TAMPEREEN TEKNILLINEN YLIOPISTO
TAMPERE UNIVERSITY OF TECHNOLOGY

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Estimation of sleep recovery in shift working long-haul truck drivers – A heart rate variability based study

MASTER OF SCIENCE THESIS

Subject approved by Department Council
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Examiners: Professor Jari Viik
Ph.D. Jussi Virkkala

ABSTRACT

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Prolonged work hours, shortened and irregular sleep patterns often leads to inadequate recovery in shift workers resulting in increased sleepiness or fatigue during the day. Heart rate and heart rate variability (HRV) have been often used in occupational health studies to examine sleep quality and recovery. The aim of the current study was to determine the factors affecting the recovery process in shift working long-haul truck drivers and to assess the impact different shifts have on the drivers' sleep health.

Of the recruited volunteers, data collected from 38 volunteers (Age: 38.46 ± 10.89 years) satisfied the inclusion criteria for this study. Driver demographics and background questionnaires were obtained prior to measurements. R-R intervals and actigraphy data were collected for three intensive measurement days (non-night shift, night shift and leisure day) and subjective measures of sleep quality, recorded on the sleep-diary, were used for the analyses. Several time- and frequency-domain HRV indices were calculated in 10-minute segments and averaged on an hourly basis and for the entire duration of sleep. All tests for statistical significance was conducted on a within-subject basis.

Comparison of HRV indices over the entire sleep duration recorded on different intensive measurement days revealed no significant differences except for LF/HF ratio (Leisure day vs. Night shift, $p < 0.05$). Sleep duration and efficiency were significantly lower on duty days. Regression analyses indicated VLF power was strong predictor of recovery and 31% of the outcome was influenced by explanatory factors. SDNN ($r = 0.555$, adjusted $r^2 = 0.248$, $F(9, 92) = 5.166$, $p < 0.001$), RMSSD ($r = 0.414$, adjusted $r^2 = 0.131$, $F(9.92) = 4.229$, $p < 0.05$) and HF power ($r = 0.460$, adjusted $r^2 = 0.165$, $F(9.92) = 4.526$, $p < 0.001$) were significantly associated with age and sleep duration. Short-term variability indices, RMSSD and HF power, were moderately influenced by diurnal variations.

The results suggest that despite the fact that shift type does not have any direct consequences on sleep recovery, the odd work hours and irregular sleep schedules pose an indirect effect. The truncated sleep length, especially seen after night shift work, have been significantly associated with the impaired recovery and is contributed to by other short-term (diurnal variations) and long-term (ageing) factors. These results provide a basis for planning shift schedules such that direct or indirect manifestations of shift type-related influence on recovery are mitigated.

PREFACE

I take immense pleasure in thanking my supervisors, Professor Jari Viik and Professor Mikael Sallinen for their motivation and encouragement throughout the course of my research. You have been my greatest source of inspiration.

This study was a part of the *An educational intervention to promote safe and economic truck driving* project, conceived and implemented at the Finnish Institute of Occupational Health, Helsinki. I am indebted to all the project members, with special mention to researchers Mia Marianne Pylkkönen and Maria Sihvola for their endless support and guidance in the project.

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Last, but not least, I wish to thank my extended family for all their faith and trust. Words can never suffice to thank my parents, Jeyanthi Pradhapan and Veerappan Pradhapan, for their unconditional love and sacrifice. To them, I am eternally grateful.

Tampere, November 12th, 2013

PARUTHI PRADHAPAN

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LIST OF TERMS AND ABBREVIATIONS

ApEn	approximate entropy
HF power	power in the high frequency band
HRV_{index}	HRV triangular index
LF power	power in the low frequency band
NN50	heart beat intervals greater than 50 ms.
pNN50	percentage of NN50
Poincaré SD1	standard deviation of point's perpendicular to the line of identity
Poincaré SD2	standard deviation of point's parallel to the line of identity
RMSSD	root mean squared difference of successive N-N intervals
SampEn	sample entropy
SDNN	standard deviation of N-N interval
TINN	triangular interpolation of N-N intervals
TP	total power in all frequency bands
UHF power	power in the ultra-low frequency band
VLF power	power in the very low frequency band

ANOVA	analysis of variance
ANS	autonomic nervous system
AR	auto-regressive (model)
AV	atrio-ventricular
BMI	body mass index
BPM	beats per minute
CGSA	coarse grain spectral analysis
CI	confidence interval
DTQ	diurnal type questionnaire
DV	dependent variables
ECG	electrocardiograph
EEG	electroencephalograph
ESS	Epworth sleepiness scale
FFT	Fast Fourier transform
FIOH	Finnish Institute of Occupational Health
HPA	hypothalamic pituitary adrenal
HRV	heart rate variability
IPFM	integral pulse frequency modulation
IQR	interquartile range
IV	independent variables
JYU	University of Jyväskylä
KSS	Karolinska sleepiness scale
N-N	normal-to-normal

NREM	non-rapid eye movement
PNS	parasympathetic nervous system
PSD	power spectral density
REM	rapid eye movement
RSA	respiratory sinus arrhythmia
RVLM	rostral ventro-lateral medulla
SA	sino-atrial
SAM	sympathetic adrenal medullary
S.D.	standard deviation
SNS	sympathetic nervous system
SWS	slow wave sleep
TUT	Tampere University of Technology
VIF	variance inflation factor
WASO	wake after sleep onset

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1. INTRODUCTION

Ever since Hon and Lee [1] first appreciated the clinical significance of the inter-beat interval variability of the fetal heart in 1963, the fluctuations of the heart beat have been investigated repeatedly by several researchers in the fields of medicine, occupational and environmental health, sports training and sleep medicine to identify the factors controlling or modulating these changes. This method of analysis has come to be known as heart rate variability (HRV) and is identified as an effective, efficient and non-invasive method of studying the variations in the autonomic nervous system (ANS). The ANS is constituted of two opposing branches called the sympathetic (SNS) and parasympathetic nervous systems (PNS). The various internal or external factors affecting the ANS, directly or indirectly, cause a shift in balance between these two branches causing abnormal variations in the HRV indices. Thus, HRV is said to contain valuable information on cardiovascular health, nervous system, levels of fitness and the effects of stress on the body.

HRV can be obtained from electrocardiograph (ECG) recordings by measuring the R-R interval time series, usually called the R-R interval tachogram. After pre-processing to remove artefacts, several conventional time, frequency and non-linear measures are computed from these tachograms using standard algorithms, which are simple to implement and is the primary reason for its popularity. The HRV indices can measure variability from recordings as short as 5 minutes to even 24 hours and hence, can be classified as short- and long-term measures. However, the significance and implications of most of these indices are complex and are not completely understood even after several years of research.

Traditionally, HRV has been used to study cardiovascular diseases such as myocardial infarction, coronary artery disease and sudden cardiac death as a predictor of mortality. In recent years the applications have been diverse. However, sleep quality and recovery analysis in shift workers is a relatively new dimension to the application of HRV in occupational and environmental health context. Bonnet and co-workers [2] studied the fluctuations in HRV as a function of sleep stages and time of the night and concluded that sympathetic and parasympathetic activation were synchronous to rapid eye movement (REM) and non-rapid eye movement (NREM) sleep stages and since, similar findings have been reported by other researchers [3]. Burton et al. [4] noted that poor sleep quality was marked by reduced HRV as a result of weak vagal influence. Another study suggested that poor sleep quality was not a result of sleep disorders but due to decrease in duration of uninterrupted sleep [5]. An alternate theory supports the view that decreased HRV was

a result of autonomic hyper vigilance [6] and the effects of daily stress [7]. In professional drivers, population with undiagnosed or untreated sleep disorders [8] and shift workers [9; 10] are most prone to sleep-related crashes which have been attributed to irregular work-sleep schedules and inadequate sleep.

The Finnish Institute of Occupational Health (FIOH), Tampere University of Technology (TUT), University of Jyväskylä (JYU) and Taipele Telematics collaborated to design and implement the project, titled *'An educational intervention to promote safe and economic truck driving'*. The overall aim of the study was *(1)* to examine the levels of sleepiness and stress in different shift types and determine the factors that cause these effects, *(2)* How the sleepiness and stress affect driving behavior, style and do they significantly affect fuel consumption and carbon emission, and *(3)* Does an educational intervention have an effect on alertness and mitigation of sleepiness at the wheel. The author's responsibility was to evaluate the physiological aspects of stress and sleepiness during different shift types and whether intervention had an effect on the drivers' overall performance.

The aim of this study was to investigate if shift type or other individual/sleep-related factors affected the process of recovery during sleep. The physiological measurements were compared to actigraphy based measures of sleep quality and subjective reports from sleep diaries for the total sleep duration to comprehend how the sleep recovery is affected by various independent and habitual factors such as age, alcohol consumption, body mass index (BMI), diurnal type, mean sleep need and type of shift. Further, core sleep and hourly analyses were performed for the HRV indices. The results will provide a basis for designing: *(a)* shift schedules so that professional drivers can utilize the time between shifts to recover from stress and work-time sleepiness more efficiently, and *(b)* interventions for occupational health therapists to address the sleep related problems faced by these populations.

2. OBJECTIVES

Physiologically, the efficiency of sleep is measured by electroencephalography (EEG) monitoring during the sleep period to assess the amount and duration of different sleep cycles. However, EEG measurements are not always possible as it needs a clinical setting and unnatural sleeping environments might affect the quality of sleep. The purpose of the present study was to determine if HRV could effectively determine factors affecting recovery during sleep. The R-R interval measurements obtained from shift working long-haul truck drivers was used as the basis for this study. Data was collected during three intensive measurement days such that each driver recorded one night shift, one non-night shift and a day off. The off day was used as baseline measurement to compare recovery during sleep after night and non-night shifts. The three main objectives of the study were:

- Quantize the outcomes from the entire sleep duration for different shift types and determine correlation outcomes for subjective and actigraphy measures with HRV.
- Compare the core sleep (4 hours from sleep onset) and optional sleep (remaining sleep until arousal) to determine how much the duration sleep is significant in the recovery process.
- Measure sleep recovery in an hourly basis to determine if the circadian cycle was a significant contributor in determining sleep efficiency in different shift types.

The hypothesis was that various environmental and occupational factors causes work stress in individuals, thereby causing a reduction in vagal tone. Without sufficient recovery from these work stressors, the cognitive performance at work is subdued. The reasons could be direct, such as sleep duration, conditions and time of the day, or indirect factors such as levels of work stress, physical exertion, direction of shift rotation, etcetera. Insufficient recovery can cause chronic fatigue and cardiovascular diseases when left untreated on the long run. Since HRV is a non-invasive measure of ANS activity, it is believed to give some insight on the differences in sleep after different shift types and the factors affecting the recovery process. By studying the sleep recovery process across different shift types, the causes for diminished sleep quality in these professional drivers can be identified and understood. This would lead to elegant planning and management of work schedules and work force, and minimize on-road incidents and accidents.

3. SLEEP

Sleep is believed to be the period of rest and restoration for bodily functions. The cognitive, regenerative and reparative functions of sleep are essential for the maintenance of homeostasis. Sleep patterns undergo evolutionary changes with age. The sleep and wake cycles for newborn children are random and can occur at any time of the day. Infants and young children (3-7 years) experience polyphasic sleep, during which sleep is fragmented into multiple sleep periods. However, with age, the pattern of sleep transforms in to a more stable monophasic sleep. An average adult requires between 7-8 hours of restorative sleep every day. Sleep deprivation results when a person sleeps less than 5-6 hours every day for a certain period. Sleep efficiency is also believed to decline with age. The changes in sleep patterns with age are depicted in Figure 3.1.

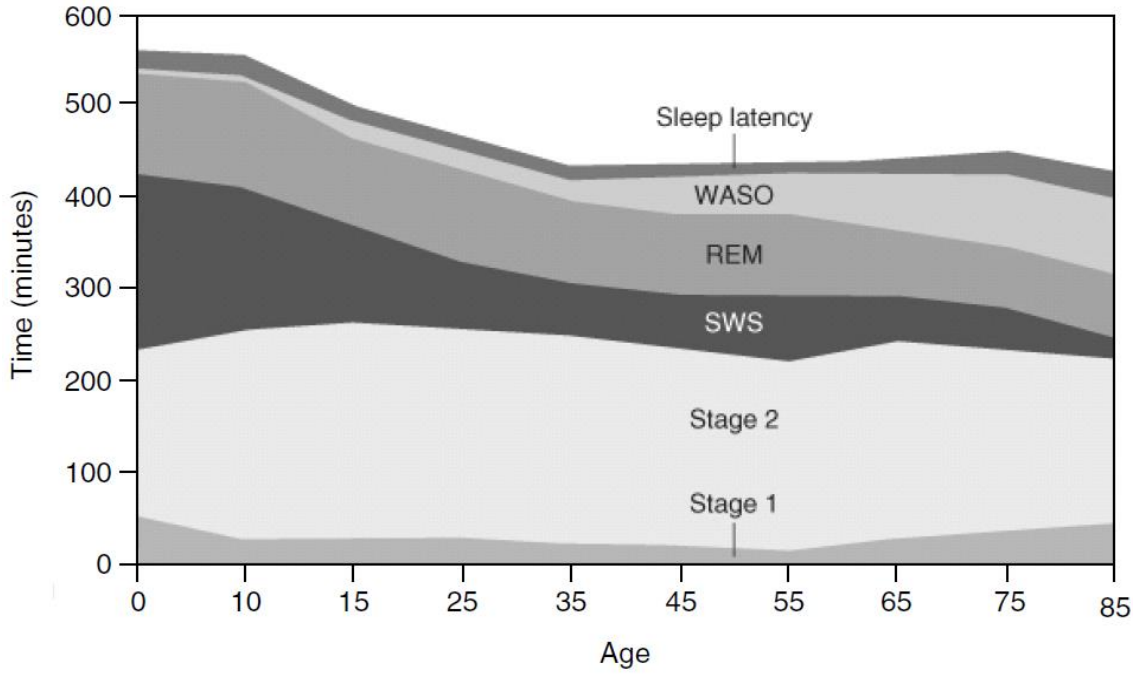

Figure 3.1. *Effect of ageing on sleep. The vertical axis represents the time for sleep latency, amount of time awake after initial bout of sleep (WASO), slow wave sleep (SWS), rapid eye movement (REM) and non-rapid eye movement (NREM) sleep stages. Source: [11].*

Loss of sleep reduces the period of NREM sleep that in turn causes daytime tiredness. During REM sleep, the postural muscle tone is absent and an EEG shows rapid and low amplitude fluctuations, similar to frequencies observed during wakefulness. This period is categorized by greater thresholds to arousal, rapid movement or twitches in the eye, head or limbs, and the occurrence of dreams. In contrast, NREM sleep is marked by deep

sleep with lower blood pressure, breathing frequency and metabolic rates. The EEG pattern are slow with larger amplitudes and no body movements are evoked. NREM sleep constitutes 75-80% of the total sleep while REM sleep fills the remaining period. The pattern of alternations between the two types of sleep is not completely understood, but irregular patterns are found to be associated with sleep disorders. [12]

3.1 Physiological changes during sleep

Onset of sleep causes significant changes in the regulation of autonomic function, which results in an evident change in the physiology of the cardiovascular system. When compared to wakefulness, there is a prominent increase in cardiac parasympathetic activity during NREM stage of sleep [13; 14] and some studies suggest a steady increase is seen across the four stages [15]. The PNS activity was found to be subdued in REM sleep stage when compared to NREM sleep [15]. Therefore, it can be ascertained that the sympathetic-parasympathetic tone is synchronous with REM-NREM sleep cycle. The PNS is also responsible for the modulation of heart rate, blood pressure and breathing rate during sleep. Brief surges in blood pressure and heart rate occur in response to arousal or body movements that are due to sporadic spiking of sympathetic modulation. Apart from cardiovascular changes, ventilation and respiratory flow become faster especially during REM sleep. [16] Hypoventilation is prevalent during both sleep stages. Endocrine functions such as melatonin secretion, growth hormone and thyroid hormone are influenced by sleep. Melatonin, the chemical factor in the body that arouses sleepiness, is induced by light-dark cycle. [17]

3.2 Effect of shift work on sleep

Shift work is associated with increased risk of cardiovascular disease, accidents, sleep disturbances and fatigue. Shift workers have reported more frequent problems due to sleep disturbances than daytime workers [18] and the effects vary according to the shift timing. Shift work has been established as the primary reason for shortened sleep in these populations [18; 19]. The principal cause perceived is the conflict between the regulation of circadian rhythm and the displaced work hours. Moreover, Sallinen et al. [20] have reported high levels of sleepiness during night as well as morning shifts. Reason for sleep loss after night shift could be the interference of circadian rise of metabolism during early morning hours whereas phase advancing bedtime to compensate for truncated sleep is linked to that of morning shift [21].

Other factors such as work stress, overtime work and physical workload could also have an effect on sleep disturbances. Reports have shown work stress due to high work demand attributed to disturbed sleep habits and fatigue. [22] Fatigue was a significant contributor to sleep disturbances but Åkerstedt et al. [21] anticipated that shift workers considered sleepiness a better description since both conditions cannot be exclusively

classified. Interestingly, less fatigue was observed in older subjects. This could be one possible explanation for higher sleep related road accidents [23] and sleepiness [24] in lower age groups. The shift types need to be investigated further to identify the impact it has on sleep pattern.

3.2.1 Night shift

Chronobiologically, the best time for an individual to easily fall asleep and benefit most from the restorative function of sleep is during the night as this is sync with the normal sleep-wake rhythm and other biological rhythms of the body. In a review, Åkerstedt [25] observed that average sleep length in night workers is 4-6 hours while in day or afternoon workers it was 7-9 hours and this was concurred by other studies [26; 27]. Sleep disruption has demonstrated alterations in the REM-NREM sleep cycle, especially in stage 2 and REM sleep stages. In one-third of the night shift workers, the decreased sleep length after the shift was found to be compensated by short afternoon naps [28], usually more than 1 hour in length [29]. The reduced length curtails the restorative function of sleep, quite often resulting in increased sleepiness during the time awake.

3.2.2 Morning shift

Folkard and co-workers [30] found that the sleep before a morning shift was more disturbed when compared to the night shift. A shortened sleep, similar to sleep after a night shift, is observed. Most subjective indications have shown difficulty awakening and not being refreshed by sleep as major reasons for displeasure amongst day shift workers, despite the fact that the quality of sleep is unaffected.

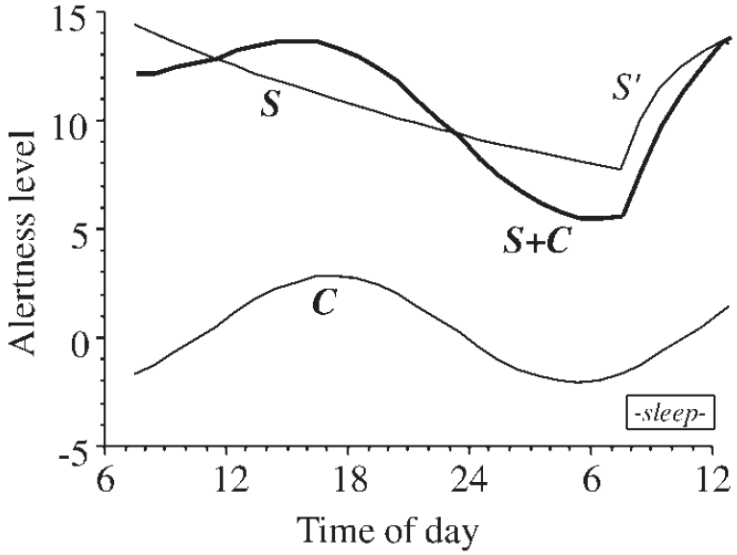

Figure 3.2. *The main components influencing sleepiness. S represents the homeostatic effect of time awake or recovery during sleep and C denotes circadian rhythms. It can be seen that the combined effect of S and C causes lowest levels of alertness during the early morning hours (0500-0900 hours). From [18].*

Early rise times are strongly associated with increased sleepiness during the day [31], which is often compensated by short naps during the afternoon, after the shift has ended [32]. The short sleep duration before a morning shift can be ascribed to early sleep termination without an advancement in bedtime due to the difficulty initiating sleep in the acrophase of the circadian cycle. Early awakening also coincides with the period in the circadian cycle when the combined influence of homeostatic and circadian factors is at a peak. As a result, levels of sleepiness are maximum (shown in Figure 3.2). The early rise time further diminishes the slow wave sleep (SWS) and increases the stress levels as a result. [33]

3.3 Factors of shift system

Various factors in the shift work system may affect the sleep quality and alertness upon awakening of individuals. Although no studies have probed the pattern of sleep in different shift rotation speeds, some theories have tried to define the amount of sleep that is permissible in certain shift schedules. Permanent night shift workers slept less when compared to permanent day shift employees. However, Wilkinson [34] in a review found that permanent night shift workers reported longer sleep (6.7 hours) when compared to weekly rotating (6.3 hours) or rapidly rotating (5.8 hours) shift workers. Härmä et al. [35] noted that a very rapidly forward rotating shift system positively influenced sleep, alertness and well-being when compared to backward rotating shifts.

