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CHARACTERIZATION OF AUTONOMIC AND CARDIORESPIRATORY CONTROL IN NEWBORN POPULATIONS AT RISK FOR SUDDEN INFANT DEATH SYNDROME

Doctoral Dissertation of:
Maristella Lucchini

Advisor:
Prof. Maria Gabriella Signorini

Co-Advisor:
Prof. William P. Fifer

Tutor:
Prof. Pietro Cerveri

Supervisor of the PhD Program:
Prof. Andrea Aliverti

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ABSTRACT

Sudden Infant Death Syndrome (SIDS) is defined as a sudden and unexplained death of an infant younger than 1 year of age. Although infrequent, SIDS is still the most common cause of infant death between one month and one year of age in developed countries. Thanks to epidemiological, animal and pathophysiological studies, possible risk factors and mechanisms leading to this death are more understood, nonetheless number of deaths has reached a plateau in the last decade and no reliable quantitative tool to assess risk exists.

The purpose of this Ph.D. thesis is to employ novel signal processing methodologies in order to accurately estimate the effect of several risk factors on the autonomic and cardiorespiratory control in newborn infants. In particular, the effects of sleep states, sleep position, prematurity and exposure to alcohol and smoking during pregnancy are investigated.

The original contribution of this thesis centers on the utilization of noninvasive methodologies, to analyze physiological signals routinely acquired in hospital and home settings. Moreover, the approach to signal analysis incorporates complex system generation and interaction, with a systemic view of the cardiorespiratory physiology. Different modes of interactions were explored, ranging from amplitude to phase modulation.

Parameters proposed proved to be capable of characterizing multiple autonomic profiles, providing information in line with pathophysiological findings. Results highlighted sleep position effects on autonomic control, with less parasympathetic activity in prone position, especially at 2 months of age. Prematurity greatly influenced the cardiorespiratory coupling, both in terms of strength and directionality, with weaker respiratory drive associated with prematurity. Lastly, neonates exposed to smoking and alcohol during pregnancy showed a blunted autonomic response when exposed to the autonomic challenge of head up tilt.

In conclusion, the methods and results of this Ph.D thesis show the utility and power of complex and multivariate signal processing techniques in the investigation of an enigmatic syndrome such as SIDS, where multiple organs and their control systems are involved.

SUMMARY

Introduction

Sudden Infant Death Syndrome (SIDS) is defined as a sudden and unexplained death of an infant younger than 1 year of age. It occurs in an infant considered healthy and it remains unexplained even after autopsy, a careful examination of the death scene and a review of the clinical history [1]. Although infrequent, SIDS is the most common cause of infant death between one month and one year of age in developed countries, with around 2000 death a year only in the United States [2].

Thanks to the information from epidemiological, animal and pathophysiological studies, we have now knowledge of risk factors and have formulated hypotheses regarding the underlying mechanisms. Currently, the most supported theory is the triple risk model proposed by Filiano and Kinney in 1994 [3]. The model views SIDS as the result of the simultaneous occurrence of three factors: an underlying vulnerability (i.e. prematurity, genetic abnormalities, etc.), a critical developmental period in homeostatic control (i.e. the first year of life), and exposure to exogenous stressor(s) (e.g. newborn placed in a prone sleep position, overheating, inadequate oxygen supply etc.).

One crucial aspect in this and many other models proposed is the involvement of autonomic control circuits and their role in cardiorespiratory regulation. Brainstem alterations and altered serotonin receptors were found in SIDS cases autopsies [4], [5], and altered heart rate (HR), breathing and arousing patterns were identified in vulnerable populations and conditions, such as prematurity and prone sleeping [6], [7].

Nonetheless, currently there is no marker for risk stratification nor measures to quantify the impact of the identified risk factors on autonomic development. This is even more relevant given that in the past

10 years death rates have reached a plateau, with no major further decline since the successful Back to Sleep campaign [8].

Thus, the principal purpose of this Ph.D. thesis is to provide quantitative and noninvasive tools to evaluate the impact of several risk factors on the functionality of cardiorespiratory and autonomic control. The ultimate objective is to provide noninvasive and early markers to assess risk.

