Morphological Changes of the QRS Complex as a Marker of Autonomic Modulation of the Heart Rate

By

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Abstract:

The aim of this project was to investigate if the autonomic cardiac modulation is reflected in the morphology of individual QRS complexes.

ECG recordings from 20 healthy subjects and 18 subjects diagnosed with CRPS type I during a tilt table test were analysed. Furthermore, ECG recordings from 11 healthy subjects with capsaicin induced pain were analysed. Two features were extracted from each QRS complex; the steepest ascending slope of the QRS complex, and the scale where a wavelet adapted to each subject had the largest correlation with the QRS complex. The outcome was compared to the mean NN interval and standard deviation of the NN intervals (SDNN).

An increase in the slope and a decrease in scale, NN interval and SDNN was expected when the sympathovagal balance was shifted towards sympathetic predominance and the opposite was expected when the sympathovagal balance was shifted towards parasympathetic predominance. A significant increase in the slope and decrease of the scale and NN interval was seen when the healthy subjects were tilted from supine to upright position (p<0.05). The CRPS patients and healthy subjects with induced pain did not show a change in slope, but they did show a significant decrease in scale (p<0.05) when they were tilted from supine to upright position and when they were induced with pain respectively. A significant increase in the standard deviation of the slope and scale was observed in the healthy subjects when they were tilted from supine to upright position (p<0.05) and in the healthy subjects before and during induced pain (p < 0.05). Although the tendency was an increase of the standard deviation of the scale when the CRPS patient were tilted, only the standard deviation of the slope increased significantly in the CRPS patients. In contrast, SDNN decreased in the healthy subjects and CRPS patients during the tilt from supine to upright.

The findings partly support that the QRS complex gets narrower and higher when the sympathovagal balance is shifted towards sympathetic predominance and the opposite when shifted towards parasympathetic predominance. The discrepancy between the decrease of SDNN and the increase in standard deviation of the slope and scale during sympathetic predominance might be explained by the fact that parasympathetic innervation of the ventricles is sparse compared to the sympethetic innervation. This would mean that the slope and scale expresses sympathetic modulation rather that parasympathetic modulation of the heart rate. Further investigation is required to fully understand the physiological correlates of the findings and the potential of morphological changes of the QRS complex as a marker of autonomic regulation of the heart rate.

Preface

This report was made by group 1085A on the 4th semester of Master of Science in Biomedical Engineering and Informatics at the Department of Health Science and Technology at Aalborg University, Denmark in the period February 2^{nd} to June 4th 2009.

The aim of this project was to reveal and extract morphological changes of the QRS complex caused by autonomic modulation of the heart rhythm. The report investigates the morphological changes of the QRS complex caused by the autonomic nervous system and documents the establishment of methodologies that extract features that represent the morphological changes of the QRS complex.

Citations marked with an asterisk means that the cite did not contain any information about the year of publication. A CD-ROM with the generated MatLab code is enclosed.

We would like to thank Ole Kæseler Andersen and John Hansen for making datasets available for the project. In addition we would like to thank José A. Biurrun Manresa for being helpful during the experimental part of the project.

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Introduction

Under normal conditions, the autonomic nervous system (ANS) regulate internal organs and circulation to maintain homeostasis, e.g. by controlling arterial pressure, gastrointestinal motility and secretion, emptying of the urinary bladder, body temperature etc. The ANS is capable of regulating visceral functions very rapidly and intensely, e.g., it can double the heart rate within 3 to 5 seconds from normal level [Guyton & Hall 2006].

The concept, sympathovagal balance refers to the autonomic state resulting from sympathetic and parasympathetic influences [Goldberger 1999]. During the last three decades, numerous studies have shown that abnormalities in the sympathovagal balance are related to different diseases [TaskForce 1996].

Experimental studies have shown significant relations between ANS and cardiovascular diseases [TaskForce 1996, Acharya et al. 2006]. It has been revealed that there is a relation between the risk of developing a lethal cardiac arrhythmia and signs of either increased sympathetic activity or decreased parasympathetic activity [TaskForce 1996, Stein et al. 1994]. Predominance of sympathetic activity and reduced parasympathetic cardiac control have been seen in patients with acute myocardial infarction [Ravenswaaij-Arts et al. 1993]. Moreover, observations of patients with congestive heart failure show alterations of the sympathovagal balance; the general consensus seems to be that there is a sympathetic overdrive rather than a parasympathetic withdrawal among these patients. An altered sympathovagal balance is also seen in patients after cardiovascular surgery and it has been related to myocardial ischemic episodes in patients who have undergone coronary artery bypass grafting [TaskForce 1996]. As a complication of diabetes mellitus, autonomic neuropathy occurs and this often affects the cardiac autonomic function. It is characterised by early and widespread neuronal degeneration of small sympathetic and parasympathetic nerve fibres [Zipes & Jalife 2004, Ravenswaaij-Arts et al. 1993]. Patients with cervical and high thoracic spinal cord lesions also have autonomic abnormalities that affect the heart. In these patients some autonomic pathways are severed, but baroreceptor afferents and parasympathetic efferents of the ANS are intact. This causes a reduced ability to regulate the cardiac activity although rise and fall in blood pressure is registrated e.g. during postural changes [Malik 1998].

The association between autonomic activity and different diseases has encouraged the development of quantitative markers of autonomic activity.

1.1 Existing Quantitative Markers of Autonomic Activity

During the last decades different methods have been applied to assess the autonomic activity; these include for example cardiovascular reflex tests and biochemical tests [Ravits 1997]. In recent years, noninvasive techniques based on electrocardiographic recordings have been used as markers of cardiac autonomic tone; among these, heart rate variability (HRV) is the most widely used and evaluated [TaskForce 1996].

1.1.1 Heart Rate Variability

The ANS regulates the heart rate; increased sympathetic activity causes acceleration whereas increased parasympathetic activity causes deceleration of the heart rate [Guyton & Hall 2006], which is the reason why HRV is used as a marker of autonomic activity. The increasing popularity of HRV as a measure of autonomic activity since the 70's and a lack of method standardisation combined with a continuous introduction of new HRV measures lead to the formation of a task force of the European Society of Cardiology and The North American Society of Pacing and Electrophysiology in 1996. The task force established standards of measurement, physiological interpretation, and clinical use of HRV [TaskForce 1996]. In order to quantify HRV, the intervals between consecutive beats originating in the SA node, also called normal to normal intervals (NN intervals) must be found in the electrocardiogram (ECG). The analysis of HRV must satisfy four conditions; 1) a satisfactory signal-to-noise ratio of the ECG is neccesary to identify the each beat properly, 2) the digital sampling must be regular and robust to identify a fiducial point for each beat, 3) morphology and rhythm characteristics for each beat must be classified to distinguish between beats originating in the sinus node and ectopic beats, and finally 4) only beats originating in the sinus node should be considered for the HRV analysis [Zipes & Jalife 2004]. Two main approaches have been used to measure the HRV; time domain analysis and frequency domain analysis. Besides these, nonlinear analysis is an emerging field within the analysis of HRV.

Time Domain Analysis

In time domain analysis, a number of parameters can be derived from NN intervals. The parameters are based on descriptive statistic and geometrical measures. An overview of the most frequently used statistical time domain parameters is given in table 1.1.

Statistical measures			
Variable	Units	Description	Remarks
SDNN ms The standard deviation of a intervals.		The standard deviation of all NN- intervals.	An estimate of overall variance. The outcome depends on the length of the recording.
SDANN	ms	The standard deviation of the average NN interval calculated over short periods, usually 5 minutes, of the entire recording.	An estimate of changes due to cycles longer than 5 minutes. The outcome depends on the length of the recording.
SDNN index	ms	Mean of the standard deviation of all 5 minute segments in the entire recording.	An estimate of changes due to cycles shorter than 5 minutes. The outcome depends on the length of the recording.
RMSSD	ms	The square root of the mean of the squares of difference between adjacent NN intervals.	An estimate of short term variations in the NN interval i.e. an estimate of high frequency variations.
NN50 count		The number of differences between adjacent NN intervals differing by more than 50 ms.	The same as above.
pNN50	%	The NN50 count divided by the total number of NN intervals in the recording.	The same as above.

 Table 1.1: An overview of the most frequently used statistical time domain parameters in HRV analysis [TaskForce 1996, Acharya et al. 2006].

An overview of the geometric time domain parameters is given in table 1.2.

Geometric measures				
Variable	Units	Description	Remarks	
HRV or RR triangular index		The total number of NN intervals divided by the number of the most frequently occurring NN interval on a discrete scale with bins with a fixed length.	Both geometric measures are estimates of overall variance. They are more influenced by lower than high frequencies. The geometric measures are less sensitive to the quality of the NN	
TINN	ms	The width of the baseline of the triangular interpolation of the NN interval histogram.	intervals compared to the statistical measures, but require at least 20 minutes of recording (preferably 24 hours).	

 Table 1.2: An overview of the most frequently used geometrical time domain parameters in HRV analysis [TaskForce 1996, Acharya et al. 2006].

The time domain measures can be divided into two groups; those dependent on the NN interval and those dependent on the difference between adjacent NN intervals [Stein et al. 1994]. The variables can be calculated for different segments of a recording and compared, e.g., during rest and during activity. Due to the high correlation between the measures, the task force of the European Society of Cardiology and The North American Society of Pacing and Electrophysiology recommended the use of four of the HRV measures; SDNN, SDANN, RMSSD, and the HRV triangular index. The task force also emphasizes that it is inappropriate to compare measures derived from recordings of different durations, due to different statistical basis [TaskForce 1996].

Frequency Domain Analysis

The frequency domain measures are based on a transform of the series of succesive registered RR intervals into the frequency domain. The series can be represented in two ways; as the RR intervals versus the beat number, as a discrete event series where the R_i - R_{i-1} interval is plotted versus the time of occurence of R_i . The latter representation is an irregularily sampled signal and the Fourier transform requires an equidistant sampled signal. Therefore, an interpolation and resampling of the signal is required prior to a Fourier transform [TaskForce 1996].

When a power spectral density analysis is made on the series, it provides information about the distribution of power (i.e. variance) as a function of frequency [Fuster et al. 2004]. There must be differentiated between short term (2-5 min) and long term (24 hours) recordings. In short term recordings, three main frequency components have been identified: a very low frequency (≤ 0.04 Hz), a low frequency (0.04-0.15 Hz), and a high frequency (0.15-0.4 Hz) component. In addition, an ultra low frequency component (≤ 0.03 Hz) has been identified in long term recordings [TaskForce 1996].

Short term recordings (5 min)				
Variable Units		Description	Remarks	
Total power ms ²		The variance of the NN intervals.	An estimate of overall variance.	
VLF ms ²		Power in the very low frequency range (≤ 0.04 Hz). Its physiologic correlates are understood, but it is assume reflect the influence of e.g. t regulation, circadian, and ne endocrine rhythms.		
LF	ms ²	Power in the low frequency range (0.04-0.15 Hz).	Is assumed to reflect both sympathetic and parasympathetic modulations of the heart rate.	
LF norm	n.u.	Power in the low frequency range (0.04-0.15 Hz) in normalised units (LF/(LF+HF)).	By some, it is assumed to be a quantitative marker for sympathetic modulations.	
HF ms ² Power in the high frequency range (0.15-0.4 Hz).		Power in the high frequency range (0.15-0.4 Hz).	Is assumed to reflect parasympa- thetic modulations of the heart rate. The respiratory rhythm contributes to the HF component.	
HF norm	n.u.	Power in the high frequency range (0.15-0.4 Hz) in normalised units. (HF/(LF+HF)).	The same as above.	
LF/HF ratio		The ratio between low and high frequency power.	By some, it is assumed to reflect the sympathovagal balance, by others the sympathetic modulations of the heart rate.	
		Long term recordings (24 h	ours)	
Variable	Units	Description	Remarks	
Total power	ms ²	The variance of the NN intervals.	An estimate of overall variance.	
ULF ms ²		Power in the ultra low frequency range (≤ 0.003 Hz).	Its physiologic correlates are not fully understood, but it is assumed to reflect the influence of e.g. thermo regulation, circadian, and neuro- endocrine rhythms.	
VLF ms ² Power in the very low frequency The same as abore range (0.003-0.04 Hz).		The same as above.		
LFms2Power in the low frequency range (0.04-0.15 Hz).Is assumed to r sympathetic an modulations of		Is assumed to reflect both sympathetic and parasympathetic modulations of the heart rate.		
HF ms ² Power in t range (0.1)		Power in the high frequency range (0.15-0.4 Hz).	Is assumed to reflect parasympa- thetic modulations of the heart rate. The respiratory rhythm contributes to the HF component.	

The frequency domain parameters for short term and long term analysis of HRV are listed in table 1.3.

 Table 1.3: An overview of the most frequently used frequency domain parameters in short term and long term HRV analysis [TaskForce 1996].

Efferent vagal activity has been shown to be a major contributor to the high frequency component in clinical and experimental observations of autonomic manoeuvres e.g. electrical vagal stimulation, muscarinic receptor blockade and vagotomy. Some researchers consider, the low frequency component as a marker of sympathetic modulation whereas others consider it as a parameter that are under both sympathetic and parasympathetic influences [TaskForce 1996]. The physiological correlate of the very low and ultra low frequency component is still an area under investigation [Acharya et al. 2006], but has been related to circadian, neuroendocrine rhythms and thermo regulation [Stein et al. 1994]. As table 1.3 also expressed, a general consensus about the physiological correlates of the different frequency measures has not been fully established [Acharya et al. 2006].

1.1.2 Limitations of Heart Rate Variability

The derivation of information about HRV requires a precise detection of R peaks in the recording, because imprecise location affects the outcome of the different measures. Furthermore, the fact that HRV is based on the NN intervals limits its use of HRV measures to individuals with a sinus rhythm and a limited number of ectopic beats [Acar et al. 2000, Mateo & Laguna 2003]. Both the statistical time domain and frequency domain measures are highly sensitive to artifacts, ectopic beats and missing beats. Ectopic and missing beats that can corrupt the results, can be handled by inserting an artificial beat or by shifting the following beats. Although this minimises the effect of the ectopic and missing beats, it is prone to subjective bias and the result will still be distorted [Mateo & Laguna 2003]. The geometrical measures are less affected by ectopic and missing beats, which reduces the need for preprocessing [Acharya et al. 2006] but they should only be performed on recordings of at least 20 minutes of duration. Thus, an optimal HRV analysis demands recordings without ectopic beats. For the analysis to be reliable, different criteria have been proposed, e.g. the number of beats originating in the SA node must be at least 70 % (some even demand 99 % of the beats to orignate in the SA node). The most strict criteria demands that the recording must not contain more than 10 ectopic beats per hour. These criteria excludes many patients from HRV analysis, e.g. 20-30 % of all highrisk patients in post acute myocardial infarct groups are excluded from HRV analysis due to frequent ectopic beats, artifacts, or arrythmia episodes [Huikuri et al. 1999]. Furthermore, when evaluating results of the HRV measures, it should be noted that it is inapropriate to compare measures calculated on ECGs of different duration [TaskForce 1996].

The different time domain measures lack the ability to discriminate between sympathetic and parasympathetic influences [Acharya et al. 2006]. In the frequency domain the HF component of the power spectrum is related to vagal activity, whereas the meaning of the LF component is more controversial; some consider it as a measure of sympathetic modulations when expressed in normalised units, others interpret it as a combination of sympathetic and parasympathetic activity. The consensus about the LF component is, that both sympathetic and parasympathetic inputs contribute to it [Notarius & Floras 2001]. The HF component can be significantly influenced by respiratory patterns [Acharya et al. 2006].

The translation of the NN interval series from the time domain to the frequency domain presupposes that there is an underlying periodicity in the signal. This is a technical limitation, since the heart rate signal is a nonstationary signal [Notarius & Floras 2001]. This stationarity issue is a frequently discussed problem in long term recordings. A signal can be considered stationary if the modulations of a certain frequency remain unchanged during the recording. If the modulations change, the interpretation of the results are not well defined. The heart rate signal can be considered as a quasi stationary signal, which justifies the transform into the frequency domain [TaskForce 1996].

There has been a lot of confusion regarding the meaning of the different measures, especially in the frequency domain. In the early studies the spectral components were regarded as a reflection of the autonomic tone [Notarius & Floras 2001], i.e the balance between the activity in the sympathetic and parasympathetic division [Martini 2004]. But studies comparing the firing rates of vagal and sympthetic cardiac fibres with the sino-atrial response shows that the heart rate variations not necessarily correspond to variations in the mean firing rate, but rather to the sino-atrial responsiveness to the changes in the autonomic tone [Notarius & Floras 2001].

1.2 Initial Problem

Among the limitations of the existing markers of ANS activity are precise location of fiducial points. For this reason, it is interesting to consider if ANS activity is reflected within beats and not only by the time interval between beats. This gives rise to the following initial problem:

Are there reasons to believe that changes in the autonomic activity lead to morphological changes within single beats in surface ECGs?

If the autonomic activity is reflected in changes in the morphology of the surface ECG, some of the limitations with the existing HRV markers can be overcome, e.g. the need for correction due to ectopic and missing beats which distorts the results can be avoided, this making it also possible to apply this technique in persons with a high degree of ectopic and/or missing beats.

Problem Analysis

In order to answer the initial problem, an understanding of the mechanisms that are responsible for the generation and appearance of an ECG is necessary. Furthermore, the ANS' effect on these mechanisms must be clarified in order to see if there is a connection between the ANS' regulation of the heart and the morphology of the ECG.

2.1 Generation of the Electrocardiogram

An ECG reflects the electrical activity of the heart. When the electrical impulse propagates through the myocardium a small portion of this electrical activity reaches the body surface, where it can be recorded by placing surface electrodes on specified places on the skin. It is the cardiac electrical system of the heart that is responsible for generating and conduction the electrical activity. [Guyton & Hall 2006].

2.1.1 The Cardiac Electrical System

The electrical system of the heart contains two types of cells; specialised cells of the conducting system and cardiac contractile cells. The former generate and conduct electrical current, while the latter respond to the electrical current and produce the contraction that propels blood into the circulatory systems [Sherwood 2004, Guyton & Hall 2006]. The components of the conduction system of the heart can be seen on figure 2.1.