Although no statistical evidence is available regarding differences in sleep quality or duration, several theories have suggested a clockwise shift rotation (morning-afternoon-night) to be ideal for effective sleep. However, the shift workers have often found it necessary to have one or two off days after a clockwise rotating night shift to recover. [35] Shift workers were found wanting a short nap (0.5-2 hours) during duty hours as a counter-measure for increased levels of sleepiness during wake time and decreased sleep duration. Despite very few experimental studies finding this useful [36], the overall impression is that napping might be a useful counter-measure for maintaining alertness levels during shifts.

3.4 Sleep recovery after shift work

The need for recovery after work is defined as the need to recover from work-induced fatigue. The recovery period is usually the time an individual requires to return to pre-stress level of functioning after the termination of stressors. [37] Long shift hours, especially with high workload, or night/early morning shifts usually demands longer recuperative periods. Insufficient recovery between two periods of work might lead to short-term effects, such as fatigue and decreased work capacity, which can also build up into adverse health effects on the long run. Sluiter et al. [38] observed that short periods of rest during work allows temporary recovery from work-related fatigue. However, this is not possible

in professional drivers since it requires long periods of sustained attention. Long work hours, time of day and monotony of work adds to the fatigue factor and need for recovery is higher in this profession [39; 40]. Moreover, the work demand varies according to type, duration, physical workload and combination of trips the drivers have to perform. Long-term planning is difficult as estimation of kind of trip and duration vary in each case. Therefore, the onus is on efficient sleep and recovery between shifts and shift types.

Sleep recovery is dependent, apart from sleep quality and quantity, on several factors such as age, gender, social activities and work demands. Kiss et al. [41] observed that the need for recovery was highest in the older age groups although a slight decrease was observed after the age of 54, which was attributed to *the healthy worker* effect. Interestingly, a moderately high need for recovery was also observed in the <25 year age group, which could possibly be due to lower level of experience on the job. Another noteworthy finding was that participating in social and leisure activities helped the recovery process. Higher physical activity was also associated with better recovery levels. [42] Sallinen et al. [43] have proposed that shift scheduling must be done in a way that at least 8 hours of sleep is available for shift workers, especially in such safety-sensitive occupations.

4. HEART RATE VARIABILITY

Cardiovascular homeostasis refers to the tendency of the body to orchestrate a regular heart rate and blood pressure under changing environmental conditions. Since no physiological parameter is absolutely stationary or periodic, spontaneous fluctuations can be observed in cardiovascular functions even when no perturbing influences are identified. This variation over time of the interval between successive heartbeats has come to be known as heart rate variability. The HRV reflects the heart's ability to adapt and respond to unpredictable stimuli and is considered the most significant, non-invasive measure of the functioning of the ANS. [44]

The normal variability in heart rate is believed to be due to the autonomic neural regulation of the heart and circulatory system [45]. In literature, relationships between autonomic function and disease states, such as cardiac dysfunction and sudden cardiac death [46-48], diabetic autonomic neuropathy [49], hypertension [50; 51], psychiatric disorders [52; 53], acute and chronic stress [54; 55], mental challenges and emotional states [56; 57] is well documented. Although HRV is simple, non-invasive and fairly accurate means of measuring sympatho-vagal balance at the sino-atrial (SA) level, interpretation of the variability under disease conditions is still not completely understood as different factors such as age, cardiac health, fatigue, fitness, gender, habits, hypertension, insulin resistance, nutritional factors, obesity, pollution, sleep recovery etcetera contribute to significant individual variations. To better comprehend the causes, a deeper insight and understanding of the physiology behind the heart rate, its regulation and variability is mandatory.

4.1 Physiology of the heart

The heartbeat originates at the SA node, which is embedded in the posterior wall of the right atrium (as seen in Figure 4.1). The SA node comprises of a group of specialized cells that are continuously generating contractile stimulus spreading to different heart muscles through specialized pathways and creating a well-synchronized heart muscle contraction, ultimately producing a heartbeat. Although several neurons of the intrinsic cardiac nervous system, including the atrio-ventricular (AV) node, are capable of exhibiting autonomous heart stimulation, the SA node, being the principal pacemaker of the heart, exhibits the highest discharge frequency thereby subjugating electrical impulses from other cardiac centres. The SA node generates between 100-120 intrinsic beats per minute (bpm) when at rest, in the absence of any neural or hormonal influences [58]. The auto-rhythmicity of the SA node is fairly constant, but is modulated by various factors

that add variability at different frequencies However, the heart rate is limited to 60-75 bpm due to the continuous influence of the ANS over the SA node activity.

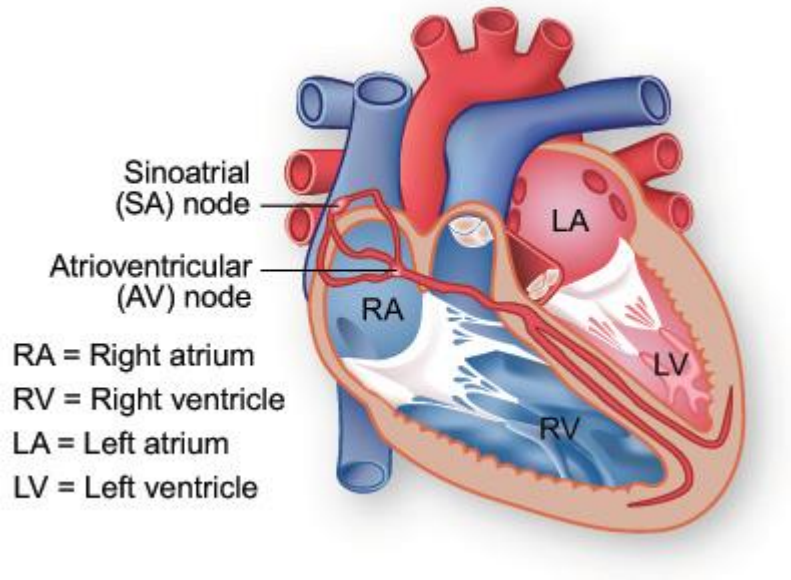

Figure 4.1. *Diagram showing stimulus centres for generating a heart beat.*

4.2 Autonomic nervous system influence on heart rate

The modulation of the ANS affects multiple organ systems and its effect has been linked to the change in the state of consciousness. Figure 4.2 shows how the two branches of ANS control various organs and their regulation. The ANS carries sensory impulses from the blood vessels, heart and all other organs in the abdomen, chest and pelvis regions to the brain (medulla, pons and hypothalamus). These impulses elicit automatic or reflex responses through efferent autonomic nerves, thereby stimulating appropriate reactions of the heart, vascular system and other organs of the body to intrinsic and extrinsic variations which each and every individual experiences. The afferent nerves serve both the branches of ANS and conveys impulses from sensory organs, muscles, circulatory system and body organs to the control centres situated in the medulla, pons and hypothalamus. The sympathetic and parasympathetic nerves then transmit the efferent impulses, originating in these brain centres, back to different parts of the body.

It is a generally accepted notion that NREM sleep is associated with an increase in cardiac parasympathetic activity. The activity of PNS progressively increases across all stages of NREM sleep [59] and decreases during REM sleep [60]. The SNS activity, by far, remains unchanged or decreases marginally during the transition from wakefulness to NREM sleep. In addition to the influence of sleep on ANS, increased incidence of cardiovascular incidents in the morning hours suggest a possible circadian influence on ANS. [61] Burgess and colleagues [62] in their study indicated that the PNS was influenced by the circadian system whereas SNS was primarily influenced by sleep and not by circadian rhythm.

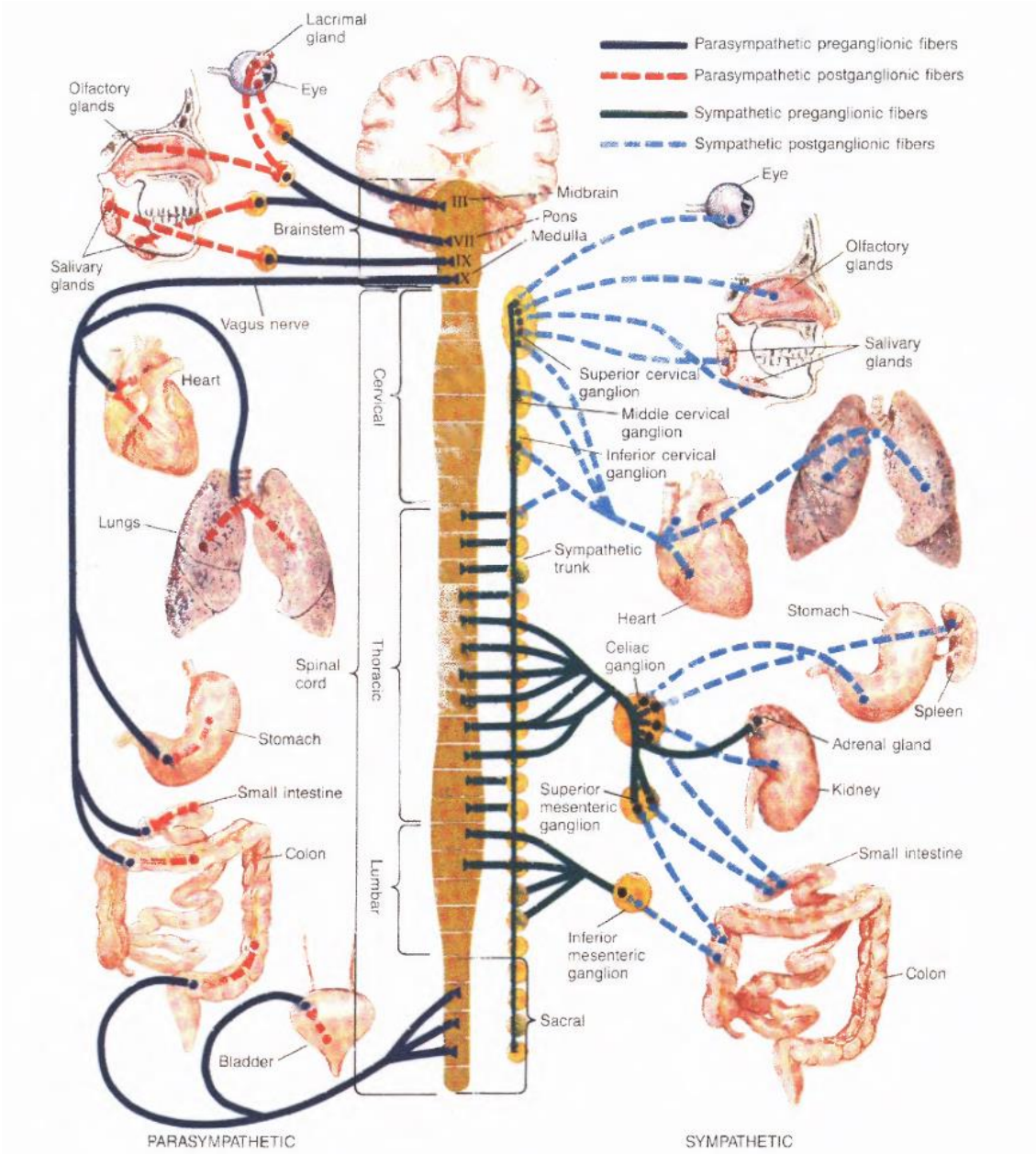

Figure 4.2. *The two branches of the autonomic nervous system [63].*

The SA node is controlled by both the sympathetic and parasympathetic (also called vagal) branches of the ANS. Sympathetic fibres innervate most of the heart including the AV, heterotrophic centres, atrial and ventricular myocardium. Both the left and right vagus nerves stimulate the SA node, AV node and atrial muscles whereas the control of the ventricle muscles is still unclear. Generally, the stimulation of the right vagus nerve results in a decrease in heart rate and activity within the right sympathetic nerve induces an increased heart rate response. Rapid changes in heart rate are usually a result of shift in vagal regulation.

The SA node response to vagal activity is typically short-lived (occurs within 5 seconds) when compared to the cardiac responses to SNS which typically takes about 20-30 seconds for maximal output. [64] The differences in response times are mainly due to the

slow exocytotic release of noradrenaline from the sympathetic nerve terminal and the involvement of a secondary messenger, adenylyl cyclase, in SNS regulation. Other anatomical disparities between the autonomic branches, such as location of the preganglionic cell bodies of the neurons (those of PNS are located within the heart whilst those of SNS are isolated in the paravertebral ganglia) and myelination of the preganglionic fibres, contribute to faster transmission of vagal signals [65]. This dynamic balance between sympathetic and parasympathetic activity causes a continuous oscillation of the heart rate ultimately resulting in HRV. Under resting conditions, the parasympathetic tone is predominant, resulting in low resting heart rate. However, several factors contribute significantly for the variability in heart rate.

4.3 Measurement and analysis

4.3.1 Measurement

Traditionally, HRV is obtained from ECG signals using a digital, high frequency, portable device such as a Holter monitor. A Holter monitor is an ambulatory device that is capable of recording ECG signals over 24-hour periods through a series of non-invasive electrodes attached to the subject's chest, allowing them to perform normal day-to-day activities without hampering degree of movement. The data is stored on digital flash memory devices that can be retrieved after the recording is complete. Despite the recommended minimum sampling frequency being 500 Hz [66], many devices have a sampling frequency ranging between 100 to several 1000 Hz. A low sampling frequency could lead to digitization errors. Although this is not a major concern in data acquired from healthy populations whose variability is greater, it could lead to computational errors and diminished prognostic value for lower variability in the heart rate, which is common in diseased populations. Grácia-González et al. [67] have shown that a lower sampling rate can affect spectral indices of HRV and such data should be treated with care. A recent study by Hejjel and colleagues [68] suggests that a minimum sampling interval of 1 millisecond (ms) without interpolation is essential for accurate HRV analysis.

To derive heart rate tachograms, it is essential to accurately identify a particular fiducial point from each heart beat complex. Generally, QRS complex or R-peaks are used for this purpose as they have distinct properties and are easy to identify. Several detection algorithms such as Hilbert transform [69], pattern recognition [70], wavelet transform [71] or other filtering methods [72-74] have been discussed in literature. The R-peak detection should be accurate to avoid false or missed peak detection. Next, the R-R interval between subsequent R-peaks is computed to produce the R-R interval tachogram or HRV signal. It has to be taken into account that the tachogram is not sampled at uniform intervals owing to the fact that the duration of adjacent heartbeats is distinct. In order to overcome the irregular sampling rate, the spectrum is either represented as a function of cycles

per minute [75], is resampled by interpolation [66] or by applying integral pulse frequency modulation (IPFM) model [76]. Of these, the interpolation technique, as illustrated in Figure 4.3, is most widely used for HRV analysis.

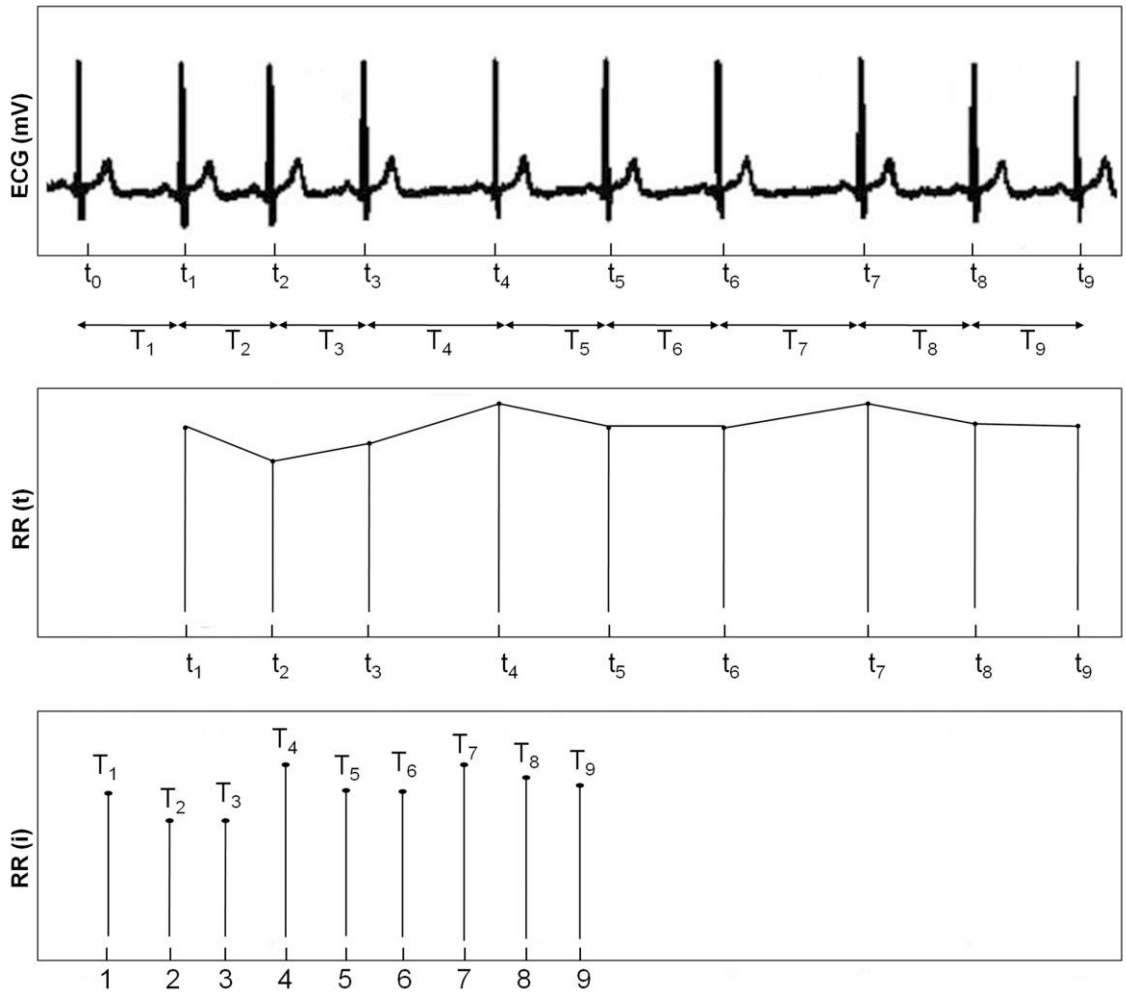

Figure 4.3. *(a) ECG with different R-peak intervals, (b) Interpolation of R-R interval time series, (c) R-R interval tachogram [77].*

4.3.2 Pre-processing

Ambulatory ECG recordings usually comprise imperfections in the form of abnormal sinus rhythms or artefacts that are of both physiological and technical origin. Physiological artefacts, such as cardiac dysrhythmias, are common in subjects suffering from cardiovascular diseases. Ectopic beats, on the other hand, are prevalent even in normal subjects. However, HRV analyses are performed only on R-R intervals resulting from normal sinus node depolarisations, termed normal-to-normal (N-N) time series, as they influence the reliability of results. For example, the presence of ectopic beats in the tachogram gives a higher band power and erroneous standard deviation of R-R intervals [78]. Technical artefacts are in the form of shortcomings of software algorithms, poor electrode adhesion or motion artefacts. Many researchers have emphasized the importance of pre-processing in order to remove these artefacts. [79-84]

Several algorithms and methods have been developed over the years for artefact correction in heart rate tachograms. Interpolation is most popular as it preserves the initial number of samples unlike the deletion method. Most interpolation algorithms, such as degree zero, linear, spline and non-linear predictive, serve as low-pass filters with different filtering capacities. Interpolation of degree zero replaces the abnormal R-R intervals with the mean of neighbouring R-R intervals. Degree one interpolation, also called linear interpolation, fits a straight line over the abnormal intervals to obtain new values. Frequently used is the cubic spline interpolation, where smooth curves are estimated by fitting a third degree polynomial. Long duration tachograms may also contain slow linear trends or non-stationarities, which stresses the importance of trend removal prior to analysis. Detrending based on polynomial models of first or higher orders [82] and smoothness priors' approach [83] have been effective.

