HEART RATE VARIABILITY MEASUREMENTS OBTAINED FROM THE ROUTINELY MONITORED NEONATAL ELECTROCARDIOGRAM

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor of Medicine by

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Declaration

This thesis is a result of my own work. The material contained within this thesis has not been presented, nor is currently being presented either wholly or in-part for any other degree or other qualification. Acknowledgements of the contribution of others are given within the thesis.

Dedications

Dedicated to my beautiful daughters

Amelia and Annie Rose

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The seeds of this project were sown by Professor Richard Cooke who commented on the vast amount of information that is before eyes that we ignore. He has taught and guided me from being a medical student, through to a junior doctor, research fellow and more recently as a consultant. I am indebted to him as a source of wisdom and inspiration.

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Abbreviations

ANOVA	Analysis of variance
ANS	Autonomic nervous system
AR	Autoregressive
AS	Active sleep
AV	Atrioventricular
BP	Blood pressure
BR	Baroreceptor
BRS	Baroreceptor sensitivity
CGA	Corrected gestational age
CPAP	Continuous positive airway pressure support
CSV	Comma separated variable
DFT	Discrete Fourier transform
ECG	Electrocardiogram
EMG	Electromyographic
FFT	Fast Fourier transform
FIR	Finite Impulse Response filter
Fs	Sampling Frequency
FT	Fourier transform
FSD	Full scale deflection
GA	Gestational Age
GP	Gaussian Process
HF	High Frequency
HF+	Additional High Frequency band
HFOV	High frequency oscillatory ventilation
HIE	Hypoxic ischaemic encephalopathy
HR	Heart rate

HRC	Heart rate characteristics
HRV	Heart rate variability
IHR	Instantaneous heart rate
IPFM	Integrated pulse-frequency modulator
IQR	Interquartile range
IUGR	Intrauterine growth restriction
IVH	Intraventricular haemorrhage
LF	Low frequency
LSP	Lomb Scargle periodogram
LTV	Long term variability
MDI	Motor developmental index
MED	Median
N	Data length in samples
NICU	Neonatal Intensive Care Unit
NN	Time interval between two successive normal R waves
NNi	Normal to normal R wave interval time series
PCSD1	Poincare standard deviation 1
PCSD2	Poincare standard deviation 1
PDA	Patent ductus arteriosus
PDI	Motor developmental index
PMA	Post menstrual age
PPi	PP interval
PRi	PR interval
PSA	Power Spectral analysis
PSD	Power Spectral density
PVL	Periventricular leucomalacia
QS	Quiet Sleep
RDS	Respiratory distress syndrome

RMSD	Root mean square deviation
RMSE	Root mean square error
ROC	Receiver operator curve
RR	Time interval between two successive R waves
RRi	RR-interval time series
RSA	Respiratory Sinus Arrhythmia
SA	Sinoatrial
SD	Standard deviation
SDANN	Standard deviation of the average Normal to Normal R time intervals
SDNN	Standard deviation of the Normal to Normal R time intervals
SGA	Small for gestational age
SIDS	Sudden infant death syndrome
SIMV	Synchronised intermittent mandatory ventilation
SNR	Signal to noise ratio
SPA	Smoothing priors approach
STV	Short term variability
Var	Variance
VLBW	Very low birth weight
VLF	Very low frequency
ULF	Ultra Low Frequency
UTI	Urinary Tract infection

Abstract: Heart rate variability measurements obtained from the routinely monitored neonatal electrocardiogram

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Advances in medical care have enabled infants born up to 18 weeks early to survive however there are significant risks associated with promoting this survival. For the smallest babies there remains high mortality and morbidity. The earlier identification of pathological processes would prompt targeted therapies and in turn hopefully improve their outcome. By monitoring the function of the autonomic nervous system (ANS), moving from a physiological to a pathological state could be identified before clinical suspicion is raised. The assessment that exists between heart beats (heart rate variability, (HRV)) provides a window into the functioning of the ANS.

For a measurement to be a useful clinical monitoring tool it must be robust, valid and practical. Methods used to analyse HRV in infants has been restricted to the research setting. The aims of this thesis were to develop a methodology that is able to use the routinely monitored electrocardiogram to produce sophisticated measures of HRV.

A novel R wave detector is presented which is validated with synthetic neonatal ECG data. It is assessed against published R wave detectors and shown to be highly accurate at correctly identifying the R wave over a range of heart rates, HRV's and increasing signal noise.

The method to determine frequency harmonics of the neonatal ECG utilises the Lomb Scargle periodogram (LSP) and is demonstrated to be much more robust than traditional Fast Fourier Transform (FFT) methods. LSP was more accurate than the FFT methods in the deriving a known LF:HF ratio across a range of (i) different HR's, (ii) different HRV's and (iii) missing data.

The developed methodology is then applied to ECG recordings of infants being cared for in the neonatal intensive care unit. When applied to the routinely monitored ECG, the method was able to measure HRV values in 97% of the recordings. The effect of removal of non-stationeries within the NNi tachogram was assessed and shown to significantly affect the HRV metrics. This is important when assessing previous published results which do not account for non-stationeries.

The stability of HRV measures in well babies was then investigated. It was demonstrated that the majority of HRV measures do fluctuate during routine care but are not statistically significantly different from when the babies are recorded in the steady state. The routinely monitored ECG is therefore able to represent the underlying autonomic state.

Finally, the method was applied to disparate populations of babies (different gestational age groups and well/unwell babies) who would demonstrate different autonomic activity. These investigations demonstrate that time domain and Poincare measures of HRV are able to detect autonomic differences in the routinely monitored ECG signal and that recordings in the real world reflect the experimental world in the preterm population but not in the term population.

This thesis presents a method which is able to determine HRV metrics from the routinely monitored ECG signal from babies undergoing intensive care treatment.

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Chapter 1

Introduction, Background and Literature review

1.1 Introduction

To sustain a healthy individual homeostasis is requisite. A pivotal component of maintaining homeostasis is the intact functioning of the autonomic nervous system (ANS), which continually fluctuates, adapting in response to both internal and external stimuli. By monitoring ANS function, individuals who remain homeostatic can be identified along with those who are changing from a physiological into a pathological state. With the progress of information technology it is possible to explore the dynamic functioning of the ANS using the variation in intervals between successive heart beats, termed "heart rate variability" (HRV).

1.2 The Premature Infant

A preterm infant is defined as one being born before 37 weeks' gestation. With medical advances it is now possible for babies born as early as 23 weeks' gestation and weighing as little as 400g to survive. The risks of promoting this survival are significant and include multiple and frequent events such as sepsis, hypotension, cerebral intraventricular haemorrhages, acute and chronic pulmonary problems including blocked endotracheal tubes, pneumothraces, apnoea and chronic lung disease[1]. There is often little warning of these deleterious events and without immediate resuscitation or treatment, death or serious morbidity may occur. Despite advances in intensive care the mortality and morbidity rates remain high with infants born below 26 weeks gestation having a mortality rate of approximately 50% and half of all survivors having moderate or severe disabilities at 6 years of age[2;3]. The aim of neonatal intensive care is to improve the intact survival of these fragile infants.

The neonatal intensive care unit (NICU) provides continuous routine monitoring of various physiological parameters which provide information on the physical state of the infant. Whilst information from these signals such as heart rate (HR) and respiratory rate are presented at the cotside there is a large volume of 'hidden' information contained within these physiological recordings which is simply discarded. In particular, information contained with the ECG has been demonstrated to provide a window into the functioning of the ANS.

1.3 The Autonomic Nervous System

The peripheral nervous system is divided into the somatic and autonomic nervous systems, the latter being represented in figure 1. The somatic nervous system controls skeletal muscle under voluntary control and the reception of external stimuli, whilst the ANS maintains homeostasis through the regulation of smooth muscle, cardiac muscle and glandular secretions. The ANS has both an efferent and afferent system which form reflex arcs that pass through the hypothalamus and medulla oblongata, enabling the transmission of impulses between the central nervous system and the peripheral organ system.

The afferent, or sensory, division of the ANS consists of primary visceral sensory neurons which monitor: (i) the levels of carbon dioxide, oxygen and glucose in the blood, (ii) arterial blood pressure and (iii) the chemical composition of the stomach and gut content. These sensory neurons propagate action potentials which pass to the spinal cord via the rami communicantes and the posterior roots. The primary sensory neuron synapses with secondary visceral sensory neurons transmitting the afferent information to the main integration and control areas of the ANS in the medulla oblongata, pons and hypothalamus. Here the impulses elicit appropriate reactions in efferent nerves via reflex arcs resulting in appropriate modification in the functioning of the cardiovascular, respiratory, enteric and other organ systems. In addition, as part of the limbic system the hypothalamus receives input from the cerebral cortex. This enables cortical activity to stimulate the hypothalamus, in turn affecting autonomic activity.

The motor neurons of the ANS are divided into two divisions, the sympathetic and parasympathetic nervous systems (SNS and PNS), on the basis of anatomical and functional differences. In general the SNS is excitatory, responsible for so called 'fight or flight' responses, whereas PNS activity is inhibitory or 'rest and digest'. Both divisions consist of myelinated preganglionic fibres which synapse with unmyelinated postganglionic fibres which innervate the effector organ. The sympathetic preganglionic fibres are in the spinal cord at thoracolumbar levels, whereas the parasympathetic preganglionic fibres are situated in the medulla oblongata and in the sacral spinal cord. The preganglionic fibres synapse with the postganglionic fibres in clusters called ganglia. The sympathetic ganglia are located in two sympathetic chains close to the spinal cord, the prevertebral and pre-aortic chains, whilst the parasympathetic ganglia are located in close proximity to the target organ, for example the submandibular ganglion for the salivary glands and the paracardiac ganglia for the heart. Fibres from both divisions of the ANS innervate most organs and typically influence in an antagonistic way, with increased activity in one branch accompanied by a reduction in the other. The resultant organ function, for example heart rate (HR), reflects this continually fluctuating balance.

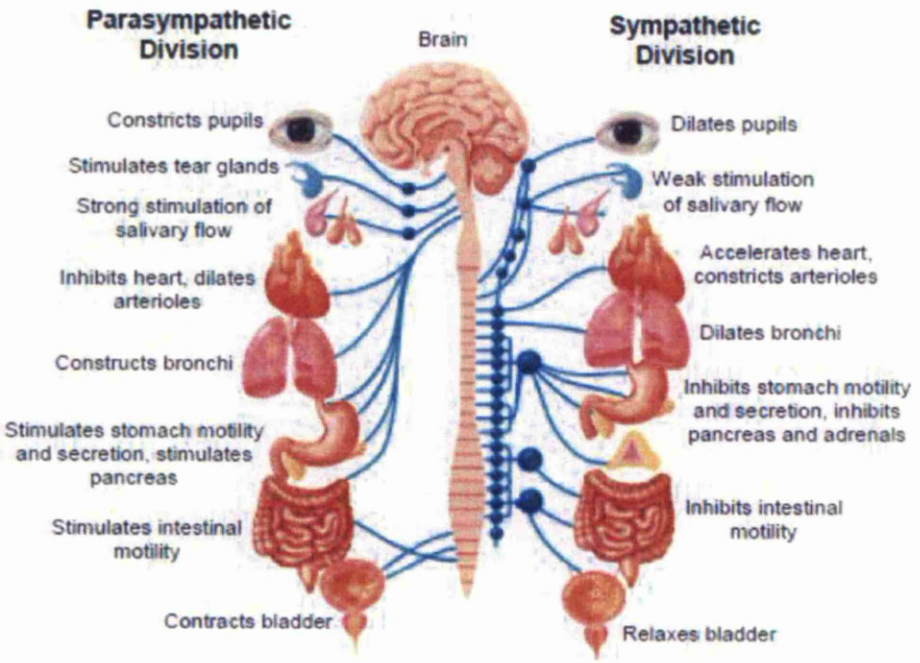


Figure 1.1 Organisation of the autonomic nervous system (ANS). The sympathetic division (right) is stimulated by stressful situations increasing the heart rate cardiac output and blood flow to the muscles and inhibiting digestive activity. Stimulation of the parasympathetic branch of the ANS (left) leads to increased digestive activity, and depresses blood pressure, heart rate and cardiac output. Taken from Morris and Maisito 2001[4].

1.4 The Control of Heart Rate

1.4.1 Sinoatrial node and autonomic innervation

Each heart beat originates in the sinoatrial (SA) node located at the junction of the superior vena cava and the right atrium. The SA node displays spontaneous pacemaker activity, in the human adult firing at a rate of between 100 and 130 action potentials per minute[5-8]. In the newborn, animal work suggests that this intrinsic firing of the SA node is at a higher rate due to shortening of the action potential[9-12]. This intrinsic firing rate is modulated by the two branches of the ANS which densely innervate the SA node[13]. During physiological conditions the two components of the autonomic nervous system work in balance, with the activation of one limb accompanied by the inhibition of the other[14]. The resultant HR reflects this balance between the sympathetic and parasympathetic impulses.

Parasympathetic activation is mediated by the synaptic release of acetylcholine from the vagus nerve which acts upon nicotinic and muscarinic receptors in the SA node, the atrioventricular (AV) conducting pathways and the atrial muscle. This slows depolarisation of the sinoatrial node and also AV conduction, resulting in a reduction in heart rate[15]. Acetylcholine has a very short latency period and a high turnover rate which results in a rapid response, enabling the PNS to regulate cardiac function on a beat to beat basis[16,17].

Sympathetic activation results in an increase in heart rate above the intrinsic level generated by the SA node and augmentation of myocardial contractility. In addition, the rate of conduction of the cardiac impulse through the heart and the duration of

contraction is shortened. These effects are mediated through synaptic release of noradrenaline which is reabsorbed and metabolised relatively slowly, resulting in changes in cardiovascular response over a longer time period[16]. Following the onset of sympathetic stimulation there is a latent period of up to 5 seconds followed by a progressive increase in HR which reaches a steady state in 20-30 seconds[15]. This time delay in affecting HR contrasts with the almost instantaneous response to parasympathetic activation.

Because of these differences in neurotransmitter function, the two divisions of the ANS operate at different frequencies and variations in heart rate related predominantly to changes sympathetic or parasympathetic activity can thus be identified and quantified[18-20].

In the adult human at rest, parasympathetic activity predominates over sympathetic resulting in a reduced SA node firing rate and in turn lowering the resting heart rate to 60-80 bpm. The opposite is true in the neonate and infant with sympathetic activity predominating until approximately 2 years of age[21,22]. This sympathetic dominated balance increases the preterm infant's heart rate to 110 – 160 bpm. The heart rate will increase or decrease depending upon the relative activity of the parasympathetic and sympathetic centres which in turn are dependent on reflexes from the afferent visceral sensors and factors such as arousal and activity levels[23-25]. Some reflexes increase heart rate through a decrease in vagal tone, an increase in sympathetic activity, or both. Other reflexes will decrease the heart rate by the opposite actions. At any one time several reflexes are likely to operate simultaneously, maintaining cardiovascular homeostasis by producing a variable HR and cardiac output.

1.4.2 Reflexes Influencing Heart Rate

1.4.2.1 Baroreceptor Reflex

The baroreceptor reflex buffers sudden changes in systemic blood pressure by adapting HR and peripheral vascular resistance, aiming to stabilise perfusion pressure in the face of disturbances of circulatory homeostasis. The baroreceptor reflex arch

consists of baroreceptors, afferent nerves, the central nervous system, and efferent nerves[26]. Arterial baroreceptors are present throughout the body with important groups found in the aortic arch[27], coronary arteries[28], splanchnic circulation and the carotid bodies. An increase in blood pressure stretches these vessels, resulting in an abrupt increase in the discharge frequency of the afferent nerves. Baroreceptor stimulation increases the efferent cardiac vagal activity and decreases sympathetic activity, resulting in a decrease in SA firing and in turn HR[15].

The reflex is typical of a negative-feedback control system as an increase in arterial blood pressure (BP) increases the baroreceptor afferent impulses, which in turn decrease the arterial pressure through the reflex arc. Heart rate responses are mediated mainly by parasympathetic efferent activity, whereas vascular resistance is adapted by sympathetic activity only. As both branches of the ANS are involved in the reflex, investigating the baroreceptor response provides information on autonomic cardiac regulation[29,30]. Because vascular resistance is difficult to measure, autonomic changes related to baroreceptor reflex control are usually studied by evaluating HR and BP fluctuations only.

Baroreceptor reflex mediated HR control has been studied more extensively than other effector mechanisms (peripheral resistance, venous return and cardiac contractility) because of the relatively easy access to the relevant signals (HR and BP). The response has been studied by transiently increasing or decreasing arterial pressure following the administration of vaso-active agents[31,32] or by applying a positive or negative pressure pulse to the neck, decreasing or increasing the transmural pressure on the carotid sinus region[33]. By injecting vasoactive drugs, arterial BP rises or falls from the equilibrium point (physiological BP) and an input-output curve can be constructed[34]. This input-output relation curve is sigmoidal, having a threshold zone in the lower BP values (where RR interval is minimal) and a saturation zone in the higher blood pressure values (where RR interval is maximal figure 1.2)[35].

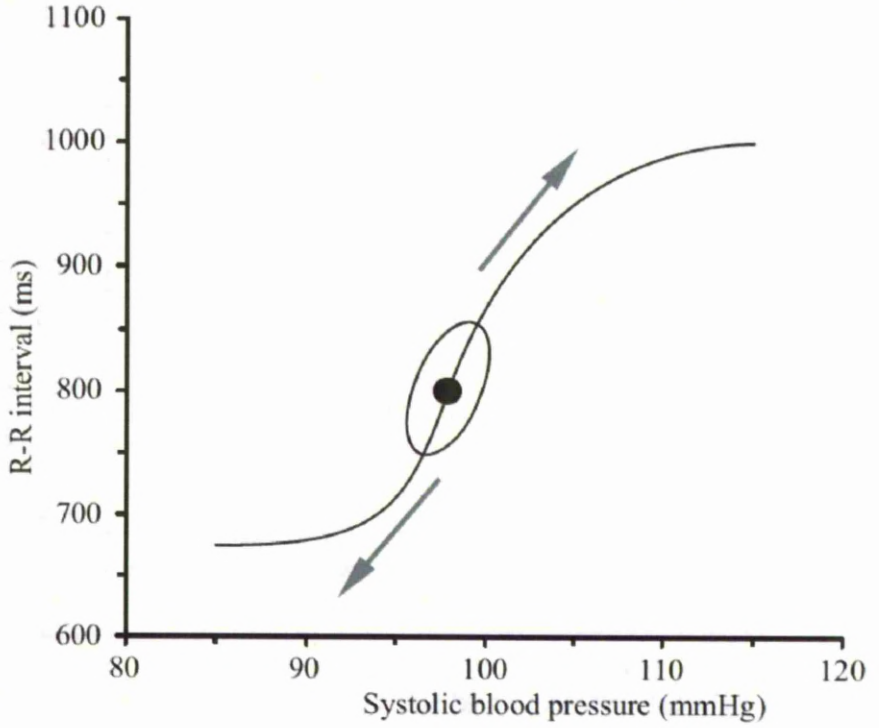


Figure 1.2 Figure representing the closed-loop relationship between arterial systolic blood pressure and R-R interval in adults. Korner 1974[34].

Two parameters within this sigmoidal relationship can be determined to estimate baroreceptor mediated heart rate control. Firstly, the level at which the reflex responds most effectively to changes in BP and secondly, the magnitude of the reflex response per unit of BP deviation from the operating point (baroreceptor sensitivity)[34].

In healthy, conscious humans, baroreceptor mediated control of HR is primarily mediated by stimulation and withdrawal of parasympathetic activity. Meanwhile, sympathetic stimulation, while influencing basal HR, plays a minor role in baroreceptor mediated HR control. Evidence for this is demonstrated by the observation that whilst HR response to rising and falling BP can be blocked by atropine, there is no effect following the administration of propanolol[36,37]. In addition, the higher baroreceptor sensitivity during BP rise than during fall (30 *vs* 10 ms $mmHg^{-1}$)[38] suggests a relative low vagal inhibitory tone during resting conditions. As

the baroreceptor reflex is mediated via the vagal nerve, the baroreceptor response is able to modulate HR on a beat to beat basis.

In summary, the baroreceptor mediated heart rate response to increased arterial BP seems to be exclusively mediated by an increase in the cardioinhibitory influence of the vagus. In addition the excitation of the sinus node that accompanies baroreceptor deactivation is also largely mediated by the vagus. Thus, although the arterial blood pressure-heart period relation curve is frequently used as a surrogate of the overall state of the baroreceptor reflex system, one should keep in mind that this is a very simple representation of the system.

1.4.2.2 Chemoreceptor reflex

Peripheral chemoreceptors are situated in the carotid and aortic bodies with their afferent activity stimulated by hypoxia, hypercapnia or acidaemia. Chemoreceptor stimulation increases the rate and depth of respiration and influences heart rate. However, because HR is also influenced by respiratory efforts, the effects of chemoreceptor stimulation may be masked by the secondary effects of the respiratory response to changes in oxygen, carbon dioxide concentrations and blood pH[15].

1.4.2.3 Other reflexes

There are several other reflexes influencing HR which whilst being studied in the adult are poorly understood in the human infant. These include reflexes initiated by; (i) Atrial stretch receptors (ii) pulmonary artery stretch receptors, (iii) coronary artery chemoreceptors, (iv) abdominal viscera receptors and (v) mesenteric vessel baroreceptors.

1.4.3 Respiratory influences on Heart Rate

In healthy adults changes in HR occur in association with respiration, termed respiratory sinus arrhythmia (RSA). In response to inspiration, HR accelerates and during expiration HR slows. The mechanism linking the variability of HR to respiration is complex and involves both central and reflex interactions. The major reflex mechanism is believed to be activation of lung stretch receptors with inspiration (Hering-Breuer reflex) and changes in baroreceptor and atrial stretch receptor activity induced by changes in intrathoracic pressure[15]. Fluctuations in HR have been observed to persist at the approximate respiratory frequency in the absence of respiration and after elimination of pulmonary reflexes. This supports the concept of a central respiratory generator, maintaining respiratory/heart period rhythmicity in the absence of peripheral input from lung stretch receptors[39]. Respiratory modulation of the HR occurs at a high frequency (approximately 0.25 Hz in the adult or 15 times per minute at rest) and can be abolished by vagal blockade[40-43] suggesting that RSA is mediated by the PNS.

Chapter 2

Heart Rate Variability

2.1 Introduction

The appreciation that the heart does not beat regularly is not a new one. Examining the peripheral pulses to detect abnormalities in HRV dates back over 2000 years with Wang Shu-He (265 - 317 A.D) writing;

"If the pattern of the heartbeat becomes as regular as the tapping of a woodpecker or the dripping of the rain on the roof the patient will be dead in four days" [44]

The study of HRV is based upon the duration of the time interval between successive heart beats, or its reciprocal the instantaneous heart rate (IHR) derived from the electrocardiogram (ECG). 6.4.3

2.2 The Electrocardiogram

Heart muscle cells contract in response to stimulation by a self propagating wave of electric field, beginning at the sinoatrial node (SA) node. Self propagation occurs because, as each myocardial cell contracts, the flow of sodium and potassium ions in and out of each cell generates a small electric field which stimulates, in turn, adjacent myocardial cells in the direct of propagation of the resulting wave of contraction. The electric field dipoles of all myocardial cells contracting / relaxing at any moment sum to produce a significant resultant electric field which extends well beyond the heart and produces measurable differences in electrical potential (voltage) between points at the surface of the body. These voltages, measured at standardised locations, form the familiar time varying ECG voltage signals.

A single normal cycle of the ECG represents the successive atrial depolarisation/repolarisation and ventricular depolarisation/repolarisation which occurs with every heartbeat. The ECG provides information regarding the overall rhythmicity of the heart as well as information about the propagation of the electrical signal through the various cardiac structures. A typical ECG record of a normal heartbeat (or cardiac cycle), comprising a P wave, a QRS complex and a T wave, is shown in Figure 2.1.

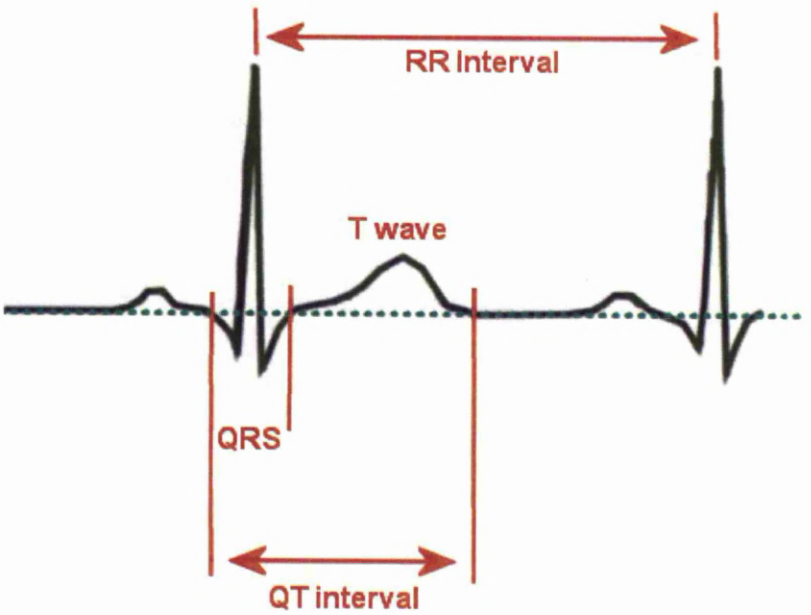


Figure 2.1 The ECG recording of a normal heart beat

The P-wave accompanies the depolarisation of the atria. The QRS complex corresponds to the depolarization of the ventricles, appearing as a relatively strong signal as they contain more muscle mass than the atria. Its complexity arises due to the intricate pathway that the spread of activation takes through the ventricles. Shortly after the peak of the R-wave the ventricles begin contracting. The T wave represents the repolarisation of the ventricles. Atrial repolarisation is not apparent as a discrete component as it occurs during the much stronger ventricular depolarisation.

Segments, or intervals, between features may also be reported (generally extending from one wave to the start of another), see Figure 2.1. For example the Q-T interval, defined as the time required for the ventricles to undergo a complete cycle of depolarisation and recovery. Interpretation of the ECG involves a sequential analysis of each component in the tracing. Since the peak of the QRS complex is a well defined feature, the time duration between two consecutive R-waves in the ECG is commonly used as the most accurate determination of HR.

2.3 Fiducial marker for beat detection in the ECG

The first step in obtaining a measurement of HRV is the accurate detection of each heart beat originating from the sinoatrial node. This is a difficult process made even more problematic in the neonate by the presence of large volumes of noise, artefacts and ectopic beats. A fiducial marker must be chosen from the ECG which accurately reflects the competing action of both branches of the ANS. The most obvious choice would be the point where the autonomic nervous system exerts its influence, the sino-atrial node represented by the p wave on the ECG. However, the p wave has no temporally well defined feature and is usually of low amplitude and is therefore often difficult to detect[45]. Conversely, the high amplitude R wave has a temporally well defined peak and which is much easier to identify and label as the fiducial marker for each heart beat. Care must be taken when using the time between successive R waves, the RR interval (RRi) as a proxy measure of the PP interval (PPi) due to the variation that exists in the PR interval with changing heart rates. Sympathetic activation of the SA node increases nodal firing and in turn heart rate but also leads to a shortening of the PR interval as well as the QT interval[46], QRS width[47] and T wave[48]. However, the magnitude of the beat to beat modulation of the PRi is correlated with, and much less significant than that of the RRi[49] and so the RRi can be used as a reliable method for interbeat measurement[50].

The RRi becomes an unreliable proxy measure of PPi if there is disruption to the normal coupling of the atria and ventricular electrical activity. This occurs in the following situations; atrial flutter, atrial fibrillation, ventricular tachycardia and third degree heart block. Fortunately all of these electrical disturbances are uncommon in

the neonatal population. However, a continuous ECG recording will rarely consist entirely of successive "normal" QRS complexes. HRV analysis is only concerned with beats that have originated from the SA node, where the ANS is exerting its influence. Therefore beats which do not originate from the SA node or signal noise which may be detected as a "beat" should be excluded from the HRV metric calculation. The corrected time series therefore includes only the normal to normal (NN) beats.

2.4 Quantifying Heart Rate Variability

The aim of HRV analysis is to quantify the autonomic status of an individual and many different HRV metrics have been proposed to achieve this[50]. The most widely used metrics can be broken down into three main categories; simple time domain statistics, frequency domain analysis and non-linear techniques. All of these techniques however have a common analytical base, the production of the NN interval tachogram (NNi).

2.4.1 NN interval Tachogram

The NN are measured from the accurate detection of each R wave in the ECG (see chapter 4) and are graphically presented on the y-axis of a tachogram against the time of occurrence. The functional value of the tachogram is therefore the duration of a single heart beat (ms) for a given moment in time. This is an unusual time series in that both axes are time intervals and are related to each other [51]. Furthermore, since the variability in HR occurs on a beat-to-beat basis, the time series is inherently unevenly spaced along the horizontal axis. The horizontal distance between each point (time stamp) is different for each adjacent pair, with the difference recorded on the vertical axis. Alternative methods of producing a tachogram to represent the heart rhythm are discussed in chapter 4.

2.4.2 Time Domain Analysis

Time domain methods use mathematically simple techniques to measure the amount of variability present in a pre-specified time period in a continuous ECG recording. After editing the ECG to remove artefacts and non-sinus beats, the normal RR intervals, termed normal-to-normal intervals (NNi) (sections 2.4.1 and 4.1), are identified and subjected to simple statistical analysis. Time series indices can be further divided into two broad categories[52];

(a) those derived from the direct measurements of the NNi such as mean NNi and standard deviation of the NNi's (SDNN)

(b) those derived from the differences between adjacent cycles, such as the proportion of differences that exceed an arbitrary limit eg 50ms.

Table 2.1 highlights the indices recommended by the Task Force of the European Society of Cardiology and the North American Society of Pacing Electrophysiology[50]

Statistical Methods	**Explanation**	**Component Measured**	**Comments**
SDNN (ms)	Standard Deviation of the normal to normal QRS complexes. (i.e. the square root of variance)	Estimate of Overall HRV	Result varies depending on length of recording. The longer the recording the more variance is observed
SDANN (ms)	Standard Deviation of the Average Normal to Normal QRS complex	Estimate of long-term components of HRV	Standard deviation of the averages of NN intervals in all 5-minute segments of the entire (eg 24-hour) recording.

Statistical Methods	Explanation	Component Measured	Comments
SDSD (ms) –	Standard deviation of differences between adjacent NN intervals		
RMSSD (ms)	Square root of the mean squared differences of successive NN intervals	Reflects short term variability	
NN50* (count)	Number of interval differences of successive NN intervals greater then 50 ms	Identifies individuals with parasympatheti c dysfunction	Identifies the number of NN intervals that exceed a pre-set threshold ie 50ms
pNN50* (%)	NN50 divided by total number of NN intervals	Reflects Short term variaibilty	Percentage of differences between adjacent NN that are greater then 50ms calculated over a 24 hour recording

*NN50 is not suitable for the assessment of neonatal HRV due to the higher mean HR. Recently, pNN25 has been suggested as a more useful measure for neonatal ECGs[53].

Table 2.1 Information regarding the different time domain measures of HRV[50].

The simplest measurement of HRV is the standard deviation of the NN interval (SDNN), i.e. the square root of variance. Since variance is mathematically equal to total power of spectral analysis, SDNN reflects all the cyclic components responsible for variability in the period of recording[50]. SDNN is often calculated over a 24-h period and therefore encompasses both high frequency and (very) low frequency

components seen in a 24-h period. As the period of monitoring decreases, SDNN estimates shorter cycle lengths and the total variance of HRV decreases[54]. It has therefore been argued that in arbitrarily selected ECGs, SDNN is not a well defined statistical quantity because of its dependence on the length of recording period and it is inappropriate to compare SDNN measures obtained from recordings of different durations[50].

Another commonly used statistical variable is the standard deviation of the average NN interval (SDANN) which is calculated from the average NNi in short segments (usually 5 minute) of the total monitoring period. An increase or decrease in SDANN reflects a relative increase or decrease in sympathetic regulation. The SDNN index is calculated from the mean of the 5-min SDNN calculated over 24 hours, which measures the variability due to cycles shorter than 5 min[50].

The most commonly used measures derived from interval differences include the square root of the mean squared differences of successive NN intervals (RMSSD). An increase or decrease in this parameter reflects a relative increase or decrease in PNS activity.

Spontaneous fluctuations with a predefined time interval can also be characterised, for example the number of interval differences of successive NN intervals greater than 50 msec NN50, or the proportion derived by dividing NN50 by the total number of NN intervals and pNN50. NN50 however is not appropriate for the neonate who has a much higher mean heart rate. Recently NN25 has been proposed as a more useful measurement for neonatal HRV[53]. These measurements of short-term variation estimate high frequency variations in heart rate and thus are highly correlated.

2.4.3 Poincare Analysis

A further option for analysing HRV data is by depicting the series in a geometric pattern, such as the Poincaré plot, (figure 2.2). The Poincaré map is a representation of a time series within an x-y coordinate system, where the values of each pair of successive elements define a point in the plot, i.e. each RRi is plotted as a functional

value of the subsequent RRi (RR versus RR, msec). This creates a correlation depiction of consecutive RRi's. Analysis of this graphical representation can be performed by a visual-qualitative inspection of the shape of the displayed point cloud, which for a normal healthy adult subject is characteristically oval shaped[55].

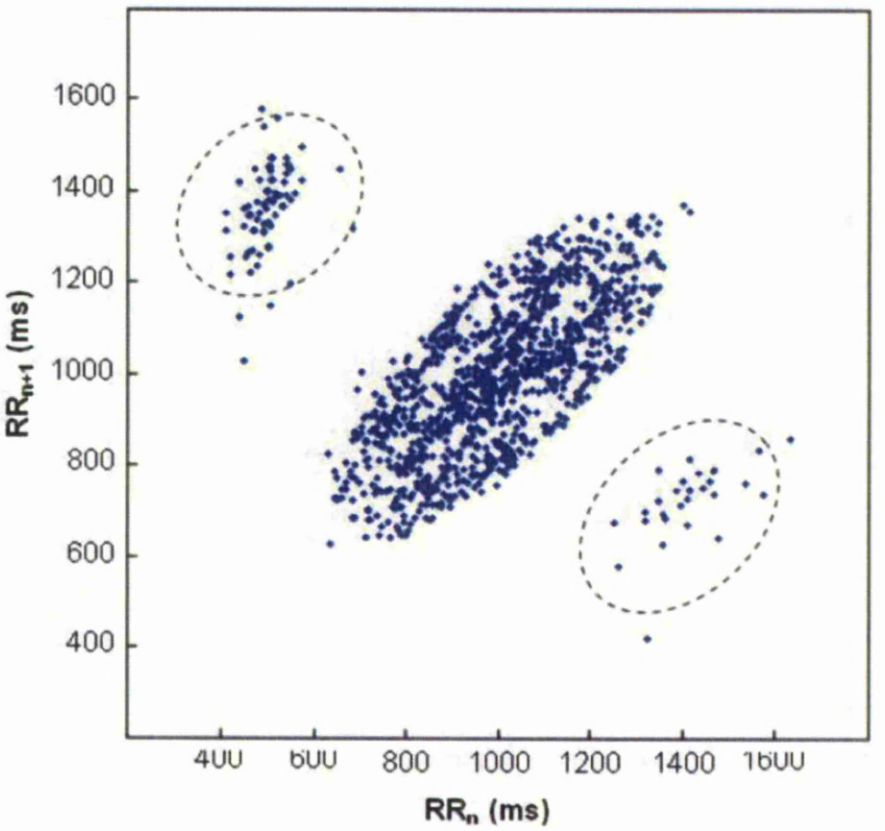


Figure 2.2 Poincaré plot of an R-R interval tachogram. Ectopic beats (circled) can be easily identified as outliers from the central point cloud which represents the "normal" beats.

There are two standard numerical descriptors of the Poincaré plot - SD1 and SD2 (Figure 1).1,2 SD1 measures the dispersion of points belonging to the PP along the line perpendicular to the line of identity and depicts short-term HRV. SD2 assesses the dispersion of points along the line of identity and portrays both long and short term HRV. The ratio SD1:SD2 thus represents the sympathovagal balance.[56-59]

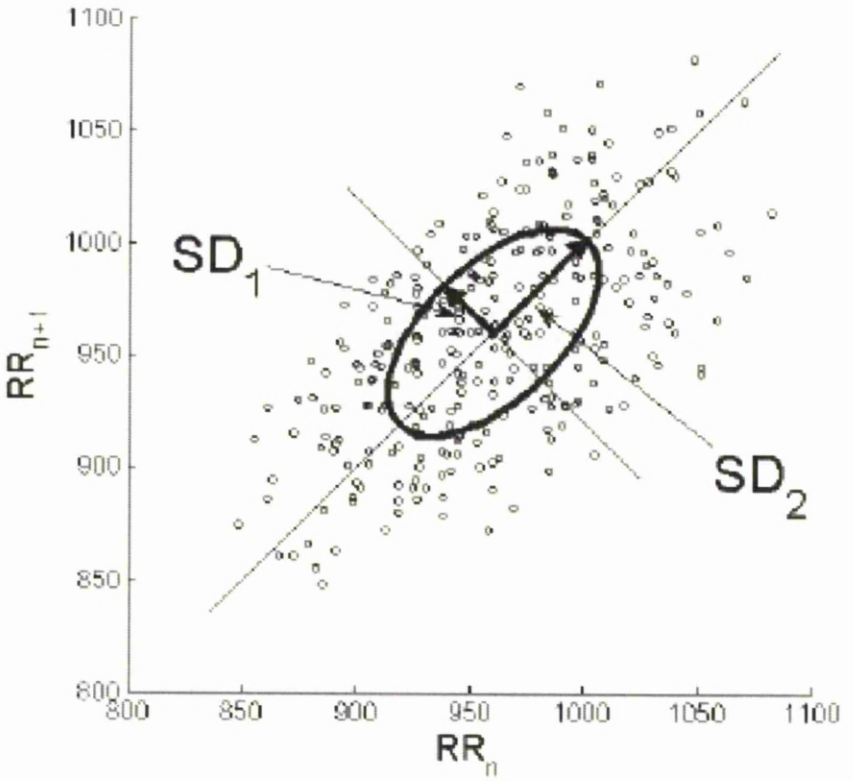


Figure 2.3 A sample of Poincaré plot with its numerical descriptors SD1 and SD2

2.4.4 Frequency Domain Analysis

Whilst time domain indices quantify the overall amount of variability present in the ECG recording, they are a non-specific measure of autonomic activity. The underlying physiological mechanisms which affect these parameters are not fully understood. In the adult it is accepted that time domain variables are dependant

primarily on vagal stimulation for the bulk of their magnitude with RSA and nocturnal bradycardia being two of the most important components[60]. A shift in the autonomic tone towards sympathetic activation will therefore reduce the magnitude of these indices[61]. The effect of vagal tone is not however linear with a greater effect of acetylcholine release at slower heart rates further complicating the interpretation of time domain indices[62]. Time domain analysis remains a useful tool however, as only minimal (and usually automated) editing of the ECG is required and therefore these techniques are easier to apply to long term ECGs with excellent reproducibility[63]. Thus, time domain metrics are useful in detecting abnormalities in autonomic activity but cannot be used to quantify specific changes in sympathetic or parasympathetic activity[16]. For this, frequency domain analysis must be performed.

Frequency domain is a term used to describe the analysis of mathematical functions or signals with respect to frequency. Although we appear to think and act in the time domain, the neurobiological processes that govern our existence are played out in this frequency domain[64]. Frequency analysis is based on the periodicity of various biological systems, i.e. a biological signal repeats itself within a determined time interval and thereby exhibits a certain frequency. To investigate and quantify these frequencies, a signal varying over time, $x(t)$, (such as the NNi tachogram) must first be transformed to amplitude varying over a range of frequencies, $X(f)$. This is achieved by spectral analysis.

Spectral analysis reduces the total HRV signal (consisting of numerous repeating oscillations) into its constituent frequency components and quantifies the relative power of various frequencies. Following transformation into the frequency domain, the amount of cyclical variation present at different frequencies can be detected and quantified by power spectral analysis (PSA)[65]. This information can be presented graphically by plotting the amount of variation present in a recording on the y axis against the frequency at which it occurs on the x axis. (fig 5) By measuring the area under the curve at different frequencies (expressed as spectral power) a numerical measure of the amount of variability present in given frequency bands can be obtained.[16] PSA was first introduced into HRV analysis in 1981 by Akselrod *et al.*[66] who demonstrated that frequency domain analysis allowed the quantification of the frequency components representing SNS and PNS activity. Since the introduction of

PSA in HRV analysis, many authors have applied a variety of power spectral estimation techniques. In order to facilitate comparisons between studies, the frequency spectrum of an RR interval tachogram has been classified into Spectral Power Density present in the following frequency bands: (1) ultra-low frequencies, ULF (≤0.003 Hz) that include circadian (daily) rhythms; (2) very-low frequencies, VLF (0.003-0.04 Hz) thought to be due thermoregulation and humoral (immune) systems; (3) low frequencies, LF (0.04-0.15 Hz) usually considered as a marker of sympathetic modulation associated with blood pressure phenomenon (baroreceptor activity), and both cardiac sympathetic and parasympathetic" nerve activity; (4) high frequencies, HF (0.04-0.15 Hz) that are well defined and synchronised to the RSA. The total power in the spectrum, regarded as an overall measure for the influence of the ANS on the cardiovascular system, is calculated as the size of the entire area within all frequencies.

These bands are, however, recommendations for the adult ECG with significant differences noted in the neonatal ECG (see section 2.5).

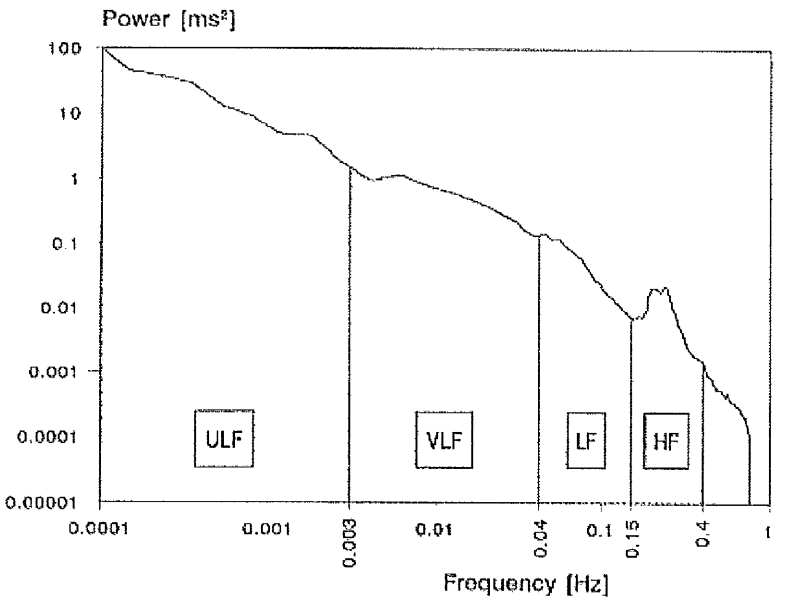


Figure 2.4 Example of an estimate of power spectral density obtained from the entire 24-hour interval of a long-term recording. Note the logarithmic scales on both axes.

Variable	Frequency measured	Component represented
Ultra-low frequency (ULF)	0 – 0.0033Hz	Circadian Rhythms
Very Low frequency (VLF)	0.0033 – 0.04 Hz	Thermoregulation, Peripeheral vasomotor RAA systems
Low Frequency (LF)	0.04 – 0.15 Hz	Sympathetic and Parasympathetic activity
High Frequency (HF)	0.15 – 0.4 Hz	Parasympathetic Activity
LF/HF ratio	N/A	Sympathetic/parasympathetic balance

Table 2.2 Frequency domain definitions used in analysis of HRV in adults.

The division of the spectrum into these frequency bands provides physiological information about the distinct biological regulatory mechanisms that contribute to HRV. There is now considerable frequency domain spectral analysis research data to demonstrate that these regulatory mechanisms act at frequencies that are confined (approximately) within these bands [14]. The HF component describes the beat to beat changes related to respiratory rhythm and is widely accepted as a marker of parasympathetic outflow[67]. LF fluctuations in heart rate are often attributed to blood pressure variations[51]. However, the physiological interpretation of the LF band is more controversial and although sympathetic and parasympathetic mechanisms can operate at these frequencies many authors ascribe fluctuations in the LF band to sympathetic activation only[14;16;68;69]. Certainly, an increase in LF power has consistently been observed as a consequence of sympathetic activation (rest –tilt manoeuvre, mental stress, haemorrhage, coronary occlusion etc)[67].

Fluctuations below the VLF and ULF bands are thought to be due to long-term regulatory mechanisms such as the thermoregulatory system, the rennin-angiotensin system (related to blood pressure and other chemical regulatory factors) and other humoral factors[67]. In 1998 Taylor *et al.* showed that the VLF fluctuations appear to depend primarily on the parasympathetic outflow[70]. In 1999 Serrador *et al.*

demonstrated that the ULF band appears to be dominated by contributions from physical activity and that HRV in this band tends to increase during exercise. They therefore assert that any study that assesses HRV using data (even partially) from this frequency band should always include an indication of physical activity patterns[71].

2.4.5 Sympathovagal balance

The ratio of power in the LF and HF can be used as a measure of the balance between the SNS and PNS[29]. Whilst controversy remains regarding the use of this metric[51] it is probably a valid estimate in a wide range of physiological situations, particularly when investigating the sympathovagal balance under various conditions[72]. In addition when compared with the individual LF and HF powers, the LF:HF ratio also offers the ability to measure whether reciprocal versus non-reciprocal changes have occurred in SNS and PNS activity[73]. It is important to recognize however that in cases where both LF and HF are powers are reduced (for example post myocardial infarction) the quotient would appear normal despite the pathological change.

2.4.6 Non-linear Analysis

A linear system is one in which the magnitude of a response is proportional to the strength of the stimulus. A non linear system does not demonstrate proportionality, rather the response is disproportionate: small changes can have dramatic and unanticipated effects[74]. Chaotic dynamics can be used to provide an explanation for the different complex and erratic patterns that appear. Chaos refers to the existence of behaviour so unpredictable as to appear random because of the inherent perturbations in the initial conditions[75]. It was originally assumed that chaotic fluctuations in cardiac electrical activity were produced in pathological conditions, such as during atrial or ventricular fibrillation[76]. However, these views have been challenged [77] and it is now accepted that the complex heart rate fluctuations observed during normal sinus rhythm in healthy subjects are due in part to deterministic chaos, and that pathological states may involve a paradoxical decrease in this type of nonlinear variability[74]. Goldberg has termed the observed loss of chaos as "decomplexification" of the

signal[78], a phenomenon which can be observed in clinical practice, for example Cheyne-Stokes respiration pattern in patients with end-stage lung disease, or the consistent heart rate in the neonate with severe hypoxic ischaemic encephalopathy.

2.5 Neonatal Heart Rate Variability

Whilst the same autonomic generators of HRV found in the adult are active in the neonate, the indices of variability are vastly different. The neonate has a different heart rate, respiratory rate and an immature autonomic nervous system which results in different parameters for both the time and frequency domain. The investigation of heart rate variability and autonomic functioning in the neonate has been hampered by studies which have used different frequency domain definitions, durations of recordings, and reported units with small sample sizes. The lack of methodological standards makes comparison between different studies difficult. In 1996 the Task Force for the European Society of Cardiology and The North American Society of Pacing and Electrophysiology produced recommendations for the standardisation of methodology, defined physiological and pathophysiological correlates and described appropriate clinical applications for the study of HRV[50]. However these recommendations are for the adult population and are not appropriate for the study of HRV in neonates[79].

2.5.1 Frequency Band Definitions

Internationally agreed recommendations exist for the defined frequency bands for human adults HRV. Frequency peaks can be detected at a LF of approximately 0.1 Hz, representing Mayer's waves, and at a HF of 0.25 Hz, representing RSA. These recommendations are however not applicable to the neonate due to a shift in the HF spectrum[50]. Neonates breathe at between 30 and 90 breaths per minute (0.5 - 1.5 Hz) with a heart rate between 100 and 180 bpm (1.7 – 3.0 Hz). Thus, different spectral peaks are observed in the frequency domain analysis of neonatal HRV. As yet there is a lack of consensus regarding the definition of frequency bands for neonatal HRV and

there are several reasons for this[35]. Firstly, preterm infants have an immature respiratory drive with periodic breathing and apnoeas, resulting in a continually fluctuating respiratory rate. Therefore the high frequency spectral peak in neonates may be dispersed. Secondly, as a consequence of this modulating HF peak the signal is only stable for a relative short time which limits appropriate signal analysis. Thirdly, because of the relatively high respiratory rate the upper spectral limit for the HF band poses a particular problem for neonates. Sampling theorem states that the maximum frequency that can be measured from spectral analysis of a signal can be no greater than half of the sampling frequency. This maximum is termed the Nyquist frequency[80]. Thus, for investigation of changes in heart rate the cut off for the upper pole of the HF band should be half the lowest normal mean heart rate. As a mean HR of less than 90 bpm is considered bradycardia in the neonate, the Nyquist criterion requires an upper limit of 0.75 Hz (ie (90/2)/60). However, neonatal respiratory rates may at times reach 90 breaths per minute. In order for respiration at such a frequency to be visible as RSA, the upper limit would have to be 1.5 Hz or twice the limit imposed by the Nyquist criterion.

This lack of consensus means that individual investigators have used several different frequency bands to investigate HRV. The majority of studies have used 0.15 or 0.2 Hz as the cut-off point between LF and HF bands (Table 2.3). However others have extended this to 0.5Hz and left a frequency band between the LF and HF band which is not measured[81-86]. The lower border of the LF band starts at 0.02 Hz but if a VLF band is also being investigated most studies use 0.04Hz to divide the LF/VLF bands[81,87-89]. The literature shows upper limits ranging from 0.8 to 2.0, according to the respiratory peak[90-93], or not stated[94,95].

	Subjects	LF band (Hz)	HF band (Hz)
Aarimaa 1988[96]	Preterm, Term	0.02 – 0.2	0..2 – 1 .0
Giddens 1985[95]	Term	0.04 – 0.2	0.2 - ?
Dykes 1986[94]	Term	0.02 – 0.2	02 - ?
Baldzer 1989[81] (i)	Term	0.04 – 0.16	> 0.2 Hz RSA*
Eiselt 1993[83]	Preterm, Term	0.03 – 0.1	0.4 – 1.0
Spassov 1994[82]	Term	0.03 – 0.1	0.4 – 1.0
Ravenswaaij[92;93] 1994, 1995 (ii)	Preterm	0.04 – 0.2	< 0.2 Hz RSA*
Chatow 1995[97]	Preterm, Term	0.02 – 0.2	0.2 – 2.0
Mazursky 1998[98]	Preterm	0.02 – 0.15	0.15 – 1.5
Sahni 1999[84]	Preterm, Term	0.05 – 0.2	0.5 – 2.0
Veerappan 2000[99]	Preterm	0.03 – 0.4	0.4 – 1.0
Andriessen 2004 [85;100]	Preterm, Term	0.04 – 0.15	0.4 – 1.5
Andriessen [90]	Preterm, Term	0.04 – 0.15	RSA*
Andriessen 2003 [91]	Preterm	0.03 – 0.2	RSA*
Khattak 2007 [101]	Preterm and Term	0.05 – 0.25	0.25 – 1.00
Watkins 1996[102]	Preterm	0.05 – 0.2	0.2 – 1.0
Longin 2005**[53]	Term	0.05 – 0.15	0.15 – 0.5
Mehta 2002[87] (iii)	Term	0.04 – 0.15	0.15 – 0.4
Nakamura 2005 [103]	Preterm	0.04 – 0.15	0.15 – 0.4
De Rogalski Landrot 2007 [104]	Preterm and Term	0.04 – 0.15	0.15 – 1.4
Longin 2006** [105]	Preterm and Term	0.05 – 0.2	0.2 – 1.0
Rassi 2004[106]	Preterm	0.05 – 0.2	0.2 – 2.0
Jean-Louis 2004[86]	Preterm	0.03 – 0.15	0.5 – 1.0
Smith 2004	Preterm	0.02 – 0.2	0.2 – 2.0
Oberlander 2002 [107]	Preterm	0.04 – 0.15	0.15 – 0.8
Franco 2000[108]	Term	0.04 – 0.15	P24 – p75 (iv)

(i) VLF 0 – 0.04Hz

(ii) VLF 0.017 – 0.04 , the respiratory sinus arrhythmia (RSA) was defined as a band within ± 0.2 Hz of the respiratory frequency obtained from peak amplitude in the respiratory power spectrum;

(iii) VLF 0.0033 – 0.04 and ULF < 0/0033 also reported from 24 hr recordings

(iv) individualised respiratory bandwidth, defined between p-25 and p-75 of the respiratory power spectrum.

RSA = Respiratory Sinus Arrhythmia

** LF was termed MF in these papers and VLF = LF, 0.01 – 0.05Hz

RSA* = freq band between 10^{th} and 90^{th} centiles of individualized respiratory frequency

Table 2.3 Frequency bands used in previous research into HRV in neonates.

Based on the available literature the frequency ranges of interest in this thesis are as follows; the whole frequency band of interest is the range between 0.0 to 1.5 Hz; the ULF frequency band is defined as < 0.017 Hz, the VLF band is defined as 0.017 - 0.04 Hz, the LF band is defined as the frequency range between 0.04 and 0.15 Hz with two HF bands being defined, one 0.15 – 1.5 Hz. and a second being 0.40 – 1.0Hz.

2.5.2 Baroreceptor response

Data about the ontogeny and function of the baroreceptor reflex and in the human infant are limited and conflicting. This is partly due to the limited experimental (pharmacological or mechanical) possibilities to challenge the baroreceptor reflex in neonates[35]. The ontogeny of the BR in the human infant has been investigated by passive head-up tilting whilst measuring responses in BP, HR and limb blood flow. Several investigators have demonstrated that head-up tilting in healthy term and preterm infants produces a consistent and significant response in HR[98,109-112] whilst

other studies did not show evidence of a well developed baroreceptor mediated response[113,114]. In healthy, term infants a fall in blood pressure associated with an increase in heart rate in response to passive head-up tilt has been demonstrated[109,110] and can be elicited as soon as 2 hours after birth, becoming more pronounced at 24 hours[112]. In preterm infants (26-37 weeks) passive head-up tilt resulted in significant vasoconstriction in the lower limbs with a fall in aortic pressure with an unchanged HR[115]. The inadequate ability to maintain BP and the absence of a tachycardia suggest that the premature infant lacks the fully integrated BR response seen in more mature infants and adults. More mature preterm infants (33-37 weeks GA) however, show a well developed HR response to tilting[109]. Moreover, by studying preterm infants (28 – 32 weeks) in a longitudinal fashion the development of the HR response, from unchanged to a demonstrable increase, has been elicited[98]. Thus, the available studies of heart rate response to passive head-up tilt in human neonates suggest, at least qualitatively, a baroreceptor reflex mediated heart rate response is present and matures with advancing postconceptual age.

Few studies have quantified BR sensitivity in neonates. Drouin and Gournay measured BRS non-invasively in term and preterm infants using an automated oscillometric device for measuring blood pressure which has previously reported as giving similar BP readings as an umbilical arterial catheter[116]. The baroreceptor sensitivity was lower in preterm infants (approximately 4 ms $mmHg^{-1}$, 29-32 weeks) than in term infants (approximately 10 ms $mmHg^{-1}$, 40-41 weeks)[117]. A subsequent longitudinal study in preterm (24 – 36 weeks gestation) and term infants demonstrated that baroreflex sensitivity at birth was lower in the preterm group and increased with gestational age. BRS also increased with postnatal age, but the values for preterm infants at term still tended to be lower than values for full term babies[118]. Andriessen et al further studied BRS by cross-spectral analysis between BP and RRi fluctuations, a method which has previously been demonstrated to be an estimate of BRS in adults[119-122]. Andriessen studied stable preterm infants in the first week of life[91] and at increasing postnatal ages[90]. The feasibility of this method in stable preterm (28-32 weeks GA) neonates was demonstrated and showed BRS to be higher in the LF band (4.2 ms $mmHg^{-1}$) then in the HF band (1.7 ms $mmHg^{-1}$) in the first week of life[91]. Further investigation at advancing post natal ages of both preterm (28 – 37 weeks) and term infants showed that BRS calculated from the LF transfer gain increased

significantly with advancing postmenstrual age from 5ms $mmHg^{-1}$ (preterm) to 15ms $mmHg^{-1}$ (term). In agreement with previous studies, an increased NNi HF power relative to total power was observed with advancing postmenstrual age suggesting increased parasympathetic involvement. Furthermore, BR function was significantly correlated with the NNi variability but not with BP variability. This close relationship between NNi variability and BRS was also demonstrated in a pharmacological study whereby atropine administered to preterm neonates undergoing intubation, reduced BRS and NNi variability but had no effect on the BP variability[100]. These results suggest that significant vagal tone is already present in preterm infants.

In summary, whilst the above studies have used different methodologies, with different patient populations, it can be concluded that in the human infant: (i) a BR mediated HR response can be demonstrated in the immediate postnatal period, (ii) BRS is limited in preterm infants but increases with advancing postmenstrual age and (iii) BRS increases in response to parasympathetic development.

2.5.3 Chemoreceptor response

In the full term neonate, the sensitivity of chemoreceptors to carbon dioxide is mature[123] with the response to oxygen quickly resetting from the in-utero to ex-utero environment over the first few days of life[124]. The response to hypoxia results in an initial increase in minute volume which is poorly sustained followed by a return to, or fall below, baseline. This response evolves with advancing postnatal age with the increase in minute volume becoming more sustained and the hypoxic depression becoming less dramatic[125]. Preterm infants however demonstrate an immature response to hypercapnia which matures with increasing postnatal age[126].

2.5.4 Respiratory Sinus Arrhythmia

The presence of RSA in the human infant is disputed. Studies in both term[81,127-131] and preterm [92,101,132,133] infants have been able to detect a respiratory peak occurring during quiet sleep (QS) and sporadically in active sleep (AS). The occurrence of RSA in these infants is attributed to regular breathing patterns in QS and associated maturity of the parasympathetic nervous system [89,95,128,129,133] In preterm infants, maturation of RSA has been demonstrated with advancing postnatal age [92,101]. It has also been argued that the occurrence of RSA is not dependant on the respiratory rate, but more on the breathing stability and amplitude[53,94,95] However, several studies have failed to detect a peak in the HF spectrum corresponding to the respiratory rate in both term[53,95] and preterm infants[105]. Reasons for this failure to detect RSA have been proposed and include;

1. Immature central control of respiration. The typical neonate displays patterns of irregular breathing even during quiet sleep with the rate and tidal volume varying with each breath[134]. Thus, a stable frequency of breathing does not exist and therefore a respiratory peak in the HF spectrum cannot be detected.
2. Immature neural pathways for the transfer function from respiration to heart rate modulation[97]. In the adult, respiratory effort modulates the heart rate at high frequencies, mediated by parasympathetic activity. Parasympathetic immaturity would diminish the propagation of respiratory driven changes in the infant. The mechanisms that lead to periodic breathing remain unclear, with some authors suggesting unstable responses of immature chemoreceptors[133].
3. High respiratory rate/small breath amplitude. The neonates high respiratory rate coupled with low amplitude makes the detection of a HF peak related to respiration difficult[95,135]. Sampling theory dictates that RSA is difficult to verify if the RR exceeds half the mean heart rate, a relatively common occurrence in neonates.

In summary, the presence of RSA in the neonate is dependent on there being present intact functioning of the ANS, in particular a mature PNS, with a relatively stable

respiratory pattern. This situation is not uncommon in the term infant, allowing for the detection of RSA, but is infrequent in the preterm infant.

2.5.5 Maturation of the ANS

In-utero ANS maturation has been assessed by longitudinal assessment of fetal HRV in a small number of studies with diverse conclusions. Prior to 20 weeks gestation there is little intrinsic HRV as neither the sympathetic or parasympathetic influences exert significant effects despite anatomic functional integrity[136]. With advancing gestation a consistent decrease in baseline heart rate with an increase in variability is observed which is attributed to the maturation of the parasympathetic nervous system[137-140]. Frequency domain analysis of fetal magnetocardiograms from 16 to 42 weeks gestation, in 49 healthy singleton pregnancies showed that total spectral power (0.003 – 1.0 Hz) increased with gestation, with the rate of increase slowing substantially beyond 32 weeks gestation. The increase in power was more pronounced in the LF band but also present in the HF band, suggesting maturation of both PNS and SNS activity[141]. This progressive increase in LF band power age has been elicited by others, but coupled with a decrease in HF power in the third trimester[139,142].