Figure 2.1: The conducting system of the heart. Edited from Guyton and Hall [2006].

The generation of the action potential leading to a contraction of the heart's chambers is initiated by a spontaneous generation of an action potential in the sinoatrial node (SA node), which is located in the posterior wall of the right atrium. The action potential is conducted through both atria to the atrioventricular node (AV node) by the internodal pathways. The propagation of the action potential through both atria excites the contractile cells of the atria and ensures their full contraction of these. The AV node conducts the impulse from the atria into the ventricular my-ocardium and causes it to contract [Guyton & Hall 2006, Sherwood 2004, Despopoulos & Silbernagl 2003]. A more thorough description of the cardiac electrical system and impulse propagation can be found in appendix A.



The action potentials generated in the different structures are somewhat different. Figure 2.2 illustrates the action potentials from different structures of the heart.

Figure 2.2: The different action potentials for each of the specialised cells in the heart. Edited from Malmivuo & Plonsey [1995].

Two main types of cells are found in the heart; pacemaker cells and contractile cells. Cardiac pacemaker cells depolarise spontaneously without any external stimulation, whereas the cardiac contractile cells only contract when stimulated [Sherwood 2004]. The SA node and AV node exhibit similar shapes of the action potential and the AV bundle, bundle branches, Purkinje fibres, and myocardium exhibit similar shapes of the action potential [Despopoulos & Silbernagl 2003]. The two action potentials' shapes and the flux of ions responsible for the shapes are illustrated in figure 2.3a and 2.3b.

Figure 2.3a shows the action potential for a pacemaker cell. The action potential for a pacemaker cell can be divided into three phases; a prepotential phase (phase 4), a depolarisation phase (phase 0), and a repolarisation phase (phase 3). Pacemaker cells do not have a constant resting potential, instead they slowly depolarise again immediately after a repolarisation. At a maximum diastolic potential (MDP) of about -70 mV, Na $^+/K^+$ channels open and causes a large influx of Na⁺ ions and a small efflux of K⁺ ions. This initiates the prepotential phase (phase 4). When the membrane potential reaches about -55 mV the transient Ca²⁺ channels (also called T-type Ca²⁺ channels) open and cause an influx of Ca²⁺ ions. As Ca²⁺ ions enter the cell, the membrane potential is further increased and reaches the threshold potential (TP) at about -40 mV. At this stage another type of calcium channels opens; the longer lasting Ca²⁺ channels (also called L-type Ca²⁺ channels). Opening of these channels increases influx of Ca²⁺ ions and depolarises the cell (phase 0). At approximately 0 mV, the L-type Ca²⁺ channels close and at the same time K⁺ channels open, which causes an efflux of K⁺ ions. This initiates the repolarisation potential (phase 3), which makes the membrane potential return to -70 mV [Sherwood 2004, Walker & Spinale 1999].



Figure 2.3: Illustration of action potentials and the flux of ions in (a) pacemaker cells and (b) cardiac contractile cells. The numbers 0-4 denotes the different phases of the action potential. TP denotes threshold potential. MDP denotes maximum diastolic potential. Edited from Despopoulos and Silbernagl [2003].

The action potential of the cardiac contractile cells is initiated by the cardiac pacemaker cells, but varies from the action potential of cardiac pacemaker cells in ionic mechanisms and shape as seen in figure 2.3b. Unlike the pacemaker cells the cardiac contractile cells have a stable resting potential at approximately -90 mV. When the cells are depolarised to approximately -70 mV, Na⁺ channels open and causes a large influx of Na⁺ ions. The large influx of Na⁺ ions causes a rapid depolarising of the membrane potential to a value of approximately 30 mV (phase 0). When the membrane potential reaches 30 mV, the Na⁺ channels close and K⁺ channels open causing an efflux of K⁺ which initiates repolarisation (phase 1). The opening of K⁺ channels is followed by opening of L-type Ca²⁺. Because of a large influx of Ca²⁺ ions simultaneously with the efflux of K⁺ ions, the repolarisation is delayed and the positivity of the membrane potential is prolonged causing the plateau (phase 2). As the L-type Ca²⁺ channels close the efflux of K⁺ makes the membrane potential to return to its initial resting potential (phase 4) [Sherwood 2004, Walker & Spinale 1999].

As seen on figure 2.3a and b, the influx and efflux of different ions are resposible for the appearance of the action potentials. Changes in the slope of prepotential, the amplitude of the TP, and the amplitude of the MDP determine the rate of impulse generation in the SA node and thereby the heart rate. The duration of myocardial action potentials are dependent on the heart rate; the higher the frequency is, the shorter will the action potential be [Despopoulos & Silbernag] 2003].

The contractile cells in the heart are connected by gap junctions, which makes them function as a syncytium i.e. as a joint unit where processes in a single cell quickly spreads to the adjacent cells, almost at if it was a single large cell. Thus, the atrial myocardium functions as a syncytium and the ventricular myocardium function as a syncytium [Guyton & Hall 2006]. Also the specialised cells of the conducting system are connected by gap junctions [Dhein 1998*b*]. Gap junctional coupling between the cells are responsible for the propagation of an action potential from its initial point in the SA node along the specialised conduction pathways to the contractile cells of the ventricles [Rohr 2004]. More information about gap junctions can be found in appendix A in section A.3 on page 59.

2.1.2 Elements of the Electrocardiogram

As mentioned it is the impulse propagation through the myocardium that is reflected in an ECG. A normal ECG exhibits three different waveforms; the P wave, the QRS complex, and the T wave. The QRS complex is made of three distinct waves; the Q wave, R wave, and the S wave [Guyton & Hall 2006]. An illustration of a normal ECG is given in figure 2.4.



Figure 2.4: A normal electrocardiogram (ECG). Edited from Guyton and Hall [2006].

Immediately after the SA node has generated an impulse the depolarisation of both atria occur. This is reflected as the P wave in the ECG. The isoelectric line between the P wave and the Q wave corresponds to the conduction of the impulse through the AV node.

The Q wave represents the depolarisation of the septum. The left part of the septum is depolarised before the right side, this causes the negativity of the Q wave. The R wave is caused by the propagation of the depolarisation wave in the septum towards the apex and afterwards depolarisation of the apex to the base of the heart. The S wave reflects the depolarisation of the posterior portion of the base of the left ventricle. Altogether, the Q, R, and S waves constitute the QRS complex, which represents the depolarisation of the ventricles [Martini 2004, Guyton & Hall 2006].

The repolarisation of the atria coincides with the QRS complex. The QRS complex is a relative strong electrical signal because the ventricular muscle is much more massive than the atrial muscle [Guyton & Hall 2006, Martini 2004]. The atrial repolarisation wave also called T_a is directly opposite in polarity to the P wave [Fuster et al. 2004]. The isoelectric line between the QRS complex and the T wave is the period where the entire ventricular myocardium is depolarised and repolarisation of the ventricles has not yet begun [Guyton & Hall 2006, Martini 2004].

The T wave is the last wave appearing in a normal ECG and represents the repolarisation of the ventricular muscle. The positivity of the T wave is due to the propagation of the repolarisation wave, which spreads in reversed direction of the depolarisation wave [Martini 2004, Guyton & Hall 2006].

2.1.3 Appearance of the Electrocardiogram

The appearance of the ECG is determined by the transmission of impulses through the heart, because, any changes in the trasmission pattern and velocity will affect the current flow around the heart and consequently affect the shape of the waves in the ECG. This can be understood by considering a vectorcardiographic representation of a depolarising muscle. During the propagation of the depolarisation wave a part of the muscle will be depolarised while the remaining part is still polarised. This causes a voltage difference between the two parts. The voltage difference is determined by the amount of depolarised and polarised muscle respectively; the largest difference is seen when half of the muscle mass is depolarised and the other half is not. The direction and magnitude of the current generated in the heart at a given instant can be depicted as a vector that points in the direction of the current flow and with length proportional to the voltage difference between depolarised and polarised muscle [Guyton & Hall 2006]. A more thorough explanation of the ECG and vectorcardiography can be found in appendix A in section A.5 on page 64.

A representation of the magnitude and direction of the instantaneous mean electrical vector of the ventricles during the depolarisation is shown in figure 2.5.



Figure 2.5: Vectorcardiographic representation during the depolarisation of the ventricular muscle mass. I, II, and III corresponds to Einthoven's bipolar leads. Edited from Guyton and Hall [2006].

By projecting the mean electrical vector onto the three bipolar leads (i.e. I, II, and III) a picture of the electrical activity measured by each lead is obtained [Sand et al. 2004, Guyton & Hall 2006]. The first step in figure 2.5 shows a short mean electrical vector because only small portion of septum is depolarised, thus all electrocardiographic voltages are low in the three bipolar leads. Next, the mean electrical vector is long because much of the ventricular muscle is depolarised, this is also shown as the shaded areas in figure 2.5. The voltage at lead II is greater comparing to the rest of the leads because the mean electrical vector extends almost in the same direction as the axis of lead II. Next, the depolarisation wave reaches the epicardium and the apex of the heart, the mean electrical vector is changing slowly toward the left side, due to the greater amount of ventricular muscle mass in the left side, thus slower depolarisation. Afterwards, the mean vector becomes shorter because only small portion of the ventricular muscle is still polarised. Furthermore the direction of the vector is toward the base of the left ventricle. In this case only lead I has positive electrical voltage. Finally, the entire ventricular muscle is depolarised and no current flows around the heart. Therefore, the mean electrical vector becomes zero and consequently the voltages measured in all leads become zero, as shown in figure 2.5.

From the above, it can be seen that the ECG is determined by the pattern, amplitude, and velocity of the impulse propagation during cardiac activity. The ANS' effect on these variables is therefore crucial to clarify whether the ANS has an effect on the morphology of the ECG or not.

2.2 Autonomic Innervation and Regulation of the Heart

The autonomic innervation of the heart is rich. Parasympathetic innervation is particularly rich to the SA node, sinoatrial conducting pathways, and the AV node, whereas the parasympathetic innervations to the ventricles are sparse [Wei-Jin et al. 2005, Malik 1998, Brodde & Michel 1999, Guyton & Hall 2006]. The sympathetic nervous system innervates all parts of the heart but is dominating in the ventricles [Wei-Jin et al. 2005, Guyton & Hall 2006]. Figure 2.6 illustrates the sympathetic and parasympathetic innervations of the heart.



Figure 2.6: The sympathetic and parasympathetic innervation of the heart. The parasympathetic innervation is dominating in the SA node, atrial myocardium, and the AV node compared to the ventricles. In contrast, sympathetic innervation dominates in the ventricles. Edited from Guyton and Hall [2006].

The two divisions have opposite effects on the heart; the sympathetic division causes excitation, whereas the parasympathetic causes inhibition. The parasympathetic division predominates in resting conditions whereas the sympathetic division dominates in more demanding situations [Martini 2004]. Additional information about the ANS can be found in appendix B on page 71.

2.2.1 Parasympathetic Effects on Cardiac Cells

The parasympathetic nervous system releases acetylcholin at the postganglionic nerve endings. Acetylcholin binds to one or more subtypes of muscarinic (M) receptors in the human heart. Five different muscarinic receptor subtypes (M_1-M_5) have been identified. M_2 is the predominating muscarinic receptor type in the heart [Brodde & Michel 1999].

An inhibitory G protein (G_i) is attached to the M₂ receptor on the membrane of the cell. When acetylcholine binds to the M₂ receptor, the G_i protein is activated, which causes an inhibition of adenylate cyclase. The inhibition of adenylate cyclase inhibits the formation of cyclic adenosine monophosphate (cAMP) from adenosine triphosphate (ATP) [Guyton & Hall 2006, Despopoulos & Silbernagl 2003]. cAMP would have activated type A protein kinase (PKA) which is capable of phosphorylating proteins. The absence of PKA causes a reduction of Ca²⁺ influx, due to inactivation of L-type Ca²⁺ channels. Furthermore, the activation of the G_i protein opens potassium channels which causes an efflux of K⁺ from the cell leading to hyperpolarisation [Brodde & Michel 1999]. An overview of the actions and effects is illustrated in figure 2.7.



Figure 2.7: Illustration of a postganglionic parasympathetic neuron releasing acetylcholine. Edited from Despopoulos and Silbernagl [2003].

In the SA node, the reduction of Ca^{2+} influx causes the slope of the prepotential to decrease and the threshold potential to shift to a more positive value. The efflux of the K⁺ ions shifts the resting potential to a more negative value, and therefore hyperpolarises the cell. Thus, the prepotential is prolonged and causes a decrease in heart rate, also called negative chronotropic effect [Despopoulos & Silbernagl 2003]. These three effects on the SA node are illustrated in figure 2.8a, b, and c respectively.



Figure 2.8: The factors that affect the action potential and thereby the heart rate. (a) A reduction in the steepness of the prepotential slope prolongs the period until the threshold potential is reached. (b) An increase in threshold potential prolongs the period until the threshold potential is reached. Finally, (c) a lowering of the resting potential also increases the period until the threshold potential is reached. Edited from Despopoulos & Silbernagl [2003].

The conduction of the action potentials in the AV node is also affected. The reduction in Ca^{2+} influx causes a slower depolarisation of the AV node and thus a reduced conduction velocity, also called negative dromotropic effect, on the AV node, cf. 2.9.



Figure 2.9: Change in depolarisation slope. Edited from Despopoulos & Silbernagl [2003].

Finally, a decreased contractility, also called negative inotropic effect, is caused by a reduction in influx of Ca^{2+} , which is responsible for the exposure of actin filaments during the contraction [Despopoulos & Silbernagl 2003].

2.2.2 Sympathetic Effects on Cardiac Cells

The sympathetic nervous system uses epinephrine and norepinephrine as primary transmitter substances. There are two adrenergic receptor types; α and β adrenoceptors. These receptors can be divided further into α_1 , α_2 , β_1 , and β_2 adrenoceptors. The presence of these four receptor types in the human heart has been shown, but the majority of the adrenergic receptors in the human heart are β_1 adrenoceptors [Brodde & Michel 1999].

The effect of stimulation of β_1 adrenoceptors is illustrated in figure 2.10a and b. When epinephrine or norepinephrine binds to the β_1 adrenoceptor it activates a G protein in a manner similar to that of acetylcholine binding to M₂ receptors. Instead of activating an inhibitory G protein, β_1 adrenoceptors activates a stimulatory G (G_s) protein. The activation of the G_s protein activates cAMP which in turn activates PKA [Sherwood 2004]. PKA phosphorylates L-type Ca²⁺ channels which activates them, and consequently increases the intracellular Ca²⁺ concentration [Kamp & Hell 2000]. This causes a positive chronotropic effect on the SA node and positive dromotropic effect on the AV node.



Figure 2.10: Illustration of what happens when β₁ adrenoceptors are stimulated by norepinephrine or epinephrine.(a) The overall process during β₁-adrenergic stimulation. (b) Illustration of the major calcium transport mechanisms in the cell. Edited from Despopoulos & Silbernagl [2003] and Bers [2002] respectively.

The increased concentration of cytosolic Ca^{2+} activates ryanodine receptors (RyR) in the sarcoplasmatic reticulum (SR). This causes a release of a large amount of Ca^{2+} from the SR to the cytosol. In the membrane of the SR, there is a Ca^{2+} pump called sarcoplasmatic reticulum Ca^{2+} -ATPase (SERCA2), which pumps the cytosolic Ca^{2+} back into the SR. In humans, the activity of SERCA determines the rate of removal of more than 80% of cytosolic Ca^{2+} [Frank et al. 2003]. SERCA2 pumps are regulated by a protein called phospholamban (PLB). Normally, PLB inhibits SERCA2, but under β_1 -adrenergic stimulation PKA phosphorylates PLB and thereby reduces the inhibitory effect of PLB on SERCA2. This causes an increase of the rate of intracellular Ca^{2+} removal from the cytosol into the SR.

The result of the described process is a rapid increase in intracellular Ca^{2+} concentration due to the RyR receptor but of short duration due to the Ca^{2+} removal mediated by SERCA2 [Fuster et al. 2004, Bers 2002]. This produces positive inotropic effects on the myocardium. The high concentration of the intracellular Ca²⁺ increase the amplitude and decrease the duration of the action potential of the cardiac contractile cells [Parilak et al. 2009]. This is illustrated in figure 2.11.



Figure 2.11: Two action potentials recorded at a heart rate on 120 bpm and 160 bpm. The increase in amplitude and decrease in duration of the action potential from 120 bpm to 160 bpm is clearly seen [Parilak et al. 2009].

Figure 2.11 clearly demonstrates how the action potential duration decreases and amplitude increases with increasing heart rate.

2.2.3 Summary of Autonomic Nervous Systems Effect on Cardiac Cells

From the above it became obvious that the ANS has great influence on the cardiac activity. In general, the parasympathetic division causes negative chronotropic, dromotropic, and inotropic effects to occur. In contrast, the sympathetic division causes positive chronotropic, dromotropic, and inotropic effects to occur. The two divisions' effect on intracellular concentrations of substances and ions is outlined in table 2.1.

Parasympathetic Division					
Agonist	Receptor	Effect on intracellular concentrations	Effect on the heart		
Acetylcholine	M ₂	↓ cAMP ↓ PKA ↓ Ca ²⁺ ↓ K ⁺	÷ Chronotropic ÷ Dromotropic ÷ Inotropic		
Sympathetic Division					
Agonist	Receptor	Effect on intracellular concentrations	Effect on the heart		
Epinephrine Norepinephrine	β1	↑ cAMP ↑ PKA ↑ Ca ²⁺	+ Chronotropic + Dromotropic + Inotropic		

 Table 2.1: Observations made regarding autonomic innervation of receptors found in the heart, their effect on intracellular concentrations when stimulated, and the resulting effect on the heart.

Besides the effects summarised in 2.1, the ANS also regulates the conductance of the gap junctions which connects adjacent cells in the heart.