Nevertheless, the application of various editing methods affect HRV analysis. These can be attributed to study settings, type of study population, length of R-R interval time series, editing methods and amount of samples edited, etcetera. Spectral parameters are sensitive to signal length and comparison of different samples should be performed only if the signals are of same length. Short-term HRV analyses are sensitive to artefacts and pre-processing whereas long-term analyses give unbiased outcomes. This is because long-term tachograms comprise a large number of samples and sustain the original beat-to-beat variability despite pre-processing. [84] Several researchers have scrutinized the numerous interpolation techniques to determine the most accurate editing routine but the results have been diverse [85; 86]. However, the disparities between different editing methods is trivial when the number of artefacts is small.

Stationarity of the signal is of utmost importance while analysing HRV. A signal is said to be truly stationary if the parameters that define the working point of the system remain constant throughout the period of measurement. However, this is extremely remote in physiological systems as there is only limited knowledge of the dynamics involved in individual processes. Stationarity of a signal is strongly linked and inversely proportional to the duration of the recording as physiological changes are inevitable for long duration measurements. This leads to complicated and insensitive measure as the physiological state is delivered as an average of interim changes during the period with considerable loss of valuable information. One approach to overcome the issue of stationarity is to divide to the time series into smaller epochs to analyse the dynamics of the signal over time. There are several approaches to adopting this technique: shorter segments are stationary and therefore more reliable. Therefore, the changes over segments can be used to estimate the statistical significance of the results.

4.3.3 Analysis

HRV is analysed by different methods, commonly classified as time domain, frequency domain and non-linear methods to assess the dynamics of the heart rate. These methods are discussed in detail in the following sections.

Time domain methods

Time domain analysis is the simplest analysis method. Variables such as mean heart rate, mean N-N interval, heart rate during wakefulness and sleep, exercise heart rate, variation in heart rhythm due to respiration, tilt, Valsalva manoeuvre etcetera, are most commonly studied. The time domain methods are further classified as *statistical* and *geometrical* parameters.

Statistical parameters:

Statistical parameters are relatively simple and calculated from instantaneous R-R intervals or from differences between consecutive samples in the tachogram. Although statistical parameters are computed for both short-term (typically 5 minutes) and long-term recordings (typically 24 hours), some parameters are dependent on the length of the segments and therefore, comparisons should always be performed between recordings of equal duration. Since these methods are sensitive to artefacts, the data is usually pre-processed and hence, most parameters include the term *NN* in the abbreviation. Standard deviation of the N-N interval (SDNN), which represents the square root of the variance, is mathematically equivalent to the total power of spectral analysis and reflects the cyclic components of the variability in the recorded series. The equation for SDNN is

$$SDNN = \left(\frac{1}{n-1} \sum_{i=1}^{n} (NN_i - NN_{mean})^2 \right)^{\frac{1}{2}} \quad (1)$$

where NN_i is the normal R-R intervals, NN_{mean} is the mean of normal R-R intervals and n is the number of samples. SDNN is measured in ms. It represents the heart's intrinsic ability to respond to hormonal influences. Theoretically, SDNN can be measured for tachograms of any length. However, as the monitoring duration decreases, SDNN measures shorter cycle lengths and the variance in heart rate is reduced. In practice, due to its dependence on length of recording, SDNN cannot be used to compare measurements of different durations. Other statistical measures include standard deviation of the average N-N interval (SDANN) calculated over short periods, which estimates the changes in heart rate due to longer cycles. The SDNN index is the mean of the 5-minute standard deviation of N-N interval calculated over 24 hours and measures the variability of cycles shorter than 5-minutes.

RMSSD denotes the square root of the mean squared differences of successive N-N intervals and is mathematically represented as

$$RMSSD = \left(\frac{1}{n-1} \sum_{i=1}^{n-1} (NN_{i+1} - NN_i)^2 \right)^{\frac{1}{2}} \quad (2)$$

where NN_{i+1} symbolises the $i+1^{th}$ term in the tachogram and is measured in ms. The RMSSD is sensitive to heart rate fluctuations and is used as an index of vagal cardiac control. Other parameters used to measure beat variations include NN50, which indicates the number of neighbouring heart rate intervals that differ by greater than 50 ms, and pNN50, the proportion of beats differing by 50 ms. NN50 and pNN50 are highly correlated with RMSSD and hence can be considered good estimates of vagal tone. Recently, the SDNN/RMSSD ratio has been proposed as a new index to quantify the sympatho-vagal balance. [87]

Geometrical parameters:

Apart from simple statistical analysis, the N-N data series can be represented as geometric patterns, which have exposed several vital characteristics of the beat intervals. [88] Classic descriptors such as skewness and kurtosis, which quantify symmetry of variables, are frequently used in HRV studies. The main advantage over statistical methods is its ability to negate the effects of anomalous data points, as they are significantly shorter or longer than normal data points and fall outside the normal range. Sample density distribution of N-N intervals, sample density distributions of differences between adjacent N-N intervals and Poincaré plots (also known as Lorenz plots) are some examples of geometric methods used to analyse HRV. All geometrical methods are based on three different principles: *(1)* basic measure of the geometric pattern obtained which translates as a measure of HRV, *(2)* interpolation of the geometric pattern by a mathematically defined shape, whose parameters define the variability, and *(3)* classification of geometric shapes into pattern based categories representing different classes of HRV. [66] However, to acquire these geometric measures, the data series first needs to be converted to a discrete scale with an optimal bin size, the most common being 8 ms.

The HRV triangular index (HRV_{index}) represents the integral of density distribution divided by the maximum of the distribution. On a discrete scale, the parameter is calculated as

$$HRV_{index} = \frac{Total\ number\ of\ N-N\ intervals}{Number\ of\ N-N\ intervals\ in\ the\ modal\ bin} \quad (3)$$

which is dependent on the precision of discrete scale. The triangular interpolation of N-N (TINN) is the baseline width of the distribution which is measured as the base of a triangle conceptualized by approximating the N-N interval distribution. The HRV_{index} and

TINN express the overall HRV measured over 24 hours and are influenced by lower frequencies. [89] Several such geometric measures can be derived but most of them are highly correlated to one another.

Poincaré plot is a map of dots on the X-Y plane where each dot represents the duration of the beat interval plotted against the preceeding beat interval. Unlike previously discussed parameters, the Poincaré plots are analyzed qualitatively where the shape of the plot is classified into functional groups that is later used to interpret the nature of the cardiac signal. A more detailed account of the significance of Poincaré plots are described by Mourot and colleagues [90]. Visual inspection of these plots reveal a complex cardiac pattern that is not otherwise described by other HRV parameters. For example, Woo *et al.* [91] have shown that different patterns are obtained from Poincaré plots obtained from 24 hour recordings of healthy subjects (seen in Figure 4.4) and heart failure patients. Qualitative analyses requires fitting an ellipse with centre coinciding with the centre of the scatter plot. The standard deviation of points perpendicular to the axis of line of identity (SD1) and along the line of identity (SD2) measure the short term and long term variability respectively. However, the use of qualitative evaluation is limited due to its subjective nature and probability of false interpretation. The frequently used quantitative measures include width- and length-derived parameters. Despite the insensitivity of geometrical parameters to analytical quality, one major disadvantage of the geometric method is its inability to quantize short term recordings. In practice, recordings at least 20 minutes in length are required to ensure accurate analysis and hence refutes short term changes in HRV. [89]

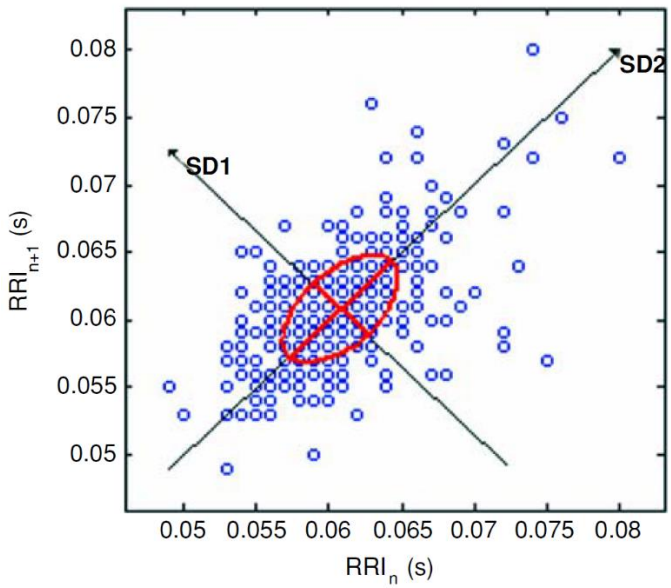

Figure 4.4. *Poincaré plot of a normal subject [92].*

Frequency domain methods

HRV analysis in the frequency domain entails the estimation of power (variance) across different frequency bands. To perform spectral analysis, the duration of the recording

should be at least ten times the wavelength of the lowest spectral component. The presentation of variance as a function of frequency is usually termed as power spectral density (PSD) or spectrogram. PSD calculation is generally performed by either parametric or non-parametric methods. No clear guidelines are available as to which method is to be followed and hence, both methods are in practice with specific recommendations. The advantages of parametric method are smoother spectral components, simpler post-processing to calculate power distribution and accurate estimation of PSD even when the number of samples is significantly small, whereas the advantages of non-parametric methods include simple fast Fourier transform (FFT) based computation and higher processing speed. [66]

In parametric method, the power spectrum estimation is based on autoregressive (AR) model where each sample is expressed as a linear combination of previous samples with an error signal, which is usually white noise. Since the poles of the spectrum can be obtained, processing of calculating power and peak frequencies is much easier as compared to non-parametric methods. An appropriate model order is chosen such that a good frequency resolution sans spurious peaks is achieved in the power spectrum. A high number may introduce noise peaks whereas a small number will result in an over-smoothed spectrum. To avoid such discrepancies, the model order is recommended to be twice as large as the number of frequency peaks and is typically 15-20 for R-R time series. The parametric method is capable of producing an accurate PSD even with smaller number of samples but might introduce errors in the estimation of certain low frequency components. Moreover, the disadvantage of parametric method is the need to verify the suitability of the chosen model and complexity. Non-parametric estimates of PSD are primarily based on FFT, which utilizes the discrete Fourier transform and reduces the computation complexity. However, the FFT method is preferred only for long R-R time series as it does not produce a good frequency resolution for shorter recordings and may cause leakage of power from main frequency band. To overcome this, windowing techniques have been adopted to obtain good resolution. The segment and window length needs to be chosen carefully in order to sustain a stationary signal. [66]

The Task Force of the European Society of Cardiology [66] have classified the various frequency bands: *ultra-low frequencies* (UHF; <0.003 Hz) that includes the circadian rhythm [93], *very low frequencies* (VLF; 0.003-0.04 Hz) affected by thermoregulation [94], *low frequencies* (LF; 0.04-0.15 Hz) that is sensitive partly to sympathetic and sympathetic modulation [95; 96] and *high frequencies* (HF; 0.15-0.4 Hz) that are primarily modulated by cardiac parasympathetic control mechanisms [97].The distribution of power across the three major frequency bands, VLF, LF and HF, have found to contain information regarding the ANS and various reflex mechanisms. It is widely accepted that power in the HF band represents vagal activity [98] whereas the VLF and LF bands are associated with sympathetic activity [99]. Long period fluctuations, thought to originate from renin-angiotensin and other humoral factors, and non-stationarities affect power in

the VLF band. The LF band comprises the slow oscillation of the heart, centred around 0.1 Hz, and is due to mechanisms controlling blood pressure [100]. Although various researchers have postulated LF power as a marker for sympathetic modulation, some other studies have suggested contributions from both parasympathetic and sympathetic modulation [101; 102]. The HF component synchronizes with respiratory frequency at around 0.25 Hz. Often, the ratio of LF to HF power, termed LF/HF ratio, is used to assess the sympatho-vagal balance and has been popular amongst researchers [103]. However, a recent study by Billman [104] claimed otherwise. According to Billman, complex nature of LF spectrum and non-linear interactions between parasympathetic and sympathetic nerve activity, amongst others, make it difficult to comprehend the physiological basis of the LF/HF ratio with certainty.

The LF and HF powers may also be expressed in normalized units to account for the inter-individual differences. For normalization, the absolute power of each component is expressed as a proportion of the total power. In addition to the above-mentioned frequency bands, the ULF band has also been investigated sporadically but the exact physiology remains unknown. The total power (TP) is the sum of power across all the frequency bands. The distribution across different frequency bands during various sleep stages is presented in Figure 4.5.

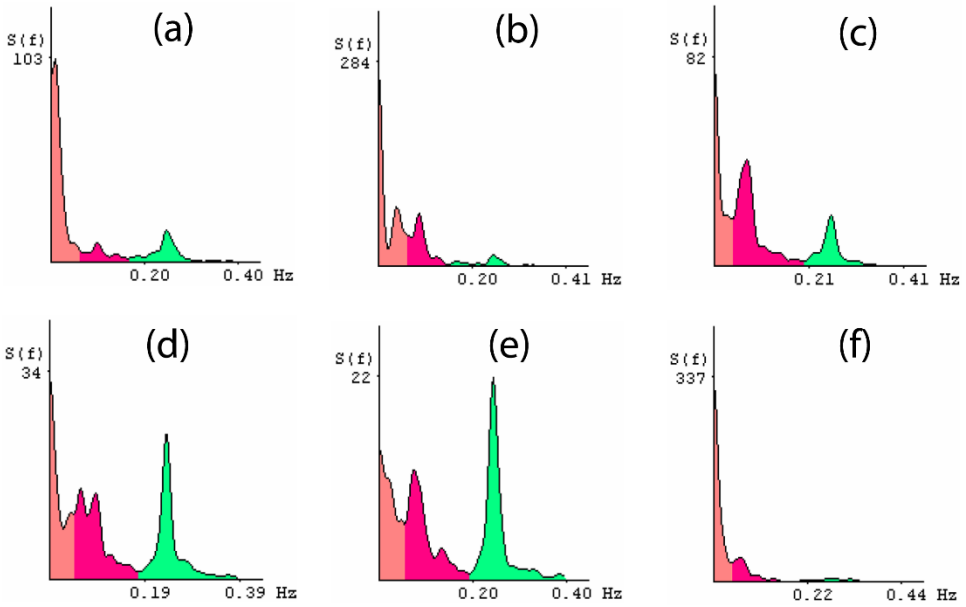

Figure 4.5. *Power distribution across frequency bands during (a) wakefulness, (b) stage 1, (c) stage 2, (d) stage 3, (e) stage 4 of non-rapid eye movement sleep, and (f) rapid eye movement sleep [105].*

Non-linear methods

The traditional time and frequency parameters measure only the linear dynamics of the variability in heart rate. As the human cardiovascular system is intrinsically non-linear, which might be of hemodynamic, electrophysiological, humoral or modulation of the

nervous system by origin [66], various non-linear methods have been researched to quantify the variability and detect the non-linear fluctuations which are otherwise not evident. Parameters derived from these non-linear analyses have been found to be sensitive indicators of changes in sympatho-vagal balance. [106; 107]

Entropy or complexity of the output from a dynamic system is studied using approximate entropy (ApEn) [108], sample entropy (SampEn) [109] and correlation dimension [110]. ApEn statistically measures system complexity and regularity of a stationary signal, thereby quantifying predictability of fluctuations. A high value suggests a lower predictability and regularity. Although it has been widely used in cardiovascular studies [111-113], there are significant demerits. The ApEn algorithm is designed such that it counts each sequence as matching itself, which leads to bias. The bias leads to dependency on record length, which produces lower than expected values of ApEn, and inconsistency in analysis. To overcome the bias of ApEn, a new statistical parameter, SampEn, was derived from approaches developed by Grassberger et al. [109], which did not include self-matches. Simpler algorithm, faster computation, independence of record length and consistency are some advantages SampEn has over ApEn [114]. Similar to ApEn, lower values indicate more self-similarity. In addition, other methods such as 1/f scaling of Fourier spectra [115], H scaling exponent and Coarse Grain Spectral Analysis (CGSA) [116], symbol dynamics [117], Lyapunov exponent analysis [118] and fractal dimension [119] have also been examined. Despite several attempts at validating the methods in experimental time series data, the scope of these methods have not been understood to utilize them in diagnosis.

4.4 Short- and long-term variability

The duration of measurement is usually determined by the aim of the study, methods used for analyses and other factors. Depending on the length of the R-R interval time series, the HRV measurement are classified as short (2-10 minutes) and long term (> 1 hour). Typically, short-term recordings are analysed in the frequency domain whilst time domain is preferred for long-term analysis. According to the Task Force [66], certain parameters based on time series duration are recommended for assessment of HRV: SDNN and HRV_{index}, estimate of overall HRV; RMSSD, estimate of short-term components of HRV. Spectral estimates are seldom used for long-term recordings, as physiological mechanisms for heart period modulation cannot be considered stationary for 24-hour periods. In order to quantize long-term signals, spectral results from shorter segments (5 or 10 minutes) are averaged over the entire length of the signal and the results are comparable to the LF and HF components obtained from spectral analyses of entire signal [120]. Likewise, certain non-linear methods are suitable for short term whereas others are more appropriate for long term. It is to be noted that HRV components only provide a measure of the degree of autonomic modulation and not the level of autonomic tone [121]. These methods cannot replace each other and should be carefully selected depending on the

study. This is because, although the mathematical method used for analyses is the same for both short and long-term recordings, the physiological interpretation is different and hence cannot be considered a surrogate for studies with differing durations.

4.5 Reproducibility and reliability

HRV reproducibility and reliability is critical in longitudinal studies where data is collected from the same individual at different times, which could be days, months or even several years apart, from several individuals under same clinical conditions or under different physiological states. Reproducibility summarizes the effects of intra- or inter-individual differences and precision accuracy. Reliability, on the other hand, evaluates the measure of homogeneity in intervention studies (such as body position, mental stress and different manoeuvres) and agreement to other methods for measuring cardiac autonomic control. [122] Although such an assessment is inconsequential from a prognostic point of view, the modulation of HRV can only be considered clinically useful if these measurements are reproducible and reliable. Reproducibility of HRV parameters depends on several factors, which include and are not limited to temperature, noise, mental stress, breathing frequency and time between measurements [123; 124]. One of the most evident and compelling reasons for poor reproducibility is the sensitivity of HRV indices to changes in duration of the R-R interval segment. McNames et al. [125] have shown that HRV metrics are biased estimates and the comparison of parameters, especially overall estimates, obtained from recordings of different lengths is inappropriate. Apart from signal duration, discrepancies could arise because of study population, conditions of examination, body position, exercise and other eminent features, some of which are discussed in detail.

4.5.1 Intra- and inter-individual differences

Intra-individual variation of HRV indices might be of importance when the effects of internal or external stimuli, such as changes in mood, alertness and mental activity are examined. It requires repeating measurements in a controlled environment and usually define the range beyond which HRV changes should be considered significant and interpreted as true effect of interventions or changes in the study setup. The contribution of within-individual variation greatly depends on the duration between measurements. Measurements taken during the same testing period (usually a few minutes/hours apart) showed that the intra-individual variation decreased with prolongation of recording length. However, measurements conducted days/months apart and prolongation of recording length led to a 30% increase in the variation of certain HRV measures [124]. In addition, the variation were considerably lower when repeated measurements were conducted over time.

Determining the inter-individual variation is necessary when population studies are performed in order to comprehend the effects of various interventions. The between-subject variation defines the range for HRV indices within the population. In such cases, the changes in mean or median of the study group should be interpreted for the definition of normal limits and determination of abnormal HRV. The inter-individual variation constitutes for 86-91% of the error in time-domain indices (independent of measurement length) and around 60-80% for spectral measures (dependent on measurement length). [124]

4.5.2 Measurement interval

Pitzalis and colleagues [126] exclusively studied time- and spectral-domain measures and demonstrated that short- and long-term time domain indices were highly reproducible although the 10-minute recordings showed only low to moderate reproducibility. Long-term spectral indices only presented an average measure of various influences that occurred during a 24-hour period and therefore, may not provide information on acute changes in the ANS. As a result, Pitzalis suggested that frequency domain measures should be conducted short-term to minimize the effects of modulating factors. Despite LF power being reproducible under all test conditions, the reproducibility of TP and HF power showed varied degree of reproducibility depending on the analyses conditions. TP was reproducible only at rest, whilst HF power only during controlled respiration. Interestingly, contrasting claims have been put forward. Sinnreich et al. [127] showed that all spectral parameters are moderately reliable in short-term evaluation. Lord et al. [128] evaluated the reproducibility of measurements performed at different times of the day for healthy subjects: morning (between 08:00 and 09:00), early afternoon (between 12:00 and 13:00) and late afternoon (between 15:00 and 16:00) and found certain parameters vary significantly in the day-to-day as well as measurements at different times of the day. However, short-term time domain indices were less contentious and were considered moderately reproducible in various studies. [126; 129]

4.5.3 Postural changes

Changes in posture from supine to orthostatic position induced changes in the HRV indices due to increase in sympathetic activity and decrease in parasympathetic influence. The HRV indices in the upright position were found to be better reproducible when compared to those of supine position. [130; 131] One explanation put forward from these studies was that subjects were susceptible to external factors such as mood, sleep, stress and anxiety in the supine position. It is believed that the modulatory influence of vagus nerve is restricted and arterial baroreceptors are activated in the upright position, thereby reducing the influence of such external factors. However, the study by Kowalewski et al. [129] suggested that HRV indices, apart from LF/HF ratio, were independent of position and reproducible when the measurements were conducted on the same day.