ANS maturation has also been assessed by studying HRV in healthy infants at different gestational and postmenstrual ages (PMA) with several studies demonstrating increasing HRV spectral power with advancing ages[92,97,105,118,143-146] Chatow et al demonstrated an increase in HF power and a decrease in LF/HF ratio in infants of 34-35 weeks GA compared with 39 - 41 weeks GA when recorded on day 1 of life (mean LF:HF ratio 71.4 vs 16.3)[97]. These findings are supported by a study by Longin et al who studied 39 infants within the first 7 days of life divided into two gestational age bands; (i) 29 – 32 weeks GA, and (ii) 32 – 35 weeks GA. Short term (10 minute) recordings demonstrated a significant increase in total, HF and LF power as well as a decrease in the LF/HF ratio when the more mature infants were compared with the more preterm ones. Furthermore, the indices reflecting PNS control showed the most significant increases[105]. The results from this study of preterm infants were also compared with a cohort of healthy term infants from a previous study by the same team[53]. The term infants demonstrated significantly increased HRV in all power

spectrum bands, with a shift in favour of parasympathetic control reflected by a decreased LF/HF ratio. Clairambault et al further defined ANS maturation by examining 24 healthy newborns whilst asleep in the first 11 days of life. The infants were divided into three postconceptual age groups (31 – 36, 37 – 38, and 39 – 41 weeks) with LF and HF power estimated over 512 beats. LF power demonstrated a steady increase between the groups whereas vagal tone showed marked maturation at 37 weeks, before plateau at term[145]. This plateau was not demonstrated however by Sahni who demonstrated a steady increase in both time and frequency domain measures of HRV in preterm infants (26-37 weeks GA) when studied in three PCA groups; (i)31 – < 34 weeks,(ii) 34 – <35 weeks, and (iii) 35 – 38 weeks[146]. Finally, Gournay et al used the baroreceptor sensitivity as an indicator of ANS development demonstrating increasing sensitivity with advancing gestational age in preterm infants of 24 – 37 weeks GA[118].

Longitudinal studies in both term and preterm infants has provided information regarding postnatal maturation of the ANS. Patzak[147] et al followed 16 healthy term infants from the first day of life through to six months of age. Increasing power in all frequency bands was observed in the first five days of life which then declined, reaching a minimum value at 1-2 months of age. The power in LF, HF and total power subsequently increased up to 6 months of age. The LF/HF ratio development followed a more irregular course but reached a maximal value at the end of the first month. The early modulation of HRV indices has also been observed in several other studies, including; (i) total power increase in infants born at 34-35 weeks gestational age (GA) when monitored in the first five days of life[132], (ii) increase in time domain[148] and spectral indices[92] in infants born at less than 33 weeks gestation during the first three days of life and, (iii) increasing time domain indices in premature infants (28 -36 weeks GA) during the first 168 hours of life[149].

Long term autonomic development has been studied by investigation of HRV indices in infants, children and adults. In a longitudinal study, Schechtman et al.[150] reported an initial decrease in HRV for all frequencies over the first month of life, followed by an increase up to 6 months of age. This pattern was also observed by Massin in a cross sectional study of 587 infants[151]. Finley et al performed a cross sectional study of HRV from 24 hour recordings taken from healthy individuals aged 1 month to 24

years. Increasing LF, HF and total spectral power was demonstrated up to 6 years, followed by a decrease in all parameters[21]. This pattern of an increase up to 6 years, followed by decreasing power was also observed by Goto who performed a similar study in children up to 15 years old[152].

2.5.6 ANS development in the preterm infant

The premature infant has less ANS activity, as demonstrated by reduced HRV, than the term infant. In order to investigate the effect that being born prematurely has on ANS development, preterm infants must be compared with term infants at a comparable postmenstrual age (PMA). Early studies compared the baseline HR of healthy preterm infants (29 – 36 weeks) with term infants at matched PMAs. Preterm infants had an elevated HR which persisted until approximately 7 months chronological age[153]. This elevation in preterm HR was also observed by Eiselt et al[83] who in addition showed that HRV, as measured by spectral power, was lower in preterm infants at term corrected gestational age (CGA) compared with term infants. Furthermore, when recordings were compared during different sleep states there was no difference in heart rate or HRV in preterm infants in contrast to the stark changes seen with term infants[83]. Similar spectral power results were demonstrated by Patural et al when ECG recordings taken at corrected term age for premature infants were compared with those taken from term infants. Parasympathetic activity, reflected by HF power, was significantly lower in the preterm infants than in the term infants and when the preterms were divided into three GA birth groups (<28, 28 – 31, 32 – 37 weeks) the observed effect was greater with increasing prematurity[154].

Studies using different methods to elicit autonomic control have reached similar conclusions. ANS activity as elicited by baroreceptor sensitivity was significantly lower at term CGA in preterm infants (<33 weeks) when compared with full term infants (5.6 vs 8.9 ms $mmHg^{-1}$)[118]. Tuladhar used reflex heart rate responses elicited by non-arousing trigeminal stimulation to assess autonomic functioning in term and preterm (26 – 32 weeks GA) infants. The increase in heart rate was significantly less in preterm infants than term infants at 2 - 3 weeks corrected postnatal age, but not at 2 - 3 months. Again, no difference between sleep state HR was observed in preterm

infants, a phenomenon which was present in the term infants. To assess the long term prognosis for ANS functioning in preterm infants Patural et al investigated a cohort of 30 preterm infants (25 – 37 weeks) and 14 term infants at theoretical term, 2-3 years and at 6-7 years of age[104]. Reduced spectral power in preterms at birth was observed at term CA. However, these differences were not present at the subsequent recordings at 2-3 and 6-7 years of age. The preterm infants ANS development demonstrated rapid development, becoming equivalent to the term controls at 2 years of age.

From the above studies, it can be seen that the ANS, and in particular parasympathetic activity, increases with gestational age and early postnatal life. The preterm infant demonstrates reduced variability, and diminished ANS activity compared to the term infant. In addition, upon reaching the age of term infants, ANS function remains lower in preterm infants than in term infants. Whilst preterm birth and the intensive care environment may be the cause for this observation, the impact of low fetal ANS activity on premature delivery is not known.

2.5.7 Influence of behavioural and sleep state

Several studies have demonstrated significant differences in heart rate variability during either active (AS) or quite sleep (QS) in term infants. Heart rate, long term variability (LTV) and low frequency spectral indices are increased during AS, whilst high frequency spectral power and short term variability is increased in QS in term infants[128,155-158] The increase in LTV in AS has been suggested to arise due to the changes in breathing patterns and /or body movements that occur in AS[79]. Increased short term variability (STV) may in part be explained by the increase in RSA accompanying slower, more regular breathing pattern in QS[89]. Studies of the effect of sleep state in the preterm infant however provide conflicting results. Whilst some investigators[92,132,145,159] have detected similar patterns in preterms (26-37 week) to those observed in term infants, the increase in STV indices are not to the same magnitude. Furthermore, others have not detected a difference in heart rate between sleep states[160,161,53,105].

The effect of sleep position on autonomic functioning has been investigated due to its relationship with sudden infant death syndrome (SIDS). The prone position is considered to be a major risk factor for SIDS[162,163] and has also been demonstrated to be associated with an increase in heart rate and a reduction in HRV in term infants[108,164-166], suggesting increased sympathetic activity. Preterm infants studied at different gestations and post natal ages have demonstrated similar results to term infants[86,146,167-169] but the observed differences are more apparent in QS than in AS [146,168]. It has been proposed that the modification of autonomic control when sleeping prone decreases the reserve for further reflex circulatory adjustments, increasing the risk for SIDS[170].

2.6 Clinical studies of pathological influences on neonatal HRV

2.6.1 Pulmonary Disease

Early observational studies performed in preterm infants demonstrated that long term HRV, as assessed by simple time domain statistics, was reduced in respiratory distress syndrome (RDS) and that the magnitude of this reduction was related to the severity of RDS[149,171-173]. Furthermore, the reappearance of HRV was associated with increased survival whereas, in infants who died, the reduced variability remained[149,172;173]. They were flaws in these early studies, particularly the lack of confounder reporting/analysis (eg ventilatory status, sepsis, birth weight, gestation) but their results were in agreement with a later studies which did correct for confounding variables[174].

The mechanism responsible for reduced HRV in RDS is not fully understood. It has been suggested that the reduced compliance of the surfactant deficient lung leads to a reduction in respiratory movements, thus lessening the mechanical respiratory effect on haemodynamics and in turn HRV[96]. No studies have been undertaken investigating the effect of exogenous surfactant administration, thus increasing lung compliance, on neonatal HRV. Central activity in the medulla oblongata may be transiently depressed in the presence of hypercarbia resulting in reduced modulation of heart rate[174]. It has

also been proposed that whilst the PNS functions properly, the SNS is attenuated possibly owing to poor regulation of blood pressure[93]. This would agree with studies that found reduced LTV but not STV in infants with RDS.

The effect of mechanical ventilation has been demonstrated in anaesthetised adult patients where reversal of the normal RSA pattern has been described[175]. That is, the heart rate decreased during mechanical lung inflation in contrast to the increase in HR related to spontaneous inspiratory effort. This phenomenon may be due to the positive intrathoracic pressure direct effect upon the aortic baroreceptors or the negative intrathoracic pressure occurring during expiration instead of inspiration (Bainbridge reflex)[93]. In ventilated, non-sedated preterm infants, this reversal of RSA has been demonstrated in some studies (<29 weeks GA)[106] but not in others (<33 weeks GA)[93]. Rassi et al observed reversed RSA in preterms who were receiving synchronised intermittent mandatory ventilation (SIMV), and a normal RSA pattern in infants who were receiving high frequency oscillatory ventilation (HFOV). The frequency of HFOV was thought to be too high to be observed within the HF band of 0.2 – 2Hz, and the infants own respiratory effort appeared to reproduce the normal RSA pattern. However, van Ravenswaaij et al did not demonstrate reversal of RSA but the presence of 'artificial' RSA which mimicked the normal pattern. Entrainment of the central respiratory activity by artificial ventilation was proposed as a possible mechanism for this occurrence, a phenomenon which has been described in both animals[176] and humans[177]. This entrainment is only present with intact vagus nerves and is mediated via the lung stretch receptors, thus, the more mature preterm infants in this study may synchronise their inspiratory activity with the ventilator, resulting in RSA, in the presence of mature PNS development.

2.6.2 Brain injury

Clinically, the most striking abnormality of reduced HRV occurs in term infants who have suffered hypoxic brain injury. Physiological trend graphs demonstrate a heart rate which shows very little variation for several days. This clinical observation is reflected by a case control study of asphyxiated term infants who demonstrated diminished RSA and HF power.[178] Moreover, Mcintosh et al have demonstrated that

in term infants the degree of reduction in HRV is proportional to the severity of hypoxic ischaemic encephalopathy (HIE)[179]. In preterm infants, reduction in HRV has also been observed in cases of; (i) asphyxia and (ii) a large (grade IV) intraventricular haemorrhage (IVH)[148].

In a small case-control study by Watkins et al, spectral analysis of very low birth weight (VLBW) infants (<1500 grams) on the first day of life showed that infants with low HF power on day 1 of life were more likely to be diagnosed with an intraventricular haemorrhage (IVH) by day 7[102]. However, this observation is only evidence of association and whilst the two groups were closely matched for birth weight and FiO_2 requirements, there is no mention of the potentially confounding variables of gestational age or illness severity.

A study by Hanna et al[180] of 19 VLBW infants recorded at 32 to 37 weeks corrected gestational age attempted to characterise the maturation of HRV indices in brain injured infants and also determine if a correlation between HRV and long term outcomes such as length of hospital stay, neurodevelopmental outcome and diagnosis of cerebral palsy exists. The recordings were taken from infants who had been previously recruited to a study investigating physiological response to sensory stimulation with simple time domain measures (SDNN, SDNN and SDANN index and RMSD) longitudinally assessed. The ECGs were recorded after receiving multisensory stimulation and the infants were followed up until 1 year of age to assess neurodevelopmental outcome. The subjects were stratified by the presence and nature of brain injury as follows; no brain injury, periventricular leucomalacia, grade III/IV IVH, or a combination of periventricular leucomalacia (PVL) and grade III/IV IVH. HRV was different for each type of brain injury. Compared to the non injured group, PVL was associated with an increase in all HRV indices, whereas IVH demonstrated a significant reduction in HRV. This may however reflect the length of time in stage 1 sleep, which was significantly less in infants with PVL. Determinates of the HRV indices were investigated by multivariate, stepwise regressive models which demonstrated that using routine demographic and physiological variables in the models could account for the observed HRV indices in the non-injured and IVH groups but not in the PVL group. Moreover, the determinants were different, with the non-injured group.

Only in the non brain injured group was a significant correlation found between HRV indices and longer term outcomes (gestational age at discharge, length of hospital stay and neurodevelopmental index scores (mental (MDI) motor (PDI) indices from the revised Bayley Scale of Infant Development (Bayley II))). Increased SDNN and SDNN index were related to increased scores for MDI, decreased total length of hospital stay and earlier gestational age at discharge. That is, the more variability present, the better the outcome at 1 year of age. This however did not hold for the brain injured group where the relationships did not reach significance. The occurrence of cerebral palsy was significantly related to the SDNN and SDNN index across all groups by using analysis of variance (ANOVA). In multivariate models that included central neural injury, gestational age at birth, birth weight, age at study, and mean heart and respiratory rates to describe the various long term outcomes, the accuracy of the models was improved significantly by the addition of HRV indices. The group concluded that in the non brain-injured infant there is the potential for HRV indices to be used as a prognostic marker for neurodevelopmental outcome. The time domain HRV indices which estimate low frequency activity were highly correlated with neurodevelopmental outcome in this group, thus suggesting parasympathetic and cerebral cortex maturity. They hypothesised that the reduction in HRV seen in cases of IVH is due to reduced sensory processing in the brain at 32-36 weeks, which may be a neuroprotective strategy[180]. However, no comment is made about the degree of ventricular dilatation or presence of cerebral oedema or hydrocephalus which may result in reduced HRV.

In summary, brain injuries in preterm and term infants appears to affect HRV with patterns that are distinguishable from the non brain injured infant and between injury types by simple time domain analyses. The relatively small number of these types of brain injuries occurring and the relatively large number of potential confounding variables makes research into using HRV analyses as a potential prognostic tool extremely difficult. However, the potential for analysing intra-individual changes in HRV for use as a monitoring tool to detect or predict the presence of intraventricular haemorrhages in the acute setting is a future possibility.

2.6.3 Sepsis

Late onset neonatal sepsis is a major cause of mortality and morbidity in preterm infants. VLBW infants with confirmed sepsis have a 2.5 fold increase in mortality and more than 30% increase in hospital stay[181]. The early diagnosis and prompt treatment of sepsis is therefore a priority. Griffin et al have, over several years, developed a potential early warning screening tool for late onset neonatal sepsis by investigating the reduced heart rate variability and transient decelerations that occur up to 24 hours before clinical signs of sepsis[182-188]. They have termed these decelerations and reduced HRV, "Heart Rate Characteristics" (HRCs) and have used various techniques to ascribe low, medium and high risk HRC's to neonatal ECG recordings. Firstly, the skewness, or the third moment about the mean, of NNi when plotted on a histogram was used[185], later refined to a more flexible measure of "skewness", termed sample asymmetry[187]. In addition, HR entropy is assessed as this has been demonstrated to be reduced prior to the onset of sepsis[189]. The authors suggest that monitoring HRC is useful as a screening test for the development of sepsis[188]. They argue that HRC monitoring has a receiver operator curve (ROC) area (0.76), positive predictive value (15% risk of sepsis, urinary tract infection (UTI), or death in next 7 days) and a significant increase in risk for the high to low risk recordings to warrant being used as a screening tool[182,188]. However, whilst these results are encouraging they do not demonstrate a tool which presently can initiate or change management. A positive predictive value (PPV) of 15% indicates a high number of false positive results, which is also commonly seen with biochemical tests for neonatal sepsis[190].

In an additional study the accuracy of HRC in predicting sepsis was further assessed by ascribing a "sepsis" risk score to HRC as well as clinical information (feed intolerance, apnoea, Immature white cell count proportion etc)[186]. It was demonstrated that whilst HRC monitoring in the preceding 24 hours is correlated to the risk of developing sepsis, provides independent information towards the diagnoses and becomes abnormal in infants who develop sepsis, it added little information to those infants deemed at high risk from their clinical features alone. That is, a low HRC did not exclude the risk of sepsis in an individual deemed at risk from their clinical features alone. It was argued that whilst HRC monitoring uses existing data, and is

associated with imminent non-specific illness, it can be used as an adjunct to clinical information in predicting late onset sepsis.

In addition to using HRC to predict the development of sepsis the research team have also included HRCs in a regression model with demographic and laboratory data, producing an HRC index to predict adverse events (sepsis, UTI or death). This HRC index was validated in two centres, demonstrating that high risk HRC measurements had a 5 to 6-fold increased risk for an adverse event in the following 24 hours, increasing to 6 to 7-fold when associated with abnormal laboratory investigations[191].

The development of the above described method is unique in that it is the only example of neonatal HRV monitoring which is available to be used in the clinical environment. HRC monitoring currently appears to be a non-specific marker of illness development, however it may lead to new monitoring strategies with future refinement and investigation. HRC monitoring has recently been approved in the USA by the FDA and the team are now investigating whether or not it improves the outcome for preterm infants in a multicentre study.

2.6.4 Patent Ductus Arteriosus

Infants with a PDA had a higher heart rate and short term variability but reduced long term variability when compared with matched controls. Interestingly, these observations did not change following successful closure with indomethacin[174]. In another study however, successful closure of the duct with indomethacin associated with an improvement in ventilation, demonstrated a reduction in mean heart rate and an increase in long term variability. Infants whose ventilatory requirements did not improve showed smaller changes in HRV, while those who deteriorated showed no change in HRV indices[192]. The authors suggested that the changes seen following successful closure are due to an improvement in brain stem oxygenation. However, no comment is made on the reduction in preload and left ventricular failure which accompanies the successful closure of a PDA.

2.6.5 Small for Gestational Age

Infants who are small for gestational age (SGA) with intra-uterine growth restriction (IUGR) are at a higher risk of perinatal mortality/morbidity[193,194], sudden infant death syndrome[195-197], have poorer neurodevelopmental outcome[198] and in adulthood have a higher risk of cardiovascular disease (the Barker hypothesis[199]). Spassov et al conducted a case control study, analysing ECG recordings taken from term infants who were either SGA (<3rd percentile for weight) or appropriate for gestational age (AGA)[82]. SGA infants had a faster resting heart rate and reduced power within all frequency bands except for LF during quiet sleep. Galland et al investigated HRV during sleep at rest and also following head up tilting in SGA (<10th centile) and AGA infants up until 3 months of age. Importantly they excluded infants whose mothers had smoked during pregnancy as this has been shown to suppress autonomic activity both in utero and in early life[200-202]. Whilst mean heart rate and 'raw' HRV were not significantly different, when the HR was included as a covariate, SDRR/SDΔRR (a measure related to the LF:HF ratio) became significantly higher in the SGA than in the AGA infants. This is suggestive of higher sympathetic and lower vagal tone in SGA infants. Following the intervention of tilting, the heart rate reflex responses were reduced in SGA infants, suggesting a less mature or compromised ANS. Moreover, these differences persisted when tested at 1 and 3 months of age. These results suggest that SGA infants are less able to respond to both intrinsic and extrinsic stressors, such as blood pressure fluctuations, and may partly explain the increased incidence of SIDS seen in these infants.

The higher sympathetic tone in SGA infants supports the hypothesis suggested by Barker[199]. Sympathetic over-activity is well recognised in association with increased cardiac mortality, stroke, diabetes, higher systolic blood pressure and increased percentage of body fat.

2.7 Previous methodologies used to investigate neonatal HRV

The above review demonstrates the heterogeneous nature of previous research into neonatal heart rate variability. However, whilst the studies vary in methods, indices and conclusions the majority have a common theme. That is, they are limited to the research environment and do not use methodologies which are applicable to clinical monitoring and utilisation. Frustratingly, the monitoring environment on the NICU already contains the raw ECG data required to obtain heart rate variability indices, yet the methods to obtain clinically applicable metrics are not presently available. Challenges to developing these methods include the quality, enormity and variety of the data produced and the equally enormous clinical issues of what to monitor, when and how to monitor it, in whom, and how it can be used to diagnose specific illnesses. The only example of HRV measurements being used in the clinical environment is in Virginia, USA. Griffin et al have used a measure of heart rate variability and transient decelerations, termed "Heart Rate Characteristics" (HRC), to continually monitor preterm infants at risk of sepsis. The result is a monitoring system which, according to the research group, allows the diagnosis and treatment of sepsis in infants who have yet to displayed signs of illness[188]. The research team must be applauded for their methodical, systematic approach to tackling the assessment of heart rate variability to detect the onset of sepsis.

The other reported methods used to assess heart rate variability vary in their applicability to be used as a clinically useful monitoring tool. For example, whilst frequency domain analysis provides information regarding the function of both branches of the autonomic nervous system it has been demonstrated to be of limited usefulness in detecting abnormal records[203] even when using the superior method of Lomb periodogram analysis suggested by Moody[204]. Whilst being recognised as a good measure of cardiac risk in adults, it is argued that Fourier analysis of ECG data is practically unreliable (and hence clinically impractical) as a single ectopic, missed or misread beat in a 15 minute sequence can change the spectral parameters by up to 400%[205]. The major problem with using the Fourier transform to provide spectral components of the ECG is that in order to provide reliable results it assumes that the signal is stationary, i.e. that the same frequency components are present throughout

the whole recording. Whilst this assumption can be overcome by paying particular attention to experimental protocols and recording conditions in physiological studies, using the Fourier transform method to monitor frequency changes in "real world" ECG recordings is impractical. The autonomic nervous systems raison d'etre is to continually adapt to both internal and external stimuli and is therefore, by definition, continually fluctuating in its activity. Methods to detect these fluctuations are thus required to accurately describe the relative functioning of both branches of the autonomic nervous system. These methods require the ability to simultaneously describe the ECG signal in both frequency and time domains.

Rassi et al have described a time domain method to quantify the frequency components within neonatal ECGs. The LF and FF components were extracted from the RRi tachogram by using Finite Impulse Response (FIR) filtering. A 256th order filter with a low cut-off of 0.2Hz and a high cut-off of 2.0 Hz was used for the HF components, with the LF components obtained by a 512th order filter with cut-offs at 0.05Hz – 0.2 Hz.[106]. Finite Impulse Response filters are a type of digital filter which are simple to implement and introduce a linear phase shift and thus a constant time delay[206]. This time delay can be easily eliminated, to preserve the original time relationship. Rassi et al studied the interrelations between LF and HF components over short time periods (resolution of one period of LF oscillations i.e. 0.2 Hz or 5 seconds) and demonstrated that in most cases an increase in the LF component is correlated with a decrease in the HF component (and vice versa) with a time delay. This observation in very short time windows is in agreement with previous studies which have examined sympathovagal balance over much longer periods (from tens of seconds to several days)[92,97]. Of most interest however is the methodology employed, which allows the near real time quantification of LF and HF components in the time domain. However, since publishing the results of this analysis on 9 preterm infants recordings of 45 minutes duration, the research team have not used this technique to investigate autonomic functioning in preterm infants in either physiological or pathological conditions.

2.8 Methodologies proposed for the investigation of neonatal HRV in this thesis

The aims of this thesis are to develop a method which is capable of taking the raw ECG data obtained from the routine monitoring of neonates in intensive care and to produce HRV metrics which can be utilised to provide clinically useful measurements of autonomic activity. This will take several steps, namely;

i. Recording the neonatal ECG
ii. Identifying each normal heart beat
iii. Quantifying both time and frequency domain parameters of HRV
iv. Comparing HRV measures in different groups of babies with expected differences in ANS activity

The methodology will be developed and validated with ECGs containing neonatal characteristics with known parameters. It will then be applied to real world data obtained by the routine monitoring of babies in the NICU.

Chapter 3

Monitoring on the Neonatal Intensive Care Unit and Methodology Development

3.0 Background

In the past decade there has been a rapid proliferation in the use of information technology on the neonatal intensive care unit. This has arisen because of the need to represent the increasing volumes of data being produced by the more sophisticated cotside monitoring and the expanding number of diagnostic tests available. To aid clinicians, patient information systems have been developed which receive information directly from the cotside monitors, ventilators, laboratories and other hospital systems. Clinical documentation can then be inputted by the attending physicians and nursing staff to provide a complete, cotside patient management system. These systems have been proven to be valuable and accurate tools for documentation, data collection, and the on-screen display of patient information[207-210]. However, whilst it is recognised that intensive care environments lend themselves particularly well to computer involvement, it is essential that computers are not used simply because they are advanced technology and imperative that they do not detrimentally interfere with direct patient care[1,211-212].

3.1 Physiological Monitoring in Neonates

Physiological parameter monitoring is of vital importance when caring for sick infants. The reasons for this include;

a. They are physically small, enclosed within incubators, sometimes inside plastic bags to maintain their temperature or under phototherapy lights to treat jaundice. This makes observation of their clinical status difficult.

b. The fragile infant requires as little handling as possible to ensure thermal and fluid stability[1] and to allow adequate rest between potentially frequent handling procedures[213].

c. Neonatal units often have a 1:2 or 1:3 nurse/patient ratio. Physiological trend screens can be viewed from a distance, enabling the attending teams to assess an unstable baby away from the cotside.

d. Sick infants deteriorate quickly, in seconds or minutes. This is due to immature homeostatic systems as well as the inability to communicate a deteriorating condition[1].

It can be appreciated that neonatal patients are uniquely disadvantaged due to their size and inability to communicate. Clinical information available from neonatal patients may frequently be limited to the physiological parameters measured on the monitors[214]. It is therefore of vital importance to obtain as much information as possible about the status of the infant from these parameters.

The information provided by physiological monitoring aids decision making regarding management and also acts as an alert to any change in condition of the baby[215]. An infant being cared for on the NICU will have the following signals monitored routinely;

i. Heart Rate. ECG electrodes placed on the infant's chest detect the intrinsic electrical activity within the heart. The ECG records the vector sum of all the electric dipole moments throughout the myocardium and produces a combined trace. The monitor will then measure the time between each R wave to calculate the instantaneous heart rate.

ii. Respiratory Rate. The chest ECG electrodes also detect impedance across the chest wall. As the chest moves with respiration, the impedance fluctuates and is recorded as a "respiration".

iii. Oxygen Saturations. A pulse oximeter is attached to the infant's limb and measures the percentage of oxygenated haemoglobin and blood flow past the probe. Oximeters are capable of distinguishing pulsatile flow from other more static signals (such as tissue or venous signals)

and so will provide only arterial saturations. The pulsatile, arterial flow can also be used to provide information regarding the heart rate.

iv. Blood Pressure. The measurement by an umbilical or peripheral arterial catheter is widely accepted as the optimum method for monitoring blood pressure[216]. Using oscillometric cuff methods may overestimate the blood pressure if it is low, providing false reassurance [217-219]. The umbilical arterial catheter is inserted through the umbilical arteries and sits in the aorta. The catheter is connected to a fluid filled line which in turn is connected to a pressure transducer. Pressure changes in the aorta are transmitted along the fluid filled line and the pressure waveform is detected by the transducer, resulting in a pressure reading as well as a blood pressure waveform.

All infants receiving intensive care will have their heart rate, respiratory rate and oxygen saturations measured. Blood pressure monitoring is reserved for the sicker babies as it requires the insertion of an invasive, intra-arterial catheter. Occasionally an individual baby may require cerebral electrical activity monitoring, either by electroencephalogram or cerebral function monitoring, but this is not, as yet, routine. Finally, more sophisticated monitoring tools are available but are currently only being utilised as research tools including; (a) Near Infrared Spectroscopy which enables the non-invasive monitoring of tissue oxygenation and haemodynamics, (b) Cerebral Impedance Tomography, which can detect real time changes in cerebral blood volume and intraventricular haemorrhage[220].

3.1.1 Physiological trend monitoring on the NICU

Physiological data is unique as it is continuously recorded, providing both instantaneous monitoring and the ability to observe time-stamped trends in physiological parameters. Longitudinal measurements obtained from continuous monitoring can be displayed in trend graphs, allowing changing pathology to be observed in real time. This trend monitoring has three main areas of use; (i) as a real time clinical aid to patient management, e.g. apnoea of the newborn; (ii) as a research

tool, demonstrating the effects of procedures on physiology; (iii) for educating members of staff about how physiological events develop.

On the NICU at LWH information obtained from standard monitoring equipment is presented by cotside computers as trend graphs. These graphs can be manipulated to provide trends over varying time periods, from minutes through to days. (figure 3.1). Figure 3.1 displays the trend graph for heart rate, oxygen saturations (SpO2), respiratory rate and blood pressure for an individual baby over a 24 hour period. On the right hand side of the figure, the numbers correspond to the instantaneous recordings of the physiological parameters. In this example it can be seen that the heart rate and blood pressure are steadily decreasing from the beginning of the recording until 02:00. At this time there is an abrupt increase in both of these parameters which corresponded with the commencement of inotropic support.

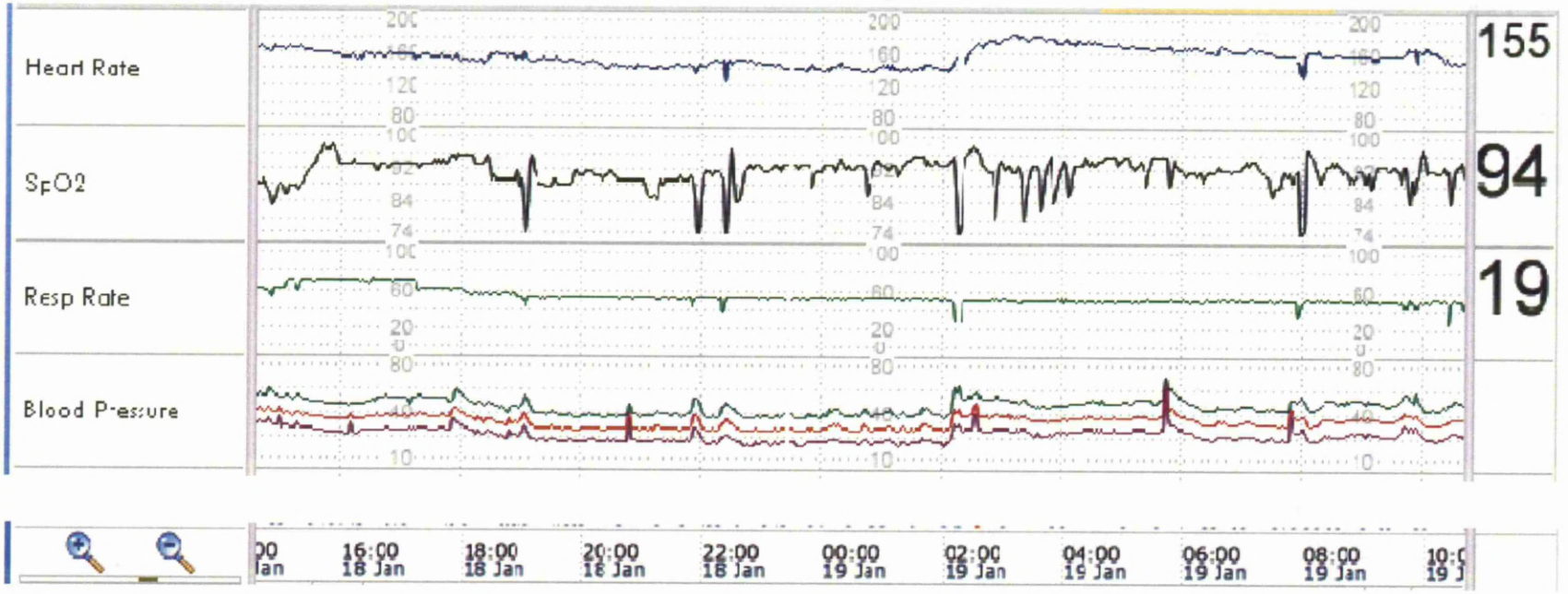


Figure 3.1 A physiological trend graph from a neonate. The heart rate, oxygen saturations (Spo2), respiratory rate and blood pressure are reported as instantaneous measurements (numbers on the right side of the graph) and also as trends over the previous 20 hours.

Figure 3.2a

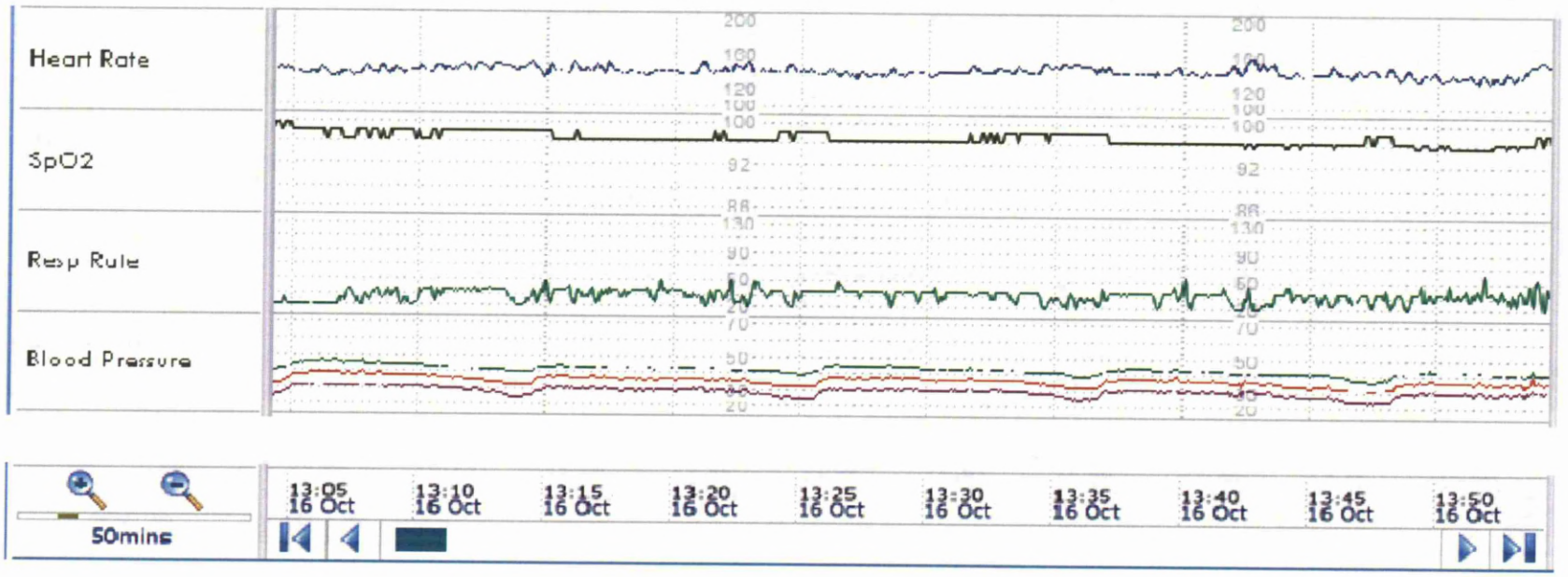

Heart Rate
SpO2
Resp Rate
Blood Pressure
50mins
13:05 16 Oct
13:10 16 Oct
13:15 16 Oct
13:20 16 Oct
13:25 16 Oct
13:30 16 Oct
13:35 16 Oct
13:40 16 Oct
13:45 16 Oct
13:50 16 Oct

Figure 3.2b

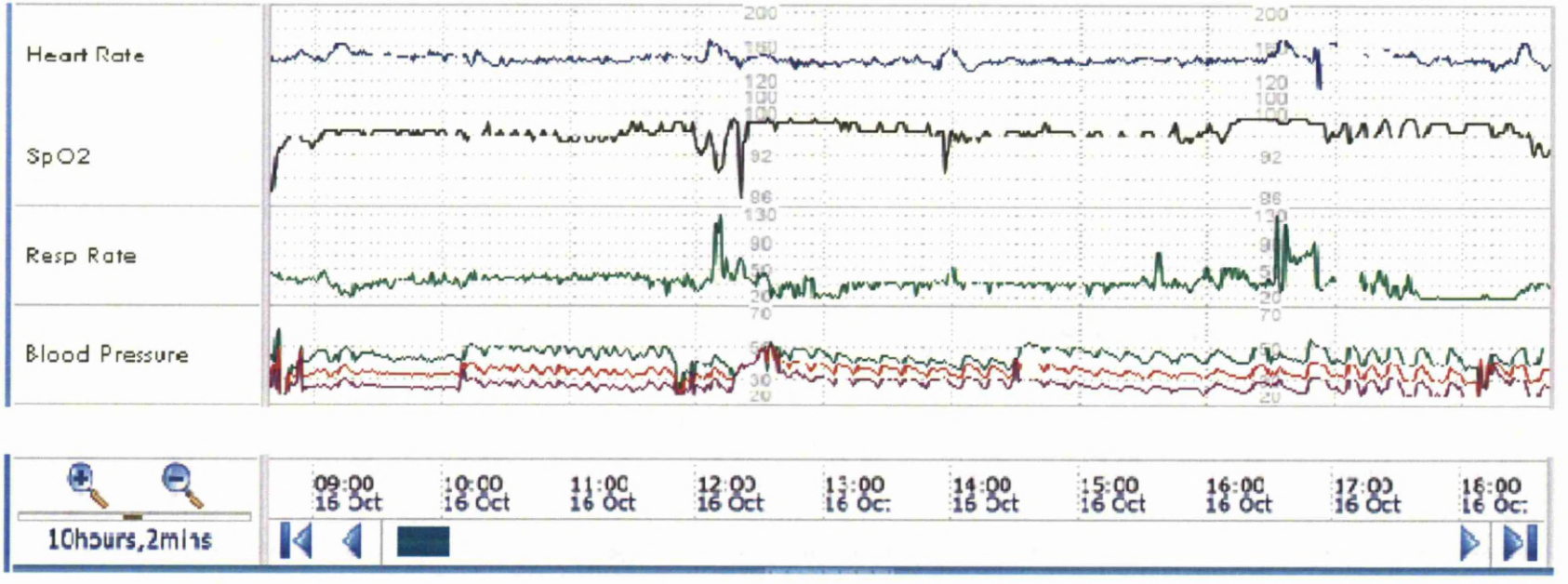


Figure 3.2a and 3.2b A physiological trend graph from a neonate. Fig 3a is over a 50 minute time period, whilst 3b is over a 10 hour time period. The rhythmic harmonics in the arterial waveform are only apparent when the time scale is adjusted.

The physiological trend graphs can be manipulated, with both the x axes (time) and y axes (measurement) having an adjustable scale. The clinical importance of this is clearly demonstrated in figure 3.2a and b, where the same recording viewed over different time scales yields different patterns. The blood pressure recording when viewed over 10 hours demonstrates rhythmic fluctuations in blood pressure which are not apparent when the trend for 50 minutes is observed.

Observation of these trend graphs is used by clinicians to aid in the clinical assessment of the infant. The accuracy of this method has not previously been assessed. In work related to this thesis, clinicians were able to determine whether or not a neonate developed sepsis with 53% sensitivity and 80% specificity when the preceding 48 hours of physiological trend graphs was observed. When clinicians were confident, their accuracy in correctly assigning the record as being from an infant who did or did not develop sepsis was 82% (IQR 67–88)[221]. (See section "Publications arising from this thesis").

3.2 The Monitoring Environment on the Neonatal Unit

The monitoring environment consists of two separate networks, the unity and hospital networks These networks are connected to a PC (termed "datacollect1") allowing the exchange of data between them. The two networks utilise a patient data management system called "The Badger System" which integrates the data and presents all laboratory results, physiological monitoring, radiological reports, ventilator information and patient notes at the cotside in a web based browser.

The unity network consists of the physiological monitors which display the physiological waveforms (ECG, plethysmography, blood pressure and respiratory rate) for each baby at the cotside. The hospital network consists of the cotside PCs and the NICU server. The NICU server stores all current patient data within the Badger System, obtains and stores physiological trend data and is responsible for the storage and retrieval of all laboratory data. The data is then provided to the Badger system for display at the cotside.

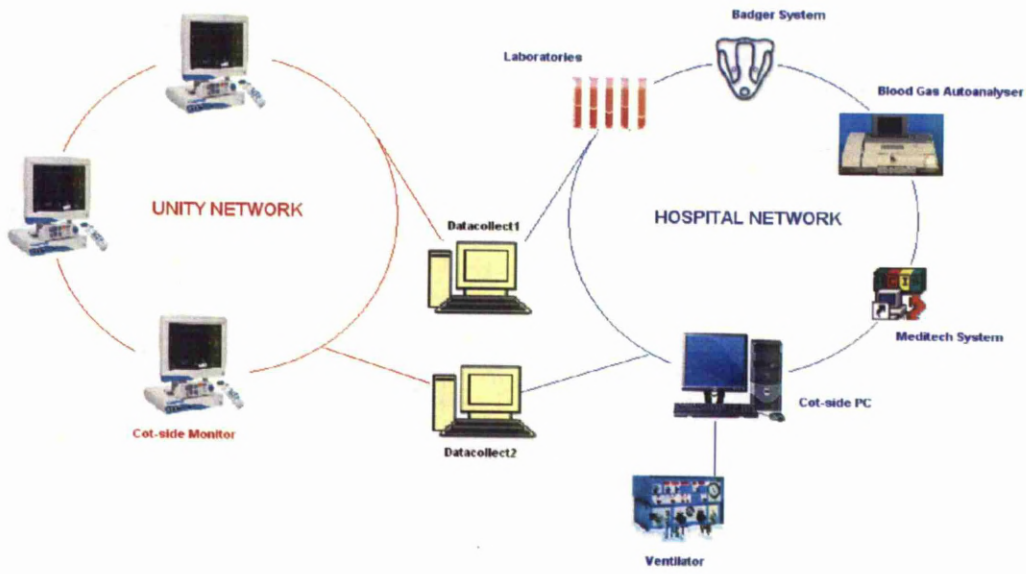


Figure 3.3 A schematic overview of the two networks and the datacollect PCs. Datacollect2 is the PC used to obtain the waveform data used in the thesis.

3.3 Acquisition of Physiological Waveform Data

The first step in analysing heart rate variability is the acquisition of the raw ECG data. All infant physiological data is sent from the solar 8000 monitors to the datacollect PC via a private Ethernet referred to as the unity network (see above). For this thesis, a separate PC was installed, termed datacollect2, which sat alongside datacollect1 and is also connected to both the unity network and the general hospital network. (figure 3.3).

A data acquisition program called "solar 8000 waveform capture" was installed on Datacollect2. This program was written by Clevermed (the team who are responsible for the development of the Badger system) using the Delphi development environment (program language based on Pascal). The data acquisition program is activated by double clicking on the "solar8000waveformcapture" icon on datacollect2. This opens up the user interface (figure 3.4).

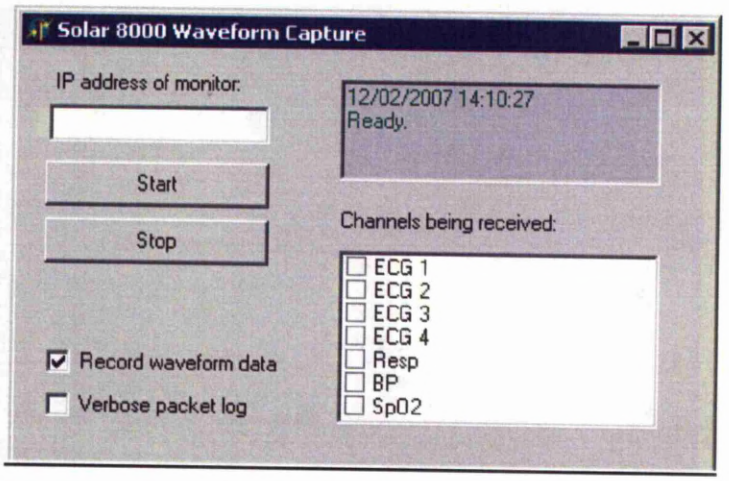


Figure 3.4 The Solar 8000 waveform capture program window

Each cotside monitor has a unique IP address. By inputting this into the window "IP address of monitor:" and selecting the start button, the program begins to acquire the data across all channels that are currently being monitored (figure 3.9).

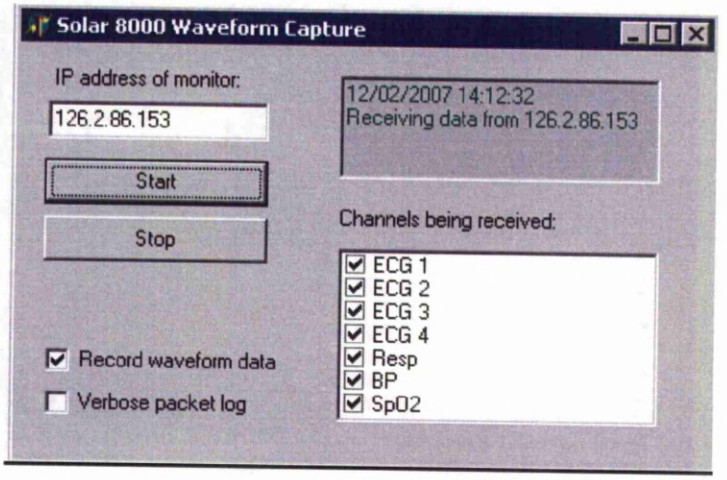
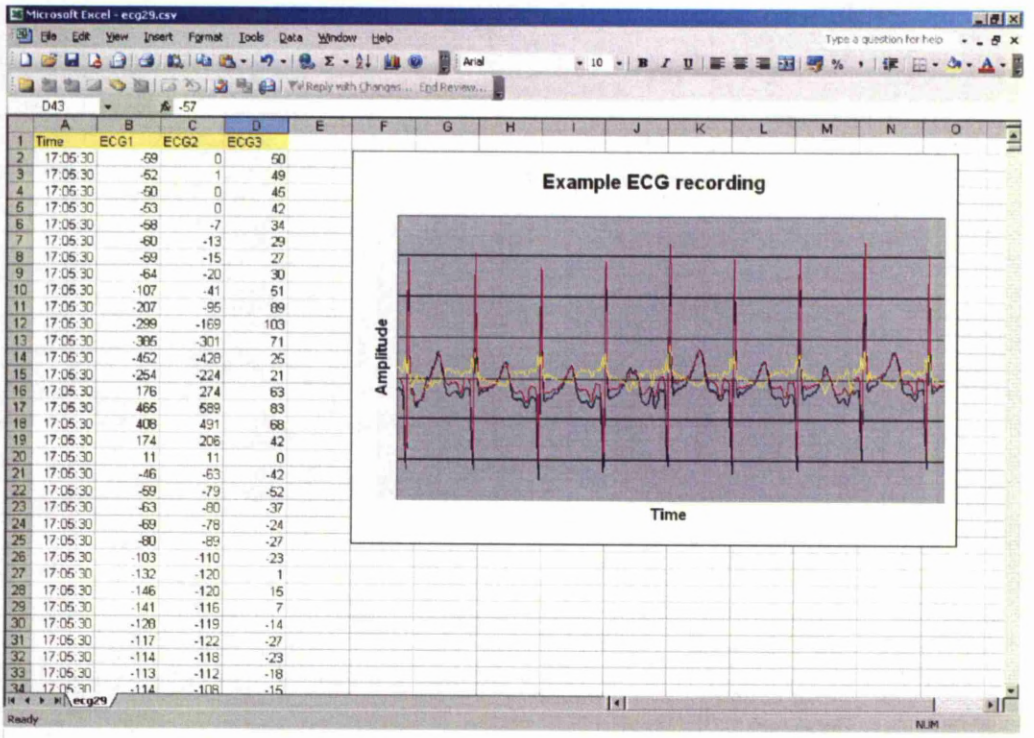


Figure 3.5 The IP address relating to a particular cotside monitor is inputted. All 7 channels are "ticked" indicating they are all being recorded.

Given the IP address of a particular monitor, the software obtains packets of wave data at 4 Hz. The monitor returns 0.25 seconds worth of waveform data for every available data channel. The monitors provide waveforms at different sampling rates depending on the channel. The ECG data are sampled at 240Hz, whilst the blood pressure (BP), respiratory pattern (Resp) and plethysmography (SpO2) waveforms are sampled at 60Hz. The ECG channels are termed "ECG 1", "ECG 2" and "ECG3" representing the different leads that are recorded from the three ECG skin probes. ECG 4 is a redundant channel.

The encoded binary data received from each monitor is then converted by the waveform acquisition software into plain text. The data packets returned by each monitor contain sequence numbers, but no timestamp. The time to which each sample corresponds is estimated by the data collection machine. Whilst in theory, sample transmission is affected by network latency and contention, the use of the dedicated unity network means that latency is of the order of milliseconds and dropped packets are rare. Multiple instances of the software can be run simultaneously to obtain waveform data from several different monitors.

The waveform data is saved as 'comma separated variable' or '.csv' text files. These files can be read by a variety of software programs including Microsoft excel and Matlab, enabling subsequent visualisation and signal processing of the recorded signal. Once imported into Microsoft excel it is possible to create a line graph of each of the three channels, which results in the typical characteristic ECG wave pattern. An example of the file imported into excel with a short sample line chart can be seen in figure 3.10.



	Time	ECG1	ECG2	ECG3
2	17:05:30	-59	0	50
3	17:05:30	-52	1	49
4	17:05:30	-50	0	46
5	17:05:30	-53	0	42
6	17:05:30	-58	-7	34
7	17:05:30	-60	-13	29
8	17:05:30	-59	-15	27
9	17:05:30	-64	-20	30
10	17:05:30	-107	-41	51
11	17:05:30	-207	-96	89
12	17:05:30	-299	-169	103
13	17:05:30	-386	-301	71
14	17:05:30	-452	-428	25
15	17:05:30	-254	-224	21
16	17:05:30	176	274	63
17	17:05:30	466	589	83
18	17:05:30	408	491	68
19	17:05:30	174	206	42
20	17:05:30	11	11	0
21	17:05:30	-46	-63	-42
22	17:05:30	-69	-79	-52
23	17:05:30	-63	-80	-37
24	17:05:30	-69	-78	-24
25	17:05:30	-80	-89	-27
26	17:05:30	-103	-110	-23
27	17:05:30	-132	-120	1
28	17:05:30	-146	-120	15
29	17:05:30	-141	-116	7
30	17:05:30	-128	-119	-14
31	17:05:30	-117	-122	-27
32	17:05:30	-114	-118	-23
33	17:05:30	-113	-112	-18
34	17:05:30	-114	-108	-15

Figure 3.6 The derived text file of ECG waveform data is imported into excel. By plotting a line chart the original wavefrom pattern can be visualised.

The CSV file format is also able to be read by a variety of programs which can apply sophisticated signal processing techniques to obtain further information from the signal, for example HRV. The program used in this thesis for signal processing is Matlab© (Matlab 7.5, The MathWorks Inc., Natick, MA, 2007). Matlab© is a software package for scientific and engineering computation which integrates numerical analysis, matrix computation, signal processing and graphics.

3.4 HRV analysis Schematic

The analysis of HRV in either the time or frequency domain consists of several steps which are summarised in figure 3.11. Further description of the HRV analysis will be demonstrated in the following chapters.

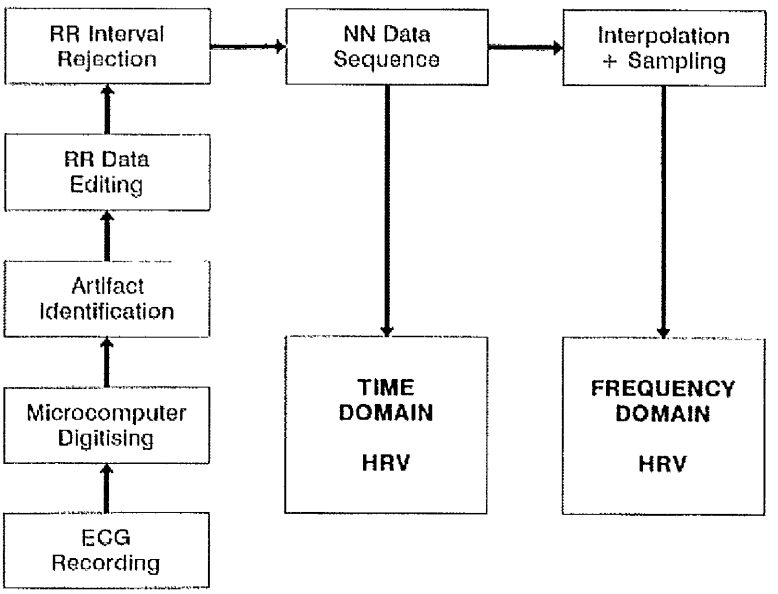


Figure 3.7 Flow chart summarizing individual steps used when recording and processing the ECG signal in order to obtain data for HRV analysis.

Chapter 4

Automatic R wave detection in Neonatal ECG recordings

4.1 Terminology used to describe R waves and their intervals

The terminology used to describe the time interval between R waves can be confusing. RR refers to the time interval between two successive R-waves in the ECG. These can include ectopic beats where an R wave is present. The normal-to-normal R-wave (NN) refers to the time interval between two successive "normal" R waves, that is R waves occurring along with a p wave, indicating normal propagation of electrical impulses through the heart muscle. The RR interval time series (RRi) plots successive RR intervals producing a graphical display of the RR intervals. This display includes RR intervals, with ectopic or missing beats being potentially identified as outliers (see figure 4.1). The normal-to-normal interval time series (NNi) applies the same rules but only includes the "normal" RR intervals.

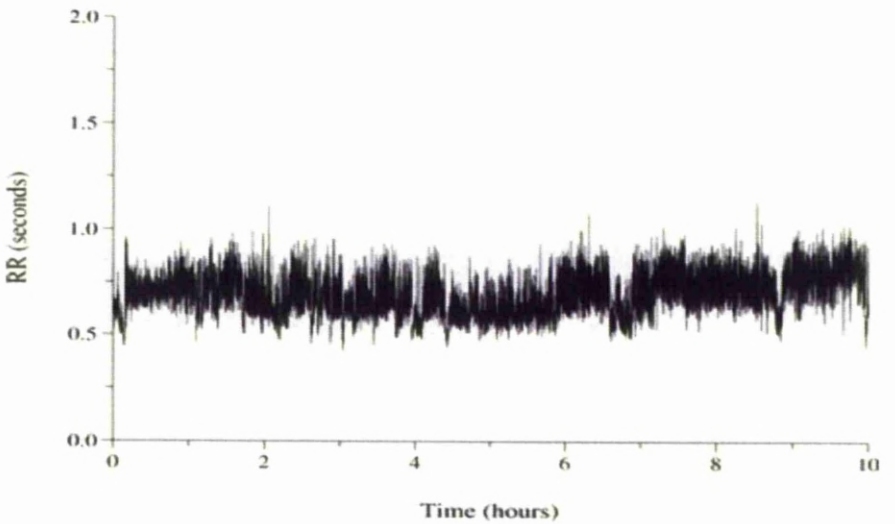


Figure 4.1 RR interval time series (RRi). Each RRi interval is plotted against time, producing a graphical display of the RRi.

4.2 Automatic R wave detection in adult ECG recordings

The first step in obtaining a measurement of HRV is the accurate detection of each heart beat originating from the sinoatrial node. As discussed in 2.3, the fiducial marker for each beat is the "normal" R wave. This is a difficult process, made even more problematic in the neonate, by the presence of large volumes of noise, artefact and ectopic beats.

Standards produced by the Task Force of the European Society of Cardiology and The North American Society of Pacing and Electrophysiology state that it is necessary to use a well tested algorithm in order to locate a stable and noise-independent reference point[50]. A large variety of methods for the accurate detection of the interbeat interval have been proposed and used, featuring high percentages of correct detection. All of these studies have been conducted using Adult derived ECGs. There are no published data on the accuracy of fiducial point detection in neonatal ECGs.

Friesen *et al.* compared nine QRS detection algorithms with different types and volumes of synthesized noise[223]. The QRS detection methods were based on: i) amplitude and first derivative, ii) first derivative only, iii) first and second derivative, and iv) digital filtering. All of these algorithms used fixed detection thresholds. The algorithms were evaluated with a "gold standard" ECG which was obtained from a human volunteer. A single cycle of the ECG was digitised, copied and appended to itself repetitively to make a 32 second ECG record strip. This gave an ECG with a constant heart rate (62bpm), QRS complex (80ms) and R-wave amplitude (1.08mV). The added noise consisted of five different representative sources: i) electromyographic noise, ii) powerline interference, iii) baseline drift due to respiration, iv) abrupt shifts in baseline and v) a composite of the above, and were added to the ECG at four different levels: 25,50,75 and 100 percent of the maximum amplitude.

No single algorithm in the study was superior for all types of noise. The digital filter algorithm adapted from Engelese and Zeelenberg[224] was able to perform well with different combinations of noise but became less accurate with noise greater than 50% of the maximum signal amplitude. However, the effectiveness of the digital filter algorithm were in part attributed to the powerline notch filter which could also be used in a pre-processing stage for any of the other algorithms. The choice of "gold standard" ECG in this study is questionable. Whilst the repetitive short segment ECG provides a constant waveform pattern with constant characteristics, the true underlying dynamics of a recorded ECG can never be known[45]. The

authors do not comment on how they determined the benchmark fiducial point for the QRS complex to which they then compare the different algorithms' accuracies. Furthermore, Daskalov *et al.* applied these algorithms to selected synthesised signals containing records with pronounced baseline drift. The results were unsatisfactory, presumably due to the use of fixed detection thresholds, whereas adaptive ones would be more appropriate[225,226].

Other studies have used digitised ECG recordings from human subjects taken from the arrhythmia database from the Massachusetts Institute of Technology and Beth Israel Hospital (MIT-BH). This database contains 48 half-hour excerpts of two-channel ambulatory ECG recordings, obtained from 47 subjects studied by the BIH Arrhythmia Laboratory between 1975 and 1979. The recordings are digitized at 360 Hz per and independently annotated by two or more cardiologists. The annotations labels point to specific locations within the recording and describe events at those locations, for example, the occurrence of each individual heart beat ("beat-by-beat annotations"). The annotations are computer-readable and are used as the "gold standard" to assess the accuracy of the various QRS detection methods[74].

Poli *et al.* used a generic algorithm for QRS detection whereby complexes were emphasised with respect to the rest of the signal by polynomial filters and compared to an adaptive threshold. The authors reported 99.60% sensitivity (Se) and 99.51% specificity (Sp)[227]. This method is however unable to perform in real time. Afonso *et al* proposed hardware filter banks for ECG signal decomposition, where several parameters were independently computed and combined in a decision rule. The authors reported Se = 99.59% and Sp = 99.56% for their real-time, single-channel beat detection algorithm[228]. Dotsinsky and Stoyanov developed a heuristic, pseudo-real-time algorithm for ventricular beat detection for a single-channel ECG recording, based on steep edges and sharp peaks evaluation criteria. They reported Se = 99.04% and Sp = 99.62%, obtained with two channel recordings[229]. Moraes *et al.* combined two different algorithms working in parallel – the first was taken from the work of Englese and Zeelenberg[224] and the other was based on Pan and Tompkins[230], and Ligtenberg and Kunt[231]. They reported Se = 99.22% and Sp = 99.73% after having excluded records of patients with a pacemaker fitted. After excluding recordings containing high amplitude noise, the statistical indices rises to Se = 99.56% and Sp = 99.82% [232]. Li *et al.* used wavelet transforms for detection. They reported 0.15% false detections in 46 files from the database[233]. Christov *et al* used two real-time detection adaptive threshold

algorithms. The threshold combined three parameters: an adaptive slew-rate value, a second value which rises when high-frequency noise occurs, and a third one intended to avoid missing of low amplitude beats. The algorithms were used with all MIT-BIH arrhythmia ECGs and demonstrated sensitivity $Se = 99.69\%$ and specificity $Sp = 99.65\%$ for Algorithm 1 and $Se = 99.74\%$ and $Sp = 99.65\%$ for Algorithm 2[226].

By using the standardised MIT-BIH database, the performance of the various algorithms can be compared; however the choice of these recordings as "gold standard" does have limitations. The annotation method relies on human input to correctly identify the instantaneous heart beat. This "expert" interpretation is flawed as human input is affected by factors apart from the presented waveform. In this thesis the chosen "gold standard" is synthetic ECG signals with known input variables. This complies with guidelines for HRV analysis which state that equipment should be tested with signals of known HRV properties rather than with databases of already digitised ECGs[50]. Furthermore, the large variety of QRS detection algorithms, and the continuous efforts for their enhancement, proves that universally acceptable solution has not yet been found[226].