2.2.4 ANS's Effects on Gap Junctions

As mentioned before, gab junctions are responsible for the impulse propagation in the heart [Rohr 2004]. Gap junctions are regulated by different substances, among these are cAMP, PKA, and Ca^{2+} [Dhein 1998*b*]. The concentration of these substances changes in the intracellular environment due to autonomic innervation (cf. the observations outlined in table 2.1).

An increase in intracellular cAMP leads to increased coupling between adjacent cells [Dhein 1998*b*]. PKA enhances the conductance of the most abundant type of gap junction found in the heart. The change is very rapid and may lasts for several minutes. A large increase in intracellular Ca^{2+} causes reduction in conductance of the gap junctions. Under normal conditions the Ca^{2+} concentration does not reach sufficient levels to cause a reduction in gap junctional conductivity [Dhein 1998*a*].

If the changes, in the mentioned substances mediated by the autonomic nervous system are high enough to cause a regulation of the cardiac gap junctions then, in general sympathetic innervation would cause an increase in gap junctional conductance, whereas the parasympathetic innervation would cause a decrease in gap junctional conductance.

The conduction velocity in tissue is determined by two factors; 1) it is directly related to the rate of rise and the

amplitude of the action potential and 2) it is inversly related to the resistance of the conducting pathway. The amplitude of action potentials is affected by the adrenergic stimulation of the heart. The resistance of the conducting pathway in the heart is composed of series of cytoplasmic and gapjunctional resistances. Thus, an increase in the gap juntional conductance leads to an increase in conduction velocity [Burt & Spray 1988]. Burt and Spray [1988] have reported that gap junctional conductance is enhanced by agents that elevate intracellular cAMP levels, and that the effect is seen within time courses comparable to those of naturally mediated inotropic effects of cAMP. Mello [1991] supports the view that the cAMP mediated phoshorylation of gap junctions happens at the basal levels of cAMP.

An increase in junctional conductance probably does not have a great impact on the conduction velocity in the electrical system of the heart, since junctional resistance is not rate limiting. Conversely, an increase in junctional conductance in areas of the heart where conduction not only occurs in the longitudinal direction of the fibres and where the junctional resistance is a limiting factor, such as the ventricles, is expected to increase conduction velocity with up to 10 % [Burt & Spray 1988].

2.3 Conclusion on the Initial problem

The analysis of the initial problem revealed that changes in the ECG are mediated by changes in the pattern of impulse propagation, the velocity of impulse propagation through the heart, and the amplitude of the voltage differences generated by the impulse.

The analysis did not give reason to believe that the pattern of impulse propagation is changed by the ANS. Interestingly, the analysis showed that sympathetic stimulation of the heart causes an increase in heart rate, an increase in conduction velocity through the AV node and myocardium, and an increased amplitude of the action potentials of cardiac contractile cells. The parasympathetic division inhibits the processes which causes these changes, thus, the parasympathetic division has the opposite effect. This encourages to believe that the morphology of the QRS complex changes with variation in ANS balance.

Hypothesis

The problem analysis described how ANS affects the ECG. It showed that there is physiological evidence that the autonomic nervous system has an effect on the conduction velocity especially in the ventricular myocardium and on the the amplitude of the action potentials in the myocardium.

Based on these findings we hypothesised that these effects are reflected in the morphology of the ECG as a decrease in width and an increase in amplitude of the QRS complex during increased sympathetic regulation of the heart and the opposite effect during parasympathetic regulation.

Therefore, the aim of this project was to develop methods to extract features which can reveal the aforementioned changes in the QRS complex. Furthermore, the extracted features were linked to the autonomic regulation of the heart.

Solution Model

In order to test our hypothesis a sequence of operations were carried out. These operations are presented in the following solution model. The solution model aimed to extract features from the morphology of the QRS complex, which reflects velocity and amplitude of the electrical impulses involved in ventricular depolarisation and finally to evaluate the extracted features' relation to the sympathetic and parasympathetic modulation of the heart. The solution model comprises of five operations which are presented in figure 4.1.



Figure 4.1: Illustration of the solution model, which is made up of five operations: ECG signal Acquisition, ECG signal Preprocessing, QRS Complex Detection, Feature Extraction, and Feature Evaluation.

The first three operations in the solution model are necessary to obtain a clean signal with a limited amount of noise and to exclude beats originating outside the SA node, before the feature extraction can take place. The specific purpose of each operation is outlined below.

ECG signal Acquisition

The first operation was to acquire the ECG signals that should be used to test the hypothesis. In this project three datasets were used; dataset A which consisted of ECG recordings from healthy subjects, dataset B which consisted of ECG recordings from subjects with chronic regional pain syndrome (CRPS), and last, data set C which consisted of ECG recordings from healthy subjects subjected to capsaicin induced pain.

ECG signal Preprocessing

The purpose of the preprocessing was to remove the noise that contaminates the ECG signal. The noise can be drift of the ECG signal baseline due to respiration, noise from the power line, motion artifacts from electrode movements, and electromyographic (EMG) interference due to muscular activity.

QRS Complex Detection

The purpose of the QRS complex detection was to locate all QRS complexes in the recordings. Only QRS complexes originating in the SA node were included.

Feature Extraction

Methods to extract features which reflect the expected morphological changes of the QRS complex were developed. The features were extracted from the QRS complexes found in the previous operation.

Feature Evaluation

The extracted features was compared to the expected changes. Furthermore, they were compared to SDNN, which is a time domain measure of HRV that reflects overall variance i.e. contributions from both divisions of the ANS to the variability are included in SDNN [Bigger et al. 1992, TaskForce 1996].

The solution was implemented in MatLab (version 7.7.0.471 - R2008b).

ECG signal Acquisition

ECGs recorded by Astrid Terkelsen at the Danish Pain Research Center at Aarhus University Hospital and ECGs recorded by ourselves have been studied. Two datasets of ECGs recorded by Astrid Terkelsen were used; dataset A with healthy subjects and dataset B with patients suffering from complex regional pain syndrom (CRPS). A tilt table test was conducted on each subject in dataset A and B. The dataset recorded by ourselves is called dataset C and includes subjects with capsaicin induced pain. The effect of the tilt table test, complex regional pain syndrome, and pain on the ANS is described below followed by a description of the three datasets.

5.1 Tilt Table Test

Tilt table tests are used to study the adaption of heart rate and blood pressure to postural changes, and thereby evaluate among other things the hemodynamic and neuroendocrine response in subjects with autonomic dysfunction [Fuster et al. 2004].

In the tilt table test, a person is shifted from supine position to upright posture. The shift in posture causes a redistribution of the central blood volume towards the lower part of the body due to gravitational forces [Zipes & Jalife 2004, Fuster et al. 2004]. An illustration of the set up is illustrated in figure 5.1. The person must be strapped to the tilt table to avoid any muscle contractions in the legs, and thus to obtain larger blood flow to the legs after the shift from supine position to upright posture.



Figure 5.1: Set up of the tilt table test. The person is strapped to the table which can be tilted [Hopkins 2009*].

The gravitational effect results in a shift of 500-1000 mL blood from the upper body to the lower body. The shift causes a drop in central blood pressure, which baroreceptors in the aortic arch and carotid sinuses register [Zipes & Jalife 2004]. This triggers a baroreflex that inhibits the parasympathetic division of the autonomic nervous system and excites the sympathetic division [Guyton & Hall 2006]. This restores and maintains the arterial blood pressure, by increasing the heart rate and constricting the blood vessels [Zipes & Jalife 2004]. Thus, the tilt table test mediates a change in the autonomic regulation of the heart; parasympathetic regulation dominates in supine position, whereas sympathetic division dominates the regulation in the upright position.

The significance of the tilt angle was investigated by Zaidi et al. [2000], they found that the heart rate was not subject to significant changes when the tilt angle exceeded 60° .

5.2 Complex Regional Pain Syndrome

CRPS is characterised by pain, abnormal regulation of blood flow and sweating, oedema of the skin and subcutaneous tissue, trophic changes of the skin, and tremor. CRPS is divided into two types; type I and II. The syndrome is most frequently precipitated by a trauma which affects distal parts of an extremity, e.g. a fracture, contusions or postsurgical conditions. CRPS type I patients have small or no obvious nerve lesions, whereas CRPS type II have nerve lesions. The patients develop the mentioned symptoms subsequently to the inciting event. Usually, the sensed pain is not proportional to the inciting event, and the symptoms occur inconsistently to the spatial distribution of nerves and at sites not related to the site of the inciting lesion, e.g. on the ipsilateral side of the body [MacMachon & Koltzenburg 2006, Jänig & Baron 2002].

The triggering trauma causes changes in the information processing in the central nervous system and thereby abnormal sympathetic activity. CRPS is far from understood, but sympathetically maintained pain is an often present clinical symptom for CRPS. Sympathetically maintained pain is a condition where pain is dependent on activity in sympathetic neurons [Jänig & Baron 2002].

5.3 Autonomic Reaction to Pain

As a reaction to pain the sympathetic nervous system immediately activates defence mechanisms e.g. increased heart rate, increased blood pressure, and increased cardiac output. Intradermal injection of capsaicin causes burning pain that last for several minutes. The pain caused by capsaicin affects the autonomic system by inhibiting the activity of the parasympathetic division and increasing the activity of the sympathetic division [Terkelsen et al. 2005, Bourguignon et al. 2005].

5.4 Dataset A: Healthy Subjects

Dataset A consisted of recordings from 20 healthy subjects (12 males and eight females, mean (\pm SD) age were 43.44 (\pm 14.00) years, mean BMI (\pm SD) 26.10 (\pm 4.33)).

A tilt table test was conducted on each subject. The subjects rested for 30 minutes prior to the test. The subjects were placed in a supine position for ten minutes, whereupon they were tilted 60° to an upright position for 20 minutes followed by ten minutes of recovery in the supine position. The upright period was divided into two segments of ten minutes each.

During the tilt table test, ECG recordings from lead II were obtained with a sampling frequency of 1 kHz. The recordings have been amplified with a gain on 1000, analog band pass filtered with cut-off frequencies at 0.08 Hz and 150 Hz.

When referring to this dataset in the following, the four sequences of ten minutes are related to their positions as supine, upright 1, upright 2, and recovery respectively.

5.5 Dataset B: Complex Regional Pain Syndrome Patients

Dataset B consisted of ECG recordings form 18 subjects suffering from CRPS (12 males and six females, mean (\pm SD) age were 42.81 (\pm 11.92) years, mean BMI 26.11 (\pm 5.63).

The included patients were all more than 18 years, diagnosed with CRPS, and have had spontaneous aroused pain for at least three months.

A tilt table test, similar to the one for the healthy subjects in dataset A, was conducted on the subjects in this dataset while ECG recordings were obtained from Einthovens lead II. The recordings were amplified with a gain on 1000, analog band pass filtered with cut-off frequencies at 0.08 Hz and 150 Hz, and sampled at 1 kHz.

5.6 Dataset C: Healthy Subjects With Induced Pain

In this dataset, capsaicin induced pain was used to mediate a change in the autonomic regulation of the heart. The dataset consisted of recordings from 11 healthy subjects (nine males and two females, mean (\pm SD) age were 23.09 (\pm 2.74) years, mean BMI (\pm SD) 22.75 (\pm 2.71)).

Initially, three Ag/AgCl surface electrodes were placed on the chest of each subject. One electrode was used as a reference electrode and the other two electrodes were placed such that ECG recordings from Einthoven's lead II was obtained. For further information regarding electrode configurations for ECG recordings see appendix A.5 on page 64.

The subjects rested 10-15 minutes before the recordings were initiated. A baseline recording of two minutes was acquired while the subject was resting in supine position. Afterwards, 10 μ g capsaicin in a volume of 0.1 ml was injected in the flexor digitorum bevis muscle, which is a muscle that lies in the middle of the sole of the foot. The subjects ECGs were recorded for two minutes after the injection.

The ECG recordings were analog high pass filtered with a cut-off frequency of 0.03 Hz to avoid half cell potentials. The digital convertion was obtained by a 12 bit A/D converter with a voltage range of ± 10 V. The recordings were sampled at 10 kHz. The high sampling frequency was chosen to achieve a high resolution of the morphological changes in the QRS complex.

ECG signal Preprocessing

<u>6</u>

The purpose of this operation was to remove noise that might corrupt the ECG signals. Noise reduction was very important, because superimposed noise could corrupt the morphology of the QRS complex. The noise comes from interference from the power line at 50 Hz, movement artifacts from electrodes, muscular activity, etc. [Webster 1998].

6.1 Frequency Analysis of the ECG signal

Before dealing with the ECG signals, the aforementioned noise elements had to be filtered out without causing a significant change of the QRS complex. The power spectrum of an ECG signal lies in the interval of 0.01-250 Hz [Webster 1998]. Hejjel & Kellenyi [2005] found that a high pass cut-off frequency above 1 Hz may distort the ECG, reduce the QRS complex's amplitude, and lead to alterations of the slope of the R peak.

6.2 Filtering of the ECG signal

Although the prior information about the frequency spectrum of an ECG signal was available the spectral components of the ECG recordings were examined before proceeding.

Figure 6.1a shows a segment from an ECG recording with a sampling frequency of 1 kHz. The power spectrum corresponding to the entire signal is shown on figure 6.1c. Additionally to the P wave, QRS complex, and T wave there were low frequency components and high frequency components. The former could be due to movement artifacts from the electrodes and the latter could be due to EMG activity and 50 Hz power line interference.



Figure 6.1: The four plots shows; (a) a segment of an ECG recording before filtering and (b) the same segment but after filtering, (c) the power spectrum for the entire raw ECG signal before filtering and (d) after filtering.

It is clearly seen on figure 6.1c that the majority of the energy in the signal is below 150 Hz. Furthermore, it is seen on figure 6.1a and figure 6.1c that the 50 Hz power line is a major contributor of noise to the signal.

The most appropriate filtering operation depends on the application. In this project the morphological changes in the QRS complexes were essential and therefore it was chosen only to filter out the 50 Hz noise. Removing the 50 Hz component does affect the ECG because of the overlap in the power spectrum. However, this effect was found to be negligible compared to the energy in the entire power spectrum of the ECG signal. Additionally, the phase distortion that is a consequence of filtering alters the temporal relation of the original signal. In order to avoid this phase distortion a zero phase filtering operation was performed.

It was chosen to use a notch filter to remove the noise from the 50 Hz power line. A notch filter is a filter that passes all frequencies except those in a stop band. The width of the stop band depends on the filters quality factor (Q value). A high Q value gives a narrow stop band and more phase distortion of the signal, while a low Q value gives a wide stop band and thereby removes more frequency components, but gives less phase distortion [Bai et al. 2004]. The Q value was chosen to 50. Using this value ensured that the stop band was narrow and that primarily noise from the

power line were removed. The filtering operation was performed by filtering the signal in the forward direction, and then the filtered signal was reversed and filtered with the same filter again. This operation eliminates phase distortion. The result of the filtering on the ECG segment in figure 6.1a is shown on figure 6.1b and the frequency spectrum of the entire signal after filtering is shown on figure 6.1d. It is seen that the 50 Hz power line was attenuated. Figure 6.2 shows that the ECG signal was not phase distorted, due to the zero phase filtering operation.



Figure 6.2: A segment of the ECG signal before (blue) and after the filtering operation (red).

After filtering the ECG signals the detection of QRS complex was applied. This operation is described in the next chapter.
QRS Complex Detection

After having filtered the ECG recordings, the QRS complexes were detected. It was chosen to implement a QRS detection algorithm based on the one developed by Pan & Tompkins [1985]. It is a popular and often used algorithm due to its high selectivity and specificity. Furthermore it is easy to implement [Wagner et al. 2003]. The algorithm detects the QRS complexes based on four steps; differentiation, squaring, moving average filtration, and threshold exceeding of the signal. Before application of the algorithm it was chosen to downsample the filtered ECG recording to reduce computational costs of the detection. After detection in the downsampled signal, the corresponding QRS complexes were recognised in the original signal from which features were extracted.

Downsampling

The original filtered ECG recording was downsampled to a samplings frequency of 250 Hz, which is enough to get a reliable detection of the QRS complex location. The downsampling was implemented as in equation 7.1.

$$y_{250}[n] = \frac{1}{N} \sum_{i=0}^{N-1} x_{f_s}[nN - i], \qquad N = \frac{f_s}{250Hz}$$
(7.1)

Where x_{f_s} is the raw ECG signal with samplings frequency fs of 1 kHz or 10 kHz. N becomes an integer and is used as an index. A segment of a downsampled ECG signal is shown in figure 7.1.



Figure 7.1: A segment of an ECG signal downsampled to 250 Hz.

Usually, prior to downsampling a low pass filter should be implemented to avoid aliasing. The implemented downsampling includes an averaging, and justifies the downsampling without further filtering bacause averaging can be regarded as a low pass filtering. After the downsampling, the four steps of the QRS detection algorithm were applied.

Differentiation

The first step in the algorithm is to differentiate the signal, hereby information about QRS complex slope is achieved. The derivative was computed within a window width of 20 ms. Equation 7.2 was used for the differentiation.

$$y'_{250}[n] = y_{250}[n] - y_{250}[n+N], \qquad N = [f_s \cdot 0.020]$$
(7.2)

N is an integer and can therefor be used as an index. The stepsize of 20 ms makes the differentiation less sensitive to noise bursts.





Figure 7.2: Result of the differentiation of the segment shown in figure 7.1.

It is clearly seen on figure 7.2 that the P and T waves are suppressed, while the QRS complex is enhanced.

Squaring

After the differentiation the signal was squared point by point, as stated in equation 7.3, where $y'_{250}[n]$ is the amplitude of the signal at sample number n.

$$y_{sa}[n] = (y'_{250}[n])^2 \tag{7.3}$$

The squaring makes all data points of the derivative positive and enhances higher frequencies, which primarily comes from the ECG [Pan & Tompkins 1985]. The result is shown on figure 7.3.



Figure 7.3: The squared of the differentiated signal shown in figure 7.2.

After the squaring it is seen that only the QRS complexes are visible. Each QRS complex is represented as a double peak; one for the ascending slope of the R peak and one for the descending slope of the R peak.