To summarize, there is no concrete evidence in literature regarding the effect of measurement interval on the reproducibility and reliability of HRV. Poor reliability has been established for resting, short measurement intervals [132] whereas, on the other hand, some of the most reliable measures of HRV have been produced for tests conducted several months apart [127]. Apart from measurement interval, the reproducibility of spectral features is debatable. Some studies have shown that LF power possesses poor reliability [132] whilst other studies exhibit HF power as the least reproducible spectral variable [133; 134]. Therefore, it is recommended the optimal data collection methods described by the Task Force [66] be followed while designing the research study.

4.6 Factors influencing HRV

HRV is a reflection of various physiological factors modulating the normal rhythm of the heart. These factors are measured and controlled by neural modulation and various receptors thereby causing fluctuation in the heart rate. Some of the most important causes are described in the subsequemt chapters.

4.6.1 Genetic factors

There is growing evidence that genetic factors contribute to heart rate and HRV control and the various genes involved have been described in literature. [135-137] Martin et al. [135], through variance decomposition linkage analysis, found that heritability of resting heart rate was 26% and was linked to chromosome 4. Further studies have revealed similar links to blood pressure, type 4 long QT syndrome, associated with bradycardia. In the Framingham Heart Study, Singh et al. [138] have furnished evidence linking LF and VLF power to chromosome 2 and 15, respectively. The combined results of Framingham Heart Study and Framingham Offspring Study shows significant correlation between siblings, which was not so prominent in the spouses, indicating the influence of genetics on HRV heritability [139]. In an ambulatory setting, a genetic contribution of 35-47% for SDNN and 40-48% for RMSSD have been reported by Kupper et al. [140]. Short-term HRV indices measuring vagal tone at rest and stress have also been associated with the same gene [141]. Thus, the genetic influence on HRV has been consistently proven.

4.6.2 Age and gender

Ageing causes complications in the assessment of HRV due to the changes in the ANS with advancing age [142]. Differences in HRV from infancy to adulthood can be due to a variety of reasons, ranging from under-developed ANS in premature infants [143] to hereditary factors [144]. Spectral parameters such as LF, HF and total power have been found to increase from 0-6 years, followed by a steady decrease to adulthood [145; 146]. Umetani and colleagues [147] have shown an exponential decline in vagal indices (RMSSD and pNN50) originating in early adulthood and by the sixth decade of life,

reaches 25-50% of the value observed in young adults. A more gradual decline was observed for SDNN obtained from 24-hour ECG recordings (seen in Figure 4.6). This is evident, as studies have shown an increase in sympathetic nervous activity with advancement in age [148; 149]. The functional changes in ANS can be attributed to a gradual increase in basal and stimulated plasma noradrenaline concentrations [150], altered adrenoreceptor function [151] and diminished responsiveness to adrenergic agonists and antagonists [152]. Although ageing attenuates the arterial baroreflex control, the increased sympathetic outflow is not associated with baroreflex sensitivity.

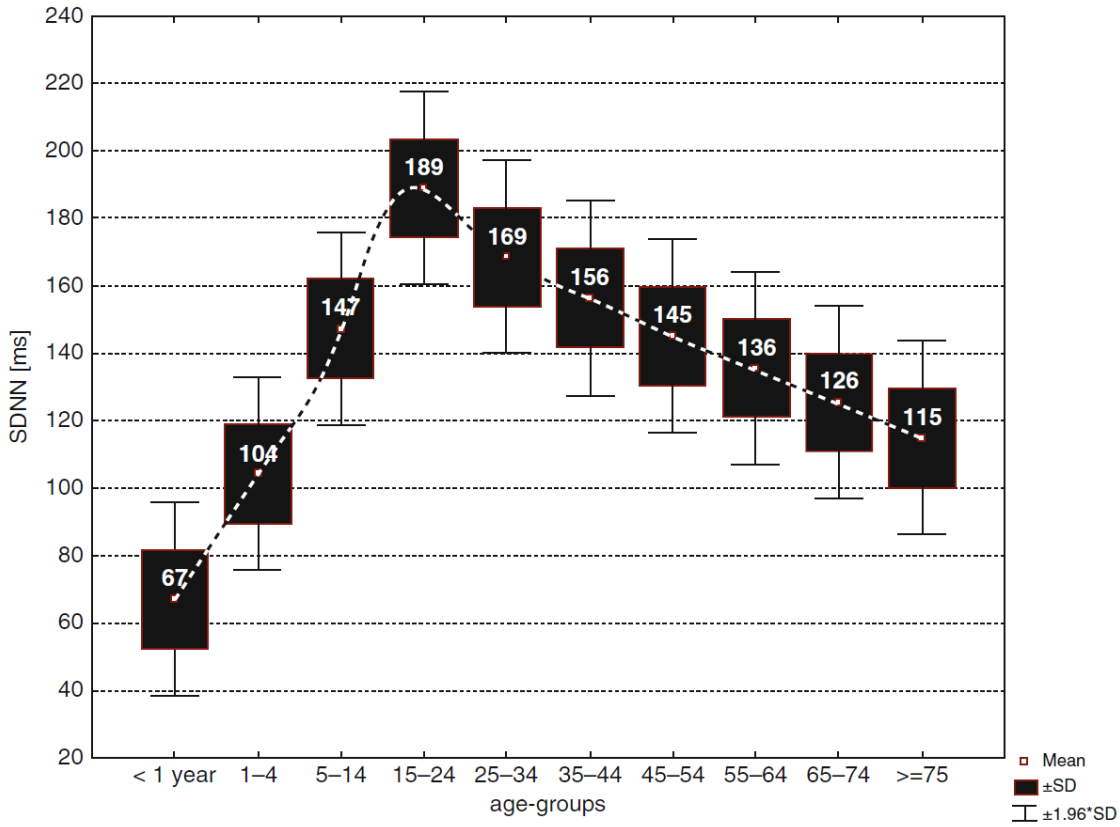

Figure 4.6. *24-hour ECG data obtained from 18 Holter-based studies shows the age dependence of SDNN in healthy subjects. The variability reaches its maximum around early adulthood and declines gradually in the later stages of life. (From [122]).*

Gender differences in autonomic control originate not only from hormonal changes, but also from constitutional and demographic, life-style, intrinsic and peripheral nervous system factors. Some studies have shown that LF and TP are slightly higher in men than in women whereas differences in HF power are variable [153]. Other studies have shown time domain features to be either similar, higher [146] or lower [147] between both genders. Therefore, the opinions related to changes in HRV arising from gender differences are diverse and not well established since they have more often led to inconclusive results.

4.6.3 Respiration

Respiratory influenced fluctuation of HRV reflects the respiratory driven vagus modulation of sinus arrhythmia. According to Rentero et al. [154], as cited by Stauss [155], the

respiratory sinus arrhythmia (RSA) is generated by central coupling of the respiratory oscillator with autonomic centres in the brain stem. Respiration affects heart rate mainly due to lung volume changes and peripheral hemodynamic reflexes. The Bainbridge reflex, initiated by atrial mechanoreceptors, causes tachycardia and bradycardia as a reflex to hypervolumia and hypovolemia respectively. Via the Bainbridge reflex, these respiratory changes causes respiratory fluctuations in cardiac autonomic nervous system.

The R-R interval oscillations caused by respiration occur in the HF band, i.e. between 0.15-0.4 Hz. It is to be noted that only the parasympathetic component of the efferent pathway contributes to RSA, as sympathetic actions are too damped [155]. As a result, the magnitude of respiratory implicated heart rate fluctuation has been described as an index for measuring vagal tone. Expiration is believed to impose vagal activity by stimulating arterial chemoreceptors and baroreceptors which is then completely subdued during the inspiratory phase [156]. The heart rate coincides with breathing frequency during inspiration. It has also been observed that the heart rate fluctuations increase when the breathing rate achieves frequencies connected to the baroreflex. Hence, it can be said that maximal RSA occurs at a respiratory frequency of 0.1 Hz [157].

4.6.4 Thermoregulation

Thermoregulation is associated with circadian rhythm of heart rate and its variation. Fleisher *et al.* [158] had previously reported an increase in the VLF component of HRV during core and skin surface cooling, suggesting a direct modulation of cardiac function by thermal stimuli. Liu *and co-workers* [159] made it evident from his study that sympathetic activity was significantly higher when the subjects experienced lower (< 21°C) or higher (> 29°C) air temperatures. Moreover, reducing temperatures decreased heart rate and markedly increased HRV, suggesting that the temperature effects on HRV are partly independent of the ANS. It is now evident that both, direct effects of the pacemaker activity on the SA node [160] and indirect effects mediated through the ANS [161], facilitate temperature-induced changes in the heart rate and HRV. Aoyagi et al. [162], by suggesting core body temperature correction for HRV, further exemplified the significance of temperature-mediated changes.

4.6.5 Blood pressure

The variations in blood pressure is considered a rich source of information to understand the mechanisms of cardiovascular control [163]. In the human body, blood pressure is maintained by a baroreflex mechanism with assistance from several baroreceptors situated in the heart, aortic arch, carotid sinus and other large vessels [164]. The baroreflex is considered a vagally mediated control mechanism between the heart rate and blood pressure. Stimulation of baroreceptors enhances vagal activity, causing a deceleration of heart rate and an increase in blood pressure. This relation between heart rate and blood pressure is known as baroreflex sensitivity. The influence of baroreflex is also the origin

of Mayer waves that correspond to pressure oscillations in the arteries at a frequency lower than respiratory frequency. [165; 166] However, a study conducted by Virtanen *et al.* [167] has shown that the association of blood pressure to HRV is independent of heart rate. Elevated blood pressure leads to autonomic imbalance, which in turn might manifest as impaired HRV. High blood pressure was found to be an independent determinant of decreased absolute HRV [168].

4.6.6 Diurnal variation

Diurnal variation of HRV is related to the circadian rhythm of sleep and wakefulness [169]. The level of variation due to circadian rhythm is best described by 24-hour HRV indices such as SDNN and HRV_{index}. More detailed estimates of HRV measured at hourly intervals has shown that indices measuring vagal tone, such as RMSSD, pNN50 and HF power, attain their maximum in the morning (around 5:00) and is independent of activity [170]. Shift workers have shown increase in short-term HRV indices during sleep, indicating a shift in circadian rhythm [171]. Augmented diurnal variation with age, reported by Bonnemeier et al. [170], also suggested that age plays a vital role in day-night rhythm changes. Other causes for irregular diurnal pattern have been due to cardiovascular diseases, hypertension or diabetes.

4.6.7 Mental stress

Traditionally, both short- and long-term HRV analyses methods have been used to comprehend the effects of mental stressors on variability of heart rate. Stress is known to subdue vagal tone by inducing several effects on sympathetic and parasympathetic components. [172] Response to reaction time tasks in healthy subjects shows an increase in LF power and is associated with respiratory changes. Anxiety and depression affect heart modulation to a certain extent. Apart from a faster heart rate in depressed individuals, a lower HF power was observed in patients who had a high Hamilton Depression Score [173]. In sleep deprived individuals, the changes in respiratory rate was found to be the fundamental cause for change in HRV indices.

4.6.8 Alcohol consumption

As opposed to general belief that alcohol consumption enhances positive effects in individuals [174], past literature has shown that alcohol intoxication reduces HRV and diminishes potential for adaptive emotional response [175-177]. In a study by Spaak et al. [178], it was observed that time and frequency domain indices of parasympathetic modulation dwindled in a dose-dependent manner whereas frequency domain indices of sympathetic HR modulation were augmented at higher doses of alcohol (greater than 30 grams). A habitual alcohol intake of 23-45grams/day every evening instigated an increase in heart rate and LF/HF ratio during sleep in a study comprising Japanese men [179]. Nonetheless, it is contested that the decrease in HRV is a result of positive association between alcohol consumption and heart rate rather than a peripheral effect on the cardiac

vagal nerve activity [180]. Despite these uncertainties, chronic alcohol dependence causes vagal neuropathy associated with the impairment of the central and peripheral nervous systems, ultimately leading to various cardiovascular ailments.

4.6.9 Tobacco use and air pollution

Active or passive exposure to tobacco smoke is one of the strongest contributors to the risk of cardiovascular diseases, which includes heart disease, stroke, sudden death, peripheral artery disease and aortic aneurysms. Changes in HRV have been associated with acute exposure to tobacco smoke as well as other respirable suspended particles in the air. [181-183] Studies have shown instances of elevated heart rate and decreased HRV indices because of smoking [184; 185] and exposure to air particulate pollution [186] within a time span ranging from a few hours to several days after inhalation. Niedermaier *et al.* [187] has reported that smoking reduces vagal cardiac nerve activity and alters arterial baroreflex response. These effects have been attributed to nicotine, which is known to enhance sympathetic activation as result of catecholamine release [188]. The effect of air pollution on HRV was dependent on the concentration rather than the constituents of the particulates. Although studies have shown that particulate matter in air resulted in a loss of parasympathetic control of heart rate, the effects of particulate pollution on autonomous regulation are not fully understood. [183; 186]

4.6.10 Pathological conditions

Various cardiac [189-191] and non-cardiac [192-194] pathological conditions cause an autonomic imbalance during which the sympathetic system is hyperactive or the parasympathetic system is hypoactive, resulting in a higher energy demand from the cardiovascular system. However, the biological mechanisms behind these autonomic imbalances remain largely unknown. One theory suggests the involvement of inhibitory effects on the rostral ventro-lateral medulla (RVLM), which is constantly modulated by neurons originating at various sources. The intermediary neurons, which have synapses with RVLM neurons, are possibly analogous to synaptic transmission and could lead to an elevated sympathetic modulation. [195] Another theory suggests the presence of cardiac chronotropic responsiveness to various pathological states leading to reduced variability in the heart rate [196]. However, such hypotheses require further investigation to understand the physiology. Sustained levels of higher sympathetic or lower parasympathetic causes increased risk of morbidity or mortality, especially in the case of cardiovascular diseases.

4.6.11 Drugs

Several drugs, especially ones used to treat cardiac ailments, caused significant changes in HRV. Atropine, a commonly used antidote to resurrect the heart during cardiac procedures, has been found to eliminate RSA and abolish or augment the HF power variability.

[197] It works by inhibiting the muscarinic action of acetylcholine on the tissues innervated by postganglionic cholinergic nerves, which ultimately paralyses vagal response. Anaesthetic drugs diminished power in the spectral domain across the HF, LF and TP bands [198]. β-blocker therapy reduces mortality by inducing a shift in autonomic tone towards parasympathetic dominance [199] and are known to subdue responsiveness to mechanical and hemodynamic stimuli of afferent sympathetic fibres. Antiepileptic drugs carbamazepine and phenytoin are sodium-channel blockers that have a membrane stabilizing effect on the central nervous system [200]. Inhibition of neuro-hormonal activation, which causes progressive autonomic impairment and baroreceptor dysfunction [201], by digoxin [202], angiotensin-converting enzyme inhibitors [203] and oral dopaminergic agents [204; 205] has shown to improve overall HRV in association with vagal control and have been beneficial in the treatment of patients with heart failure. Several other studies have reported effects on HRV due to antiarrhythmic, chemotherapeutic, narcotics and sedative drugs.

5. MATERIALS AND METHODS

5.1 Study population

The protocol and data used in this study were designed and collected by FIOH. Sample size was determined using SAS power calculations with subjective sleepiness, measured using a nine-level Karolinska Sleepiness Scale (KSS) [206], which correlates with EEG, as an outcome measure. The criterion set for selection was that the drivers had to be between 20 and 65 years of age, have at least two years of truck driving experience and feel healthy enough for the job. 54 volunteers, working both day and night shifts, were recruited from four middle-sized haulage companies. The companies were initially provided with oral and written information about the project and given a time span of two to three weeks to recruit volunteers who were interested and met the above criteria. Detailed information was provided to all the recruited participants regarding the aims, methods, timetable, potential inconvenience and the option to opt out at any point of time during the study. Informed consent was obtained from all volunteers by FIOH before the data collection phase. As compensation for the time and effort for the recordings, each participant received Euro 150 for successful completion of measurements.

5.2 Study design

The study was part of a larger intervention study titled '*An educational intervention to promote safe and economic truck driving*', with emphasis on assessment of driver sleepiness and the factors contributing to the same. The original study design covered a measurement phase of two weeks each for pre-intervention and post-intervention phase during which duty and off days were covered by *basic* and *intensive* measurements which includes sleep/work routines, questionnaires, video recording and vehicle tracking to mention a few. The study design and data collection methods are described in Figure 5.1. Intervention was designed to account for circadian rhythms, sleepiness and countermeasures. Theoretical material was delivered through a 90-minute lecture, followed by a 2-hour workshop to discuss and solve the issues pertaining to driver sleepiness. In this study, HRV measurements recorded on intensive measurement days along with actigraphy and subjective ratings recorded on the sleep diary from the pre-intervention phase were used for the analyses.

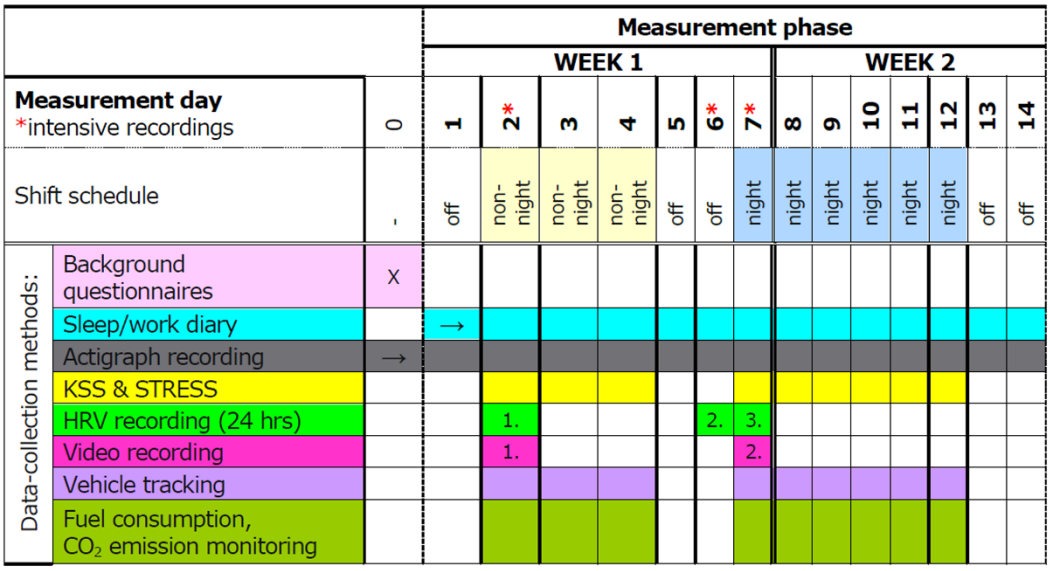

Figure 5.1. *Study design describing data collection for basic and intensive measurements. In this study, recordings performed on intensive recording days during pre-intervention measurements, comprising of one non-night shift, night shift and day off were analysed [207].*

5.3 Data collection

The data collection phase was preceded by asking the drivers to fill a background questionnaire, which included demographics, health statistics, diurnal type (DTQ) [208], FIOH Sleep Questionnaire and other material measuring habitual sleep. The questionnaires and measurement devices were sent to the participants few days prior to the measurement phase. The basic measurements covered actigraphy and sleep/wake diary, which included sleep timing, duration and quality information. In addition to basic measurements, the intensive recordings (HRV measurements) were conducted on two duty days (one on a night shift and another during a non-night shift) and one day off. The drivers were intimated over phone or in person to accustom them to the measurement procedure and were prompted by a text message approximately 12 hours before each intensive measurement day and at the beginning and end of the measurement phase. The drivers were encouraged to report sleep/wake patterns during the entire data collection procedure using the sleep diary.