4.3 Accurate R wave detection in Neonatal ECG recordings

The accurate detection of the fiducial R wave is made more difficult in the neonatal ECG by the increased presence of noise and artefact in the ECG. Noise can be referred to as persistent contaminant of the signal whereas artefacts indicate transient interruption such as limb movement[45]. Noise and artefact contamination are a particular problem in the analysis of neonatal ECGs due to the intensive care environment, their small physical size and immature, frequent movements. Sources of contaminants include[223]:

I. Power line interference. Mains electricity is provided at 50Hz with an amplitude of up to 0.5 of full scale deflection (FSD) of the ECG amplitude;
II. Electrode contact noise/artefact. Loss of contact or movement between the electrode and skin results in either complete or partial loss, saturation at FSD levels or baseline jumps in the ECG signal. This is a particular problem in premature neonates who are initially cared for in a humid environment and have immature, thin skin which makes constant electrode attachment difficult;

III. Electromyographic (EMG) noise. Electrical activity due to muscle contractions is visible between 50 and 10,000 Hz in adult patients with a mean amplitude of 0.1 of the FSD level. In premature neonates movements are primitive, non-purposeful and can be myoclonic in nature. These result in EMG noise and can also disrupt the contact between the ECG electrodes leads and skin;

IV. Baseline drift. This usually occurs from respiration at approximately 0.15 of FSD at frequencies between 1 to 2 Hz in neonates;

V. Electrical device noise. Noise generated by other medical equipment present in the patient care environment at frequencies between 100kHz and 1MHz. Neonatal Intensive Care units have a large number of electrical devices including cotside PC's, drug infusion pumps and monitoring equipment.

VI. Quantisation noise and aliasing. This is the distortion of the signal that occurs when the continuous, analogue ECG is converted to a digital signal for processing.

It can be seen that detecting characteristics in the neonatal ECG provides a significant challenge due to the high levels of signal to noise ratio (SNR). Also, the problem of missing data due to loss of electrode contact and ectopic beats increased the complexity in accurately identifying each normal heart beat. An example of a typical neonatal ECG can be seen in figure 2

There have been no reported comparisons of beat detection algorithms for ECGs with neonatal characteristics. Neonatal ECG characteristics and morphology differ greatly from adult ECG recordings (see section 2.5). The preterm neonatal heart beats at 100 – 180 beats per minute and has a morphology which alters with increasing age. The QRS axis in preterm neonates lies between 65 and 174 degrees, which is "right deviated" when compared with adult ECGs, and is narrow (80ms), increasing with age. QRS morphology in the newborn may have more notches and direction changes than in adults. The Q wave may have a large amplitude (0.55mV in lead III) and secondary R waves may be present[234]. As the work in this thesis was to use noisy, routinely monitored ECG recordings from neonates resident in the intensive care unit a novel R wave detector was required.

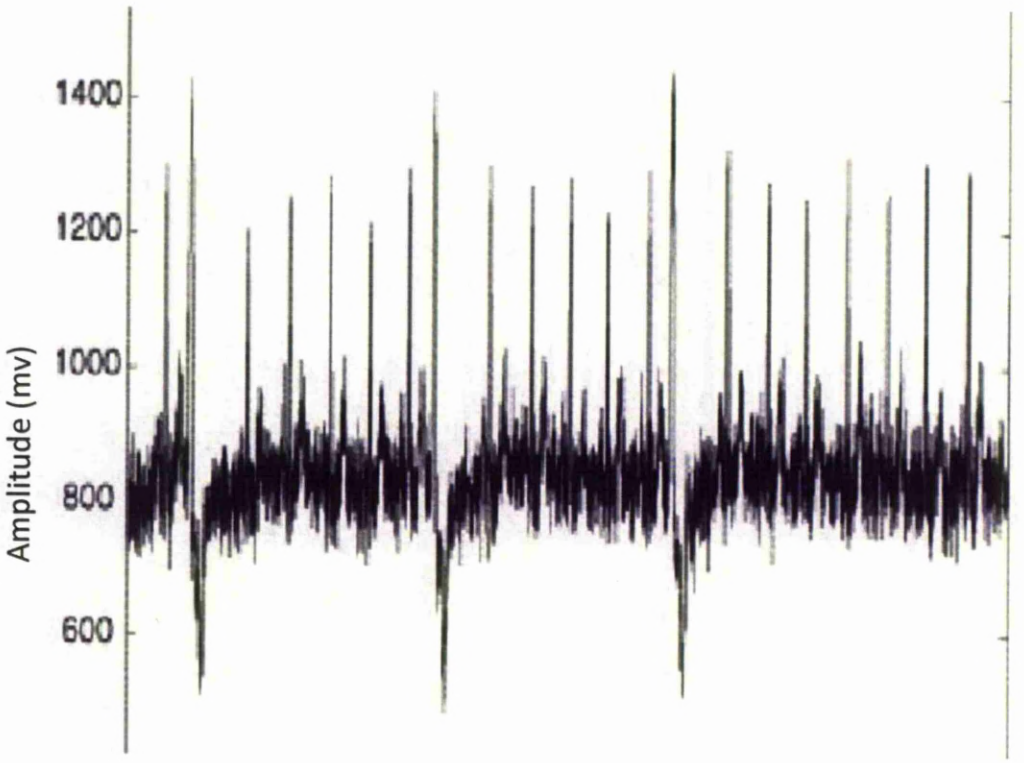


Figure 4.2 Example of a typical routinely monitored neonatal ECG demonstrating the high volume of signal noise.

4.4 Development of an R wave detector

The R wave detector was developed with Professor A.C. Fisher, Department of Medical Physics and Clinical Engineering at the Royal Liverpool University Hospital. All the HRV analysis tools were developed in the MatLab® language extended with the Signal Processing and Statistics Toolboxes (Mathworks Inc., Nantucket). The RR detector relies on the energy in the R wave component of the ECG in the limited bandwidth around 16 to 18Hz being by far greater than in any other part of the cardiac cycle[235]. The main features of the RR detection algorithm are presented in figure 4.3.

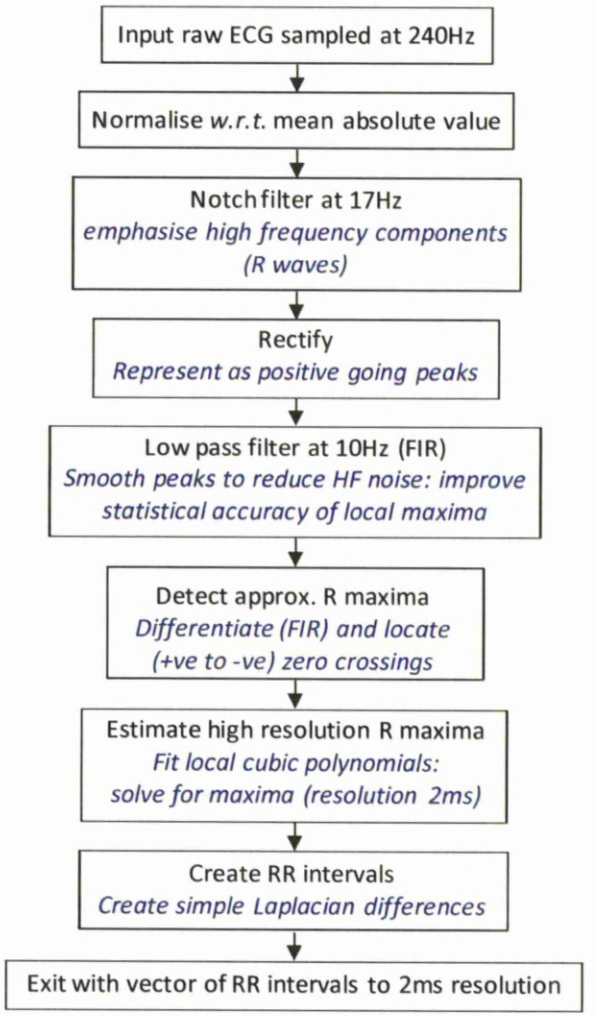


Figure 4.3 Schematic representing the steps in identifying the R wave

The raw ECG when sampled at 240Hz (Fs) is assumed to have a bandwidth of 120Hz *i.e.* up to the Nyquist frequency at 0.5Fs. By normalising the data with respect to its mean absolute value and notch pass-band filtering using a high performance design (128-coefficient least-squares Finite Impulse Response (FIR)filter), the R wave energy is effectively isolated as a bipolar feature. This is rectified to positive-going only by computing its absolute value and smoothing the result with a 10Hz Infinite Impulse Response (IIR) Butterworth filter which results in a series of 'low noise' peaks which statistically imply the occurrence of 'R events'. The temporal locations of the maxima of these peaks are relatively easily determined by low-pass differentiation (using a 64 coefficient FIR implementation) and locating the +ve to –ve zero-crossing transitions. The resolution of this stage is limited by the rather conservative Fs of the ECG recording (240Hz). By resampling (up-sampling by x10) the data segments +/- 10

ms around the *approximate* temporal positions of the R events implied by the differentiation and zero-crossing operations (as above), and then determining the maxima of the local least-squares cubic polynomials fitted to these 20ms intervals, 'R events' can be inferred to a resolution of ~2ms. The RR intervals are subsequently determined from the simple Laplacian difference of the R event vector.

4.5 Validation of the method

The above methodology resulted in the development of two R-wave detectors: i) an 'approximate' detector which detects the peak position of the QRS complex from the zero crossings of difference vectors and ii) an 'accurate' detector which, in addition, interpolates around the QRS complex to find the R wave with greater resolution. A third R wave detector was obtained from the PhysioNet forum[74].

PhysioNet is an internet based forum which belongs to the Research Resource for Complex Physiologic Signals, a cooperative project between Boston's Beth Israel Deaconess Medical Centre/Harvard Medical School, Boston University, McGill University and Massachusetts Institute of Technology. This resource consists of three main components[74];

1. PhysioNet. An online forum for the dissemination and exchange of recorded biomedical signals and open-source software for signal analysis.

2. PhysioBank. An archive of digital recordings of physiologic signals, including ECGs, for use by the biomedical research community. PhysioBank contains signals from healthy adult subjects and patients with a variety of medical conditions including sudden cardiac death, congestive heart failure, and sleep apnoea. There are currently no ECG recordings from infants or children.

3. PhysioToolkit. Contains a library of open source software for signal processing and analysis which is free to download and use. It includes software which can detect physiologically significant events using both simple techniques and more novel methods such as nonlinear dynamics and also allow the interactive display and characterisation of signals.

An R wave detector developed by Dr. Gari Clifford, Massachusetts Institute of Technology, was obtained from the PhysioToolkit. This R wave detector is based on Pan and Tompkins[230] algorithm. Dr Clifford was contacted and assisted in this thesis by altering the R wave detector to better suit the characteristics of the neonatal ECG recordings. A dynamical model for generating synthetic ECG signals termed ECGSYN[238] was also downloaded from the PhysioToolkit and used to produce 'gold standard' ECGs with known parameters.

It is of vital importance that any signal processing technique used in the analysis of HRV is evaluated and quantified in its performance. Whilst ECG recordings from human subjects are available in databases such as PhysioBank the lack of internationally agreed upon benchmarks means that it is impossible to compare competing signal processing techniques. The definition of such benchmarks is hindered by the fact that the true underlying dynamics of a real ECG can never be known. This void in the field of biomedical research requires a *gold standard*, where an ECG with well understood dynamics and known characteristics is made freely available[238].

This need to evaluate signal processing techniques motivated McSharry and Clifford to develop ECGSYN, a dynamical model for generating synthetic ECG signals where the user has the flexibility to input desired characteristics[238]. ECGSYN is freely available through the PhyisoNet website[74] in Matlab and C open source code or can be utilised in a Java applet. ECGSYN is based upon time-varying differential equations and is continuous with convincing beat-to beat variations in morphology and interbeat timing. Extremely realistic ECG signals with complete flexibility over the choice of parameters that govern the structure of these ECG signals in both the temporal and spectral domains are produced. In addition the morphology of the P, QRS and T complexes can be fully specified.

The key features of ECGSYN include[45];

i. Ability to generate multiple ECGs at a range of heart rates and HRV parameters. The heart rate standard deviation and spectral components within two frequency bands are fully user defined. Algorithms can therefore be tested on a vast range of ECGs, some of which can be extremely rare and therefore underrepresented in databases.
ii. The sampling frequency can be varied and the response of an algorithm can be evaluated.

iii. The signal is noise free, so noise can be incrementally added and a filter response at different frequencies and noise levels can be evaluated for differing physiological events.

For evaluating and quantifying the performance of the R wave detectors, ECGSYN was utilised to produce a database of synthetic "neonatal" ECGs. These ECGs included normal values for heart rate and HRV (standard deviation of heart rate) obtained from previous studies of term and preterm infants[53,87,149]. White noise was then added to the ECG recordings in increasing increments to assess the performance of the R wave detectors with increasing SNRs.

Two hundred and forty ECGs were synthesized each with unique input characteristics. The following parameters were used:

1. Internal Sampling Frequency. This is the frequency at which the underlying ECG is produced. This was set at 480Hz.
2. External Sampling Frequency. The frequency which the underlying ECG is sampled. Set at 240 Hz, the same sampling frequency that the Solar8000waveform program samples ECG data on the neonatal unit.
3. Heart Rate. ECGs were produced with a range of different heart rates reflecting rates seen in previous studies of term and preterm infants[53,87,149].
4. Standard Deviation of heart rate. Normal values obtained from previous studies informed the range of input standard deviations
5. Time of recording. Each ECG recording lasted for 60 minutes.
6. LF:HF ratio was fixed at 0.5 as for this assessment of the R wave detectors the frequency components were not of interest.

Following the production of 40 ECGs with different heart rates and standard deviations, random white noise was added using the Matlab function "awgn" at 6 uniform fractions of the amplitude of the ECGSYN produced waveforms of 1.4. The accuracy of the three R wave detectors in detecting each QRS complex was then assessed.

Parameter	Input Values
Heart Rate	60, 80, 100, 120, 140, 160, 180, 200 bpm
HRV (stdev of HR)	0, 1, 2, 4, 6, 8
SNR	0.0, 0.1, 0.2, 0.3, 0.4, 0.5

Table 4.1 Input parameters for investigation of R wave detector methodologies on synthesized neonatal ECGs.

The exact location for each R wave peak was determined by the ECGSYN programme and this was the 'gold standard' position of the R wave. The three R wave detectors (Liverpool approximate, Liverpool accurate and Clifford) were then assessed in their ability to detect each QRS complex by comparing the estimated position of the R wave with the real position of the R wave. The root mean square error (RMSE) for each estimated R wave was then calculated, with the mean RMSE results for the entire recording being used to compare the different methods.

The three R wave detectors were then assessed and compared in their ability to correctly identify these three parameters with: i) increasing heart rate, ii) increasing standard deviation of heart rate and iii) increasing noise levels within the ECG.

Of the 240 ECGs, 56 were unable to be analysed as the three methods were unable to detect any R waves. All the "failed" ECGs had a SNR of 0.5. The three methods were however able to detect R waves in 13/67 (24%) of the ECGs with an SNR of 0.5 though with a low degree of accuracy.

	RR detect accurate	RR detect approx	Physionet RR detect
RMSE	0.9 (0.5 – 1.3)	0.9 (0.7 – 1.4)	39.3 (0.6 – 63.2)

Table 4.2 Accuracy of the three methods for R wave detection. Values are the median of the Root Mean Square Errors for the 184 synthetic ECG recordings.

The R wave detectors accuracy was also compared in their accuracy when confronted with changes in heart rate, HRV and noise. The median RMSE for each of the R wave detectors is summarised in Figures 4.2 to 4.7.

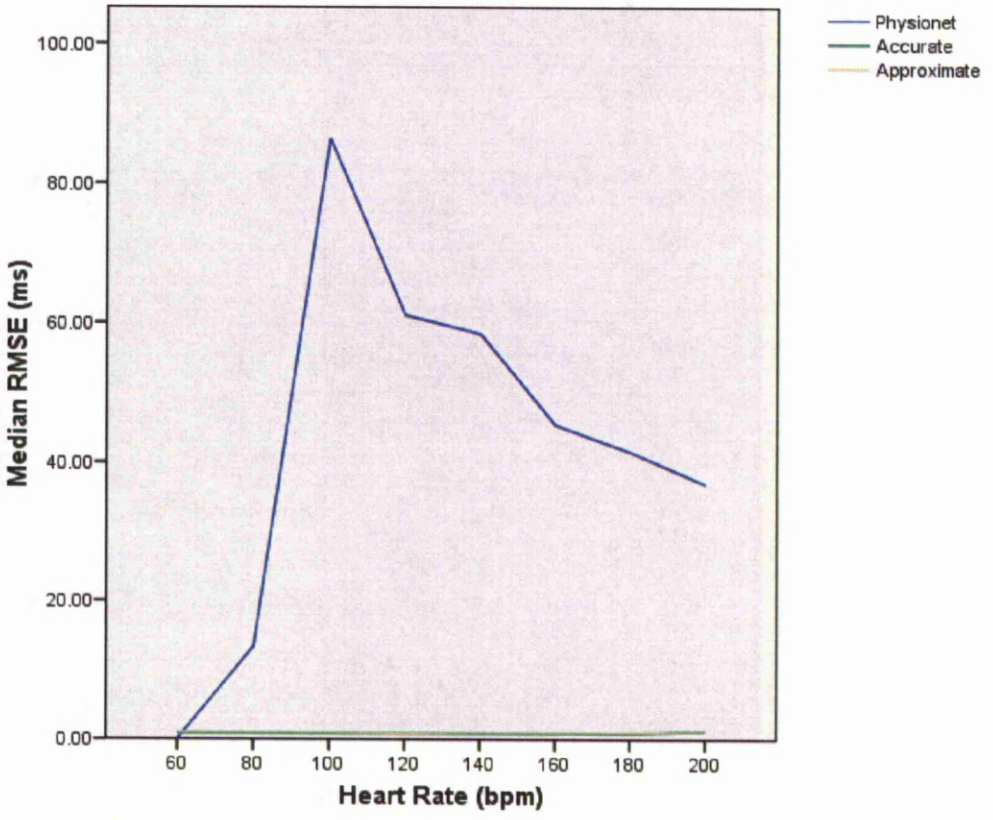


Figure 4.4 Line chart demonstrating the median RMSE for the three R wave detection methods with increasing heart rate

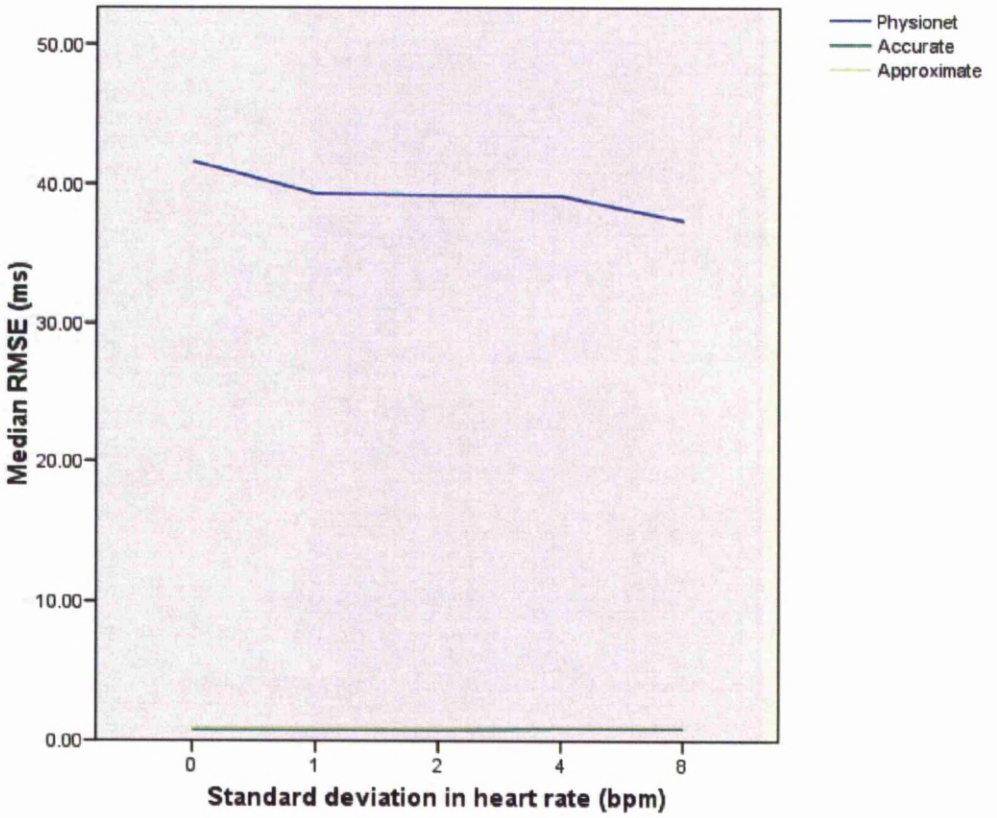


Figure 4.5 Line chart demonstrating the median RMSE for the three R wave detection methods with increasing heart rate variability

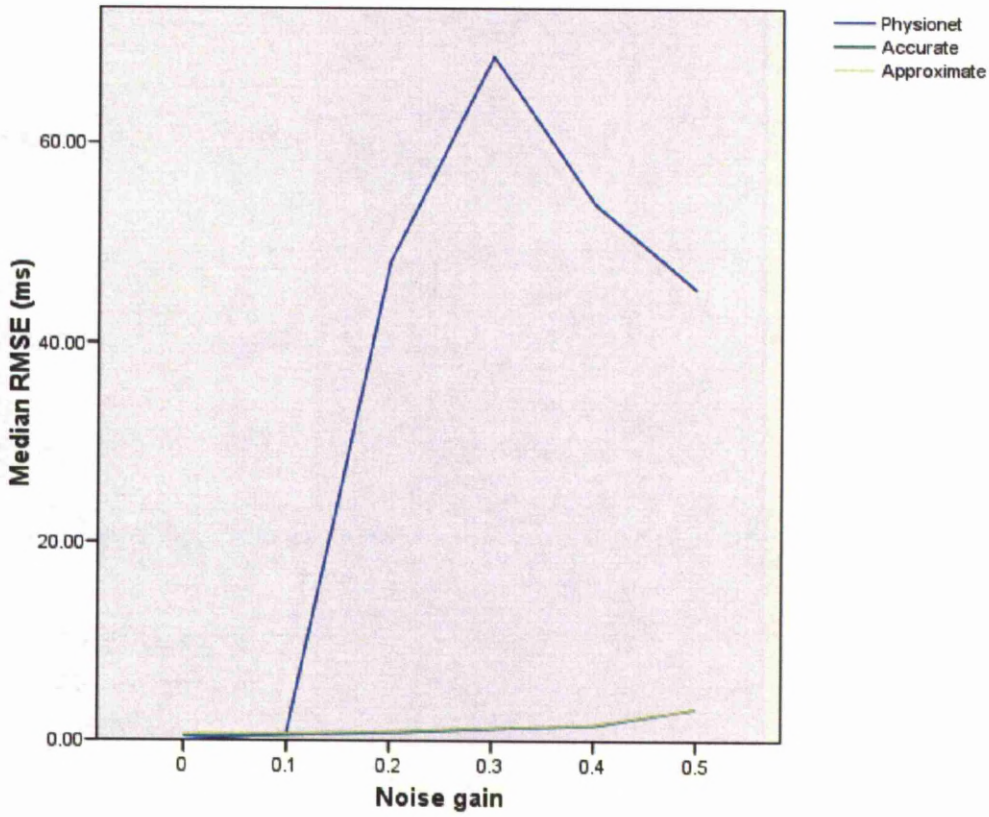


Figure 4.6 Line chart demonstrating the median RMSE for the three R wave detection methods with increasing levels of noise

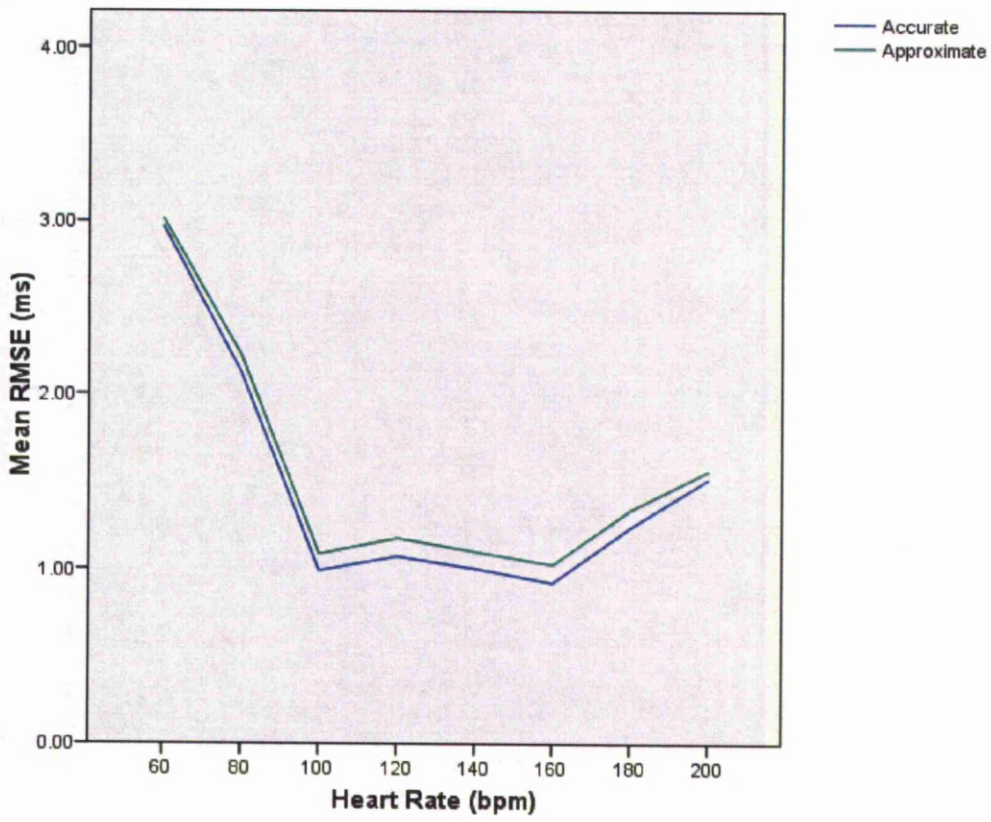


Figure 4.7 Line chart demonstrating the median RMSE for the two R wave detection methods developed for this thesis with increasing heart rate

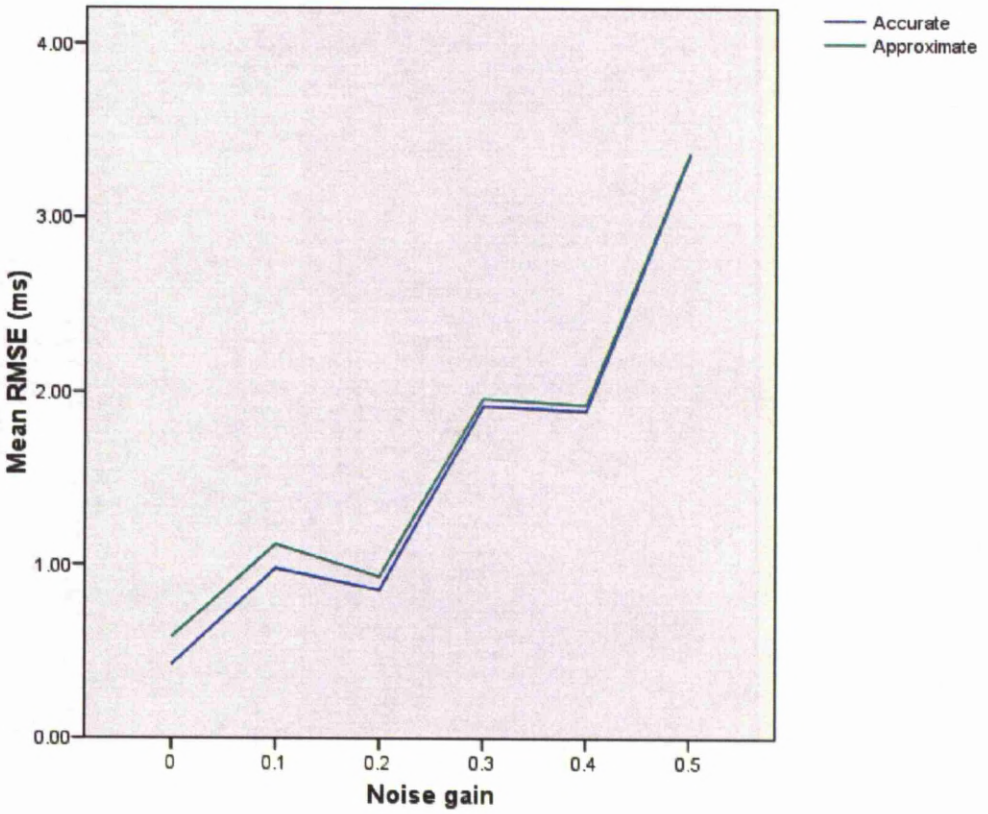


Figure 4.8 Line chart demonstrating the median RMSE for the two R wave detection methods developed for this thesis with increasing levels of noise.

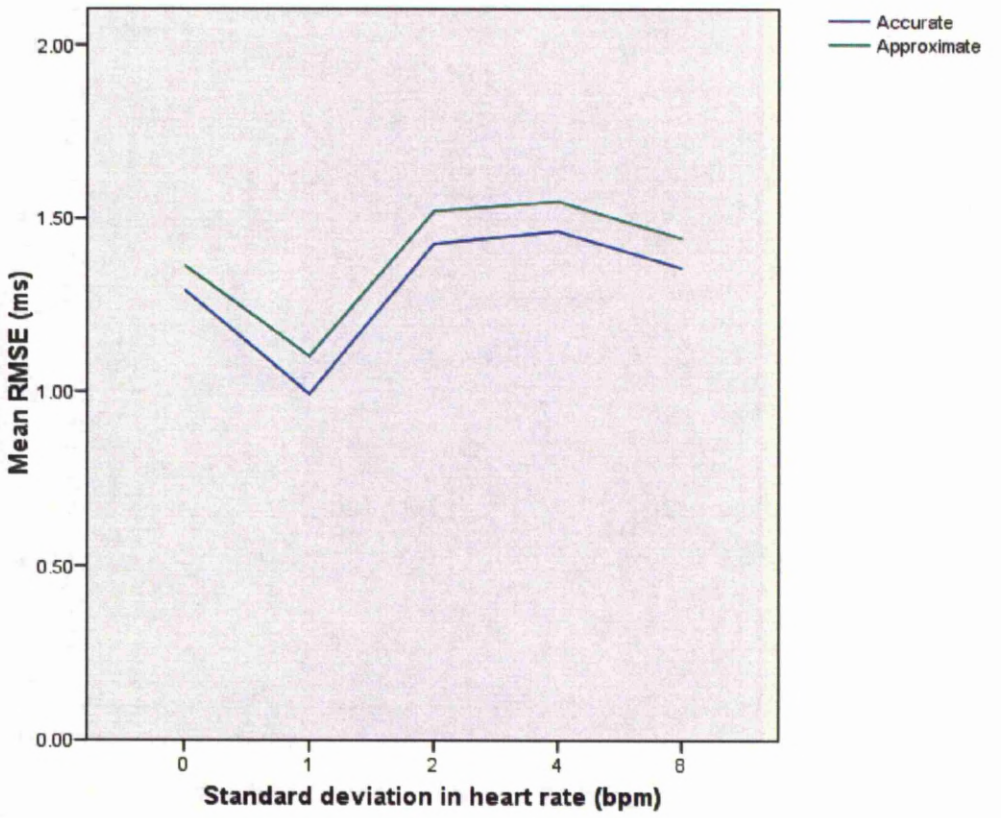


Figure 4.9 Line chart demonstrating the median RMSE for the two R wave detection methods developed for this thesis with increasing heart rate variability

It can clearly be seen that the R wave detectors developed for this thesis (Liv RR detect accurate and approx) were much more accurate in correctly identifying the position of each R wave than those developed by the Physionet team, even after adjustments had been made to better suit the characteristics of the neonatal ECG. The RMSE for both the Liv RR detectors had a mean of 0.9, with the Physionet RR detect method being highly inaccurate with mean RMSE being 39.3. This would make the Physionet RR detector unusable in detecting R waves in the neonatal ECG. Interestingly, the Liverpool RR approximate estimator demonstrated a narrower interquartile range than the accurate R detector. The additional step of interpolation adds no benefit in correctly identifying the exact position of the R wave and was thus abandoned.

Accurate detection of the R wave is absolutely crucial as the first step in determining HRV indices. In this chapter it has been demonstrated that the available R wave detectors are not suitable for accurate R wave detection in ECGs with characteristics of those recorded from infants being monitored in the NICU.

Chapter 5

Frequency domain analysis of RR time series

Frequency domain is a term used to describe the domain for analysis of mathematical functions or signals with respect to frequency, rather than time[239]. A time-domain graph shows how a signal changes over time, whereas a frequency-domain graph shows how much of the signal lies within each given frequency band over a range of frequencies. A given function or signal can be converted between the time and frequency domains with a pair of mathematical operators called a transform. An example is the Fourier transform, which decomposes a function into the sum of a (potentially infinite) number of sine wave frequency components. The 'spectrum' of frequency components is the frequency domain representation of the signal. The inverse Fourier transform converts the frequency domain function back to a time function. There are several different transforms used for this, though the commonest being those based on the Fourier transform (FT) (or its discrete equivalent the DFT). Prior to performing spectral analysis, the HRV must be represented by a signal to represent the heart rhythm.

5.1 A signal to represent the heart rhythm

The NNi's derived from the ECG must first be combined to construct a representative signal of the HRV. As discussed in 1.5.3.1, this is usually in the form of the NNi tachogram whereby each NNi is plotted against the time of occurrence. Alternatively, the instantaneous heart rate can be plotted. Furthermore, as well as the cardiac event series (NNi or IHR) being plotted against the time of occurrence, they can also be plotted as a function of beat number. Each of these methods of constructing the HRV signal introduces complications for performing spectral analysis in the frequency domain and is discussed below.

5.1.1 Interval time function (Classic NNi tachogram)

The most intuitive approach is to plot the interval event series as a function of time, whereby the duration of each NN interval is represented on the vertical axis and plotted against the time of occurrence on the horizontal axis. This is an unusual event series given that both axes are related to each other, representing time between beats, and results in an inherently unevenly sampled signal. This form of the heart rate signal is therefore not well suited to the standard Fourier analysis (see 5.2) which demands a constant time interval.

In order to overcome this, a signal must be derived from the original event series that can be defined at all times and hence transformed onto a prescribed (regularly spaced) time axis. The simplest way to achieve this is by tachogram resampling, which involves applying interpolation methods to the original time series to obtain a new representative signal. The regularly sampled form can now be further processed using standard Fourier analysis. Resampling results in a semi-continuous tachogram whereby the rate is assumed constant within each event interval[240]. This resulting signal exhibits unnatural abrupt changes at the R-wave occurrence and since it is biologically unfeasible that the signal is discontinuous, linear and higher order, resampling methods are preferred and are just as simple to apply. The distortion which resampling introduces is generally underappreciated when reporting HRV metrics. The action of replacing the known data with evenly sampled points requires the assumption of some underlying model which describes the relationship between each point[241]. Linear interpolation, although stable, leads to rough approximations which are biologically unrealistic. Higher order schemes on the other hand are prone to instabilities and in general fail to fully capture the causal dynamics of the signal. Resampled spectra may often be comparable by visual inspection[240] yet even subtle degrees of smoothing can manifest as large differences in the frequency spectrum and thus misrepresent the derived HRV metrics[241].

5.1.2 Interval beat series

This is the sequence of NN intervals plotted regularly as a function of beat number. This approach produces an equi-spaced event series which can be considered as a regularly sampled waveform, and is useful in that it can be directly processed using standard

procedures for spectral analysis (e.g. FT). However since the heart beat series is a function of beat number rather than time, spectral estimation results in an analysis not strictly in the frequency domain as we normally consider it but in the beat domain, also known as the sequency or beatquency domain[212,242]. Cycles-per-beat is substituted for cycles-per-second (Hertz) in the equivalent frequency representation making the resulting spectrum somewhat difficult to interpret. DeBoer et al compared HRV signals as a function of beat number and time[240]. They found that the resulting spectra exhibited similar characteristics in appearance, however did not compare quantitatively derived values of HRV metrics.

5.1.3 Instantaneous heart rate

A related series may also be derived from the heart period by taking the series of the reciprocals of the NN intervals and plotting them as a function of time. This signal is representative of the instantaneous heart rate. Few investigators question the choice between heart period and heart rate, however interestingly both Mohn and Santos found the spectrum of inverse intervals to be superior (exhibiting decreased spectral leakage)[243,244]. Although the relation between the two may seem trivial and the signals hold the same information content, comparison between the two has shown considerable discrepancies, and the selection between heart rate and period can significantly affect the interpretation of the results[245]. The IHR must also be submitted to resampling schemes to obtain an evenly sampled waveform amenable to spectral analysis.

5.1.4 Low pass filtered event series (spectrum of counts)

More complicated (physiologically based) schemes to reconstruct the heart rate modulating signal have also been considered. The most widely used is the so-called low pass filtered event series (LPFES) introduced by Hyndman and Mohn in 1975 who arrived at the method by employing an integrated pulse-frequency modulator (IPFM) as an underlying model for pacemaker activity[246].

The IPFM model simulates beat occurrence times by imitating the function of the sino-atrial node. An input signal is integrated until a preset reference value is reached at which point the

device sends out a pulse ('heartbeat') and the integrator is reset to zero before repeating the process. By assuming an underlying IPFM model, evenly sampled data can be obtained by passing the cardiac event series through a LP-filter. The resulting continuous signal is subsequently sampled and the spectrum calculated by a DFT. However, it is difficult to design a low-pass filter with an ideal frequency response, and many implementations lose fidelity of the heart rate reconstruction as they fail to preserve the high frequency components[247]. Reconstructing the modulating signal based on the IPFM model is still an active area of research yet there remains controversy in the accuracy of such an approach.

All four ECG derived signals are quite closely related however, each has its inherent limitations when performing spectral analysis with the FT[248]. There is however an alternative to the FT that avoids the resampling conundrum and for which no underlying model needs to be formulated, the Lomb-Scargle periodogram (section 5.4).

5.2 Fourier Transform

The Fourier transform fits harmonic equations to time-varying data, yielding the frequency components of the original signal. (Figure 5.1).

There are several common conventions for defining the Fourier transform ($\hat{f}$) of an integrable function f: R → C. A simple definition is thus:

$$\hat{f}(\xi) = \int_{-\infty}^{\infty} f(x)\, e^{-2\pi i x \xi}\, dx$$

When the independent variable x represents *time* (with SI unit of seconds), the transform variable ξ represents frequency (in hertz).

For HRV analysis, the time varying NNi tachogram (with units in seconds) is transformed to the frequency domain with the resultant function being with units s^{-1} or Hertz (Hz). The fast Fourier transform (FFT) is merely a discrete Fourier transform (a specific type of Fourier

transform used when sampling a continuous function) that greatly speeds computation and is highly efficient[249].

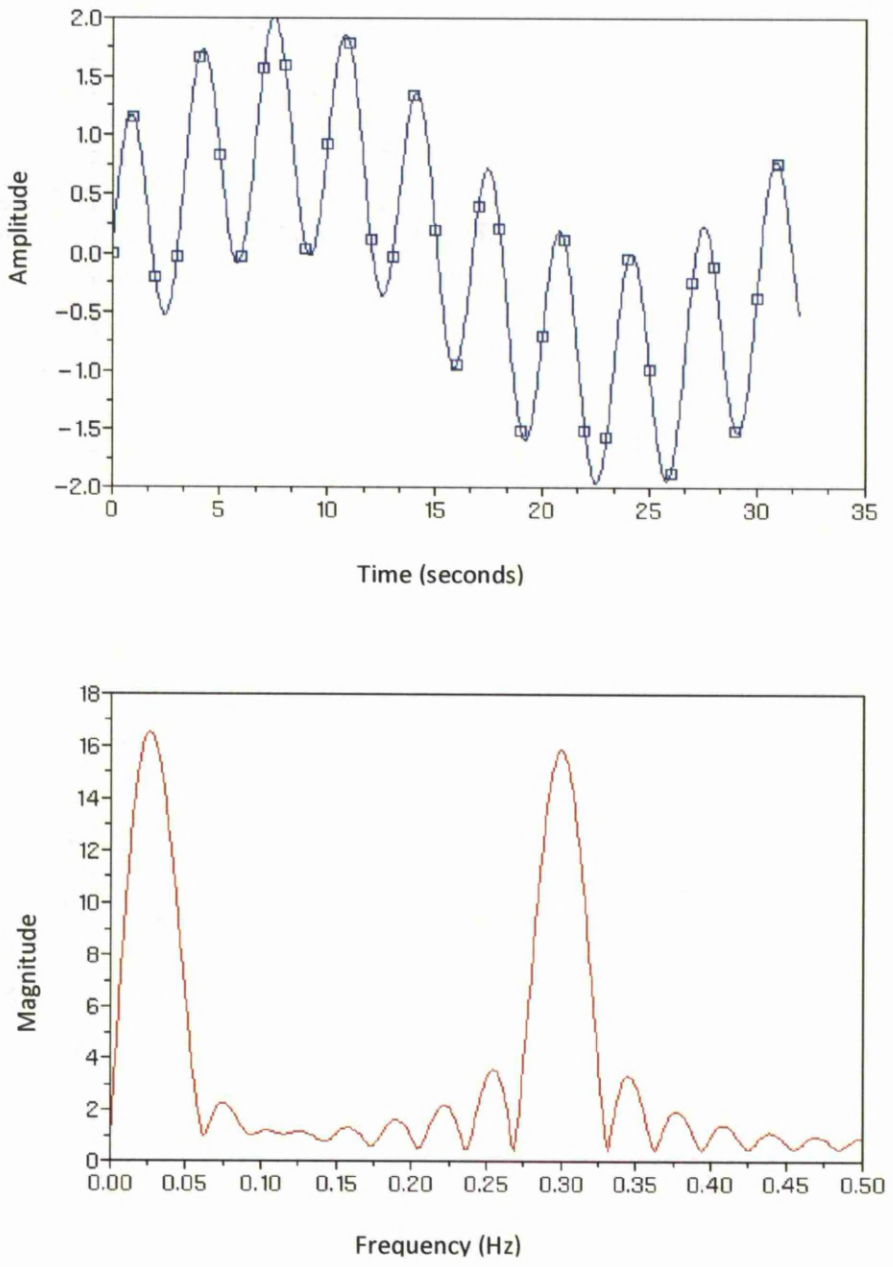


Figure 5.1 Example of a Fourier transform. The original signal in the time domain can be demonstrated to have two major frequency components at approximately 0.025Hz and 0.3 Hz. following the Fourier transform.

Four common distortions may arise in using the fast Fourier transform[250]:

1. Aliasing. The sampling theorem states that an accurate determination of spectral components is possibly only at frequencies no greater than the Nyquist critical frequency, determined as one-half of the sampling rate. Therefore if fluctuations in heart rate are under investigation, the high frequency cut-off point should be half the lowest normal mean heart rate. Signals at frequencies above the Nyquist critical frequency will be erroneously translated to below the Nyquist limit in *X(f)*. Assuming the heart rate to be 150 beats per minute (equal to 2.5 Hz), the Nyquist criterion requires an upper limit of the respiratory frequency of 75 breaths per minute (equal to 1.25 Hz).

2. Spectral Leakage. Oscillations at frequencies that produce exactly 1, 2, 3, etc., cycles over the record duration will produce narrow peaks at the corresponding frequencies. However, any other frequency oscillations present in the signal will produce broad peaks. Tapering the end of the time-domain sample removes the discrepancy between the start and end of the record, minimising spectral leakage. Tapering is achieved by using a time-domain sampling window (*e.g.* a triangle shaped Parzen window).

3. Errors due to non-equidistant sampling. Time-domain data for transformation by the Fourier transform are assumed to be sampled at a constant interval. However, the raw heart rate can only be computed after a beat occurs. Because beats occur at varying rates, new heart rates are available at irregular intervals. To overcome this issue, interpolation and resampling is used to produce equidistant sampling of the signal. (see 5.2.)

4. Errors due to lack of stationarity. The Fourier transform assumes stationarity of the frequencies components within the signal. That is, the data segment is assumed to have the same spectral characteristics throughout. However, this is difficult to assure in physiological studies. The compromise is between the reduced resolution of short collection periods and the spectral distortions from non-stationarity over longer periods.

Thus, FFT techniques require the cardiac event series to be;

1. Equidistantly sampled
2. Within the nyquist frequency and
3. Stationary

These limitations in the use of FFTs hinder their utilisation in the production of PSDs of HRV from the cardiac event series, particularly those derived from "real world" recordings obtained from the NICU where significant noise and missing beats are present and where stationarity is not commonplace. There is however an alternative to the FFT which avoids the problems of resampling and for which no underlying model needs to be formulated, the Lomb-Scargle periodogram. (see 5.4)

5.3 Resampling for Spectral estimation

Resampling transforms an irregularly sampled process, such as the NNi tachogram, into an equidistantly sampled signal. This signal can then be utilised by Fourier techniques to produce PSDs. Resampling always causes spectral bias, due to aliasing and to shifting of the observation times and results in an inaccurate representation of the cardiac event series. Figure 5.2 illustrates how resampling schemes can distort an underlying signal. In this example the unevenly sampled points (marked by *) are equivalent to each heart beat with the underlying signal taking the form of a sinusoid (blue line). Resampling at 7Hz using linear and (red) and cubic spline interpolation (green) interpolation[241]. Linear resampling results in a very crude approximation of the signal whereas the cubic spline passes closely through the real data points and generates a reasonably accurate representation of the underlying signal. However, it can be seen that even this method underestimates the peaks and troughs. The accuracy will further decline if the true form of the signal is not sinusoidal in nature[51].

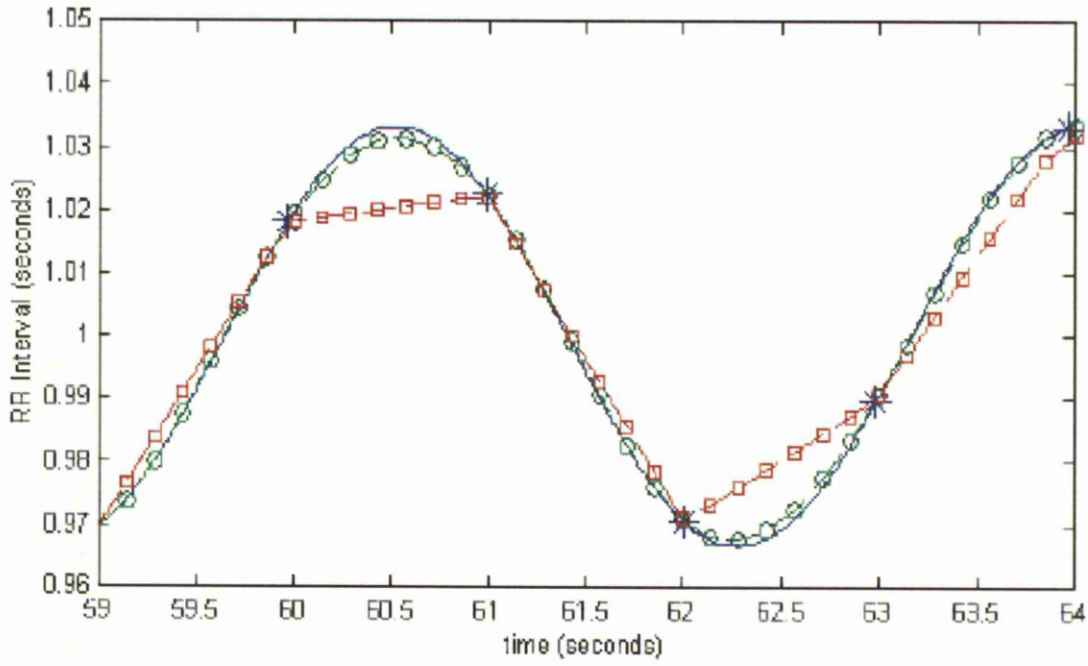


Figure 5.2 The effect of interpolative resampling on a perfect sinusoid. The ideal waveform represented by the blue solid line is unevenly sampled at points * physiologically representative of the beat occurrence. The linearly resampled signal is represented by -□- and the cubic spline by -o- .

5.4 The Lomb-Scargle periodogram

The Lomb-Scargle periodogram was introduced as a method for deriving the PSD of unevenly sampled signal in 1976 by astronomer Nicholas Lomb[251]. In using the Lomb method, resampling onto an even timescale is unnecessary and the pitfalls introduced therein (see above) are avoided. The principle is based on a least squares method of fitting sinusoids to samples of data; a sine wave can be fitted to a set of observations and thus obtain a single point of a spectrum. That component can then be subtracted and the process repeated until a spectrum results with the desired frequency resolution. Previously the high computational burden of the Lomb method proved a major obstacle to its general use and thus its application for HRV analysis[252] . In 1989, Press and Rybicki, developed a fast algorithm for implementing the Lomb periodogram[253]. Given the advantages of such an approach, several

papers have presented PSD analyses using the Lomb periodogram on unevenly sampled heart rate signals [51,241,254,255]. Moody applied the Lomb method to an artificial heart rate time series demonstrating that for noise-free HR signals AR, FFT (of cubic resampled R-R interval series) and the Lomb methods provided equivalent spectral estimates[51]; yet the Lomb proved to be more robust in the presence of random noise and ectopic beats. Laguna et al similarly concluded the superior performance of the Lomb estimate when compared with classical methods of PSD estimation with resampling (cubic spline and linear)[255].

More recently, Clifford and Tarassenko, published a set of systematic experiments to quantify the exact errors resulting from the resampling process in the spectral estimation of HRV signals, focusing on different levels of noise and ectopy[241]. They found that the FFT overestimated the LF:HF ratio by approximately 50% with linear resampling and 10% with cubic spline resampling whereas the Lomb was found to be more accurate on both artificial and real data[75]. Furthermore replacement or removal of artefactual beats compounded these errors, whereas the Lomb method performed consistently up to a high percentage (20%) of beat removal.

5.5 Dealing with missing data and ectopic beats

It is seldom possible to acquire a HR time series for a sizeable length of time without the occurrence of cardiac ectopies (abnormal beats that occur with an unusual timing) or beats that cannot be reliably detected (missing beats). Ectopic beats can be regarded as such when they have intervals of less than or equal to 80% of the previous sinus beat[256]. Since this phenomenon will result in beats which occur substantially earlier than that of a normal event, the shortened R-R intervals will create higher frequencies in the spectrum. This may lead to an overestimation of the HF components of the HRV metrics. On the contrary the effect of missing beats will lead to extended R-R interval durations which will lead to extended RRi's, leading to an overestimation of the LF components. These artefacts within the ECG must be detected and either corrected or removed. Fourier based methods require interpolation of these missing or ectopic beats otherwise stationarity is lost. However the spectral estimates derived from the heart rate data may be distorted in the process.

Neonatal ECG recordings are more susceptible to corruption due to the reasons set out in 3.1.1. Using neonatal (and adult) ECGs with simulated (single and multiple beat) artefact removal, Peters et al found that the VLF and LF powers could be calculated consistently for up to 25% of corrected data, but the HF (adult: 0.15-0.4 Hz, newborns: 0.4-1.5 Hz) power estimates were underestimated particularly for newborns due to the effect of the resampling[257]. In this study, the R-R intervals were also deleted at random for up to 50% of the dataset. Spectral analysis was performed using the FFT on evenly resampled form of the data. They concluded that only short data segments free of artefact should be used if the HF is to be considered, or that only the LF region be used in fetal monitoring[258]. They also warned against the use of normalised values which incorporate the HF components. The investigators fail to consider however their information limits, for example the validity of the VLF power metric due to the short amount of data analysed (192 seconds), which is significantly less than that that recommended by the Task Force (300 seconds) (assuming a sampling frequency of 1000Hz). They also attempt to resolve frequencies up to 1.5 Hz which would require an average of 3 bps (576 beats in 192 seconds) which is rather a high expectation even for neonatal data. It is therefore no surprise that the HF components were severely affected by the missing data which effectively reduces the sampling rate to unacceptable levels. Resampling at 4Hz merely creates the illusion of a high temporal resolution whereas in reality we are constrained to obtain glimpses of the (ideal) signal once per beat at most[259] .

In contrast with the above findings the LF power component has also shown to be unreliable with inconsistent data as the loss of stationarity induced by removal of the missing segments preferentially affects the lower frequency components. Birkett et al compared the effects of dealing with missing segments by beat replacement or removal[260]. Either the intervals corresponding to ectopic beats (and the three beats following) were estimated by interpolation (linear or cubic-spline) or the segment was completely discarded. It was found that the low frequency power calculations (by FFT) were significantly higher when interpolative resampling was used as opposed to removal, whereas the HF were relatively unchanged between the two. Lippman et al also concluded that removal of sections worked better than beat replacement by interpolation but found that the HF component was underestimated, in addition to the overestimation of the low frequencies[261]. This may be due to the differences in length of data used; the former use 24 hour recordings[260] whereas the latter use 5 minutes[261]. It is not recommended that metrics derived from different signal durations be compared[50].

The results in the aforementioned study by Clfford and Tarassenko are in agreement with these observations demonstrating that cubic resampling results in an overestimation of the LF/HF-ratio and reported that the LF over-estimation is the dominant error[241]. The PSD estimate using interpolation prior to an FFT was found to grow linearly with the number of ectopics removed. In addition to the FFT they also look at the performance of the LSP on incomplete data and found that the accuracy remained even when 20% of the data points were missing.

Alternative schemes to resampling have also been proposed to deal with missing data. Albrecht and Cohen carried out experiments to examine two different methods for characterising the HR power spectrum of data over bad intervals containing (low incidences of) ectopic or missing beats[262]. They compared two schemes for dealing with ectopic beats. The first method made no explicit assumptions about the specific value of the HR in the bad intervals and thus used only the original data points. Missing beats were dealt with by constructing a windowing function (varying from 1 to 0 to 1) over the bad interval and then estimating the position of the R wave on the product of the windowing function and the HR. The second method simply constructed linear splines across bad intervals to replace ectopic beats. Against the theoretical reasoning the first method performed quite badly (for many typical R-R tachograms) in the HF power estimates whereas the ad-hoc method of splining performed better, demonstrating substantially lower variance. This is due to the fact that the linear spline has essentially no high frequency content and therefore adds little noise to the HF band of the spectrum.

Time domain HRV measures are also susceptible to corruption due to missing data and noise. Kim et al looked at the effect of noisy data and missing data on the associated time domain metrics[263]. They compared several statistical measures derived from the time series for various amounts of missing data from (length 0 - 100 seconds) from 5 minute tachograms to demonstrate the errors that arose between the original and incomplete tachograms. The mean NN was found to be the most robust parameter to missing data and remained practically uninfluenced by corrupt data.

The presence of ectopic beats has been shown to (falsely) elevate the standard deviation (SDNN), masking the detection of a depressed SDNN which may be indicative of congestive heart failure[264].

5.6 Zero-meaning, detrending and signal stationarity

In any time series which has non-zero mean, such as the heart rate, a windowing function will introduce significant distortion of the lower frequencies in the spectrum by artificially reducing the values near the beginning and end of the series to zero. Such artefact is easily corrected for by subtracting the mean value of the time series from each sample point, a process called zero-meaning[51,259].

PSD estimation inherently assumes that the signal is at least weakly stationary, however real HRV series rarely conform to this ideal. R-R interval series of longer durations often show baseline trends representative of long term variations (for example demonstrative of differences between sleep and wake states). If the modulations are not stable then the results of frequency analysis are less well defined obscuring detailed information about autonomic regulation[50]. Any methods that attempt to characterise specific periodicities over time may be distorted by slow linear or complex trends. This is a challenging issue because non-stationarities in HRV are not uncommon[265]. If a section of the data exhibits significant changes in the mean or variance over the length of the window, the HRV estimation technique can no longer be trusted. A number of methods have been proposed to correct for this.

The initial approach to the problem should be to prevent (or more realistically to minimise) non-stationarities by ensuring that test conditions (and subject conditions) remain stable throughout the recording period. Whilst this is applicable to the research setting this does not lend itself to using HRV analysis as a clinical tool in the "real world" setting. Even when stability of the test conditions is obtained, non-stationarities may still appear in the heart period series. Some investigators argue that all such changes should be regarded as relevant and part of the HRV and do not apply any non-linear detrending[51]. However, many consider such pre-processing to be absolutely necessary and deal with persistent trends by other means[266].

Weber et al suggest that HRV data should be systematically tested for non-stationarities and only segments within which (weak) stationarity is satisfied should be chosen for spectral analysis[265]. However, this introduces selection bias and the conclusions drawn may not be representative of the data as a whole.

Alternatively, it is more common practice to detrend the signal by removing the baseline trend from the window prior to analysis based on linear[267] or polynomial models[268]. Ashkenazy et al found that distinction between healthy subjects and patients, using the (time domain) SDNN as a measure of HRV, was more successful when detrending was first applied to the R-R interval series (7-8 minutes in length) by subtracting a running local average[269]. In frequency domain analysis, the strong VLF component has been found to distort other frequency bands, especially the LF component of the spectrum[270]. The application of band-pass filters[267,268] to isolate a frequency range of interest can improve the accuracy of estimating a constant periodicity superimposed on slower trends. However, the target itself may not be stationary (such as RSA) and cannot simply be removed or extracted[271]. Tarvainen et al presented a detrending method based on the smoothness priors formulation[270] which operates like a time-varying FIR high pass filter. The frequency response can thus be adjusted which allows the RSA component to be separated by adjusting a single parameter. De Beer et al used a customised filtering technique applicable to neonatal data in order to reach an agreeable compromise between LF frequency resolution and HF time resolution[272]. As, with regard to the duration of the recording, the optimum lengths for accurate analysis of the LF and HF components are conflicting: the higher the frequency of interest, the shorter should be the duration of the recording and the lower the frequency of interest, a longer duration of recording is required[50]. This is of particular importance for neonatal data where the difference between the HF and LF bands is greater. The relevant portions of the spectrum are thus analysed individually (using appropriate window lengths) by filtering the respective bands separately before FFT spectral analysis.

Although moderate violations of stationarity may not seriously affect spectral estimation of HRV[266], the issue of stationarity is important and should not be ignored[273]. This is particularly so in the case of premature infants who, due to an immature autonomic regulation, demonstrate high incidences of signal non-stationarity due to a rapidly fluctuating heart rate and blood pressure[274]. One must however, check the frequency response of any filter (detrending scheme) to ensure that the spectral components of interest are not adversely affected in the process.

In summary, a large number of HRV studies use resampled R-R interval series for spectral analysis by conventional means (FT). However, there is no consistent approach to the resampling scheme (or sampling rate) used. Furthermore, when resampling schemes are

employed in the presence of missing data we have to stretch even further the assumptions made about the underlying signal which often has undesirable effects in the frequency domain.

It is apparent that ectopic beats must be removed but it remains unclear what level of artefact correction is tolerable. Furthermore the error is dependent on the spectral estimation and pre-processing method employed, therefore one should always be cautious when projecting any findings from individual studies to investigations in general.

The Lomb-Scargle periodogram is regarded as a reliable method for performing spectral analysis of unevenly sampled data, and is thus ideal for HRV analysis. Moreover the method is fairly tolerant of occasional gaps in data (missing beats), a phenomenon which has been shown to significantly confound the standard FT methods. In this thesis, the Lomb-Scargle periodogram will be used to produce PSDs of neonatal ECGs.

5.7 Development of a new method of frequency domain analysis appropriate for analysis of neonatal RR interval times series

In the conventional HRV tachogram, the time axis is represented either as the actual time taken directly from the ECG record at which each RR-interval (or NN-interval) is calculated or the indirect time as calculated as the running cumulative sum of RR-intervals. Thus, each RR-interval is time-stamped, and significantly, the time axis is irregularly sampled. The majority of published spectral HRV analysis work employs interpolative resampling to remap the RR interval tachogram onto a regularly-spaced time axis at, typically, 250ms (milli-seconds) intervals to facilitate analysis.