Moving Average

The result after the differentiation and squaring operation is a double peak within the duration of a single QRS complex. By moving average filtering, the two peaks of a QRS complex can be joined in a single wide peak representing the QRS complex. The algorithm for the moving average filter was implemented by calculating the average amplitude of samples in a window. As a principal rule, the width of the window must be as wide as the widest QRS complex; if it is too wide, it will merge the QRS complex and T wave together, but as seen on figure 7.3 only the QRS complex is visible, so merging of the QRS complex and the adjacent waves are not actually an issue. If the window width is too

narrow, then the QRS complex will still exhibit several peaks [Pan & Tompkins 1985]. Therefore, the window width was set to 80 ms which is within the range of the width of a QRS complex [Despopoulos & Silbernagl 2003]. The mathmatical implementation of the moving average window, with a width of 80 ms at a sampling frequency of 250, is written in equation 7.4:

$$y_{MA}[n] = \frac{1}{N} \sum_{i=1}^{N} y_{sq}[[n-1] \cdot N + i], \qquad N = f_s \cdot 0.080s$$
(7.4)

where N is the number of samples in the width of the moving average window. The result of the moving average operation is shown on figure 7.4.



Figure 7.4: The signal segment in figure 7.3 after moving average filtering.

As seen on figure 7.4 the signal was not smooth as expected after a traditional moving average operation. This is because the moving average window was shifted N indexes between each averaging.

Threshold Exceeding

After the moving average operation a threshold was implemented. The value of the threshold was set to the standard deviation of the amplitudes within the areas of interest. This value was chosen in order to adapt the threshold to each subject and minimize the effect of outliers compared to a threshold defined as e.g. a percentage of the maximum value in the signal.

Every time the moving average filtered signal exceeded the threshold, a QRS candidate was located. The index in the original ECG, corresponding to the index of the threshold exceeding, was used to locate the fiducial point of the QRS complex in the original ECG signal. The QRS candidate was identified as the index of maximum amplitude within 100 ms on each side of the exceeding index. The QRS candidate detection is illustrated in figure 7.5.



Figure 7.5: The upper panel shows where the moving average filtered signal (blue) exceeds the threshold (red). The index corresponding to the index from the upper panel was found in the original ECG signal with samplings frequency of 1 kHz in the lower panel (dashed green line). The QRS candidate was identified as the index of maximum amplitude (marked with a red circle) within 100 ms on each side of the exceeding index (marked with a yellow dashed line).

The entire ECG signal was dragged up/down toward zero by subtracting the mean value of the segment between the P wave and the QRS complex. This ensured that the QRS complexes are aligned around the isoelectric segment.

Validation of QRS Candidates

After the QRS detection algorithm, all the detected QRS complexes were validated to ensure that only the correct QRS complex candidates were located. The validation was only done for the QRS complexes within the areas of interest e.g. supine, upright 1, upright 2, and recovery. It was done by ensuring that no candidates were closer than physiologically possible (200 ms [Pan & Tompkins 1985]). At the same time QRS candidates located with an interbeat interval larger than 1000 ms (corresponding to a heart rate on 60 bpm) were identified and subjectively validated. Finally, consecutive beats resulting in more than 20 % change of the NN interval have been eliminated by others [Huikuri et al. 1999], therefore, it was chosen to validate all beats resulting in a 20 % or larger change between consecutive NN intervals.

By validating all the QRS candidates it was assured that no QRS complexes originating outside the SA node were analysed further, since only the true QRS complexes should be used in the feature extraction. The maximum number of excluded QRS complexes in a single recording was two. The effect of the removal of two complexes was considered neglectable.

Due to technical problems with the amplification of the signal prior to the A/D conversion, some of the recordings were saturated, this resulted in a cut-off of the tip of the R peak. This complicated exact detection R peak of fiducial points for the saturated QRS complexes. It was chosen to use the center of the area where the R peak saturated as fiducial point in order to standardise the error it will impose on the heart rate signal.

A window on 150 ms centered around the located fiducial points was used to represent each QRS complex. 150 ms were chosen to ensure that only the QRS complex was contained in the stored without and not the P and T waves.

Feature Extraction

After the QRS complexes were detected and validated, two features were extracted, both based on the demonstrated relation between ANS's regulation of the myocardial conduction velocity and amplitude of the electrical impulses. As stated in section 2.2 on page 12, increased sympathetic regulation of the heart should cause an increase in the amplitude of the QRS complex and velocity of the electrical impulse propagation, whereas the parasympathetic division should have the opposite effect.

8.1 Feature Background

The mean QRS complex for four randomly chosen subjects in dataset A for the supine, upright 1, upright 2, and recovery recording is shown on figure 8.1. By visual inspection of the mean QRS complexes it was seen that the morphology changed.



Figure 8.1: The mean QRS complex for four different subjects in dataset A for the supine (blue), upright 1 (red), upright 2 (yellow), and recovery (green) recording.

In figure 8.1a, b, c, and d it seems that the amplitude of QRS complex was increased from supine (blue) to upright 1 (red) and increased further in upright 2 (yellow). The amplitude decreased again in recovery (green), except in the example in figure 8.1b, where it stayed at the same level as upright 2. The last upright position (yellow) seemed to cause the highest amplitude in all four examples. The mean QRS for the supine position (blue) had the lowest peak amplitude in figure 8.1a, b, and d. Even though the standard QRS complex is rather well defined, as seen on figure 8.1

the QRS complex of different subjects may differ significantly.

The mean QRS complex for all 20 subjects from dataset A is shown in figure 8.2. It can be seen that the QRS complex was narrow during the upright positions (red and yellow) compared to the mean QRS complexes for supine and recovery (blue and green). There was also an increase in the amplitude from supine position to upright 1 and a further increase in upright 2. The amplitude decreased at recovery, but was higher than the amplitude in the initial supine position.



Figure 8.2: The mean QRS complex for all 20 subjects in dataset A for the supine (blue), upright 1 (red), upright 2 (yellow), and recovery (green) recording.

The mentioned observations partly supported what we expected, because the QRS complexes became narrower and increased in amplitude in the upright position compared to supine.

In an attempt to accentuate the morphological changes related to velocity of the impulse propagation and amplitude of the QRS complex, two features were extracted. The first feature was very intuitive and simple and reflected the slope of the QRS complex. The second feature was more complex and used wavelets in an attempt to reveal morphological changes.

8.1.1 Feature Evaluation Method

After extraction of the features, it was investigated whether the features reflected the expected changes in autonomic modulation of the heart during postural changes and pain. The features were compared to the classic time domain measure, SDNN. SDNN was calculated as stated in equation 8.1.

$$SDNN = \sqrt{\frac{1}{N} \sum_{i=1}^{N} (NN_i - m)^2}$$
 (8.1)

where NN_i is the i^{th} NN interval and m is the mean NN interval length and N is the number of NN intervals in the ECG recording.

8.2 Feature 1: Slope of the QRS Complex

The idea behind this feature was to find the steepest ascending slope of the QRS complex. It was a simple feature based on the demonstrated effect of the ANS on the amplitude and width of the QRS complexes. The steepest ascending slope is on the R peak, this is illustrated in figure 8.3.



Figure 8.3: The steepest ascending slope of the QRS complex is found on the R peak.

8.2.1 Method

The slope was defined as the difference between two averages. The first average value was the average of adjacent samples within three ms, the second average was calculated for samples 10 ms later and was also the average of adjacent samples within three ms. This was done for all points. The averaging and distance between the averaging on 10 ms was chosen to reduce the effect of noise peak and at the same time ensure that the steepest slope was on the ascending site of the R peak. The size of the inter-point distance should not be chosen too small since this would make the feature more sensitive to noise. On the other hand if the inter-point distance was set too large it could attenuate the measured slope. Equation 8.2 was used to calcluate the slope.

$$QRS'[n] = \frac{1}{M} \sum_{i=0}^{M-1} \left(x[n+N+i] - x[n+i] \right) \qquad N = f_s \cdot 0.010$$
(8.2)

M is the amount of samples that were averaged. The samplings frequency f_s determined the value of M; M equaled three for dataset A and B, and 30 for dataset C. N was the number of samples between the two averages; 10 samples for dataset A and B, and 100 samples for dataset C. The steepest ascending was found by locating maximum and minimum in the vector QRS' for each QRS complex.

8.3 Feature 2: Morphology Changes Demonstrated with Wavelets

The slope of the QRS complex represents the morphological changes at on specific place in the QRS complex. A second feature dependent on the shape of the entire QRS complex was extracted. This feature was based in the contineous wavelet transform.

8.3.1 Theory

A wavelet is a limited wave in time and frequency. A wavelet denoted $\psi(t)$ is a mother wavelet. The mother wavelet is used to create daughter wavelets which are similar to the mother wavelet but dialated or compressed compared to the mother wavelet [Addison 2002]. There are nummerous wavelet functions which can be used as the mother wavelet, and the appropriate choice of mother wavelet depends on the application. See appendix C on page 85 for further information. A wavelet analysis can be used to examine the signal in both time and frequency, i.e., which frequency does the signal contain over a limited period of time. The CWT enables this time-frequency representations of a given signal, and is given by the convolution integral of a signal x(t) by a wavelet and is defined as follows [Addison 2002]:

$$T(s,\tau) = \frac{1}{\sqrt{s}} \int_{-\infty}^{+\infty} x(t) \psi\left(\frac{t-\tau}{s}\right) dt$$
(8.3)

The CWT returns the correlation, *T*, between the signal, x(t), and a scaled version of the mother wavelet. *s* denotes the scaling factor which either dilates or compresses the mother wavelet. A scaling factor *s* results in a daugther wavelet with a width on *s* times the width of the mother wavelet. Thus, low scales correspond to compressed mother wavelets, while large scales correspond to dilated mother wavelets. The factor $\frac{1}{\sqrt{s}}$ is for energy normalization at different scales [Addison 2002, Kumar et al. 2003]. The translation parameter τ is used to shift the wavelet across the signal and is related to the time information in the signal in the transform domain. The relation between scale and frequency is that low scales correlate with high frequencies and vice versa [Addison 2002, Kumar et al. 2003].

The result from equation 8.3 is a measure of how good the correlation between the mother wavelet with a given scale factor *s*, on a given translation τ and the signal x(t) is. The higher the absolute value of the correlation is, the better is the correlation between the signal and the scaled and translated mother wavelet. If the output is 0 then there is no correlation between the signal and the scaled, translated wavelet [Addison 2002, Kumar et al. 2003].

A wavelet it must satisfy the following conditions [Addison 2002, Yang et al. 2007]:

1) A wavelet must be a finite length signal, and therfore a wavelet must have finite energy:

$$E = \int_{-\infty}^{+\infty} |\Psi(t)|^2 dt < \infty$$
(8.4)

2) If $\hat{\psi}(f)$ is the Fourier transform of the wavelet $\psi(t)$, then:

$$\hat{\Psi}(f) = \int_{-\infty}^{+\infty} \Psi(t) e^{-i(2\pi f)t} dt$$
(8.5)

and then the admissibility condition must hold:

$$C_g = \int_0^{+\infty} \frac{|\hat{\psi}(f)|^2}{f} df < \infty$$
(8.6)

This implies that the wavelets has no zero frequency component, i.e. $\hat{\psi}(0) = 0$, equivalent to the wavelet having zero mean in the time domain. And therefore it must be oscillatory [Yang et al. 2007].

Based on the capabilities of the CWT it was assumed that the CWT could reveal morphological changes of the QRS complex, by investigating which scales return the best correlation between each QRS complex and the wavelet.

8.3.2 Method

As mentioned in the last section there are plenty of predefined mother wavelets which can be applied, but since the characteristics of the QRS complex vary between subjects as it was seen in figure 8.1 on page 35, it was chosen to adapt the mother wavelet to each subject to achieve high correlation for all subjects. The average QRS complex from the baseline recording, i.e. the recording obtained in supine position for dataset A and B and the recording obtained during rest for the subjects in dataset C, was used as a template for each adapted wavelet.

The beginning of the Q wave and the end of the S wave in the average QRS complex were found visually. The

criteria for the beginning of the QRS complex was defined as the time where the signal left the isoelectric line at the beginning of the Q wave, and the end of the QRS complex was defined as the time where the signal returned to the isoelectric line after the S wave. For some QRS complexes the beginning and end points were not explicitly pronounced, in these cases a narrower interval within the QRS complex was chosen.

In order to use the adapted wavelets for CWT, the wavelets must fullfil the requirements in equation 8.4 and 8.5. The adapted wavelets approximates the average QRS complexes by least squares fitting while fulfilling the requirements. Figure 8.4 shows two adapted wavelets from two subjects, it clearly demonstrate the difference between the subjects.



Figure 8.4: Two adapted wavelets from two different subjects. The wavelets have an effective support from t = 0 to 1. The difference in shape of the adaptive mother wavelets between the two subjects is clearly demonstrated.

The adaptive mother wavelets were created in MatLab using the wavelet toolbox with an effective support from 0 to 1, meaning that the mother wavelets were defined in this interval. After creating the adapted mother wavelet for each subject, the CWT was performed separately on each QRS complex.

The correlation $(T(s,\tau))$ was obtained by the following process: The adapted wavelet with one scale was shifted along the ECG signal and for each value of time shifting τ the correlation was computed. Afterwards, the wavelet was scaled with another scale and then shifted along the signal again while computing the correlation. This process was repeated until all desired scales had been investigated. The stepsize of the scales can be adjusted and was chosen to 50 μ s. The smaller the stepsize is the higher is the resolution of best correlated wavelets.

Finally, the scales with the maximum correlation coefficients was identified. By this approach the scale resulting in the best match between the wavelet and each QRS complex was achieved.

Results

The expectations and results of the extracted features for each dataset are presented in the following chapter. It was tested if the extracted features differed significantly between the recordings where parasympathetic dominance was expected and recordings where sympathetic dominance was expected.

A Friedman test was applied to the data from dataset A and B. The Friedman test does not rely on the assumption that the data follows any probability distribution. When significant differences were found (p<0.05), post hoc analysis with Wilcoxon's signed rank test was carried out and differences were considered significant only following the Bonferroni correction, i.e. p<0.0125 was considered significant in the post hoc analysis. Since, only two groups are considered in dataset C, the Wilcoxon signed rank test was applied at once. A p<0.05 was considered significant.

The results are presented in box plots that shows the median, the 25^{th} and 75^{th} percentiles, and whiskers that extends to the most extreme data value which is within the 95 % confidence interval if the data would have followed a normal distribution.

9.1 Results for Dataset A

As mentioned, dataset A consisted of 20 healthy subjects who were subjected to a tilt table test. Four recordings were analysed for each subject, the recordings were denoted: supine, upright 1, upright 2, and recovery according to the postures.

We expected a rise in heart rate and a fall in SDNN in situations with sympathetic dominance and vice versa. Regarding the slope, we expected an increase in the steepness when going from a situation with parasympathetic dominance to sympathetic dominance, the opposite was expected with the scale i.e. a decrease in scale when going from a situation with parasympathetic dominance to sympathetic dominance.

Figure 9.1 shows the results for a healthy subject during the tilt table test. It can be seen in the upper panel that the NN interval decreases from supine to upright 1 and 2, and increases again in recovery. In the second panel, an increase in the slope was seen in the upright periods compared to supine and recovery. The third panel, shows the extracted scale for each QRS complex. It can be seen that the scale decreases from supine to upright position and increases again from upright to recovery. The fourth panel shows the maximum correlation between the wavelet and each QRS complex, and the lower panel shows the time lag where the maximum correlation was found. There were two time lags within the supine recording that was more than 65 ms away from the fiducial point for the corresponding QRS complex. It can be seen that the scale (the third panel) where maximum correlation was found (the fourth panel), and at the same time the correlation was decreased for these two time lags (the fifth panel). Except from the two outliers, all time lags were in the range from 82 to 92 ms.



Figure 9.1: Result from a representative subject from dataset A. The coloured segments represents the four segments which were analysed. The upper panel shows the subject's tachogram. The second panel shows the steepest ascending slope as a function of the time of occurence. The third, fourth and fifth panel shows the scale where maximum correlation was found, the correlation matching the scale, and the time lag where the maximum correlation was found respectively, all as a function of the time of the QRS complex's occurence.

In the entire dataset A, there was a single recording (the one pictured in figure 9.1) where two of the scale measures within the areas of interest were attributed to a time lag more than 65 ms away from the fiducial point of the QRS complex. The fiducial point for each QRS complex was located at the time 75 ms. The overall effect of two outliers

was considered negligible. In general, the time lags, where maximum correlation was found, were in average within a range of 6.5 ms for the recordings and the largest range within a single recording was 15 ms. The smallest time lag which resulted in maximum correlation between the scaled wavelet and a QRS complex was 51 ms and the largest time lag was 96 ms.

9.1.1 Comparison of the Mean Level of the Extracted Features

As the example in figure 9.1 also illustrates, a decrease in the NN interval, an increase in slope steepness, and an increase scale were observed when the subjects were tilted from supine to upright and the opposit tendency was seen when the subjects were tilted back into supine position for recovery. Figure 9.2 shows three boxplot of the average NN interval, slope steepness, and scale. The p-value in the top comes from the Friedman test. The result of the post hoc test is also shown; groups marked with asterisk differs significantly from the ones to which a line is drawn.



Figure 9.2: Box plots of (a) the average NN interval, (b) the ascending slope, and (c) the scale for dataset A. The p-value obtained with the Friedman test is stated above the plots. p < 0.05 indicates that at least one of the groups (supine, upright 1, upright 2, or recovery) differ from the remaining. A group marked with asterisk differs significantly from the ones to which a line is drawn, or from all the remaining groups if no line is drawn.

Figure 9.2 shows that the three measures all resulted in significant differences between the groups. The post hoc analysis revealed that the NN interval differed significantly between all the groups. The duration decreased from supine to upright 1 and upright 2, whereas it increased again in recovery.

The ascending slope increased from supine to upright 1 and 2 and the slope for supine differed significantly from upright 2 and recovery. Furthermore, the slope differed significantly between upright 1 and 2. The slope decreased from upright to recovery but not significantly.

As expected, the scale decreased from supine to the upright position and increased again in recovery. The scale for supine and recovery differed significantly from the scale for upright 1 and 2.