The tools employed are time series methods and analysis of neonatal HR and breathing, obtained from noninvasive collection in hospital. Heart rate variability (HRV) was a primary direction given the plethora of publications linking risk with HRV measures and sympatho-vagal autonomic activity. Given the relevant role of the cardiorespiratory interaction in maintaining homeostasis and responding to variable physiological demands, in our analysis we added breathing and its interaction with HRV to provide a more complete approach.

The research was conducted under the guidance of several collaborators from two institutions, the Politecnico di Milano and the Columbia University Medical Center. Datasets for the analyses were used in collaboration with Drs. Rakesh Sahni and Nina Burtchen, and the PASS Research Network. The design and realization of the analytic and statistical part were developed under the supervision of Professors William P. Fifer, Michael M. Myers and Maria G. Signorini.

Advanced methods of analysis.

The autonomic nervous system (ANS) is a part of the peripheral nervous system that acts to control physiology of many bodily functions. It is divided in two branches, sympathetic and parasympathetic, acting synergistically for the regulation of physiology and behavior crucial for survival. Primary autonomic functions include the regulation of HR, blood pressure, rate of respiration, body temperature, sweating, gastrointestinal motility and secretion. One of the most interesting function relevant to this thesis is the control over the heart muscle: sympathetic fibers increase HR, atrioventricular conduction, and contractility of cardiac muscle, while they dilate the coronary arteries. In contrast, parasympathetic nerves slow down the HR and reduce heart contractility, favoring conservation of energy. These effects are integrated with several other factors, such as the spontaneous contractility of the heart itself, the peripheral resistance of the vascular tree, the effects of circulating hormones and the metabolic supply to the heart.

This complex interplay generates a range of heart rate variability (HRV) patterns, which reflects ANS mediated change in the time intervals between adjacent heartbeats. For this reason, HRV has been for long time considered a powerful tool, providing a window to observe non-invasively the interaction between the sympathetic and parasympathetic nervous systems and their capability to respond properly to internal and external challenges [9]. HRV is also influenced by respiration; the neuronal control of breathing and HR are closely linked, functionally as well as anatomically. The close interaction between cardiac and respiratory control is critical for survival. This synergy is crucial for the homeostatic regulation of blood gases, and essential for regulating central nervous functions, such as arousal [10], [11].

Thus, breathing and HR are the output of a complex network of controlling mechanisms, which constantly adapt to the ever-changing need of the organism. Since the 1960s, many efforts have been spent to describe the systems behind these signals, starting with time domain and following with frequency domain approaches [12]. Nonetheless, the complex origin of these signals often makes traditional linear signal processing approaches unsuitable or only partially capable of characterizing the systems generating the data. More parameters coming from nonlinear chaos theory and the information theory can provide alternative approaches to extract meaningful information and describe the behavior of the systems generating the data under analysis [13]. Furthermore, advanced bivariate techniques have been proposed in order to highlight different types of interactions among systems and unveil their relationships.

Previous studies had addressed the questions of autonomic and cardiorespiratory regulation in the context of SIDS, but mostly employing traditional spectral techniques to asses cardiac and respiratory functioning and their interaction [14]–[17]. One of the objectives of this thesis is to implement complex signal processing methods to characterize newborn HRV and cardiorespiratory activity. For this reason, we selected entropies and phase rectified signal averaging (PRSA) techniques, given previous positive applications in adults, newborns and fetuses.

In 1993, Pincus was the first to introduce a practical measure of complexity, namely Approximate Entropy (ApEn), showing that infants who had an apparent life threatening event presented greater ApEn instability across quiet sleep than any normal infant exhibited, with incidents of extremely low values [18]. Following this work, the group of Moorman applied a modified version of ApEn, called Sample Entropy, to detect sepsis in newborns [19]. These entropy measures aim to quantify the

regularity, defined as the presence of repetitive patterns in a time series within a certain tolerance *r* and at different lags [19], [20]. They provide entropy estimation indices even with relatively short segments and without making any assumptions about the underlying structure of the system. A challenging problem for entropy estimation is the choice of the tolerance *r*, to assess if two patterns could be defined similar. In 2006, Lake proposed a new parameter called quadratic sample entropy (QSE) [21]. It removes the dependency on *r*, which can be optimally varied for each data record. One way to optimally vary *r,* is that of setting a minimum number of matches (MCM), while minimizing *r*, in order to have a stable and consistent entropy estimate. We also discuss ways to optimally select the MCM.