Resampling introduces noise and distortion, corrupting both the fine detail of the higher frequency components, whereas detrending significantly attenuates the estimate of low frequency power. The conventional approach to the spectral representation of the HRV tachogram consists of firstly, detrending in the time domain, and secondly, power estimation in the frequency domain. The detrending operation, as a time domain operation, is a high-

pass filter, usually with a cut-off at very low frequency, such as ~0.005Hz. There is no universally accepted formal justification for such an operation other than that it minimises the effects of medium term non-stationarity within the immediate time epoch (observation window) of interest. To accommodate the requirements of these conventional processing techniques, the data must be resampled onto a regular (uniform) time axis. This is readily achieved by cubic spline interpolation but does so at the expense of introducing low frequency noise and distortion by 'smoothing' higher frequencies which compromises accuracy and is therefore inappropriate in this present study.

Stationarity is an axiomatic requirement in estimating the power distribution with respect to frequency. The data of a section of the HRV tachogram are informally assumed stationary if their variance is unchanging. The most commonly adopted practice is to establish the frequency limit of stationarity as the reciprocal of the length of the sampling window. Whilst this might be justifiable as being pragmatic, it is in fact difficult to apply if the detrending method cannot be characterised explicitly by its frequency response.

A number of methods have been described which identify a trend component in the HRV tachogram such that it can be removed by simple subtraction. These methods include fixed coefficient low-order polynomials[267,275] adaptive high-order polynomials[268] and more recently, the smoothing priors approach[270,276] (SPA). Whilst polynomials can be efficiently applied directly to the HRV tachogram, their frequency response is effectively poor. The SPA exhibits a well-described second-order response but does require the tachogram to be resampled onto a regular time axis. The SPA approach is based on the definition of a Gaussian Process (GP) with a stationary and continuous covariance function, evaluated at regular intervals on the time axis. The GP effectively defines a class of functions which act as smoothers.

For the purposes of this neonatal study and the challenging nature of the ECG data, a more robust and accurate method of determining the conventionally defined spectral HRV components was needed than is currently available in commercial systems designed, predominantly, for the analysis of adult HRV.

In parallel with this thesis additional work by Professor Tony Fisher, Professor David Groves and Katie Sanders within the department of medical physics and clinical engineering at the Royal Liverpool University Hospital developed and investigated a novel method for

determining the frequency components within the NNi tachogram using the LSP. When the LSP method was compared to two FFT methods (FFT with linear interpolation and FFT with cubic spline interpolation), the LSP was demonstrated to be superior in the following;

i. Deriving a known LF:HF ratio across a range of different HRs
ii. Deriving a known LF:HF ratio across a range of different HRVs
iii. Deriving a known LF:HF ratio across a range of beat removals, remaining within 15% of the true LF:HF ratio even when 50% of the RRi data points are removed.

The LSP was less accurate when compared with the FFT methods in deriving a known LF:HF ratio when ectopic beats were present. This demonstrates the importance of accurate beat detection as the presence of a single ectopic beat causes unacceptable distortion of the frequency spectrum. With the LSP providing LF:HF ratios within 15% even when 50% of data points are missing, it is safer to be over cautious when determining the R wave as a missing beat will affect the spectral analysis much less than the inclusion of an ectopic beat in the NNi time series.

The improvement in accuracy is achieved by avoiding any requirement to resample the data at both stages of processing, either for detrending the RR tachogram of for estimating spectral power from the detrended tachogram. Further, the combination of techniques is far less sensitive to 'missing' and 'false' RR intervals and, together with improved accuracy of measurement, are more appropriate to the unavoidably 'noisy' neonatal ECG recordings. The two new analysis methods developed are described in Appendix B with the comparisomn between the LSP and FFT methods described in Apppendix C.

5.8 Summary

Frequency domain analysis is essential to determine the functioning of both branches of the ANS. FFT methods have been used to determine the frequency components within the NNi tachogram however they are unsuitable for the routinely monitored neonatal ECG signal. A novel method for frequency domain analysis was therefore required. This method avoids the pitfalls of the FFT methods by using the LSP without resampling of the NNi tachogram.

The "real world" neonatal ECG contains high levels of artefact and missing data. Whilst the FFT techniques can only be performed on continuous data, the LSP can be calculated over the entire length of the signal, despite missing data, and is able to interpret frequencies well below those detected by the FFT. This allows a more complete picture of the underlying HR signal.

The LSP has thus been demonstrated to provide a much more accurate and robust method for producing frequency domain HRV analysis of real world ECG's and is the method of choice for determining the PSD of the routinely monitored neonatal ECG.

Chapter 6

Methods for assessment of Neonatal ECGs

6.1 The Liverpool Neonatal ECG Recording Bank

ECG's were recorded from neonates who were receiving care on the NICU at LWH. Routine neonatal ECG monitoring uses a three lead ECG configuration to form the Einthoven triangle - two electrodes are placed onto either side of the chest and a third on the outer aspect of one thigh. The electrodes are relatively large in size, compared to the size of a preterm infant, and there is therefore no "standard" lead positioning as in the full 12 lead ECG.

For this thesis, ECGs were recorded for different time durations on a variety of well and unwell babies. These included:

1. Twenty minute, observed recordings.
2. Two hour unobserved recordings.
3. Longer term recordings, lasting from 12 hours up to several days.

Recordings consisted of the routinely monitored ECG signal over 3 channels. These recordings were stored on datacollect2 PC (section 3.3) before being transferred on to a password protected external hard disk drive. The recordings contained no identifiable information. They were subsequently downloaded from the hard disk drive to the computer servers in the medical physics and clinical engineering department and processed using the methodology described in chapters 4 and 5.

These recordings formed a bank of different lengths of recordings on a variety of babies. The following demographic and clinical data was collected for each recording in the bank:

i. Gestational age at birth
ii. Age at recording
iii. Birth weight and weight at recording
iv. Antenatal details (maternal smoking, drugs used, steroid administration, presence of pregnancy induced hypertension)
v. Birth details (Apgar score and cord pH if available)
vi. Clinical information (respiratory support, method of feeding presence/absence of; PDA on echocardiogram, intraventricular haemorrhage or periverintricular leucomalcia on cranial ultrasound scan)
vii. Current medications (inotropes, morphine, caffeine, midazolam, phenobarbitone)
viii. Septic status – free from infection, sepsis in subsequent 24 hours, currently suspected sepsis, currently confirmed sepsis
ix. Outcome (died, chronic lung disease, retinopathy of prematurity, necrotising enterocolitis) (see Appendix A)

The Liverpool Neonatal ECG Bank consisted of 315 recordings, varying in length from 20 minutes to 154 hours. These recordings were taken over a 2 year period by the author. The full details of the Liverpool neonatal ECG recording bank are recorded in Appendix A. The recordings were divided into durations of recording: 20 minute, 2 hours and longer term recordings.

6.1.1 20 minute recordings

These recordings were made whilst the baby was observed to ensure they were not handled, had continuous ECG measurements and suffered no clinical deterioration during the recording. Recordings were performed whilst the infants were asleep at least 30 minutes after a feed and where they were not disturbed throughout the duration of the recording. Prior to the recording the electrodes were adjusted so as to provide a positive R wave in lead II. No other manipulation of the ECG leads occurred and the morphology of the ECG on channels I

and III was not considered. Recordings were discontinued if the clinical condition of the baby necessitated handling, care givers entered the incubator or the ECG leads became detached. Recordings continued if the baby had a bradycardic event or if there was visible noise on the ECG. The observer noted the sleep state (quiet or active) based upon criteria as described by Sheldon[222]. The 20 minute recordings represented the "experimental" environment for babies in the resting or steady state. Demographic data for all of the observed 20 minute recordings are presented in table 6.1.

Gestational Age at birth (weeks)	28.2 (24.1 – 41.9)
Birth Weight (grams)	1000 (530 – 3350)
Sex	43 male, 35 female
Age at recording (days)	8 (0-96)
Corrected GA at recording (weeks)	31.3 (24.9 – 41.9)

Table 6.1 Demographic data (median (range)) for all of the 78 observed (20 minute) recordings

6.1.2 2 hour recordings

The recordings of 2 hours duration were made from infants routine receiving care on the neonatal unit. These recordings were not observed and reflect the "real world" neonatal ECG. Recordings were commenced at any point in the infant's care routine and continued if the infants required any intervention or the ECG leads became disconnected. The full range of gestational and postnatal ages of infants were included. A subgroup of infants had unobserved recordings made immediately following an observered recording, allowing the comparison between HRV recorded in the "real world" and the "experimental" environement to be made (Chapter 9).

Within the 2 hour recordings, HRV measures were obtained from 20 minute time epochs. Each 20 minute epoch would overlap the previous epoch by 10 minutes. For example, in a 2 hour recording there would be eleven 20 minute epochs (0 – 20 mins, 10 – 30 mins , 20 –

40mins etc.). The resultant HRV metric for the 2 hour recording is the median value of each 20 minute epoch within the recording.

Demographic data for all of the unobserved 2 hour recordings is given in table 6.2

Gestational Age at birth (weeks)	28.4 (23.1 – 41.9)
Birth Weight (grams)	1077 (515 – 4620)
Sex	52 male, 48 female
Age at recording (days)	8 (0-120)
Corrected GA at recording (weeks)	31.9 (24.7 – 42.0)

Table 6.2 Demographic data (median (range)) for all of the 100 unobserved (2 hour) recordings

6.1.3 Longer term recordings

Unobserved recordings from2 hours up to 154 hours were also made. These recordings were not used for analysis in this thesis but remain in the Liverpool Neonatal ECG bank for future study.

6.1.4 Rejected Recordings

Prior to any processing of any 20 minute epoch the percentage of missing NNi data for each of the three ECG channels was calculated from the total record. Any record that had in excess of 10% of the NNi data missing was excluded from further analysis. From methodology development it is known that with 10% of data points missing the LSP is accurate in identifying the frequency bands within 3% (Appendix C, table C.5). If more than one channel contained less than 10% missing data then all available channels were analysed with the resultant HRV metrics representing the mean values across all channels.

6.2 HRV analysis

HRV analysis was performed on 20 minute epochs within the NNi tachogram. The HRV metrics obtained were:

1. Time Domain measures
    a. Variance of the NNi (Var) (ms^2)
    b. Standard deviation of the NNi (SD) (ms)
    c. Mean NNi (ms)
    d. Kurtosis (kurt)
    e. Skewness (skew)

2. Frequency Domain measures
    a. Ultra Low Frequency (ULF) (<0.017Hz)
    b. Very Low Frequency (VLF) (0.017 - 0.04 Hz)
    c. Low Frequency (LF) (0.04 and 0.15 Hz)
    d. High Frequency$_a$ (HF_a) (0.15 – 1.5 Hz)
    e. High Frequency$_a$ (HF_b) (0.40 – 1.0Hz)
    f. Low Freqency to High Frequency$_a$ ratio (LF: HF_a)
    g. Low Freqency to High Frequency$_b$ ratio (LF: HF_b)

3. Non linear measures
    a. Poincaré SD1 (PCSD1)
    b. Poincaré SD2 (PCSD2)
    c. Poincaré SD1: Poincaré SD2 ratio (PCSD1:SD2)

6.3 Statistical Analysis

For non-related samples, the Mann Witney test was used for continuous data and Fishers exact test used for categorical data. To compare the HRV metrics before and after the removal of non-stationaries the Wilcoxon signed rank test was used.

For recordings which were longer than 20 minutes duration and therefore contained overlapping epochs, the data, by definition, were not independent. Thus simple statistical methods were not suitable for data analysis. The data were analysed using a statistical model which is described below. Firstly the data was transformed to better match the Gaussianity assumption required by the analysis. The method of analysis used consisted of fitting a regression line y=a*x+b, where y represents each of the measured quantities, and x is a binary variable denoting the gestational age category the infant belonged to.

After the fit, the estimated variances of the parameters a and b were corrected non-parametrically (using Huber's method) to take into account the possible correlations due to overlapping data segments and repeated measurements. The null hypothesis of no effect corresponds to the null hypothesis that the parameter a=0. The method implemented an unbalanced ANOVA design (variance corrected), which in turn can be thought of as a generalisation of the t-test. As the analysis required transformation and modelling of the data, a p value <0.05 cannot be used. Instead a p value <0.01 is considered statistically significant.

6.4 Experiments performed

In chapters 7-12 the results of several experiments using the recordings in the Liverpool Neonatal ECG bank are presented. A brief summary of these experiments is provided below.

6.4.1 Ability to produce HRV metrics (Chapter 7)

This thesis has thus far demonstrated that it is possible to produce HRV metrics from synthesized neonatal ECGs (chapter 4, Appendix C). In Chapter 7, the developed method is applied to routinely monitored neonatal ECGs to determine its ability to produce HRV metrics from these recordings. For this experiment, all of the 20 minute and 2 hour ECGs within the recording bank were selected. For demographic details of the babies which these recordings were taken from see table 6.1 and 6.2.

6.4.2 Determining the effect of removal of non-stationeries (Chapter 8)

Non-stationaries within the NNi tachogram distub the underlying harmonic[273]. In chapter 8, HRV measures were obtained from all of the 20 minute recordings both before and after the removal of non–stationaries. The results after removal were compared to those before removal to assess the impact of removing non-stationaries on HRV measures. For demographic details the babies which these recordings were taken from see table see table 6.1.

6.4.3 Stability of the HRV metrics (Chapter 9)

To assess how the HRV metrics fluctuate during routine neonatal care, several "well" infants had a "real world" (unobserved) recording made immediately after having an "experimental" (observed) recording.

Well infants were defined as those who were spontaneously breathing, free of sepsis or did not develop sepsis in the subsequent 48 hours, were not on morphine or sedative medication (midazolam or phenobarbitone), had no evidence of a patent ductus arteriosus and did not have an intraventricular haemorrhage larger greater than grade 2.

The observed, 20 minute recording represented the "experimental" or "steady" state. The unobserved, 2 hour recording represented the "real world" state. For each baby, the HRV values obtained in the "real world" state were compared to the HRV value obtained during the "steady" state, providing a ratio thus allowing the total variation for each HRV metric throughout the 2 hour recording to be demonstrated. The demographic data for the 16 babies who were used in this experiment are presented in table 6.3. The HRV results for this experiment are presented in chapter 9.

Baby Ref. No.	Sex	GA at birth (weeks)	Age at recording (days)	CGA at recording	No. of epochs contributing to HRV measure (%)
1	m	27.4	47	34.1	6 (55)
2	f	31.3	1	31.4	11 (100)
3	f	31.7	8	32.9	11 (100)
4	m	30.7	8	31.9	8 (73)
5	m	36.9	1	36.9	9 (82)
6	m	25.7	9	28.3	11 (100)
7	f	41.9	1	42.0	10 (91)
8	m	28.6	34	33.4	7 (64)
9	f	31.9	57	40.0	11 (100)
10	f	31.3	7	32.3	11 (100)
11	m	31.3	10	32.7	11 (100)
12	m	32.3	8	33.4	11 (100)
13	f	26.1	3	26.6	10 (91)
14	m	27.6	4	28.1	11 (100)
15	f	30.4	1	30.6	11 (100)
16	f	27.3	11	28.9	11 (100)

Table 6.3 Demographic data and the number of 20 minute epochs contributing to the 2 hour recordings for each observed/unobserved pair.

6.4.4 Comparing HRV in different gestational age groups (Chapters 10, 11 and 12)

Previous work has demonstrated that more mature infants have increased variability, with differences in frequency power distributions when compared with less mature infants[92,97,105,118,143-146]. To determine if the methodology developed for this thesis was able to detect similar differences, infants who were less than 10 days of age and "well" (see 6.2.3) were selected from the Liverpool Neonatal ECG bank. Infants less than 10 days of age were selected to minimise the deleterious effect that intensive care has on the developing autonomic nervous system.

The first comparison used 20 minute observed recordings, comparing HRV measures in babies ≤ 32 weeks and > 32 weeks corrected gestational age (CGA) at the time of recording (demographics in table 6.3). This experiment was repeated using the recordings of 2 hours duration (demographics in table 6.4). These experiments determined if the HRV measures, and in turn autonomic activity, were measurably different in the two gestational age groups.

	32 weeks (n=18)	**> 32weeks (n=14)**	p value
Gestation at birth (weeks)	27.1 (26.0 – 30.4)	34.7 (32.4 – 35.5)	<0.0005
Birth Weight (grams)	1070 (870 – 1305)	2183 (1401 – 2880)	<0.0005
Age recorded (days)	3.0 (1.0 – 5.8)	3.5 (1.0 – 6.8)	0.954
Gestation Age recorded (weeks)	28.1 (26.9 -31.0)	34.7 (33.0 – 35.8)	<0.0005
CPAP (%)	8 (44)	0 (0)	0.004
Caffeine (%)	16 (89)	1 (7)	<0.0005
AN Steroids (%)	17 (94)	9 (64)	0.064

Table 6.4 Demographic and clinical data for the observed recordings from well preterm (≤32 weeks GAA) and near term or term babies. Mann Witney test used for continuous data and Fishers exact test used for categorical data.

	≤ 32 weeks (n=21)	> 32 weeks (n=14)	P value
Gestation at birth (weeks)	27.9 (26.7 – 30.1)	32.6 (32.0 – 34.2)	<0.0001
Birth Weight (grams)	1020 (768 – 1349)	1816 (1647 – 2361)	<0.0001
Age recorded (days)	3 (2 – 7)	4 (1 – 8)	0.708
Gestation Age recorded (weeks)	28.4 (26.7 – 30.3)	33.6 (32.7 – 35.2)	<0.0001
CPAP (%)	5 (48)	0 (0)	0.0689
Caffeine (%)	13 (62)	3 (21)	0.0364
AN Steroids (%)	17 (81)	12 (86)	1.0000

Table 6.5 Demographic and clinical data for the unobserved recordings from well preterm (≤ 32 weeks GAA) and near term or term babies (> 32 weeks). Mann Witney test used for continuous data and Fishers exact test used for categorical data.

6.4.5 Comparing HRV in "experimental" and "real world" recordings in well babies (Chapters 10, 11 and 12)

Subsequent to comparison in 6.2.4, for each of the gestational age groups (≤ 32 weeks and > 32 weeks) the HRV results from the observed 20 minute recordings (experimental state) and the unobserved 2 hour recordings (real world state) were compared. This experiment determined if the HRV measures in the real world were the same as when measured in the experimental state.

To ensure that the "experimental" and "real world" populations were similar, demographic and clinical data were compared. This demonstrates that the groups were similar (table 6.5 and 6.6).

	Observed (18)	Unobserved (21)	P value
Gestation at birth (weeks)	27.1 (26.0 – 30.4)	27.9 (26.7 – 30.1)	0.78
Birth Weight (grams)	1070 (870 – 1305)	1020 (768 – 1349)	0.51
Age recorded (days)	3 (1 – 6)	3 (2 – 7)	0.71
Gestation Age recorded (weeks)	28.1 (26.9 -31.0)	28.4 (26.7 – 30.3)	0.89
CPAP (%)	8 (44)	5 (48)	0.20
Caffeine (%)	16 (89)	13 (62)	0.07
AN Steroids (%)	17 (94)	17 (81)	0.35

Table 6.6 Comparison of demographic and clinical data for the observed and unobserved recordings taken from well preterm (≤ 32 weeks) babies. Mann Witney test used for continuous data and Fishers exact test used for categorical data.

	Observed (14)	Unobserved (14)	P value
Gestation at birth (weeks)	34.7 (32.4 – 35.5)	32.6 (32.0 – 34.2)	0.44
Birth Weight (grams)	2183 (1401 – 2880)	1816 (1647 – 2361)	0.58
Age recorded (days)	3.5 (1.0 – 6.8)	4 (1 – 8)	0.80
Gestation Age recorded (weeks)	34.7 (33.0 – 35.8)	33.6 (32.7 – 35.2)	0.44
CPAP (%)	0 (0)	0 (0)	1.00
Caffeine (%)	1 (7)	3 (21)	0.60
AN Steroids (%)	9 (64)	12 (86)	0.39

Table 6.7 Comparison of demographic and clinical data for the observed and unobserved recordings taken from well term and near term (> 32 weeks) babies. Mann Witney test used for continuous data and Fishers exact test used for categorical data.

6.4.6 Comparing HRV in "well" and "unwell" babies (Chapters 10,11 and 12)

To assess if the HRV measures were able to detect differences between two distinct groups of babies, HRV results obtained from 2 hour recordings of "well" babies were compared to those who were "unwell".

Well babies are defined in 6.2.3. It would be expected that the "unwell" babies would have differences in autonomic functioning and would thus have differences in HRV measures.

The comparison between the two different populations of babies described in 6.2.4 and 6.2.5 is presented in chapters 10,11 and 12. The chapters are divided into time domain, frequency domain and Poincaré measures of HRV. The same babies recordings are used for these three chapters.

	Well, Spontaneously breathing (N=8)	**Unwell, Ventilated (N = 8)**	**P value**
GA at birth (weeks)	28.4 (25.6 – 33.1)	28.6 (25.4 – 33.8)	0.958
Birth Weight	972 (770 – 2080)	1070 (810 – 1952)	1.000
Age at recording (days)	12.5 (3.5 – 2.5)	15.5 (2.5 – 28.5)	0.958
CGA at recording	32.4 (29.1 – 34.4)	32.5 (29.0 – 35.9)	0.958
Sepsis (%)	0	5 (63)	0.100
IVH > Grade 2	0	5 (63)	0.100
PDA	0	1 (13)	0.500
Morphine	0	6 (75)	0.004
Caffeine	5 (63)	1 (13)	0.059

Table 6.8 Demographic and clinical data for unobserved recordings from well, spontaneously breathing babies matched with unwell, ventilated babies for gestational and post natal age. Mann Witney test used for continuous data and Fishers exact test used for categorical data.

To aid in presentation of the results, the results from the experiments listed in 6.2.4, 6.2.5 and 6.2.6 are presented over three chapters (10, 11 and 12), divided by the nature of the HRV measure: Time domain (chapter 10), frequency domain (chapter 11) and Poincare measure (chapter 12).

Chapter 7

Ability to obtain HRV metrics from the routinely monitored neonatal ECG

7.1 Introduction

This thesis has demonstrated that the developed methodology is able to produce HRV metrics from synthesized neonatal ECG's (4.5 and appendix C). Before any clinical investigation can be undertaken it is important that the method is also capable of producing HRV measures from the routinely monitored neonatal ECG. The first assessment applied the method of determining HRV measures to the observed, "experimental" 20 minute ECG recordings Subsequently, the method was applied to the "real world" recordings of infants undergoing routine care on the NICU who were not observed. This demonstrated the ability of the method to produce HRV metrics from the routinely monitored, "real world" neonatal ECG.

7.2 Methods

From the Liverpool Neonatal ECG bank observed recordings of 20 minutes duration and 2 hour, unobserved recordings were obtained. For the 20 minute recordings, each of the three ECG channels recorded were assessed to determine if they contained in excess of 10% of "missing" NNi data and were therefore unsuitable for analysis (6.1.4). For example, if a channel within the 20 minute recording contained $\geq$ 2 minutes where a R wave could not be detected, then it was rejected for analysis. Any channels with $\geq$ 90% of data available were then subject to the methodology to determine the HRV components within the ECG.

Each 2 hour recording consists of 11, overlapping 20 minute time epochs (6.1.2). For each 2 hour recording the number of 20 minute epochs containing $\geq$ 90% of NNi data, and therefore contributing to the overall HRV result, was determined.

7.3 Results

7.3.1 "Experimental" observed recordings

Seventy eight observed recordings were made from forty nine infants. The median gestation age at birth of the infants was 28.2 weeks (range 24.1 to 41.9 weeks), birth weight 1000g (range 530 to 3350g) with the median CGA of recording being 31.3 weeks (range 24.9 to 42.0 weeks). (table 6.1)

Of the 78 recordings, 76 provided HRV metrics. Two recordings were unable to produce any HRV metrics as all 3 channels within these recordings had missing data in excess of 10% of the total data for that channel. The "best" channel for these two rejected recordings had 10.9% and 11.5% of the data missing. For the remainder, 27 (34.6%) recordings provided one suitable channel, 27 (34.6%) provided two channels, and 22 (28.2%) provided 3 channels (table 7.1). The median percentage of data present for the "best" channel for each recording was 98.7% (IQR 97.0 – 99.6%). The HRV metric for each recording is determined from averaging the result from all channels where there is less than 10% of data missing. If only those channels which were used to produce HRV metrics are considered, the median percent of data present was 97.4%v (IQR 94.3 to 99.0%). (Table 7.1)

No. recordings with 0 channels >90% data	2 (2.6%)
No. recordings with 1 channel >90% data	27 (34.6%)
No. recordings with 2 channels >90% data	27 (34.6%)
No. recordings with 3 channels >90% data	22 (28.2%)
"Best" channel % data present (median(IQR))	98.7% (97.0 – 99.6%)
% Data present for channels used for HRV	97.4% (94.3 – 99.0%)

Table 7.1 Details of the number of channels available to produce HRV measures from the 76 20 minute ECG recordings

7.3.2 "Real World" unobserved recordings

One hundred recordings were made from 97 babies. The median gestation age at birth of the infants was 28.4 weeks (range 23.1 to 41.9 weeks), birth weight 1077g (range 515 to 4620g) with the median age of recording being 31.9 weeks (range 24.7 to 42.0 weeks). (table 6.2)

97 out of the 100 recordings were able to provide a HRV measure within the 2 hour window, that is there was at least one 20 minute time epoch which was suitable for analysis. The median number of epochs included to produce the HRV metric was 10 (IQR 8 – 11) with nearly half (47%) of all 2 hour recordings having all 11 time epochs contributing.

No. recordings producing HRV metric	97 (97%)
Median number of time epochs producing a HRV metric	10 (8 - 11)
No. recordings with HRV metrics from all 11 time epochs	47 (47%)
No. recordings with HRV metric from ≥ 6 time epochs	87 (87%)

Table 7.2 Details of the number of time epochs available to produce HRV measures from the 100 2 hour ECG recordings

7.4 Discussion

The developed methodology was able to produce HRV metrics in 97% (observed) and 98% (unobserved) of recordings. The developed method is thus demonstrated to be able to produce HRV measures from routinely monitored neonatal ECG signals. Under "experimental" (observed) conditions, approximately $1/3^{rd}$ of recordings will provide HRV data from all 3 ECG channels, one third from 2 channels and one third from all 3 channels. The two recordings which were unable to provide HRV metrics contained < 12% of missing data. Given that the LSP is accurate to within 4.4% even when 15% of data points are removed (appendix C), it could be argued that the cut off at 10% of missing data points is over cautious.

With the "real world" unobserved recordings, a 2 hour time window will provide a HRV measurement 97% of the time. The majority of these (87%) will provide HRV metrics more than half the time whilst nearly half will provide a HRV metric at all times. Given that the method is producing sophisticated frequency domain measures of HRV these are encouraging results in the development of using HRV as a monitoring tool.

7.5 Conclusion

The method developed for use in this thesis is effective at producing HRV measures from neonatal ECG recordings from babies who are receiving routine neonatal care in the NICU. Ninety seven per cent of recordings of 2 hours duration were able to provide HRV metrics, demonstrating that the method is robust enough to be used to monitor HRV using the "real world" ECG recorded in the NICU.

Chapter 8

The effect of removing non-stationaries

8.1 Introduction

The underlying HRV harmonic is disturbed by the presence of non-stationaries[273]. How these non-stationaries are dealt with prior to producing HRV metrics is rarely discussed in the published research with there being no assessment of the impact they may have on overall HRV reported in the neonatal literature. Whist there is important information within these non-stationaries, such as the presence of bradycardic events prior to the onset of neonatal sepsis, they disturb the underlying harmonic within the NNi tachogram. In this chapter, HRV metrics before and after the removal of non-stationaries will be compared to assess the impact of removing non-stationaries.

8.2 Methods

Recordings of 20 minute duration were selected from the Liverpool Neonatal ECG bank and analysed to produce HRV metrics before the removal of non-stationaries. Following the removal of non-stationaries the same recordings produced a second set of HRV metrics. The results before and after removal of removal of the non-stationaries were compared using the Wilcoxon signed rank test with a p value <0.05 indicating a statistically significant difference.

8.3 Results

Seventy six 20 minute ECG recordings and were subjected to the developed methodology to produce HRV metrics. The demographics for these babies is in table 6.1. Following the removal of non-stationaries overall variability is, unsurprisingly, reduced (standard deviation of RRi 16ms *vs.* 13.5ms, $p<0.0005$). Removing non-stationaries also significantly reduced the kurtosis, Poincare SD1 and SD2, the frequency components VLF, LF, HF and the LF:HF+ ratio. (Table 8.1). Examples of the non-stationary data removed can be seen in figures 6.2, the removed data highlighted in red.

	Raw RRi tachogram	**Processed RRi tachogram**	**%diff**	**p value**
Variability (ms^2)	255.9 (113.9 - 407.8)	183.5 (99.4 - 268.1)	-28.3	<0.0005
Standard deviation (ms)	16.0 (10.7 - 20.2)	13.5 (10.0 - 16.4)	-15.3	<0.0005
Mean RRi (ms)	390.2 (367.4 - 423.8)	388.7 (366.3 - 424.0)	-0.4	0.424
Kurtosis	6.49 (3.38 - 18.60)	3.90 (3.16 - 5.15)	-39.9	<0.0005
Skew	-0.133 (-0.922 - 0.424)	0.000 (-0.393 - 0.338)	-99.9	0.295
Poincarre SD1	3.92 (3.18 - 4.58)	3.37 (2.77 - 4.15)	-13.9	<0.0005
Poincarre SD2	19.73 (13.65 - 26.38)	15.97 (12.42 - 20.49)	-19.1	<0.0005
ULF (Hz)	0.302 (0.191 - 0.375)	0.307 (0.175 - 0.407)	1.4	0.261
VLF (Hz)	0.631 (0.535 - 0.757)	0.606 (0.474 - 0.721)	-3.9	0.001
LF (Hz)	0.244 (0.142 - 0.416)	0.177 (0.123 - 0.261)	-27.4	<0.0005
HF (Hz)	0.046 (0.026 - 0.089)	0.036 (0.022 - 0.055)	-22.0	<0.0005
HF+ (1.0Hz)	0.058 (0.031 - 0.096)	0.067 (0.038 - 0.093)	14.7	0.263
LF:HF ratio	4.98 (4.11 - 6.60)	5.37 (3.55 - 7.34)	7.9	0.897
LF:HF+ ratio	2.38 (1.57 - 3.23)	1.84 (1.19 - 2.80)	-22.8	<0.0005

Table 8.1 Effect of removing non-stationarities within the neonatal ECG on the derived HRV metrics (Median and IQR). Wilcoxon signed rank test used for analysis.

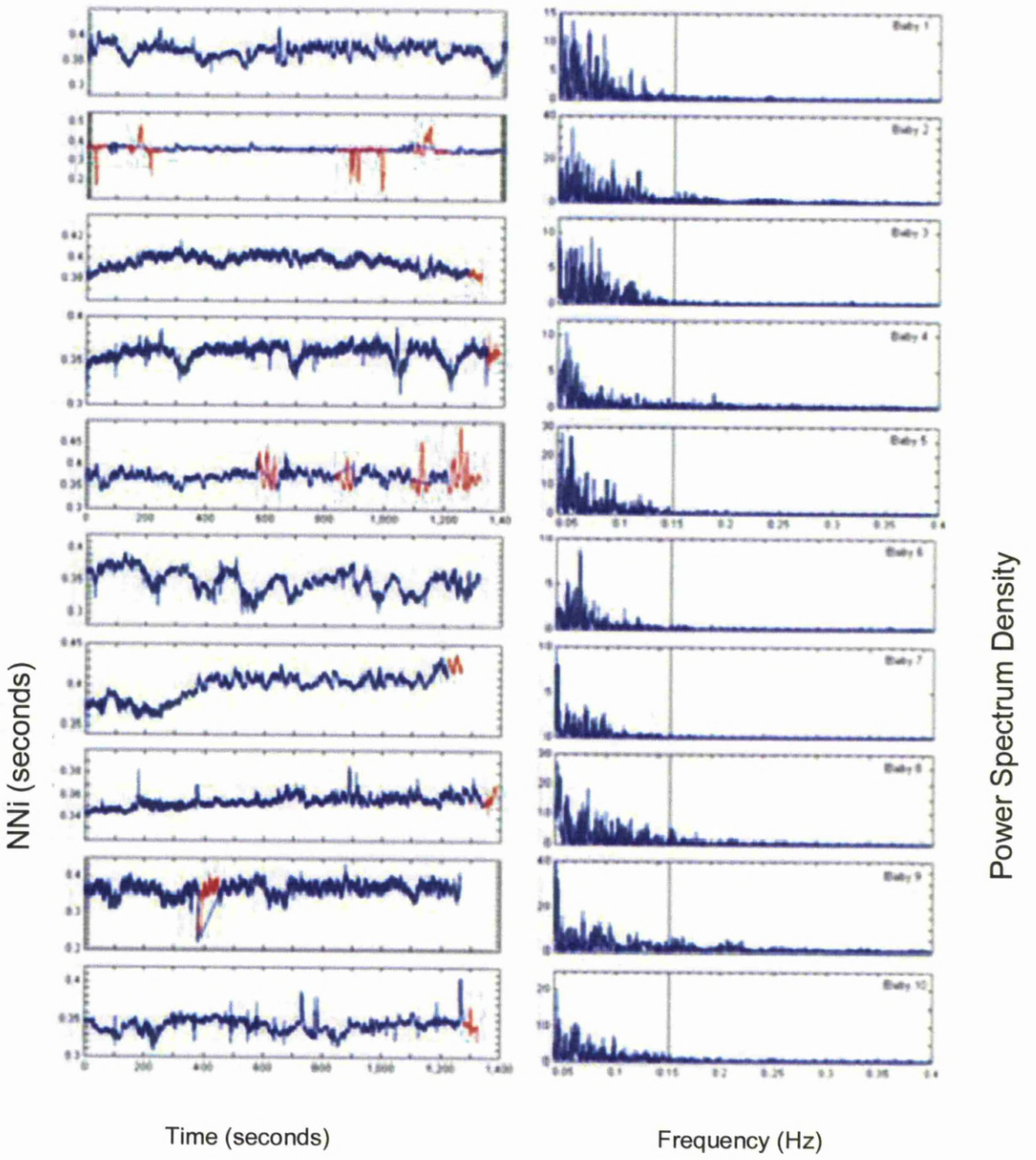


Figure 8.1 Example RRi tachograms with their corresponding power spectrum densities from 20 minute recordings. The signals in red within the RRi tachograms represent non-stationaries that are removed.

8.4 Discussion

Overall variability, as measured by the standard deviation of the RR interval, Poincare SD1 and SD2, is unsurprisingly reduced when non-stationaries are removed from the NNi tachogram. In addition, the power in each of the VLF, LF and HF frequency bands is reduced. The non-stationaries contribute a significant amount of the variation seen in HRV.

The neonate is particularly prone to the presence of non-stationaries with the majority of published reports of neonatal HRV failing to acknowledge their presence. It is important that the characteristics of these non-staionaries are presented as they may contain important clinical information about the current homeostatic state of the infant. This is particularly prevalent, for example, in the septic infant who demonstrates increasing episodes of bradycardia. If the RR intervals within these bradycardic events are used to calculate HRV metrics, they may overestimate the true underlying HRV harmonic.

8.5 Conclusion

The presence of non-stationeries has a significant effect on the resulting HRV metrics. It is assumed that the removal of these non-stationeries allows the underlying HRV harmonic to be assessed and allows the underlying ANS activity to be measured. Caution must be undertaken when results from previous studies are interpreted where non-stationeries have not been taken into consideration as the resultant HRV metrics may reflect the effect of these non-stationeries and not the underlying HRV harmonic. Comparisons between the results obtained in this thesis and from other studies must also be interpreted with caution as non-stationeries will be removed from the NNi tachogram when subjected to investigations in the following chapters.

Chapter 9

Stability of HRV metrics during routine neonatal care

9.1 Introduction

Traditional HRV analysis has used recordings of infants in ideal, "laboratory" conditions. That is when they are asleep, undisturbed with electrodes remained constantly attached in the ideal position. Infants being cared for in the neonatal intensive care unit undergo frequent disturbances to their steady state. Painful procedures, routine hygiene cares, feeds and noise will all affect the infant causing changes in the infant's autonomic functioning. For a HRV metric to be a useful clinical monitoring measurement, this background clinical "noise" must have minimum effect on the measurement. The HRV measurement recorded during normal intensive care management must closely resemble that recorded in the observed steady state. In this chapter, the stability of each HRV metric measured during routine intensive will be determined.

9.2 Methods

The observed, 20 minute recording represented the "experimental" state. The unobserved, 2 hour recording represented the "real world" state. Sixteen "well" babies (see 6.2.3) had unobserved ("real world") recordings of 2 hours duration taken immediately after the observed 20 minute ("steady state") recordings had been completed (table 6.3). For each baby, the HRV values obtained from the "real world" recording were compared to those from the "experimental" recording providing a ratio. This ratio compared the median HRV measure in the "real world" recording with the HRV measure from the 20 minute "experimental" recording. In addition, the HRV metric for each individual 20 minute epoch within the 2 hour "real world" recording was compared to the value obtained from the 20

minute "experimental" recording, allowing the total variation throughout the 2 hour recording to be demonstrated.

As the comparison was between one 20 minute epoch and several, overlapping 20 minute epochs (see 6.1.2) and multiple analyses were undertaken, simple statistical analysis was not suitable to determine significant differences. The statistical methods used are described in 6.3.

To determine if individual babies demonstrated more variability than others, a summary statistic for all of the HRV measures in the 2 hour recording for each baby was produced. The ratio for each HRV metric was made positive and then the median HRV ratio for each baby was determined.

9.3 Results

Sixteen well infants had observed/unobserved paired ECG recordings. The median gestational age at birth was 31 weeks (IQR 27.5 – 31.7), age at recording 8 days (3 – 10) with corrected gestational age at recording 32.5 weeks (30.1 – 33.6) (table 6.3). All of the 16 two hour recordings provided HRV measurements. The mean number of 20 minute epochs contributing to the 2 hour median HRV metric was 10 (standard deviation 1.7, range 6 - 11) with 10 out of the 16 recordings being of sufficient quality to produce HRV metrics for all 11 20 minute epoch. Demographic data and information regarding the number of time epochs contributing to the unobserved measurements is listed in table 6.3.

Recording Pair No.	Var	SD	Mean	Kurt	Skew	PC SD1	PC SD2	PCsd1 :sd2	ULF	VLF	LF	HF	HF+	LF:HF	LF:HF+	Median Ratio
1	3.46	1.86	0.93	3.14	-18.85	2.35	1.30	2.10	0.68	1.91	0.70	1.86	2.30	0.40	0.39	**1.86**
2	1.60	1.26	0.82	1.13	0.66	0.83	1.28	0.58	2.88	1.09	1.17	2.06	0.50	0.73	1.66	**1.15**
3	0.79	0.89	1.02	2.64	1.75	1.22	0.80	1.49	1.28	0.81	1.31	1.13	1.10	1.05	0.93	**1.07**
4	1.08	1.04	1.01	1.01	-4.88	1.12	1.03	0.98	0.67	0.76	1.42	1.39	0.85	1.02	1.29	**1.02**
5	2.21	1.49	0.98	0.81	0.77	0.93	1.30	0.75	2.78	1.17	0.52	1.18	1.13	0.49	0.56	**1.06**
6	4.77	2.18	1.04	2.09	1.57	0.57	1.10	0.57	0.57	1.01	1.27	1.17	0.48	0.71	1.01	**1.03**
7	4.34	2.08	0.94	3.42	-22.77	1.05	1.89	0.57	1.39	1.87	0.98	0.84	0.65	1.03	1.47	**1.22**
8	0.71	0.84	0.96	1.15	1.81	0.86	0.81	1.02	1.17	1.19	0.71	0.84	0.95	0.82	0.80	**0.85**
9	3.77	1.94	0.95	3.68	1.80	0.83	1.01	0.86	0.85	0.97	0.88	1.55	1.31	0.68	0.82	**0.96**
10	9.93	3.15	1.13	0.59	2.02	1.53	2.20	0.70	0.68	0.86	1.24	0.89	0.23	1.44	2.61	**1.19**
11	2.01	1.42	0.91	1.97	0.99	0.95	1.49	0.64	1.51	1.55	0.81	0.41	0.48	2.13	2.20	**1.45**
12	2.64	1.63	0.94	0.73	0.63	0.79	1.40	0.56	0.70	1.84	0.74	0.48	0.36	1.42	1.75	**0.86**
13	0.22	0.47	1.05	5.15	-1.73	0.97	0.45	2.58	0.28	1.31	3.75	6.54	2.71	0.57	1.00	**1.02**
14	1.76	1.33	0.99	1.13	-3.71	0.87	1.17	0.68	1.40	1.23	1.24	1.00	0.68	1.23	1.76	**1.20**
15	5.00	2.24	1.07	12.20	-2.77	1.10	1.49	0.73	0.84	0.71	1.83	3.64	1.16	0.61	1.10	**1.13**
16	0.09	0.30	0.78	0.16	-69.67	0.42	0.28	0.90	0.12	0.45	0.31	0.23	0.08	0.19	0.07	**0.25**

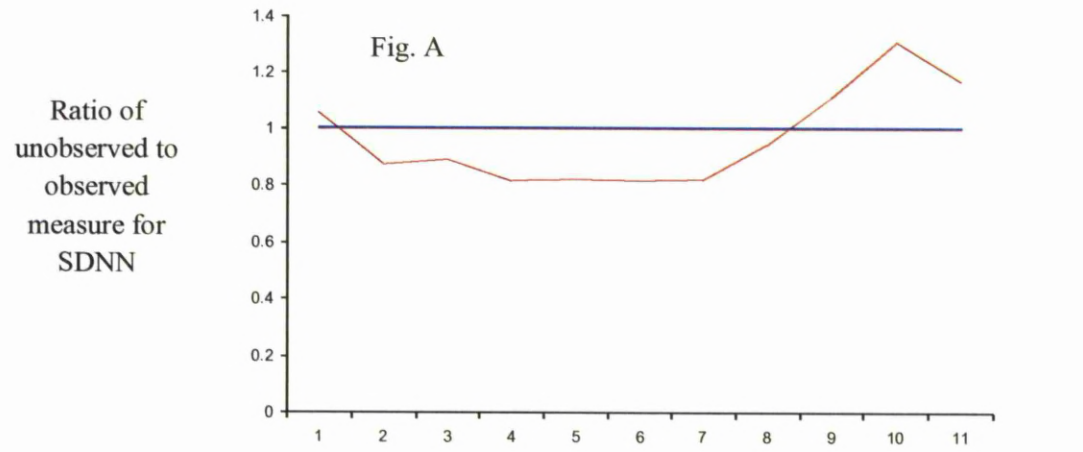
Table 9.1 HRV ratio for each recording pair (1-16). The median ratio is a summary statistic for the ratio observed across all HRV measures for each individual baby.

There was a wide range of variation between babies in the unobserved:observed ratios observed. Some babies showed a marked reduction 0.25 (baby 16, table 9.2) when "real world" were compared with "experimental" HRV measures, whilst others showed an overall increase in the HRV measures. Correlating the median ratio for each baby with gestational age at birth (r^2 = 0.06), age at recording (r^2 = 0.03) and CGA at recording (r^2 = 0.06) showed no significant correlation

An example of the variation seen in the 20 minute epochs for the SD of the RRi for two unobserved recordings is in figure 9.1.

The HRV metrics which remained most stable when the unobserved recordings were compared to the observed recordings were mean RRi (median ratio 0.98 (IQR 0.94 – 1.03)) and PCSD1 (0.94 (0.83 – 1.10)). The HRV metrics which demonstrated stability with an IQR of 0.5 – 1.5 when the unobserved recordings were compared to the observed recordings were mean PCSD2 (1.22 (0.96 -1.43)), PCSD1:SD2 ratio (0.74 (0.62 – 0.99)), ULF (0.85 (0.67 – 1.39)), VLF (1.18 (0.84 – 1.37)), LF (1.20 (0.73 – 1.28)), and the ratio LF:HF (0.92 (0.60 – 1.10)). (Table 9.3).

When the statistical model described above was applied, overall variability (reflected in Variance and standard deviation of the NNi) and kurtosis demonstrated statistically significant differences between observed and non-observed recordings (table 9.3). The other HRV measures were not statistically significantly different between the "real world" and "experimental" recordings.



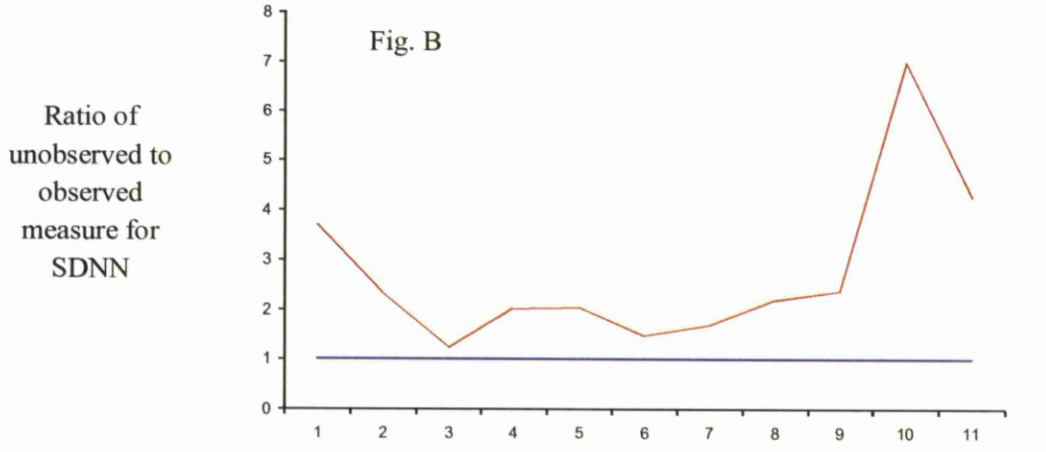


Figure 9.1 Line chart demonstrating variation in the standard deviation in heart rate over a 2 hour period (red line). The blue line represents the value obtained from the observed recording. (ECG 64 and 58). It can be seen in fig a (ECG 64 and 58, pair 3) that the SD shows little variation from the observed recording. In fig B (ECG 68 and 73, pair 10) the SD fluctuates between 1.2 to 7.0 times the observed data.

	Unobserved:Observed Ratio Median (IQR)	**F Stat**	**p-value**
Variability	2.11 (1.01 – 3.91)	7.81	0.014
Standard deviation	1.45 (1.00 – 1.98)	7.81	0.014
Mean RRi	0.98 (0.94 – 1.03)	1.71	0.21
Kurtosis	1.94 (0.96 – 3.21)	7.7	0.014
Skew	0.72 (-4.0 – 1.62)	2.16	0.16
Poincare SD1	0.94 (0.83 – 1.10)	0.062	0.81
Poincare SD2	1.22 (0.96 -1.43)	1.97	0.18
Poincare SD1:SD2	0.74 (0.62 – 0.99)	1.91	0.19
ULF	0.85 (0.67 – 1.39)	0.12	0.73
VLF	1.18 (0.84 – 1.37)	3.54	0.08
LF	1.20 (0.73 – 1.28)	1.62	0.22
HF	1.18 (0.84 – 1.63)	3.27	0.091
HF+ (1.0Hz)	0.90 (0.48 – 1.14)	1.2	0.29
LF:HF ratio	0.92 (0.60 – 1.10)	1.76	0.2
LF:HF+ ratio	1.20 (0.81 – 1.68)	1.05	0.31

Table 9.2 Ratios of unobserved:observed HRV measures for the entire recording with statistical anlaysis (see text) to determine if the unobserved results were artistically significantly different to the observed results.

9.4 Discussion

This chapter has explored the fluctuation of the HRV measures in well babies. For a HRV metric to be a useful clinical monitoring tool it must perform well in the real world environment of the neonatal intensive care unit. That is, be able to detect the underlying autonomic activity of the neonate and not be altered by routince care practises. Only the SDNN and the kurtosis of the NNi is significantly different when "real world" recordings are compared to those recorded in the "experimental" state. The HRV metrics which most closely reflect the resting autonomic status are mean RRi and Poincare SD1 (ratio 0.98 (0.94 – 1.03) and 0.94 (0.83 – 1.1).

The continually modulating HRV metrics may simply reflect the fluctuating activity of the ANS in response to undetermined stressors. However, one would also expect that the underlying homeostatic autonomic functioning to remain constant over a 2 hour period in a well baby. Babies who are moving from the "well" to "unwell" state may manifest this in either (or both) of the following ways;

i. change in the absolute median value of the HRV metric reflecting change in "quantity" of autonomic functioning
ii. change in the modulation of the HRV metric reflecting a change in the ability of the ANS to respond to different stressors.

The results demonstrate that the HRV metrics fluctuate to varying degrees during routine neonatal care. Those most closely reflecting the steady state are the Poincare measures SD1, SD2, and the ratio (SD1:SD2) with the LF:HF ratio also being relatively static. The low and high frequency bands, standard deviation, skew and kurtosis showed most variability. Standard deviation and kurtosis demonstrated statistically significant differences when observed and unobserved recordings were compared. This is expected as these metrics will increase with increasing length of recording; the more RRi measured the more variability will be observed. The statistical significant differences between these metrics is evidence that they are affected by events occurring whilst the baby is not being observed during measurement. Therefore changes in these in any experiment conducted with the baby not being observed may be due to 'unobserved events' rather than that being tested by the particular experimental design. However,

for the other HRV metrics, the non-significant differences between observed and unobserved recordings indicate that unobserved recordings do reflect the "resting" ANS state. Thus it is valid to use unobserved, "real world" recordings of neonatal ECGs to produce HRV metrics in further experiments. Due to the small sample size however, caution should be exercised in the interpretation of p-values in this chapter.

In addition to the fluctuation of the HRV metrics, individual babies demonstrated different degrees to which their HRV measures fluctuated during routine neonatal care. There was no correlation to gestational age at birth or CGA at recording with these fluctuations.

9.5 Conclusion

In this small observational cohort most HRV metrics recorded in the "real world" reflected those measured in the "steady state". The expected increase in overall variability and kurtosis with increasing length of recording is demonstrated. Other HRV measures however were not statistically significantly different when real world HRV measures were compared to those in the steady state. Caution must be taken due to the small sample size and further work with larger populations would be able to determine if the results of this investigation remain valid.

Chapter 10

Time domain measures

10.1 Introduction

To determine if the developed methodology is able to distinguish between different ANS activity states, recordings of different populations of babies were selected from the Liverpool Neonatal ECG bank. These populations consisted of:

1. Different gestational age groups. Previous work has demonstrated that more mature infants demonstrate increased variability, with differences in frequency power distributions when compared with less mature infants[92,97,105,118,143-146]. To determine if the observed recordings and the methodology developed for this thesis was able to detect similar differences, the HRV results obtained from preterm (≤32 weeks gestational age) and near term/term infants (>32 weeks gestational age) were compared.

2. "Well" and "Unwell" babies. For a metric to be a useful monitoring tool it must be able to determine between the physiological and pathological state with differences measurable both inter and intra individual. The developed methodology was applied to two distinct groups of babies ("well" and "unwell", see 6.1.2) to determine if the measured variables were able to distinguish between the two groups. If any of the metrics are potential clinically useful monitoring tools they should be able to detect differences in HRV indices

Time domain HRV measures were compared within these distinct clinical groups to determine if statistically significant differences were present.

10.2 Methods

To determine the HRV differences between babies at different gestational ages, infants who were less than 10 days of age and "well" were selected. Well infants are defined in 6.2.3. Infants less than 10 days of age were selected to minimise the deleterious effect that intensive care has on the developing autonomic nervous system. The HRV results obtained from preterm (≤32 weeks gestational age) and near term/term infants (>32 weeks gestational age) were compared.

Two methods of recording were used – the observed 20 minute recordings and the unobserved 2 hour recordings. To compare the results between the gestational age groups from 20 minute recordings the Mann-Whitney test was used for comparison. As the 2 hour recordings contained overlapping time window epochs (6.1.2) the data, by definition, were not independent. Thus simple statistical methods were not suitable for data analysis. Statistical analyses are described in 6.3.

To determine if the HRV measures in the "real world" were similar to those in the "steady state", the results from the unobserved recordings were compared with those from the observed recordings for each of the gestational age groups (≤ 32 weeks and <32 weeks

The comparison between "well" and "unwell" babies only used recordings of 2 hours duration. This was because there were limited numbers of observed recordings of well babies as they underwent more frequent clinical interventions and handling episodes. Unwell, ventilated babies were matched with the spontaneously breathing well babies for gestational and post natal age at time of recording. Ventilated babies were included if they had any of the above conditions and were thus deemed "unwell". The statistical analysis described above to compare gestational age groups with 2 hour recordings was applied for this comparison.

10.3 Results

For the comparison between different gestational age groups thirty two 20 minute observed recordings and thirty five 2 hour unobserved recordings were obtained from well infants in the first 10 days of life. Demographic data for both the observed and unobserved is presented in tables 6.4 and 6.5 respectively.

For both observed and unobserved infants the birth weight was significantly lower for the more immature infants as expected. Also, the infants less than 32 weeks gestational age were more likely to be receiving caffeine (16 *vs.* 1, p = <0.0005 and 13 *vs.* 3, p = 0.0364) and/or continuous positive airway pressure support (CPAP) (8 *vs.* 0, p=0.004, 5 *vs.* 0 0.0689). There was a non-significant trend toward increased administration of antenatal steroids in the more immature infants. (Table 6.4 and 6.5)

Eight 2 hour recordings were identified from babies who were ventilated and were defined as "unwell". For each unwell case, a well control baby was matched for gestational and post natal age. The characteristics of the "well" and "unwell" babies are shown in table 10.3.

10.3.1 Comparing HRV in different gestational age groups

From the observed 20 minute recordings, mature infants demonstrated increased mean RRi (i.e. slower heart rates). There was no difference in the variability, kurtosis or skew between different GA groups. (table 10.4).

	≤ 32 weeks (n = 18)	> 32 weeks (n=14)	P value
Variability (ms^2)	150.5 (98.7 – 242.5)	274.0 (108.9 – 570.8)	0.145
Standard deviation (ms)	12.3 (9.9 – 15.6)	16.6 (10.4 – 23.9)	0.145
Mean RRi (ms)	393 (365 – 421)	454 (405 – 474)	0.016
Kurtosis	3.8 (3.0 – 5.8)	4.0 (3.3 – 4.6)	0.866
Skew	-0.28 (-0.46 – 0.45)	-0.18 (-0.67 – 0.08)	0.512

Table 10.1 Median and IQR for the time domain HRV values obtained from 20 minute observed recordings in well babies comparing preterm (<32 weeks GAA) with infants near term or term.

When the longer, two hour recordings were analysed the mean RRi was increased in the more mature babies (426.2 ms *vs.* 387.9 ms) but did not reach the statistical significance required of p <0.001 for the statistical analysis. (table 10.5)

	≤ 32 weeks (n=21)	>32 weeks (n=14)	F stat	P value
Variability (ms^2)	132.9 (98.3 – 236.0)	467.0 (407.1 – 606.7)	22.12	<0.0001
Standard deviation (ms)	11.6 (9.9 – 15.4)	21.6 (20.2 – 24.4)	5.59	<0.0001
Mean RRi (ms)	387.9 (366.9 – 403.1)	426.2 (410 .7 – 446.2)	5.89	0.016
Kurtosis	4.47 (3.30 – 7.16)	2.98 (2.93 – 3.23)	18.59	<0.0001
Skew	0.272 (-0.109 – 0.564)	0.070 (-0.262 – 0.145)	0.92	0.34

Table 10.2 Median and IQR for the time domain HRV values obtained from 2 hour unobserved recordings in well babies comparing preterm (<32 weeks GAA) with infants near term or term. See main text for statistical analysis used.

10.3.2 Comparing HRV in "experimental" and "real world" recordings in well babies

In preterm babies (≤ 32 weeks GA) none of the time domain HRV measures were statistically significantly different between the "experimental" (observed) and "real wolrd" (unobserved) recordings. (table 10.6).

	Experimental (n = 18)	Real World(n = 21)	P value
Variability (ms^2)	150.5 (98.7 – 242.5)	132.9 (98.3 – 236.0)	0.96
Standard deviation (ms)	12.3 (9.9 – 15.6)	11.6 (9.9 – 15.4)	0.99
Mean RRi (ms)	393 (365 – 421)	387.9 (366.9 – 403.1)	0.99
Kurtosis	3.8 (3.0 – 5.8)	4.47 (3.30 – 7.16)	0.28
Skew	-0.28 (-0.46 – 0.45)	0.272 (-0.109 – 0.564)	0.90

Table 10.3 Median and IQR for the time domain HRV values obtained from preterm babies (≤ 32 weeks) in observed and unobserved recordings. See main text for statistical analysis used.

In the term and near term babies increased SD of the NNi, mean RRi and skewness to the right of the NNi were present in the unobserved recordings compared to the observed recordings and were highly statistically significantly different (p>0.0001). (table 10.7)

	Experimental (n = 14)	Real World (n = 14)	P value
Variability (ms^2)	274.0 (108.9 – 570.8)	467.0 (407.1 – 606.7)	<0.0001
Standard deviation (ms)	16.6 (10.4 – 23.9)	21.6 (20.2 – 24.4)	<0.0001
Mean RRi (ms)	454 (405 – 474)	426.2 (410 .7 – 446.2)	<0.0001
Kurtosis	4.0 (3.3 – 4.6)	2.98 (2.93 – 3.23)	0.12
Skew	-0.18 (-0.67 – 0.08)	0.070 (-0.262 – 0.145)	<0.0001

Table 10.4 Median and IQR for the time domain HRV values obtained from term and near term babies (> 32 weeks) in observed and unobserved recordings. See main text for statistical analysis used.

10.3.3 Comparing HRV in "well" and "unwell" babies

Comparing well with unwell babies demonstrated a statistically significant difference in skewness of the RRi only with the well babies demonstrating a negative skew (-0.35 (-0.46 - -0.28)) and the unwell babies a positive skew (0.16 (-0.06 – 0.76). (Table 10.6)

	"Well" (n = 8)	"Unwell" (n = 8)	Trans	F stat	P value
Variability (ms^2)	272.7 (197.3 – 401.6)	227.3 (166.4 – 303.7)	Log	1.3	0.26
Standard deviation (ms)	16.5 (14.0 – 20.0)	15.1 (12.9 – 17.4)	Id	2.05	0.26
Mean RRi (ms)	381.9 (373.8 – 387.2)	397.5 (384.6 – 439.2)	Log	1.58	0.21
Kurtosis	3.39 (3.12 – 3.62)	3.78 (3.50 – 5.07)	Arc	3.89	0.051
Skew	-0.35 (-0.46 - -0.28)	0.16 (-0.06 – 0.76)	Arc	14.5	0.0002

Table 10.5 Median and IQR for the time domain HRV values obtained from 2 hour unobserved recordings in spontaneously breathing compared with ventilated babies. See text for statistical methods applied. Trans* = Transformation applied to data, logarithmic, arcan or identity.

10.4 Discussion

Comparing infants recorded at different gestational ages demonstrates differences in time domain HRV metrics. More mature infants (>32 weeks corrected gestational age at the time of recording) had increased mean RRi (i.e. faster heart rates) in both the observed and unobserved groups. Increased variability (increased standard deviation of RRi) with reduced kurtosis (indicating a wider spread) was also present in the more mature babies during unobserved recordings, common with previous investigations of HRV in different gestational age groups (section 2.5.5).

For preterm babies the time domain measures of HRV were similar when the results of those recorded in the "real world" were compared with those recorded in the "experimental" state. This demonstrates that the real world measures of HRV reflect the underlying autonomic activity and are not influenced by the routine care given. However, the more mature babies showed highly significant differences when the real world measures were compared to the experimental measures. This statistically significant difference may reflect a more mature and active ANS, which is fluctuating physiologically in response to minor stressors experienced during routine

care. This compares with the more immature ANS which does not respond to these minor stressors. This increased fluctuation/responsiveness requires further investigation to determine its cause. In addition, this "variability of the HRV" should be investigated to determine if it provides useful clinical information over and above the individual HRV measures.

In the well babies skewness was toward the left and skewed to the right in the unwell babies. Skewness is a measure of the asymmetry of variables: with a negative skew the left tail is longer with the mass of the distribution concentrated on the right of the median, i.e. there are relatively few low values; with a positive skew the right tail is longer with the mass of the distribution concentrated on the left of the median i.e. there are relatively few high values. In the context of this study it means that the unwell babies had more NNi's that were shorter than the median, resulting in a higher heart rate. This skewness was also detected by Griffin and Moorman's team when analaysing HRV in septic infants and is utilised in their production of their heart rate characteristics measure[286].

10.5 Conclusion

In this chapter, time domain measures of HRV were compared to determine if there were differences between two distinct groups of babies. As expected, mean RRi were statistically significantly different between preterm and more mature infants. Kurtosis was also different between these babies when recordings were taken from babies undergoing routine care.