9.1.2 Comparison of the Standard Deviation of the Extracted Features

As the example in figure 9.1 showed, the fluctuations was larger in some areas of the recording compared to others. Box plots of SDNN and the standard deviation of the extracted features can be seen in figure 9.3. The p-value in the top comes from the Friedman test. The result of the post hoc test is also shown; groups marked with asterisk differs significantly from the ones to which a line is drawn.



Figure 9.3: Box plots of (a) SDNN, (b) the standard deviation of the slope, and (c) the standard deviation of the scale for dataset A. The p-value obtained with the Friedman test is stated above the plots. p < 0.05 indicates that at least one of the groups (supine, upright 1, upright 2, or recovery) differ from the remaining. A group marked with asterisk differs significantly from the ones to which a line is drawn.</p>

SDNN decreased from suine to the upright periods and increased again from upright to recovery. The SDNN for the two upright periods differed significantly from recovery, SDNN for supine did not differ from any of the other groups.

The standard deviation of both slope and scale showed the opposite tendency of SDNN, i.e. the standard deviation of the slope and scale increased when the subjects werw tilted from supine to upright and decreased again when they were tilted back into supine position for recovery. The standard deviation of the slope for the supine position differed significantly from the remaining groups. The slopes for upright 1 and 2 also differed significantly from recovery. The standard deviation of the scale in supine differed significantly from the standard deviation of the scale in the two upright periods.

9.2 Results for Dataset B

Dataset B consisted of 18 subjects diagnosed with CRPS and with sympathetically maintained pain. These subjects were subjected to a tilt table test identical to the one conducted on the healthy subjects in dataset A. The result from a subject with CRPS is seen in figure 9.4.



Figure 9.4: Result from a subject from dataset B. The coloured segments represents the four segments which were analysed. The upper panel shows the subject's tachogram. The second panel shows the steepest ascending slope as a function of the time of occurence. The third, fourth and fifth panel shows the scale where maximum correlation was found, the correlation matching the scale, and the time lag where the maximum correlation was found respectively, all as a function of the time of the QRS complex's occurence.

The results from the subject in figure 9.4 showed a decrease in the NN interval when the subject was tilted to upright position from supine and an increase in the NN interval when the subject were tilted back into supine position for recovery. Compared to the healthy subject in figure 9.1, the NN interval was shorter for the CRPS patient in the supine position. The slope increased in the upright positions compared to supine and recovery. Compared to the healthy subject in figure 9.1, the CRPS patient's slope in supine was larger than the healthy subject's supine slope. The scale decreased when the CRPS patient's position was changed from supine to upright and increased again when the patient was returned to supine position for recovery. The time lag where maximum correlation was seen varied from 80 to 86 in the areas of interest.

Like dataset A, there was a single recording in dataset B where two of the scale measures within the areas of interest were attributed to a time lag more than 60 ms away from the fiducial point of the QRS complex. The fiducial point for each QRS complex was located at the time 75 ms. Also here, the overall effect of two outliers was considered negligible. In general, the time lags, where maximum correlation was found, were in average within a range of 6.4 ms for the recordings and the largest range within a single recording was 12 ms. The smallest time lag, which resulted in maximum correlation between the scaled wavelet and a QRS complex, was 61 ms and the largest time lag was 93 ms.

9.2.1 Comparison of the Mean Level of the Extracted Features

As the example in figure 9.4 also showed, a change in the NN interval and scale was seen when the subjects in dataset B were tilted from supine to upright an again when they were tilted back into supine position for recovery. Figure 9.5 shows a box plot of the average NN interval, slope steepness, and scale. The result from the Friedman test is stated above each plot. The result of the post hoc test is also shown; groups marked with asterisk differs significantly from the ones to which a line is drawn.



Figure 9.5: Box plots of (a) the average NN interval, (b) the ascending slope, and (c) the scale for dataset B. The p-value obtained with the Friedman test is stated above the plots. p < 0.05 indicates that at least one of the groups (supine, upright 1, upright 2, or recovery) differ from the remaining. A group marked with asterisk differs significantly from the ones to which a line is drawn.

The NN interval decreased from the supine position to the two upright positions and increased again from the upright periods to recovery. All groups except supine and recovery differed significantly.

The slope did not differ significantly between any of the positions. In contrast, the scale for the supine and recovery differed significantly from the scale during upright position.

9.2.2 Comparison of the Standard Deviation of the Extracted Features

Just as for dataset A, the variations in the different positions were compared. Figure 9.6 shows box plots of the results. The p-value of the Friedman test is stated above each plot. The results of the post hoc tests are also illustrated; a group marked with asterisk differs significantly from the groups to which a line is drawn.



Figure 9.6: Box plots of (a) SDNN, (b) the standard deviation of the slope, and (c) the standard deviation of the scale for dataset B. The p-value obtained with the Friedman test is stated above the plots. p < 0.05 indicates that at least one of the groups (supine, upright 1, upright 2, or recovery) differ from the remaining. A group marked with asterisk differs significantly from the ones to which a line is drawn.</p>

As the box plots show, SDNN decreases from supine to upright 1 and 2 thereafter it is increased again to recovery. SDNN in supine position differed significantly from SDNN in upright 2 and recovery. Upright 1 differed significantly from recovery. In contrast, the standard deviation of the scale did not differ between any of the groups.

9.3 Results for Dataset C

As mentioned, dataset C consisted of recordings from 11 healthy subjects before and during capsaicin induced pain. The recordings obtained prior to pain were denoted rest and the recordings obtained during pain were denoted pain.

As mentioned, the detection of fiducial points were affected by the technical problems with the amplification prior to A/D conversion. This should be considered when evaluating the results. A decrease in NN interval and a fall in SDNN during pain compared to rest was expected. The slope was expected to increase during pain and the scale was expected to decrease during pain.





Figure 9.7: Result from a subject in dataset C. The panels to the left shows the extracted values of the recording obtained during rest and the panels to the right shows the extracted values for the recording obtained during pain. The upper panels show the NN interval. The second panels show the scale where maximum correlation was found. The third and fourth panels show the correlation matching the scale, and the time lag where the maximum correlation was found respectively, all as a function of the time of the QRS complex's occurrence.

When induced with pain, the results from the subject in figure 9.7, showed a decrease in the NN interval and the scale. The slope values fluctuated more during pain compared to before pain. The correlation between the wavelet and the

QRS complexes decreased in pain compared to before. The time lags where the maximum correlation was found were all in the range 68.1 ms to 75.9 ms

None of the time lags found in dataset C were considered as outliers. The fiducial point for each QRS complex was located at the time 75 ms. The time lags, where maximum correlation was found, were in average within a range of 7.7 ms for a single recording and the largest range within a single recording was 12.3 ms. The smallest time lag which resulted in maximum correlation between the scaled wavelet and a QRS complex was at 57.6 ms and the largest time lag was 89.4 ms.

9.3.1 Comparison of the NN Interval and the Scale

Figure 9.8 shows box plots of the NN interval, slope, and scale during rest and pain. The p-value stated above is the result of the Wilcoxon signed rank test.



Figure 9.8: Box plots of (a) the average NN interval, (b) the slope, and (c) the scale for dataset C. The p-value obtained with the Wilcoxon signed rank test is stated above the plots. p < 0.05 indicates that the groups differ significantly.

As expected, both NN interval and scale decreased from the resting period to the period with induced pain. The difference was significant for both NN interval and scale. The slope did not change significantly from the rest period to the pain period.

9.3.2 Comparison of SDNN and the Standard Deviation of the Scale

SDNN and the standard deviation of the slope and the scale were also compared. Figure 9.9 shows box plots for SDNN, the standard deviation of the slope, and the standard deviation of the scales.



Figure 9.9: Box plots of (a) SDNN, (b) the standard deviation of the slopes, and (c) the standard deviation of the scale for dataset C. The p-value obtained with the Wilcoxon signed rank test is stated above the plots. p < 0.05 indicates that the groups differ significantly.

The comparisons of SDNN, the standard deviation of the slopes, and the standard deviation of the scales all showed a significant difference from the recordings during rest to the recordings during pain. The change in SDNN was opposite of what was expected, i.e. SDNN increased during pain. The standard deviation of the slope and the scale increased during pain.

Discussion

10

Analysis of variations in NN interval (also so called HRV) have been used as quantitative markers of autonomic modulation of cardiac rhythm. These markers have a common limitation, which is the dependency of accurate detection of a fiducial point for each heart beat, since inaccurate detection will lead to distortion of the results. Furthermore, the different measures of HRV is sensitive to ectopic beats and missing beats, which needs to be handled prior to HRV analysis. Regardless of how the ectopic and missing beats are handled, it will impose an error on the analysis [Mateo & Laguna 2003, Acar et al. 2000]. Thus, HRV analysis is sensitive to exact location of fiducial points and missing and ectopic beats. In addition, different frequency measures used to express HRV requires a resampling of the heart rate signal prior to the analysis, which demands a reliable heart rate signal. Therefore, markers independent on exact location of fiducial points, ectopic and missing beats are desirable. The aim of this project was to investigate if the autonomic cardiac regulation is reflected in the morphology of individual QRS complexes.

In a study, Parilak et al. [2009] showed that action potential duration decreased and action potential amplitude increased when the heart rate increased. Spray & Burt [1990] stated that gap junctional conductance are increased by cAMP, which is a second messenger in adrenergic stimulation of the heart. This was assumed to cause a narrowing of the QRS complex, due to increased conduction velocity, and an increase in the amplitude of the QRS complex during sympathetic stimulation and the opposit effect was expected during parasympathetic stimulation.

10.1 Methodology

Surface ECG recordings, obtained using Einthoven's lead II configuration, from three datasets were analysed in this project; Dataset A and B, consisting of 20 healthy subjects and 18 patients suffering from CRPS I, going through a tilt table test, and dataset C, consisting of 11 healthy subjects before and during capsaicin induced pain. Dataset A and B were acquired by Astrid Terkelsen at the Danish Pain Research Center, Aarhus University Hospital, whereas dataset C was obtained by us.

The frequency content of surface ECGs ranges from 0.01 to 250 Hz [Webster 1998]. Dataset A and B were analog band pass filtered from 0.08 to 150 Hz and sampled at 1 kHz. Since the band pass filter filtered away some of the power at frequencies expected to be related to the ECG it is unknown how much the filtering affected the morphology of the QRS complex. In general, filtering of frequencies within the area of interest will always remove parts of the signal that are desirable to preserve. When it is the morphological changes that are important, and filtering should be kept to a minimum.

The recordings in dataset C were high pass filtered with a cut-off frequency on 0.03 Hz and recorded with a sampling frequency of 10 kHz. This was done to ensure a high time resolution of the morphology of the QRS complex. Due to technical problems with the amplification of the signal prior to the A/D conversion, some of the recordings were saturated, resulting in a cut-off of the tip of the R peak. This complicated the exact detection of fiducial points for the saturated QRS complexes. It was chosen to use the center of the area where the R peak saturated as fiducial point in order to standardise the error it imposed on the heart rate signal. Other ways to locate a fiducial point could include filtering or estimation of the intersection point between the two sides of the R peak. No matter which method was used, it would impose an error on the heart rate signal.

Two features were extracted from each QRS complex. The first feature was the steepest ascending slope of the QRS complex. This feature is simple to extract, but at the same time sensitive to noise peaks. The second feature was more complex but at the same time less sensitive to noise. A contineous wavelet transform of each QRS complex was made with an adapted wavelet. An average QRS complex from a baseline session was used as template for the adapted wavelet for each person. The slope is completely independent of an exact location of the fiducial point. The

second feature is partly independent, because the average QRS complex, utilised to generate the mother wavelet, is dependent on the location of fiducial points. The actual extraction of the feature only rely on how well the adapted wavelet resembles each QRS complex, and not on the location of the individual QRS complex's fiducial point. This independency of exact location of the fiducial point is desirable together with the fact that ectopic beats can be ignored without affecting the outcome of the measures.

The simplicity of the slope feature makes it sensitive to noise and it is a measure of how the QRS complex behaves in a single point of the entire complex, but in contrast to the scale it is capable of expressing the sum of the morphological changes of the width and amplitude. The scale is less sensitive to noise and reflects the shape of the entire QRS complex and not just a single point, but the scale resulting in the highest correlation is a result of a trade off between the width and the amplitude of the QRS complex. It is unknown how well the amplitude correction $(1/\sqrt{s})$ of the wavelet amplitude at a given scale correlates to the amplitude changes of the QRS complex at different widths. Optimally, the extracted feature should represent the entire shape of the QRS complex, and at the same time avoid the trade off between width and amplitude.

The extracted features were compared to the mean NN interval and SDNN, which is a measure of overall variance. It can be questioned which HRV measure is the most appropriate to compare with. SDNN was chosen because it is one of the most frequently used HRV measures and it is simple to calculate [Acharya et al. 2006]. SDNN is considered to reflect both the sympathetic and the parasympathetic influence on HRV [Bigger et al. 1992, TaskForce 1996]. Other measures of HRV which either reflect sympathetic or parasympathetic influence on HRV could have been used. One of these measures could for example be the square root of the mean squared differences of successive RR intervals (RMSSD), which is a measure of HRV that is considered to be a reliable index of parasympathetic activity. Additionally, frequency domain analysis could also be used as a measure of HRV [Stein et al. 1994].

10.2 Autonomic Modulation of the QRS Complex

Healthy subjects were expected to show a decrease in NN interval and SDNN when their sympathovagal balance shifted from parasympathetic dominance towards sympathetic dominance [Malik 1998]. Based on the results from Parilak et al. [2009] and Spray & Burt [1990], the slopes of the QRS complexes were expected to increase, and the scales were expected to decrease, when the sympathovagal balance shifted towards sympathetic predominance and the opposit effect was expected when the sympathovagal balance was shiftet towards parasympathetic predominance.

Dataset A: Healthy Subjects

As expected, the healthy subjects (dataset A) showed an increase in the steepness of the slope, from the supine period to upright 1, and again from upright 1 to upright 2. Thus, a gradual increase was observed from supine to upright 1 and to upright 2. The gradual increase could be explained by the fact that sympathetic innervation of the ventricles is plentiful compared to the parasympathetic innervation, which is sparse [Wei-Jin et al. 2005]. Therefore, the parasympathetic withdrawal in the upright period could be assumed not to have the same pronounced effect on the ventricles. This means that the QRS complex increases gradually in amplitude and/or gradually gets narrower. The average steepness of the slope in recovery did not decrease to the average steepness in supine position, this could possibly be explained by circulating cathecholamins released to the blood stream from the adrenal medullae during the upright periods. According to Guyton & Hall [2006], the removal of cathecholamins from the blood stream takes 2 to 4 minutes.

Also as expected, the scale of the adapted wavelet decreased significantly from the supine position to the two upright positions and increased significantly from the upright positions to recovery. In contrast to the former observation, the scale did not reveal the same gradual change as the slope did. It is unknown what underlies this discrepancy. In order to be in agreement with the results of the slope it could be explained by an abrupt increase in conduction velocity and thereby narrowing of the QRS complex and a more gradual increase of the amplitude over time.

The standard deviation of the slope and the standard deviation of the scale exhibited the opposite behavior of SDNN, i.e. the standard deviation of the slope and the scale increased in the periods where sympathetic dominance were expected. It is speculated if this can be explained by the parasympathetic innervation of the ventricles which is sparse compared to the sympathetic innervation [Wei-Jin et al. 2005]. This would mean that a low sympathetic modulation of the heart is reflected as a low variation in slope and scale compared to situations with higher sympathetic modulation.

Dataset B: Complex Regional Pain Syndrome Patients

The subjects in dataset B had all been diagnosed with CRPS and suffered from sympathethically maintained pain. CRPS patients are thought to have a sympathetic overdrive. A study by Harden et al. [2004] showed that persons suffering from CRPS with sympathetically maintained pain have a significantly higher concentration of plasma norepinephrine and to a lesser extend epinephrine compared to healthy control subjects. Circulating catecholamins have similar effect to direct sympathetic stimulation, and could therefore be assumed to cause a narrowing and an increase in amplitude of the QRS complex.

Like the healthy subjects, the CRPS patients NN interval and scale decreased as expected when they were tilted from supine to upright. But in contrast to the healthy subjects, the slope did not reveal any differences between the different postures. The standard deviation of the slope increased when they were tilted from supine to upright and decreased when they were tilted back into supine position. The scale showed the exact opposite outcome, i.e. no difference in the scale during the postural changes, but a significant increase in the standard deviation of the scale when they were tilted to upright posture.

Unfortunately, CRPS is far from fully understood. To us, it is unknown how the long term effect of the high level of circulating catecholamins and sympathetic hyperactivity found in CRPS patients affect the heart. More knowledge about CRPS might explain the observed behaviour of the slope and scale measures. The choice of subjects with CRPS could be reconsidered for feature validation, because of the lack of information regarding CRPS. Other diseases where a change in the HRV has been indicated can also be used. This could be patients with congestive heart failure or patients who have had a myocardial infact [TaskForce 1996].

Dataset C: Healthy Subjects with Induced Pain

As expected, a significant reduction in both NN interval and scale were seen from the recordings during rest to the recordings during pain. Opposite to what was expected, an increase in SDNN occured when the subjects were induced with pain [Terkelsen et al. 2005, Appelhans & Luecken 2008]. Unfortunately, SDNN can be affected by the performed interpolation of fiducial points. Furthermore, the short duration (2 min) might also influence the outcome.

The slope of the QRS complex did not differ significantly between the recording made during rest compared to the recording made during pain, but the scale did. The standard deviation of the slope and scale increased significantly during pain compared to before pain. This is in agreement with what was observed for the healthy subjects in dataset A.

Observations across Datasets

In general, the expected morphological changes were observed in healthy subjects. When SDNN was compared to the standard deviation of the ascending slope and the scale an opposite effect was seen, i.e. when SDNN increased the standard deviation of the other measures decreased and vice versa. Previous findings regarding HRV suggests that parasympathetic regulation happens more rapidly than changes mediated by the sympathetic division, and therefore causes more variation of the heart rate during parasympathetic dominance compared to sympathetic dominance. It can be speculated what causes this opposite effect. Maybe it can be explained by the fact that sympathetic innervation of the ventricles is rich, whereas the parasympathetic innervation is sparse [Wei-Jin et al. 2005]. This would mean that the morphological changes of the QRS complex is mainly a marker of the sympathetic modulation of the heart and not so much of the parasympathetic modulation.