5.3.1 HRV measurement

A heartbeat measurement device (shown in Figure 5.2) with two snap electrodes were attached to the driver's chest to record the R-R interval time series. On duty days, the measurements started approximately one hour before the beginning of the shift and on days off, about one hour after waking up. The duration of measurement was about 24 hours for each intensive recording. The HRV measurements on the day off were considered as a reference for comparison with data acquired on duty days, assuming they were not under the influence of any physical exertion during that period.

Figure 5.2. *Device used to measure the heart rate of the subjects participating in the study [207].*

To determine the HRV parameters for the sleep period, the 24-hour R-R interval measurements were segmented using sleep start and sleep end times, recorded by the drivers in the sleep/wake diary, as markers for segmentation. An example of the segmentation process is presented in Figure 5.3. All data processing was performed using Matlab R2012b (The MathWorks Inc., Natick, Massachusetts, United States). The segmented sections were then sent to the Biosignal Analysis and Medical Imaging Group, University of Eastern Finland for HRV analyses and the methods are described elsewhere [209]. Although the Task Force guidelines [66] recommend 5-minute interval length for short-term analysis, our data was analysed for 10-minute intervals to ensure an acceptable number of samples were available for examination. The use of longer interval for analysis was justified by the fact that the data might contain artefacts which when excluded, reduces the number of R-R interval data points available for analysis. However, the overall estimates are prone to alterations in physical and mental states of the subjects [210] and environmental changes [211].

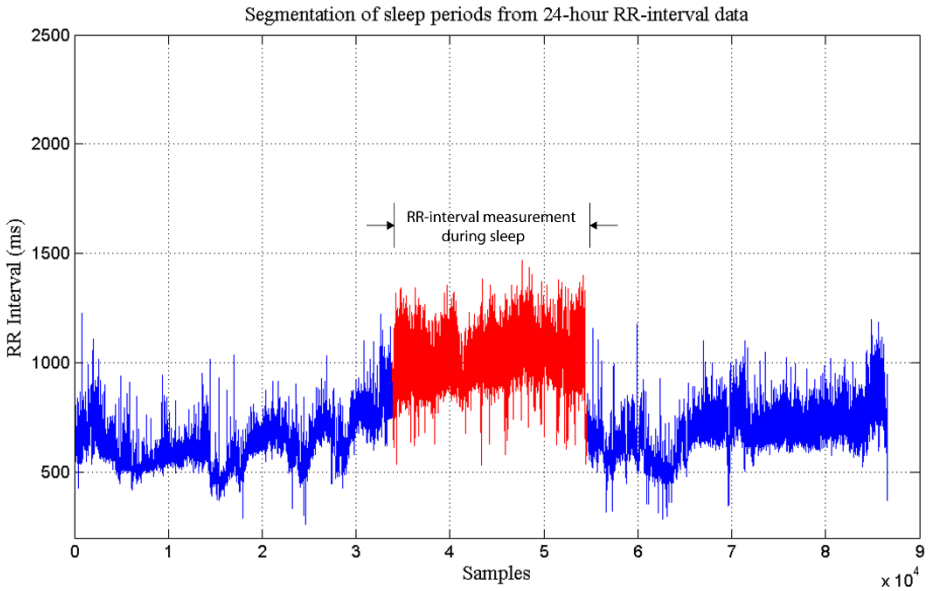

Figure 5.3. *24-hour R-R interval measurements. Region marked in red denotes sleep periods segmented using bed and wake-up times indicated in the sleep diary.*

Artefacts in the R-R interval time series interfere with the outcomes and therefore, suitable pre-processing techniques were implemented to ensure only artefact free segments were utilised. If sufficient data points were not available after artefact removal, interpolation techniques were adopted [212]. In addition, an advanced de-trending procedure [213] based on smoothness prior regularization was used due to its simplicity and minimal data distortion.

HRV indices recommended by the Task Force [66] for short-term measures, or have been used frequently in HRV studies to quantify the sympatho-vagal balance of ANS, were selected for quantifying the data. The list of variables used in this study are presented in Table 5.1. SDNN is considered to determine parasympathetic activity of long-term variations [214] and RMSSD has shown to be a useful indicator of vagal tone [215]. Frequency domain parameters VLF power, HF power and LF/HF ratio, which are associated with thermal regulation and parasympathetic tone, were also chosen as primitive factors for analyses. All frequency parameters were calculated using auto-regressive method.

Table 5.1. *HRV parameters used in the study (as described in [216]).*

Parameter	Units	Description
		Time domain
SDNN	s	Standard deviation of the normal R-R interval time series
Mean HR	1/min	Mean instantaneous heart rate
RMSSD	ms	Root mean squared difference of successive R-R interval
		Frequency domain
VLF Power	ms^2	Power in the 0.002-0.04 Hz frequency band
HF Power	ms^2	Power in the 0.15-0.4 Hz (high) frequency band
LF/HF ratio		Ratio of power in the LF and HF frequency bands

After pre-processing to remove artefacts and de-trending to account for non-stationarities, HRV measured in 10-minute intervals were scanned for intervals with poor signal quality or outliers. Segments with error percentage greater than 5% [217] or outliers detected using the Interquartile range (IQR) method were discarded. If the number of outlier segments in the entire sleep period were more than 20 or if more than 50% of the recording was attenuated by noise or missing, the data was considered unsuitable and excluded from analyses. The 10-minute segments were then averaged *(a)* on an hourly basis to give an estimate of the changes in HRV indices for every hour of sleep, and *(b)* for entire sleep duration. Averaging the values obtained from sequential 10-minute intervals minimizes errors imposed by noisy data or external factors. If the number of 10-minute intervals available for analysis in any particular hour fell below 3 (which represents less than 30 minutes of data available in the particular hour), the values for that hour were excluded since sufficient data was not available to authentically represent the autonomic changes occurring in that hour.

5.3.2 Actigraphy measurement

Actigraphy was measured using Actiwatch, a wrist-worn actigraphy device with movement detectors and sufficient memory to store data for the measurement period. Due to its non-invasive and comfortable measurement protocol, actigraphy has been widely accepted as an alternative to conventional polysomnography in the measurement of sleep quality and recovery pattern [218; 219]. The technology is based on the fact that there is less movement during sleep and more during wakefulness. The activity is measured using piezo-electric accelerometer that measures the amount, intensity and duration of movement. Activity is measured in counts and is proportional to the intensity if movement. The corresponding voltage is then stored at a sampling frequency of 32 Hz. Using this information, periods of sleep and wakefulness are determined by pre-conceived algorithms.

The device was worn on the dominant arm during the recording based on a standard protocol. The collected data were then downloaded to a computer and analysed using the Actiwatch device software and several variables measuring sleep quality were computed for the duration between sleep start and end times. Samples were collected in 15-second epochs. Some basic parameters such as levels of activity/inactivity, rhythm patterns and sleep/wake parameters (total sleep time, sleep efficiency, fragmentation index) were derived. Total sleep time is the measure of the time asleep between sleep start and end as determined by the algorithm. Sleep efficiency is the percentage of time asleep during the total bedtime. Fragmentation index is used as an indicator of movement or restlessness during sleep and is the percentage of time movement was detected using certain pre-determined thresholds. Total activity score is the sum of all counts recorded during each epoch between sleep start and sleep end. [207]

5.4 Data and statistical analyses

Prior to data and statistical analyses, an assessment of the distribution of the data is essential, as normal distribution is an underlying assumption in parametric testing. Due to small sample size, the Shapiro-Wilk numerical test method was adopted to check normality of the distribution for HRV and actigraphy measures. Based on the distribution, parametric or non-parametric methods were chosen for statistical evaluation. The recovery analyses was categorized in three study modules, *(a)* Total sleep analysis, *(b)* core and non-core sleep analysis, and *(c)* Hourly analysis for different intensive measurement days. All statistical tests were performed using IBM SPSS version 21.0 for Windows platform (SPSS Inc., Chicago, IL, United States). Probability values of $p < 0.05$ were considered significant.

5.4.1 Total sleep analysis

Sleep after shifts were compared to recovery sleep on the day off and between workdays without controlling for individual, sleep or habitual factors. A within-subject test for significance was performed to determine the effect of shift type on sleep recovery using paired two-tailed T-tests or Wilcoxon sign rank test for the measured HRV and actigraphy parameters. If the means of the same measurement variable across different shifts were significantly different, the null hypothesis was rejected and differences in sleep recovery after different work schedules inferred.

The association of various independent variables (IV; individual, sleep- and shift-related factors) on the dependent variables (DV; HRV measures) influencing or predicting the level of recovery during sleep were assessed by linear regression. First, a linear relationship between the DV and IV had to be established. If a non-linear trend existed, curve estimations were performed for the corresponding variables and based on the curvilinear association, variables were suitably transformed to attain linearity. Curve estimations were performed with linear, logarithmic and quadratic models. A summary of the transformations used is presented in Table 5.2. Univariate regression of explanatory factors for each of the DVs were then established with R^2 indicating the level of dependence of the DV to the IVs.

Table 5.2. *Transformation models to obtain linear relationship between independent and dependent variables.*

Method	Transformation	Regression equation
Linear model	None	$y = b_0 + b_1 x$
Logarithmic model	$IV = \log(x)$	$y = b_0 + b_1 \log(x)$
Quadratic model	$DV = \mathrm{sqrt}(y)$	$sqrt(y) = b_0 + b_1 x$

IV and DV denote independent variable and dependent variable, respectively.

For forward selection, the *p* value for inclusion was set at <0.05 and the explanatory factors that satisfied this condition were identified and examined in a multivariate regression model with forced entries of age, alcohol consumption and shift type. Multi-collinearity is undesirable in regression analyses as it increases the standard error of coefficients. To eliminate paradoxical results from multiple regression analyses, the variance inflation factors (VIF) associated with each individual and explanatory variable were examined. A VIF of 5 or more indicates correlation between two more variables. In such cases, only one of these were included to the model.

5.4.2 Core and non-core sleep analysis

Horne [220] hypothesized that night sleep was constituted of two types of sleep: core sleep and optional sleep. Core sleep is said to occur in the initial stages of sleep and comprises a high proportion of SWS and is considered the most restorative phase for body

mechanisms and neurological functions. Uninterrupted phase of core sleep is required to maintain adequate levels of daytime alertness and cognitive functioning. The additional sleep that occurs beyond the core sleep was *optional* sleep. This phase is termed *non-core* in the present study to scrutinize this hypothesis. It is believed that non-core sleep does not contribute to the recovery process and was supported by a mathematical model of sleep and waking functions, which suggested that neurobehavioral functions were primarily restored during SWS. Both phases of sleep commence at sleep onset. However, core sleep is stronger during the first few hours of sleep and declines during the later stages.

The variability in heart rate during both sleep stages was established in two dimensions. First, the core and non-core periods of sleep were individually assessed across shift types to determine any significant differences in the recovery. Next, all shifts were pooled together to investigate the magnitude of recovery during core sleep, during non-core sleep and then the entire duration of sleep. Test for significance was performed on a within-subject basis.

5.4.3 Hourly analysis

Hourly means of HRV indices without controlling for individual factors such as age, diurnal type or habitual factors were assessed to understand the pattern of recovery with time. To avoid discrepancies, the last hour of sleep was included based on the criterion that at least 30 minutes of sleep was recorded. In computing means of each hour, a minimum of 10 samples in each shift type was required to be considered for analysis. By comparing hourly means across different shift types, the effect of pre-sleep stressors at work or the effect of time of the day on the recovery process is quantified. Due to unequal sleep periods across different shift types, the length of sleep was trimmed to the mean sleep duration across all shift types for the purpose of comparison.

6. RESULTS

Among the recruited 54 volunteers, three drivers discontinued the study during the measurement phase. For all the drivers whose recordings were successfully completed, the raw R-R interval measurements were segmented with the start time and end time of sleep, recorded on the sleep diary, for the intensive recording days. The heart rate measurements of three drivers were noisy and considered unsuitable for analysis. Data from ten drivers were discarded due to incomplete or unavailable information due to broken device or missing heart rate or actigraphy data. The HRV indices, complemented by actigraphy and subjective ratings, and statistical significance of the differences were evaluated primarily across the three shift types: non-night shift, night shift and the leisure day.

6.1 Driver demographics and sleep times

Thirty-eight drivers (one female) met all the selection criteria and were included in the study. Drivers' mean age was 38.46 ± 10.89 years. Based on DTQ, more drivers considered themselves to be morning type (22.2%) than evening type (16.7%) while the majority rated themselves intermediate (61.1%, not clearly morning or evening type). Driver experience on the job ranged between 3 to 39 years with a mean professional experience of 15.47 years. According to the FIOH sleep questionnaire, the drivers reported a mean sleep need of 07:49 hours a day. Detailed driver demographics and measures from questionnaires are presented in Table 6.1.

Table 6.1. *Descriptive statistics of volunteers participating in the study.*

Individual factors	Mean ± S.D.	Minimum	Maximum
Age (years)	38.46 ± 10.89	22.50	58.30
Body mass index (BMI)	27.11 ± 4.49	18.80	41.70
Diurnal type			
Morning type (%)	22.2		
Evening type (%)	16.7		
Intermediate type (%)	61.1		
Sleep need (hours)	7:49 ± 0:48	6:00	10:00
ESS score	6.88 ± 3.99	0.00	13.00
Trucking experience (years)	15.47 ± 10.84	3.00	39.00

Normal range of BMI: 19-25; Graded diurnal type (1-3): 1 – Evening type, 2 – Intermediate, 3 – Morning type; Epworth sleepiness score (ESS) scale: 0-24

Figure 6.1 represents the observed duration and time of the day the drivers slept after the shifts. The mean sleep duration was considerably shorter on duty days as compared

to the day off. Sleep after non-night and night shift were 01:22 hours and 02:13 hours shorter than leisure day sleep, respectively.

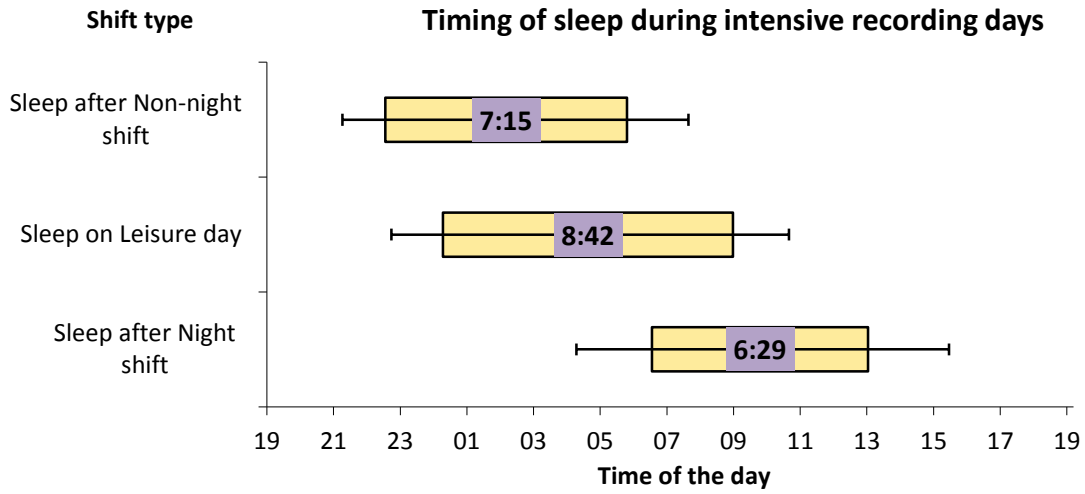

Figure 6.1. *Chart illustrating the duration and time of the day for sleep on the days of intensive recording. Horizontal line denotes the S.D. of the start and end of each sleep period.*

Except SDNN, the Shapiro-Wilk test for normality revealed a skewed distribution for all HRV parameters irrespective of the type of intensive measurement. Sleep efficiency and total activity scores also demonstrated non-normal distribution. The results of the test are presented in Appendix 1. Therefore, non-parametric tests were preferred for measures of statistical significance throughout the analyses. The relative strength of subjective and objective measures of sleep quality towards HRV are presented in Table 6.2. None of the bivariate correlations between physiological and actigraphy measures of sleep quality achieved substantial relevance. VLF power showed strongest resemblance to sleep efficiency (r = -0.345, p <0.001) and total activity score (r = 0.387, p <0.001). Weak correlation between SDNN and subjective sleep quality (r = 0.197, p <0.05), and VLF power and alertness upon awakening (r = 0.191, p <0.05) was observed. Since none of the sleep or subjective estimates achieved significant correlation to HRV, these parameters were included as surrogates in the analyses.

Table 6.2. *Spearman's correlation coefficients for HRV parameters against actigraphy and subjective measures of sleep quality for all shift types.*

	Sleep efficiency	**Fragmentation index**	**Total activity score**	**Subjective alertness upon awakening**	**Estimated sleep quality**
Heart rate	0.181	-0.132	-0.201 *	-0.071	-0.140
SDNN	-0.247 *	0.199 *	0.272 *	0.182	0.197 *
RMSSD	-0.180	0.086	0.210 *	0.153	0.148
VLF power	-0.345 **	0.275 *	0.387 **	0.191 *	0.169
HF power	-0.116	0.032	0.147	0.134	0.115
LF/HF ratio	0.027	0.081	-0.066	-0.055	0.037

Statistically significant measures of correlation are denoted as: ***** for p <0.05 and ** for p <0.001.

6.2 Total sleep duration

The mean of all HRV parameters, actigraphy and subjective measures were categorized and calculated based on shift type and are presented in Table 6.3. The test for significance was performed using Wilcoxon sign-rank test and the results are presented in Appendix 2. Apart from LF/HF ratio between day off and sleep after night shift (p <0.05), the baseline comparisons of HRV indices revealed no significant differences between the intensive measurement days. A lower heart rate and marginally higher mean SDNN, RMSSD, VLF power and LF/HF ratio were observed on sleep after night duty compared to other intensive recording days but no significance was achieved. Sleep duration and efficiency were healthier on day off as compared to duty days, being significantly higher (p <0.05) on leisure day.

Table 6.3. *Mean and S.D. of different variables for sleep after non-night shift, night shift and leisure day for whole night's sleep period.*

	Non-night shift	Leisure day	Night shift
Heart rate variability parameters			
Heart rate (beats/min)	61.13 ± 6.34	62.12 ± 9.29	59.82 ± 6.30
SDNN (ms)	65.29 ± 24.85	65.98 ± 27.72	67.18 ± 20.67
RMSSD (ms)	51.95 ± 29.12	52.37 ± 31.87	52.88 ± 24.30
VLF power (ms^2)	1358.61 ± 806.55	1402.48 ± 896.81	1413.68 ± 691.01
HF power (ms^2)	1234.13 ± 1311.32	1304.29 ± 1407.29	1168.29 ± 1024.48
LF/HF ratio	3.26 ± 2.32	3.91 ± 3.51	3.04 ± 2.12
Actigraphy parameters			
Time in bed (h:min)	07:15 ± 01:13	08:42 ± 01:24	06:29 ± 01:28
Actual sleep (h:min)	06:25 ± 01:12	07:46 ± 01:08	05:43 ± 01:22
Sleep efficiency (%)	88.43 ± 5.99	89.69 ± 4.37	87.97 ± 4.41
Fragmentation index	26.21 ± 12.67	23.30 ± 12.15	25.43 ± 11.24
Total activity score	4479.55 ± 2758.37	4982.89 ± 3763.08	4142.53 ± 2814.01
Subjective parameters			
Subjective alertness after waking			
Poor (%)	0 (0)	0 (0)	0 (0)
Poor – Average (%)	2 (5.3)	2 (5.3)	3 (7.9)
Average (%)	13 (34.2)	8 (21.1)	17 (44.7)
Average – Good (%)	17 (44.7)	24 (63.2)	14 (36.8)
Good (%)	5 (13.2)	4 (10.5)	2 (5.3)
Estimated sleep quality			
Poor (%)	1 (2.6)	0 (0)	0 (0)
Poor – Average (%)	2 (5.3)	0 (0)	2 (5.3)
Average (%)	6 (15.8)	3 (7.9)	8 (21.1)
Average – Good (%)	16 (42.1)	20 (52.6)	17 (44.7)
Good (%)	12 (31.6)	15 (39.5)	9 (23.7)
Alcohol consumption (yes/no, %)	2 (5.3)	16 (42.1)	1 (2.3)
Napping off duty (yes/no, %)	0 (0)	2 (5.3)	3 (7.9)

In addition, subjective measures of sleep indicated significant differences between shift types (Figure 6.2). Sleep quality and alertness were perceived to be better on leisure day (p <0.05) when compared to duty days. In the subjective ratings of alertness upon awakening, majority of the drivers rated their sleep to be between *Average – Good* during non-night shift (44.7%) and leisure days (63.2%), whereas only *Average* after night shifts (44.7%). The rating of alertness was significantly different for sleep after a night shift in

comparison to leisure day sleep (p <0.05). The ratings were reflected in sleep quality estimates in which a higher percentage of drivers gave a *Good* rating during the non-night shift (31.6%) and day off (39.5%) as compared to night shifts (23.7%). However, sleep quality on both duty days were rated significantly lower when compared to leisure day (p <0.05). *Poor-Average* ratings are not shown in Figure 6.2 due to insufficient samples (N<10).