HRV measures in preterm babies were the same when measured in the real world and experimental state. However, in term babies, real world measures of HRV were significantly different from those recorded in the experimental state, possibly indicating the increased maturation of the ANS.

Only skewness of the RRi was statistically significantly different between the two groups when well and unwell babies were compared. The usefulness of time domain measures of HRV using "real world" neonatal ECG as a descriptor of autonomic activity are demonstrated in this

chapter. Time domain measures warrant further investigating as a useful measure of HRV using routinely monitored neonatal ECG signals.

Chapter 11

Frequency domain HRV Measures

11.1 Introduction

In chapter 10, time domain measures of HRV were demonstrated to be statistically significantly different between distinct groups of babies and when "real world" HRV was compared with "experimental" HRV in mature babies but not in more preterm babies. Frequency domain measures quantify the activity of both branches of the ANS activity and therefore provide direct information on the relative activity of the PNS and SNS (2.4.4). However, traditional FFT methods are unusable for routinely monitored signals (5.2). In this chapter, the developed method which utilises the LSP will be assessed to determine if frequency domain measures of HRV values are different between different populations of babies and between "real world" and "experimental" recordings

11.2 Methods

From the Liverpool Neonatal ECG bank, the same recordings as in chapter 10 were selected, that is recordings taken from different gestational age groups (≤32 weeks and > 32 weeks) and well/unwell babies. The two gestational age groups included observed 20 minute and unobserved 2 hour recordings. The frequency domain HRV measures from the gestational age groups and the well/unwell pairs were compared using the statistical analyses described in section 10.2.

To determine if the HRV measures in the "real world" were similar to those in the "steady state", the results from the unobserved recordings were compared with those from the observed recordings for each of the gestational age groups (≤ 32 weeks and <32 weeks).

11.3 Results

The patient demographics for the three compared populations of babies are presented in section 10.3.1.

11.3.1 Comparing HRV in different gestational age groups

For the short term recordings the frequency bands of more mature infants demonstrated decreased power in the VLF (0.49 *vs.* 0.64) and increased power in the LF (0.33 *vs.* 0.15) and HF (0.06 *vs.* 0.03) power band (table 11.1). In the longer term recordings there were no statistically significant differences between either the two gestational age groups (table 11.2) or the well and unwell babies (table 11.9) with any of the frequency measure of HRV.

	≤32 weeks (n=18)	> 32 weeks (n=14)	P value
ULF	0.29 (0.16 – 0.43)	0.20 (0.10 – 0.30)	0.168
VLF	0.64 (0.48 – 0.74)	0.49 (0.31 – 0.58)	0.009
LF	0.15 (0.09 – 0.23)	0.33 (0.22 – 0.38)	0.002
HF	0.03 (0.02 – 0.06)	0.06 (0.02 – 0.07)	0.045
HF+ (1.0Hz)	0.06 (0.04 – 0.10)	0.07 (0.05 – 0.12)	0.613
LF:HF ratio	6.40 (4.01 – 8.60)	5.25 (3.35 – 8.48)	0.613
LF:HF+ ratio	1.93 (1.09 – 2.65)	2.35 (1.23 – 3.70)	0.357

Table 11.1 Median and IQR for the frequency domain HRV values obtained from 20 minute observed recordings in well babies comparing preterm (<32 weeks GA) with infants near term or term.

	≤32 weeks (n=21)	>32 weeks (n=14)	F stat	P value
ULF	0.31 (0.22 – 0.43)	0.310 (0.155 – 0.336)	4.55	0.034
VLF	0.64 (0.55 – 0.70)	0.619 (0.532 – 0.651)	0.96	0.33
LF	0.21 (0.17 – 0.28)	0.218 (0.199 – 0.228)	0.66	0.42
HF	0.07 (0.03 – 0.10)	0.043 (0.033 – 0.052)	0.14	0.71
HF+ (1.0Hz)	3.99^{-4} (0.29^{-4} – 9.92^{-4})	1.59 (1.39 – 1.78)	4.55	0.034
LF:HF ratio	3.68 (2.11 – 5.85)	5.32 (4.72 – 6.03)	0.07	0.79
LF:HF+ ratio	5.20 (2.21 – 12.7)	14.72 (11.83 – 17.3)	6.55	0.011

Table 11.2 Median and IQR for the frequency domain HRV values obtained from 2 hour unobserved recordings in well babies comparing preterm (<32 weeks GAA) with infants near term or term. See main text for statistical analysis used.

11.3.2 Comparing HRV in "experimental" and "real world" recordings in well babies

Similar results to those seen for the time domain measures (10.3.2) were obtained when the frequency measures were compared between the observed an unobserved recordings. The preterm population showed no statistically significant differences (table 11.3) however the more mature babies had increased power in the ULF and HP bands along with an increase in the LF:HF ratios. (table 11.4)

	Experimental (n=18)	Real world (n=21)	P value
ULF	0.29 (0.16 – 0.43)	0.31 (0.22 – 0.43)	0.40
VLF	0.64 (0.48 – 0.74)	0.64 (0.55 – 0.70)	0.83
LF	0.15 (0.09 – 0.23)	0.21 (0.17 – 0.28)	0.29
HF	0.03 (0.02 – 0.06)	0.07 (0.03 – 0.10)	0.27
HF+ (1.0Hz)	0.06 (0.04 – 0.10)	3.99^{-4} (0.29^{-4} – 9.92^{-4})	0.72
LF:HF ratio	6.40 (4.01 – 8.60)	3.68 (2.11 – 5.85)	0.95
LF:HF+ ratio	1.93 (1.09 – 2.65)	5.20 (2.21 – 12.7)	0.96

Table 11.3 Median and IQR for the frequency domain HRV values obtained from preterm babies (≤ 32 weeks) in observed and unobserved recordings. See main text for statistical analysis used.

	Experimental (n=14)	Real world (n=14)	P value
ULF	0.20 (0.10 – 0.30)	0.310 (0.155 – 0.336)	<0.0001
VLF	0.49 (0.31 – 0.58)	0.619 (0.532 – 0.651)	0.57
LF	0.33 (0.22 – 0.38)	0.218 (0.199 – 0.228)	0.38
HF	0.06 (0.02 – 0.07)	0.043 (0.033 – 0.052)	<0.0001
HF+ (1.0Hz)	0.07 (0.05 – 0.12)	1.59 (1.39 – 1.78)	0.70
LF:HF ratio	5.25 (3.35 – 8.48)	5.32 (4.72 – 6.03)	<0.0001
LF:HF+ ratio	2.35 (1.23 – 3.70)	14.72 (11.83 – 17.3)	<0.0001

Table 11.4 Median and IQR for the frequency domain HRV values obtained from term and near term babies (> 32 weeks) in observed and unobserved recordings. See main text for statistical analysis used.

11.3.3 Comparing HRV in "well" and "unwell" infants

There were no statistically significant differences in frequency domain measures when well babies were compared with unwell babies. (table 11.3). VLF was approaching a statistically significant difference but did not meet the requirement of a pvalus <0.01 as required by the statistical analysis (see 6.3)

	"Well" (n=8)	"Unwell" (n=8)	Trans.	F-stat	P value
ULF	0.380 (0.309 – 0.475)	0.26 (0.23 – 0.54)	Log	0.73	0.39
VLF	0.75 (0.66 – 0.82)	0.68 (0.61 – 0.72)	Log	4	0.047
LF	0.15 (0.12 – 0.16)	0.16 (0.13 – 0.18)	Log	0.6	0.44
HF	0.03 (0.02 – 0.04)	0.04 (0.03 – 0.07)	Log	0.62	0.43
HF+ (1.0Hz)	0.002.(0.001 – 0.005)	0.004 (0.001 – 0.005)	Log	0.21	0.65
LF:HF ratio	4.93 (3.31 – 5.89)	3.75 (1.84 – 6.10)	Log	0.2	0.65
LF:HF+ ratio	611.0 (204.7 – 752.4)	408.5 (209.6 – 1625.8)	Log	0.56	0.45

Table 11.5 Median and IQR for the frequency domain HRV values obtained from 2 hour unobserved recordings in spontaneously breathing compared with ventilated babies. See text for statistical methods applied. Trans* = Transformation applied to data.

11.3 Discussion

Statistically significant differences were detected in frequency domain measures when more mature babies were compared with more premature babies in the short term recordings. More mature babies demonstrated decreased power in the VLF and increased power in the LF and HF power band. VLF HRV represents numerous influences on the heart, including thermoregulation, the renin-angiotensin system, and endothelial factors[423]. The VLF power band has not been extensively studied in the newborn infant and the increased power seen in the more immature babies requires further investigation to determine its ontogeny and if this is a reflection of a more

immature ANS. LF, representing sympathetic control, and HF representing parasympathetic control showed increasing power when more mature babies were compared with preterm babies. This is in common with the findings of Longin et al[105] and reflects the increased activity within the ANS with advancing gestational age. The LF:HF ratio did not differ between gestational age categories.

The same pattern was seen when the frequency domain measures in the unobserved and observed recordings were compared as was seen when the time domain measures were compared in 10.3.2; the preterm babies showed no difference with the term babies having significant differences in the ULF and HF band, and in the LF:HF ratio. This difference between preterm and more mature babies supports the results in chapter 10 that the more mature babies are demonstrating an increase in ANS maturation activity by having increased fluctuating HRV measures.

When the longer term recordings were analysed there were no statistically significant differences between either the gestational ages or between the well and unwell babies. This may reflect the small sample sizes or the sampled population. However as statistically significant differences were detected in the observed recordings it may be that the continually fluctuating ANS activity when the babies are awake and receiving routine neonatal care masks the more subtle changes which occur between the two groups.

11.4 Conclusion

Frequency domain measures were significantly different between different gestational age groups when observed short term recordings were compared but not when longer term recordings were compared. Though the sample sizes are small, the null hypothesis cannot be rejected and the data in this thesis do not support the use of frequency domain measures as a useful measure of HRV in the routinely monitored neonatal ECG.

Chapter 12

Poincaré measures

12.1 Introduction

Poincaré measures offer an attractive option when considering the routinely monitored ECG as they inherently deal with ectopics and missing data. Poincaré SD1 represents fast beat to beat variability and has been shown to correlate highly with both RMSSD and HF power[284,285]. Poincaré SD2 reflects longer term HRV changes and has been show to correlate with the LF:HF ratio[285]. In addition, in chapter 8 it was demonstrated that Poincaré SD1 and SD2 measures taken during routine neonatal care closely match the steady, resting state when infants are asleep.

12.2 Methods

From the Liverpool Neonatal ECG bank, the same recordings as in chapter 10 and 11 were selected, that is recordings taken from different gestational age groups (≤32 weeks and > 32 weeks) and well/unwell babies. The two gestational age groups included observed 20 minute and unobserved 2 hour recordings. The Poincaré HRV measures from the gestational age groups and the well/unwell pairs were compared using the statistical analyses described in section 10.2.

To determine if the HRV measures in the "real world" were similar to those in the "steady state", the results from the unobserved recordings were compared with those from the observed recordings for each of the gestational age groups (≤ 32 weeks and <32 weeks

12.3 Results

The patient demographics for the three compared populations of babies are presented in section 10.3.1.

12.3.1 Comparing HRV in different gestational age groups

In the 20 minute recordings, Poincaré SD1 was greater in the more mature group, suggesting increased short term variability, but this did not reach statistical significance (4.4 *vs.* 3.4, p = 0.059). There was no difference between either Poincaré SD2 or the ratio between Poincare SD1 and SD2. For the 2 hour recordings, Poincaré SD2 was increased in the more mature babies (23.3 *vs.* 13.5, p = 0.0019). (table 12.2) The other Poincaré measures showed no significant differences between the two gestational age groups.

	≤ 32 weeks (n=18)	**> 32 weeks (n=14)**	**P value**
PCSD1	3.4 (2.7 – 4.2)	4.4 (3.4 – 5.4)	0.059
PCSD2	15.7 (12.3 – 20.8)	18.4 (13.7 – 23.2)	0.464
PCSD1:SD2	0.22 (0.14 – 0.31)	0.20 (0.17 – 0.34)	0.424

Table 12.1 Median and IQR for the Poincaré HRV values obtained from 20 minute observed recordings in well babies comparing preterm (<32 weeks GA) with infants near term or term.

	≤32 weeks (n=21)	>32 weeks (n=14)	F stat	P value
PCSD1	3.2 (2.41 – 3.9)	3.6 (3.2 – 3.9)	2.7	0.1
PCSD2	13.5 (10.6 – 17.9)	23.3 (20.5 – 25.4)	9.87	0.0019
PCSD1:SD2	0.22 (0.17 – 0.33)	0.15 (0.14 – 0.18)	3.95	0.048

Table 12.2 Median and IQR for the Poincaré HRV values obtained from 2 hour unobserved recordings in well babies comparing preterm (<32 weeks GA) with infants near term or term. See main text for statistical analysis used.

12.3.2 Comparing HRV in "experimental" and "real world" recordings in well babies

When the Poincaré measures were compared between the observed and unobserved recordings they demonstrated the same differences as seen in Chapters 10 and 11, namely that the results from the preterm babies were the same (table 12.3) but the more mature babies demonstrated statistically significant differences (table 12.4). In the more mature babies there was an increase in PCSD2 with a decrease in PCSD1 and the SD1:SD2 ratio.

	Experimental (n = 18)	Real World (n=21)	P value
PCSD1	3.4 (2.7 – 4.2)	3.2 (2.41 – 3.9)	0.05
PCSD2	15.7 (12.3 – 20.8)	13.5 (10.6 – 17.9)	0.44
PCSD1:SD2	0.22 (0.14 – 0.31)	0.22 (0.17 – 0.33)	0.84

Table 12.3 Median and IQR for the Poincaré HRV values obtained from preterm babies (≤ 32 weeks) in observed and unobserved recordings. See main text for statistical analysis used.

	Experimental (n = 14)	Real World (n=14)	P value
PCSD1	4.4 (3.4 – 5.4)	3.6 (3.2 – 3.9)	<0.0001
PCSD2	18.4 (13.7 – 23.2)	23.3 (20.5 – 25.4)	<0.0001
PCSD1:SD2	0.20 (0.17 – 0.34)	0.15 (0.14 – 0.18)	<0.0001

Table 12.4 Median and IQR for the Poincaré HRV values obtained from term and near term babies (> 32 weeks) in observed and unobserved recordings. See main text for statistical analysis used.

12.3.3 Comparing HRV in "well" and "unwell" infants

There were no statistically significant differneces between the Poincaré measures in well and unwell babies (table 12.3).

	"Well" (n=8)	"Unwell" (n=8)	Trans.	F-stat	P value
R PC SD1	3.39 (2.86 – 4.13)	3.28 (2.79 – 4.54)	Log	0.02	0.88
R PC SD2	20.2 (17.2 – 26.4)	17.5 (16.0 – 20.2)	Identity	2.72	0.1
PCSD1:PCSD2	0.16 (0.121 – 0.217)	0.17 (0.165 – 0.262)	Log	0.53	0.47

Table 12.5 Median and IQR for the Poincare HRV values obtained from 2 hour unobserved recordings in spontaneously breathing compared with ventilated babies. See text for statistical methods applied. Trans* = Transformation applied to data.

12.4 Discussion

In the observed recordings, there was a non-significant trend towards increased Poincaré SD1 in the more mature babies reflecting increased short term variability and parasympathetic input. Poincaré SD2 and the ratio PCSD1:SD2 were not significantly different.. In the longer term recordings SD2 was statistically significantly greater in the more mature babies. SD2 is a measure of both long and short term HRV and correlates with the LF:HF ratio, reflecting sympathovagal balance[285]. The increased SD2 in this study demonstrates the increased activity of the ANS in more mature babies. Whilst there was an observed increase in the SD1 in more mature babies this did not reach statistical significance in the longer term unobserved recordings.

The statistical significant differences in the unobserved versus observed recordings being present in the more mature babies and not in the preterm babies again supports the possibility that the more mature babies manifest there advancing activity with an increase in the fluctuation of the HRV measures.

None of the Poincaré measures were different between well and unwell babies, suggesting that Poincaré measures whilst perhaps being useful in detecting the change sin HRV in matrutring ANS are not able to detect the different changes seen in well and unwell babies.

12.5 Conclusions

Poincaré HRV measures were not statistically significantly different between the short term observed recordings at different gestational ages. The limited number of RRi in the 20 minute segment may be insufficient to produce HRV Poincaré measures and future work should investigate to determine if longer recording periods are more suitable to Poincaré analysis. In the longer term recordings Poincaré SD2 measures of HRV are able to detect differences in autonomic functioning between different gestational age groups. This is encouraging for Poincaré measures being used as a HRV monitoring tool.

The difference between preterm and more mature babies was again manifested by increased variability of the HRV measures in the more mature babies during routine care.

Chapter 13

Discussion and Conclusions

Investigating the autonomic nervous system activity by determining heart rate variability is not a new research tool. However, despite the technique being developed over the past three decades, its utilisation as a clinical monitoring tool remains elusive. The first step toward this development is the proof of concept that the sophisticated measures of HRV can be determined from the routinely monitored neonatal ECG signal. The thesis has demonstrated that this can be achieved. The thesis describes a complete process from acquisition of the ECG signal (chapter 3), identification of the fiducial marker for the heart beat (R interval) (chapter 4), filtering the RRi tachogram and utilising the Lomb Scargle periodogram to produce the power within the various frequency bands (Chapter 5 and Appendices B and C). The developed methodology was validated using 240 synthetic ECGs and 500 synthetic RRi tachograms (Chapter 4 and appendix C). Synthetic data was chosen to validate the method as it allows "known" inputs to be determined by the methodology, providing a true gold standard. This is in contrast to validating the method with "real world" ECGs whereby the true fiducial marker for the heart beat is unknown.

The developed methodology for R wave detection was compared with a widely available R wave detector from Physionet. The developed R wave detector was significantly more accurate in determining the R wave across increasing heart rates, heart rate variability and noise within the signal (section 4.5).

One of the greatest challenges to "real world" data is the presence of both missing data or ectopic beats. To produce spectral estimates of HRV in this thesis, the Lomb Scargle Periodogram was compared with the more traditional fast Fourier Transform. The LSP was accurate at determining the relative powers within the synthetic RRi data across various heart rates and consistently outperformed the FFT with increasing proportions of missing or ectopic beats. The findings demonstrating that the LSP is able to remains within 5% accuracy when up to 20% of the data are missing, and within 15% when up to 50% of the data are missing. The cubic spline fast

Fourier transform has this level of accuracy when only 5-10% of data are missing. Thus the LSP is much more ideally suited to use when investigating routinely monitored and was subsequently employed for this (Appendix C).

During the development of the methodology, over 300 ECG recordings were obtained from babies on the NICU. These ranged from 20 minute recordings from babies who remained undisturbed through to recordings from extremely sick infants which lasted several days. These recordings along with the corresponding demographic and clinical data formed the Liverpool Neonatal ECG bank. This is the only bank of neonatal ECGs which exists and is available for future research work.

The developed methodology was then applied to recordings from this bank to determine if the method could potentially be used as a clinically useful monitoring tool. In the first instance, the ability of the method to produce HRV measures from routinely monitored neonatal ECG recordings was determined. When applied to these ECG signals, the method was able to produce HRV measures from 97% of recordings thus demonstrating that the method is robust enough to be used to monitor HRV using the "real world" ECG data (Chapter 7).

Subsequently, whilst acknowledging the important clinical information non stationaries contain within the NNi tachogram, in this thesis they were removed so as to concentrate on the underlying harmonic fluctuation within the NNi. It is demonstrated that the removal of non-stationeries has a large and significant effect on the measured HRV metrics (Chapter 8). This highlighted the importance of presenting the method of dealing with non-stationaries when results from HRV analysis are reported.

Following the removal of non-stationaries, the fluctuation of HRV measures during routine neonatal care was investigated. Most HRV metrics recorded in the "real world" reflect those recorded in the "experimental" state. As expected, the standard deviation of NNi and kurtosis did demonstrate statistical significance between the "real world" and "experimental" measurements. The lack of statistically significant differences between the other HRV measures demonstrates that routine monitoring can be used to produce HRV metrics. (Chapter 9)

HRV measures were then compared in different populations of babies. Two distinct populations were chosen where an expected difference in autonomic functioning would be expected and has been demonstrated in previous research studies. Babies in two different gestational age groups and "well" and "unwell" babies were compared. The different HRV measures were compared within these groups to determine if significant differences were detectable. The studies demonstrated that in the routinely monitored neonatal ECG both time domain and Poincaré measures were statistically significantly different between both the gestational age groups and the "well" and "unwell" babies. Increased HRV with advancing gestational age as well as increased skewness of NNi's in unwell babies was demonstrated. This supports the methodology developed as being robust as the results tally with those reported by other authors. Frequency measures, whilst demonstrating increased maturity of the ANS when the "experimental" recordings were compared, was not statistically significantly different when the real world ECGs were compared. Whilst the small numbers included in this thesis do not exclude frequency domain measures from being used to monitor HRV from routinely monitored signal, the results do not promote its use currently (Chapters 10,11 and 12).

Using the 20 minute and 2 hour recordings from well babies, a striking difference was seen between preterm and more mature babies. The HRV values for preterm babies were the same if the babies were recorded for one 20 minute "experimental" style recording or if they were recorded over 2 hours in the "real world". The preterm babies HRV measures remain relatively constant whilst they are well. HRV measures from the routinely monitored ECG in babies < 32 weeks can be used to quantify steady state autonomic activity. This contrasts with the more mature babies where the majority of HRV measures fluctuate during routine care. (Chapters 10, 11 and 12) . This may reflect the more mature ANS which is responding to minor stressors and fluctuating physiologically.

The long term aim for this area of research was to produce a continuous metric from babies being routinely monitored in the neonatal intensive care unit. This metric could then be investigated as an "early warning system" for the development of neonatal illness and/or be a predictor of outcome for a variety of clinical conditions. A continually produced number with a relative risk of "illness" within the next 24-48 hours is envisaged. During the writing of this thesis Moorman's research team from Virginia presented and subsequently published their

clinical monitoring system which followed the above plan. They use relatively simple measures of HRV (standard deviation, sample entropy, and asymmetry function analysis) to produce a summary statistic for the HRV sepsis (termed the HeRO™ score). The HeRO™ monitoring system was subjected to a randomised controlled trial involving 3003 VLBW, demonstrating a 22% reduction in mortality when HeRO scores were available to the clinical team[286]. The team of approximately 30 engineers, mathematicians and clinicians have been working on this system for approximately 12 years and must be applauded for the excellent work in bringing this system from the research setting to a commercially available product.

The HeRO™ monitoring system is now the "gold standard" (and only) HRV monitoring system used in the NICU. I have made contact with Randall Moorman and his team who have visited the NICU at LWH. They too have been aware of the work in this thesis and collaborating with LWH into future work investigating HRV as a clinically useful monitoring tool.

This thesis has been the first step towards developing sophisticated measures of HRV as a useful clinical monitoring tool. It can be used as a "manual" for future researchers, demonstrating how to obtain, store, pre-process and obtain the NNi from the routinely monitored neonatal ECG. It has been demonstrated that time domain and Poincaré measures show most promise as a monitoring tool whereas frequency domain measures are probably better suited to the experimental environment. The fluctuation of HRV measures from 20 minute time periods has been shown to occur in more mature babies and not in preterm babies. This requires further investigation as this is likely to reflect the advancing maturation of the ANS.

Chapter 14

Further Work

Chapter 8 demonstrated that the presence of non-stationeries had a significant effect on the resulting HRV metrics. It is assumed that the removal of these non-stationeries allows the underlying HRV harmonic to be assessed, thus allowing the underlying ANS activity to be measured[273]. Whilst the removal may be warranted for frequency domain analysis, the information contained within the removed data may provide additional information. For example, the clinical observation of increased bradycardic events prior to a diagnosis of sepsis would support the view that the non-stationaries do contain relevant clinical information. The information in these non-stationaries was not used in this thesis and further investigation of the "spikes" within the NNi tachogram is required. If babies have continual HRV measures recorded, the frequency and power within these spikes in the 48 hours prior to the onset of illness could be determined. In addition, for the development of a HRV monitoring tool it may be wise to leave the spikes in the NNi tachogram for time domain and Poincare but remove for frequency domain anlaysis. Frequency domain analysis methodology is not able to provide accurate results when these spikes are present. However, time domain analyses methodology will allow the presence of these spikes to affect the resulting metrics, such as skewness and SDNNi. For example, the presence of bradycardic events prior to the onset of sepsis would cause an increase in SDNNi and skewness to move to the left, potentially providing early information that the baby was becoming unwell..

Most HRV metrics measured in the "real world" state were not statistically significantly different from those when measured in the "experimental state" (Chapter 9). However, caution must be taken due to the small sample size with the possibility of a type II error. Further investigation with a larger sample size is required to if the results of the experiment in this thesis remain valid.

The description of the continually modulating HRV measures in Chapter 9 and the fluctuating HRV measures in the more mature babies in chapters 10,11 and 12 are purely observational and require further research to determine the source of the fluctuations. HRV measures taken over

longer time periods (2-6 hours) whilst the babies are observed would help determine of the fluctuations are physiological or represent disturbances experienced by the babies. The observer could record any disturbances witnessed and then correlate these to the fluctuating HRV measures. In addition, babies who moving from the "well" to "unwell" state could be recorded to determine the effect that moving to the pathological state has on the stability of HRV measures.

The fluctuating HRV measures in the more mature babies and the relatively static nature of the HRV measures in more preterm babies (chapters 10,11 and 12) warrant further investigation . ANS maturation may be manifested in two ways, either by a change in the absolute value of the HRV measure in a 20 minute window, or by an increase in fluctuation of the HRV values over longer time periods. This "variability of the variability" requires further investigation to determine if it is a useful marker of ANS activity and maturation. Longitudinal analysis of 20 minute HRV values will demonstrate if the HRV measures fluctuate more as the baby matures or they reduce prior to the baby becoming unwell.

For each of the HRV measures, normal reference ranges should be determined as well as longitudinal analysis of intra-individual measures undertaken to determine which metrics demonstrate changes preceding the onset of illness. Correlation of abnormal HRV measures with clinical outcomes is then required. Clinical outcomes should be both short term (presence of sepsis, intraventricular haemorrhage, mortality) and long term (neurodevelopmental outcome in childhood). Of particular interest would be the HRV measures which would lead to an intervention (such as a septic screen and antibiotics) which could potentially improve the long term outcome for sick and/or preterm infants. Once this is determined, providing clinicans with the HRV measures can be subjected to a randomised controlled trial to determine if the extra information provided by HRV measurement improves mortality and long term neurodevelopmental outcome in preterm infants. If the HRV measures in this study are demonstrated to improve mortality and/or morbidity in preterm infants then a comparison randomised control trial between the HRV metrics in this study and the HeRO system would be warranted.

Use of HRV monitoring would also be useful as a research tool. Whilst the HeRO system is now available as a monitoring tool, the methods used in this thesis remain pertinent. By their own

admission, the HeRO system uses quite "simple" measures of HRV and detects only changes that would likely be visible in routine physiological monitoring to the trained human eye. The methods in this thesis may detect more subtle changes in HRV. Of particular interest would be to investigate the clinical observation of abnormal HRV patterns in infants with HIE. Cot side anecdotal observation of babies with HIE shows that the sickest babies have little or no variability, occasionally with sinusoidal heart rate patterns. Determining if these heart rate patterns are correlated with neurodevelopmental outcome at 2 years of age would provide additional prognostic information. In addition, obtaining HRV measures from the routinely monitored ECG signal is now relatively straightforward. HRV measures can be used as a biomarker to investigate ANS activity in research studies including therapeutic interventional studies.

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Study Number	Observed	lenghth of rec	Status	Unit Number	Rec No	Sex	DOB	Time of Birth	Gestation in weeks	Birth Weight	Date recorded	Time recorded	Recording Gestation
1	0	2		1232404	1	f	05/08/2006	21:57:00	23.14	515	18/08/2006	10:30:00	24.86
3	0	2		1240043	1	f	15/08/2006	10:00:00	27.00	1160	18/08/2006	10:30:00	27.43
4	0	2		1232809	1	f	10/08/2006	02:00:00	28.43	1040	18/08/2006	10:30:00	29.57
5	0	2		1233317	1	m	16/08/2006	00:00:00	33.57	1975	18/08/2006	10:30:00	33.86
6	0	2		1230720	1	m	16/07/2006	12:22:00	28.86	1050	18/08/2006	19:30:00	33.57
7	0	2		1230531	1	f	13/07/2006	08:47:00	29.86	1405	18/08/2006	19:30:00	35.00
8	0	2		1224075	1	f	20/04/2006	20:46:00	24.29	620	18/08/2006	19:30:00	41.00
9	0	2		1233387	1	f	17/08/2006	04:06:00	40.57	4028	18/08/2006	19:30:00	40.71
10	0	2		1240041	1	f	25/08/2006	10:37:00	26.43	980	18/08/2006	19:30:00	29.86
11	0	2		1232754	1	m	30/01/1900	00:00:00	30.57	1080	18/08/2006	19:30:00	31.86
12	0	2		1233562	1	f	18/08/2006	22:31:00	33.14	1865	21/08/2006	03:00:00	33.43
13	0	2		1233560	1	m	18/08/2006	20:10:00	31.86	1640	21/08/2006	03:00:00	32.14
14	0	2		1231283	1	m	21/07/2006	00:00:00	28.14	1150	21/08/2006	03:00:00	32.57
15	0	2		1233704	1	m	22/08/2006	09:56:00	38.29	3400	23/08/2006	02:00:00	38.29
16	0	2		1230531	2	f	13/07/2006	08:47:00	29.86	1405	23/08/2006	02:00:00	35.57
17	0	2		1230528	1	m	13/07/2006	10:57:00	26.71	950	23/08/2006	02:00:00	32.43
18	0	2		1135715	1	m	20/08/2006	15:33:00	32.00	1775	23/08/2006	02:00:00	32.29
19	0	2		1233524	1	m	21/08/2006	12:43:00	39.00	3240	23/08/2006	02:00:00	39.14
20	0	2		1233560	1	m	18/08/2006	20:10:00	31.86	1640	23/08/2006	02:00:00	32.43
21	0	2		1240043	1	f	15/08/2006	10:00:00	27.00	1160	23/08/2006	03:00:00	28.00
22	0	2		1236142	1	m	22/09/2006	15:14:00	30.29	1610	25/09/2006	12:00:00	30.57
23	0	2		1236182	1	m	23/09/2006	00:00:00	25.29	810	25/09/2006	13:00:00	25.57
25	0	2		1240035	1	m	15/10/2006	00:57:00	25.00	760	16/10/2006	19:00:00	25.14
51	0	2		1239944	1		12/11/2006	19:30:00	24.71	640	17/11/2006	12:00:00	25.29
52	0	2		1238430	1	f	15/11/2006	09:50:00	31.29	1390	17/11/2006	12:00:00	31.57
53	0	2		1240207	1	f	14/11/2006	16:46:00	26.71	870	17/11/2006	12:00:00	27.00
54	0	2		1240363	1	m	16/11/2006	14:52:00	36.86	2215	17/11/2006	12:00:00	36.86
55	0	2		1236578	1	m	01/10/2006	14:34:00	27.43	880	17/11/2006	12:00:00	34.14
56	0	2		1239300	1	m	09/11/2006	06:21:00	30.71	1960	17/11/2006	12:00:00	31.86
57	0	2		1240327	1	f	16/11/2006	06:13:00	37.86	3080	17/11/2006	12:00:00	38.00
58	0	2		1239299	1	f	09/11/2006	06:05:00	31.71	1200	17/11/2006	12:00:00	32.86
71	0	2		1242243	1		12/12/2006	05:21:00	28.29	1095	14/12/2006	16:00:00	28.71
72	0	2		1260001		f	03/12/2006	00:00:00	27.29	1028	14/12/2006	13:00:00	28.86
73	0	2		1241247		m	04/12/2006	14:38:00	25.71	760	14/12/2006	13:00:00	28.29
74	0	2		1240207	2	f	14/11/2006	16:46:00	26.71	870	14/12/2006	13:00:00	30.86

Study Number	Observed	lenghth of rec	Status	Unit Number	Rec No	Sex	DOB	Time of Birth	Gestation in weeks	Birth Weight	Date recorded	Time recorded	Recording Gestation
75	0	2		1242303	1	f	13/12/2006	15:02:00	32.57	1837	14/12/2006	16:00:00	32.71
76	0	2		1242240	1	m	12/12/2006	01:36:00	29.00	910	14/12/2006	13:00:00	29.29
77	0	2		1238528		f	24/10/2006	14:23:00	26.00	530	14/12/2006	16:00:00	33.29
84	0	2		1242240		m	12/12/2006	01:36:00	29.00	910	18/12/2006	13:00:00	29.86
85	0	2		1240549		m	20/11/2006	19:41:00	24.14	700	18/12/2006	13:00:00	28.00
86	0	2		1260001		f	03/12/2006	00:00:00	27.29	1028	18/12/2006	13:00:00	29.43
87	0	2		1240207	2	f	14/11/2006	16:46:00	26.71	870	18/12/2006	13:00:00	31.57
88	0	2		1242568		f	17/12/2006	05:35:00	41.86	3220	18/12/2006	13:00:00	42.00
99	0	20		1244597	1	m	16/01/2007	18:21:00	26.00	550	16/01/2007	20:00:00	26.00
109	0	2		1244729		f	18/01/2007	10:01:00	31.86	1690	02/02/2007	14:00:00	34.00
110	0	2		1242518		m	18/12/2006	19:25:00	25.29	850	02/02/2007	17:00:00	31.86
111	0	2		1243905		f	08/01/2007	00:00:00	25.86	830	02/02/2007	15:00:00	29.43
112	0	3		1243905		f	08/01/2007	00:00:00	25.86	830	05/02/2007	11:00:00	29.86
113	0	2		1245464		f	28/01/2007	15:41:00	31.29	1905	05/02/2007	11:00:00	32.29
114	0	2		1243447		m	01/01/2007	09:24:00	28.57	1030	05/02/2007	11:00:00	33.43
117	0	2		1244923		m	20/01/2007	23:33:00	25.43	710	05/02/2007	11:00:00	27.57
118	0	2		1244923		m	20/01/2007	23:33:00	25.43	710	13/02/2007	16:31:00	28.71
119	0	2		1245973		m	03/02/2007	00:35:00	26.00	720	13/02/2007	13:00:00	27.43
120	0	2		1244729		f	18/01/2007	10:01:00	31.86	1690	13/02/2007	14:00:00	35.57
121	0	2		1242518		m	18/12/2006	19:25:00	25.29	850	13/02/2007	12:00:00	33.29
122	0	2		1246357		m	08/02/2007	05:11:00	29.14	1480	14/02/2007	10:30:00	29.86
123	0	2		1243905		f	08/01/2007	00:00:00	25.86	830	14/02/2007	15:00:00	31.14
124	0	2		1243904		f	08/01/2007	01:50:00	25.86	790	14/02/2007	12:00:00	31.14
125	0	2		1245995		f	07/02/2007	15:26:00	25.86	810	15/02/2007	10:00:00	26.86
126	0	6		1248441		m	08/03/2007	11:50:00	32.29	1740	08/03/2007	11:00:00	32.29
127	0	24		1248442		m	08/03/2007	12:45:00	32.29	1130	08/03/2007	13:00:00	32.29
129	0	2		1248125		m	06/03/2007	12:18:00	31.29	1464	08/03/2007	12:20:00	31.43
130	0	4		1248125		m	06/03/2007	12:18:00	31.29	1464	06/03/2007	18:40:00	31.29
131	0	2		1246714		m	13/02/2007	09:40:00	31.29	2050	15/02/2007	16:00:00	31.57
133	0	4		1260016		f	15/02/2007	05:00:00	25.29	625	06/03/2007	18:00:00	29.29
134	0	6		1244800		m	18/01/2007	18:30:00	26.71	1040	02/03/2007	02:00:00	32.86

Study Number	Observed	lenghth of rec	Status	Unit Number	Rec No	Sex	DOB	Time of Birth	Gestation in weeks	Birth Weight	Date recorded	Time recorded	Recording Gestation
135	0	5		1244729		f	18/01/2007	10:01:00	31.86	1690	28/02/2007	00:00:00	37.57
136	0	2		1248368		m	07/03/2007	15:38:00	28.43	680	08/03/2007	15:00:00	28.57
137	0	2		1245484		f	29/01/2007	11:03:00	28.14	1180	15/02/2007	13:40:00	30.57
138	0	4		1243904		f	08/01/2007	01:50:00	25.86	790	02/03/2007	02:30:00	33.43
139	0	5		1243905		f	08/01/2007	00:00:00	25.86	830	02/03/2007	02:34:00	33.43
140	0	2		1243905		f	08/01/2007	00:00:00	25.86	830	15/03/2007	10:30:00	35.29
141	0	5		1247573		f	25/02/2007	21:40:00	28.14	1210	28/02/2007	00:00:00	28.43
142	0	24		1247573		f	25/02/2007	21:40:00	28.14	1210	25/02/2007	22:00:00	28.43
143	0	2		1248638		f	11/03/2007	14:49:00	26.57	940	14/03/2007	09:00:00	27.00
144	0	4		1247950		m	01/03/2007	15:45:00	35.71	2240	01/03/2007	22:00:00	35.71
145	0	2		1248441		m	08/03/2007	11:50:00	32.29	1740	15/03/2007	15:00:00	33.29
146	0	2		1248442		m	08/03/2007	12:45:00	32.29	1130	15/03/2007	15:00:00	33.29
147	0	2		1249499		m	22/03/2007	11:48:00	23.71	600	30/03/2007	12:00:00	24.71
149	0	2		1244597		m	16/01/2007	18:21:00	26.00	550	16/03/2007	11:00:00	34.43
150	0	2		1244597		m	16/01/2007	18:21:00	26.00	550	29/03/2007	12:00:00	36.14
152	0	2		1249714		f	25/03/2007	14:26:00	26.14	1000	29/03/2007	11:00:00	26.57
153	0	2		1243904		f	08/01/2007	01:50:00	25.86	790	16/03/2007	14:00:00	35.43
156	0	2		1244729		f	18/01/2007	10:01:00	31.86	1690	16/03/2007	10:30:00	40.00
158	0	2		1250297		f	01/04/2007	11:40:00	30.43	1310	02/04/2007	16:30:00	30.57
160	0	2		1250161		m	29/03/2007	17:40:00	27.57	1200	02/04/2007	16:30:00	28.14
162	0	2		1248442		m	08/03/2007	12:45:00	32.29	1130	16/03/2007	10:30:00	33.43
164	0	2		1248125		m	06/03/2007	12:18:00	31.29	1464	16/03/2007	13:30:00	32.71
166	0	2		1248977		m	14/03/2007	21:25:00	35.71	2870	16/03/2007	13:30:00	36.00
167	0	2		1249294		f	20/03/2007	00:12:00	34.00	1670	29/03/2007	12:00:00	35.29
168	0	2		1249735		m	26/03/2007	06:35:00	28.57	1360	07/04/2007	21:00:00	30.29
169	0	4		1260016		f	15/02/2007	05:00:00	25.29	625	06/04/2007	17:45:00	32.43
170	0	2		1250745		m	09/04/2007	00:01:00	35.71	2390	11/04/2007	00:45:00	36.14
171	0	2		1250746			09/04/2007	00:10:00	35.71	2245	11/04/2007	00:45:00	36.14
172	0	2		1250461		m	04/04/2007	07:20:00	41.14	4620	04/04/2007	09:45:00	41.14
173	0	2		1250408		m	02/04/2007	22:35:00	30.00	1560	03/04/2007	15:00:00	30.14
174	0	2		1249714		f	25/03/2007	14:26:00	26.14	1000	03/04/2007	10:00:00	27.29
175	0	23		1249714		f	25/03/2007	14:26:00	26.14	1000	06/04/2007	22:00:00	27.86

Study Number	Observed	lenghth of rec	Status	Unit Number	Rec No	Sex	DOB	Time of Birth	Gestation in weeks	Birth Weight	Date recorded	Time recorded	Recording Gestation
176	0	2		1248368		m	07/03/2007	15:38:00	28.43	680	07/04/2007	21:30:00	32.86
177	0	2		1244597		m	16/01/2007	18:21:00	26.00	550	07/04/2007	21:45:00	37.57
178	0	29		1251367		m	17/04/2007	10:45:00	29.57	1380	19/04/2007	15:50:00	29.86
179	0	24		1251367		m	17/04/2007	10:45:00	29.57	1380	17/04/2007	11:00:00	29.57
181	0	2		1250297		f	01/04/2007	11:40:00	30.43	1310	03/04/2007	14:40:00	30.57
182	0	23		1250297		f	01/04/2007	11:40:00	30.43	1310	06/04/2007	22:30:00	31.14
183	0	23		1250063		f	31/03/2007	02:46:00	25.14	770	06/04/2007	22:30:00	26.00
184	0	2		1250063		f	31/03/2007	02:46:00	25.14	770	04/04/2007	10:43:00	25.71
185	0	2		1249701		m	30/03/2007	22:45:00	32.57	2400	08/04/2007	21:30:00	33.71
186	0	2		1270005		f	26/03/2007	00:00:00	28.00	1270	08/04/2007	21:30:00	29.86
187	0	2		1260016		f	15/02/2007	05:00:00	25.29	625	01/05/2007	15:13:00	36.00
188	0	2		1251557		f	23/04/2007	09:17:00	34.29	2470	27/04/2007	10:00:00	34.86
189	0	2		1251196		m	14/04/2007	15:05:00	27.14	1060	01/05/2007	15:15:00	29.43
190	0	2		1251212		m	14/04/2007	11:21:00	29.29	1280	01/02/2007	15:15:00	31.71
191	0	2		1250063		f	31/03/2007	02:46:00	25.14	770	27/04/2007	09:45:00	29.86
192	0	3		1250160		m	29/03/2007	17:00:00	27.57	1080	02/04/2004	16:30:00	28.14
193	0	5		1250161		m	29/03/2007	17:38:00	27.57	1200	06/04/2007	17:00:00	28.71
194	0	18		1252307		m	02/05/2007	11:59:00	25.29	980	10/05/2007	15:30:00	26.29
195	0	48		1253410		m	15/05/2007	17:38:00	30.86	1360	15/05/2007	19:20:00	30.86
196	0	2		1253627		f	16/05/2007	20:56:00	32.00	1795	17/05/2007	21:00:00	32.14
197	0	2		1260016		f	15/02/2007	05:00:00	25.29	625	17/05/2007	22:00:00	38.29
198	0	20		1280014	2	f	02/06/2007	19:20:00	25.43	990	05/06/2007	09:00:00	25.71
199	0	20		1254914		f	04/06/2007	01:55:00	28.00	1100	05/06/2007	09:00:00	28.14
200	0	24		1253212		f	11/05/2007	00:000	26.43	900	17/05/2007	23:00:00	27.29
201	0	10		1251196		m	14/04/2007	15:05:00	27.14	1060	19/05/2007	00:00:00	32.14
202	0	28		1252621		m	02/05/2007	18:47:00	28.00	1070	03/05/2007	09:00:00	28.00
206	0	2		1280014	4	f	02/06/2007	19:20:00	25.43	990	21/07/2007	12:00:00	32.43
207	0	58		1260110		f	11/08/2007	22:10:00	32.29	1900	11/08/2007	23:30:00	32.29
208	0	22		1260111		f	11/08/2007	22:12:00	35.29	1620	11/08/2007	23:30:00	32.29
209	0	72		1260719		m	20/08/2007	14:33:00	30.57	1705	21/08/2007	08:30:00	30.57
210	0	54		1259198		m	01/08/2007	18:28:00	35.71	2270	02/08/2007	09:00:00	35.71
211	0	2		1260989	2	m	24/08/2007	13:40:00	24.00	710	04/09/2007	18:00:00	25.43
212	0	24		1260989	3	m	24/08/2007	13:40:00	24.00	710	06/09/2007	18:00:00	25.71
213	0	60		1260989	4	m	24/08/2007	13:40:00	24.00	710	14/09/2007	16:00:00	26.86
214	0	18		1260989	5	m	24/08/2007	13:40:00	24.00	710	18/09/2007	15:00:00	27.57

Study Number	Observed	lenghth of rec	Status	Unit Number	Rec No	Sex	DOB	Time of Birth	Gestation in weeks	Birth Weight	Date recorded	Time recorded	Recording Gestation
215	0	75		1260918	1	f	24/08/2007	09:54:00	28.00	1000	24/08/2007	10:42:00	28.00
216	0	50		1260918	2	f	24/08/2007	09:54:00	28.00	1000	03/09/2007	00:00:00	29.29
217	0	24		1260918	3	f	24/08/2007	09:54:00	28.00	1000	06/09/2007	00:00:00	29.71
218	0	18		1260918	4	f	24/08/2007	09:54:00	28.00	1000	13/09/2007	00:00:00	30.71
219	0	16		1260918	5	f	24/08/2007	09:54:00	28.00	1000	17/09/2007	17:00:00	31.29
220	0	16		1260675	3	f	25/08/2007	11:33:00	29.29	1350	13/09/2007	15:00:00	31.86
221	0	16		1260675	4	f	25/08/2007	11:33:00	29.29	1350	17/09/2007	15:00:00	32.43
222	0	16		1260675	5	f	25/08/2007	11:33:00	29.29	1350	19/09/2007	17:00:00	32.71
223	0	24		1260675	2	f	25/08/2007	11:33:00	29.29	1350	04/09/2007	16:00:00	30.57
224	0	26		1260719	1	m	20/08/2007	14:33:00	30.57	1705	29/08/2007	14:00:00	31.71
225	0	28		1260719	2	m	20/08/2007	14:33:00	30.57	1705	05/09/2007	16:45:00	32.71
226	0	16		1260719	3	m	20/08/2007	14:33:00	30.57	1705	13/09/2007	16:00:00	33.86
227	0	16		1260719	4	m	20/08/2007	14:33:00	30.57	1705	17/09/2007	17:00:00	34.43
228	0	94		1261127	1	f	24/08/2007	22:00:00	26.71	800	25/08/2007	09:00:00	26.71
229	0	24		1261127	2	f	24/08/2007	22:00:00	26.71	800	06/09/2007	21:00:00	28.57
230	0	16		1261127	3	f	24/08/2007	22:00:00	26.71	800	13/09/2007	16:00:00	29.57
231	0	19		1261127	4	f	24/08/2007	22:00:00	26.71	800	17/09/2007	17:00:00	30.14
232	0	13		1261127	5	f	24/08/2007	22:00:00	26.71	800	19/09/2007	16:30:00	30.43
233	0	28		1261127	6	f	24/08/2007	22:00:00	26.71	800	25/09/2007	17:00:00	31.29
234	0	25		1261058	2	m	24/08/2007	04:13:00	29.14	1435	29/08/2007	14:00:00	29.86
235	0	14		1261058	3	m	24/08/2007	04:13:00	29.14	1435	07/09/2007	21:00:00	31.14
236	0	19		1261058	4	m	24/08/2007	04:13:00	29.14	1435	18/09/2007	16:00:00	32.71
237	0	15		1258497	2	m	22/07/2007	12:14:00	26.00	630	06/08/2007	20:00:00	28.00
238	0	72		1258497	4	m	22/07/2007	12:14:00	26.00	630	12/08/2007	23:00:00	29.29

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Study Number	Observed	lenghth of rec	Status	Unit Number	Rec No	Sex	DOB	Time of Birth	Gestation in weeks	Birth Weight	Date recorded	Time recorded	Recording Gestation
239	0	20		1258497	5	m	22/07/2007	12:14:00	26.00	630	23/08/2007	12:00:00	30.43
240	0	24		1258497	3	m	22/07/2007	12:14:00	26.00	630	10/08/2007	21:00:00	28.57
241	0	27		1260675	5	f	25/08/2007	11:33:00	29.29	1350	25/09/2007	17:00:00	33.71
242	0	24		1285008	1	m	26/08/2007	02:00:00	24.00	650	03/09/2007	10:00:00	25.14
243	0	15		1285008	2	m	26/08/2007	02:00:00	24.00	650	07/09/2007	21:00:00	25.71
244	0			1262860		f	18/09/2007	12:55:00	39.71	3340	19/07/2007	17:00:00	39.86
245	0	51		1261765	1	m	04/09/2007	18:45:00	29.86	1090	04/09/2007	20:00:00	29.86
246	0	65		1261765	2	m	04/09/2007	18:45:00	29.86	1091	14/09/2007	16:00:00	31.14
247	0	12		1261765	3	m	04/09/2007	18:45:00	29.86	1092	21/09/2007	12:00:00	32.14
248	0	17		1261765	4	m	04/09/2007	18:45:00	29.86	1093	25/09/2007	00:00:00	32.71
249	0	24		1261765	5	m	04/09/2007	18:45:00	29.86	1094	01/10/2007	09:00:00	33.57
250	0	72		1261767	1	m	04/09/2007	18:47:00	29.86	1490	04/09/2007	20:00:00	29.86
251	0	65		1261767	2	m	04/09/2007	18:47:00	29.86	1490	14/09/2007	16:00:00	31.14
252	0	12		1261767	3	m	04/09/2007	18:47:00	29.86	1490	21/09/2007	12:00:00	32.14
253	0	24		1261767	4	m	04/09/2007	18:47:00	29.86	1490	24/09/2007	00:00:00	32.57
254	0	24		1261767	5	m	04/09/2007	18:47:00	29.86	1490	01/10/2007	09:00:00	33.57
255	0	19		1261767	6	m	04/09/2007	18:47:00	29.86	1490	11/10/2007	21:00:00	35.00
256	0	61		1261827	1	m	06/09/2007	06:33:00	27.00	790	06/09/2007	20:00:00	27.00
257	0	90		1261827	2	m	06/09/2007	06:33:00	27.00	790	14/09/2007	20:00:00	28.14
258	0	16		1261827	3	m	06/09/2007	06:33:00	27.00	790	18/09/2007	16:00:00	28.71
259	0	28		1261827	4	m	06/09/2007	06:33:00	27.00	790	25/09/2007	17:00:00	29.71
260	0	44		1261827	5	m	06/09/2007	06:33:00	27.00	190	29/09/2007	12:00:00	30.29
261	0	18		1261827	6	m	06/09/2007	06:33:00	27.00	790	04/10/2007	15:00:00	31.00

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Study Number	Observed	lenghth of rec	Status	Unit Number	Rec No	Sex	DOB	Time of Birth	Gestation in weeks	Birth Weight	Date recorded	Time recorded	Recording Gestation
262	0	8		1261827	7	m	06/09/2007	06:33:00	27.00	790	10/10/2007	00:00:00	31.86
263	0	100		1257876		m	13/07/2007	08:48:00	24.86	650	21/09/2007	13:00:00	34.86
264	0	20		1260989	4	m	24/08/2007	13:40:00	24.00	710	09/10/2007	12:00:00	30.57
265	0	22		1260989	5	m	24/08/2007	13:40:00	24.00	710	18/10/2007	12:00:00	31.86
266	0	24		1260989	6	m	24/08/2007	13:40:00	24.00	710	22/10/2007	09:00:00	32.29
267	0	12		1263605	1	m	27/09/2007	19:54:00	40.29	3450	27/09/2007	21:00:00	40.29
268	0	17		1264997	1	m	22/10/2007	12:20:00	34.14	2110	22/10/2007	14:00:00	34.14
269	0	24		1263966		m	03/10/2007	19:10:00	34.00	1910	04/10/2007	09:15:00	34.00
270	0	21		1265727		f	27/10/2007	18:05:00	29.71	750	28/10/2005	15:55:00	29.86
271	0	76		1265207	1	m	21/10/2007	19:53:00	36.14	3080	22/10/2007	08:00:00	36.14
272	0	24		1264725		m	14/10/2007	11:45:00	28.29	1150	26/10/2007	10:00:00	30.00
273	0	14		1258462		m	22/07/2007	16:23:00	25.14	830	06/08/2007	20:00:00	27.43
274	0	24		1258462		m	22/07/2007	16:23:00	25.14	830	10/08/2007	21:00:00	27.71
275	0	22		1258462		m	22/07/2007	16:23:00	25.14	830	14/08/2007	21:00:00	28.29
276	0	22		1258462		m	22/07/2007	16:23:00	25.14	830	23/08/2007	12:00:00	29.57
277	0	14		1258462		m	22/07/2007	16:23:00	25.14	830	07/09/2007	20:00:00	31.71
278	0	24		1258462		m	22/07/2007	16:23:00	25.14	830	01/01/2007	09:00:00	35.29
279	0	19		1258462		m	22/07/2007	16:23:00	25.14	830	11/10/2007	21:00:00	36.57
280	0	10		1258462		m	22/07/2007	16:23:00	25.14	830	23/01/2007	10:00:00	38.29
281	0	7		1265727		f	27/10/2007	18:05:00	29.71	750	30/01/2007	18:00:00	30.14
282	0	41		1290003		m	27/10/2007	18:49:00	23.43	575	28/10/2007	15:00:00	23.43
283	0	26		1265400	2	m	23/10/2007	19:30:00	28.14	1190	02/11/2007	12:00:00	29.57
284	0	154		1265400	1	m	23/10/2007	19:30:00	28.14	1190	23/10/2007	22:30:00	28.14
285	0	78		1265958	1	f	31/10/2007	04:32:00	26.71	860	31/10/2007	09:00:00	26.71
286	0	120		1265724	1	f	27/10/2007	19:38:00	32.00	1920	28/10/2007	15:00:00	32.00
287	0	4		1265724	2	f	27/10/2007	19:38:00	32.00	1920	06/11/2007	13:00:00	33.29
288	0	2.5		1265428	1	f	24/10/2007	02:07:00	32.57	1074	02/11/2007	12:00:00	33.86
289	0	22		1265727	1	f	27/10/2007	18:25:00	29.71	750	02/11/2007	16:00:00	30.57

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Study Number	Observed	lenghth of rec	Status	Unit Number	Rec No	Sex	DOB	Time of Birth	Gestation in weeks	Birth Weight	Date recorded	Time recorded	Recording Gestation
290	0	20		1265727	2	f	27/10/2007	18:25:00	29.71	750	06/11/2007	13:30:00	30.57
291	0	24		1261127	7	f	24/08/2007	22:00:00	26.71	800	01/10/2007	10:00:00	32.14
292	0	19		1261127	8	f	24/08/2007	22:00:00	26.71	800	04/10/2007	14:00:00	32.57
293	0	24		1261127	9	f	24/08/2007	22:00:00	26.71	800	10/10/2007	19:00:00	33.29
294	0	22		1261127	10	f	24/08/2007	22:00:00	26.71	800	18/10/2007	10:00:00	34.57
295	0	9		1261127	11	f	24/08/2007	22:00:00	26.71	800	23/10/2007	11:00:00	35.29
296	0	15		1261127	12	f	24/08/2007	22:00:00	26.71	800	02/11/2007	16:00:00	36.71
297	0	100		1285018		f	29/10/2007	19:45:00	28.00	1030	09/11/2007	09:00:00	29.57
298	0	27		1267410			19/11/2007	00:32:00	34.00	2760	19/11/2007	09:00:00	34.00
299	0	27		1266631			18/11/2007	04:19:00	34.43	2180	19/11/2007	09:00:00	34.43
300	0	96		1266902	1	f	13/11/2007	10:37:00	32.71	1570	13/11/2007	14:45:00	32.71
301	0	27		1266902	2	f	13/11/2007	10:37:00	32.71	1570	19/11/2007	09:00:00	33.57
302	0	96		1266903	1	f	13/11/2007	10:39:00	32.71	1575	13/11/2007	14:45:00	32.71
303	0	27		1266903	2	f	13/11/2007	10:39:00	32.71	1575	19/11/2007	09:00:00	34.43
305	0	96		1269444		m	13/12/2007	20:07:00	28.14	1140	15/12/2007	09:00:00	28.43
306	0	21		1269405		m	13/12/2007	13:15:00	33.71	2110	16/12/2007	00:00:00	34.14
307	0	46		1269358		m	12/12/2007	00:00:00	30.86	1110	17/12/2007	13:00:00	31.57
313	0	10		1274356	1	m	17/02/2008	12:00:00	31.57	1830	17/02/2008	14:00:00	31.57
314	0	48		1273059	1	m	01/02/2008	22:06:00	34.57	2320	02/02/2008	01:00:00	34.57
315	0	36		1270346	1	m	26/12/2008	14:43:00	29.43	1580	28/12/2008	11:00:00	29.71
316	0	72		1290201	1	m	07/12/2007	14:48:00	26.86	960	12/12/2007	11:00:00	27.57
24	1	2		1237527	1	f	10/10/2006	22:16:00	31.57	795	16/10/2006	16:00:00	32.43
26	1	20	as	1236578	1	m	01/10/2006	14:34:00	27.43	880	20/10/2006	18:00:00	29.71
27	1	20	qs	1238211	1	f	20/10/2006	11:37:00	34.71	2150	20/10/2006	18:00:00	34.71
28	1	20	as	1237523	1	f	10/10/2006	18:16:00	32.00	1485	20/10/2006	18:00:00	33.43
29	1	20	as	1236810	1	m	03/10/2006	10:06:00	30.00	905	20/10/2006	18:00:00	32.43
30	1	20	qs	1238330	1	f	20/10/2006	12:45:00	34.71	2245	20/10/2006	18:00:00	34.71
31	1	20	qs	1237670	1	f	12/10/2006	06:43:00	26.00	870	22/10/2006	22:00:00	27.43
32	1	20	qs	1238326	1	m	23/10/2006	12:04:00	39.00	3350	23/10/2006	22:00:00	39.00
33	1	20	qs	1238435	1	m	22/10/2006	19:37:00	35.71	3120	23/10/2006	22:00:00	35.86
34	1	20	qs	1236810	2	m	03/10/2006	10:06:00	30.00	905	23/10/2006	23:30:00	32.86
35	1	20	qs	1238387	1	m	23/10/2006	08:47:00	25.57	1000	23/10/2006	23:00:00	25.57
36	1	20	qs	1236578	2	m	01/10/2006	14:34:00	27.43	880	23/10/2006	23:30:00	30.43
37	1	20	qs	1238387	2	m	23/10/2006	08:47:00	25.57	1000	24/10/2006	23:00:00	25.71

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Study Number	Observed	lenghth of rec	Status	Unit Number	Rec No	Sex	DOB	Time of Birth	Gestation in weeks	Birth Weight	Date recorded	Time recorded	Recording Gestation
38	1	20	qs	1238419	1	f	22/10/2006	04:15:00	26.14	955	24/10/2006	23:00:00	26.43
39	1	20	qs	1238528	1	f	24/10/2006	14:23:00	26.00	530	25/10/2006	22:00:00	26.14
40	1	20	qs	1231783	2	m	28/07/2006	00:21:00	24.43	705	31/10/2006	21:00:00	38.14
41	1	20	qs	1238528	2	f	24/10/2006	14:23:00	26.00	530	31/10/2006	21:00:00	27.00
42	1	20	qs	1238419	1	f	22/10/2006	04:15:00	26.14	955	31/10/2006	20:00:00	27.29
43	1	20	qs	1238971	1	f	29/10/2006	23:41:00	32.71	1380	31/10/2006	20:00:00	32.29
44	1	20	as	1239360	1	m	06/11/2006	23:05:00	24.43	925	09/11/2006	12:00:00	24.86
45	1	20	as	1239386	1	m	05/11/2006	18:31:00	36.14	2390	09/11/2006	12:00:00	36.57
46	1	20	qs	1238528	3	f	24/10/2006	14:23:00	26.00	530	09/11/2006	12:00:00	28.29
47	1	20	as	1239299	1	f	09/11/2006	06:05:00	31.71	1200	09/11/2006	12:00:00	31.71
48	1	20	as	1236578	3	m	01/10/2006	14:34:00	27.43	880	09/11/2006	12:00:00	32.57
49	1	20	as	1238430	1	f	15/11/2006	09:50:00	31.29	1390	16/11/2006	17:00:00	31.43
50	1	20	as	1240207	1	f	14/11/2006	16:46:00	26.71	870	16/11/2006	17:00:00	26.86
59	1	20	qs	1240079	1	m	16/11/2006	07:56:00	28.00	1290	17/11/2006	16:00:00	28.14
60	1	20	qs	1240207	1	f	14/11/2006	16:46:00	26.71	870	17/11/2006	16:00:00	27.00
61	1	20	qs	1240363	1	m	16/11/2006	14:52:00	36.86	2215	17/11/2006	16:00:00	37.00
62	1	20	qs	1236578		m	01/10/2006	14:34:00	27.43	880	17/11/2006	16:00:00	34.14
63	1	20	qs	1239300		m	09/11/2006	06:21:00	30.71	1960	17/11/2006	12:00:00	31.86
64	1	20	as	1239299	1	f	09/11/2006	06:05:00	31.71	1200	17/11/2006	12:00:00	32.86
65	1	25	qs	1240959	1	f	24/11/2006	00:56:00	33.71	1380	30/11/2006	16:00:00	34.57
66	1	20	qs	1240549	1	m	20/11/2006	19:41:00	24.14	700	30/11/2006		25.57
67	1	20	as	1260001		f	03/12/2006	00:00:00	27.29	1028	14/12/2006	13:00:00	28.86
68	1	20	qs	1241247		m	04/12/2006	14:38:00	25.71	760	14/12/2006	13:00:00	28.29
69	1	20	qs	1240207	2	f	14/11/2006	16:46:00	26.71	870	14/12/2006	13:00:00	30.86
70	1	20	as	1242240	1	m	12/12/2006	01:36:00	29.00	910	14/12/2006	13:00:00	29.29
78	1	20	qs	1240549		m	20/11/2006	19:41:00	24.14	700	18/12/2006	13:00:00	28.00
79	1	20	qs	1260001		f	03/12/2006	00:00:00	27.29	1028	18/12/2006	13:00:00	29.43
80	1	20	as	1241247		m	04/12/2006	14:38:00	25.71	760	18/12/2006	15:00:00	27.71
81	1	20	qs	1240207	2	f	14/11/2006	16:46:00	26.71	870	18/12/2006	13:00:00	31.57