The observations in the CRPS patients were more difficult to interpret, both for SDNN and the extracted features. Both the CRPS patients and the healthy subjects subjected to capsaicin induced pain did not show a significant difference between the slopes in the different situations. In general, the healthy subjects had a relative high resting pulse, this could be due to anxiety which causes an increased sympathetic modulation an increased plasma catecholamin concentration. It is known that CRPS patients have a constant elevated plasma catecholamin concentration [Harden et al. 2004]. This could account for the similarity between the CRPS patients and the healthy subjects who were induced with pain. Unfortunately, no measures of anxiety were obtained.

In general, morphological changes of the QRS complex is a novel approach to quantifying the autonomic modulation of the heart rate. Therefore, further investigation of the physiological correlates of the findings are necessary to reveal the potential of morphological changes as a marker of autonomic modulation of the heart rate.

Conclusion

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Through a litterature study it was shown that the ANS regulates factors which affects the appearance of the QRS complex. Sympathetic stimulation was shown to increase the amplitude of action potentials and increase the velocity of impulse propagation in the myocardium. Parasympathetic stimulation was shown to have opposite effects. It was hypothesised that these effects are reflected in the morphology of the ECG as a decrease in width and an increase in amplitude of the QRS complex during dominating sympathetic regulation of the heart and vice versa during parasympathetic regulation.

It was demonstrated that the steepest ascending slope of the QRS complex increased along with a decrease in NN interval, during periods with parasympathetic withdrawal and sympathetic dominance and vice versa. A contineous wavelet transform of the QRS complexes using an adapted mother wavelet also demonstrated an increase in amplitude and a narrowing of the QRS complex during parasympathetic withdrawal and sympathetic dominance and vice versa.

In contrast to SDNN, the standard deviation of the changes in the steepest ascending and descending slopes and standard deviation of the changes illustrated through the contineous wavelet transform were increased during sympathetic dominance compared to during parasympathetic dominance.

In conclusion, these findings support the hypothesis that autonomic modulation of the heart is reflected in the morphology of the QRS complex. Further investigation is required to fully understand the physiological correlates of the findings.

The Heart



A.1 The Heart and the Blood Circulation

The human heart is muscular organ that consists of two pumps; a right and a left pump. Each of these constantly pumps the blood through vessels to all the tissues in the body. The right pump propels the blood to the lungs through the pulmonary circuit and the left pump propels it to the rest of the body through the systemic circuit of the body [Guyton & Hall 2006]. An illustration of the heart and the direction of blood circulation in the heart is shown on figure A.1.



Figure A.1: Anatomical structure of the heart and the direction of the circulating blood [Guyton & Hall 2006].

The two pumps of the heart consists of two chambers each; an atrium and a ventricle. The blood that comes from the systemic circuit arrives in the right atrium of the heart and pumps the blood into the right ventricle. The right ventricle pumps the blood into the pulmonary circuit. The blood that comes from the pulmonary circuit enters the left atrium which propel the blood into the left ventricle which pumps it into the systemic circuit. The tricuspid and the mitral valve are one-way valves which separate the atria from the ventricles. The cardiac cycle consists of two phases, the relaxation phase, in which the heart is filled with blood and a contraction phase, in which the blood is ejected into circulation; these two phases are termed diastole and systole respectively [Guyton & Hall 2006, Martini 2004].

A.2 The Cardiac Muscle

The pumping of blood into the pulmonary and the systemic circulations is accomplished by systematic contraction and relaxation of the cardiac muscle. The cardiac muscle is striated and multinucleate and makes up the wall of the heart. The wall of the heart is made of three distinct layers; the endocardium, the myocardium, and the epicardium. The atrial and the ventricular types of muscle contract in the same way as skeletal muscle, but the duration of the contraction can

be up to 15 times longer due to longer action potentials. The atrial muscle and the ventricular muscle are separated by the electrically nonconductive fibrous tissue that surrounds the atrioventricular valvular openings between the atria and the ventricles. This fibrous tissue is termed the base of the heart [Martini 2004, Guyton & Hall 2006]. Figure A.2 shows the branched interconnection structure between the cardiac muscle cells.



Figure A.2: Organisation of cardiac muscle fibers [Boron & Boulpaep 2005].

The cardiac muscle cells are connected by specialised structures known as intercalated discs. Figure A.2 shows that at the intercalated disc, the cell membranes of two adjacent cardiac muscle cells are tangled and bound together by two types of membrane junctions; desmosomes and gab junctions. The desmosome is a type of adhering junction that mechanically holds the cardiac muscle cells and helps to stabilise the relative positions of adjacent cells during contraction. A gap junction is an electrical synapse that allows almost free diffusion of ions to flow between adjacent cells. Because of the low resistance in the gap junctions the ions move with ease in the intracellular fluid along the longitudinal axes of the cardiac muscle fibers, as a result action potential spreads easily from one cardiac muscle cells becomes excited, the action potential spreads from cell to cell throughout the branched interconnections and causes a synchronised muscle contraction of the entire muscle [Martini 2004, Guyton & Hall 2006].

A.3 Gap Junctions

Gap junctions are channels found in the intercalated discs, that separates adjacent cardiac muscle fibres. Gap junctions are fusions between the two cell's cell membranes which allow transport of ions and small molecules between the cells [Guyton & Hall 2006]. The conduction of electrical signals from the SA node to the myocardium, which generates a coordinated contraction of the heart, is highly dependent on gap junctions [Kwak & Jongsma 1996]. Cells connected by gap junctions form a close electric and metabolic unit, also called a syncytium. Thus, the heart is a large syncytium, and the excitation of a single cell spreads to the rest of the cardiac myocardium via gap junctions [Guyton & Hall 2006]. Through their coupling of the myocardial cells, gap junctions are responsible for the biophysical properties of the cardiac tissue [Dhein 1998*a*].

A gap junction is made up by two connexons which fuses to create a common channel between two cells. Each connexon is made of six connexins. A connexin is a polypeptide which has four transmembrane domains, two extracellular, and one intracellular loop. The ends of the polypeptide are located on the intracellular side. The two extracellular loops connect the connexons. Thus, the extracellular loops are responsible for the forming of the complete gap junctional channel [Dhein 1998*a*]. On figure A.3 a single connexin and two fused connexons are illustrated.



Figure A.3: Illustration of a connexin and its four transmembrane domains and intra- and extracellular loops. Six connexins form a connexon and two connexons from adjacent cells form a gap junction. Edited from Despopoulos and Silbernagl [2003].

The amino acid sequence of the connexins vary and it is known that at least 20 different isoforms exists in the human genome [Zipes & Jalife 2004]. The four transmembrane domains and two extracellular loops are highly homologous, whereas the intracellular part displays a higher diversity among the isoforms [Kwak & Jongsma 1996].

The connexins are named after their molecular weight e.g. connexin43 also denoted Cx43 weights 43 kD. The isoforms found in mammalian cardiovascular tissue are Cx37, Cx40, Cx43, Cx45, and Cx46. However, Cx40, Cx43, and low levels of Cx45 is predominant in the human heart [Dhein 1998*a*].

A.3.1 Distribution of Cardiac Gap Junctions

Type and distribution of gap junctions in the human heart varies dependent on location. In the SA node, the centre should be distinguished from the periphery. The centre zone does not contain gap junctions whereas the periphery does [Dhein 1998*a*]. In figure A.4 the distribution of cardiac gap junctions is illustrated.



Figure A.4: The distribution of different gap junctions in the cardiovascular structures. Cx43 is the most frequently occurring type [Dhein 1998b]

The ratio between the occurrences of the different connexins can be altered by the course of cardiac disease. Cx43 is the type found most abundantly [Dhein 1998*a*].

A.3.2 Regulation of Cardiac Gap Junctions

Opening and closing of the channels is possible because an α -helix, in the transmembrane domains of the connexins, is inclined with respect to the entire connexin. This makes a twisting motion which closes channel possible [Dhein 1998*a*]. The opening and closing of a gap junction is illustrated in figure A.5.



Figure A.5: A twisting of the connexins open and closes the gap junctional channel. Edited from Despopoulos and Silbernagl [2003].

The physiological opening and closing of gap junctions is dependent on calcium, sodium, magnesium, cAMP, ATP, pH, temperature, leukotrienes, cathecholamines, and acetylcholine. The effect of the different substances and conditions on the gap junctions conductance is different from tissue to tissue and from species to species [Dhein 1998*a*].

A large increase in intracellular Ca^{2+} causes an reduction in conductance of Cx43. Under normal conditions the Ca^{2+} concentration is not high enough to cause a reduction in conductivity. The same is applies to Mg²⁺ [Dhein 1998*a*]. A rise in intracellular Na⁺ has been reported to lead to a reduction in conductivity [Dhein 1998*b*].

An increase in intracellular cAMP leads to increased coupling between adjacent cells [Dhein 1998b].

ATP plays an important role in the physiological intracellular regulation of the gap junctional conductance. A reduction of the intracellular ATP concentration causes a rapid decline in conductance [Dhein 1998*b*].

Physical conditions such as pH and temperature also plays a role in the regulation of gap junctions. A reduction in pH leads to uncoupling, so does a decrease in temperature [Dhein 1998*b*].

Leukotrines are natural substances, responsible for inflammatory responses. In relation to gap junctions, their presence causes a decrease in the coupling between cells [Dhein 1998b].

Cathecholamines and acetylcholin, e.g. released by the sympathetic nervous system, causes an activation of protein kinases within the cell. Cathecholamines activates protein kinase A (PKA) and protein kinase C (PKC). PKA enhances the conductance of Cx43. The change is very rapid and lasts for several minutes. PKC is activated by diacylglycerol (DAG) which is accompanied by inositol triphosphate (IP₃) release. IP₃ mediates a transient rise in intracellular Ca²⁺ levels while PKC phosphorylates specific amino acid residues at specific sites in the connexins. The phosphorylation and the rise in intracellular Ca²⁺ both affect the gap junction function. PKC increases the permeability and electrical conductivity of Cx43 [Dhein 1998*a*]. Acetylcholin, which is a parasympathetic transmitter substance, activates protein kinase G (PKG) which in turn reduces the electrical coupling through gap junctions [Kwak & Jongsma 1996].

A.4 The Cardiac Electrical System

Two types of muscle cells are involved in a normal heart beat; specialised cardiac muscle cells of the conducting system, which generate and conduct electrical current, and cardiac contractile cells, which respond to the electrical current and produce the contraction that propels blood [Guyton & Hall 2006, Despopoulos & Silbernagl 2003]. The hearts conducting system can be seen on figure A.6.



Figure A.6: The conducting system of the heart [Guyton & Hall 2006].

The conduction system consists of the following elements [Martini 2004, Despopoulos & Silbernagl 2003]:

- The sinoatrial node (SA node), located in the posterior wall of the right atrium, near the entrance of the superior vena cava.
- The atrioventricular node (AV node), located on the interatrial septum.

- The anterior, medial and posterior internodal pathways which conduct impulses from the SA node to the AV node.
- The AV bundle (bundle of His), the bundle branches, and the Purkinje fibers, which spread the stimulus to the ventricular myocardium.

A heartbeat begins with a spontaneous generation of an action potential in the SA node. The cells that generate the action potential are called pacemaker cells. Each action potential generated in the SA node normally prompts a heartbeat, meaning that the heart rate is determined by the rate of generation of action potentials in the SA node. Thus the control and coordination of the heart rate is guarded in the SA node and for that reason the SA node is also called the natural pacemaker of the heart. The intrinsic rate impulse generation in the SA node is between 60-100 beats per minute. The AV node and the inferior structures produce a spontaneous action potential if they are not excited by other electrical activity. Their intrinsic rate is between 40-55 and 25-40 beats per minute respectively under normal conditions [Despopoulos & Silbernagl 2003]. The cells in the AV node and the inferior structures, i.e. the AV bundle, bundle branches, and purkinje fibres, are unable to assume their own natural slower rates, because they are triggered by action potentials originating in the SA node before they are able to reach threshold at their own slower rhythm. The action potentials is distributed rapidly through both atria by the internodal pathways toward the AV node. The action potential passes the stimulus to the contractile cells of both atria, which in turn contract. The ventricular myocardium is not affected by the stimulus since it is isolated from the atrial myocardium at the base of the heart. The propagation of the stimulus across the atria until it reaches the AV node takes 30-50 ms. In the AV node the impulse is delayed for about 90-100 ms. This delay causes the atria to contract before the ventricles. After the AV node the signal travels along the interventricular septum within the AV bundle and the bundle branches to the Purkinje fibers, which distribute the impulse to the ventricular myocardium. At this point the atrial contraction is completed, and the ventricular contraction is initiated. The propagation of the stimulus across the bundle branches to the Purkinje fibers takes about 30-75 ms [Guyton & Hall 2006, Martini 2004, Sherwood 2004]. The velocity of the impulse conduction is regulated by efferent nerves which modulates the activity of the heart dependent on the needs [Despopoulos & Silbernagl 2003].

A.4.1 The Action Potentials in Cardiac Muscle

As written previously the pacemaker cells in the conduction systems generate and control the heartbeat and the cardiac contractile cells produce contractions that propel the blood. The cardiac contractile cells contracts when stimulated, while the cardiac pacemaker cells contract without any external stimulation. As seen in figure A.7 that the action potentials that occur in these two types of cells are a bit different [Despopoulos & Silbernagl 2003, Martini 2004, Sherwood 2004].



Figure A.7: Illustration of action potential in (a) pacemaker cells and (b) cardiac contractile cells. P stands for ion permeability through the cell membrane. Edited from Sherwood [2004].

In pacemaker cells the membrane potential slowly depolarises (pacemaker potential) until threshold is reached and an action potential is initiated. After repolarising, the membrane potential once again depolarises to threshold, cyclically

continuing in this manner to self generate action potentials, which are spread throughout the heart to make it beat rhythmically without any nervous stimulation [Sherwood 2004].

Figure A.7a shows the action potential in a pacemaker cell. The action potential contains three main phases; called phase 4, 0, and 3 [Sherwood 2004]:

- **Phase 4 Pacemaker potential** The pacemaker cells do not have a constant resting potential, the most negative value the membrane potential reach is about -70 mV. This is due to a continuous outflow of potassium ions (K⁺). The most important changes in ion movement that give rise to the pacemaker potential are; a decreased potassium permeability, slow influx flow of sodium ions (Na⁺) and an increased influx of calcium ions (Ca²⁺). The K⁺-channels slowly close at negative potentials, this slowly closure gradually reduces the outflow of positive potassium ions. The pacemaker cells do not have voltage gated Na⁺-channels. Instead they have channels that are always open and thus permeable to Na⁺ at negative potentials. The pacemaker cells have two types of voltage-gated Ca²⁺-channels. One type is transient voltage-gated Ca²⁺-channels that opens during the pacemaker potential and give rise to an increased influx of Ca²⁺ shortly. The slow depolarisation continues until the threshold potential is reached at about -40 mV.
- Phase 0 Depolarisation When the threshold potential is reached, the rising phase of the action potential occurs in response to activation of the second type of the voltage-gated Ca²⁺-channels which is the longer-lasting Ca²⁺channels that give rise to larger influx of calcium ions.
- **Phase 3 Repolarisation** The voltage-gated K^+ -channels are activated and the potassium permeability increases and causes efflux of K^+ from the cell. During this phase the Ca²⁺-channels are rapidly inactivated and the permeability of sodium is decreased. When the action potential is over, slow closure of the K^+ channels initiate the next pacemaker potential.

The action potential in the cardiac contractile cells, is initiated by the cardiac pacemaker cells, but varies in ionic mechanisms and shape, from the action potential of cardiac pacemaker cells as seen in figure A.7b. The action potential of a contractile cell constitutes of four phases [Sherwood 2004, Martini 2004, Guyton & Hall 2006]:

- **Phase 4 Resting membrane potential** In contrast to the cardiac pacemaker cells, the membrane of the cardiac contractile cells remains at rest at about -90 mV until it is stimulated by electrical activity. This phase of the action potential is related with the diastole of the ventricles of the heart.
- **Phase 0 Rapid depolarisation** When the threshold potential at approximately -70 mV is reached, voltage-gated Na⁺-channels are activated and the sodium permeability increases. This results in a large influx of sodium ions which changes the membrane potential rapidly from -70 mV to a positive value of 30 mV. The duration of this phase is between 3 and 5 ms.
- **Phase 1 and 2 The plateau** When the action potential reaches the value of 30 mV, the sodium permeability begins to decrease and the calcium permeability begins to increase. The longer-lasting Ca²⁺-channels are activated and a large influx of Ca²⁺ and a decrease in potassium permeability prolong the positivity inside the cell and cause the plateau. The duration of the plateau is approximately 175 ms, but it is dependent on the permeability which in turn is dependent on the heart rate.
- **Phase 3 Repolarisation** The longer-lasting Ca^{2+} channels close, while the K⁺ channels are activated. The closure of the Ca^{2+} channels and the efflux of potassium ions makes the inside of the cell more negative than the outside and restore the resting membrane potential. The duration of the repolarisation phase is approximately 75 ms.

After an action potential is initiated, the cardiac contractile cell is unable to respond to another stimulus for 250 ms. This period is termed the refractory period. Because of the long duration of the refractory period tetanic contractions cannot occur in a normal cardiac muscle. This feature is vital, because a heart in tetany can not pump blood [Martini 2004].

A.5 The Electrocardiogram

The electrocardiogram (ECG) is a recording of the electrical activity of the heart; it is a recording of fluctuations in extracellular voltage created by the movement of action potentials through the cardiac myocytes. The fluctuations vary from fractions of a millivolt to several millivolts. The voltage fluctuations appear as waves on the ECG. A normal ECG is composed of five waves; P, Q, R, S, and T wave. A normal ECG is displayed on figure A.8 [Boron & Boulpaep 2005].



Figure A.8: A normal ECG recording. The components of the ECG is shown [Boron & Boulpaep 2005].