Another approach for nonlinear characterization of HRV is PRSA, which offers the possibility to analyze separately HR accelerations and decelerations and select parameters to specify the frequencies of interest. This affords the opportunity to investigate rapid parasympathetic influences as well as slower sympathetic ANS mechanisms. This technique requires relatively long recordings since it is based on averaging many segments in order to discard irrelevant data. It was applied successfully in the identification of intra uterine growth restricted fetuses and detection of cardiovascular risk in adults [22], [23].

Our second objective was to characterize and quantify the mutual influence of cardiovascular and respiratory rhythms. Many contributions were found in the literature, ranging from cross-spectral analysis to nonlinear methods, e.g., mutual information or time delay stability [24], [25]. These include linear and nonlinear relations between HR and respiration signal. Nonetheless, a limitation of all these techniques was that they did not measure the directionality of the relationships, and thus, they could only partially reveal the underlying interacting mechanisms responsible for the changes in complexity, especially when knowledge of the underlying physiology was limited.

Transfer Entropy (TE) was developed to address precisely this issue. Its focus is on tracking the information flow between two systems. Specifically, TE can enhance the quantification of the directional coupling between respiration and HR in order to incorporate both sympathetic and parasympathetic regulatory influences. The main advantages of this method are that it captures both linear and nonlinear contributions to information flow, and it can differentiate the directionality of transfer. Applying TE on shifted signals we can also evaluate how long the effect of a system on the other lasts and thus inquire their relationships at different time scales. This is relevant since we know

that different time scales might reflect the effect of different autonomic branches on the cardiac regulation.

Another approach is that of describing the cardiorespiratory coupling as a dynamic synchronization process, meaning an interaction between two subsystems which can be modeled as two weakly self-sustained chaotic oscillators [26]–[28]. The basic idea is that given two weakly coupled systems, the amplitude of their oscillations may remain uncorrelated whereas their phases do mutually perturb each other. With this assumption, it becomes possible to investigate cardiorespiratory synchronization by means of a phase analysis of RR series and respiratory signal with a method called Phase Locking, rather than applying a classical amplitude analysis. The behavior of the cardiorespiratory system can be seen as synergetic, i.e. a multi-stable system switching between several phase configurations, with a preference for a specific set of phase relations, which can be seen as attracting frequency ratios [29]. In this context, Rosenblum et al. also proposed a method which exploits the notion of phase synchronization of irregular oscillators in order to reveal whether the interaction is bi- or unidirectional and to quantify the degree of asymmetry in the systems' coupling [30].

Interestingly, different modes of interaction are not exclusive, rather they may simultaneously coexist, representing different aspects of neural regulation and acting on different time scales [31], [32].

The performance of these novel techniques were compared with that of traditional approaches, proposed by the Task Force of 1995 [33]. Specific guidelines for newborns are lacking, and their different degree of maturation has a big impact on many variables. As a matter of fact, newborns' HR is double adults' HR. For this reason, we utilized three minute segments, which contain roughly the same number of beats as the five minutes, usually used for adults. Additionally, we tested the performance of fetal parameters, given the developmental overlap in timing of nervous system development, especially for premature populations. Parameters estimated for the time domain include mean RR, Standard Deviation of Normal to Normal RR intervals (SDNN) and Root Mean Square of the Successive Differences (RMSSD), Long-Term Irregularity (LTI) and Short-Term Variability (STV). Spectral and cross-spectral analyses were also performed, extracting areas under the curve (signal variance) for Low and High frequency bands.

These techniques were employed to quantify how SIDS risk factors may alter cardiorespiratory interaction and autonomic activity. In particular, we investigated the influence of sleep state, sleep

position, prematurity and the combined effect of prenatal smoking and alcohol exposure on baseline physiology and a physiological challenge, i.e. the head up tilt. These conditions were chosen to cover both intrinsic and extrinsic risk factors, as proposed by the triple risk model, as well as their interaction.

HRV and Cardiorespiratory Analysis Results

As a preliminary step, we decided to address the question of how sleep states affect cardiorespiratory measures, in particular the novel measures introduced in this study, in order to be able to account for this influence in larger databases and following studies. From the literature we know that HR and its long term variability, measured by parameters such SDNN, are generally higher in active sleep (AS), while short term variability, for instance measured by RMSSD, is higher in quiet sleep (QS). These findings have been attributed to the increased vagal activity in QS and increased body movement and sympathetic activity in AS. With respect to the quantification of cardiorespiratory interaction as a function of sleep state, QS generally presents higher values of coherence at high frequencies (HF), indicating well established linear coupling in that frequency range.