Appendix 1 HRV Patient Database

Study Number	Observed	lenghth of rec	Status	Unit Number	Rec No	Sex	DOB	Time of Birth	Gestation in weeks	Birth Weight	Date recorded	Time recorded	Recording Gestation
82	1	20	qs	1242568	term baby	f	17/12/2006	05:35:00	41.86	3220	18/12/2006	13:00:00	42.00
83	1	20	qs	1242240		m	12/12/2006	01:36:00	29.00	910	18/12/2006	13:00:00	29.86
89	1	20	qs	1240549		m	20/11/2006	19:41:00	24.14	700	02/01/2007	20:00:00	30.14
90	1	20	qs	1241247		m	04/12/2006	14:38:00	25.71	760	02/01/2007	20:00:00	30.86
91	1	20	qs	1243435		m	31/12/2006	14:32:00	28.43	1100	02/01/2007	20:00:00	28.71
92	1	20	qs	1243543		m	03/01/2007	05:17:00	31.29	1524	15/01/2007	11:00:00	33.00
93	1	20	qs	1244111		m	10/01/2007	05:30:00	34.71	2880	15/01/2007	11:00:00	35.43
94	1	20	qs	1240207		f	14/11/2006	16:46:00	26.71	870	15/01/2007	11:00:00	35.43
95	1	20	qs	1244111		m	10/01/2007	05:30:00	34.71	2880	15/01/2007	12:00:00	35.43
96	1	20	qs	1243905	1	f	08/01/2007	00:00:00	25.86	830	15/01/2007	17:00:00	26.86
97	1	20	qs	1243447	1	m	01/01/2007	09:24:00	28.57	1030	15/01/2007	17:00:00	30.43
98	1	20	qs	1243448		m	01/01/2007	09:29:00	28.57	950	15/01/2007	17:00:00	30.43
100	1	20	as	1244800	1	m	18/01/2007	18:30:00	26.71	1040	23/01/2007	09:30:00	27.43
101	1	20	as	1244597		m	16/01/2007	18:21:00	26.00	550	18/01/2007	19:10:00	26.29
102	1	20	qs	1243905		f	08/01/2007	00:00:00	25.86	830	23/01/2007	11:00:00	28.00
103	1	20	qs	1242240		m	12/12/2006	01:55:00	29.00	910	23/01/2007	09:30:00	35.00
104	1	20	as	1242518		m	18/12/2006	19:25:00	25.29	850	29/01/2007	11:30:00	31.14
105	1	20	qs	1245009		m	22/01/2007	19:12:00	25.00	730	29/01/2007	11:00:00	25.86
106	1	20	qs	1243447		m	01/01/2007	09:24:00	28.57	1030	22/01/2007	16:00:00	32.57
107	1	20	qs	1243448		m	01/01/2007	09:29:00	28.57	950	22/01/2007	16:00:00	32.57
108	1	20	as	1244729		f	18/01/2007	10:01:00	31.86	1690	02/02/2007	14:00:00	34.00
115	1	20	qs	1243447		m	01/01/2007	09:24:00	28.57	1030	05/02/2007	11:00:00	33.43
116	1	20	qs	1245464		f	28/01/2007	15:41:00	31.29	1905	05/02/2007	11:00:00	32.29
128	1	20	qs	1248125		m	06/03/2007	12:18:00	31.29	1464	06/03/2007	18:40:00	31.29
132	1	20	qs	1260016		f	15/02/2007	05:00:00	25.29	625	06/03/2007	18:00:00	29.29
148	1	20	qs	1244597		m	16/01/2007	18:21:00	26.00	550	16/03/2007	11:00:00	34.43
151	1	20	qs	1249714		f	25/03/2007	14:26:00	26.14	1000	29/03/2007	16:30:00	26.57
154	1	20	qs	1243904		f	08/01/2007	01:50:00	25.86	790	16/03/2007	14:00:00	35.43
155	1	20	qs	1244729		f	18/01/2007	10:01:00	31.86	1690	16/03/2007	10:30:00	40.00
157	1	20	qs	1250297		f	01/04/2007	11:40:00	30.43	1310	02/04/2007	20:00:00	30.57
159	1	20	qs	1250161		m	29/03/2007	17:40:00	27.57	1200	02/04/2007	20:00:00	28.14
161	1	20	qs	1248442		m	08/03/2007	12:45:00	32.29	1130	16/03/2007	10:30:00	33.43
163	1	20	qs	1248125		m	06/03/2007	12:18:00	31.29	1464	16/03/2007	13:30:00	32.71

Appendix 1 HRV Patient Database

Study Number	Observed	lenghth of rec	Status	Unit Number	Rec No	Sex	DOB	Time of Birth	Gestation in weeks	Birth Weight	Date recorded	Time recorded	Recording Gestation
165	1	20	as	1248977		m	14/03/2007	21:25:00	35.71	2870	16/03/2007	13:30:00	36.00
180	1	20	qs	1250297		f	01/04/2007	11:40:00	30.43	1310	06/04/2007	22:30:00	31.14
203	1	20	qs	1257452		f	22/07/2007	06:34:00	34.14	2240	22/07/2007	14:00:00	34.14
204	1	20	qs	1257452		f	22/07/2007	06:34:00	34.14	2240	22/07/2007	19:00:00	34.14
205	1	20	qs	1280014	3	f	02/06/2007	19:20:00	25.43	990	21/07/2007	12:00:00	32.43
304		96		1264725		m	14/10/2007	11:45:00	28.29	1150	15/10/2007	10:00:00	28.29
308		44		1268663		m	19/12/2007	00:00:00	31.71	1790	19/12/2007	14:00:00	31.71
309		50		1270570		m	30/12/2007	19:51:00	32.57	2060	30/12/2007	21:00:00	32.57
310		96		1269450		f	13/12/2007	22:35:00	27.14	1230	15/12/2007	09:00:00	27.43
311		96		1269452		f	13/12/2007	22:50:00	27.14	1040	15/12/2007	09:00:00	27.43
312		72		1269726		m	21/12/2007	02:37:00	26.86	1000	21/12/2007	08:00:00	26.86

Appendix 1 HRV Patient Database

Study Number	Age at recording	Apgar 1	Apgar 5	AN steroids	Chorioamnionitis	Drugs in pregnancy	Smoking	PIH	cord ph	No resp support	CPAP	Ventilated	PIP	PEEP	MAP	Rate	FIO2
1	12			1	0	0	0	1	7.14	0	1	0					24.00
3	3	7	9	0	0	0	1	0		1	0	0					21.00
4	8	8	8	1	0	0	0	0		1	0	0					21.00
5	2	9	9	1	1	0	0	0	7.26	1	0	0					21.00
6	33	9	8	1	0	0	1	0	7.30	1	0	0					21.00
7	36	6	10	1	1	0	0	0	7.28	1	0	0					21.00
8	120	7	9	1	0	0	0	0		1	0	0					0.2 nc
9	1	8	10	0	0	0	0	0		1	0	0					21.00
10	24	7	8	1	0	0	1	0	7.47	0	1	0					21.00
11	9	4	8	1	0	1	1	0		1	0	0					21.00
12	2	8	9	1	0	0	1	0	7.28	1	0	0					21.00
13	2	9	9	1	0	0	1	0		1	0	0					21.00
14	31	0	5	1	0	0	1	0	7.33	1	0	0					21.00
15	0	8	10	0	0	0	0	0	7.20	1	0	0					24.00
16	40	6	10	1	1	0	0	0	7.28	1	0	0					21.00
17	40	6	6	1	1	0	0	0	7.23	1	0	0					0.12 nc
18	2	8	10	1	0	0	1	0	7.28	1	0	0					21.00
19	1	5	9	0	0	0	0	0	7.16	1	0	0					21.00
20	4	9	9	1	0	0	1	0		1	0	0					21.00
21	7	7	9	0	0	0	1	0		0	1	0					22.00
22	2	9	9	1	0	0	0	0	7.03	0	1	0					23.00
23	2	5	9	1	0	0	0	0		0	0	1	14	6	4	35	32.00
25	1	8	9	1	0	0	1	0		0	0	1	14	5	7	40	30.00
51	4	1	5	1	0	0	1	0	7.11	0	1	0					30.00
52	2	7	7	1	0	0	0	1	7.21	1	0	0					21.00
53	2	4	6	1	1	0	0	0		1	0	0					21.00
54	1	9	9	0	0	0	1		7.32	1	0	0					21.00
55	19	7	10	1	0	0	0	0		1	0	0					35.00
56	8	10	10	1	0	0	0	0		1	0	0					21.00
57	1	7	8	1	0	0	0	0	7.34	1	0	0					21.00
58	8	7	8	1	0	0	0	0	7.25	1	0	0					21.00
71	3	7	9	0	0	0	0	0	7.26	1	0	0					21.00
72	11	6	8	1	0	0	1	0	7.24	0	1	0					41.00
73	9	6	6	1	0	0	1	0	7.34	1	0	0					28.00
74	29	4	6	1	1	0	0	0		0	1	0					26.00

Appendix 1 HRV Patient Database

Study Number	Age at recording	Apgar 1	Apgar 5	AN steroids	Chorioamnionitis	Drugs in pregnancy	Smoking	PIH	cord ph	No resp support	CPAP	Ventilated	PIP	PEEP	MAP	Rate	FiO2
75	1	8	9	1	0	0	0	0	7.27	1	0	0					38.00
76	2	5	8	1	0	0	0	0		0	1						21.00
77	51	6	9	1	0	0	0	1	7.25	1	0	0					21.00
84	6	5	8	1	0	0	0	0		0	1	0					21.00
85	27	5	6	1	1	0	0	0		0	0	1	22	6	10	40	54.00
86	15	6	8	1	0	0	1	0	7.24	0	1	0					26.00
87	34	4	6	1	1	0	0	0		1	1	0					37.00
88	1	9	10	0	0	0	0	0	7.30	1	0	0					21.00
99	0	9	10	1	0	0	0	1		0	0	1	14	5	6	40 - 10	21.00
109	15	5	5	1	0	0	0	0	7.25	1	0	0					36.00
110	46	7	10	1	1	0	1	0		0	1	0					80.00
111	25	6	8	1	0	0	0	0	7.26	1	0	0					0.05 nc
112	28	6	8	1	0	0	0	0	7.26	1	0	0					0.12 nc
113	7	3	4	0	0	0	1	0		1	0	0					21.00
114	34	9	10	1	0	0	1	0	7.31	1	0	0					0.2nc
117	15	7	10	0	0	0	0	0	7.32	1	0	0					28.00
118	23	7	10	0	0	0	0	0	7.32	0	1	0					22.00
119	10	6	8	1	0	0	1	0		0	1	0					25.00
120	26	5	5	1	0	0	0	0	7.25	1	0	0					0.30 nc
121	56	7	10	1	1	0	1	0		0	1	0					70.00
122	5	7	10	1	0	0	0	0	7.30	0	1	0					21.00
123	37	6	8	1	0	0	0	0	7.26	1	0	0					0.02 nc
124	37	6	8	1	0	0	0	0		1	0	0					29.00
125	7	5	10	1	0	0	0	0		0	0	1	14	4	5	20	21.00
126	0	5	9	1	0	0	0	0	7.08	0	0	1	18	5	8	40-20	21.00
127	0	5	9	1	0	0	0	0		1	0	0					21.00
129	1	9	9	1	0	0	0	0	7.28	0	0	1	14	4	6	50-35	21.00
130	0	9	9	1	0	0	0	0	7.28	1	0	0					21.00
131	2	8	10	1	0	0	0	0	7.37	1	0	0					21.00
133	28	1	5	0	0	0	0	0		0	0	1	18	4	8	50	50.00
134	54	8	8	1	0	0	1	0		1	0	0					

Appendix 1 HRV Patient Database

Study Number	Age at recording	Apgar 1	Apgar 5	AN steroids	Chorioamnionitis	Drugs in pregnancy	Smoking	PIH	cord ph	No resp support	CPAP	Ventilated	PIP	PEEP	MAP	Rate	FIO2
135	40	5	5	1	0	0	0	0	7.25	1	0	0					60.00
136	1	9	9	1	0	0	0	0	7.30	0	1	0					21.00
137	17	8	10	1	0	0	0	0		1	0	0					21.00
138	53	6	8	1	0	0	0	0		1	0	0					0.08 nc
139	53	6	8	1	0	0	0	0	7.26	1	0	0					21.00
140	66	6	8	1	0	0	0	0	7.26	1	0	0					0.02nc
141	2	10	10	1	0	0	0	0	7.39	1	0	0					21.00
142	2	10	10	1	0	0	0	0	7.39	1	0	0					21.00
143	3	9	10	1	1	0	0	0		0	1	0					37.00
144	0	7	9	0	0	0	1	0	7.37	1	0	1	16	4		20	21.00
145	7	5	9	1	0	0	0	0	7.08	1	0	0					21.00
146	7	5	9	1	0	0	0	0		1	0	0					21.00
147	8	2	7	1	0	0	0	0	6.99	0	0	1	16	4	7	50	40.00
149	59	9	10	1	0	0	1	1		0	1	0					30.00
150	71	9	10	1	0	0	1	1		1	0	0					65.00
152	3	6	9	1	0	0	1	0	7.39	0	1	0					21.00
153	67	6	8	1	0	0	0	0		1	0	0					0.03nc
156	57	5	5	1	0	0	0	0	7.25	1	0	0					40.00
158	1	9	10	1	0	0	0	0		1	0	0					21.00
160	4	5	7	0	0	0	1	0		0	1	0					22.00
162	8	5	9	1	0	0	0	0		1	0	0					21.00
164	10	9	9	1	0	0	0	0	7.28	1	0	0					21.00
166	2	5	9	1	0	0	1	0	7.16	1	0	0					21.00
167	9	5	3	1	0	0	0	1		1	0	0					21.00
168	12	6	10	1	0	0	1	0	7.27	1	0	0					21.00
169	71	1	5	0	0	0	0	0		0	1	0					37.00
170	3	4	5	0	0	0	0	0	6.80	1	0	0					21.00
171	3	4	5	0	0	0	0	0	7.10	1	0	0					21.00
172	0	0	4	0	0	0	0	0	7.20	1	0	0					21.00
173	1	9	9	1	0	0	1	0	7.34	1	0	0					21.00
174	8	6	9	1	0	0	1	0	7.39	0	0	1	19	5	8	40	28.00
175	12	6	9	1	0	0	1	0	7.39	0	0	1	22	6	9	30	34.00

Appendix 1 HRV Patient Database

Study Number	Age at recording	Apgar 1	Apgar 5	AN steroids	Chorioamnionitis	Drugs in pregnancy	Smoking	PIH	cord ph	No resp support	CPAP	Ventilated	PIP	PEEP	MAP	Rate	FIO2
176	31	9	9	1	0	0	0	0	7.30	1	0	0					25.00
177	81	9	10	1	0	0	1	1		1	0	0					0.05nc
178	2	6	10	1	0	0	0	0		1	0	0					21.00
179	0	6	10	1	0	0	0	0		1	1	1					21.00
181	1	9	10	1	0	0	0	0		0	0	1	20	5	9	60	31.00
182	5	9	10	1	0	0	0	0		1	0	0					21.00
183	6	9	10	1	0	0	1	0		0	0	1	14	5	6	15	28.00
184	4	9	10	1	0	0	1	0		0	0	1	14	5	6	15	28.00
185	8	9	10	1	0	0	0	0		1	0	0					21.00
186	13	3	8	1	0	0	0	0	7.03	0	0	1	19	5	7	15	21.00
187	75	1	5	0	0	0	0	0		0	1	0					36.00
188	4	9	10	1	0	0	0	0	7.30	1	0	0					0.02nc
189	16	3	6	1	0	0	0	0	7.19	0	1	0					40.00
190	17	5	8	0	0	0	1	0		1	0	0					21.00
191	33	9	10	1	0	0	1	0		0	1	0					30.00
192	4	4	7	1	0	0	1	0		0	0	1	18	5	8	40	34.00
193	7	5	7	1	0	0	1	0		0	1	0					24.00
194	7	7	10	1	0	0	0	0	7.16	0	0	1					60 -90
195	0	5	8	1	0	0	0	0		0	0	0					21.00
196	1	9	10	1	0	0	0	0	7.19	1	0	0					21.00
197	91	1	5	0	0	0	0	0		0	1	0					39.00
198	2	3	7	1	0	0	0	0		0	0	1		5	9	50-60	39.00
199	1	3	7	1	0	0	0	0		0	0	0					21.00
200	6			1	0	amitryptilline	0	0		0	1	0					35.00
201	35	3	6	1	0	0	0	0	7.19	0	1	0					21.00
202	0	9	10	1	0	0	1	0		1	0	0					21.00
206	49	3	7	1	0	0	0	0		0	1	0					0.5 nc
207	0	8	9	1	0	0	0	0	7.39	1	0	0					21.00
208	0	2	9	1	0	0	0	0	7.33	1	0	0					21.00
209	0	7	9	1	0	1 - asthma+	0	0	7.48	0	1	1					
210	0	3	4	1	0	0	1	0		1	0	0					21.00
211	10	8	9	1	0	0	0	0	7.40	0	0	1				60	
212	12	8	9	1	0	0	0	0	7.40	0	0	1				40	
213	20	8	9	1	0	0	0	0	7.40	0	0	1				30 - 50	
214	25	8	9	1	0	0	0	0	7.40	0	0	1				30 - 50	

Appendix 1 HRV Patient Database

Study Number	Age at recording	Apgar 1	Apgar 5	AN steroids	Chorioamnionitis	Drugs in pregnancy	Smoking	PIH	cord ph	No resp support	CPAP	Ventilated	PIP	PEEP	MAP	Rate	FIO2
215	0	8	9	1	0	0	0	0	7.38	1	0	0					
216	9	8	9	1	0	0	0	0	7.38	1	0	0					
217	12	8	9	1	0	0	0	0	7.38	1	0	0					
218	19	8	9	1	0	0	0	0	7.38	1	0	0					
219	23	8	9	1	0	0	0	0	7.38	1	0	0					
220	18	9	9	1	0	0	0	0	7.38	1	0	0					0.00
221	22	9	9	1	0	0	0	0	7.38	1	0	0					0.00
222	24	9	9	1	0	0	0	0	7.38	1	0	0					0.00
223	9	9	9	1	0	0	0	0	7.38	1	0	0					0.00
224	8	7	9	1	0	asthma	0	0	7.48	1	0	0					0.00
225	15	7	9	1	0	asthma	0	0	7.48	1	0	0					0.00
226	23	7	9	1	0	asthma	0	0	7.48	1	0	0					0.00
227	27	7	9	1	0	asthma	0	0	7.48	1	0	0					0.00
228	0	6	8	1	0	citalopram	0	1	7.39	0	1	0					
229	13	6	8	1	0	citalopram	0	1	7.39	0	1	0					
230	20	6	8	1	0	citalopram	0	1	7.39	0	1	0					
231	24	6	8	1	0	citalopram	0	1	7.39	0	1	0					
232	26	6	8	1	0	citalopram	0	1	7.39	0	0	1					
233	32	6	8	1	0	citalopram	0	1	7.39	0	0	1					
234	5	8	9	1	0	0	0	0		1	0	0					
235	14	8	9	1	0	0	0	0		1	0	0					
236	25	8	9	1	0	0	0	0		1	0	0					
237	14	1	3	1	0	0	0	1		1	1	0					
238	23	1	3	1	0	0	0	1		0	0	1					

Appendix 1 HRV Patient Database

Study Number	Age at recording	Apgar 1	Apgar 5	AN steroids	Chorioamnionitis	Drugs in pregnancy	Smoking	PIH	cord ph	No resp support	CPAP	Ventilated	PIP	PEEP	MAP	Rate	FIO2
239	31	1	3	1	0	0	0	1		1	1	0					
240	18	1	3	1	0	0	0	1		0	0	1					
241	31	9	9	1	0	0	0	0	7.38	1	0	0					0.00
242	8	nr	nr		1	0	1	0	0	0	1	0					
243	12	nr	nr		1	0	1	0	0	0	1	1					
244	1	9	10	0	0	0	0	0	6.94	1	0	0					
245	0	9	10	1	0	0	0	0	7.32	1	0	1					
246	9	9	10	1	0	0	0	0	7.32	1	0	0					
247	16	9	10	1	0	0	0	0	7.32	1	1	0					
248	20	9	10	1	0	0	0	0	7.32	1	0	0					
249	26	9	10	1	0	0	0	0	7.32	1	0	0					
250	0	9	10	1	0	0	0	0	7.32	1	0	0					
251	9	9	10	1	0	0	0	0	7.32	1	0	0					
252	16	9	10	1	0	0	0	0	7.32	1	0	0					
253	19	9	10	1	0	0	0	0	7.32	1	0	0					
254	26	9	10	1	0	0	0	0	7.32	1	0	0					
255	36	9	10	1	0	0	0	0	7.32	1	0	0					
256	0	6	8	1	0	labetalol	0	1	7.25	0	0	1					
257	8	6	8	1	0	labetalol	0	1	7.25	1	1	0					
258	12	6	8	1	0	labetalol	0	1	7.25	0	0	1					
259	19	6	8	1	0	labetalol	0	1	7.25	1	1	0					
260	29	6	8	1	0	labetalol	0	1	7.25	1	1	0					
261	28	6	8	1	0	labetalol	0	1	7.25	1	1	0					

Appendix 1 HRV Patient Database

Study Number	Age at recording	Apgar 1	Apgar 5	AN steroids	Chorioamnionitis	Drugs in pregnancy	Smoking	PIH	cord ph	No resp support	CPAP	Ventilated	PIP	PEEP	MAP	Rate	FIO2
262	34	6	8	1	0	labetalol	0	1	7.25	1	1	0					
263	70	nr	nr	1	1	0	0	0		1	1	0					
264	46	8	9	1	0	0	0	0	7.40	1	1	0					
265	55	8	9	1	0	0	0	0	7.40	1	1	0					
266	58	8	9	1	0	0	0	0	7.40	1	1	0					
267	0	9	9	0	0	0	0	0	7.22	1	0	0					
268	0	9	10	0	0	0	0	0		1	0	0					
269	0	2	4	1	0	0	1	0		1	0	0					
270	1	9	9	1	0	0	0	0	7.34	1	0	0					
271	1	9	10	0	0	0	0		7.29	1	0	0					28.00
272	12	5	10	1	0	0	0	0	6.97	1	1	0					
273	16	8	10	1	0	0	0	0	7.34	0	0	1					
274	18	8	10	1	0	0	0	0	7.34	0	0	1					
275	22	8	10	1	0	0	0	0	7.34	0	1	0					
276	31	8	10	1	0	0	0	0	7.34	0	1	0					
277	36	8	10	1	0	0	0	0	7.34	0	1	0					
278	71	8	10	1	0	0	0	0	7.34	0	1	0					
279	80	8	10	1	0	0	0	0	7.34	1	1	0					
280	92	8	10	1	0	0	0	0	7.34	1	0	0					
281	3	9	9	1	0	0	0	0	7.34	1	0	0					
282	0	nr	nr	1	0	0	0	0		0	0	1					
283	10	6	8	1	0	0	0	0		1	1	0					
284	0	6	8	1	0	0	0	0		1	1	1					
285	0	8	10	0	1	0	1	0	7.31	0	1	1					
286	0	7	10	0	0	carbamzepine	1	0	7.22	1	0	0					
287	9	7	10	0	0	carbamzepine	1	0	7.22	1	0	0					
288	9	6	10	1	0	0	1		7.32	1	0	0					
289	6	9	9	1	0	0	1		7.34	1	0	0					

Appendix 1 HRV Patient Database

Study Number	Age at recording	Apgar 1	Apgar 5	AN steroids	Chorioamnionitis	Drugs in pregnancy	Smoking	PIH	cord ph	No resp support	CPAP	Ventilated	PIP	PEEP	MAP	Rate	FIO2
290	6	9	9	1	0	0	1		7.34	1	0	0					
291	38	6	8	1	0	citalopram	0	1	7.39	1	1	0					
292	41	6	8	1	0	citalopram	0	1	7.39	1	1	0					
293	47	6	8	1	0	citalopram	0	1	7.39	1	1	0					
294	55	6	8	1	0	citalopram	0	1	7.39	1	0	0					
295	60	6	8	1	0	citalopram	0	1	7.39	1	0	0					
296	70	6	8	1	0	citalopram	0	1	7.39	1	0	0					
297	12	5	6	1	0	0	1	0		1	1	1					
298	0	9	10	1	0	amiloride	0	0	7.24	1	0	0					
299	1	5	9	0	0	0	0	0	7.01	1	0	0					
300	0	9	9	1	0	0	0	0	7.36	1	0	0					25.00
301	7	9	9	1	0	0	0	0	7.36	1	0	0					25.00
302	0	8	9	1	0	0	0	0	7.36	1	0	0					
303	7	8	9	1	0	0	0	0	7.36	1	0	0					
305	2	9	9	1	0	0	1	0		0	0	1					
306	3	8	9	1	0	0	0	0		1	0	0					
307	5	6	9	1	0	0	0	1		1	0	0					
313	0	3	7	1	0	0	0	0		1	0						
314	0	9	9	1	0	0	0	0	7.30	1	0	0					
315	3	9	9	1	0	0	0	0		1	0						
316	5	nr	nr		1	0	0	0	0		0	1	1					
24	6	7	10	1	0	0	0	0	7.27	1	0	0					21.00
26	19	7	10	1	0	0	0	0		1	0	0					21.00
27	0	8	9	1	0	0	1	0		1	0	0					21.00
28	10	6	10	0	0	1	1	0	7.23	1	0	0					21.00
29	17	9	9	1	0	0	0	0	7.39	1	0	0					21.00
30	0	9	10	1	0	0	1	0		1	0	0					21.00
31	10	6	7	1	0	0	1	0		0	1	0					25.00
32	0	9	9	0	0	0	0	0	7.29	1	0	0					21.00
33	1	10	10	0	0	0	1	0	7.35	1	0	0					21.00
34	20	9	9	1	0	0	0	0	7.39	1	0	0					21.00
35	0	9	9	1	0	0	1	0		0	0	1	14	5	6	20	25.00
36	22	7	10	1	0	0	0	0		1	0	0					21.00
37	1	9	9	1	0	0	1	0		0	1	0					40.00

Appendix 1 HRV Patient Database

Study Number	Age at recording	Apgar 1	Apgar 5	AN steroids	Chorioamnionitis	Drugs in pregnancy	Smoking	PIH	cord ph	No resp support	CPAP	Ventilated	PIP	PEEP	MAP	Rate	FIO2
38	3	7	9	1	1	0	0	0	7.23	0	1	0					24.00
39	1	6	9	1	0	0	0	1	7.25	0	1	0					21.00
40	96	6	9	1	0	0	0	0	7.33	1	0	0					0.03 nc
41	7	6	9	1	0	0	0	1	7.25	0	1	0					21.00
42	10	7	9	1	1	0	0	0	7.23	0	1	0					21.00
43	2	7	9	1	0	0	0	0	7.26	1	0	0					21.00
44	3	7	10	1	0	0	0	0		0	1	0					38.00
45	3	9	10	0	0	0	1	0	7.26	1	0	0					22.00
46	16	6	9	1	0	0	0	1	7.25	0	1	0					21.00
47	0	7	8	1	0	0	0	0	7.25	1	0	0					21.00
48	39	7	10	1	0	0	0	0		1	0	0					36.00
49	1	7	7	1	0	0	0	1	7.21	1	0	0					21.00
50	1	4	6	1	1	0	0	0		1	0	0					21.00
59	1	6	7	1	0	0	0	0		0	1	0					21.00
60	2	4	6	1	1	0	0	0		1	0	0					21.00
61	1	9	9	0	0	0	1	0	7.32	1	0	0					21.00
62	47	7	10	1	0	0	0	0		1	0	0					35.00
63	8	10	10	1	0	0	0	0		1	0	0					21.00
64	8	7	8	1	0	0	0	0	7.25	1	0	0					21.00
65	6	7	10	1	0	0	1	0	7.25	1	0	0					21.00
66	10	5	6	1	1	0	0	0		0	1	0					60.00
67	11	6	8	1	0	0	1	0	7.24	0	1	0					41.00
68	9	6	6	1	0	0	1	0	7.34	1	0	0					28.00
69	29	4	6	1	1	0	0	0		0	1	0					26.00
70	2	5	8	1	0	0	0	0		0	1						21.00
78	27	5	6	1	1	0	0	0		0	0	1	22	6	10	40	54.00
79	15	6	8	1	0	0	1	0	7.24	0	1	0					26.00
80	14	6	6	1	0	0	1	0	7.34	0	1	0					31.00
81	34	4	6	1	1	0	0	0		1	0	0					37.00

Appendix 1 HRV Patient Database

Study Number	Age at recording	Apgar 1	Apgar 5	AN steroids	Chorioamnionitis	Drugs in pregnancy	Smoking	PIH	cord ph	No resp support	CPAP	Ventilated	PIP	PEEP	MAP	Rate	FIO2
82	1	9	10	0	0	0	0	0	7.30	1	0	0					21.00
83	6	5	8	1	0	0	0	0		0	1						21.00
89	42	5	6	1	1	0	0	0		0	1	0					40.00
90	36	6	6	1	0	0	1	0	7.34	1	0	0					34.00
91	2	10	10	1	0	0	0	0		1	0	0					21.00
92	12	9	10	1	0	0	1	0	7.37	1	0	0					21.00
93	5	9	10	1	0	0	0	0	7.35	1	0	0					21.00
94	61	4	6	1	1	0	0	0		1	0	0					0.12 nc
95	5	9	10	1	0	0	0	0	7.35	1	0	0					21.00
96	7	6	8	1	0	0	0	0	7.26	0	1	0					21.00
97	13	9	10	1	0	0	1	0	7.31	1	0	0					26.00
98	13	9	9	1	0	0	1	0	7.35	1	0	0					24.00
100	5	8	8	1	0	0	0	0		1	0	0					21.00
101	2	9	10	1	0	0	0	1		0	0	1	14	4	5	10	30.00
102	15	6	8	1	0	0	0	0	7.26	0	1	0					23.00
103	42	5	8	1	0	0	0	1		1	0	0					0.03 nc
104	41	7	10	1	1	0	1	0		0	1	0					96.00
105	6	1	5	1	0	0	1	0		0	1	0					21.00
106	21	9	10	1	0	0	1	0	7.31	1	0	0					0.2nc
107	21	9	9	1	0	0	1	0	7.35	1	0	0					0.2nc
108	15	5	5	1	0	0	0	0	7.25	1	0	0					36.00
115	34	9	10	1	0	0	1	0	7.31	1	0	0					0.2nc
116	7	3	4	0	0	0	1	0		1	0	0					21.00
128	0	9	9	1	0	0	0	0	7.28	1	0	0					21.00
132	28	1	5	0	0	0	0	0		0	0	1	18	4	8	50	50.00
148	59	9	10	1	0	0	1	1		0	1	0					30.00
151	3	6	9	1	0	0	1	0	7.39	0	1	0					25.00
154	67	6	8	1	0	0	0	0		1	0	0					0.03nc
155	57	5	5	1	0	0	0	0	7.25	1	0	0					40.00
157	1	9	10	1	0	0	0	0		1	0	0					21.00
159	4	5	7	0	0	0	1	0		0	1	0					22.00
161	8	5	9	1	0	0	0	0		1	0	0					21.00
163	10	9	9	1	0	0	0	0	7.28	1	0	0					21.00

Appendix 1 HRV Patient Database

Study Number	Age at recording	Apgar 1	Apgar 5	AN steroids	Chorioamnionitis	Drugs in pregnancy	Smoking	PIH	cord ph	No resp support	CPAP	Ventilated	PIP	PEEP	MAP	Rate	FiO2
165	2	5	9	1	0	0	1	0	7.16	1	0	0					21.00
180	5	9	10	1	0	0	0	0		1	0	0					21.00
203	0	6	8	1	0	0	0	0	7.30	1	0	0					60.00
204	0	6	8	1	0	0	0	0	7.30	0	0	1					60.00
205	49	3	7	1	0	0	0	0		1	0	0					0.5 nc
304	0	5	10	1	0	0	0	0	6.97	0	0	0					
308	0	5	9	1	0	0	0	0	7.18	1	0	0					
309	0	9	10	1	0	0	0	0		1	0	0					
310	2	7	9	1	0	0	0	0	7.30	1	1	0					
311	2	8	9	1	0	0	0	0	7.15	1	1	1					
312	0	5	7	1	0	0	0	0	7.15	1	1	1					

Appendix 1 HRV Patient Database

Study Number	Current Weight	On abx	Suspected sepsis	Confirmed Sepsis	Subsequent sepsis (48 hr)	IVH Grade	IVH subsequently	Other Brain abn	PVL	PVL subsequent	Other Brain abn subsequent	Dopamine	Dobutamine	Inotropes in next 48 hours
1		1	0	1	0	0	0	0	0	0	0	0	0	0
3	1100.00	0	0	0	0	0	0	0	0	0	0	0	0	0
4	1074.00	1	0	1	0	0	0	0	0	0	0	0	0	0
5	1900.00	0	0	0	0	0	0	0	0	0	0	0	0	0
6	1485.00	0	0	0	0	0	0	0	0	0	0	0	0	0
7	2100.00	0	0	0	0	0	0	0	0	0	0	0	0	0
8	2875.00	0	0	0	0	0	0	0	0	0	0	0	0	0
9	4280.00	1	0	0	0	0	0	0	0	0	0	0	0	0
10	1030.00	1	0	0	0	0	0	0	0	0	0	0	0	0
11	1090.00	0	0	0	0	0	0	0	0	0	0	0	0	0
12	1865.00	1	1	0	0	0	0	0	0	0	0	0	0	0
13	1640.00	0	0	0	0	0	1	0	0	0	0	0	0	0
14	1540.00	0	0	0	0	0	0	0	0	0	0	0	0	0
15	3400.00	1	1	0	0	0	0	0	0	0	0	0	0	0
16	1405.00	0	0	0	0	0	0	0	0	0	0	0	0	0
17	1510.00	0	0	0	0	0	2	0	0	0	0	0	0	0
18	1785.00	0	0	0	0	0	0	0	0	0	0	0	0	0
19	3240.00	0	0	0	0	0	0	0	0	0	0	0	0	0
20	1640.00	0	0	0	0	0	1	0	0	0	0	0	0	0
21	1140.00	0	0	0	0	1	0	0	0	0	0	0	0	0
22	1540.00	0	0	0	0	1	0	0	0	0	0	0	0	0
23	810.00	1	1	0	0	0	2	0	0	0	0	0	0	0
25	740.00	1	0	0	0	0	0	1	0	1	0	0	0	0
51	645.00	0	0	0	0	0	2	0	0	0	0	0	0	0
52	1390.00	0	0	0	0	0	0	0	0	0	0	0	0	0
53	870.00	0	0	0	0	0	0	0	0	0	0	0	0	0
54	2215.00	0	0	0	0	0	0	0	0	0	0	0	0	0
55	1930.00	0	0	0	0	1	0	0	0	0	0	0	0	0
56	0.00	0	0	0	0	0	0	0	0	0	0	0	0	0
57	3080.00	1	0	0	0	0	0	0	0	0	0	0	0	0
58	1200.00	0	0	0	0	0	0	0	0	0	0	0	0	0
71		0	0	0	0	0	0	0	0	0	0	0	0	0
72	1100.00	0	0	0	0	0	0	0	0	0	0	0	0	0
73	790.00	0	0	0	0	1	2	0	0	0	0	0	0	0
74	1132.00	0	0	0	0	0	0	0	0	0	0	0	0	0

Appendix 1 HRV Patient Database

Study Number	Current Weight	On abx	Suspected sepsis	Confirmed Sepsis	Subsequent sepsis (48 hr)	IVH Grade	IVH subsequently	Other Brain abn	PVL	PVL subsequent	Other Brain abn subsequent	Dopamine	Dobutamine	Inotropes in next 48 hours
75	1830.00	1	1	0	0	0	0	0	0	0	0	0	0	0
76	910.00	0	0	0	0	1	0	0	0	0	0	0	0	0
77	1095.00	0	0	0	0	0	0	0	0	0	0	0	0	0
84	1051.00	0	0	0	0	0	0	0	0	0	0	0	0	0
85	910.00	1	0	1	0	1	1	0	0	0	0	0	0	0
86	1190.00	0	0	0	0	0	0	0	0	0	0	0	0	0
87	1250.00	0	0	0	0	0	0	0	0	0	0	0	0	0
88	3220.00	1	0	0	0	0	0	1	0	0	0	0	0	0
99		1	0	0	0	0	1	0	0	1	0	0	0	0
109	1640.00	0	0	0	0	0	0	0	0	0	0	0	0	0
110	1425.00	0	0	0	0	0	0	0	0	0	0	0	0	0
111	910.00	0	0	0	0	1	1	0	0	0	0	0	0	0
112	1000.00	0	0	0	0	1	1	0	0	0	0	0	0	0
113	1720.00	0	0	0	0	0	0	0	0	0	0	0	0	0
114	1735.00	0	0	0	0	0	0	0	0	0	0	0	0	0
117	780.00	0	0	0	0	0	0	0	0	0	0	0	0	0
118	845.00	0	0	0	0	0	0	0	0	0	0	0	0	0
119	860.00	0	0	0	0	0	0	0	0	0	0	0	0	0
120	1690.00	0	0	0	0	0	0	0	0	0	0	0	0	0
121	1770.00	0	0	0	0	0	0	0	0	0	0	0	0	0
122	1461.00	0	0	0	0	1	4	1	0	0	0	0	0	0
123	1065.00	0	0	0	0	1	1	0	0	0	0	0	0	0
124	1125.00	0	0	0	0	0	0	0	0	0	0	0	0	0
125	760.00	1	0	1	0	0	0	0	0	0	0	0	0	0
126	1740.00	1	1	0	0	0	0	0	0	0	0	0	0	0
127	1130.00	1	0	0	0	0	0	0	0	0	0	0	0	0
129	1464.00	0	0	0	0	0	0	0	0	0	0	0	0	0
130	1464.00	0	0	0	0	0	0	0	0	0	0	0	0	0
131	1760.00	0	0	0	0	0	0	0	0	0	0	0	0	0
133	757.00	0	0	0	0	2	0	0	0	0	0	0	0	0
134	2060.00	0	0	0	0	0	0		0	0	0	0	0	0

Appendix 1 HRV Patient Database

Study Number	Current Weight	On abx	Suspected sepsis	Confirmed Sepsis	Subsequent sepsis (48 hr)	IVH Grade	IVH subsequently	Other Brain abn	PVL	PVL subsequent	Other Brain abn subsequent	Dopamine	Dobutamine	Inotropes in next 48 hours
135	2085.00	0	0	0	0	0	0	0	0	0	0	0	0	0
136	680.00	0	1	0	0	2	2	0	0	0	0	0	0	0
137	1132.00	1	0	1	0	3	3	0	0	0	0	0	0	0
138	1330.00	0	0	0	0	0	0	0	0	0	0	0	0	0
139	1335.00	0	0	0	0	1	1	0	0	0	0	0	0	0
140	1790.00	0	0	0	0	1	1	0	0	0	0	0	0	0
141	1112.00	1	1	0	0	0	0	0	0	0	0	0	0	0
142	1210.00	1	1	0	0	0	0	0	0	0	0	0	0	0
143	860.00	0	0	0	0	0	0	0	0	0	0	0	0	0
144	2240.00	1	0	0	0	0	0	0	0	0	0	0	0	0
145	1562.00	0	0	0	0	0	0	0	0	0	0	0	0	0
146	1072.00	0	0	0	0	0	0	0	0	0	0	0	0	0
147	640.00	1	0	1	0	4	4	1	0	0	1	0	0	0
149	1134.00	0	0	0	0	1	1	0	1	1	0	0	0	0
150	1134.00	0	0	0	0	1	1	0	1	1	0	0	0	0
152	810.00	0	0	0	0	1	1	0	0	0	0	0	0	0
153	1840.00	0	0	0	0	0	0	0	0	0	0	0	0	0
156	2711.00	0	0	0	0	0	0	0	0	0	0	0	0	0
158	1310.00	0	0	0	0	0	0	0	0	0	0	0	0	0
160	1030.00	0	0	0	1	0	0	0	0	0	0	0	0	0
162	1132.00	0	0	0	0	0	0	0	0	0	0	0	0	0
164	1464.00	0	0	0	0	0	0	0	0	0	0	0	0	0
166	2870.00	1	1	0	0	0	0	0	0	0	0	0	0	0
167	1640.00	0	0	0	0	0	0	0	0	0	0	0	0	0
168	1230.00	0	0	0	0	0	0	0	0	0	0	0	0	0
169	1185.00	0	0	0	0	2	0	0	0	0	0	0	0	0
170	2380.00	1	1	0	0	0	0	0	0	0	0	0	0	0
171	2245.00	0	0	0	0	0	0	0	0	0	0	0	0	0
172	4620.00	0	0	0	0	0	0	0	0	0	0	0	0	0
173	1560.00	1	0	0	0	0	0	0	0	0	0	0	0	0
174	1020.00	0	0	0	0	0	0	0	0	0	0	0	0	0
175	1046.00	1	0	1	0	0	0	0	0	0	0	0	0	0

Appendix 1 HRV Patient Database

Study Number	Current Weight	On abx	Suspected sepsis	Confirmed Sepsis	Subsequent sepsis (48 hr)	IVH Grade	IVH subsequently	Other Brain abn	PVL	PVL subsequent	Other Brain abn subsequent	Dopamine	Dobutamine	Inotropes in next 48 hours
176	1510.00	0	0	0	0	2	2	0	0	0	0	0	0	0
177	1482.00	0	0	0	0	1	0	0	1	1	0	0	0	0
178	1380.00	0	0	0	0	0	0	0	0	0	0	0	0	0
179	1380.00	0	0	0	0	0	0	0	0	0	0	0	0	0
181	1250.00	1	0	0	0	0	0	0	0	0	0	0	0	0
182	1205.00	0	0	0	0	0	0	0	0	0	0	0	0	0
183	690.00	1	0	1	0	1	1	0	0	0	0	0	0	0
184	700.00	0	0	0	0	1	1	0	0	0	0	0	0	0
185	2345.00	0	0	0	0	0	0	0	0	0	0	0	0	0
186	1300.00	0	0	0	0	0	0	0	0	0	0	0	0	0
187	2046.00	0	0	0	0	2	0	0	0	0	0	0	0	0
188	2330.00	0	0	0	0	0	0	0	0	0	0	0	0	0
189	1160.00	0	0	0	0	3	3	0	0	0	0	0	0	0
190	1290.00	0	0	0	0	0	0	0	0	0	0	0	0	0
191	940.00	0	0	0	0	1	1	0	0	0	0	0	0	0
192	1330.00	0	0	0	1	2	3	0	0	1	0	1	0	0
193	970.00	0	0	0	0	0	0	0	0	0	0	0	0	0
194	1030.00	1	0	1	0	2	3	0	0	0	0	0	0	0
195	1360.00	1	0	0	0	0	0	0	0	0	0	0	0	0
196	1795.00	1	0	0	0	0	0	0	0	0	0	0	0	0
197	2738.00	0	0	0	0	2	2	0	0	0	0	0	0	0
198	932.00	1	0	0	0	1	3	0	0	0	0	0	0	0
199	1100.00	1	0	0	0	0	0	0	0	0	0	0	0	0
200	997.00	0	0	0	0	0	1	0	0	0	0	0	0	0
201	1245.00	0	0	0	0	3	3	0	0	0	0	0	0	0
202	1070.00	0	0	0	0	0	0	0	0	0	0	0	0	0
206	1822.00	0	0	0	0	3	3	0	0	0	0	0	0	0
207	1900.00	1	0	0	0	0	0	0	0	0	0	0	0	0
208	1620.00	1	0	0	0	0	0	0	0	0	0	0	0	0
209		1	1	0	0	0	0	0	0	0	0	0	0	0
210		1	0	0	0	0	0	0	0	0	0	0	0	0
211	0.00	0	0	0	0	1	1	0	0	1	0	0	0	0
212	0.00	1	0	1	0	1	1	0	0	1	0	0	0	0
213	0.00	1	0	1	0	1	1	0	0	1	0	0	0	0
214	0.00	1	0	1	0	1	1	0	0	1	0	0	0	0

Appendix 1 HRV Patient Database

Study Number	Current Weight	On abx	Suspected sepsis	Confirmed Sepsis	Subsequent sepsis (48 hr)	IVH Grade	IVH subsequently	Other Brain abn	PVL	PVL subsequent	Other Brain abn subsequent	Dopamine	Dobutamine	Inotropes in next 48 hours
215		1	0	0	0	0	0	0	0	0	0	0	0	0
216		0	0	0	0	0	0	0	0	0	0	0	0	0
217		0	0	0	0	0	0	0	0	0	0	0	0	0
218		0	0	0	0	0	0	0	0	0	0	0	0	0
219		0	0	0	0	0	0	0	0	0	0	0	0	0
220	0.00	0	0	0	0	1	0	0	0	0	0	0	0	0
221	0.00	0	0	0	1	1	0	0	0	0	0	0	0	0
222	0.00	1	1	0	1	1	0	0	0	0	0	0	0	0
223	0.00	0	0	0	0	1	0	0	0	0	0	0	0	0
224	0.00	0	0	0	0	0	0	0	0	0	0	0	0	0
225	0.00	0	0	0	0	0	0	0	0	0	0	0	0	0
226	0.00	0	0	0	0	0	0	0	0	0	0	0	0	0
227	0.00	0	0	0	0	0	0	0	0	0	0	0	0	0
228		1	0	0	0	0	3	0	0	1	0	0	0	0
229		0	0	0	0	3	3	0	0	1	0	0	0	0
230		0	0	0	0	3	3	0	0	1	0	0	0	0
231		0	0	0	0	3	3	0	0	1	0	0	0	0
232		1	0	0	0	3	3	0	0	1	0	0	0	0
233		1	0	1	0	3	3	0	0	1	0	0	0	0
234		0	0	0	1	0	0	0	0	0	0	0	0	0
235		1	1	0	0	0	0	0	0	0	0	0	0	0
236		0	0	0	0	0	0	0	0	0	0	0	0	0
237		0	0	0	0	1	1	0	0	0	0	0	0	0
238		1	0	1	0	1	1	0	0	0	0	0	0	0

Appendix 1 HRV Patient Database

Study Number	Current Weight	On abx	Suspected sepsis	Confirmed Sepsis	Subsequent sepsis (48 hr)	IVH Grade	IVH subsequently	Other Brain abn	PVL	PVL subsequent	Other Brain abn subsequent	Dopamine	Dobutamine	Inotropes in next 48 hours
239		0	0	0	0	1	1	0	0	0	0	0	0	0
240		1	0	1	0	1	1	0	0	0	0	0	0	0
241	0.00	0	0	0	0	1	0	0	0	0	0	0	0	0
242	0.00	0	0	0	0	0	2	0	0	0	0	0	0	0
243	0.00	0	0	0	0	0	2	0	0	0	0	0	0	0
244		1	1	0	0	0	0	0	0	0	0	0	0	0
245		1	0	0	0	0	0	0	0	0	0	0	0	0
246		1	0	1	0	0	0	0	0	0	0	0	0	0
247		1	0	1	0	0	0	0	0	0	0	0	0	0
248		1	0	1	0	0	0	0	0	0	0	0	0	0
249		0	0	0	0	0	0	0	0	0	0	0	0	0
250		1	0	0	0	0	0	0	0	0	0	0	0	0
251		0	0	0	0	0	0	0	0	0	0	0	0	0
252		0	0	0	0	0	0	0	0	0	0	0	0	0
253		1	0	1	0	0	0	0	0	0	0	0	0	0
254		1	0	1	0	0	0	0	0	0	0	0	0	0
255		1	0	1	0	0	0	0	0	0	0	0	0	0
256		1	0	0	0	0	0	0	0	0	0	0	0	0
257		1	0	1	0	0	0	0	0	0	0	0	0	0
258		1	0	1	0	0	0	0	0	0	0	0	0	0
259		1	0	1	0	0	0	0	0	0	0	0	0	0
260		0	0	0	0	0	0	0	0	0	0	0	0	0
261		0	0	0	0	0	0	0	0	0	0	0	0	0

Appendix 1 HRV Patient Database

Study Number	Current Weight	On abx	Suspected sepsis	Confirmed Sepsis	Subsequent sepsis (48 hr)	IVH Grade	IVH subsequently	Other Brain abn	PVL	PVL subsequent	Other Brain abn subsequent	Dopamine	Dobutamine	Inotropes in next 48 hours	
262		0	0	0	0	0	0	0	0	0	0	0	0	0	
263		0	0	0	0	0	0	0	0	0	0	0	0	0	
264		0	0	0	0	1	1	0	0	1	0	0	0	0	
265		0	0	0	0	1	1	0	0	1	0	0	0	0	
266		0	0	0	0	1	1	0	0	1	0	0	0	0	
267		1	0	0	0	0	0	0	0	0	0	0	0	0	
268		1	0	0	0	0	0	0	0	0	0	0	0	0	
269		1	0	0	0	0	0	0	0	0	0	0	0	0	
270		1	0	0	0	0	0	0	0	0	0	0	0	0	
271		1	0	0	0	0	0	0	0	0	0	0	0	0	
272		0	0	0	0	0	0	0	0	0	0	0	0	0	
273		0	0	0	0	3	3	0	0	0	0	0	0	0	
274		0	0	0	0	3	3	0	0	0	0	0	0	0	
275		0	0	0	0	3	3	0	0	0	0	0	0	0	
276		0	0	0	1	3	3	0	0	0	0	0	0	0	
277		1	1	0	0	3	3	0	0	0	0	0	0	0	
278		1	1	0	0	3	3	0	0	0	0	0	0	0	
279		0	0	0	0	3	3	0	0	0	0	0	0	0	
280		1	1	0	0	3	3	0	0	0	0	0	0	0	
281		0	0	0	0	0	0	0	0	0	0	0	0	0	
282		1	1	0	0	2	2	0	0	0	0	1	0	0	
283		0	0	0	0	0	0	0	0	0	0	0	0	0	
284		1	0	0	0	0	0	0	0	0	0	0	0	0	
285		1	0	0	0	0	0	0	0	0	0	0	0	0	
286		1	0	0	0	0	0	0	0	0	0	0	0	0	
287		0	0	0	0	0	0	0	0	0	0	0	0	0	
288	0.00	0	0	0	0	0	0	0	0	0	0	0	0	0	
289	0.00	0	0	0	0	0	0	0	0	0	0	0	0	0	

Appendix 1 HRV Patient Database

Study Number	Current Weight	On abx	Suspected sepsis	Confirmed Sepsis	Subsequent sepsis (48 hr)	IVH Grade	IVH subsequently	Other Brain abn	PVL	PVL subsequent	Other Brain abn subsequent	Dopamine	Dobutamine	Inotropes in next 48 hours	
290	0.00	0	0	0	0	0	0	0	0	0	0	0	0	0	
291	0.00	0	0	0	0	0	3	0	0	1	0	0	0	0	
292		0	0	0	0	0	3	0	0	1	0	0	0	0	
293		0	0	0	0	0	3	0	0	1	0	0	0	0	
294		0	0	0	0	0	3	0	1	1	0	0	0	0	
295		0	0	0	0	0	3	0	1	1	0	0	0	0	
296		0	0	0	0	0	3	0	1	1	0	0	0	0	
297		1	0	1	0	0	0	0	0	0	0	0	0	0	
298		1	0	0	0	0	0	0	0	0	0	0	0	0	
299		1	0	0	0	0	0	0	0	0	0	0	0	0	
300		1	0	0	0	0	0	0	0	0	0	0	0	0	
301		0	0	0	0	0	0	0	0	0	0	0	0	0	
302		1	0	0	0	0	0	0	0	0	0	0	0	0	
303		0	0	0	0	0	0	0	0	0	0	0	0	0	
305		1	1	0	0	0	0	0	0	0	0	0	0	0	
306		0	0	0	0	0	0	0	0	0	0	0	0	0	
307		1	1	0	0	0	0	0	0	0	0	0	0	0	
313	0.00	1	0	0	0	0	0	0	0	0	0	0	0	0	
314	0.00	1	0	0	0	0	0	0	0	0	0	0	0	0	
315		1	0	0	0	0	0	0	0	0	0	0	0	0	
316		1	0	0	0	0	0	0	0	0	0	0	0	0	
24	880.00	0	0	0	0	0	0	0	0	0	0	0	0	0	
26	1060.00	0	0	0	0	1	0	0	0	0	0	0	0	0	
27	2150.00	1	0	0	0	0	0	0	0	0	0	0	0	0	
28	1312.00	0	0	0	0	0	0	0	0	0	0	0	0	0	
29	980.00	0	0	0	0	0	0	0	0	0	0	0	0	0	
30	2245.00	1	0	0	0	0	0	0	0	0	0	0	0	0	
31	820.00	0	0	0	0	0	0	0	0	0	0	0	0	0	
32	3350.00	1	0	0	0	0	0	0	0	0	0	0	0	0	
33	3120.00	1	0	0	0	0	0	0	0	0	0	0	0	0	
34	980.00	0	0	0	0	0	0	0	0	0	0	0	0	0	
35	1000.00	1	1	0	0	1	0	0	0	0	0	0	0	0	
36	1060.00	0	0	0	0	1	0	0	0	0	0	0	0	0	
37	1000.00	1	1	0	0	1	0	0	0	0	0	0	0	0	

Appendix 1 HRV Patient Database

Study Number	Current Weight	On abx	Suspected sepsis	Confirmed Sepsis	Subsequent sepsis (48 hr)	IVH Grade	IVH subsequently	Other Brain abn	PVL	PVL subsequent	Other Brain abn subsequent	Dopamine	Dobutamine	Inotropes in next 48 hours
38	955.00	1	1	0	0	4	4	0	0	0	0	0	0	0
39	530.00	1	0	0	0	0	0	0	0	0	0	0	0	0
40	2225.00	0	0	0	0	0	0	0	0	0	0	0	0	0
41	530.00	0	0	0	0	0	0	0	0	0	0	0	0	0
42	955.00	1	1	0	0	4	4	0	0	0	0	0	0	0
43	1380.00	1	0	0	0	0	0	0	0	0	0	0	0	0
44	925.00	0	0	0	0	0	0	1	0	0	0	0	0	0
45	2390.00	0	0	0	0	0	0	0	0	0	0	0	0	0
46	580.00	0	0	0	0	0	0	0	0	0	0	0	0	0
47	1200.00	1	0	0	0	0	0	0	0	0	0	0	0	0
48	1592.00	0	0	0	0	1	0	0	0	0	0	0	0	0
49	1390.00	0	0	0	0	0	0	0	0	0	0	0	0	0
50	870.00	0	0	0	0	0	0	0	0	0	0	0	0	0
59	1290.00	1	0	0	0	0	3	0	0	0	0	0	0	0
60	870.00	0	0	0	0	0	0	0	0	0	0	0	0	0
61	2215.00	0	0	0	0	0	0	0	0	0	0	0	0	0
62	1930.00	0	0	0	0	1	0	0	0	0	0	0	0	0
63	0.00	0	0	0	0	0	0	0	0	0	0	0	0	0
64	1200.00	0	0	0	0	0	0	0	0	0	0	0	0	0
65	1380.00	0	0	0	0	0	0	0	0	0	0	0	0	0
66	700.00	1	0	1	0	1	1	0	0	0	0	0	0	0
67	1100.00	0	0	0	0	0	0	0	0	0	0	0	0	0
68	790.00	0	0	0	0	1	2	0	0	0	0	0	0	0
69	1132.00	0	0	0	0	0	0	0	0	0	0	0	0	0
70	910.00	0	0	0	0	1	0	0	0	0	0	0	0	0
78	910.00	1	0	1	0	1	1	0	0	0	0	0	0	0
79	1190.00	0	0	0	0	0	0	0	0	0	0	0	0	0
80	860.00	0	0	0	0	1	2	0	0	0	0	0	0	0
81	1250.00	0	0	0	0	0	0	0	0	0	0	0	0	0

Appendix 1 HRV Patient Database

Study Number	Current Weight	On abx	Suspected sepsis	Confirmed Sepsis	Subsequent sepsis (48 hr)	IVH Grade	IVH subsequently	Other Brain abn	PVL	PVL subsequent	Other Brain abn subsequent	Dopamine	Dobutamine	Inotropes in next 48 hours
82	3220.00	1	0	0	0	0	0	1	0	0	0	0	0	0
83	1051.00	0	0	0	0	0	0	0	0	0	0	0	0	0
89	1035.00	1	1	0	0	1	1	0	0	0	0	0	0	0
90	1020.00	0	0	0	0	1	1	0	0	0	0	0	0	0
91	1070.00	0	0	0	0	0	0	0	0	0	0	0	0	0
92	1534.00	0	0	0	0	0	0	0	0	0	0	0	0	0
93	2800.00	0	0	0	0	0	0	0	0	0	0	0	0	0
94	2090.00	0	0	0	0	0	0	0	0	0	0	0	0	0
95	2880.00	0	0	0	0	0	0	0	0	0	0	0	0	0
96	760.00	0	0	0	0	0	1	0	0	0	0	0	0	0
97	1090.00	1	0	1	0	0	0	0	0	0	0	0	0	0
98	1050.00	0	0	0	0	1	1	0	0	0	0	0	0	0
100	1040.00	0	0	0	0	0	0	0	0	0	0	0	0	0
101	550.00	1	0	0	0	0	1	0	0	1	0	0	0	0
102	860.00	0	0	0	0	1	1	0	0	0	0	0	0	0
103	1650.00	0	0	0	0	0	0	0	0	0	0	0	0	0
104	1390.00	0	0	0	0	0	0	0	0	0	0	0	0	0
105	640.00	0	0	0	0	0	2	0	0	0	0	0	0	0
106	1460.00	0	0	0	0	0	0	0	0	0	0	0	0	0
107	1155.00	0	0	0	0	1	1	0	0	0	0	0	0	0
108	1640.00	0	0	0	0	0	0	0	0	0	0	0	0	0
115	1735.00	0	0	0	0	0	0	0	0	0	0	0	0	0
116	1720.00	0	0	0	0	0	0	0	0	0	0	0	0	0
128	1464.00	0	0	0	0	0	0	0	0	0	0	0	0	0
132	757.00	0	0	0	0	2	0	0	0	0	0	0	0	0
148	1134.00	0	0	0	0	1	1	0	1	1	0	0	0	0
151	810.00	0	0	0	0	1	1	0	0	0	0	0	0	0
154	1840.00	0	0	0	0	0	0	0	0	0	0	0	0	0
155	2711.00	0	0	0	0	0	0	0	0	0	0	0	0	0
157	1310.00	0	0	0	0	0	0	0	0	0	0	0	0	0
159	1030.00	0	0	0	1	0	0	0	0	0	0	0	0	0
161	1132.00	0	0	0	0	0	0	0	0	0	0	0	0	0
163	1464.00	0	0	0	0	0	0	0	0	0	0	0	0	0