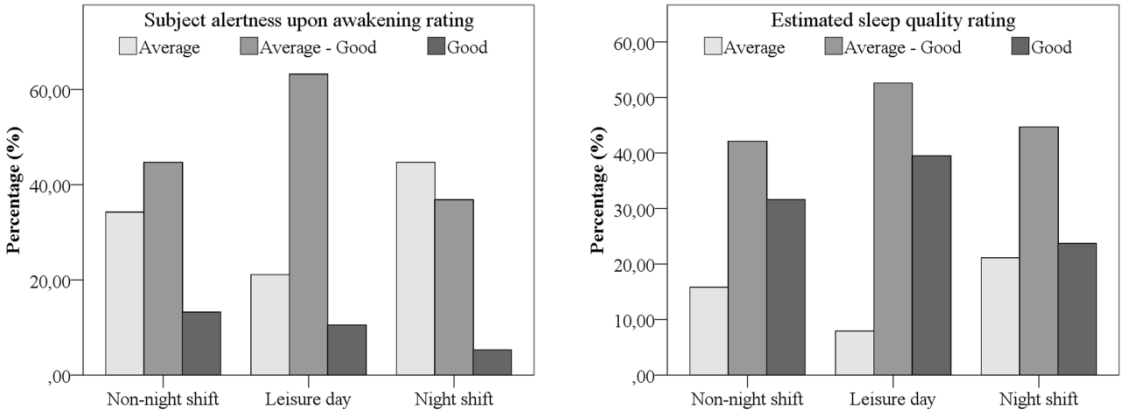

Figure 6.2. *Mean subjective ratings of alertness upon awakening and sleep quality across the different shift types.*

Table 6.4. *Measurements classified by subjective alertness report (Mean ± S.D.).*

	Poor – Average N = 7	**Average** N = 38	**Average – Good** N = 55	**Good** N = 11
Individual factors				
Age (years)	38.18 ± 9.59	38.81 ± 10.13	39.58 ± 11.35	32.12 ± 8.26
BMI	26.42 ± 4.16	25.57 ± 3.23	28.19 ± 5.03	26.50 ± 3.42
Diurnal type				
Morning type (%)	2.8	4.8	11.4	3.8
Intermediate (%)	1.9	21.9	32.4	3.8
Evening type (%)	1.9	8.6	6.7	0
Mean sleep need	7:42 ± 1:00	08:00 ± 0:36	07:54 ± 0:48	7:06 ± 1:00
ESS score	10.00 ± 3.00	7.00 ± 4.00	6.00 ± 4.00	9.00 ± 2.00
Trucking experience	15.8 ± 10.5	15.9 ± 9.9	16.2 ± 11.1	9.0 ± 8.7
HRV parameters				
Heart rate (bpm)	64.41 ± 12.57	61.68 ± 8.22	60.81 ± 6.07	57.40 ± 7.46
SDNN (ms)	63.05 ± 30.72	60.93 ± 23.96	68.98 ± 24.56	74.84 ± 21.78
RMSSD (ms)	50.62 ± 35.28	47.97 ± 28.65	54.70 ± 28.10	59.48 ± 28.32
VLF power (ms^2)	1274.4 ± 854.1	1172.5 ± 651.7	1515.4 ± 870.6	1697.9 ± 789.9
HF power (ms^2)	1336.2 ± 1509.7	1196.9 ± 1405.4	1251.1 ± 1146.4	1376.9 ± 1296.4
LF/HF ratio	6.55 ± 6.02	3.11 ± 2.12	3.29 ± 2.34	3.26 ± 2.78
Actigraphy parameters				
Time in bed (h:min)	6:06 ± 1:16	6:58 ± 1:40	7:57 ± 1:22	7:59 ± 2:11
Actual sleep (h:min)	5:12 ± 0:59	6:13 ± 1:29	7:04 ± 1:14	7:09 ± 2:07
Sleep efficiency (%)	85.86 ± 8.57	89.32 ± 4.81	88.79 ± 4.70	89.05 ± 4.35
Fragmentation index	24.70 ± 16.36	24.82 ± 11.05	25.39 ± 13.08	24.47 ± 7.41
Total activity score	4809 ± 3385	3851 ± 3091	4815 ± 3195	5123 ± 3233

Table 6.5. *Measurements classified by estimated sleep quality (Mean ± S.D.).*

	Poor – Average N = 4	**Average** N = 17	**Average – Good** N = 53	**Good** N = 36
Individual factors				
Age (years)	42.85 ± 7.87	35.53 ± 9.54	39.76 ± 10.86	37.48 ± 11.18
BMI	26.85 ± 2.81	26.41 ± 2.51	27.62 ± 5.60	26.76 ± 3.42
Diurnal type				
Morning type (%)	1	3.8	9.6	8.7
Intermediate (%)	1.8	9.6	27.9	21.2
Evening type (%)	1	2.8	9.6	2.8
Mean sleep need	7:48 ± 0:30	7:36 ± 0:36	8:00 ± 0:42	07:49 ± 1:00
ESS score	10.00 ± 3.00	06.00 ± 4.00	06.00 ± 4.00	8.00 ± 4.00
Trucking experience	20.5 ± 7.9	11.5 ± 8.9	16.3 ± 11.0	15.5 ± 10.9
HRV parameters				
Heart rate (bpm)	59.25 ± 6.08	63.99 ± 8.55	61.51 ± 7.59	59.02 ± 6.85
SDNN (ms)	58.25 ± 15.31	57.83 ± 23.08	65.19 ± 25.49	72.57 ± 23.94
RMSSD (ms)	36.00 ± 8.79	45.89 ± 27.64	52.33 ± 29.18	57.34 ± 29.30
VLF power (ms^2)	1341.2 ± 715.8	1038.1 ± 566.7	1423.4 ± 911.6	1527.9 ± 714.9
HF power (ms^2)	407.8 ± 167.7	1152.6 ± 1349.3	1279.9 ± 1321.4	1304.6 ± 1188.3
LF/HF ratio	5.00 ± 2.83	3.51 ± 3.39	3.18 ± 2.48	3.66 ± 2.83
Actigraphy parameters				
Time in bed (h:min)	6:33 ± 1:13	7:04 ± 1:50	7:33 ± 1:38	7:44 ± 1:38
Actual sleep (h:min)	5:47 ± 1:08	6:23 ± 1:37	6:40 ± 1:28	6:54 ± 1:32
Sleep efficiency (%)	88.25 ± 4.48	90.62 ± 4.09	88.40 ± 5.23	89.15 ± 4.06
Fragmentation index	30.65 ± 18.44	23.36 ± 9.24	25.99 ± 13.46	23.86 ± 10.49
Total activity score	3136 ± 1102	4096 ± 3987	4750 ± 3410	4355 ± 2341

The mean and S.D. across different subjective ratings are described in Table 6.4 and 6.5. The distribution of individual and sleep factors were also assessed to determine any bias. Drivers' who rated their sleep quality and alertness upon awakening as *Good* had distinctively better HRV measures and longer sleep times although the sleep efficiency, fragmentation of sleep and activity score were similar across all categories. No distinct differences were observed in the mean age or BMI across each category. Comparison of mean sleep need and sleep duration revealed that those who tend to sleep lesser than their perceived sleep need did not associate good alertness levels upon awakening and rated their sleep quality to be lower. This suggests that lower sleep duration, irrespective of shift type, is one reason for lower levels of recovery. However, sufficient power was not reached for statistical test of significance between subjective rating categories.

To determine the associations of various individual-, sleep- and shift-related factors on HRV measures, regression analyses was performed. Prior to regression analysis, test for linearity between DVs (HRV parameters) and IVs (individual, actigraphy and subjective factors) were established. Table 6.6 represents the results of the test for linearity with $p < 0.05$ indicating a non-linear association in the test pair.

Table 6.6. *Test for linearity of IV factors (individual, actigraphy and subjective) with DV (HRV indices) using test for ANOVA. Null hypothesis is rejected if p-value of less than 0.05 and a non-linear relationship existed between the DV-IV pair.*

	Heart rate		**SDNN**		**RMSSD**		**VLF power**		**HF power**		**LF/HF ratio**	
	F	***p***	**F**	***p***	**F**	***p***	**F**	***p***	**F**	***p***	**F**	***p***
Individual factors												
Age	4.916	<0.001	8.584	<0.001	10.521	<0.001	8.148	<0.001	10.671	<0.001	6.382	<0.001
BMI	4.205	<0.001	7.865	<0.001	8.078	<0.001	8.471	<0.001	7.306	<0.001	5.078	<0.001
Diurnal type	1.389	0.241	5.865	0.017	4.069	0.046	4.498	0.036	3.467	0.065	0.022	0.882
Mean sleep need	4.580	0.001	10.561	<0.001	12.355	<0.001	8.505	<0.001	12.037	<0.001	2.297	0.049
ESS Score	2.852	0.003	5.271	<0.001	4.938	<0.001	4.811	<0.001	4.421	<0.001	3.941	<0.001
Trucking experience	4.774	<0.001	3.286	<0.001	3.564	<0.001	3.840	<0.001	3.415	<0.001	2.878	<0.001
Actigraphy parameters												
Time in bed	1.527	0.178	0.915	0.626	0.871	0.674	1.005	0.534	0.795	0.756	0.801	0.750
Actual sleep	1.703	0.152	0.917	0.623	0.911	0.630	1.288	0.327	1.013	0.533	1.715	0.149
Sleep efficiency	0.955	0.579	0.995	0.527	0.914	0.634	1.043	0.467	0.878	0.683	1.261	0.248
Fragmentation index	0.748	0.790	0.865	0.674	0.759	0.780	0.560	0.939	0.662	0.868	2.598	0.034
Total activity score	0.628	0.790	0.453	0.860	0.665	0.777	0.365	0.899	1.874	0.533	15.044	0.203
Subjective parameters												
Shift type	0.909	0.406	0.057	0.944	0.010	0.990	0.050	0.951	0.111	0.895	1.06	0.349
Alcohol usage (yes/no)	2.664	0.105	2.027	0.157	1.587	0.210	2.860	0.094	0.569	0.452	5.667	0.059

Table 6.7. *Spearman's correlation coefficients for explanatory factors and subjective ratings of sleep. Significant levels of correlation (0.5 – 0.99) are represented in bold.*

	BMI	**Diurnal type**	**Mean sleep need**	**ESS score**	**Trucking experience**	**Sleep efficiency**	**Fragmentation index**	**Total activity score**	**Subjective alertness upon awakening**	**Estimated sleep quality**
Individual factors										
Age	0.439**	0.013	-0.246*	0.219*	**0.934****	-0.151	0.047	0.056	-0.100	-0.044
BMI	-	0.215*	-0.205*	-0.036	0.313*	-0.269*	0.143	0.239*	0.109	-0.040
Diurnal type		-	-0.412**	0.164	0.019	0.129	-0.184	-0.146	0.184	0.119
Mean sleep need			-	-0.115	-0.325*	-0.151	0.017	0.121	-0.156	-0.084
ESS Score				-	-0.243*	0.017	0.012	-0.022	-0.059	0.082
Trucking experience					-	-0.139	0.095	0.031	-0.108	0.019
Actigraphy parameters										
Sleep efficiency						-	**-0.551****	**-0.739****	0.029	0.006
Fragmentation index							-	**0.556****	-0.016	-0.058
Total activity score								-	0.141	0.063
Subjective parameters										
Subjective alertness upon awakening									-	**0.542****

Statistically significant measures of correlation are denoted as: ***** for $p < 0.05$ and ** for $p < 0.001$

With the exception of mean sleep need and LF/HF ratio, a non-linear relationship existed between all HRV indices and age, BMI, sleep need, Epworth sleepiness score (ESS) and trucking experience. All sleep and subjective measures showed significant linear associations. Curve estimates for each non-linearly associated IV-DV pair were performed (Appendix 3). Depending on the association, suitable transformations were applied to achieve linearity prior to regression estimates. Linearity for age could not be established for heart rate, VLF power or LF/HF ratio. A logarithmic curve estimate was established for SDNN with age. A quadratic line equation was fitted to SDNN, RMSSD and HF power to achieve linearity with BMI. Similarly, curve estimates showed a quadratic relation for mean sleep need and SDNN. Apart from LF/HF ratio, the ESS score did not achieve any linear, logarithmic or quadratic association to other HRV parameters.

The correlation outcomes for individual-, sleep- and subjective-factors are presented in Table 6.7. Driver's age and trucking experience were significantly correlated, $r = 0.934$, $p < 0.001$. Sleep efficiency showed moderate negative correlation to total activity score ($r = -0.551$, $p < 0.001$) and a weak negative relation to fragmentation index ($r = -0.551$, $p < 0.001$). None of the inter-measurement variables' correlation coefficients achieved significance. The associations of all individual, actigraphy and subjective factors with HRV were first assessed in a univariate linear model. Trucking experience and totally activity score were discarded due to their moderate-high correlation to age and sleep efficiency, respectively. When controlled for other covariates, regression estimates showed a 2 bpm ($p < 0.05$) increase in heart rate during sleep with the consumption of alcohol. LF/HF ratio was unaffected by any individual or sleep measures. The results of univariate regression for heart rate and LF/HF ratio are presented in Appendix 4.

Summary of the factors associated with SDNN, RMSSD, VLF and HF power are presented in Table 6.8. Age, diurnal type, sleep duration and measure of sleep efficiency were very good predictors of sleep estimates with SDNN. With every year increase in age, SDNN was lower by around 5 ms ($p < 0.05$). The unstandardized slope for sleep efficiency ($B = -1.548$) was significantly different from 0 ($p < 0.05$). Diurnal variations ($B = -8.747$, $p < 0.05$) was a significant contributor to SDNN levels during recovery. Likewise, RMSSD showed similar associations with diurnal type ($r^2 = 0.074$, $F(1,112) = 9.496$, $p < 0.05$) and sleep efficiency ($r^2 = 0.052$, $F(1,112) = 6.195$, $p < 0.05$) but only a subtle negative gradient with age ($B = -0.490$, $p < 0.05$). Both the time domain estimates of vagal tone showed significant association with sleep duration. VLF power was substantially associated with sleep efficiency ($r^2 = 0.169$, $F(1,112) = 22.845$, $p < 0.001$) and fragmentation score ($r^2 = 0.133$, $F(1,112) = 17.181$, $p < 0.001$). Interestingly, VLF power was the only HRV parameter to be associated with BMI ($B = 38.146$, $p < 0.05$). Similar to other estimates of vagal tone, diurnal type was an efficient predictor of power in the HF band ($r^2 = 0.129$, $F(1,112) = 15.694$, $p < 0.001$). It is to be noted that shift type did not assume any level of significance for predicting the outcome of the HRV measures.

Table 6.8. *Univariate linear regression with HRV indices as dependent variables.*

SDNN	**Model Summary**		**ANOVA**		**Coefficients**	
	R	**R^2**	**F**	***p***	**B**	**β**
Individual factors						
$Age_{transformed}$	0.213	0.045	5.317	0.023	-4.891	-0.213
$BMI_{transformed}$	0.028	0.001	0.072	0.788	-1.637	-0.028
Diurnal type	0.221	0.049	5.464	0.021	-8.747	-0.221
Mean sleep $need_{transformed}$	0.132	0.018	1.891	0.172	21.950	0.132
Actigraphy parameters						
Time in bed	0.210	0.044	5.166	0.025	0.001	0.210
Actual sleep	0.112	0.013	1.424	0.235	0.001	0.112
Sleep efficiency	0.317	0.100	12.497	0.001	-1.548	-0.317
Fragmentation index	0.273	0.075	9.021	0.003	0.555	0.273
Subjective parameters						
Shift type	0.032	0.001	0.113	0.737	0.944	0.032
Alcohol consumption	0.103	0.011	1.212	0.273	-3.299	-0.103
RMSSD	**Model Summary**		**ANOVA**		**Coefficients**	
	R	**R^2**	**F**	***p***	**B**	**β**
Individual factors						
$Age_{transformed}$	0.187	0.035	4.038	0.047	-0.490	-0.187
$BMI_{transformed}$	0.019	<0.001	0.033	0.857	-1.251	-0.019
Diurnal type	0.287	0.074	9.496	0.003	-13.261	-0.287
Actigraphy parameters						
Time in bed	0.176	0.031	3.567	0.062	0.001	0.176
Actual sleep	0.100	0.010	1.136	0.289	0.001	0.100
Sleep efficiency	0.229	0.052	6.195	0.014	-1.301	-0.229
Fragmentation index	0.177	0.031	3.604	0.060	0.417	0.177
Subjective parameters						
Shift type	0.014	<0.001	0.021	0.886	0.468	0.014
Alcohol consumption	0.100	0.010	1.125	0.291	-3.696	-0.100
VLF power	**Model Summary**		**ANOVA**		**Coefficients**	
	R	**R^2**	**F**	***p***	**B**	**β**
Individual factors						
$BMI_{transformed}$	0.205	0.042	4.142	0.045	38.146	0.205
Diurnal type	0.228	0.052	5.792	0.018	-294.669	-0.228
Mean sleep $need_{transformed}$	0.210	0.044	4.903	0.029	207.881	0.210
Actigraphy parameters						
Time in bed	0.268	0.072	8.699	0.004	0.036	0.268
Actual sleep	0.144	0.021	2.381	0.126	0.021	0.144
Sleep efficiency	0.412	0.169	22.845	<0.001	-65.659	-0.412
Fragmentation index	0.365	0.133	17.181	<0.001	24.200	0.365
Subjective parameters						
Shift type	0.028	0.001	0.090	0.764	27.538	0.028
Alcohol consumption	0.108	0.012	1.316	0.254	-112.173	-0.108
HF power	**Model Summary**		**ANOVA**		**Coefficients**	
	R	**R^2**	**F**	***p***	**B**	**β**
Individual factors						
$Age_{transformed}$	0.205	0.034	4.929	0.028	-23.738	-0.205
$BMI_{transformed}$	0.109	0.012	1.136	0.289	-292.599	-0.109
Diurnal type	0.359	0.129	15.694	<0.001	-733.373	-0.359
Actigraphy parameters						
Time in bed	0.211	0.044	5.200	0.024	0.044	0.211
Actual sleep	0.141	0.020	2.279	0.134	0.033	0.141
Sleep efficiency	0.194	0.038	4.386	0.038	-48.578	-0.194
Fragmentation index	0.160	0.017	2.954	0.088	16.686	0.160
Subjective parameters						
Shift type	0.022	<0.001	0.052	0.819	-32.919	-0.022
Alcohol consumption	0.023	0.001	0.057	0.811	-36.957	-0.023

Suffix *transform* indicates transformation has been performed to achieve linearity. *R* indicates the correlation, R^2 represents coefficient of distribution, which measures the total proportion of variation in the DV about its mean by the IV. *F* hypothesizes that none of the IV's represent the DV about its mean with $p < 0.05$ and $p < 0.001$ rejecting the null hypothesis. *B* and β represent unstandardized and standardized coefficients. Parameters with non-linear relationship with DV were excluded.

Multiple regression for explanatory factors showing significant association to the outcome of HRV measures were included with forced entries of age, shift type and alcohol consumption (provided linearity was established by suitable transformation, else these factors were excluded from the model) to evaluate how well the combination of these factors influenced recovery during sleep. The summary of the models for HRV indices and explanatory factors is presented in Table 6.9.

Table 6.9. *Model summary and model fit parameters.*

	Model summary			ANOVA	
	R	**R-square**	**Adjusted R-square**	**F**	**Sig.**
Heart rate (bpm)	0.323	0.104	0.070	3.000	0.022
SDNN (ms)	0.555	0.308	0.248	5.166	<0.001
RMSSD (ms)	0.414	0.172	0.131	4.229	0.002
VLF power (ms^2)	0.605	0.366	0.308	6.291	<0.001
HF power (ms^2)	0.460	0.212	0.165	4.526	<0.001

R indicates the correlation, R^2 represents coefficient of distribution, *adjusted* R^2 accounts for extraneous predictors to the model, *F* is mean square (regression) divided by the mean square (residual).