We tested the *effect of sleep state* on complex measures of cardiorespiratory coupling in 151 full term newborns studied at birth and at one month of age [34], [35]. Entropy values (SampEn and QSE) were higher in QS. TE values showed that in QS coupling is stronger both with HR driving breathing and vice versa. Moreover, only in QS information flow from in the direction of respiration to HR was dominant. TE implemented on shifted signals showed how different directionalities of interaction act on different time scales, probably driven by the different branches of the ANS.

Our results indicate that, while QS generally presents lower global HRV, higher complexity values were observed probably due to increased interactions among physiological systems, as higher TE values seem to indicate. Moreover, a difference in directionality balance based on sleep state was observed, which could be driven by differences in the average breathing frequency. That is when breathing frequency is higher there is less opportunity for the respiration to dynamically modulate HR. Higher breathing rates occur more often in AS, and this could account for an absence of a dominant directionality. With respect to the cardiorespiratory synchronization, our findings showed a significantly higher value both in percentage of time spent in phase-coupling and in length of the coupled epochs in QS, both at the newborn and one-month stage.

When looking at the parameters behavior as a function of age, time domain parameters showed an increase in HR and a decrease in variability. In TE the major differences occurred in QS, with an increase in information flow in both directions with age. Moreover, the ratio of the dominant synchronization shifted from a majority of 3:1 (3 beats in 1 breath) in newborns, to 4:1 (4 beats in 1 breath) in one-month infants. This is of particular interest, since the peak of SIDS incidence is between 2-4 month of age.

Following the characterization of autonomic and cardiorespiratory activity by state, we addressed the effects of risk factors in three populations.

First, we investigated the *effect of position during sleep in a group of 35 premature infants*, with data acquired prior to discharge from the hospital (gestational age (GA) at birth 28.7 ±2 weeks and post menstrual age at time of study 37.7 ±2.4 weeks). Twenty four of these infants were studied again two months after discharge (GA at birth 27.9 ±1.6 weeks and post menstrual age at time of study 49.2±3.2 weeks) [36]. All infants underwent the same study protocol, which included baseline sleep recordings in both prone and a baseline in supine positions. ECG was acquired at 500 Hz and RR series were extracted.

Analysis by sleep position in the first study showed clear differences both in the long-term variability parameters (LTI and PRSA) and in the short-term variability parameters (SampEn and QSE), with supine position being characterized by higher variability but lower complexity. At 2 months of age, sleep position influenced mostly short-term variability parameters, specifically RMSSD and STV, and entropies, all parameters generally associated with parasympathetic regulation. With increasing post-natal age, infant parasympathetic control should dominate HR autonomic regulation: the fact that at 2 months of age measures of vagal activity were found to be diminished in prone position might suggest a suppressive effect of this position, changing the sympatho-vagal balance.

Next, we addressed the *effect of prematurity in a dataset of 329 subjects*, from 35 to 40 weeks GA [37], [38]. Infants were tested 12 to-84 hours after delivery, with a 10-minute baseline recording of ECG and respiration, at 500 Hz and 200 Hz respectively. Following the guidelines of the American College of Obstetricians and Gynecologists, infants were divided into three groups: newborns whose GA was (1) 35-36 weeks, labelled Late Preterm (LPT); (2) GA 37-38 weeks, Early Term (ET) and (3) GA 39-40 weeks, Full Term (FT). Results showed increasing mean RR intervals, short-term HRV (RMSSD), HR complexity

(QSE) and linear cardiorespiratory coupling (spectral HF content) as a function of GA, indicating a significant increasing cardiorespiratory coupling and autonomic control as a function of GA at birth. Measures of cardiorespiratory coupling and directionality indicated no relationship between time spent in phase-synchronized state and GA group, but GA at birth influenced significantly directionality of interaction. In QS all the three GA groups showed the dominant influence of breathing on HR, and this relationship grew with GA. In AS a balanced relationship was present in LPT and it moved toward a dominant relationship from breathing to HR in FT.