Appendix 1 HRV Patient Database

Study Number	Current Weight	On abx	Suspected sepsis	Confirmed Sepsis	Subsequent sepsis (48 hr)	IVH Grade	IVH subsequently	Other Brain abn	PVL	PVL subsequent	Other Brain abn subsequent	Dopamine	Dobutamine	Inotropes in next 48 hours
165	2870.00	1	1	0	0	0	0	0	0	0	0	0	0	0
180	1205.00	0	0	0	0	0	0	0	0	0	0	0	0	0
203		1	1	0	0	0	0	0	0	0	0	0	0	0
204		1	1	0	0	0	0	0	0	0	0	0	0	0
205	1822.00	0	0	0	0	3	3	0	0	0	0	0	0	0
304		1	0	0	0	0	0	0	0	0	0	0	0	0
308		0	0	0	0	0	0	0	0	0	0	0	0	0
309		1	0	0	0	0	0	0	0	0	0	0	0	0
310		0	0	0	0	1	0	0	0	0	0	0	0	0
311		0	0	0	0	0	0	0	0	0	0	0	0	0
312		1	1	0	0	1	2	0	0	0	0	0	0	0

Appendix 1 HRV Patient Database

Study Number	PDA suspected	PDA confimred	PDA subsequen	Morphine	Caffeine	Midazolam	Phenobarbitone	NBM	NGT	Bradycardia/Cluster	Died	CLD	ROP	NEC	
1	0	0	0	0	1	0	0	0	1	0		1	0	0	0
3	0	0	0	0	1	0	0	0	1	0		0	0	0	0
4	1	0	1	0	1	0	0	0	1	0		0	0	0	0
5	0	0	0	0	0	0	0	0	1	0		0	0	0	0
6	0	0	0	0	1	0	0	0	1	0		0	0	0	0
7	0	0	0	0	1	0	0	0	1	0		0	0	0	0
8	0	0	0	0	0	0	0	0	1	1		0	1	3	0
9	0	0	0	0	0	0	0	0	0	1		0	0	0	0
10	0	1	0	0	1	0	0	0	1	0		0	1	0	0
11	0	0	0	0	1	0	0	0	1	0		0	0	3	0
12	0	0	0	0	0	0	0	0	1	0		0	0	0	0
13	0	0	0	0	1	0	0	0	1	0		0	0	0	0
14	0	0	0	0	1	0	0	0	1	0		0	0	0	0
15	0	0	0	0	0	0	0	1	0	0		0	0	0	0
16	0	0	0	0	1	0	0	0	1	0		0	0	0	0
17	0	0	1	0	1	0	0	0	1	0		0	1	2	0
18	0	0	0	0	1	0	0	0	1	0		0	0	0	0
19	0	0	0	0	0	0	0	0	1	0		0	0	0	0
20	0	0	0	0	1	0	0	0	1	0		0	0	0	0
21	0	0	0	0	1	0	0	0	1	0		0	0	0	0
22	0	0	1	0	1	0	0	1	0	0		0	0	0	0
23	0	0	0	1	0	0	0	1	0	0		1	0	0	0
25	0	0	1	1	0	0	0	1	0	0		0	1	3	1
51	0	0	0	0	1	0	0	1	0	0		1	0	0	0
52	0	0	0	0	1	0	0	0	1	0		0	0	0	0
53	0	0	0	0	1	0	0	0	1	0		0	1	2	0
54	0	0	0	0	0	0	0	0	1	0		0	0	0	0
55	0	0	0	0	1	0	0	0	1	0		0	1	0	0
56	0	0	0	0	1	0	0	0	1	0		0	0	0	0
57	0	0	0	0	1	0	0	0	0	1		0	0	0	0
58	0	0	0	0	0	0	0	0	1	0		0	0	0	0
71	0	0	1	0	1	0	0	0	1	0		0	0	0	0
72	0	0	1	0	1	0	0	0	1	0		0	disch in 02 at 321 weeks	0	0
73	0	0	0	0	1	0	0	0	1	0	disch at31 weeks		disch at31 weeks	disch at31 weeks	disch at31 weeks
74	0	0	0	0	1	0	0	0	1	0		0	1	2	0

Appendix 1 HRV Patient Database

Study Number	PDA suspected	PDA confimred	PDA subsequen	Morphine	Caffeine	Midazolam	Phenobarbitone	NBM	NGT	Breast/Bottle /Cup	Died	CLD	ROP	NEC
75	0	0	0	0	0	0	0	1	0	0	0	0	0	0
76	0	0	0	0	1	0	0	1	0	0	0	1	0	0
77	0	0	0	0	1	0	0	0	1	0	0	0	3	0
84	0	0	0	0	1	0	0	0	1	0	0	1	0	0
85	0	0	0	1	0	0	0	0	1	0	0	disch at 31 weeks	disch at 31 weeks	disch at 31 weeks
86	1	0	1	0	1	0	0	0	1	0	0	disch in 02 at 321 weeks	0	0
87	0	0	0	0	1	0	0	0	1	0	0	1	2	0
88	0	0	0	0	0	0	1	0	0	1	0	0	0	0
99	0	0	0	1	0	0	0	1	0	0	0	1	3	0
109	0	0	0	0	0	0	0	0	1	0	0	1		0
110	0	0	0	0	1	0	0	0	1	0	0	1	0	0
111	0	0	1	0	1	0	0	0	1	0	0	1		0
112	0	1	1	0	1	0	0	0	1	0	0	1		0
113	0	0	0	0	1	0	0	0	1	0	0	0	0	0
114	0	0	0	0	1	0	0	0	1	1	0	1		0
117	0	0	0	0	1	0	0	0	1	0	0	disch at 32 weeks in 02	0	0
118	0	0	0	0	1	0	0	0	1	0	0	disch at 32 weeks in oxy	0	0
119	0	0	0	0	1	0	0	0	1	0	0	0	0	0
120	0	0	0	0	0	0	0	0	1	1	0	1		0
121	0	0	0	0	1	0	0	0	1	0	0	1	0	0
122	0	0	0	0	1	0	0	0	1	0	0	0	0	0
123	0	1	1	0	1	0	0	0	1	0	0	1		0
124	0	1	1	0	1	0	0	0	1	0	0	1		0
125	0	0	0	0	1	0	0	0	1	0	0	1	0	0
126	0	0	0	0	1	0	0	1	0	0	0	0	0	0
127	0	0	0	0	0	0	0	1	0	0	0	0	0	0
129	0	0	0	0	0	0	0	1	0	0	0	0	0	0
130	0	0	0	0	0	0	0	1	0	0	0	0	0	0
131	0	0	0	0	0	0	0	0	1	0	0	0	0	0
133	0	0	0	0	0	0	0	0	1	0	0	1	2	0
134	0	0	0	0	1	0	0	0	1	1	0	0	0	0

Appendix 1 HRV Patient Database

Study Number	PDA suspected	PDA confimred	PDA subsequen	Morphine	Caffeine	Midazolam	Phenobarbitone	DAM	NGT	Breast/Bottle /Cup	Died	CLD	ROP	NEC	
135	0	0	0	0	0	0	0	0	1	1	0	1			0
136	0	0	0	0	1	0	0	1	0	0	0	1	0		0
137	0	0	1	0	1	0	0	0	1	0	0	0	0		0
138	0	0	1	0	1	0	0	0	1	0	0	1			0
139	0	0	1	0	1	0	0	0	1	0	0	1			0
140	0	0	1	0	1	0	0	0	1	1	0	1			0
141	0	0	0	0	1	0	0	0	1	0	0	0	0		0
142	0	0	0	0	1	0	0	1	0	0	0	0	0		0
143	0	0	0	0	1	0	0	0	1	0	0	1	0		0
144	0	0	0	0	0	0	0	1	0	0	0	0	0		0
145	0	0	0	0	0	0	0	0	1	0	0	0	0		0
146	0	0	0	0	0	0	0	0	1	0	0	0	0		0
147	0	1	1	1	0	0	0	0	1	0	1	0	0		0
149	0	0	0	0	1	0	0	0	1	0	0	1	3		0
150	0	0	0	0	0	0	0	0	1	0	0	1	3		0
152	0	0	0	0	1	0	0	0	1	0	0	disch at 31 weeks in 03	0		0
153	0	1 -not sig nov	1	0	1	0	0	0	1	1	0	1			0
156	0	0	0	0	0	0	0	0	1	1	0	1			0
158	0	0	0	0	1	0	0	0	1	0	0	0	0		0
160	0	0	1	0	1	0	0	0	1	0	0	1	0		0
162	0	0	0	0	0	0	0	0	1	0	0	0	0		0
164	0	0	0	0	0	0	0	0	1	1	0	0	0		0
166	0	0	0	0	0	0	0	0	0	1	0	0	0		0
167	0	0	0	0	0	0	0	0	1	1	0	0	0		0
168	0	0	0	0	1	0	0	0	1	0	0	1	0		0
169	0	0	0	0	1	0	0	0	1	0	0	1	2		0
170	0	0	0	0	0	0	0	0	1	0	0	0	0		0
171	0	0	0	0	0	0	0	0	1	0	0	0	0		0
172	0	0	0	0	0	0	0	1	0	0	0	0	0		0
173	0	0	0	0	1	0	0	0	1	0	0	0	0		0
174	0	0	0	1	0	0	0	0	1	0	0	disch at 31 weeks in 04	0		0
175	0	0	0	0	0	0	0	0	1	0	0	disch at 31 weeks in 05	0		0

Appendix 1 HRV Patient Database

Study Number	PDA suspected	PDA confimred	PDA subsequen	Morphine	Caffeine	Midazolam	Phenobarbitone	NBM	NGT	Breast/Bottle/Cup	Died	CLD	ROP	NEC
176	0	0	0	0	1	0	0	0	1	0	0	1	0	0
177	0	0	0	0	0	0	0	0	1	0	0	1	3	0
178	0	0	0	0	1	0	0	0	1	0	0	0	0	0
179	0	0	0	1	1	0	0	1	0	0	0	0	0	0
181	1	0	0	1	0	0	0	1	0	0	0	0	0	0
182	0	0	0	0	1	0	0	0	1	0	0	0	0	0
183	1	0	1	0	0	0	0	0	1	0	1	0	0	1
184	1	0	1	0	1	0	0	0	1	0	1	0	0	1
185	0	0	0	0	0	0	0	0	1	1	0	0	0	0
186	1	1	1	0	1	0	0	0	1	0	0	0	0	0
187	0	0	0	0	0	0	0	0	1	0	0	1	2	0
188	0	0	0	0	0	0	0	0	1	0	0	0	0	0
189	0	0	1	0	1	0	0	0	1	0	0	1	0	0
190	0	0	0	0	1	0	0	0	1	0	0	0	0	0
191	0	1	1	0	1	0	0	0	1	0	1	0	0	1
192	0	0	0	1	0	1	0	1	0	0	1	0	0	0
193	1	0	1	0	1	0	0	0	1	0	0	1	0	0
194	1	0	0	1	0	1	0	1	0	0	1	0	0	1
195	0	0	0	0	0	0	0	1	0	0	0	0	0	0
196	0	0	0	0	0	0	0	0	1	0	0	0	0	0
197	0	0	0	0	0	0	0	0	1	0	0	1	2	0
198	0	0	1	1	0	0	0	0	1	0	0	1	0	0
199	0	0	0	0	1	0	0	0	1	0	0	0	0	0
200	0	0	0	0	1	0	0	0	1	0	0	0	1	0
201	1	1	1	0	1	0	0	0	1	0	0	1	0	0
202	0	0	0	0	1	0	0	1	0	0	0	0	0	0
206	0	1 not sig	0	0	1	0	0	0	1	0	0	1	0	0
207	0	0	0	0	0	0	0	0	1	0	0	0	0	0
208	0	0	0	0	0	0	0	0	1	0	0	0	0	0
209	0	0	0	0	0	0	0	0	1	0	0	0	0	0
210	0	0	0	0	0	0	0	0	1	1	0	0	0	0
211	0	1	1	1	0	0	0	0	1	0	0	1	3	0
212	0	1	1	1	0	0	0	0	1	0	0	1	3	0
213	0	1	1	0	1	0	0	0	1	0	0	1	3	0
214	0	1	1	1	0	0	0	0	1	0	0	1	3	0

Appendix 1 HRV Patient Database

Study Number	PDA suspected	PDA confimred	PDA subsequen	Morphine	Caffeine	Midazolam	Phenobarbitone	Nasal	NCPAP	Breastfeeding plus	Died	CLD	ROP	NEC
215	0	0	0	0	1	0	0	1	1	0	transfer in o2 at 32 weeks	transfer in o2 at 32 weeks	transfer in o2 at 32 weeks	transfer in o2 at 32 weeks
216	0	0	0	0	1	0	0	0	1	0	transfer in o2 at 32 weeks	transfer in o2 at 32 weeks	transfer in o2 at 32 weeks	transfer in o2 at 32 weeks
217	0	0	0	0	1	0	0	0	1	0	transfer in o2 at 32 weeks	transfer in o2 at 32 weeks	transfer in o2 at 32 weeks	transfer in o2 at 32 weeks
218	0	0	0	0	1	0	0	0	1	0	transfer in o2 at 32 weeks	transfer in o2 at 32 weeks	transfer in o2 at 32 weeks	transfer in o2 at 32 weeks
219	0	0	0	0	1	0	0	0	1	0	transfer in o2 at 32 weeks	transfer in o2 at 32 weeks	transfer in o2 at 32 weeks	transfer in o2 at 32 weeks
220	0	0	0	0	1	0	0	0	1	0	0	0	0	0
221	0	0	0	0	0	0	0	0	1	0	0	0	0	0
222	0	0	0	0	0	0	0	0	1	0	0	0	0	0
223	0	0	0	0	0	0	0	0	1	0	0	0	0	0
224	0	0	0	0	0	0	0	0	1	0	0	0	0	0
225	0	0	0	0	0	0	0	0	1	0	0	0	0	0
226	0	0	0	0	0	0	0	0	1	0	0	0	0	0
227	0	0	0	0	0	0	0	0	1	0	0	0	0	0
228	0	0	1	0	1	0	0	1	0	0	0	1	1	0
229	1	0	1	0	1	0	0	0	1	0	0	1	1	0
230	1	1	1	0	1	0	0	0	1	0	0	1	1	0
231	1	1	1	0	1	0	0	0	1	0	0	1	1	0
232	1	1	1	1	0	0	0	0	1	0	0	1	1	0
233	1	1	1	1	0	1	0	0	1	0	0	1	1	0
234	0	0	0	0	1	0	0	0	1	0	0	0	0	0
235	0	0	0	0	1	0	0	0	1	0	0	0	0	0
236	0	0	0	0	0	0	0	0	1	0	0	0	0	0
237	0	0	0	0	1	0	0	0	1	0	transfer in o2 at 32 weeks	transfer in o2 at 32 weeks	transfer in o2 at 32 weeks	transfer in o2 at 32 weeks
238	0	0	0	1	0	0	0	0	1	0	transfer in o2 at 32 weeks	transfer in o2 at 32 weeks	transfer in o2 at 32 weeks	transfer in o2 at 32 weeks

Appendix 1 HRV Patient Database

Study Number	PDA suspected	PDA confimred	PDA subsequen	Morphine	Caffeine	Midazolam	Phenobarbitone	NBM	NGT	Breast/Bottle/Gavage	Died	CLD	ROP	NEC
239	0	0	0	0	1	0	0	0	1	0	transfer in o2 at 32 weeks	transfer in o2 at 32 weeks	transfer in o2 at 32 weeks	transfer in o2 at 32 weeks
240	0	0	0	0	0	0	0	0	1	0	transfer in o2 at 32 weeks	transfer in o2 at 32 weeks	transfer in o2 at 32 weeks	transfer in o2 at 32 weeks
241	0	0	0	0	0	0	0	0	1	0	0	0	0	0
242	0	0	0	0	1	0	0	0	1	0	13/09/2007	0	0	0
243	0	0	0	1	1	1	0	0	1	0	13/09/2007	0	0	0
244	0	0	0	0	0	0	0	0	0	1	0	0	0	0
245	0	0	0	1	1	0	0	0	1	0	0	0	0	0
246	0	0	0	0	1	0	0	0	1	0	0	0	0	0
247	0	0	0	0	1	0	0	0	1	0	0	0	0	0
248	0	0	0	0	1	0	0	0	1	0	0	0	0	0
249	0	0	0	0		0	0	0	1	0	0	0	0	0
250	0	0	0	0	1	0	0	1	0	0	0	0	0	0
251	0	0	0	0	1	0	0	0	1	0	0	0	0	0
252	0	0	0	0	1	0	0	0	1	0	0	0	0	0
253	0	0	0	0	1	0	0	0	1	0	0	0	0	0
254	0	0	0	0	0	0	0	0	1	0	0	0	0	0
255	0	0	0	0	0	0	0	0	1	0	0	0	0	0
256	0	0	0	1	1	0	0	1	0	0	0	discharged prior to36 weeks	0	0
257	0	0	0	0	1	0	0	1	0	0	0	discharged prior to36 weeks	0	0
258	0	0	0	1	0	0	0	1	0	0	0	discharged prior to36 weeks	0	0
259	0	0	0	0	1	0	0	1	0	0	0	discharged prior to36 weeks	0	0
260	0	0	0	0	1	0	0	0	1	0	0	discharged prior to36 weeks	0	0
261	0	0	0	0	1	0	0	1	0	0	0	discharged prior to36 weeks	0	0

Appendix 1 HRV Patient Database

Study Number	PDA suspected	PDA confimred	PDA subsequen	Morphine	Caffeine	Midazolam	Phenobarbitone	DBM	NGT	Breast/Bottle /Cup	Died	CLD	ROP	NEC
262	0	0	0	0	1	0	0	1	0	0	0	discharged prior to36 weeks	0	0
263	0	0	0	0	1	0	0	0	1	0	0	1	0	1
264	0	1	1	0	1	0	0	0	1	0	0	1	3	0
265	0	1	1	0	1	0	0	0	1	0	0	1	3	0
266	0	1	1	0	1	0	0	0	1	0	0	1	3	0
267	0	0	0	0	0	0	0	0	0	1	0	0	0	0
268	0	0	0	0	0	0	0	0	1	0	0	0	0	0
269	0	0	0	0	0	0	0	0	1	0	0	0	0	0
270	0	0	0	0	1	0	0	0	1	0	transfer back at 29 weeks	transfer back at 29 weeks	transfer back at 29 weeks	transfer back at 29 weeks
271	0	0	0	0	0	0	0	0	1	1	0	0	0	0
272	0	1	0	0	1	0	0	0	1	0	0	0	0	0
273	1	1	1	1	0	1	0	1	0	0	0	1	2	1
274	1	1	1	0	0	1	0	1	0	0	0	1	2	1
275	1	1	1	0	1	0	0	0	1	0	0	1	2	1
276	1	1	1	0	1	0	0	0	1	0	0	1	2	1
277	1	1	1	0	1	0	0	0	1	0	0	1	2	1
278	1	1	1	0	1	0	0	0	1	0	0	1	2	1
279	1	1	1	0	0	0	0	0	1	0	0	1	2	1
280	1	1	1	0	0	0	0	0	1	0	0	1	2	1
281	0	0	0	0	1	0	0	0	1	0	transfer back at 29 weeks	transfer back at 29 weeks	transfer back at 29 weeks	transfer back at 29 weeks
282	0	0	0	1	0	1	0	1	0	0	died on 2/11/7	0	0	00/01/1900
283	0	0	0	0	1	0	0	0	1	0	discharged to arrowe	discharged to arrowe	discharged to arrowe	discharged to arrowe
284	0	0	0	0	1	0	0	1	1	0	discharged to arrowe	discharged to arrowe	discharged to arrowe	discharged to arrowe
285	0	0	0	1	1	0	0	1	1	0	0	1	1	1
286	0	0	0	0	0	0	0	0	1	0	0	0	0	0
287	0	0	0	0	0	0	0	0	1	0	0	0	0	0
288	0	0	0	0	0	0	0	0	1	1	0	0	0	0
289	0	0	0	0	1	0	0	0	1	0	transfer back at 29 weeks	transfer back at 29 weeks	transfer back at 29 weeks	transfer back at 29 weeks

Appendix 1 HRV Patient Database

Study Number	PDA suspected	PDA confimred	PDA subsequen	Morphine	Caffeine	Midazolam	Phenobarbitone	[illegible]	[illegible]	[illegible]	Died	CLD	ROP	NEC
290	0	0	0	0	1	0	0	0	1	0	transfer back at 29 weeks	transfer back at 29 weeks	transfer back at 29 weeks	transfer back at 29 weeks
291	1	1	1	0	1	0	0	0	1	0	0	1	1	0
292	1	1	1	0	1	0	0	0	1	0	0	1	1	0
293	1	1	1	0	1	0	0	0	1	0	0	1	1	0
294	1	1	1	0	0	0	0	0	1	0	0	1	1	0
295	1	1	1	0	0	0	0	0	1	0	0	1	1	0
296	1	1	1	0	0	0	0	0	1	0	0	1	1	0
297	1	1	1	1	0	0	0	0	1	0	0	1	1	0
298	0	0	0	0	0	0	0	0	1	0	0	0	0	0
299	0	0	0	0	0	0	0	0	1	0	0	0	0	0
300	0	0	0	0	0	0	0	0	1	0	0	0	0	0
301	0	0	0	0	0	0	0	0	1	0	0	0	0	0
302	0	0	0	0	0	0	0	0	1	0	0	0	0	0
303	0	0	0	0	0	0	0	0	1	0	0	0	0	0
305	0	0	1	1	0	1	0	1	1	0	0	0	0	0
306	0	0	0	0	0	0	0	0	1	0	0	0	0	0
307	0	0	0	0	0	0	0	0	1	0	0		0	0
313	0	0	0	0	0	0	0	0	1	0	0	0	0	0
314	0	0	0	0	0	0	0	0	1	0	0	0	0	0
315	0	0	0	0	0	0	0	0	1	0	0	0	0	0
316	0	0	0	0	1	0	0	0	1	0	0	disch at 33weeks	0	0
24	0	0	0	0	1	0	0	0	1	0	0	0	0	0
26	0	0	0	0	1	0	0	0	1	0	0	1	0	0
27	0	0	0	0	0	0	0	0	1	1	0	0	0	0
28	0	0	0	0	1	0	0	0	1	0	0	0	0	0
29	0	0	0	0	1	0	0	0	1	0	0	0	0	0
30	0	0	0	0	0	0	0	0	1	0	0	0	0	0
31	0	0	0	0	1	0	0	0	1	0	0	1	0	0
32	0	0	0	0	0	0	0	1	0	0	0	0	0	0
33	0	0	0	0	0	0	0	0	1	0	0	0	0	0
34	0	0	0	0	1	0	0	0	1	0	0	0	0	0
35	0	0	1	0	1	0	0	1	0	0	1	0	0	0
36	0	0	0	0	1	0	0	0	1	0	0	1	0	0
37	0	0	1	0	1	0	0	1	0	0	1	0	0	0

Appendix 1 HRV Patient Database

Study Number	PDA suspected	PDA confimred	PDA subsequen	Morphine	Caffeine	Midazolam	Phenobarbitone	NBM	NGT	Breast/bottle [illegible]	Died	CLD	ROP	NEC
38	0	0	0	0	1	0	0	1	0	0	0	trans at 30 weeks	?	?
39	0	0	0	0	1	0	0	1	0	0	0	0	3	0
40	0	0	0	0	0	0	0	0	1	0	0	0	0	0
41	0	0	0	0	1	0	0	0	1	0	0	0	3	0
42	0	0	0	0	1	0	0	0	1	0	0	trans at 30 weeks	?	?
43	0	0	0	0	0	0	0	0	1	1	0	0	0	0
44	0	0	1	0	1	0	0	1	0	0	1	1	3	0
45	0	0	0	0	0	0	0	0	1	0	0	0	0	0
46	0	0	0	0	1	0	0	0	1	0	0	0	3	0
47	0	0	0	0	0	0	0	0	1	0	0	0	0	0
48	0	0	0	0	1	0	0	0	1	0	0	1	0	0
49	0	0	0	0	1	0	0	0	1	0	0	0	0	0
50	0	0	0	0	1	0	0	0	1	0	0	1	2	0
59	0	0	1	0	1	0	0	0	1	0	0	1	0	0
60	0	0	0	0	1	0	0	0	1	0	0	1	2	0
61	0	0	0	0	0	0	0	0	1	0	0	0	0	0
62	0	0	0	0	1	0	0	0	1	0	0	1	0	0
63	0	0	0	0	1	0	0	0	1	0	0	0	0	0
64	0	0	0	0	0	0	0	0	1	0	0	0	0	0
65	0	0	0	0	0	0	0	0	1	0	0	0	0	0
66	0	0	0	0	1	0	0	0	1	0	0	disch at 31 weeks	disch at 31 weeks	disch at 31 weeks
67	0	0	1	0	1	0	0	0	1	0	0	disch in 02 at 321 weeks	0	0
68	0	0	0	0	1	0	0	0	1	0	disch at31 weeks	disch at31 weeks	disch at31 weeks	disch at31 weeks
69	0	0	0	0	1	0	0	0	1	0	0	1	2	0
70	0	0	0	0	1	0	0	1	0	0	0	1	0	0
78	0	0	0	1	0	0	0	0	1	0	0	disch at 31 weeks	disch at 31 weeks	disch at 31 weeks
79	1	0	1	0	1	0	0	0	1	0	0	disch in 02 at 321 weeks	0	0
80	1	0	0	0	1	0	0	0	1	0	disch at31 weeks	disch at31 weeks	disch at31 weeks	disch at31 weeks
81	0	0	0	0	1	0	0	0	1	0	0	1	2	0

Appendix 1 HRV Patient Database

Study Number	PDA suspected	PDA confimred	PDA subsequen	Morphine	Caffeine	Midazolam	Phenobarbitone	NBM	NGT	breast bottle /Cup	Died	CLD	ROP	NEC
82	0	0	0	0	0	0	1	0	0	1	0	0	0	0
83	0	0	0	0	1	0	0	0	1	0	0	1	0	0
89	0	0	0	0	1	0	0	0	1	0	0	disch at 31 weeks	disch at 31 weeks	disch at 31 weeks
90	0	0	0	0	1	0	0	0	1	0	disch at31 weeks	disch at31 weeks	disch at31 weeks	disch at31 weeks
91	0	0	0	0	1	0	0	0	1	0	0	disch at 29 weeks	disch at 29 weeks	disch at 29 weeks
92	0	0	0	0	1	0	0	0	1	1	0	0		0
93	0	0	0	0	0	0	0	0	1	1	0	0	0	0
94	0	0	0	0	1	0	0	0	1	0	0	1	2	0
95	0	0	0	0	0	0	0	0	1	1	0	0	0	0
96	0	0	1	0	1	0	0	0	1	0	0	1		0
97	1	1	0	0	1	0	0	0	1	0	0	1		0
98	0	0	0	0	1	0	0	0	1	0	0	1		0
100	0	0	0	0	1	0	0	0	1	0	0	0	0	0
101	0	0	0	0	1	0	0	1	0	0	0	1	3	0
102	0	0	1	0	1	0	0	0	1	0	0	1		0
103	0	0	0	0	0	0	0	0	1	1	0	1	0	0
104	0	0	0	0	1	0	0	0	1	0	0	1	0	0
105	0	0	0	0	1	0	0	0	1	0	0	1		0
106	0	0	0	0	1	0	0	0	1	0	0	1		0
107	0	0	0	0	1	0	0	0	1	0	0	1		0
108	0	0	0	0	0	0	0	0	1	0	0	1		0
115	0	0	0	0	1	0	0	0	1	1	0	1		0
116	0	0	0	0	1	0	0	0	1	0	0	0	0	0
128	0	0	0	0	0	0	0	1	0	0	0	0	0	0
132	0	0	0	0	0	0	0	0	1	0	0	1	2	0
148	0	0	0	0	1	0	0	0	1	0	0	1	3	0
151	0	0	0	0	1	0	0	0	1	0	0	disch at 31 weeks in 02	0	0
154	0	1	1	0	1	0	0	0	1	1	0	1		0
155	0	0	0	0	0	0	0	0	1	1	0	1		0
157	0	0	0	0	1	0	0	0	1	0	0	0	0	0
159	0	0	1	0	1	0	0	0	1	0	0	1	0	0
161	0	0	0	0	0	0	0	0	1	0	0	0	0	0
163	0	0	0	0	0	0	0	0	1	1	0	0	0	0

Appendix 1 HRV Patient Database

Study Number	PDA suspected	PDA confimred	PDA subsequen	Morphine	Caffeine	Midazolam	Phenobarbitone	NeIM	NGT	Breast/Bottle /Cup	Died	CLD	ROP	NEC
165	0	0	0	0	0	0	0	0	0	1	0	0	0	0
180	0	0	0	0	1	0	0	0	1	0	0	0	0	0
203	0	0	0	0	0	0	0	1	0	0	0	0	0	0
204	0	0	0	0	0	1	0	1	0	0	0	0	0	0
205	0	1	0	0	1	0	0	0	1	0	0	1	0	0
304	0	0	0	0	1	1		0	1	0	0	0	0	0
308	0	0	0	0	0	0	0	0	1	0	0	0	0	0
309	0	0	0	0	0	0	0	0	1	0	0	0	0	0
310	0	0	0	0	1	0	0	1	1	0	0	0	0	0
311	0	0	0	0	1	0	0	1	1	0	0	0	0	0
312	0	0	1	0	1	0	0	1	1	0	0	1	1	0

Appendix B

Method for determining Frequency Domain measures from the NNi tachogram

Detrending by time domain estimation of the spectral HRV components without resampling using the Ornstein-Uhlenbeck Gaussian Process (OUGP) Filter

Consider a Gaussian Process $x(t)$ with the exponential covariance function:

$$k(t_i, t_j) = \exp(-\gamma|t_i - t_j|). \qquad (1a)$$

This stationary covariance function describes the Ornstein-Uhlenbeck process which was originally introduced as the model of the velocity of a particle undergoing Brownian motion. The process is *mean-square* (MS) continuous:

$$\lim_{\varepsilon \to 0} \mathbf{E}\left[(x(t+\varepsilon) - x(t))^2\right] = 0, \qquad (1b)$$

but not MS differentiable:

$$\lim_{\varepsilon \to 0} \mathbf{E}\left[\left(\frac{x(t+\varepsilon) - x(t)}{\varepsilon} - \dot{x}(t)\right)^2\right] \neq 0. \qquad (1c)$$

The properties above are expressed in terms of expectations.

In the following, the basic theory of the OUGP filter is outlined, full details being available in the section "publications arising from this thesis"[277].

Consider the Gram matrix K, obtained by evaluating the covariance function at a (not necessarily uniform) sequence of ordered times $t_1 < t_2 < \cdots < t_n$. At first sight, the matrix K does not seem a good candidate for a low-pass filter, because the discontinuity in the slope on the diagonal (due to the property in Equation (1c)) introduces potentially significant high-frequency leakage. A fundamental modification, however, is to define γ as a complex number, so the filter can be considered as the real part of the result. It is then possible to show that there exists a *unique* exponential filter with the following properties:

i. the derivative of the impulse response is continuous;

ii. the frequency response is unity at 0 and flat up to the third derivative;

iii. the frequency response falls off at 24 dB per octave when $f > f_c$, where f_c is the cut-off frequency at 3 dB (frequencies normalised *w.r.t.* Nyquist).

Defining values for γ as γ_l and γ_h, for low-pass and high-pass filters respectively,

$$\gamma_l \equiv \sqrt{2}\pi(\sqrt{2}-1)^{-1/4}(1+i)f_c \qquad (2a)$$

$$\gamma_h \equiv \sqrt{2}\pi(\sqrt{2}-1)^{1/4}(1+i)f_c \qquad (2b)$$

with $i = \sqrt{-1}$, scales the respective 3 dB points of the filter to the cut-off frequency f_c.

Defining

$j = 1 \ldots n-1$, where, as previously defined, γ stands for γ_l or γ_h:

$$w_j \equiv \gamma(t_{j+1} - t_j)$$
$$r_j \equiv \exp(-w_j)$$
$$e_j \equiv (r_j^{-1} - r_j)^{-1}.$$

It can then be proved that the inverse of the covariance matrix K is tridiagonal with the following entries:

$$T_{ij} = \begin{cases} 1 + r_1 e_1 & i = j = 1 \\ -e_i & 1 \le i = j - 1 \le n-1 \\ 1 + r_i e_i + r_{i-1} e_{i-1} & 1 < i = j < n \\ -e_j & 1 \le j = i - 1 \le n-1 \\ 1 + r_{n-1} e_{n-1} & i = j = n \\ 0 & \text{otherwise.} \end{cases}$$

Note that to evaluate the T matrix it is not necessary to store and calculate the entries of the K matrix. This is crucial for large data sets, since the space complexity of the algorithm is linear in the sample size rather than quadratic or higher ordered.

Hence, filtering a sequence s_j produces an output:

$$u_i = \sum_j K_{ij} s_j, \; i = 1 \ldots n \qquad (3a)$$

which can be more efficiently evaluated in terms of the solution of a sparse tridiagonal system involving the matrix T as:

$$\sum_j T_{ij} u_j = s_i . \qquad (3b)$$

Tridiagonal systems can be solved in linear time[50] requiring only $8n$ arithmetic operations, so the above operation is actually faster than for a regular matrix-vector product.

Given a set of measurements y_j at times t_j, $j = 1 \ldots n$, the above equation specialises as:

$$\sum_j T_{ij} u_j = \begin{cases} (y_1 - y_2)/2w_1 & i = 1 \\ (y_i - y_{i+1})/2w_i + (y_i - y_{i-1})/2w_{i-1} & i = 2, \ldots, n-1 . \\ (y_n - y_{n-1})/2w_{n-1} & i = n \end{cases}$$

It should be noted that the data have been transformed by a piecewise-linear quadrature formula, the rational for and details of which are described elsewhere[278].

The high-pass (Hy) or low-pass (Ly) filtered data can finally be obtained by the following in which $\Re(\cdot)$ denotes the real part:

$$\mathrm{H}\mathbf{y} = \Re(\mathbf{u}), \quad \mathrm{L}\mathbf{y} = \mathbf{y} - \Re(\mathbf{u}) \qquad (4)$$

Equations (3a) and (4) describe the operation of a non-causal filter; in particular, the matrix K can be seen as its impulse response, though the data are further processed by a piecewise-linear quadrature. It can also be shown that the filter has zero phase, since its frequency response is a real, even and positive function of frequency in the pass-band.

Applying Equation (4) to a time series in sequence, a third-order bandpass filter is achieved. This technique is validated elsewhere[277].

Estimation of the spectral power HRV components without resampling using the Lomb Scargle Periodogram (LSP)

The distribution of power in the RR tachogram with respect to frequency can be estimated from its power spectrum density (PSD). The two most commonly used PSD's are the Welch Periodogram based on the Discrete Fourier Transform (DFT) and the autoregressive (AR) spectrum which assumes a generative autoregressive process model. Both methods require the tachogram to be resampled at regular intervals on the time axis. This limitation is obviated in the Lomb Scargle Periodogram (LSP) which can be seen as a generalisation of the DFT[251]. The statistical-sampling approach of the LSP allows for sparse (discontinuous) time data which is not feasible with DFT methods. This enables the estimation of the PSD from a sequence concatenated from discrete data segments with the proviso that data remain aligned to the original time-stamp. This latter property is exploited in this study.

The principle of the LSP follows: for observations of the HRV tachogram X sampled in time t,

$$X_j = X(t_j) \quad (\amalg t = t_{j+1} - t_j \neq \text{constant}) \qquad (5)$$

The N-point *generalised* discrete Fourier transform is

$$F{:}T_{X(\omega)} = \left(\frac{N}{2}\right)^{\frac{1}{2}} \sum_{j=0}^{N-1} X\left(t_j\right)\left[A\cos\left(\omega t_j\right) - iB\sin\left(\omega t_j\right)\right] \quad (6)$$

where $i = \sqrt{-1}$ with A and B functions of angular frequency ω

The normalised tachogram is given by

$$P_{X(\omega)} = \frac{1}{N}\left|FT_X\left(\omega\right)\right|^2 \quad (7a)$$

$$= \frac{A^2}{2}\left[\sum_j X\left(t_j\right)\cos\left(\omega t_j\right)\right]^2 + \frac{B^2}{2}\left[\sum_j X\left(t_j\right)\sin\left(\omega t_j\right)\right]^2 \quad (7b)$$

This is further refined by Scargle such that $P_{LSP_{X(\omega)}}$ tends to the solution arrived at by the least squares fitting of the familiar function

$$x\left(t\right) = A\cos\left(\omega t\right) + B\sin\left(\omega t\right). \quad (9)$$

A fuller description of the Lomb Scargle Periodogram is given by Moody[204] and Laguna[255] after Press and Rybicki[253]. In this study, an efficient vectorised implementation of this algorithm was developed (fLSPw)[277].

Theoretically, HRV data can be represented interchangeably in the frequency and time domains. The total power of the tachogram power spectrum, as determined by integration, equals the total power (as variance) of the raw tachogram as a record in time (Parseval's Theorem). However, this is true only in the statistical limit and a case can be made for time domain processing being more robust and applicable than any form of power spectral analysis[279]. Potentially, frequency-band power estimation by directly reducing the tachogram using high-ordered bandpass filters is attractive. However, conventional linear implementations of infinite impulse and finite impulse filters require the data to be regularly sampled. The high-pass Ornstein-Uhlenbeck Gaussian Process filter, initially developed as a detrending process, does not suffer this limitation and can be implemented to give a bandpass response in a simple two-pass process which does not compromise fidelity.

Appendix C

Validation of the new method of frequency domain analysis

Lomb-Scargle periodogram versus Fast Fourier Transform

In parallel with this thesis additional work by Professor Tony Fisher and Katie Sanders within the department of medical physics and clinical engineering at the Royal Liverpool University Hospital investigated the efficacy of the Lomb-Scargle periodogram when compared to FFT with resampling of the cardiac event time series.

The three spectral estimation techniques used were;

i. LSP of the unevenly sampled NNi series
ii. FFT PSD of the resampled NNi series using linear interpolation at a sampling rate of 7Hz
iii. FFT PSD of the resampled time series using cubic spline interpolation at a sampling rate of 7Hz

To assess the accuracy of each method synthesized a randomly generated NNi time series signal with a known LF:HF ratio (0.44) was produced. In order to approximate the error for each of the resulting spectra from the three methods, the derived mean LF:HF ratio and the variance of its estimate was calculated (using a Monte Carlo method of averaging the results of a 1000 randomly generated realizations of the actual NNi series[241] for different mean heart rates (55,75 and 95 bpm) and ranges of overall variability (5, 10 and 15 bpm).

	LF:HF ratio (55±5bpm)	LF:HF ratio (75±5bpm)	LF:HF ratio (95±5bpm)
LSP	0.446 (0.092)	0.446 (0.097)	0.445 (0.095)
FFT_{cub}	0.551 (0.107)	0.456 (0.100)	0.448 (0.095)
FFT_{lin}	0.763 (0.161)	0.598 (0.132)	0.535 (0.114)

Table C.1 Comparison of three methods for deriving a known LF:HF ratio of 0.44 for different mean heart rates.

	LF:HF ratio (70±5bpm)	LF:HF ratio (70±10bpm)	LF:HF ratio (70±15bpm)
LSP	0.447 (0.094)	0.444 (0.090)	0.444 (0.087)
FFT_{cub}	0.463 (0.098)	0.458 (0.093)	0.453 (0.090)
FFT_{lin}	0.626 (0.135)	0.630 (0.129)	0.064 (0.128)

Table C.2 Comparison of three different methods (Lomb Scargle periodogram, Fast Fourier transform (cubic spline) and Fast Fourier transform (linear interpolation)) deriving a known LF:HF ratio of 0.44 for different degrees of overall variability

From these simple experiments it can be seen that the LSP estimate is very close to the ideal result and performs consistently for a range of heart rates and over a wide range of variabilities.

To further investigate the accuracy of each method, an idealised artificial NNi tachogram was generated by mixing two sine waves with defined frequencies within a LF (0.04 – 0.15Hz) and HF (0.15 to 0.4Hz) band. The baseline heart rate was set at 60 with a HF amplitude of 2.5 bpm and LF amplitude of 2 bpm, in common with previous work by Clifford G.D et al[241]. The LF:HF ratio is therefore 0.64 ($=2^2/2.5^2$). Comparison of the derived LF:HF ratio varying over heart rates from 40bpm to 120bpm, demonstrated that both resampling methods resulted in an overestimation of the LF:HF ratio where as the LSP estimate is consistently reliable, provided the mean heart rate is below the nyquist criterion (in this example, 48bpm) (Table 5.3).

PSD →	LSP			FFT_{cub}			FFT_{lin}		
HR_o (bpm) ↓	LF	HF	LF:HF	LF	HF	LF:HF	LF	HF	LF:HF
40	0.19	0.59	0.32	0.24	0.25	0.96	0.29	0.20	1.46
50	0.19	0.30	0.64	0.21	0.29	0.72	0.27	0.22	1.24
60	0.19	0.30	0.64	0.20	0.30	0.66	0.25	0.25	1.00
70	0.19	0.30	0.64	0.20	0.30	0.65	0.23	0.26	0.89
80	0.19	0.30	0.64	0.20	0.30	0.64	0.22	0.27	0.82
90	0.19	0.30	0.64	0.19	0.30	0.64	0.22	0.28	0.78
100	0.19	0.30	0.64	0.19	0.30	0.64	0.21	0.28	0.75
110	0.19	0.30	0.64	0.19	0.30	0.64	0.21	0.29	0.73
120	0.19	0.30	0.64	0.19	0.30	0.64	0.21	0.29	0.71

Table C.3 Comparison of three different methods (Lomb Scargle Periodogram (LSP), Fast Fourier transform (cubic spline) (FFT_{cub}) and Fast Fourier transform (linear interpolation) (FFT_{lin})) in producing frequency domain measures of HRV. The input LF/HF ratio is 0.64

The three spectral estimates when then assessed with both ectopic and missing beats. An ectopic beat was inserted into the NNi time series at random. The timing of this ectopic beat is the fraction, ε, of the previous NNi and varied from 0.9 to 0.5. Note that decreasing values of ε correspond to earlier arrival of the ectopic beat. The overall trend is one of a decreasing LF:HF ratio with earlier arrival of the ectopic beat. This is a manifest of the increase in the HF component caused by the sharp peak in the time series. In this example the cubic spline FFT performs better than the LSP due to the smoothing effect of the cubic spline. This example demonstrates the importance of accurate beat detection as the presence of a single ectopic beat causes unacceptable distortion of the frequency spectrum. (table 5.4)

Timing of Ectopic beat ε	LF:HF Ratio error (%)		
	LSP	FFT_{cub}	FFT_{lin}
0.90	-3.0	-1.8	24.8
0.85	-6.5	-4.7	20.8
0.80	-11.4	-8.5	15.6
0.75	-17.3	-12.9	9.5
0.70	-23.8	-17.9	2.9
0.65	-30.5	-23.1	-4.0
0.60	-36.9	-28.5	-10.7
0.55	-42.9	-33.9	-17.0
0.50	-48.3	-39.5	-23.0

Table C.4 Comparison of three different methods (Lomb Scargle periodogram, Fast Fourier transform (cubic spline) and Fast Fourier transform (linear interpolation)) in correctly determining the LF:HF ratio for different timings of ectopic beats.

The neonatal ECG has frequent periods of undetected beats or missing sections of information as discussed above (section 3.1). In some studies up to 50% of the datasets are found unsuitable for analysis[280]. To determine the accuracy for each method when dealing with missing data, the NNi time series was subjected to deleting a randomly selected percentage of data points within a 1000 NNi beat time series. For the LF:HF ratio spectral estimates the relative deviation from their theoretical value was determined and is presented in table 5.6 .

Percentage beat removal (%)	LF:HF Ratio error (%)		
	LSP	FFT_{cub}	FFT_{lin}
0	-0.5	3.7	56.4
5	-1.7 (2.6)	10.7 (4.5)	70.5 (8.4)
10	-3.0 (3.6)	21.4 (8.6)	87.9 (13.1)
15	-4.4 (4.6)	35.6 (13.9)	107.5 (18.8)
20	-5.7 (5.6)	56.1 (20.1)	131.0 (24.6)
25	-7.2 (6.2)	80.6 (26.5)	156.5 (31.6)
30	-8.8 (6.6)	112.3 (34.7)	187.2 (38.8)
35	-10.1 (7.2)	153.0 (46.8)	222.8 (46.7)
40	-12.2 (8.0)	198.3 (60.7)	261.5 (59.4)
45	-12.8 (8.5)	263.0 (82.4)	310.1 (69.9)
50	-14.8 (9.0)	345.1 (102.7)	364.0 (86.2)

Table C.5 Comparison of three different methods (Lomb Scargle periodogram, Fast Fourier transform (cubic spline) and Fast Fourier transform (linear interpolation)) in correctly determining the LF:HF ratio with increasing levels of beat removal.

It can be seen that the even small amounts of missing data cause the FFT methods to grossly overestimate the LF:HF ratio; when 10% of data points are missing even cubic spline interpolation results in an error greater than 10%. The LSP consistently outperforms the FFT, and remains within 15% of the true LF:HF ratio even when 50% of the data points are absent. (Table 5.6). Figure 5.3 demonstrates the stability of the LSP derived LF:HF ratio compared with the inaccurate FFT methods.

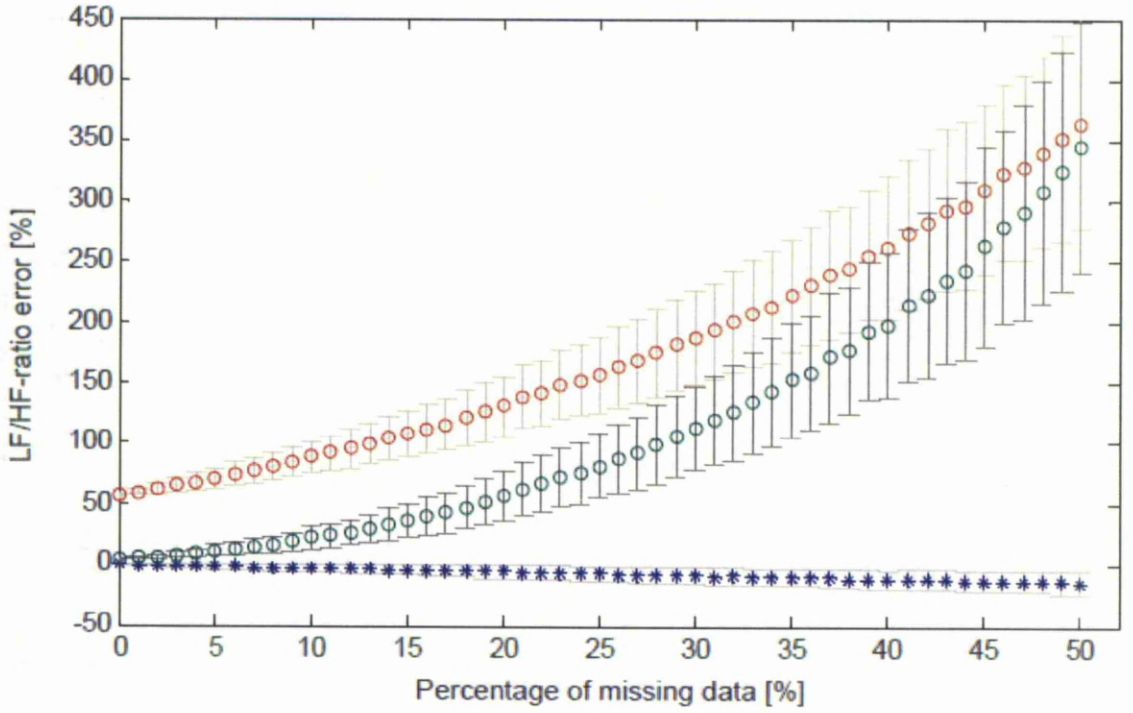

Figure C.3 Percentage error in the LF:HF ratio estimates with increasing missing beats in a 5 minute tachogram. using the LSP * and FFT with linear (-□-) and cubic spline (-o-) .interpolation. Each point represents an average of 1000 randomly seeded runs with error bars representing ± 1 standard error.

Further work examined the affect of non-stationarities and frequencies which are outside the recording time frame. For example, from examination of a typical 24 hour HRV PSD (figure 1.5) a significant proportion of the signals exists in the range below 0.04Hz (the LF band). These longer term variations are only partially captured in the short term recordings and so any components with a cycle longer than the length of the signal may be misinterpreted in the PSD. To investigate the lower frequency limit an NNi time series was created with a frequency of 1/300Hz. (F) (the minimum frequency that can theoretically be resolved from a duration of 5 minutes). The LSP was then used on recordings from 1 to 6 minutes to determine how accurately (F) could be determined. (table 5.7)

Length (mins)	Peak Location (Hz)
1	0.0043
2	0.0073
3	0.0052
4	0.0036
5	0.0033
6	0.0033

Table C.6 Estimated frequency peak location determined from the LSP for signals of 1 – 6 minutes. The input frequency is 0.0033 Hz

The addition of missing data makes accurately estimating frequencies below those within the time frame of the signal even more problematic. To test this, a series of HR signals was generated each with a single modulating frequency (F) ranging from $1x10^{-5}$Hz and 0.005 Hz). For each value of F, 1000 forms of the corresponding NNi time series were created. Sections of each time series were then removed at random such that 4 continuous sections, each of 5 minutes duration remained. The length of the signal was set to 40 minutes, so in essence only 50% of the original time signal was "visible" for the LSP to analyse at any one time. The LSP was calculated from each NNi time series and the ratio between the mean estimated value of F, F_e, and its actual value was determined. Figure 5.4 demonstrates the accuracy of the LSP. The *theoretical* low frequency limits for a 5, 20 and 40 minute signal are demonstrated by the vertical lines. It can be seen that the LSP can realistically represent frequencies above 0.0002Hz. This demonstrates that whilst the FFT techniques can only be performed on continuous data, the LSP can be calculated over the entire length of the signal, despite missing data, and is able to interpret frequencies well below those detected by the FFT. This allows a more complete picture of the underlying HR signal.

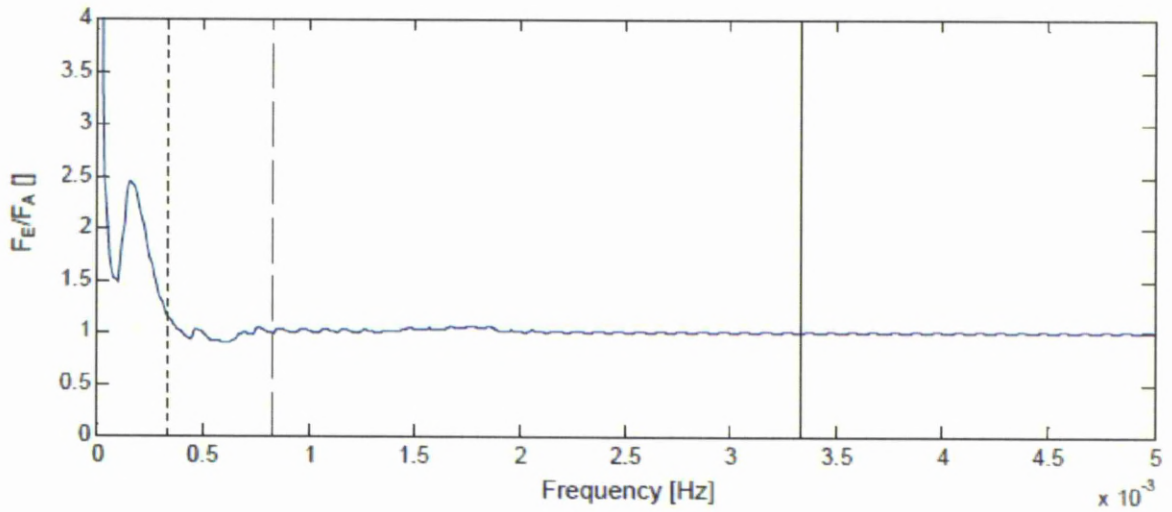

Figure C.4 Plot of F_E/F_A (mean estimated frequency/actual frequency) for different values of F. The vertical lines represent the theoretical low frequency limits for 5 (solid) 20 (dashed) and 40 (dotted) minute signals.

Validation of the method to measure frequency components with synthetic neonatal NNi tachograms

To investigate the validity of the methodology with ECGs containing neonatal parameters, 500 NNi tachograms each of one hour duration were synthesized using the described methodod. Each NNi tachogram had input parameters, P_I, of heart rate, HRV (standard deviation of the heart rate) and the VLF:LF and LF:HF ratio. Table 5.8 shows the mean and range prescribed for each parameter.

Parameter	Prescribed mean (range)
Heart rate	153 (120 – 205)
HRV	13.9 (0.3 – 28.4)
VLF:LF	7.8 (0.49 – 618)
LF:HF	13.3 (0.02 – 1802)

Table C.7 Prescribed parameters for investigation of the methodology for obtaining HRV parameters from synthesized neonatal ECGs.

Each NNi time series was then subject to investigation with the methodology described. The hour long time series was divided into 20 minute time windows, each overlapping by 10 minutes. Thus five 20 minute epochs were produced for each NNi time series. The mean and standard deviation for each parameter within these five epochs was obtained. The parameter estimated mean, P_E, was compared with the prescribed parameter P_I, producing the ratio; P_E: P_I. Table 13 presents the summary statistics for the 500 NNi time series.

Parameter	Ratio P_E: P_I. Mean (standard deviation)
Heart rate	1.00 (0.10)
HRV	1.00 (0.08)
VLF:LF	0.98 (0.14)
LF:HF	1.05 (0.24)

Table C.8 Ratio of the estimated and input parameter for synthesized neonatal RRi time series.

It can be seen that with synthesized neonatal NNi time series data the methodology is highly accurate in estimating the HR and HRV and also the VLF:LF ratio. The LF:HF ratio estimates are also accurate but have a larger standard deviation. On further examination, the inaccurate estimations of the LF:HF ratio occurred at the extremes as demonstrated in table 5.10. Therefore, LF:HF ratio values obtained at these extremes must be interpreted with caution.

Parameter	Ratio $P_{E:}\, P_{I}$. Mean (standard deviation)
LF:HF <1.0	1.49 (0.56)
LH:HF 1.0 - 40	1.03 (0.13)
LF:HF >40.1	0.49 (0.26)

Table C.9 Ratio of the estimated and input LF:HF ratio parameter for different LF:HF values.

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REGULAR ARTICLE

Clinician observation of physiological trend monitoring to identify late-onset sepsis in preterm infants

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Abstract

Aim: To determine whether trends in routinely collected physiological variables can be used retrospectively to classify infants according to the presence or absence of late-onset neonatal sepsis.
Methods: Case control study. Thirty infants born ≤32 weeks of gestation who developed late-onset sepsis were matched with 30 controls for gestational and postnatal age but remained sepsis free. For each infant, 25 clinicians inspected 48 h of routine monitoring of heart rate, respiratory rate and oxygen saturation. Clinicians were asked to determine whether the recording was obtained from an infant who did or did not develop sepsis and also indicate how confident they were in their judgement. Clinicians were stratified into three groups by professional role.
Results: The median correct assignment of infant's recordings was 67% (IQR 62–72). When very confident, this improved to 82% (IQR 67–88). Overall sensitivity was 53% (IQR 43–63) and specificity 80% (IQR 67–87). Advanced neonatal nurse practitioners consistently assigned babies to the correct group more often than other professional groups.

Conclusion: The simple observation physiological trend graphs can classify infants according to the presence or absence of late-onset neonatal sepsis. The accuracy of this method is good to strong but varies with experience of neonatal intensive care.

INTRODUCTION

Late-onset sepsis is a major cause of mortality and morbidity in preterm infants (1) with a 2.5-fold increase in mortality and a more than 30% increase in the length of hospital stay in culture-proven sepsis (2). Attempts to reduce both the incidence and impact of neonatal sepsis are therefore of great importance. The early identification of infants who are developing sepsis allows prompt therapy with antimicrobial agents, improving outcome. However, the diagnosis of neonatal sepsis is difficult as clinical signs/symptoms can be subtle and laboratory tests are of limited value (3). To be of practical use, any diagnostic test must fulfil certain criteria: it should accurately indicate the presence or absence of infection and be reliable; it should be simple to perform; results should be available quickly and it should be cost-effective.

Physiological data could provide an ideal screening tool for late-onset sepsis. Physiological data are continuously recorded, providing both instantaneous monitoring and the ability to observe time-stamped trends in physiological parameters. Longitudinal measurements obtained from continuous monitoring can be displayed in trend graphs, allowing pathology to be observed in real time as it occurs. Many clinicians believe that changes in these parameters can be used in the diagnosis of sepsis. Despite its widespread use in clinical practice, to our knowledge the accuracy of classification based on the simple observation of physiological trend graphs by clinicians caring for infants has not been assessed.

On the neonatal unit at Liverpool Women's Hospital (LWH), information obtained from standard monitoring equipment is presented in trend graphs at the cotside by computers (Fig. 1). The graphs represent the heart rate, respiratory rate, oxygen saturations and, if available, the intra-arterial blood pressure. These variables are captured at 1 Hz. This system has allowed us to perform the first step in a programme of research designed to evaluate the utility of clinician observation in the recognition of sepsis. We designed a study to examine the performance of trend analysis by clinicians under idealized conditions. The primary aim of this study was to examine the accuracy of classifying cases according to the presence or absence of late neonatal onset sepsis using retrospective examination of physiological data by clinicians. The secondary aim of this study was to gather data that would inform further research into this area.

METHODS

Patient population

Premature infants ≤32 weeks gestation and ≥7 postnatal days old who were monitored on the neonatal unit were eligible for entry. Cases were identified from our computer-based patient data information system if they developed a C-reactive protein (CRP) rise from ≤4 mg/L to ≥10 mg/L and were subsequently treated with 5 or more days IV antibiotics. Results of blood cultures were also obtained for each case. Controls were matched by gestational and postnatal age but had a CRP that remained ≤4 mg/L. Infants

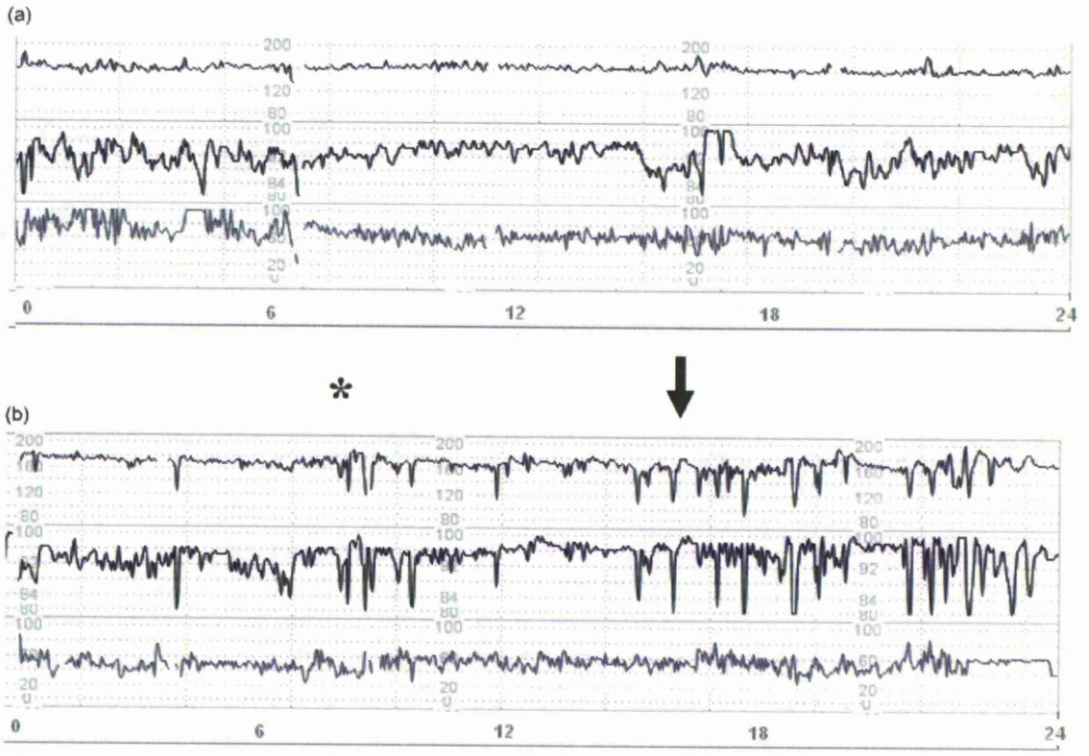

Figure 1 Physiological trend graphs showing heart rate (top), oxygen saturations and respiratory rate from two 25-week-old infants over a 24-h period. Figure 1A is from an infant who remained sepsis free. Figure 1B demonstrates an initial change in the pattern of the trend graphs approximately 16 h before a rise in CRP was noted and a clinical diagnosis of sepsis was made (asterisk), with an intensification in the changes about 8 h before (arrow).

were excluded if they had a diagnosis of grade III/IV intra-ventricular haemorrhage, periventricular leucomalacia, seizures, hypoxic ischaemic encephalopathy or were receiving midazolam or phenobarbitone therapy. Ethical approval was obtained from the Liverpool Paediatric Research Ethics Committee.