The linear combination of VLF power and explanatory factors significantly predicted sleep recovery, $F(9,92) = 6.291$, $p < 0.001$ and the factors associated with it. The sample multi-collinearity coefficient (r) was 0.605, indicating that approximately 31% of the variance (r^2) in sleep recovery can be accounted for by the linear combination of individual, sleep and subjective factors in the model.

Despite achieving zero-order collinearity to HRV variables, only age and sleep efficiency showed significant ($p < 0.05$) partial associations with parameters measuring vagal tone in the multivariate model. Long-term variability in vagal tone was predominantly influenced by age ($\beta = -0.257$) and sleep efficiency ($\beta = -0.321$), which contributed around 25% to the variance in the SDNN model ($F(9, 92) = 5.166$, $p < 0.001$). RMSSD ($r = 0.414$, adjusted $r^2 = 0.131$, $F(9.92) = 4.229$, $p < 0.05$) and HF power ($r = 0.460$, adjusted $r^2 = 0.165$, $F(9.92) = 4.526$, $p < 0.001$) models projected a weak influence of explanatory factors on sleep recovery. LF/HF ratio model was not included either due to non-linear or insignificant associations with explanatory factors. None of the models prophesied significance of shift type in recovery. Regression coefficients with 95% confidence interval (CI) are summarized in Table 6.10.

Table 6.10. *Multivariate linear regression for dependent variables.*

SDNN	**B**	**95 % CI**		**β**	***p***
		Lower	Upper		
Individual factors					
Constant	207.387	49.905	368.870	-	0.012
$Age_{transformed}$	-0.556	-0.950	-0.162	-0.257	0.006
Diurnal type	-4.780	-12.666	3.105	-0.117	0.232
Actigraphy parameters					
Time in bed	0.001	0.000	0.002	0.198	0.039
Sleep efficiency	-1.502	-2.556	-0.448	-0.321	0.006
Fragmentation index	0.166	-0.273	0.606	0.084	0.454
Subjective parameters					
Shift type	1.856	-3.318	7.030	0.063	0.478
Alcohol consumption	-4.670	-10.298	0.957	-0.145	0.103
RMSSD	**B**	**95 % CI**		**β**	***p***
		Lower	Upper		
Individual factors					
Constant	200.365	101.430	299.300		<0.001
$Age_{transofrmed}$	-0.526	-1.004	-0.048	-0.199	0.031
Diurnal type	-11.380	-19.935	-2.826	-0.246	0.010
Actigraphy parameters					
Sleep efficiency	-1.194	-2.264	-0.123	-0.210	0.029
Subjective parameters					
Shift type	-0.114	-6.413	6.185	-0.003	0.971
Alcohol consumption	-4.662	-11.593	2.270	-0.122	0.185
VLF power	**B**	**95 % CI**		**β**	***p***
		Lower	Upper		
Individual factors					
Constant	2659.542	-1574.941	6894.024	-	0.215
$BMI_{transformed}$	21.774	-12.056	55.604	0.117	0.204
Diurnal type	-125.499	-398.903	147.905	-0.090	0.364
Mean sleep need	114.807	-91.815	321.429	0.110	0.272
Actigraphy parameters					
Time in bed	0.035	0.010	0.061	0.255	0.007
Sleep efficiency	-44.550	-80.051	-9.049	-0.287	0.015
Fragmentation index	9.157	-5.697	24.012	0.139	0.224
Subjective parameters					
Shift type	88.555	-84.970	262.081	0.088	0.313
Alcohol consumption	-150.836	-340.032	38.360	-0.138	0.117
HF power	**B**	**95 % CI**		**β**	***p***
		Lower	Upper		
Individual factors					
Constant	6035.178	1465.790	10604.566		0.010
$Age_{transformed}$	-21.222	-42.341	-0.102	-0.182	0.049
Diurnal type	-636.861	-1008.204	-265.518	-0.312	0.001
Actigraphy parameters					
Time in bed	0.031	-0.009	0.071	0.144	0.124
Sleep efficiency	-39.890	-86.274	6.494	-0.159	0.091
Subjective parameters					
Shift type	-7.545	-285.446	270.356	-0.064	0.957
Alcohol consumption	-107.822	-409.389	193.744	0.064	0.480

Suffix *transform* indicates transformation has been performed to achieve linearity. $p < 0.05$ and $p < 0.001$ rejecting the null hypothesis that none of the IV's represent the DV about its mean. *CI* denotes confidence interval.

6.3 Core and non-core sleep

The duration of sleep was split into core and non-core sleep to establish whether recovery is bound only to certain phase during sleep. Table 6.11 presents the mean and S.D. for both sleep episodes across all intensive recording days. Except for the ratio of LF to HF power, all HRV indices showed improvement during non-core sleep irrespective of the intensive measurement type or duration.

Due to non-normal distribution of the data, median level and distribution over the 25^{th}-75^{th} percentile is regarded more appropriate for purpose of comparison. Box representations of the distribution of HRV indices during core and non-core sleep are presented in Figure 6.3. The median heart rate during all shift types and sleep phases oscillated between 60-65 bpm, although a variable beating frequency was recorded more often on night shift. Comparisons between night and non-night shift sleep achieved statistical significance (p <0.05) during both sleep phases.

Measures of vagal tone were marginally higher after night shifts although only SDNN between the duty days accounted for a significant difference (p <0.05). Despite 50% of the drivers recording similar RMSSD outcomes, a more diverse range was observed in the positive quartile across both sleep phases, specifically after non-night duties and leisure days. VLF power was significantly higher (p <0.05) after night shift during core sleep but reached higher levels on non-night and leisure days for the period of non-core sleep. Core sleep HF power on day off and non-core HF power after non-night shift illustrated a greater positive skew but no significance was attained. The LF/HF ratio exhibited a more dynamic range and was significantly (p <0.05) different from that of night shift during initial sleep phase.

The tangible length of sleep for which restoration of body mechanisms and functions are attained were compared against three sleep stages: start of sleep, end of core sleep and end of sleep without controlling for shift type or other factors. A within-subject test for significance for all HRV outcomes but LF/HF ratio, established a significant level of recovery is associated with both core and non-core sleep phases, irrespective of phase length (shown in Figure 6.4). This is contrary to description in literature that restorative functions are confined to initial phase of sleep.

Table 6.11. *Mean and S.D. of different variables for core and non-core sleep for intensive recording days.*

	Non-night shift		**Leisure day**		**Night shift**	
	Core sleep	**Non-core sleep**	**Core sleep**	**Non-core sleep**	**Core sleep**	**Non-core sleep**
Heart rate (bpm)	62.87 ± 6.76	57.89 ± 7.53	63.35 ± 9.15	61.19 ± 9.66	60.11 ± 6.73	59.62 ± 6.60
SDNN (ms)	60.52 ± 23.93	69.69 ± 25.93	62.42 ± 28.05	68.94 ± 28.01	65.42 ± 20.66	70.37 ± 22.62
RMSSD (ms)	47.72 ± 27.30	55.37 ± 31.05	50.56 ± 31.86	53.77 ± 32.80	52.15 ± 23.64	54.10 ± 27.35
VLF power (ms^2)	1125.62±688.94	1653.61±1010.74	1161.14±806.93	1611.42±999.57	1315.28±697.24	1560.48±797.42
HF power (ms^2)	1068.70 ± 1199.94	1397.32 ± 1409.29	1224.18 ± 1347.10	1368.18 ± 1545.51	1137.68 ± 977.03	1260.89 ± 1202.40
LF/HF ratio	3.30 ± 2.26	3.11 ± 2.53	3.92 ± 3.49	4.02 ± 3.69	2.92 ± 2.13	3.12 ± 2.47

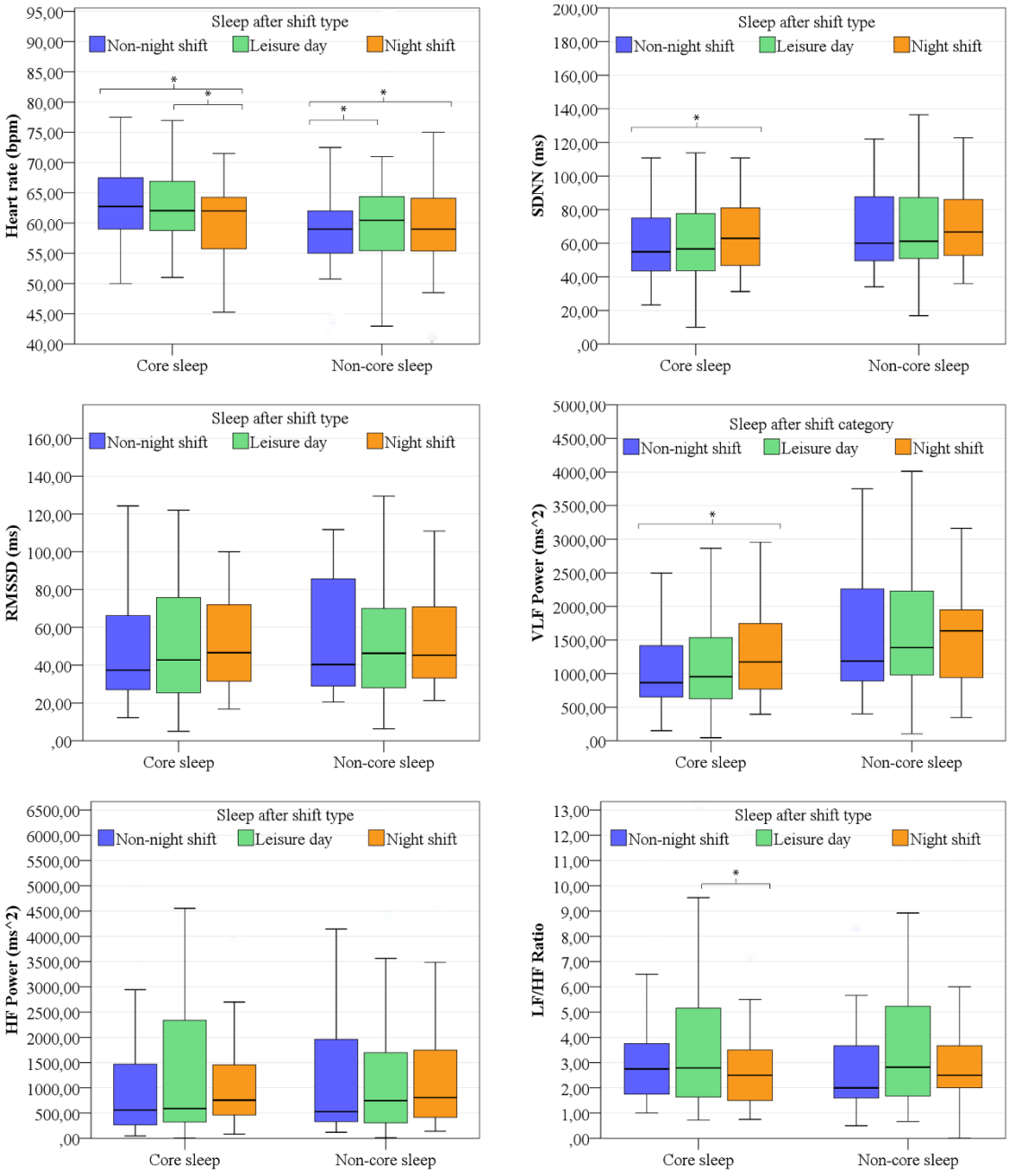

Figure 6.3. *Box plots representing median and middle 50% distribution of the shift-specific HRV outcomes measuring sleep recovery between the two phases of sleep. Wilcoxon Sign rank test was used to measure significance and are denoted as * for p <0.05.*

From Figure 6.4, SDNN, RMSSD and VLF power showed the highest outcomes after a night shift in all three stages of sleep. Some of the HRV indices indicated shift type differences existed during the start of sleep but assumed similar recovery levels during the latter half. Despite the indices of variability in heart rate being much lower at the start of sleep on non-night shifts as compared to night shifts, the means for total sleep duration of all, barring LF/HF ratio, were similar across both work days. LF/HF ratio was subdued during duty days when compared to day off.

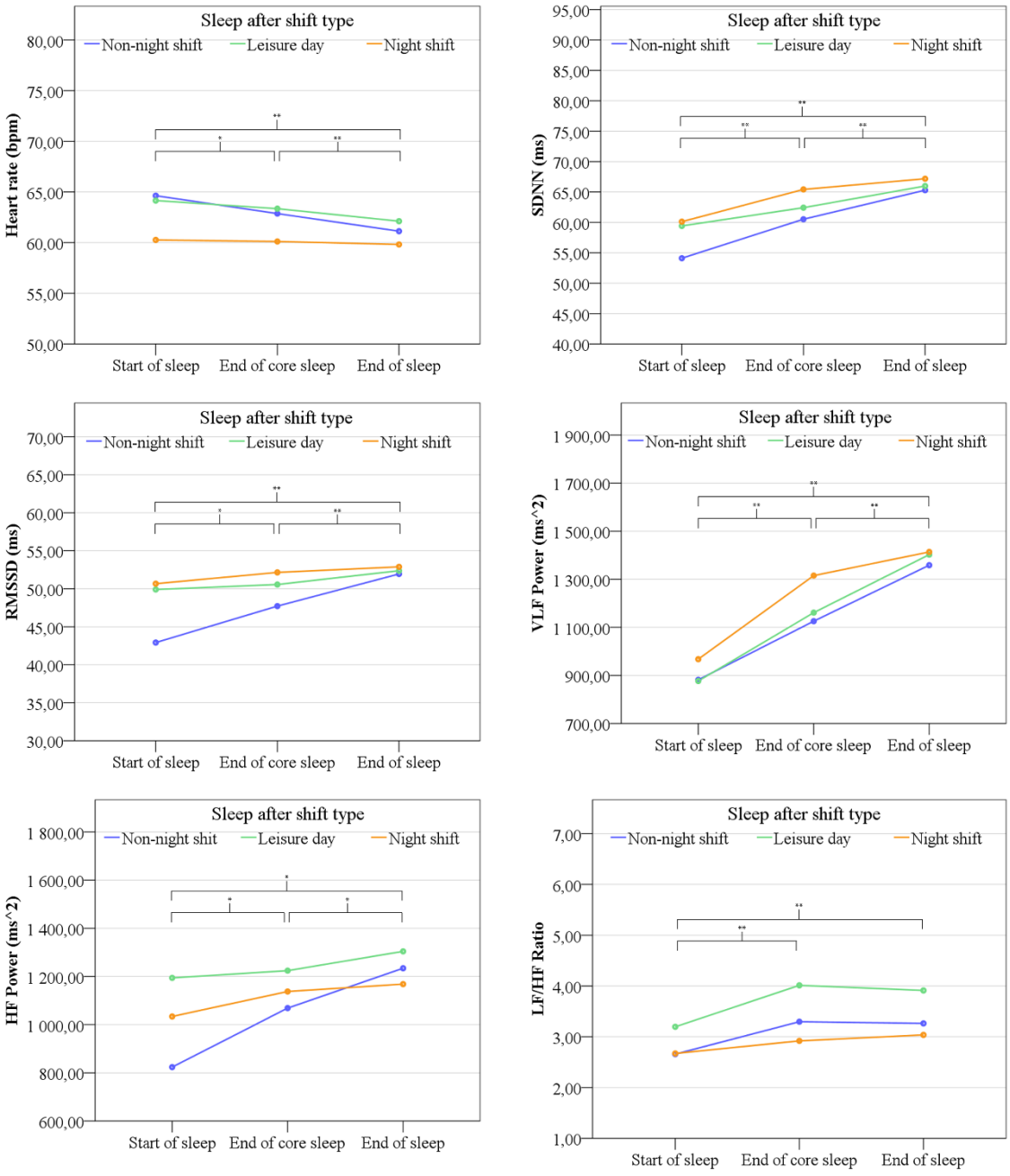

Figure 6.4. *Line charts illustrating levels of recovery attained at various phases of sleep. Significance is denoted by: * for p <0.05 and ** for p <0.001.*

6.4 Hourly analysis

Hourly means revealed no distinct recovery pattern among any intensive measurement days. Mean heart rate during night shifts was significantly lower than non-night shifts (p <0.001) and leisure days (p <0.05) during initial hours of sleep. Once again, the heart rate was negatively skewed towards the lower 25th quartile and gradually assumed a more normal distribution during the later stages. Non-night and leisure day heart rate were relatively lower during the end stages when compared to sleep after night shift. Measures of SDNN and RMSSD retained similar levels during sleep after night shifts.

However, non-night and leisure days showed subtle increase with time throughout sleep periods but no significant variation from night shifts were observed. VLF and HF power showed gradual uptrend with sleep hours and increased variations in the 75th quartile. LF/HF ratio was predominantly higher on leisure days sleep as compared to work days and was significantly different from night shift during the 2nd and 3rd hours of sleep. The sleep patterns are illustrated in the Figure 6.5. Classification by age did not discern any visible changes in the heart rate across the different age groups (Appendix 5). However, the drivers in older age group (50 – 64.9 years) showed lowest SDNN and RMSSD levels throughout the sleep period irrespective of the type of shift. Power in the HF band reflected low parasympathetic activity during sleep in this age group when compared to the younger and middle-aged population. LF/HF ratio was visibly higher across the entire sleep duration, suggesting higher sympathetic activity during sleep when compared to other categories.

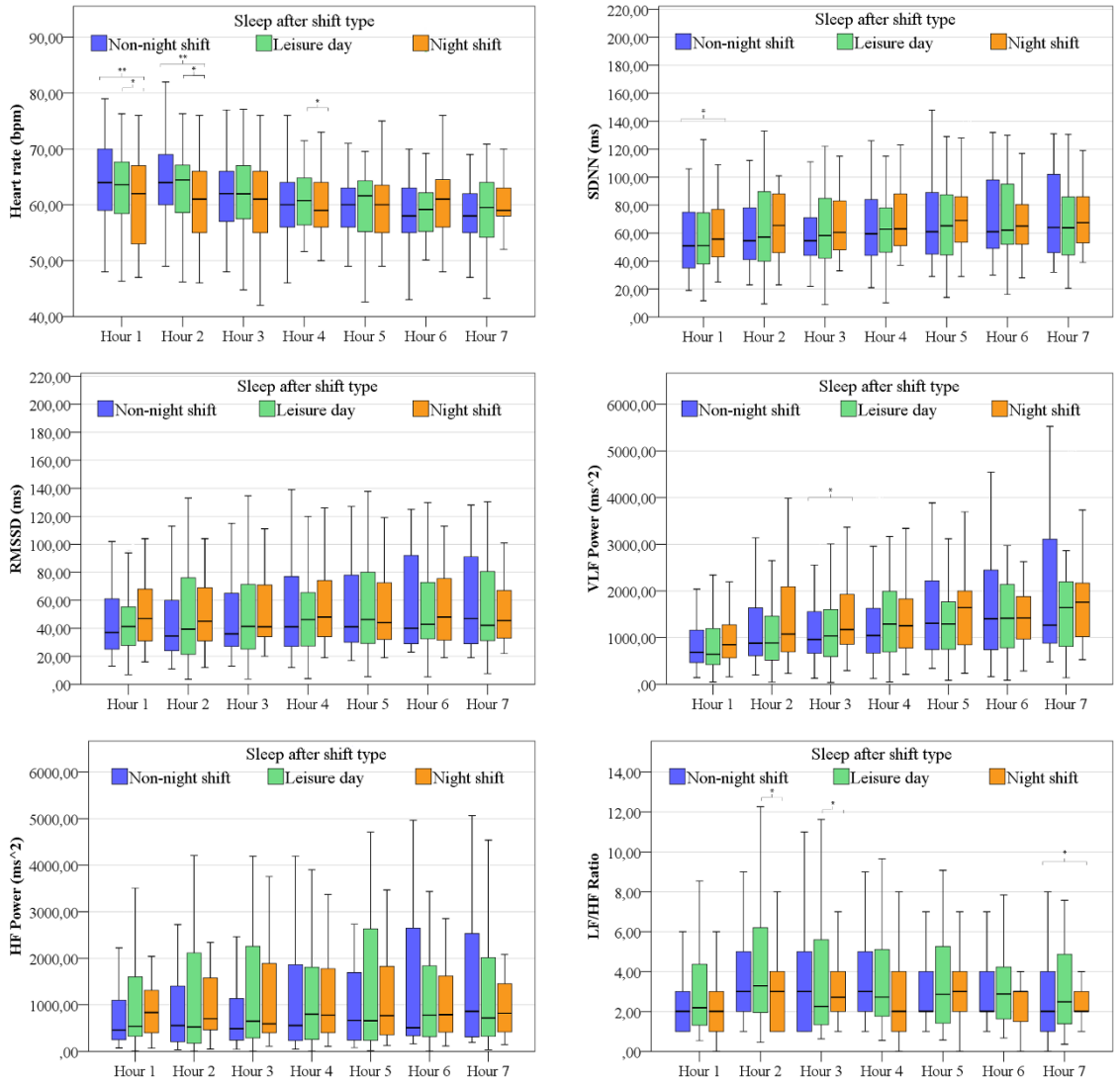

Figure 6.5. *Hourly recovery pattern of HRV parameters across various shift types. Median and distribution over upper (75%) and lower (25%) quartiles are represented. Significance is denoted by: * for p <0.05 and ** for p <0.001.*

7. DISCUSSION

Exposure to high work demands activates stress systems such as the sympathetic adrenal medullary (SAM) and hypothalamic pituitary adrenal (HPA) resulting in increased hormone secretion and cardiovascular stress. Without adequate recovery from these work stressors, the homeostatic balance is affected. This homeostatic balance here refers to the balance between SNS, dominant during energy demands of the body, and PNS, during rest and recovery. A tilt in balance between the two branches of ANS affects body processes and deteriorates performance. [221] It has been shown that apart from sleep, leisure activities without much physical exertion have helped in the recovery process. To summarize, recovery does not depend entirely on sleep but is contributed to by other factors that help minimize sympathetic activation.