Mrowka et al. had hypothesized that directionality could depend on respiratory frequency. In particular, respiration rate would act as a low pass filter, i.e. below a set respiratory frequency (proposed to 0.6 Hz) directionality would mainly be from respiration to HR, whereas above it the interaction would become bidirectional. We tested this hypothesis in our dataset and found the occurrence of this bimodal influence: concurrently with breathing frequency <0.6 Hz, an increased polarization toward values of directionality from breathing to HR occurred, especially in ET and FT. Nonetheless, the mean breathing frequency did not change significantly in the GA window analyzed (35-40 weeks); thus, the significant change in directionality with GA at birth could not be explained solely by breathing frequency. Our explanation for this phenomenon is that the threshold for the low pass filter effect is still adapting between 35-40 weeks GA. At a younger GA, for instance LPT, the value for the cutoff frequency might be lower when compared to more mature conditions, such as FT. Given that this cutoff frequency is potentially related to vagal nerve regulation, these findings would be consistent with previous studies showing immature vagal function in premature infants. Further confirmation of this hypothesis comes from the fact that we found directionality to be negatively correlated with measures of parasympathetic activity.

Lastly, the *effects of prenatal alcohol and smoking exposure on tilt response in newborn infants* were investigated in a population of 28 subjects (GA 39 ±1 weeks) , enrolled in the Safe Passage study [39]. Data was acquired within 48-96 hours of birth, with ECG and breathing for 10-minutes baseline period and a 45° head-up tilt, while the infant was sleeping prone. The tilt session was divided in: 30-sec just prior tilt (B); 15-seconds block right after the infant reached head-up position (I); three per 30-seconds blocks (T1, T2, T3) before returning to flat position. A modification of the Timeline Follow-Back Interview was employed to guide mothers' self-reported estimation of their tobacco and alcohol consumption during pregnancy [39]. Based on the assessment, subjects were divided in a control group (no prenatal

exposure to alcohol or tobacco smoke), an exposed group (heavy prenatal exposure to both substances during the first, second or third trimester of pregnancy).

Graded head-up tilt has been widely used as a test to observe and quantify autonomic control. The tilt should elicit a shift of the sympatho-vagal balance toward a sympathetic activation and parasympathetic withdrawal. In this protocol, breathing signals were of very low quality due to movement during tilt, so the analysis focused on HR parameters. It was decided to estimate vagal withdrawal with RMSSD, HF power, QSE and RSA amplitude, all parameters which had been linked with vagal activity by previous literature [40], [41]. This expected behavior was displayed by the control group, with decreases of all the parameters selected after tilt, in particular at T2. The exposed group instead showed a blunted response, with no significant differences across the blocks.

In literature, prenatal exposure to smoke has been reported to effectively impair receptors in the medullary 5-HT system, as well as alter in cardiorespiratory control mechanisms and induce abnormalities in the pathogenesis of the parasympathetic systems [42]. It is possible to speculate that the illustrated difference in vagal activity comparing the control and the exposed groups may effectively arise as a consequence of prenatal exposure and may contribute to the decreased infant's ANS capability to maintain homeostatic control when exposed to a direct physiological challenge.

From a methodological perspective, an important finding was that we showed that the introduction of QSE calculated with the Minimum Count of Matches (MCM) approach led to a significant improvement in entropy estimation because QSE was less dependent on the length of the segment analyzed and this technique allowed to adjust the tolerance *r* in order to obtain more stable and consistent results. Moreover, QSE was less influenced by artifacts than other traditional measures, and thus could be applied with minimal preprocessing, with great advantage in real time applications. Secondly, we showed the usefulness of estimating PRSA curves by incorporating adjustments to the parameter T and, thus, vary the upper frequency limit of the detectable periodicities. TE implemented on shifted signals was useful to assess systems memory when interacting with each other.

Lastly, we compared measures of vagal activity on short segments and showed that even when parameters are highly correlated they are not interchangeable due to different statistical characteristics.

Conclusions

The main purpose of this Ph.D. thesis was to characterize autonomic and cardiorespiratory regulation in newborn populations at risk for SIDS, who are particularly vulnerable to failure of the autonomic and cardiorespiratory control systems. We showed how complex parameters and techniques for bivariate analysis of breathing and HR provide additional information with respect to traditional techniques in the description of how physiological systems dynamically interact to maintain an optimal health status. Sleeping position, prematurity and exposure to smoke and alcohol during pregnancy all impaired physiological control, generally leading to an altered sympatho-vagal balance and diminished cardiorespiratory interaction.