Each wave is related to a specific event in the cardiac cycle [Martini 2004, Guyton & Hall 2006, Sand et al. 2004, Fuster et al. 2004]:

- **The P wave** is caused by the depolarisation of the right and left atrial muscle, which takes place immediately after the SA node has generated an impulse. The actual contraction of the atria begins after approximately 100 ms after the start of the P wave.
- **The Q wave** represents the depolarisation of the septum. The left part of the septum is depolarised before the right side, this is attributed to the fact that the left bundle branch is shorter than the right bundle branch. This results in the negativity of the Q wave.
- **The R wave** is caused by the propagation of the depolarisation wave in the septum towards the apex and the following depolarisation of the outer walls from the apex to the base of the heart. The depolarisation of the outer walls starts at the endocardium and spreads outward to the epicardium.

The S wave represents the depolarisation of the posterobasal region of the left ventricle.

The Q, R, and S waves constitute the QRS complex, which effectively represents the depolarisation of the ventricles. The entire QRS complex lasts from 0.06-0.12 s and is heart rate dependent. The ventricles start contracting shortly after the peak of the R wave [Martini 2004].

The repolarisation of the atria takes place about 0.15-0.2 s after the termination of the P wave. This corresponds

to the time where the QRS complex occurs. Therefore, the QRS complex coincides with the atrial repolarisation. The QRS complex is a relative strong electrical signal because the ventricular muscle is much more massive than the atrial muscle [Guyton & Hall 2006, Martini 2004]. The atrial repolarisation wave also called T_a is directly opposite in polarity to the P wave [Fuster et al. 2004].

The T wave represents the repolarisation of the ventricular muscles. The repolarisation of some ventricular muscle fibres starts about 0.20 s after the beginning of the depolarisation (the QRS complex) while other fibres take as long as 0.35 s. Thus, the ventricular repolarisation spreads over a long period, about 0.15 s. The prolonged duration partly causes the voltage to be considerably less than the QRS complex's. The positivity of the T wave is due to the propagation of the repolarisation wave that goes from the epicardium towards the endocardium and septum. This sequence of repolarization is thought to be caused due the high blood pressure inside the ventricles during contraction, which reduces coronary blood flow to the endocardium, thereby slowing repolarisation in the endocardial areas and septum.

The time elapsed between the start of the P wave and the start of the QRS complex is called the PR interval. The duration of the PR interval is normally about 0.12-0.20 s. The QT interval lasts from the start of the Q wave to the end of the T wave; ordinarily 0.35 s but it is heart rate dependent [Despopoulos & Silbernagl 2003].

The segment between the P and Q wave (PQ segment) and the segment between the S and T wave (ST segment) lies approximately on the isoelectric line (0 mV). The duration of the ST segment is about 0.35 s [Despopoulos & Silbernagl 2003].

A.5.1 Recording of the ECG

The ECG is typically recorded from the surface of the skin with electrodes. The electrodes can be placed in different configurations, which provide different details about the hearts performance in the different cardiac phases. The generally used configurations are: bipolar and unipolar frontal plane leads and unipolar transversal plane leads.

Frontal Plane Leads

Bipolar configurations continuously record the voltage difference between two surface electrodes. There are three standard bipolar configurations; these are Einthoven's leads I, II and III, which are placed in the frontal plane. Lead I records potentials between the left and right arm, lead II records between the right arm and left leg, and lead III between the left arm and leg [Despopoulos & Silbernagl 2003]. A fourth electrode placed on the right leg is used for electrical grounding [Boron & Boulpaep 2005]. The three bipolar standard leads are shown in figure A.9.

Since the body is a volume conductor, the same result can be achieved by placing the electrodes on the shoulder joints instead of the arms and by convention in the groin instead of the leg [Boron & Boulpaep 2005].

The direction from the negative to the positive electrode is called the lead axis. By convention, the lead axis direction for lead I is defined as 0° , the succeeding leads are named clockwise meaning that lead II is at 60° and lead III is at 120° . For simplicity, imagine it as an equilateral triangle placed in the frontal plane with the electrodes placed on the shoulder joints and the groin [Sand et al. 2004].


Figure A.9: Einthoven's bipolar leads I, II, and III [Boron & Boulpaep 2005].

The electrode placement for the unipolar frontal plane leads is similar to the one for bipolar frontal plane leads. The difference is that two of the electrodes are connected and serves as a reference to the third exploring electrode. This configuration is also called Goldberger leads [Boron & Boulpaep 2005, Despopoulos & Silbernagl 2003]. The configuration of the unipolar frontal plane leads can be seen on figure A.10.



Figure A.10: Goldberger's unipolar leads aVR, aVL, and aVF [Boron & Boulpaep 2005].

The electronic reconstruction of the three limb leads defines an electrical reference point in the middle of the heart and three new lead axes, cf. figure A.11a. The leads are called augmented leads and are named after placement. The aVR lead represents the augmented vector/voltage of the right arm i.e. positive connection to the right arm and negative to the centre of the heart. The aVR lead axis is -150 $^{\circ}$ in the frontal plane. Correspondingly, the aVL lead represents the augmented vector/voltage of the left arm and aVF the augmented vector/voltage of the left arm and aVF the augmented vector/voltage of the left [g (foot)]. The lead axes for aVL and aVF are -30 $^{\circ}$ and 90 $^{\circ}$ respectively [Boron & Boulpaep 2005, Despopoulos & Silbernagl 2003].

Altogether, the negative and positive ends of the six frontal plane leads define axes every 30 $^{\circ}$ in the frontal plan [Boron & Boulpaep 2005]. This is illustrated in figure A.11b.



Figure A.11: The frontal plane leads consisting of; (a) Einthoven's bipolar leads and Godberger's unipolar leads. (b) Canbrera cirlce which represents the six lead axes [Boron & Boulpaep 2005].

The circle on figure A.11b is called the Canbrera circle and represents the six lead axes. Synchronous measurement of two or more of the leads can be used to calculate the integral vector in the frontal plane [Despopoulos & Silbernagl 2003]

Transversal Plane Leads

Another set of unipolar leads has been defined in the transversal plane; Wilsons leads. These are also called precordial leads. The positive connection is one of six electrodes on the chest wall and the negative connection is defined in the middle of the heart by averaging the three aforementioned limb electrodes. The resultant leads are named V₁ through V₆. The placement of the precordial electrodes is illustrated in figure A.12.



Figure A.12: Placement and lead axes for the unipolar transversal plane leads [Boron & Boulpaep 2005].

The placement of the electrodes is as follow [Boron & Boulpaep 2005]:

- V₁: Fourth intercostal space to the right of the sternum.
- V₂: Fourth intercostal space to the left of the sternum.

- V₄: Fifth intercostal space at the midclavicular line.
- V₃: Halfway between V₂ and V₄.
- V₆: Fifth intercostal space at the midaxillary line.
- V₅: Halfway between V₄ and V₆.

When the Wilson leads are used in combination with the frontal plane leads they provide a three-dimensional view of the electrical activity of the heart [Despopoulos & Silbernagl 2003].

A.5.2 Vectorcardiography

When a muscle fibre depolarises, the outside of the membrane turns negative while the inside turns positive as the depolarising wave propagates along the muscle fibre [Sand et al. 2004]. Figure A.13 illustrates the voltage differences occurring on the outside of the membrane of a muscle fibre undergoing depolarising and repolarising.



Figure A.13: Illustration of a muscle fibre undergoing depolarisation and repolarisation. Two electrodes on the surface of the membrane measures the voltage difference between the two electrodes during the depolarization and repolarisation [Guyton & Hall 2006].

No voltage difference is registered when the entire fibre is either depolarised or polarised. When the right (positive) electrode measures a positive voltage compared to the voltage at the left (negative) electrode it results in a positive voltage difference [Sand et al. 2004].

An area, consisting of a partial positive charge and an adjacent partial negative charge, is called a dipole. When a dipole is surrounded by a conductive fluid, like the extracellular fluid, a current will pass through the fluid from the positive to the negative pole. The current consists of positive ions moving towards the negative pole and vice versa.

The current between the two poles follows ellipse shaped trajectories, if the extracellular impedance is homogeneous, and decreases with increasing distance to the source, but it can still be measured at the skin surface. The strength and direction of a dipole can be demonstrated as a vector. The length of the vector must be proportional to the voltage difference between the poles and the direction should be towards the positive pole [Sand et al. 2004]. A small area of the cardiac muscle can be regarded as a dipole, when it is being depolarised or repolarised. Each small area can be depicted as a vector as described above. Each vector contributes to a single large integral vector. Dipoles with opposite directions counteract. During the heart's depolarisation and repolarisation, the strength and direction of the electrical activity measured by that lead is obtained [Sand et al. 2004]. The amplitude of the ECG in a lead is largest when the integral vector is parallel to the lead axis. The amplitude is positive when the integral vector points toward the positive electrode [Sand et al. 2004]. Figure A.14 demonstrates how the integral vector changes during the ventricular repolarisation and how this is reflected in the bipolar limb leads.



Figure A.14: Illustration of the integralvector and its projections on the bipolar limb leads during ventricular repolarisation [Guyton & Hall 2006].

The majority of vectors of the ventricles points toward the apex during ventricular depolarisation. This means that the direction of the electrical potential is from the base of the heart towards the apex. This preponderant direction is called the mean electrical axis of ventricles or the QRS axis. The QRS axis roughly corresponds to the anatomic longitudinal axis of the heart, and normally lies between -30 $^{\circ}$ and +90 $^{\circ}$. Pathological conditions can alter the orientation of the QRS axis [Guyton & Hall 2006, Despopoulos & Silbernagl 2003].

The portion of the nervous system that controls most visceral functions of the body is called the autonomic nervous system (ANS). The ANS is not under the same degree of conscious and voluntary control as the somatic nervous system. The ANS is mainly concerned with homeostatic adjustments. It coordinates cardiovascular, respiratory, digestive, urinary, and reproductive functions. All this is done without instructions from the conscious mind [Martini 2004].

B.1 Organisation of the Autonomic Nervous System

The ANS is primarily activated by centres located in the spinal cord, brain stem, and hypothalamus. In addition, portions of the cerebral cortex, especially the limbic cortex, can affect the lower centres and influence the autonomic tone in this way [Guyton & Hall 2006].

The ANS is divided in two subdivisions; the sympathetic, and the parasympathetic division. The ANS also includes the enteric nervous system (ENS), which is an independent part of the ANS that regulates activity of the digestive tract, whereas the two other subdivisions integrate and coordinate visceral functions throughout the body [Martini 2004]. The sympathetic and parasympathetic division regulates the cardiac activity and therefore the ENS is not described further.

Usually, the effect of the sympathetic and parasympathetic divisions are opposing, i.e., when the sympathetic division causes excitation, the parasympathetic division causes inhibition. The parasympathetic division is dominating in resting conditions whereas the sympathetic division dominates in periods of exertion, stress, and emergency [Martini 2004].

B.1.1 Physiologic Anatomy of the Sympathetic and the Parasympathetic Nervous System

In contrast to the somatic nervous system, the ANS has two neurons between the central nervous system (CNS) and the effector organ; a preganglionic and a postganglionic neuron [Martini 2004]. The cell bodies of the first neurons lie within the CNS and are called preganglionic neurons. The preganglionic neurons synapse on postganglionic neurons, outside the CNS, that project to the target organ [Boron & Boulpaep 2005].

The preganglionic cell bodies of the sympathetic neurons are located in the thoracic and upper lumbar regions of the spinal cord, i.e., in segment T1 to L3, in the intermediolateral horn. The axons of the preganglionic sympathetic neurons exit the spinal cord at the ventral root and passes through the white ramus into one of the ganglia of the sympathetic chain, also called paravertebral ganglia. The paravertebral ganglia lie adjacent to the vertebral column and extend all the way from the upper neck to the coccyx. When the preganglionic neuron enters one of the ganglia three things can happen; it can synapse with postsynaptic neurons in the ganglia it enters, it can travel upward or downward to another ganglion and synapse with postganglionic neurons here, or it can exit the chain ganglia through a sympathetic nerve projecting to a peripheral sympathetic ganglion, also called a prevertebral ganglia [Guyton & Hall 2006, Boron & Boulpaep 2005]. Figure B.1 illustrates the sympathetic nervous system.



Figure B.1: A cross section of the thoracic spinal cord, the nearby paravertebral ganglia, and a prevertebral ganglion. The sympathetic preganglionic neurons originate in the intermediolateral horn and projects via the dorsal root and the white ramus to ganglia in the paravertebral and prevertebral ganglia, where it synapses with postganglionic neurons. Afferent (sensory) pathways are also shown [Boron & Boulpaep 2005].

The postganglionic sympathetic neurons thus originate in paravertebral or in prevertebral ganglia. The postganglionic fibres travel from their origin to their target organs [Guyton & Hall 2006].

The preganglionic parasympathetic neurons are located in the medulla, pons, and midbrain and in the sacral level (segment S2 to S4) of the spinal cord. Thus, unlike the thoracolumbar sympathetic preganglionic neurons, the parasympathetic preganglionic cell bodies are cranial or sacral (craniosacral). The preganglionic parasympathetic neurons originating in the brain travels with four cranial nerves; the oculomotor nerve (III), the facial nerve (VII), the glossopharyngeal nerve (IX), and the vagus nerve (X). The parasympathetic preganglionic neurons originating in S2-S4 distribute with the pelvic splanchnic nerves [Boron & Boulpaep 2005]. About 75% of the parasympathetic nerve fibres are in the vagus nerve [Guyton & Hall 2006]. In contrast to the sympathetic nervous system, the synapse between the preganglionic and postganglionic parasympathetic fibres is located in ganglia near (terminal ganglia) or in the target organ (intramural ganglia) [Martini 2004]. Figure B.2 illustrates the parasympathetic nervous system.



Figure B.2: The parasympathetic nervous system. It is demonstrated how parasympathetic fibres leave the CNS via cranial nerves III, VII, IIX, and X; additionally parasympathetic fibres leaving the sacral part of the spinal cord is demonstrated. Figure edited from [Guyton & Hall 2006].

Each preganglionic sympathetic fibre synapses on many postganglionic sympathetic neurons that are located within one or several nearby paravertebral and prevertebral ganglia. It has been estimated that a single preganglionic neuron synapses on up to 200 postganglionic fibres. The preganglionic parasympathetic fibres typically synapse in six to eight ganglia and the postganglionic fibres all influence on the same organ. Thus, the effects of parasympathetic stimulation are much more specific and localised than those of the sympathetic nervous system [Martini 2004, Boron & Boulpaep 2005]. An overview of the organisation of the sympathetic and the parasympathetic division is shown on figure B.3.



Figure B.3: An overview of the organisation of the sympathetic and parasympathetic nervous system [Boron & Boulpaep 2005].

B.1.2 Neurotransmitters and Receptors

The sympathetic and parasympathetic fibres mainly secrete one or the other of two synaptic transmitter substances; these are acetylcholine or norepinephrine. Those that secrete acetylcholine are called cholinergic, whereas the ones secreting norepinephrine or epinephrine are called adrenergic. The preganglionic neurons in both divisions are cholinergic. Thus, acetylcholine or acetylcholine-like substances applied to the preganglionic neurons causes both sympathetic and parasympathetic postganglionic neurons to be excited. In contrary, almost every postganglionic neurons of the sympathetic division are adrenergic while the majority of postganglionic neurons of the parasympathetic division are cholinergic [Guyton & Hall 2006].

To stimulate an effector organ, the transmitter substances must first bind with specific receptors on the effector cells. The receptors are located on the outside of the cell membrane. When the transmitter substances bind to a receptor the cell is inhibited or excited. This usually happens through an alteration of the membrane permeability or through enzymatic changes within the cell [Guyton & Hall 2006].

There are two principal types of acetylcholine receptors; these are muscarinic and nicotinic receptors. Muscarinic receptors are found in all effector cells stimulated by the postganglionic cholinergic neurons of the sympathetic and parasympathetic nervous system. The nicotinic receptors are found in the ganglia at the synapses between the preganglionic and postganglionic neurons of both the sympathetic and parasympathetic nervous system [Guyton & Hall 2006].

There are also two main types of adrenergic receptors; these are α and β receptors. These receptors further divided into α_1 , α_2 , β_1 , and β_2 receptors. Norepinephrine mainly excites α receptors. β receptors are also excited but in a lesser extent. Epinephrine excites both receptor types equally. Therefore, the type of receptors in the effector organ determines the strength of the response to a specific substance [Guyton & Hall 2006].

B.1.3 The Adrenal Medullae

Preganglionic sympathetic neurons innervates postsynaptic target cells in the adrenal medullae directly. These cells are called chromaffin cells, and are analogous to postganglionic neurons. They have nicotinic receptors which make them cholinergic. They are located near blood vessels to which they release norepinephrine and epinephrine. The release of epinephrine to the blood stream enhances the sympathetic nervous system's ability to reach the entire body [Boron & Boulpaep 2005]. On average, 80% of the secretion is epinephrine and 20% is norepinephrine. The effect of the circulating transmitter substances is almost the same as the effects caused by direct sympathetic innervations with these substances, except that the effects are more long lasting; up to 5-10 times as long. The prolonged effect is due to a slow removal of the hormones from the blood stream [Guyton & Hall 2006].

B.1.4 Autonomic Tone

Normally, the sympathetic and the parasympathetic divisions are both active. This background level of activity in the two divisions is called sympathetic tone and parasympathetic tone respectively. By maintaining a background level of activity, the nerve can increase or decrease activity of a stimulated organ. The balance between the sympathetic and the parasympathetic nervous system is called autonomic tone. The autonomic tone is significant for organs which receive innervations from both divisions. The heart is an example of an organ receiving dual innervations [Martini 2004].

B.2 The ANS's Effect on the Heart

The heart rate is influenced by the parasympathetic and the sympathetic nervous system. The parasympathetic nerve to the heart, the vagus nerve and the cardiac sympathetic nerves primary supply the atrium, especially the SA node and the AV node. The parasympathetic innervation of the ventricles is sparse, while the sympathetic innervation is rich as illustrated in figure B.4 [Wei-Jin et al. 2005, Guyton & Hall 2006].



