, and hence SNS activity, has not been published by Biovotion yet, the interpretation and value of the BPW measurement remains to be done with caution.

We analysed the cardiovascular parameters dependent on stage and part of the night or within NREM sleep, but without consideration of sleep cycles. First, analysis of part of the night was based on total bedtime and therefore, may be not as accurate as considering the time for N1 latency until the sleep offset in the morning. Moreover, parasympathetic function has been shown to increase across successive sleep cycles, whereas SWA has been found to decrease throughout the course of the night[15, 63]. Additionally, research suggests different underlying ANS functions dependent on if N2 is before or after a period of SWS[79], which cannot be assessed without considering cycles. Furthermore, we calculated the mean of the different slow wave parameters for five minutes windows, without taking the sleep cycles into account as well. The analysis of the NREM correlations was performed for the complete NREM episodes of one night and therefore, influence of part of the night or effects of sleep cycles might have distorted the correlations with the cardiovascular parameters. Therefore, our findings just reflect the overall correlation within NREM or with sleep stages without considering the underlying processes during sleep.

Even though our research showed promising results, the biological relevance of the significant changes in the cardiovascular parameters remains unclear. Therefore, future research is needed to determine, if the discovered changes between sleep stages and parts of the nights imply biological importance by e.g. discovering the relationship of altered cardiac autonomic regulation and higher odds for cardiovascular disease generation or progression.

Lastly, changes in breathing patterns are known to influence HRV[24,47,48]. Unfortunately, the data quality of the Everion measurement of the respiratory rate was low and therefore, could not been included into the analysis. Hence, we were not able to control our results for one major influence factor of HRV.

4.4 Outlook

To gain more insight about the interaction of both SNS and PNS activity within sleep, frequency domain HRV measurements, as for instance HF and LF power, should be added to the time-domain measurements. Those established indirect measurements of ANS function could also be used for comparison with BPW as measurement for stress, to further confirm our findings. Additionally, non-linear HRV analyses, like entropy derived measurements, could reveal further insight of the underlying autonomic regulation[69]. However, the used cardiovascular parameters and additional HRV measurements are only indirect measurements for cardiac autonomic regulation. Therefore, direct measurements of PNS activity or SNS activity like microneurography or invasive animal studies could provide deeper insight. Furthermore, the analysis of more subjects is needed to reduce inconsistent findings, which might have been caused by the unequally distributed SWS duration and resting heart rate.

Although the analysis of the parts of the nights already revealed significant differences, splitting the night into two parts will never perfectly account for the cyclic behaviour of NREM and REM throughout the course of the night. To further investigate the mechanisms and coupling of the different ANS activity representatives, within-cycle and between cycle-analysis is indispensable. Nested models for analysis of the sleep stages to control for more factors like gender, heart rate, stress, part of the night or even cycle within the night could possibly reveal additional results. Furthermore, analysis of for example the number of arousals within one cycle could expose the possibility of arousals interfering with the sleep and disturbing a continuous process of restoration, and therefore result in sympathovagal imbalance.

Finally, comparison of nights with different conditions could be added. The setup of the WESA study allows comparison of the sham nights used for this research with verum nights of the same participants, in which SWS should have been enhanced by auditory stimulation. This may reveal important and novel insights for the relationship and possible causality of brain activity with cardiac autonomic regulation during sleep.

Chapter 5

Conclusion

This is the first study showing that part of the night and underlying sleep stage differentially affect nocturnal cardiac autonomic regulation in elderly. We confirmed significant differences between heart rate and sympathovagal balance across different sleep stages with higher sympathetic activity during REM and wake periods and increased parasympathetic activity during NREM. In the first part of the night, SWS was the sleep stage with the highest parasympathetic and lowest sympathetic modulation, which is favourable for body energy conservation and indicates the most favourable state for restoration. However, SWS in the second part coincided with increased sympathetic modulation, which was even higher than during REM. Moreover, we showed that lower frequency slow waves are associated with higher HRV and and lower BPW, which coincided with decreased heart rate. Hence, low frequency slow waves might be more restorative, indicated by lower sympathetic activity and elevated parasympathetic activity. Additionally, heart rate and BPW were positively correlated with amplitude and slope of slow waves, as well as SWA, indicating a higher sympathetic activity in deeper sleep, which is contradictory to previous findings. Our findings therefore suggest, that there might be a shift in cardiac autonomic regulation throughout the course of night in elderly, which might be associated with the increasing risks for cardiovascular diseases with age. Nevertheless, more subjects need to be included in future research. Additionally, within-cycle and between-cycle comparison of the relationship of the cardiovascular parameters with the underlying sleep stage and with NREM sleep parameters might provide conclusive evidence about cardiac autonomic regulation during sleep. Moreover, future research should include sleep modulating interventions to reveal not only an associative relationship, but to identify a possible causal role of sleep in changes in cardiovascular autonomic regulation.

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Acronyms

ANS	Autonomic nervous system
bpm	beats per minutes
BPW	Blood pulse wave
EEG	Electroencephalography
EMG	Electromyography
EOG	Electrooculography
HF	High frequency band of HRV measurement
HFnu	High frequency band in normalised units
HR	Heart rate
HRV	Heart rate variability
HV	Homevist
LF	Low frequency band of HRV measurement
LFnu	Low frequency band in normalised units
NREM	Non-rapid eye movement sleep
N1	Stage 1 of non-rapid eye movement sleep
N2	Stage 2 of non-rapid eye movement sleep
N3	Stage 3 of non-rapid eye movement sleep, also referred to as SWS
PNS	Parasympathetic nervous system
PPG	Photoplethysmography
PSG	Polysomnography
REM	Rapid eye movement sleep
RMSSD	Root-mean square of successive differences of successive NN intervals (HRV measurement)
RSA	Respiratory sinus arrhythmia
SDNN	Standard deviation of NN intervals (HRV measurement)
SNS	Sympathetic nervous system
SWA	Slow wave activity (power spectral density in frequency range of 0.75 - 4.5 Hz)
SWS	Slow wave sleep (stage 3 of non-rapid eye movement sleep) or deep sleep
WESA	Wearable enhancement of sleep in aging study

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