Results show how traditional indices routinely employed in clinical studies often only scratch the surface of a more complex picture. The proposed novel techniques are advantageous for addressing specific time scales and different modes of interaction for data collected under standard clinical conditions with artifacts and noise. Moreover, these techniques were capable of characterizing systems interactions. This supports our aim to utilize nonlinear advanced parameters to obtain reliable physiological and clinical indices. This approach could lead to a quantitative autonomic profile to assess vulnerability in populations at risk for SIDS. This could grant the possibility of the definition of an "elastic" triple risk model, where the contributions of the various risk factors could be weighted and updated based on infants physical and environmental conditions, hopefully improving predictability and generating novel monitoring solutions.

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1.1. Introduction to Sudden Infant Death Syndrome

Sudden Infant Death Syndrome (SIDS) is defined as a sudden and unexplained death of an infant younger than 1 year of age. It occurs in an infant considered healthy and it remains unexplained even after an autopsy, a careful examination of the death scene and a review of the clinical history [1]. Although infrequent, SIDS is still the most common cause of infant death between one month and one year of age in developed countries, with around 2000 death a year in the United States [2].

Despite years of research, SIDS remains an enigma. Over the past few decades, numerous, world-wide, epidemiological studies have identified factors that appear to contribute to SIDS deaths, a summary of which will be presented in first part of this chapter. A review of the main risk factors interaction and proposed models will be then described, with particular attention to the most well know triple risk model. The last section is focused on the development of sleep, autonomic control and cardiorespiratory interaction in newborns. These elements are all believed to play a crucial role in the cascade of events leading to SIDS.

1.2. SIDS Risk Factors

Multiple epidemiological studies have determined that several factors, both modifiable and not, have significant associations with SIDS, both using case-control and cohort designs. A summary of these factors is presented in Table 1-1, and a detailed description follows.

Table 1-1: Summary of risk factors for SIDS, categorized in two groups. On the left are listed maternal and antenatal risk factors, with infant risk factors listed on the right

Maternal and antenatal risk factors	Infant risk factors
• Smoking, alcohol, illegal drug use • Inadequate antenatal care • Low socioeconomic status • Low maternal age • Single marital status • Low education level • Fetal growth retardation	• Male sex • Age (peak 2-4 months) • Race/ ethnic background (e.g. black, Native Indians, etc.) • Prematurity/low birth weight • Prone/side sleeping • Overheating • Soft bedding • Bed sharing

1.2.1. Socio-Demographic

- SIDS affects infants from all social backgrounds, nevertheless lower socioeconomic status, younger maternal age, lower maternal education level and single marital status are consistently associated with a higher risk of SIDS. For instance, in the United States, SIDS rates are highest for non-Hispanic black and American Indian mothers—2.1 and 1.9 times those for non-Hispanic white mothers, respectively [43]. Following educational campaigns in the last few decades we have witnessed a decline in SIDS across all social and racial groups, but recent trends indicate that there are now even greater social and racial disparities [44], [45].
- SIDS can occur at any time during the first year of age, but approximately 90% of cases happen in the first six months of life, with increased incidence between 2 and 4 months of age, as portrayed in Figure 1-1 [46].
- Boys are 30%–50% more likely than girls to be affected, and this ratio was not influenced by the reduction of SIDS incidence in the last decades, as shown in Figure 1-2 [1], [47].

1.2.2. Pregnancy Related

- Low birth weight, preterm birth, intrauterine growth retardation and shorter intervals between pregnancies affect SIDS incidence [48]–[50].

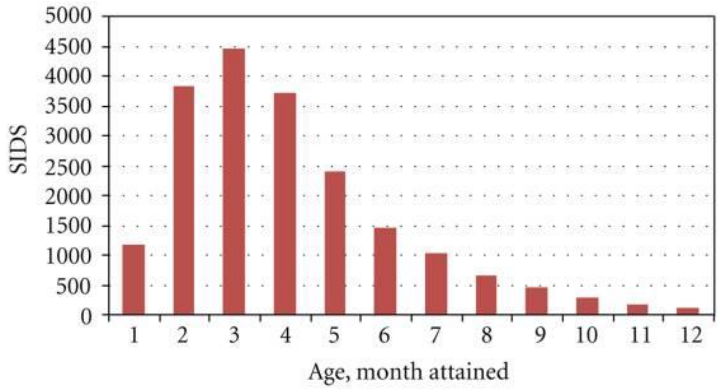

Figure 1-1: Age distribution by month of age from 15 global data sets comprising 19,949 SIDS cases [223]