Study design

Study participants were presented with a print out of the physiological trend graph containing heart rate, respiratory rate, oxygen saturations and, when available, blood pressure measurements that had been prepared for each infant. We were not able to include temperature measurements because they are not recorded continuously on the database. The print out was produced using the electronic patient data management system (Badger 3.0, Clevermed, Edinburgh, UK). The time period chosen for the cases was 48 h prior to the postnatal age when the CRP initially rose to $\geq$10 mg/L. For each control the time period presented was 48 h prior to the postnatal age when the CRP was noted to be $\geq$4 mg/L in the corresponding case. The group membership of each infant was concealed. Printouts containing each infant's physiological trend graph along with its gestational and postnatal age were distributed to 25 clinicians who were from a single regional neonatal unit and were involved in the routine day-to-day care of neonates in intensive care (the unit had 36 doctors/ANNPs in post at the time of the study). Instructions for completion of the task were provided, which included example recordings from a baby who developed sepsis and one who remained sepsis free. Each clinician was then asked to make a global judgement about whether each printout came from a baby who developed a CRP $\geq$ 10 mg/L or not. ('Yes' or 'No') and record that judgement on a standardized form. This was similar to methods used to test computer algorithms that determine whether or not sepsis is present. They were also asked how confident they were in their judgement; very confident, medium confident or not confident. The clinicians were stratified into three groups for sub-group analysis; (i) Junior medical staff with less than 10 years of experience of neonatal intensive care (i.e. senior house officers and registrars), n = 10 (19 in post) (ii) Senior medical staff with $\geq$10 years experience (i.e. consultants), n = 6 (7 in post) and (iii) Advanced neonatal nurse practitioners (ANNP) with $\geq$10-years experience, n = 9 (10 in post). Clinicians reviewed the traces independently of one another. All clinicians approached about the study had completed the task.

Statistical analysis

The extent to which clinicians correctly assigned the traces was assessed by overall score, sensitivity and specificity, positive and negative predictive value. The median values and

inter-quartile ranges were calculated for the three stratified groups of clinicians. These values were compared between the groups using the Kruskal–Wallis test. Agreement between each group was assessed using the Fleiss Kappa test (4) SPSS v 14.0 (SPSS Inc., Chicago IL, USA) was used for all analyses except the Fleiss Kappa test that was calculated using Microsoft Excel 2003.

RESULTS

Patient population

Table 1 shows the baseline characteristics for both cases/controls.

Test statistics

For all assessors the median correct response was 67% (Inter-quartile range 62–72) of printouts. The assessors were very confident in their decision median 20% (IQR 8–40) of the time. When very confident, the correct response rate improved to 82% (IQR 67–88). The number of correct responses by an individual did not correlate with the number of very confident responses by that individual. Overall sensitivity and specificity was 53% (IQR 43–63) and 80% (IQR 67–87).). Positive predictive value was 75% (IQR 66–82) with negative predictive value being 63% (IQR 60–69). (Table 2).

The three professional sub-groups were analyzed separately, with the ANNPs consistently showing better accuracy at correctly assigning the recordings as coming from infants who developed a CRP rise or not (Table 2 and Fig. 2). Inter-observer agreement was assessed by measurement of the Fleiss Kappa. Overall, Fleiss Kappa was 0.29 reflecting fair agreement. Senior doctors showed the least agreement (Kappa = 0.20, slight agreement), junior doctors were intermediate (Kappa = 0.23) while ANNPs showed the most agreement (Kappa = 0.47, moderate agreement).

The cases with CRP ≥ 10 mg/L had 17 positive blood cultures: 13 coagulase negative staphylococci (CONS), 4 other species (one each of staph aureus, pseudomonas, klebsiella and enterobacter). As expected for a test with relatively low sensitivity, the rate of correct response was lower for sepsis cases than non-sepsis cases. For sepsis cases, the rate of correct response did not vary with the blood culture results: for blood culture negative cases median (IQR) correct response was 56%(48–72); for CONS 58%(48–72) and for other 48%(44–55).

The diagnostic category was more often correctly identified from some infants' recordings than for others. Thirty-one (51.7%) infant recordings were correctly assigned by over 75% of the clinicians, 15 (25%) recordings were correctly identified by 50% to 74.9% of clinicians, 10 (16.7%) recordings were correctly assigned by 20–49% and 4 (6.7%)

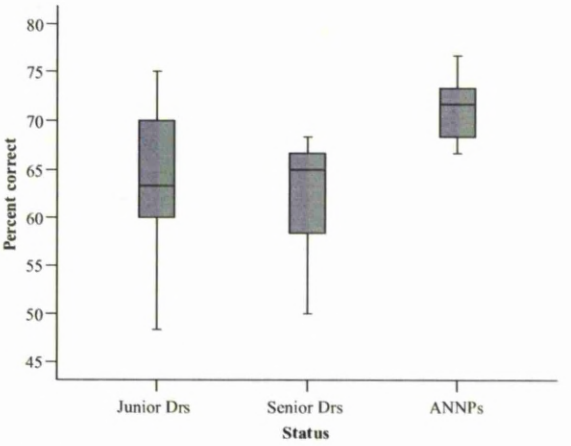

Figure 2 Percentage of responses that correctly identified sepsis status by professional group. ANNPs = Advanced Neonatal Nurse Practitioners.

Table 1 Baseline characteristics of cases and controls

	Sepsis	No sepsis
Number of infants	30	30
Gestational age weeks at birth	27 (25.3–29.8)	27.5 (25.3–29)
Corrected gestational age at time of determination of case or control status	29.8 (28.8–31.2)	30.5 (27.6–31.9)
CRP at time of determination of case or control status (mg/L)	22 (14.5–41)	≤4
Positive blood culture at time of recording	17	n/a

Median (inter-quartile range).
n/a = not applicable.

Table 2 Overall results

	Overall	Senior doctors	Junior doctors	Advanced nurse practitioners	p-value between groups*
Correct	67 (62–72)	65 (56–67)	63 (59–70)	71 (68–74)	0.011
Correct when very confident	82 (67–88)	71 (56–85)	83 (62 -88)	82 (73–96)	0.430
Sensitivity†	53 (43–63)	50 (42–56)	52 (43–58)	60 (50–65)	0.206
Specificity†	80 (67–87)	77 (63–88)	75 (63–87)	87 (80–90)	0.249
PPV†	75 (66–82)	72 (58–79)	70 (60–76)	81 (74–85)	0.086
NPV†	63 (60–69)	60 (55–64)	60 (56–68)	69 (64–70)	0.025

Values are median (Inter-quartile range).
*Kruskal–Wallis test.
†For all responses.
PPV = positive predictive value; NPV = negative predictive value.

recordings were correctly assigned by less than 20% of clinicians. Two recordings were correctly assigned by all assessors; two of the printouts were correctly assigned by only one assessor.

Post hoc interpretation of the recordings that were shown to be easy to categorize showed that infants with sepsis had frequent desaturations/bradycardias, and a steady increase in heart rate. Infants who were easy to recognize as not having sepsis had no specific heart rate patterns and no desaturations or bradycardias. In recordings that were incorrectly assigned by the majority of assessors, non-septic infants had desaturations with bradycardias (perhaps related to other conditions such as chronic lung disease of prematurity) whereas infants with sepsis that was difficult to recognize had no specific patterns in heart rate and desaturations without bradycardias.

DISCUSSION

The main findings of this study are that the simple observation of physiological trend graphs displaying heart rate, respiratory rate and oxygen saturations can classify infants according to the presence or absence of late-onset neonatal sepsis. In two-thirds of cases, clinicians were able to identify correctly which infants developed sepsis or remained sepsis free. Patterns within the trend graphs associated with the absence of late-onset sepsis can be detected and correctly interpreted by clinicians as represented by the relatively high degree of specificity. However, the sensitivity of the assessment was low being 53% across all assessors. This pattern of high specificity/low sensitivity is seen with other tests for neonatal sepsis such as high/low white cell count and CRP (3).

Several studies have reported sophisticated signal processing techniques that have used changes in heart rate to provide an early alert of the onset of sepsis (5–12). In particular Griffin et al. have developed a methodology of continually measuring 'heart rate characteristics' (HRC) (reduced variability and transient decelerations) (6–12). Our work provides an estimate of the accuracy provided by clinical observation. To be useful, more sophisticated techniques must outperform clinical observation. Clinical observation appears to have low sensitivity to sepsis (in common with other techniques) but similar specificity to other, more sophisticated approaches. Overcoming low sensitivity should be an important target for new techniques to diagnose or exclude sepsis and could be an important criterion during method development.

This is the first study to examine the strengths and weaknesses of clinician observation. This study was designed to capture issues that are important early in the developmental pipeline of a novel diagnostic test rather than be a definitive test of the effectiveness of this technique. As such, there were several limitations to this study, i.e. the subjects were only offered two diagnoses: 'sepsis' or 'not sepsis'. This was done to allow us to compare the findings of our work with that of computerized algorithms. Before assessing the traces, each subject was shown what we considered to be a clear example of 'sepsis' and a clear example of 'not sepsis'. In this exploratory study, this was necessary in order to give the subjects a consistent focus on the task in hand. It is possible that this could have privileged certain features of sepsis and future work will need to explore the accuracy of this technique when physiological data are presented in a less structured manner. This study was underpowered to detect differences in response rate according to the nature of the blood culture results. This study was done in a single centre and further work will need to examine this technique in other settings. Our definition of sepsis of a CRP rise from less than 4 mg/L to greater than 10 mg/L and where a clinical decision was made to continue antibiotics for 5 days is a pragmatic one as individual clinicians have different thresholds for continuing antibiotic therapy and may have resulted in a heterogeneous study group. Despite these issues, we believe this study has clearly demonstrated 'proof-of-principle' and suggests the importance of further work on this topic.

Further research about clinician observation of physiological trends will need to take account of several sources of variation in the accuracy of classification that we have identified. Firstly, professional group was associated with accuracy of classification: ANNPs performed consistently better than the two groups of medical doctors. We suspect that the explanation for this difference relates to the fact that each of the ANNPs on our unit has at least 15 years of experience with infants in the neonatal intensive care unit and continue to do so on a day-to-day basis. Secondly, there was marked variation within groups of professionals. At the same level of experience, some individuals were clearly better than others at recognizing patterns associated with sepsis. Thirdly, a person's self-rated confidence was not generally associated with the accuracy of their judgements. Fourthly, informal observation as the clinicians evaluated the printouts suggested that the clinicians used a range of strategies to make their judgements. These included a quick, global observation of all three signals to a more detailed interpretation of the heart rate parameter. Fifthly, some recordings appeared to be relatively easy to categorize as 'sepsis' or 'no sepsis' with half of the recordings being correctly assigned by over 75% of the clinicians. Forty percent of the recordings proved more difficult and the assessors were split in their judgement on whether the baby developed sepsis or not. A small number of readouts were assigned to the incorrect category by the vast majority of clinicians. Further research could define and evaluate which strategies are most useful, using formal approaches such as 'thinking in action' (13) and other qualitative methods informed by our observations (e.g. with respect to purposive sampling to achieve theoretical saturation efficiently). Standardized recordings could be used to identify where observers are on the continuum between novice, competent and expert. This would allow detailed exploration of how clinicians gain expertise in interpreting physiological trends. It is possible that printouts of physiological information also have educational value.

The early diagnosis of sepsis is difficult and previous work has shown that laboratory tests are neither specific nor sensitive (3). Clinicians using physiological measurements to diagnose sepsis should be aware that observation of

physiological trends has similar limitations. Some cases of sepsis were consistently classified incorrectly by these observers. In clinical practice, this suggests that some cases of sepsis may have minimal clinical features and would not be diagnosed until another screening test for sepsis is done.

The mechanism by which sepsis results in the observed changes in physiological parameters is not fully understood. In sepsis, the initiation and maintenance of the inflammatory response is under the control of circulating cytokines and cytokine levels have been demonstrated to correlate with the severity of illness (14–16). Cytokines have widespread effects on signal transduction and they may interfere with normal physiological parameter control by the sympathetic and parasympathetic nervous system (9). In particular, heart rate is known to be affected by several different cytokines (17,18). Sepsis has also been demonstrated to alter the number and distribution of β-adrenergic receptors on cardiac myocytes (19,20). Furthermore, elevated levels of circulating cytokines have been found up to 2 days before the clinical diagnosis of neonatal sepsis (21), which may explain why the observed changes in physiological monitoring are apparent before the diagnosis is made.

Finally, it is important to acknowledge that in the clinical setting, clinicians do not assess the likelihood of sepsis from a single observation of a single test. The clinical state of the infant, haematological and biochemical test results and routine monitoring such as temperature control are all taken into account when assessing whether an infant requires further investigations to determine the presence of sepsis and the commencement of antibiotics. Nevertheless, this study provides the first data to support, and quantify the value of the simple observation of physiological trend graphs during the assessment of an infant who may be developing sepsis.

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TECHNICAL NOTE

The Ornstein–Uhlenbeck third-order Gaussian process (OUGP) applied directly to the un-resampled heart rate variability (HRV) tachogram for detrending and low-pass filtering

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Abstract The heart rate variability signal derived from the ECG is a beat-to-beat record of RR-intervals and is, as a time series, irregularly sampled. It is common engineering practice to resample this record, typically at 4 Hz, onto a regular time axis for conventional analysis using IIR and FIR filters, and power spectral estimators, in the time and frequency domain, respectively. However, such interpolative resampling introduces noise into the signal and the information quality is compromised. Here, the Ornstein–Uhlenbeck third-order band-pass filter is presented which operates on data sampled at arbitrary time and preserves fidelity. The algorithm is available as open source code for MATLAB® (MathWorks™ Inc.) and supported by an interactive website at http://clinengnhs.liv.ac.uk/OUGP.htm.

Keywords HRV · ECG · EKG · RR-intervals · PSD · Ornstein–Uhlenbeck · Spectral analysis · Bandpass filter

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1 Introduction

The heart rate variability signal (HRV tachogram) derived from the ECG is a beat-to-beat record of RR-intervals and is, as a time series, irregularly sampled. It is common engineering practice to resample this record, typically at 4 Hz, onto a regular time axis for conventional analysis using IIR and FIR filters, and power spectral estimators (PSD's), in the time and frequency domain, respectively. However, such interpolative resampling introduces noise into the signal and the information quality is compromised [2, 9].

The information content of the HRV tachogram is limited at high frequencies by Shannon–Nyquist criterion. For both regularly and irregularly sampled data, the upper limiting frequency is simply half the mean sampling rate (the Nyquist frequency). In real life HRV tachograms, which are irregularly sampled and subject to noise (e.g. ectopic beats and recording artefacts), a robust estimate, such as the reciprocal of the median sampling interval, is appropriate. The low frequency information limit is determined by the assumed stationarity. Formal definitions of stationarity can be found elsewhere. The assumption of stationarity is an axiomatic requirement in the estimation of the power spectrum. The Task Force [11] recommendation is that stationarity beyond 20 min should not be assumed, although this limit is quite arbitrary. Hence, the common practice is to detrend the HRV tachogram, either by identifying and removing by subtraction a low frequency content trend component or by high-pass filtering. Trend component identification methods using fixed low-order polynomials [10, 13] and adaptive high-order polynomials [16] are readily applied to the raw irregularly sampled data but with poorly described cut-off frequencies. Reported methods using high-pass filtering, necessarily resampled

onto a regular time axis, include Butterworth-response IIR's [7], locally applied FIR's [1] and more recently, a Gaussian process-based smoothing priors (GPSP) approach [14, 15, 18]. The latter method was extended in [3] (ext-GPSP), to handle non-uniformly sampled data, resulting in a time-varying IIR filter with a second order amplitude response.

2 Methods

2.1 The Ornstein–Uhlenbeck third-order Gaussian process (OUGP) filter

Consider a Gaussian process $x(t)$ with the exponential covariance function:

$$k(t_i, t_j) = \exp(-\gamma|t_i - t_j|). \tag{1}$$

This stationary covariance function describes the Ornstein–Uhlenbeck process which was originally introduced as the model of the velocity of a particle undergoing Brownian motion. The process is mean-square (MS) continuous:

$$\lim_{\varepsilon \to 0} \mathrm{E}\left[(x(t+\varepsilon) - x(t))^2\right] = 0 \tag{1a}$$

but not MS differentiable:

$$\lim_{\varepsilon \to 0} \mathrm{E}\left[\left(\frac{x(t+\varepsilon) - x(t)}{\varepsilon} - \dot{x}(t)\right)^2\right] \neq 0 \tag{1b}$$

The properties above are expressed in terms of expectations.

In the following, the basic theory of the OUGP filter is outlined: for details, the Reader is referred to [17].

Let us consider the Gram matrix K, obtained by evaluating the covariance function at a (not necessarily uniform) sequence of ordered times $t_1 < t_2 < \cdots < t_n$. At first sight, the matrix K does not seem a good candidate for a low-pass filter, because the discontinuity in the slope on the diagonal [due to property in Eq. (1b)] introduces potentially significant high-frequency leakage. A fundamental modification, however, is to define γ as a complex number, so we can consider the filter as the real part of the result. It is then possible to show [17] that there exists a unique exponential filter with the following properties:

- The derivative of the impulse response is continuous;
- The frequency response is unity at 0 and flat up to the third derivative;
- The frequency response falls off at 24 dB per octave when $f > f_c$, where f_c is the cut-off frequency at -3 dB (frequencies normalised w.r.t. Nyquist).

Defining values for γ as γ_l and γ_h, for low-pass and high-pass filters, respectively, with $i = \mathrm{sqrt}(-1)$,

$$\begin{aligned} \gamma_l &\equiv \sqrt{2}\pi(\sqrt{2}-1)^{-1/4}(1+i)f_c \\ \gamma_h &\equiv \sqrt{2}\pi(\sqrt{2}-1)^{1/4}(1+i)f_c \end{aligned} \tag{2}$$

scales the respective 3 dB points of the filter to the cut-off frequency f_c.

Let us define for

$j = 1 \ldots n-1$, where, as previously defined, γ stands for γ_l or γ_h:

$$\begin{aligned} w_j &\equiv \gamma(t_{j+1} - t_j) \\ r_j &\equiv \exp(-w_j) \\ e_j &\equiv (r_j^{-1} - r_j)^{-1}. \end{aligned}$$

It can then be proved that the inverse of the covariance matrix K is tridiagonal with the following entries:

$$T_{ij} = \begin{cases} 1 + r_1 e_1 & i = j = 1 \\ -e_i & 1 \leq i = j - 1 \leq n-1 \\ 1 + r_i e_i + r_{i-1} e_{i-1} & 1 < i = j < n \\ -e_j & 1 \leq j = i - 1 \leq n-1 \\ 1 + r_{n-1} e_{n-1} & i = j = n \\ 0 & \text{otherwise.} \end{cases}$$

Note that to evaluate and store the T matrix, it is not necessary to store and calculate the entries of the K matrix. This is crucial for large data sets, since the space complexity of the algorithm is linear in the sample size rather than quadratic or higher ordered.

Hence, filtering a sequence s_j produces an output:

$$u_i = \sum_j K_{ij} s_j, \quad i = 1 \ldots n \tag{3}$$

which can be more efficiently evaluated in terms of the solution of a sparse tridiagonal system involving the matrix T as:

$$\sum_j T_{ij} u_j = s_i.$$

It is known [8] that tridiagonal systems can be solved in linear time, requiring only $8n$ arithmetic operations, so the above operation is actually faster than for a regular matrix–vector product.

Given a set of measurements y_j at the times $t_j, j = 1 \ldots n$, the above equation specialises as:

$$\sum_j T_{ij} u_j = \begin{cases} (y_1 - y_2)/2w_1 & i = 1 \\ (y_i - y_{i+1})/2w_i + (y_i - y_{i-1})/2w_{i-1} & i = 2, \ldots, n-1 \\ (y_n - y_{n-1})/2w_{n-1} & i = n \end{cases}.$$

It should be noted that the data have been transformed by a piecewise-linear quadrature formula. For a rationale of the choice of this transform, see [17].

The high-pass (H) or low-pass (L) filtered data can finally be obtained by the following [$\Re(\cdot)$ denotes the real part]:

$$\mathrm{Hy} = \Re(\mathrm{u}), \quad \mathrm{Ly} = \mathrm{y} - \Re(\mathrm{u}) \tag{4}$$

Equations (3) and (4) describe the operation of a non-causal filter; in particular, the matrix K can be seen as its impulse response, though the data are further processed by a piecewise-linear quadrature. It can also be shown that the filter has zero phase, since its frequency response is a real, even and positive function of frequency in the pass band [14].

It is useful to compare this method with the ext-GPSP as proposed in [3]. Whereas the former directly specifies a covariance function, the latter specifies an inverse covariance function, which is somewhat less intuitive and the associated Gaussian process prior is also improper [14]. Table 1 explicitly compares the theoretical characteristics of previous ext-GPSP with the present OUGP filter. The comparative stop band roll-off performance is illustrated in Fig. 1.

3 Results and discussion

3.1 Frequency response

A frequency-of-interest (FoI) vector was defined as:

$$\mathrm{FoI} = [0.0005 \ldots 0.003,\ 0.003 \ldots 0.01,\ 0.04 \ldots 0.15,\ 0.15 \ldots 0.40]\ \mathrm{Hz},$$

corresponding to the standard frequency ranges [11] ultra-low (ULF), very-low (VLF), low-frequency (LF) and high-frequency (HF), and additionally the range ultra-low-star (ULF*):

$$\mathrm{FoI}* = [0.002 \ldots 0.01]\ \mathrm{Hz}.$$

In 5 series of 1,000 realisations, the mean amplitude responses to white Gaussian noise (amplitudes), projected onto an irregular time axis formed as the running cumulative sum of amplitudes (as in an HRV tachogram) [2, 9], were determined using the OUGP filter in band-pass configuration (see website resources [6]). The performance is illustrated in Fig. 2 and the agreement between theory and its practical realization were shown in Table 2.

Table 1 Comparison of previous extended Gaussian process smoothing priors filter (ext-GPSP) [3] and the present Ornstein–Uhlenbeck Gaussian process filter (OUGP)

Feature	Filter model	
	ext-GPSP	OUGP
Filter order	Second	Third
Definition terms	Inverse covariance function	Explicit covariance function
Gaussian process dynamics	Non-stationary	Stationary
Definition of Gaussian process prior	Improper, i.e., it cannot be integrated (but the posterior expectation is well-defined nonetheless)	Proper probability density function

3.2 Detrending and low-pass filtering

A 30 min synthetic HRV tachogram with a median frequency of 1 Hz was realised from the sum of four sinusoids

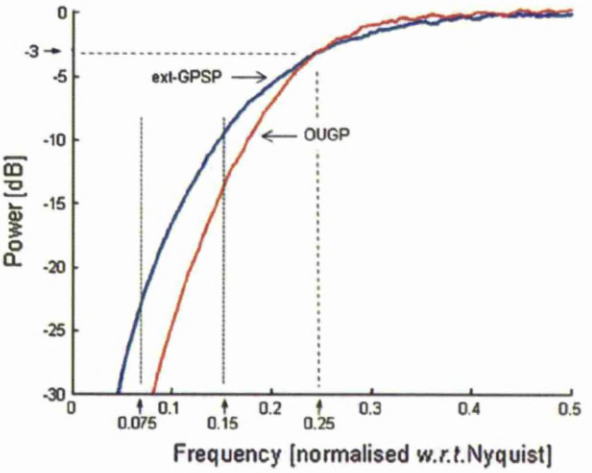

Fig. 1 Comparison of ext-GPSP and OUGP stop-band roll off in high-pass configuration with a normalised Fc of 0.25 in the octave [0.075 … 0.15] (average of 1,000 Monte Carlo realisations)

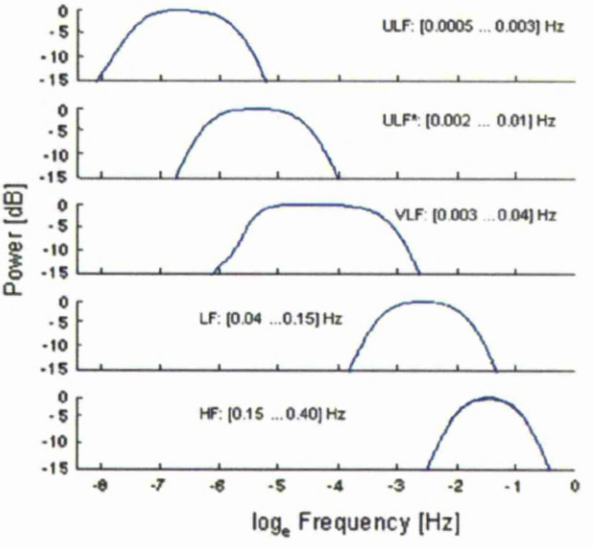

Fig. 2 Power band-pass characteristics achievable using the OUGP band-pass filter as estimated from Monte Carlo analysis of Gaussian random noise tachogram (pass-band gain set to 1)

of Gaussian-distributed centre frequency and zero phase, viz:

$$\mathrm{HRV}(t) = \sum_{i\in\{\mathrm{ulf,vlf,lf,hf}\}} G_i \sin(2\pi f_{i,\sigma_i} t) \quad (4)$$

where G is the amplitude gain, f and σ are centre frequency and standard deviation (Hz).

The sinusoidal components were defined as in Table 3.

A non-stationary feature was added as a 4 dB (w.r.t. total sinusoidal power) Brownian component constructed as the cumulative sum of a random Gaussian series.

Table 2 Comparison of theoretical and simulated −3 dB bandwidths

Frequency band	Design (Hz)	Realisation (Hz)
ULF	[0.00050 ... 0.00300]	[0.00054 ... 0.00300]
VLF	[0.00200 ... 0.01000]	[0.00205 ... 0.00990]
LF	[0.00300 ... 0.04000]	[0.00305 ... 0.03990]
HF	[0.15000 ... 0.40000]	[0.14000 ... 0.40660]

Table 3 Components of synthetic HRV tachogram

Frequency band	Amplitude gain, G	Centre frequency, f (Hz)	STD frequency, σ (Hz)
ULF	2	0.002	0.15
VLF	1	0.025	0.15
LF	1	0.01	0.5
HF	1	0.25	0.5

This tachogram was band-pass filtered with OUGP to achieve detrending (high-pass response) and low-pass band-limiting at 0.003 and 0.4 Hz −3 dB points, respectively. The time domain and frequency domain (as the Lomb Scargle PSD [2, 9]) performance is shown in Fig. 3. The decomposition in the time domain by a series band-pass operations corresponding to the VLF, LF and HF frequency bands is shown in Fig. 4.

3.3 GPOU interactive web pages

The internet-accessible GPOU website provides an interactive GUI in which the User can apply the GPOU band-pass filter to any one of six HRV data sets:

Synthetic 3 peak ~[0.045, 0.12, 0.25] Hz
Above with addition of 4 dB Brownian noise (see Performance)
Gaussian white noise
Normal subject at heart rate ~0.85 Hz
Premature baby at heart rate ~2.5 Hz
Textbook [12]: LF at ~0.1 (SD 0.010) Hz and HF at ~0.25 (SD 0.015) Hz

Bandwidths are user-selectable (at −3 dB points):

High-pass (detrending): {0.001, 0.002, 0.005, [0.001 ... 0.1]} Hz
Low-pass: {0.0125, 0.025, [0.3...1.0]} Hz.

Results are displayed both as the time series decomposition or as the Lomb Scargle PSD. The MATLAB

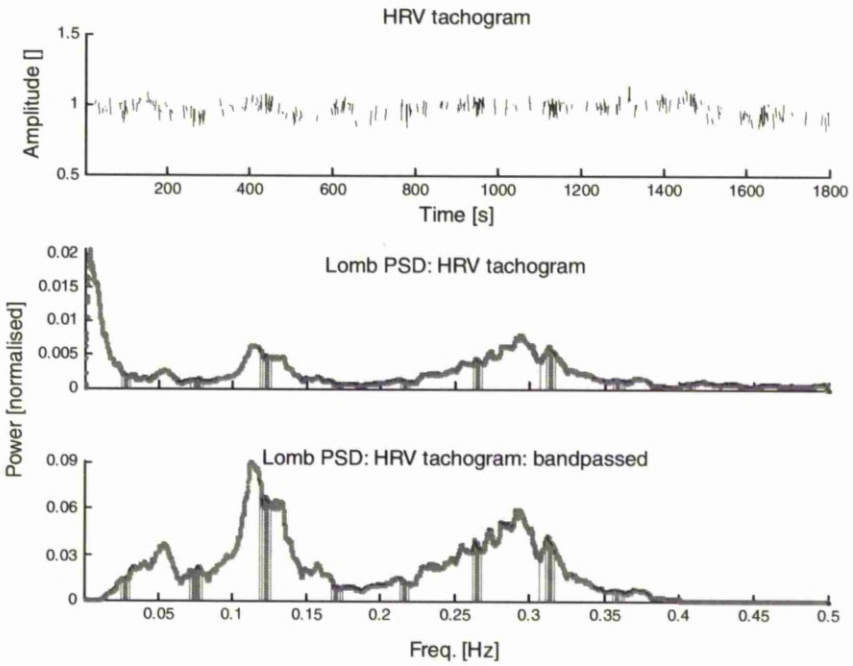

Fig. 3 Artificial HRV tachogram as a time series (*top pane*) with its Lomb Scargle PSD (*middle pane*). The PSD after OUGP band-pass filtering [0.003 ... 0.35] Hz for detrending and low-pass filtering (*bottom pane*). Note: the 0.35 Hz low-pass effect reduces the amplitude of the local peak around 0.29 Hz: this is consistent with the implicit third-order response

(version $\geq$ 2006b) code fully commented in HTML for the GPOU filter (gpsmooth_2.m) and the supporting code for the optimized Lomb Scargle (fLSPw.m) are downloadable. A guide is given to the rationale for setting the bandwidth for the exemplar datasets. An example session is given as Fig. 5.

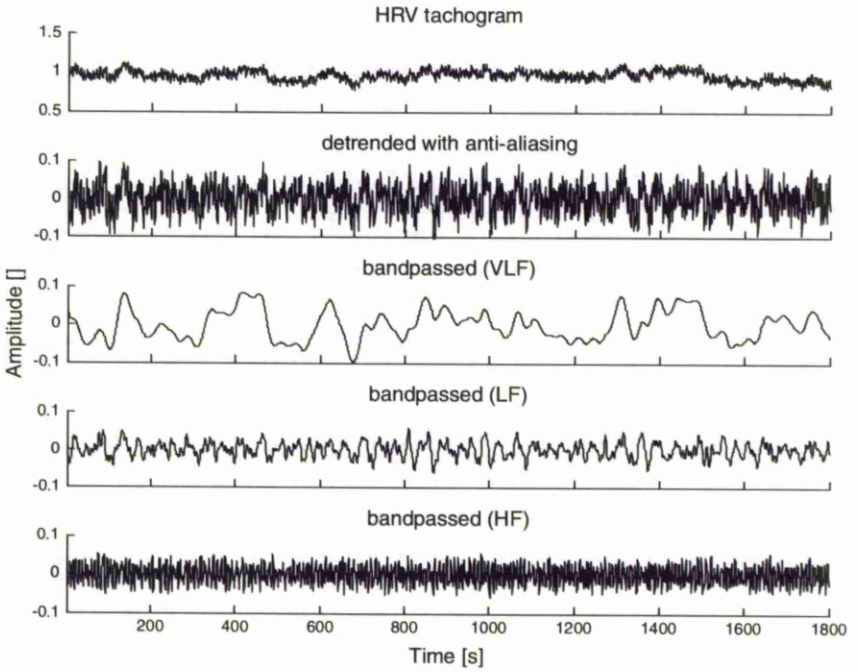

Fig. 4 Decomposition of the artificial tachogram by a series of OUGP band-pass filters

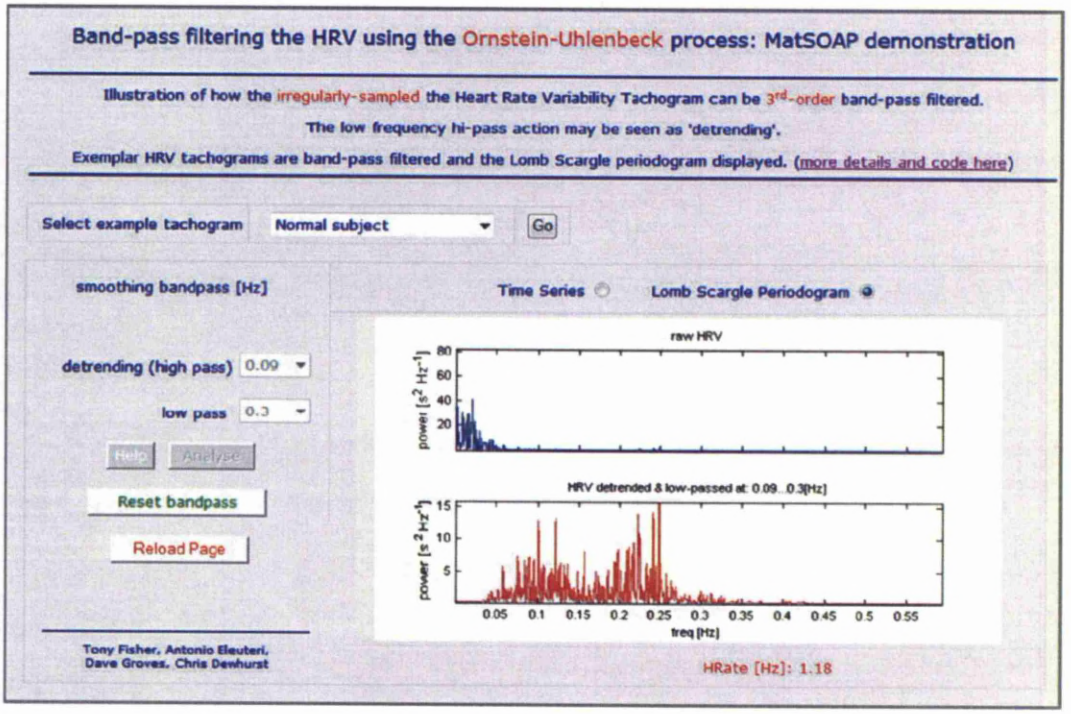

Fig. 5 Internet-accessible interactive demonstration of the OUGP band-pass filter. The User has selected the 'Normal subject' data set, detrended at 0.09 Hz, low-pass filtered at 0.3 Hz and enabled the Lomb Scargle PSD display

The web pages require JavaScript to be enabled and are optimized for the Microsoft Internet Explorer® but run adequately in Firefox® and Safari® under Microsoft Windows® and Linux operating systems. The GPOU and its associated applications execute on a MatSOAP© server which provides access to automation instances of MATLAB over the internet using the Simple Object Access Protocol (SOAP) [5]. Details of MatSOAP can be obtained from the authors [4].

A comparison of GPOU with the extended Gaussian-process smoothing model (ext-GPSP) [3] can be made interactively by referring to http://clinengnhs.liv.ac.uk/links.htm.

4 Conclusion

The OUGP filter is efficiently implemented in MATLAB. As a time domain band-pass filter, it exhibits a predictable and stable third-order zero-phase frequency response with explicit −3 dB points. It can be applied to both regularly and irregularly spaced data without the requirement for resampling. The latter property is suitable to analysis of the HRV tachogram, either as a pre-processing operation prior to PSD estimation by, for example, the Lomb Scargle method (with detrending and low-pass filtering), or directly in the time domain as a series of band-pass filters. The open source code and an interactive demonstration webpage with five exemplar HRV tachograms are maintained at http://clinengnhs.liv.ac.uk/links1.htm [6].

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Research Article

An Efficient Time-Varying Filter for Detrending and Bandwidth Limiting the Heart Rate Variability Tachogram without Resampling: MATLAB Open-Source Code and Internet Web-Based Implementation

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The heart rate variability (HRV) signal derived from the ECG is a beat-to-beat record of RR intervals and is, as a time series, irregularly sampled. It is common engineering practice to resample this record, typically at 4 Hz, onto a regular time axis for analysis in advance of time domain filtering and spectral analysis based on the DFT. However, it is recognised that resampling introduces noise and frequency bias. The present work describes the implementation of a time-varying filter using a smoothing priors approach based on a Gaussian process model, which does not require data to be regular in time. Its output is directly compatible with the Lomb-Scargle algorithm for power density estimation. A web-based demonstration is available over the Internet for exemplar data. The MATLAB (MathWorks Inc.) code can be downloaded as open source.

1. Introduction

A time record consisting of beat-to-beat RR intervals is referred to as the heart rate tachogram. This forms the basis for a number of metrics of heart rate variability (HRV). The simplest measures of HRV are based on variance determined over a range of time periods. More complex measures can be derived from power spectrum density (PSD) estimations. The two most commonly used PSDs are the Welch Periodogram, based on the DFT, and the AR Spectrum, based on an autoregressive process model [1]. Both approaches require the data to be sampled regularly. Resampling the raw HRV data onto a regular time axis introduces noise into the signal and the information quality is compromised [1]. Conventionally, the HRV power is reported over 3 bandwidths: [0.01 · · · 0.04] Hz (Very Low Frequency, VLF) [0.04 · · · 0.15] Hz (Low Frequency, LF), and [0.15 · · · 0.4] Hz (High Frequency, HF) [1, 2].

Prior to transformation into the frequency domain, normal practice requires that the time series data are "detrended" or "high-pass filtered" at a very low frequency, say ~0.005 Hz. There is no universally formal justification for such detrending other than it minimises the effects of medium-term nonstationarity within the immediate time epoch (window) of interest [2]. Stationarity is an axiomatic assumption in conventional time-to-frequency transformation of the PSD (see Appendix B).

A number of methods have been described to identify the trend component in the tachogram such that it can be simply removed by subtraction. These methods include fixed low-order polynomials [3, 4], adaptive higher-order polynomials [5, 6], and, more recently, the smoothing by priors approach (SPA) proposed by [7] which they describe as a time-varying finite impulse high-pass filter. The SPA uses a technique well-established in modern time series analysis and it addresses directly the phenomenon of nonstationarity.

However, the Tarvainen approach suffers two limitations. The first is conceptual: the algorithm requires resampling by interpolation onto a regular time axis. The second is practical: the MATLAB implementation is computationally inefficient and expensive and consequently very slow. In practice, its application is limited to relatively short tachograms [7].

In the present work, a novel algorithm is introduced which obviates these limitations by extending the SPA. The Smoothing by Gaussian process Priors (SGP) method described here explicitly does not require resampling and executes in MATLAB at least an order of magnitude faster than the SPA. By employing the SGP twice in sequence, the bandpass effect achieves detrending (high-pass) and low-pass filtering which is directly compatible with the Lomb Scargle Periodogram (LSP) [8].

2. The Smoothing Priors Approach

The SPA method considers the problem of modelling the trend component in a time series with a linear observation model:

$$z_{\text{trend}} = \mathbf{H}y + v, \tag{1}$$

where $\mathbf{H}$ is the observation matrix, v is observation error, and y are parameters to be determined. The solution to estimating the trend is then expressed in terms of minimisation of a regularised least squares problem:

$$\hat{y}_\sigma = \arg\min_y \|\mathbf{H}y - z\|^2 + \sigma^2 \|\mathbf{D}_d(\mathbf{H}y)\|^2, \tag{2}$$

where σ is a regularisation parameter and $\mathbf{D}_d$ is the discrete approximation to the dth derivative operator.

By choosing $\mathbf{H}$ as the identity matrix, and $d = 2$, the solution can be written as

$$\hat{y}_\sigma = \left(\mathbf{I} + \sigma^2 \mathbf{D}_2^{\mathrm{T}} \mathbf{D}_2\right)^{-1} z. \tag{3}$$

Tarvainen et al. argue that selection of the observation matrix is done to simplify things, in the context of estimating parameters in a finite-dimensional space. A Bayesian interpretation of (2) is given, but always in the context of finite-dimensional parameter spaces. It is interesting and useful to give a different interpretation in the context of Gaussian Process (GP) priors, which implies a function-space view, rather than a parametric view, of the regression problem. In passing it is noted that the SPA, as published, is markedly inefficient and potentially unstable in using matrix inversion. A more efficient approach is presented as Appendix C.

3. An Alternative Smoothing Prior Operator

Use of the $\mathbf{D}_2$ operator implies uniform sampling of the data and in the case of the HRV tachogram requires that the raw data be projected onto a regular time axis using some means of interpolation. Such a projection is frequently referred to as *resampling* which is undesirable in that it corrupts, preferentially, the higher frequency components [2]. In the present development, it is proposed that resampling can be avoided by using a different approximation for the second-order derivative operator. The usual approximation is based on a centred formula:

$$f''(x_i) = \frac{f(x_{i+1}) - 2f(x_i) + f(x_{i-1})}{h^2} + O(h^2), \tag{4}$$

which implies that each row of the $\mathbf{D}_2$ matrix is the constant vector $[1,-2,1]$.

A different approximation formula to the derivative, which does not imply uniform sampling, can also be obtained by Taylor expansion with nonuniform increments. After some algebra,

$$f''(x_i) = 2\frac{f(x_{i+1})(x_{i-1} - x_i) - f(x_i)(x_{i-1} - x_{i+1}) + f(x_{i-1})(x_i - x_{i+1})}{(x_{i-1} - x_i)(x_{i-1} - x_{i+1})(x_i - x_{i+1})} + O(h), \tag{5}$$

where h is now the maximum local grid spacing.

The rows of the operator now explicitly depend on the x values as desired:

$$\left[\frac{2}{(x_{i+1} - x_{i-1})(x_{i+1} - x_i)}, -\frac{2}{(x_{i+1} - x_i)(x_i - x_{i-1})}, \frac{2}{(x_{i+1} - x_{i-1})(x_i - x_{i-1})}\right]. \tag{6}$$

The operator is denoted by the symbol $\hat{\mathbf{D}}_2$.

An efficient implementation of the above algorithm (MATLAB) is the following:

```
T = length(x);
id = 2 : (T − 1);
idp1 = id + 1;
idm1 = id − 1;
V1 = 2./((x(idm1) − x(idp1)). *(x(id) − x(idp1)));
V2 = −2./((x(idm1) − x(id)). *(x(id) − x(idp1)));
V3 = 2./((x(idm1) − x(id)). *(x(idm1) − x(idp1)));
D2hat = spdiags ([V1, V2, V3] \ V1(1), [0 : 2], T −
2, T);
L = chol(speye(T) + sigma ^2 *D2hat′ *D2hat, 'low-
er');
z_stat = z − L′ \ (L \ z);
```

Note that to reduce the possibility of numerical instabilities in the solution of the linear systems, the D2hat matrix is normalised by the first element of vector V1.

4. Equivalent Kernel and Smoothing

The operation of the smoothing priors can be understood by looking at the following simplified form:

$$y = \mathbf{H}z, \tag{7}$$

where z is the vector of data and $\mathbf{H}$ is the matrix coefficient of (3). The smoother acts as a linear filter.

Since each element of z and y can be thought of as placed at a distinct time point, it is seen that each row of the $\mathbf{H}$ matrix acts over all the elements of z to produce a single element of y. Consequently, the filter is noncausal. In fact, each row of $\mathbf{H}$ defines a *weighting function*. Each weighting function is localised around a specific time, and its bandwidth determines how many samples from the past and from the future contribute to the estimate. The wider the weighting function, the smoother the resulting estimates.

In the case of uniformly sampled data, the weighting functions have the same shape (except at the boundaries), which can be imagined as a sliding window translating in time: this is a consequence of the definition of the $\mathbf{D}_2$ operator, which is time independent. Figure 1 shows some weight functions implied by the $\mathbf{D}_2$ operator.

However, for the case of arbitrarily (irregularly) sampled data of the HRV tachogram, the $\hat{\mathbf{D}}_2$ operator actually depends on time; therefore the weighting functions will take on a different shape. This makes the resulting filter effectively a time-variant filter. It is possible to calculate the transfer function of the filter $\mathbf{H}$ in the limit as the number of data points tends to infinity. It can be shown [2] that the (non-stationary) spectral density of the Gaussian process prior is

$$S(f) \propto \frac{1}{4\pi^2 f^4}. \tag{8}$$

From the above, the power spectral density of the equivalent kernel filter is derived as

$$h(f) = \frac{1}{\sigma^2 4\pi^2 f^4 + 1}. \tag{9}$$

In Figure 2 it is shown an example of the transfer function of the equivalent kernel filter (with $\sigma^2 = 1$): the phase is constant zero.

5. Estimation of the Filter Bandwidth

Although the approximation in (9) is only valid in the limit as the number of data points goes to infinity, it is still useful for calculating the approximate -3 dB bandwidth of the finite-sample approximation of the filter in terms of the smoothing parameter σ^2. Whereas the SPA as presented [7] does not provide an effective bandwidth estimate but only the qualitative behaviour of the filter, the following approximation provides a quantitative tool.

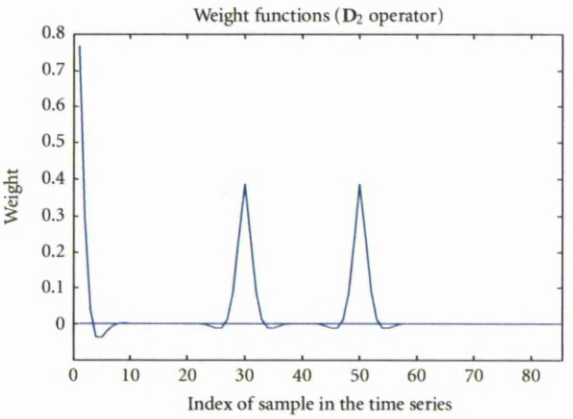

FIGURE 1: Weight functions (*viz.* $\mathbf{D}_2$ operator).

TABLE 1: approximation of -3 dB point [Hz].

True -3 dB cut-off frequency	Approximate frequency
0.05	0.049
0.1	0.102
0.2	0.208
0.3	0.34

Inverting (9) and applying the bilinear transformation of the continuous frequencies, we get

$$\sigma^2 = \left(\sqrt{2} - 1\right)\left(2\tan\left(\frac{\omega_c \pi}{2}\right)\right)^{-4}, \tag{10}$$

where ω_c is the normalised cut-off frequency (namely, the Nyquist frequency = 1).

Since the number of data points mostly impacts the estimation of low frequencies, the expectation is that the approximation is good in the low-frequency range.

In a Monte Carlo simulation, 1000 replications of the Welch periodogram estimates were made of white Gaussian noise coloured through the equivalent filter $\mathbf{H}$. Each noise sequence was composed of 5000 regularly spaced samples. In Table 1, it is seen that this approximation is good and, predictably, deteriorates as the cut-off frequency increases.

Figure 3 shows the transfer function of the digital equivalent kernel filter.

There is very little phase distortion, except at very high frequencies close to the Nyquist frequency.

6. Illustrative Performance with Synthetic and Real Data Sets

A synthetic data set of was generated (MATLAB) as series of normallydistributed random numbers of mean 0.85(1) s (equivalent to a heart rate of ~75 bpm) and std 0.025 s: this was low-pass filtered at 1 Hz (3rd-order phase-less IIR).

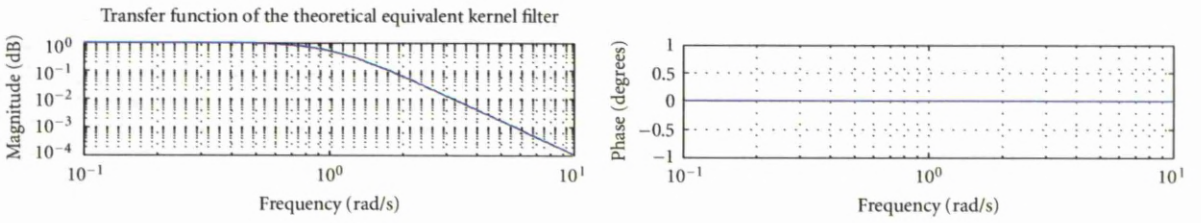

FIGURE 2: Bode plot of theoretical transfer function of equivalent kernel filter.

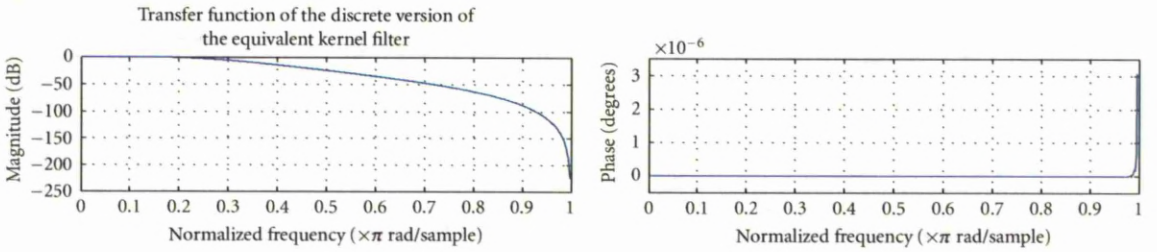

FIGURE 3: Bode plot of discrete transfer function of equivalent kernel filter.

These data were projected by interpolation, onto an irregular time axis of mean interval 0.86(1) s and variance $0.01s^2$. The resulting synthetic HRV record, as a time record of band-limited Gaussian noise, was of 30 s duration, average sampling frequency of 1.15(6) Hz and had no significant power above 1 Hz.

Clinical ECG data from a Lead II configuration were recorded from a healthy adult seated for a period of 60 minutes using a Spacelabs Medical Pathfinder Holter system. RR intervals were available with 1 ms resolution.

The time domain and frequency domain (as the Lomb Scargle periodogram) representations of the synthetic data set and the clinical data set are shown in Figure 4 to illustrate the band-pass filtering effect achieved using sequential SGP. The synthetic HRV data and the clinical HRV data are filtered in the band-pass [0.025 · · · 0.5] Hz and [0.025 · · · 0.35] Hz, respectively.

7. Internet Resources and Open-Source Code

Resources relevant to this work are located at http://clinengnhs.liv.ac.uk/links.htm and include the following.

(1) A website demonstration of SGP running on an automation instance of MATLAB 2008a. Developed for JavaScript-enabled MS IE6+ and FireFox browsers.

(2) MATLAB open-source code:

(i) Smoothing by Gaussian process Priors (SGP): gpsmooth_3.m,

(ii) Optimized Lomb Scargle Periodogram (fLSPw: *fastest Lomb Scargle Periodogram in the West*): fLSPw.m.

8. Conclusion

The SGP (Smoothing by Gaussian process Priors) algorithm is a second-order response time-varying filter which operates on irregularly sampled data without compromising low-frequency fidelity. In the context of Heart Rate Variability analysis, it provides detrending (high-pass) and low-pass filtering with explicitly specified −3 dB cut-off points. A small limitation is the implicit requirement to assume a *representative* sampling frequency to establish the frequency interval: here this is taken as the reciprocal of the median sampling interval. The SGP MATLAB code is available as open source via a comprehensive website and is directly compatible with an optimised implementation of the Lomb Scargle Periodogram (fLSPw) estimator.

Appendices

A. Gaussian Process Interpretation of Smoothing Priors

Consider the posterior expectation of a GP regressor (2) at a set of training data points z:

$$\hat{y}_\sigma = \mathbf{K}(\mathbf{K} + \sigma^2\mathbf{I})^{-1}z, \tag{A.1}$$

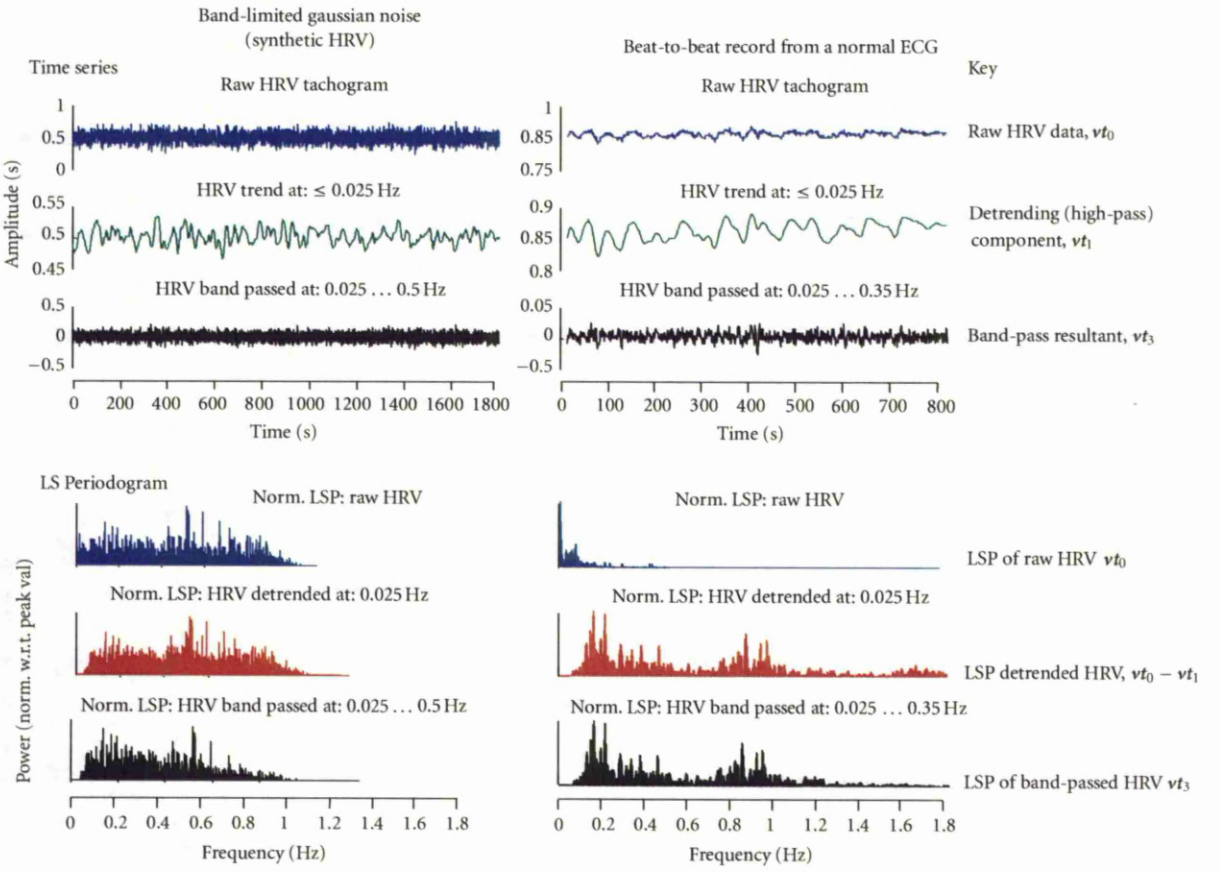

FIGURE 4: Synthetic and clinical HRV records band-pass filtered by sequential application of SGP: raw data vt_0 "smoothed" to give vt_1; $vt_2 = vt_0 - vt_1$ (not shown); vt_2 "smoothed" to give vt_3. Lomb Scargle Periodograms (LSPs) are for vt_0, vt_2, and vt_3.

where $\mathbf{K}$ is the covariance matrix of the GP y and σ is the standard deviation of the white (Gaussian) noise corrupting the data z. By algebraic manipulation of (A.1), it follows:

$$\hat{y}_\sigma = \left[(\mathbf{K} + \sigma^2\mathbf{I})\mathbf{K}^{-1}\right]^{-1} z \equiv \left(\mathbf{K}\mathbf{K}^{-1} + \sigma^2\mathbf{K}^{-1}\right)^{-1} z \equiv \left(\mathbf{I} + \sigma^2\mathbf{K}^{-1}\right)^{-1} z. \tag{A.2}$$

Comparing the above with (3),

$$\mathbf{D}_d^{\mathrm{T}}\mathbf{D}_d = \mathbf{K}^{-1}. \tag{A.3}$$

The above derivations show some important facts about the solution of the problem.

(1) The parameter σ describes the amount of (Gaussian) white noise, which affects the data. As σ gets smaller, the filtering process gets smoother.

(2) The smoothness properties of the resulting estimator depend not only on σ, but also on the choice of the covariance matrix $\mathbf{K}$. Note that polynomials *up to* (and including) 1st degree are in the null space of the regularization operator (i.e., they are both mapped to constants), which means that they are not penalized at all. This implies that the Gaussian Process prior is not stationary (see Appendix B for a definition).

B. Stationarity

A Gaussian process is completely described by its mean function and covariance function. Given a real process $f(t)$, these functions are specified as the following expectations:

$$m(t) = \mathrm{E}[f(t)],$$
$$k(t,t') = \mathrm{E}\left[\left(f(t) - m(t)\right)\left(f(t') - m(t')\right)\right]. \tag{B.1}$$

For a fixed t, $f(t)$ is a Gaussian random variable with mean $m(t)$ and variance $k(t, t)$, so that a Gaussian process can be defined as a collection of random variables, any finite number of which have a joint Gaussian distribution.

A stationary covariance function is a function of $t - t'$, that is, it is invariant to translations. The above definitions can be used to define stationarity for Gaussian processes. A process which has constant mean and whose covariance function is stationary is called *weakly stationary* (or *wide-sense stationary, WSS*). A process whose joint distributions are invariant to translations, that is, the statistics of $f(t)$ and $f(t + c)$ are the same for any c, is called *strictly stationary* (or *strict-sense stationary, SSS*). It can be shown that as SSS process is also WSS, and if the process is Gaussian, then the converse is also true.

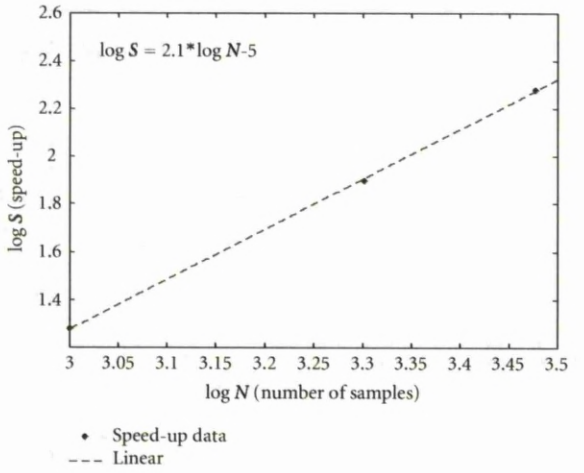

FIGURE 5: Speed-up of SGP over SPA with increasing data set size.

If any of the above conditions are violated, then the process is non-stationary; an example is the Gaussian process whose inverse covariance matrix is given by (4) and (5).

C. Improving the Speed and Stability of the SPA Smoothing Process

In general, matrix inversion is very computationally expensive and should be avoided whenever possible A more efficient solution uses the *backslash* operator \, which in MATLAB implements the solution of a linear system by Gaussian elimination. However, the matrix $(\mathbf{I}+\sigma^2\mathbf{D}_2^{\mathrm{T}}\mathbf{D}_2)$ can be nearly singular and ill conditioned, depending on values of the parameter σ^2. To circumvent this risk, the lower Cholesky factor $\mathbf{L}$ (the *square root*) of this matrix is derived, so that

$$\mathbf{L}\mathbf{L}^{\mathrm{T}} = \left(\mathbf{I}+\sigma^2\mathbf{D}_2^{\mathrm{T}}\mathbf{D}_2\right). \tag{C.1}$$

With this decomposition, matrix inversion can then simply be written as the solution, in sequence, of two triangular systems of linear equations, which is a very fast and numerically stable operation:

$$\hat{y}_\sigma = \mathbf{L}^{\mathrm{T}} \setminus (\mathbf{L} \setminus z). \tag{C.2}$$

Although the theoretical computational complexity of straight matrix inversion and the above (seemingly more complex) steps is the same, the *hidden factors* of the actual numerical computations make a very significant difference [9]. The speed-up is illustrated by performing the above computations on a sequence of varying length (from 1000 to 3000 samples), repeating the execution of both algorithms 100 times. Figure 5 shows the speed-up as a function of the data set size.

It is clear that, as the dimension of the data set increases, the speed-up increases quadratically, showing the inefficiency of the matrix inversion-based smoothing. The following code (MATLAB R006b) was used:

```
T = length(z);
D2 = spdiags(ones(T - 2,1) *[1 -2 1, 0 : 2], T -
2, T);
L = chol(speye(T) + sigma ^2 *D2' *D2, 'lower');
% warning: potential bottleneck!
z_stat = z - L' \ (L \ z);
```

It should be noted that in MATLAB R2006a, and possibly previous versions, multiplication of the σ^2 coefficient by the sparse matrix is anomalously a very slow operation.

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