Figure B.4: The sympathetic and parasympathetic innervation of the heart [Guyton & Hall 2006].

The two divisions have opposite effects on the heart; the sympathetic division causes excitation, whereas the parasympathetic causes inhibition. The parasympathetic division predominates in resting conditions whereas the sympathetic division dominates in more demanding situations [Martini 2004].

B.2.1 Parasympathetic Effects

Stimulation by the parasympathetic divisions decreases the heart rate, the conduction velocity of the action potential, and the contractility of the heart.

The transmission and effect of the parasympathetic division, on the cells is illustrated in figure B.5. The neurotransmitter acetylcholine (Ach) is released from the postganglionic nerve ending and binds to a muscarinic receptor, termed M₂-cholinoceptor. The binding to the receptor causes a disassociation of subunits of inhibitory G-protein (G_i). The disassociated subunits are: α_i , β and γ [Despopoulos & Silbernagl 2003, Brodde & Michel 1999].



Figure B.5: The transmission and effect of Acetylcholine (Ach) in the heart. Edited from Despopoulos and Silbernagl [2006].

An inhibitory G protein (G_i) is attached to the M₂ receptor on the inside of the cell. It consists of three components; an inhibitory α_i component, a β component, and a γ component [Despopoulos & Silbernagl 2003, Guyton & Hall 2006]. Guanosine diphosphate (GDP) is bound to the α_i component when the G_i protein is inactive. When acetylcholine binds to the M₂ receptor, the G_i protein is activated. The GDP bound to the α component is replaced with cytosolic guanosine triphosphate (GTP) and the α_i -GTP complex dissociate from the remaining components. The dissociated α_i -GTP complex inhibits adenylate cyclase [Despopoulos & Silbernagl 2003]. The activation of the G_i protein is illustrated in figure B.6.



Figure B.6: Activation of inhibitory G protein (G_i) by acetylcholine. Binding of acetylcholin to a M₂ receptor in the heart causes a dissociation of the inhibitory α (α_i) component and its associated guanosine diphosphate (GDP) from the protein. Guanosine diphosphate (GDP) is replaced with cytosolic guanosine triphosphate (GTP). The α_i -GTP complex inhibits adenylate cyclase. Edited from Despopoulos and Sibernagl [2003].

The α_i subunit binds with and inhibits adenylyl cyclase, which is an enzyme that, when active converts adenosine triphosphate (ATP) into cyclic adenosine monophosphate (cAMP). cAMP is a second messenger that directly activates and opens the calcium channels of the cell, when it react with the protein kinase A (PKA). Thus, by inhibition of adenylyl cyclase, cAMP will decrease and for that reason fewer calcium channels will open and they will not remain open as long. This causes a reduced calcium influx, which decreases the steepness of the pacemaker potential of the action potential (phase 4) in the SA-node and shifts the threshold potential to a less negative value. These effects are

seen in figure B.7a and B.7b [Despopoulos & Silbernagl 2003, Brodde & Michel 1999].

The β and γ subunits of the dissociated G-protein bind to and thereby activate potassium channels. This results in an increased efflux of potassium, which in turn causes a faster repolarisation phase. The membrane potential of the cardiac pacemaker cells becomes hyperpolarised, because more positive potassium ions leave the cell than normal. This shifts the resting potential to a more negative value which causes a prolonged duration of the time it takes for the pacemaker potential to reach the threshold potential level, as it is seen on figure B.7c [Despopoulos & Silbernagl 2003, Brodde & Michel 1999].



Figure B.7: The factors that affect the action potential and thereby the heart rate. Edited from Despopoulos and Silbernagl [2006].

Altogether, these effects cooperate to prolong the time for the SA node to depolarise to threshold and the net effect is a lowering of the heart rate [Despopoulos & Silbernagl 2003, Boron & Boulpaep 2005, Sherwood 2004]. The physiological effect on the AV node is similar; due to inhibition of Ca^{2+} influx, the depolarisation will be slow and the conduction velocity will be slowed as well, this is seen on figure B.7d [Despopoulos & Silbernagl 2003, Brodde & Michel 1999].

B.2.2 Sympathetic Effects

Stimulation by the sympathetic divisions increases the heart rate, the conduction velocity of the action potential, and the contractility of the heart [Despopoulos & Silbernagl 2003, Brodde & Michel 1999].

The sympathetic nervous system releases norepinephrine and epinephrine that bind to an adrenergic receptors. This receptor acts on a stimulatory G-protein (G_s) and makes it disassociate into three subunits; α_s , β , and γ .

There are two adrenergic receptor types; α and β adrenoceptors. These receptors can be divided further into α_1 , α_2 , β_1 , and β_2 adrenoceptors. The presence of these four receptor types in the human heart have been shown, but the majority constitutes of the β_1 adrenoceptors [Brodde & Michel 1999]. α_1 is 30 times more abundant than α_2 adrenoceptors in the heart, but β adrenoceptors are far more abundant than both α receptor types. The β_1/β_2 ratio in the heart is 60-70% to 40-30% in the atria and 70-80% to 30-20% in the ventricles. In the SA node, the density of β adrenoceptors is three times higher than in the surrounding atrial myocardium, and the β_2 density is also 2.5 times larger in the SA node compared to the surrounding atrial myocardium. Although, the quantity of β_1 adrenoceptors exceeds the quantity of β_2 adrenoceptors, the response mediated by the two receptor types is not necessarily different because β_2 adrenoceptors are more effectively coupled to adenylate cyclase than β_1 adrenoceptors. But other studies have shown that stimulation of β_1 causes a larger increase in contraction force than stimulation of β_2 adrenoceptors. Thus, stimulation of both β adrenoceptors are involved in positive chronotropic and inotropic effects [Brodde & Michel 1999]. Norepinephrine mainly excites α adrenoceptors. β adrenoceptors are also excited by norepinephrine, but in a lesser extent especially β_2 adrenoceptors. Epinephrine excites both adrenoceptor groups [Despopoulos & Silbernag] 2003].

 α_1 adrenoceptors works through a G_q protein which activate phospholipase C β (PLC β). This causes a formation of inositrol triphosphate (IP₃) diacyl glycerol (DAG) from inositrol biphosphate (PIP₂) [Despopoulos & Silbernagl 2003]. IP₃ activates calcium channels in the sarcoplasmatic reticulum an, this causes an increase in intracellular Ca²⁺ concentration. The produced DAG activates protein kinase C, which, like PKA, is capable of phosphorylating proteins. The G_q protein also causes an efflux of K⁺ from the cell [Despopoulos & Silbernagl 2003]. The effect of stimulation of α_1 adrenoceptors is illustrated in figure B.8.



Figure B.8: Illustration of what happens when α_1 adrenoceptors are stimulated by norepinephrine or epinephrine. Edited from Despopoulos and Silbernagl [2003].

Stimulation of α_2 adrenoceptors activates a G_i protein which inhibits adenylate cyclase and thereby the formation of cAMP from ATP. The G_i protein increases the open-probability of K⁺ channels, which causes a hyperpolarisation of the cell. If the α_2 adrenoceptor also is coupled to a G₀ protein, it can cause an inhibition of Ca²⁺ channels, i.e.



a reduction in intracellular Ca^{2+} concentration [Despopoulos & Silbernagl 2003]. The effect of stimulation of α_2 adrenoceptors is illustrated in figure B.9.

Figure B.9: Illustration of what happens when α_2 adrenoceptors are stimulated by norepinephrine or epinephrine. Edited from Despopoulos and Silbernagl [2003].

 β_1 adrenoceptors, that predominate the adrenoceptor types in the heart, activates a stimulatory G (G_s) protein. The stimulatory α component α_i of the activated G_s protein is bound to GTP. The α -GTP complex activates cAMP which in turn activates PKA. The activated G_s protein and the increased intracellular PKA level opens longer lasting Ca²⁺ channels which increases the intracellular Ca²⁺ concentration. In addition, the increase of the intracellular Ca²⁺ concentration activates calcium channels in the sarcoplasmatic reticulum (SR). This results in a rapid release of a large amount of Ca²⁺ from the SR to the cytosol [Fuster et al. 2004]. The increase in intracellular Ca²⁺ concentration produces positive chronotropic effect on the SA node, positive dromotropic effect on the AV node, and positive inotropic effects on the myocardium. The β and γ subunits of the dissociated stimulatory G-protein bind to and deactivate the potassium channels. This results in a decreased efflux of potassium ions and thereby a steeper slope of the pacemaker potential, which means faster depolarisation, and thus faster heart rate cf. figure B.7a. The conduction velocity in the AV node is increased due to the change of the slope and amplitude of the depolarisation phase of the action potential. This can be seen in figure B.7d [Despopoulos & Silbernagl 2003]. The effect of stimulation of β_1 adrenoceptors is illustrated in figure B.10.



Figure B.10: Illustration of what happens when β_1 adrenoceptors are stimulated by norepinephrine or epinephrine. Edited from Despopoulos and Silbernagl [2003].

 β_2 adrenoceptors also increases cAMP levels when stimulated by epinephrine, but does not lead to an increase in Ca²⁺. The reason for this is unknown [Despopoulos & Silbernagl 2003, Brodde & Michel 1999]. The effect of stimulation of β_1 adrenoceptors is illustrated in figure B.11.



Figure B.11: Illustration of what happens when β_2 adrenoceptors are stimulated by norepinephrine or epinephrine. Edited from Despopoulos and Silbernagl [2003].

The ANS's regulation of intracellular concentrations affect gap junctions, which are responsible for the electrical cell to cell coupling in the myocardium.

B.2.3 ANS's Effects on Gap Junctions

From the above it became obvious that the ANS has great influence on the cardiac activity. In general, the parasympathetic division causes negative chronotropic, dromotropic, and inotropic effects to occur. In contrast, the sympathetic division causes positive chronotropic, dromotropic, and inotropic effects to occur. The two divisions' effect on intracellular concentrations of substances and ions is outlined in table B.1. Note that β_1 is the predominant adrenoceptor in the heart and that the others only excists in a limited scale compared to β_1 .

Parasympathic Division		
Agonist	Receptor	Effect on
		intracellular
		concentrations
Acetylcholine	M ₂	↓ cAMP
		↓ PKA
		↓ Ca ²⁺
		$\downarrow K^+$
Sympathetic Division		
Agonist	Receptor	Effect on
		intracellular
		concentrations
		↑ IP ₃
NE ≥ E	α1	↑ DAG
		↑ PKC
		↑ Ca ²⁺
		↑ K ⁺
		↑ cAMP
	0.	↑ PKA
	u ₂	↑ Ca ²⁺
		↑ K ⁺
		↑ cAMP
E > NE	β_1	↑ PKA
		↑ Ca ²⁺
		↑ cAMP
E > NE	β2	↑ PKA
		↑ Ca ²⁺

Table B.1: Observations made regarding autonomic innervation of receptors found in the heart, and their effect on intracellular concentrations when stimulated. Note that β_1 is the predominant adrenoceptor found in the heart.

Gap junctions in the heart are regulated by different substances, among these are cAMP, PKA, PKC, and Ca²⁺ [Dhein 1998*b*]. The concentration of these substances changes in the intracellular environment due to autonomic innervation cf. the observations outlined in table B.1.

An increase in intracellular cAMP leads to increased coupling between adjacent cells [Dhein 1998*b*]. PKA and PKC both enhance the conductance of the most abundant type of gap junction found in the heart. The change is very rapid and lasts for several minutes. A large increase in intracellular Ca^{2+} causes an reduction in conductance. Under normal conditions the Ca^{2+} concentration does not reach high enough levels to cause a reduction in gap junctional conductivity [Dhein 1998*a*].

If the changes, in the mentioned substances mediated by the autonomic nervous system are large enough to cause a regulation of the cardiac gap junctions, then, in general sympathetic innervation would cause an increase in gap junctional conductance whereas the parasympathetic innervation would cause a decrease in gap junctional conductance.

The conduction velocity in tissue is determined by two factors; 1) it is directly related to the rate of rise and the amplitude of the action potential and 2) it is inversly related to the resistance of the conducting pathway. The rate of rise and amplitude of action potentials are not affected by the adrenergic stimulation of the heart. The resistance of the conducting pathway in the heart is composed of series of cytoplasmic and gapjunctional resistances. Thus, an increase in the gap juntional conductance leads to an increase in conduction velocity [Burt & Spray 1988]. Burt and Spray [1988] have reported that gap junctional conductance is enhanced by agents that elevate intracellular cAMP levels, and that the effect is seen within time courses comparable to those of naturally mediated inotropic effects of cAMP.

An increase in junctional conductance probably does not have a great impact on the conduction velocity in the electrical system of the heart, since junctional resistance is not rate limiting here. Conversely, an increase in junctional conductance in areas of the heart where conduction not only occurs in the longitudinal direction of the fibres and where the junctional resistance is a limiting factor, such as the ventricles, is expected to increase conduction velocity with up to 10 % [Burt & Spray 1988].

C.1 Introduction

In the analysis of biological signals it is often desirable to provide information related to the time-frequency variation of the signals. For this purpose, the wavelet transform and to some extent the Fourier transform are applicable.

The result of a Fourier transformation, is only related to the frequency components with out relation to time. This is because the Fourier transformation is an integral over all times and therefore it makes no sense to use this kind of transformation when interested in the time-frequency variation in nonstationary signals, i.e., where there are time-varying frequencies [Kumar et al. 2003, Addison 2002]. Instead, the short time Fourier transform (STFT) can be used. With STFT the signal is divided into subdivisions by a window and a Fourier transformation is applied on each subdivision of the signal. By this, a time-frequency representation of the signal is obtained. The drawback of using the STFT is that it is not possible to obtain an optimal time or frequency resolution, due to the use of only one window of the same size for all the subdivisions in the time-frequency plane [Kumar et al. 2003].

A wavelet analysis can be used to examine the signal in both time and frequency, i.e., which frequency does the signal contain over a period of time. Compared to the STFT, the wavelet analysis has good time resolution for high frequencies and good frequency resolution for low frequencies [Kumar et al. 2003, Addison 2002].

C.2 Wavelets

A wavelet is a limited wave in time and frequency. There is a large selection of wavelets that can be used in the wavelet analysis as long as they satisfy the following mathematical criteria [Addison 2002]:

1) A wavelet must have finite energy:

$$E = \int_{-\infty}^{+\infty} |\Psi(t)|^2 dt < \infty$$
(C.1)

2) If $\hat{\psi}(f)$ is the Fourier transform of the wavelet $\psi(t)$, then:

$$\hat{\Psi}(f) = \int_{-\infty}^{+\infty} \Psi(t) e^{-i(2\pi f)t} dt$$
(C.2)

and then the following condition must hold:

$$C_g = \int_0^{+\infty} \frac{|\hat{\psi}(f)|^2}{f} df < \infty$$
(C.3)

This implies that the wavelets has no zero frequency component, i.e. $\hat{\psi}(0) = 0$, equivalent to the wavelet having zero mean. Equation C.3 is called the admissibility condition and C_g is the admissibility constant [Addison 2002].

If the above mentioned criteria are fulfilled, the function can be used for wavelet transformation. A wavelet denoted $\psi(t)$ is a mother wavelet. There are a number of basis functions that can be used as mother wavelets. The appropriate choice of mother wavelet depends on the application. In figure C.1 four different wavelets can be seen: The Haar wavelet, the Mexican Hat, the Daubechies 6, and the Morlet wavelet.



Figure C.1: Four different types of wavelets.

C.3 The Continuous Wavelet Transform

The continuous wavelet transform (CWT) was developed as an alternative approach to the short time Fourier transform to overcome the resolution problem. A wavelet transform applied to a time domain signal x(t) is defined as [Kumar et al. 2003]:

$$W(s,\tau) = \int_{-\infty}^{\infty} x(t) \psi_{s,\tau}^{*}(t) dt$$
(C.4)

The asterisk indicates that the complex conjugate of the wavelet function is used in the transform. This enables the possibility of obtaining information from the signal at different frequencies and times respectively. The mother wavelet, ψ , is scaled by the factor *s*, and translated by τ . Hence, the translation parameter is used to shift the mother wavelet across the signal x(t), i.e., the translation gives the location of the wavelet, which is the time information in the signal. The scale factor gives the frequency information from the signal. By increasing or decreasing the scaling factor the frequency components of the signal can be obtained, i.e., large *s* correlates with the low frequency components and vice versa [Kumar et al. 2003]. Scaling and translation of a wavelet is illustrated on figure C.2.



Figure C.2: Illustration of (a) translation and (b) scaling. Inspired by [Addison 2002]

The scaled and translated version of a continuous mother wavelet is defined as [Addison 2002]:

$$\Psi_{s,\tau}(t) = \frac{1}{\sqrt{s}} \Psi\left(\frac{t-\tau}{s}\right) \tag{C.5}$$

The factor $\frac{1}{\sqrt{s}}$ is used to preserve the same amount of energy in the wavelet for every scale

The continuous wavelet transform is thereby defined as the combination of equation C.4 and C.5 [Addison 2002]:

$$T(s,\tau) = \frac{1}{\sqrt{s}} \int_{-\infty}^{+\infty} x(t) \psi\left(\frac{t-\tau}{s}\right) dt$$
(C.6)

The output from equation C.6 is a measure of how good the correlation between the mother wavelet with a given scale factor *s*, on a given translation τ and the signal is. If the output is 0 then there is no correlation between the signal and the scaled and translated wavelet. If the output is close to either ± 1 , then there is a good correlation [Addison 2002].

After the wavelet transformation the information is presented in 3D. The axis consist of the scaling factor *s*, the translation parameter τ and the output from the CWT, which gives an amplitude for the correlation between the signal and the wavelet. The principle of the wavelet transform is illustrated in figure C.3.



Figure C.3: Illustration of the principle behind the wavelet transform. First a wavelet is chosen and the CWT is calculated. Afterwards the wavelet is shifted until the whole signal has been covered and for each τ the CWT is calculated. The same procedure is done, with another scale, *s*. Large *s* correlates with low frequency components of the signal and vice versa. The result is shown to the right, where the output of the wavelet transform is illustrated in three dimensions [Addison 2002].

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