Recovery, as an outcome measure from different activities, can be assessed either as differences in absolute levels or relative levels of recovery. Most often, relative measures are used to assess the level of recovery after a period of sleep and the comparison of outcome is between the point stressor has ended and the levels attained after sleep. This approach is preferred over the other since absolute values may be influenced by other variables such as health status of the particular individual. Scores, be it physiological, subjective or affective, that represent differences between start and end of a recovery period reflect more closely the concept of recovery. These measures, however, can be influenced by factors such as time of the day, circadian rhythms, and work stressors during different shift types etcetera. Moreover, it relies on the baseline scores but the *baseline* cannot be practically defined with certainty. Another method of comparison with a reference or baseline measurements, usually done on a day with minimum effect of work stressors. This accounts for the individual differences and gives a measure of factors that are lacking in the former. [221]

In this study, both methods have been utilized to scrutinize levels of recovery after different shift types and to assess the other contributing factors in this process. This chapter summarizes the major findings of the current study. 38 drivers, who recorded successful measurements across all three intensive measurement phases, were included for the study. Due to the low sample size, all studies were conducted on an intra-individual basis to avoid individualistic variances.

7.1 HRV and sleep quality

Many studies have associated a higher HRV to be associated with better sleep quality. Although the mechanism of HRV changes are not clear, it has been found that autonomic regulation is tilted towards increased parasympathetic activity during sleep. The findings from this study have shown similar results. The subjective indices of sleep quality and alertness upon awakening indicate that individuals who perceived an *Average-Good* or *Good* sleep were found to have a higher vagal tone when compared to others who reported *Poor* or *Average* ratings (shown in Table 6.4 and 6.5). Despite having a homogenous pool of shift-working volunteers, the reasons for differences in sleep quality maybe diverse.

The disturbed sleep has been linked to high work demand and increased physical workload [26]. Physical workload has proven to have a negative effect on sleep. A study by Hall and colleagues [60] reported acute levels of stress to be associated with lower parasympathetic modulation and increased sympatho-vagal balance. It also indicated a higher level of wakefulness (sleep fragmentation) and lower index of deep sleep (efficiency). Although this is concurs to the findings reported in this study, a linear relation between subjective and objective measures of sleep quality could not be established. This gives reason to believe the influence of other factors in the perceived sleep quality amongst the study population. Although no indicators for physical work stress have been recorded, the drivers indicated lower sleep quality and alertness after night shift work. The possible effect of shift type is discussed in the subsequent section.

7.2 Contribution of shift type on recovery

Ludovic et al. [222] reported shift workers with irregular shift schedules having different work tasks during night and day shifts. Eating habits, napping during shifts, coffee and smoking, alcohol consumption and physical activity are some other sources of bias as these are bound to differ during different shift types. Acute stress has also been associated with lower parasympathetic modulation. On road incidents and traffic situations require constant vigil during daytime driving and contributes to higher stress during the shift. The effects are even more severe in rotating shift workers as the body is constantly adapting to different work and sleep schedules, thereby requiring more time for recovery between different shift types.

In this study, the measures of HRV failed to substantiate an obvious difference in recovery during different shift types. All intensive measurement days achieved similar cardiovascular modulations during sleep. The only noticeable difference between shift types was the reduced sleep hours on duty days when compared to the day off (p <0.05). The overall estimates of HRV for the entire sleep period was only marginally different between duty days and day off. Despite similar HRV during all shift types, the reasons

for a lower subjective assessment of sleep after a night shift and sources of fatigue or stress during non-night shift are discussed.

7.2.1 Night shifts

Researchers have shown that sleep after a night shift is generally shorter by 2-4 hours compared to other shift types [223] and similar differences in sleep duration were observed in the current study. One possible explanation for reduced sleep after a night shift was the time of the day and the influence of circadian rhythm. The mean bedtime for drivers after a night shift was 06:33 (S.D. ±02:16 hours). The combined effect of circadian cycle and homeostasis increased alertness levels between 06:00 – 12:00 (refer Figure 3.2). The increased wakefulness during late morning/noon results in premature awakenings and sleep termination, resulting in insufficient recovery from stressors. This explanation is supported by a reduced sleep efficiency (87.97 ± 4.41 %) and increased sleep fragmentation (25.43 ± 11.24) when combined with shorter sleep length resulted in weaker perceived recovery compared to other shift types. In addition, the reason for lower sleep recovery after a night shift may not be directly attributed to sleep alone and the consequences of working during the night should be taken into account.

7.2.2 Non-night shifts

A lower parasympathetic tone during initial sleep hours after a non-night shift was observed, indicative of higher stress or fatigue associated with the shift type. One possible explanation for the perceived levels of sympathetic activation is the increased length of wakefulness between recovery sleep before and after shifts. Day shifts are usually followed by hours of wakefulness and this contributes to increased stress levels. However, sufficient recovery was achieved to mitigate any differences due to workday stress and similar baseline levels were observed across all categories at the end of sleep.

Day/afternoon shifts are less demanding with respect to altering sleep schedules as the shift in sleep times is only marginal in comparison to night shifts. However, morning shifts require individuals to wake up very early (04:00 – 05:00), which coincides with the nadir point of the circadian cycle during which levels of alertness are at a minimum. The difficulty awakening and subjective feeling of not being refreshed contributes to the fatigue factor and the demands of high alertness during the shift results in reporting lower sleep quality after a non-night shift. Interestingly, the morning shifts are associated with reduced SWS [224], a factor that might be a significant contributor to levels of recovery.

7.3 Other factors affecting recovery

Regression analyses provided evidence of several other factors contributing to the quality of sleep and levels of recovery achieved. VLF power showed strongest association to sleep efficiency. A Spearman coefficient for VLF power and sleep efficiency showed

weak negative correlation (p <0.05). Although the modulation of VLF power has been strongly associated renin-angiotensin system, thermoregulation and regulation of heart beat intervals, the actual origins of the mechanism are still elusive. Due to its long cycle length, it could also be associated with sleep stages, especially REM sleep. Bušek et al. [225] concurred this hypothesis and suggested that VLF power increases when SWS has ceased. Another study indicated that an increased VLF power indicated increased physical activity [226]. Going by this evidence and its association with sleep duration and efficiency, VLF can be considered as a marker for sleep disturbances rather a measure of autonomic tone.

The association between HRV measures of vagal tone remained significant after corrections for possible confounding factors such as age, BMI, diurnal variations, reported mean sleep need and use of alcohol. The influence of physical and mental activity were subdued as only sleep time measures were evaluated in these models. Among time domain measures, SDNN and RMSSD reflected parasympathetic modulation of the heart and smaller values indicated a reduced vagal tone. SDNN was attenuated by age, sleep efficiency and short sleep duration. RMSSD, on the other hand, was influenced by diurnal type, indicating the role of circadian alterations in the recovery process. Age and sleep efficiency were other significant covariates. HF power was only affected by age and diurnal type in the multivariate model. From the results, it can be said with certainty that both short- and long-term measures of vagal tone are associated with several external factors that determine the sleep quality, irrespective of the shift type.

7.3.1 Age

The toleration of work demands in ageing shift workers is less compared to their younger counterparts. Despite mean sleep requirements being higher, sleep is shorter and more fragmented with increase in age and this was concurred in the present study. Interpreting the reasons for reduced vagal tone, our results indicated that recovery was passive with increasing age. This is in agreement to other findings [227], which have shown occurrence of disturbed sleep in older age groups. Despite disturbed sleep encountered in objective measures, subjective ratings were usually indicative of good sleep quality, suggesting that even shorter sleep duration are adequate for perceived levels of good sleep in older shift workers. One reason for decreased sleep lengths is due to the strong circadian influence, especially during the morning hours, which results in easy termination of sleep. However, this does not imply a better sleep quality as it generally deteriorates with age [228].

7.3.2 Circadian cycle

In a general population, a higher sympathetic tone is observed during daytime and parasympathetic domination is prevalent during the night when periods of sleep occur. However, the circadian cycle in shift workers is altered, often failing to indicate day/night

differences due to rotating or permanent (night) shift types. From the present study, diurnal variations have shown to affect sleep quality and this can be associated with the shift in circadian rhythm. In addition to the circadian cycle, maintaining sleep during unusual times, such as after a night shift, can be interrupted by other factors such as noise, brightness of day and other social aspects. The circadian cycle and core body temperatures also affect sleep duration, which corresponds to the obtained results. As suggested by Bonnemeier and colleagues [170], the diurnal variations are strongly associated with age.

7.4 Significance of core and non-core sleep

The extended periods after core sleep showed significant level of recovery, irrespective of the duration of extension. This is contrary to the hypothesis of core sleep being the most restorative and the remaining period of sleep not contributing towards recovery. SWS occurs during NREM sleep stages, which is usually longest during initial sleep hours. With increasing sleep times, the percentage of NREM sleep becomes shorter and REM sleep is more dominant, especially in periods prior to awakening. [16] Despite shorter NREM duration beyond the core sleep period, there exists no scientific evidence to suggest that the restorative functions are limited beyond core sleep. With evidence to support lower sleep durations are significant predictors of lower sleep recovery, the entire duration of sleep is important, irrespective of the degree of recovery achieved during the extended sleep hours. In addition, no significant differences were observed in either shift categories.

7.5 Study limitations

Interpretation of the current study was marked by certain limitations. First, the study population represented largely male participants (only 1 female) and hence, failed to report any differences in the recovery process owing to gender. The cardiac health was not assessed prior to the study and this could have a significant impact on the measures of HRV. In-depth evaluation of habitual factors such as alcohol consumption prior to sleep and napping during the day could not be performed as most drivers failed/refused to mention this information in their sleep diaries.

Measurement of different shift types occurred on different days from the start of a new cycle and the speed or direction of rotation were not accounted for. The amount and type of physical workload involved during the shift was not recorded. In addition, food habits, caffeine intake, smoking and other countermeasures to mitigate sleepiness during the shift were not controlled for during statistical analyses and these may have a significant impact on the results. All the above-mentioned factors need to be considered in future examinations to accurately determine the levels of post-shift recovery and determine if shift work influenced the overall well-being of the participants.

8. CONCLUSION

Subjective measures to assess the need for recovery after a strenuous work shift have been proposed and they have been successfully tested by many researchers. However, the subjective assessments are usually found to be biased and depend on individual factors. In the current study, HRV based evaluation of sleep recovery has been performed to assess the sleep quality in shift working truck drivers. The results indicate that there are no direct influences of shift type on recovery, but other factors such as sleep duration, efficiency and diurnal variations significantly affect recovery in these populations, which are indirect manifestations of irregular sleep. The quality and duration of sleep decreased with age but were seldom reflected in subjective assessments, probably due to the healthy worker effect or accustomization to shift work with experience.

Both sleep periods, first 4 hours from sleep onset and beyond, showed signs of recovery based on the HRV indices, suggesting that the entire duration of sleep contributes to recovery process or the feeling of being refreshed upon awakening. Although the initial hours showed significant differences, hourly patterns did not provide conclusive evidence of variation in recovery in either shift type. Thus, to conclude, working different shift types did not have an influence on recovery, but indirect effects such as reduced sleep duration and circadian shifts contributed significantly. Shift schedules should be designed in such a way to allow sufficient time for recuperation in rotating shift workers so that recovery achieves baseline levels and physical exertion does not hamper performance.

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APPENDICES

Appendix 1. Shapiro-Wilk test for normal distribution of HRV, actigraphy and subjective measures. p <0.05 indicates a skewed distribution of the data.

	Shift type	Statistic	Df	*p*
HRV parameters				
Heart rate	Non-night shift	0.980	38	0.731
	Night shift	0869	38	<0.001
	Day off	0.975	38	0.557
SDNN	Non-night shift	0.936	38	0.032
	Night shift	0.958	38	0.163
	Day off	0.964	38	0.250
RMSSD	Non-night shift	0.892	38	0.002
	Night shift	0.902	38	0.003
	Day off	0.916	38	0.007
VLF power	Non-night shift	0.912	38	0.006
	Night shift	0.918	38	0.009
	Day off	0.951	38	0.097
HF power	Non-night shift	0.788	38	<0.001
	Night shift	0.792	38	<0.001
	Day off	0.836	38	<0.001
LF/HF ratio	Non-night shift	0.793	38	<0.001
	Night shift	0.764	38	<0.001
	Day off	0.752	38	<0.001
Actigraphy parameters				
Time in bed	Non-night shift	0.943	38	0.052
	Night shift	0.958	38	0.158
	Day off	0.980	37	0.707
Actual sleep	Non-night shift	0.968	38	0.333
	Night shift	0.974	38	0.521
	Day off	0.977	37	0.613
Sleep efficiency	Non-night shift	0.908	38	0.004
	Night shift	0.900	38	0.003
	Day off	0.964	37	0.261
Fragmentation index	Non-night shift	0.947	38	0.070
	Night shift	0.956	38	0.136
	Day off	0.947	37	0.078
Total activity score	Non-night shift	0.884	38	0.001
	Night shift	0.760	38	<0.001
	Day off	0.761	37	<0.001
Subjective parameters				
Subjective alertness upon awakening	Non-night shift	0.831	38	<0.001
	Night shift	0.793	38	<0.001
	Day off	0.824	37	<0.001
Estimated sleep quality	Non-night shift	0.811	38	<0.001
	Night shift	0.761	38	<0.001
	Day off	0.822	37	<0.001

Appendix 2. Dependent t-tests for significance between shift categories. p <0.05 rejects the null hypothesis and indicates significant differences between shift categories.

	Non-night (vs.) Night	Non-night (vs.) Leisure day	Night (vs.) Leisure day
HRV Parameters			
Heart rate	0.267	0.811	0.081
SDNN	0.338	0.811	0.777
RMSSD	0.634	0.913	0.617
VLF power	0.350	0.637	0.755
HF power	0.873	0.902	0.994
LF/HF ratio	0.454	0.316	0.047
Actigraphy parameters			
Time in bed	0.011	< 0.001	< 0.001
Actual sleep	0.010	< 0.001	< 0.001
Sleep efficiency (%)	0.369	0.053	0.010
Fragmentation index	0.612	0.180	0.701
Subjective parameters			
Subjective alertness upon awakening	0.305	0.243	0.003
Estimated sleep quality	0.833	0.030	0.012

Appendix 3. Non-linear IV variables were subjected to 3 curve estimates (linear, logarithmic and quadratic) to determine the mathematical function that fits the distribution.

Age (years)	**Linear**		**Logarithmic**		**Quadratic**	
	R^2	**F**	**R^2**	**F**	**R^2**	**F**
Heart rate	<0.001	0.015	<0.001	<0.001	0.010	0.555
SDNN	0.048	5.663*	0.045	5.317*	0.051	2.957
RMSSD	0.035	4.038*	0.034	3.893	0.035	2.017
VLF power	0.007	0.753	0.005	0.564	0.019	1.064
HF power	0.042	4.929*	0.040	4.629*	0.044	2.558
LF/HF ratio	0.009	1.052	0.013	1.428	0.031	1.761
BMI	**Linear**		**Logarithmic**		**Quadratic**	
	R^2	**F**	**R^2**	**F**	**R^2**	**F**
Heart rate	0.011	1.004	0.009	0.838	0.015	0.684
SDNN	<0.001	0.003	0.002	0.223	0.123	6.531*
RMSSD	<0.001	0.001	0.002	0.143	0.119	6.307*
VLF power	0.042	4.142*	0.025	2.424	0.182	10.377**
HF power	0.008	0.753	0.016	1.569	0.116	6.099*
LF/HF ratio	<0.001	0.008	<0.001	0.026	0.039	1.865
Mean sleep need	**Linear**		**Logarithmic**		**Quadratic**	
	R^2	**F**	**R^2**	**F**	**R^2**	**F**
Heart rate	0.013	1.409	0.016	1.713	0.031	1.695
SDNN	0.020	2.200	0.015	1.598	0.074	4.211*
RMSSD	0.013	1.411	0.010	1.079	0.035	1.931
VLF power	0.044	4.903*	0.035	3.852	0.115	6.795*
HF power	0.035	3.897	0.031	3.397	0.050	2.756
LF/HF ratio	0.002	0.182	0.002	0.183	0.002	0.114
ESS score	**Linear**		**Logarithmic**		**Quadratic**	
	R^2	**F**	**R^2**	**F**	**R^2**	**F**
Heart rate	0.012	1.148	-	-	0.030	1.419
SDNN	0.001	0.107	-	-	0.005	0.236
RMSSD	0.001	0.074	-	-	0.001	0.036
VLF power	0.001	0.089	-	-	0.030	1.432
HF power	0.003	0.248	-	-	0.007	0.335
LF/HF ratio	0.051	5.046*	-	-	0.057	2.788

R^2 represents coefficient of distribution, which measures the total proportion of variation in the DV about its mean by the IV. F hypothesizes that none of the IV's represent the DV about its mean with * (p <0.05) and ** (p <0.001) rejecting the null hypothesis.

Appendix 4. Univariate linear regression for dependent variables heart rate and LF/HF ratio.

Heart rate	Model Summary		ANOVA		Coefficients	
	R	R^2	F	Sig.	B	Beta
Individual factors						
Age	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
BMI	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Diurnal type	0.083	0.007	0.735	0.393	0.991	0.083
Mean sleep need	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
ESS score	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Actigraphy parameters						
Time in bed	0.025	0.001	0.068	0.795	$-3.9E^{-5}$	-0.025
Actual sleep time	0.028	0.001	0.090	0.765	$3.9E^{-5}$	0.028
Sleep efficiency	0.198	0.039	5.575	0.035	0.295	0.198
Fragmentation index	0.137	0.019	2.132	0.147	-0.085	-0.137
Subjective parameters						
Shift type	0.072	0.005	0.587	0.445	-0.655	-0.072
Alcohol consumption	0.217	0.047	5.521	0.021	2.108	0.217

LF/HF ratio	Model Summary		ANOVA		Coefficients	
	R	R^2	F	Sig.	B	Beta
Individual factors						
Age	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
BMI	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Diurnal type	0.173	0.030	3.274	0.073	0.772	0.173
Mean sleep need	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
ESS score	0.226	0.051	5.046	0.067	0.164	0.226
Actigraphy parameters						
Time in bed	0.112	0.013	1.419	0.236	<0.001	-0.112
Actual sleep time	0.114	0.013	1.477	0.227	<0.001	-0.114
Sleep efficiency	0.044	0.002	0.216	0.643	-0.024	-0.044
Fragmentation index	0.136	0.018	2.097	0.150	0.031	0.136
Subjective parameters						
Shift type	0.034	0.001	0.131	0.718	-0.113	-0.034
Alcohol consumption	0.182	0.033	3.820	0.053	0.646	0.182

Suffix *transform* indicates transformation has been performed to achieve linearity. *R* indicates the correlation, R^2 represents coefficient of distribution, which measures the total proportion of variation in the DV about its mean by the IV. *F* hypothesizes that none of the IV's represent the DV about its mean with $p < 0.05$ and $p < 0.001$ rejecting the null hypothesis. *B* and β represent unstandardized and standardized coefficients.

Appendix 5. Hourly recovery pattern of HRV parameters across different age categories.