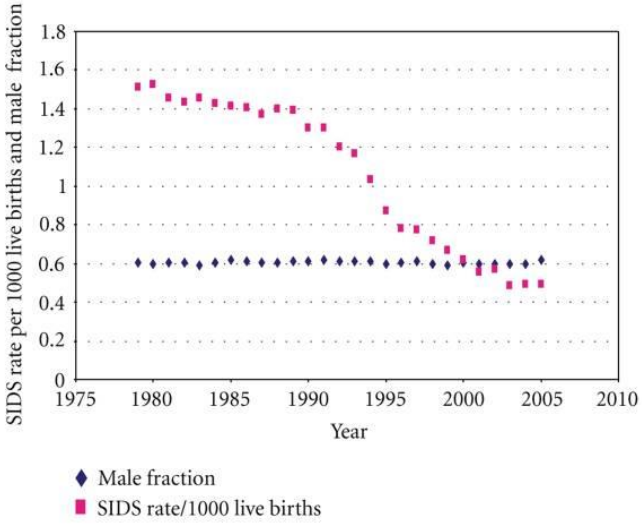

Figure 1-2: US SIDS death count during transition period following the introduction of supine vs prone sleep recommendations, showing that the male fraction remains constant at about 0.61 [222]

- Studies have shown that mothers of SIDS infants generally receive less prenatal care and initiate care later in pregnancy than do mothers of living control infants [47].

1.2.3. Maternal Substance Abuse

- There is a major association between intrauterine and postnatal exposure to cigarette smoking and risk of SIDS. The main limitations are related to the accuracy of self-reported cigarette smoking data and the difficulty in disentangling the independent effects of pre vs postnatal exposure to

environmental tobacco smoke because parental smoking behaviors during and after pregnancy are highly correlated [51], [52].

- The evidence linking prenatal illegal drug use and SIDS is conflicting. Overall, studies do link maternal prenatal drug use, especially opiates, with an increased risk of SIDS ranging from 2- to 15-fold [53], [54].
- Studies regarding the association between SIDS and maternal alcohol use prenatally or postnatally are still inconclusive. In one study of Northern Plains American Indians, periconceptual maternal alcohol consumption was associated with a 6-fold increased risk of SIDS, and binge drinking during the first trimester of pregnancy was associated with an 8-fold increase [55], [56]. It remains to be established whether these effects are primarily attributed to true biological effects, or to sociodemographic and lifestyle factors that co-occur with pregnancy drinking, or a combination and possibly synergistic effect.

1.2.4. Infant Sleep Practices and Environment

- The prone sleep position was noted in multiple case-control studies to be associated with SIDS, as early as 1965 in the United Kingdom. Following numerous public health campaigns in Europe, Australia and New Zealand in the 1980s a decline in SIDS rate occurred. In 1992 the American Academy of Pediatrics (AAP) recommended that healthy term infants be placed to sleep in the non-prone position until 6 months of age. Since the 1992 AAP recommendation and the following 1994 Back to Sleep campaign, the US percentage of SIDS has diminished more than 40% [57].
- Soft bedding such as comforters, has been associated with a 2–3-fold increased risk of SIDS. Combinations of sleep related risk factors result in even higher risk; for example, prone sleeping in soft bedding has been associated with a 20-fold increased risk of SIDS [58], [59].
- Overheating has been associated with increased risk of SIDS based on indicators such as increased room temperature, high body temperature, sweating, and excessive clothing or bedding [60].
- Several studies have suggested bed sharing as a risk factor for SIDS. Earlier case–control studies in England and New Zealand showed a 5–9-fold increased risk associated with bed sharing only among infants of mothers who smoked. More recent studies showed that bed sharing was associated with increased risk of SIDS even when mothers did not smoke or if they breastfed, particularly among younger infants [61], [62].

1.2.5. Genetic

- In postmortem analysis of brainstems of infants who died of SIDS, serotonin receptor abnormalities were found throughout the ventral medulla [5]. Several studies have identified polymorphisms in the promoter region of serotonin transporter (5-HTT) protein gene in infants who have died of SIDS [63].

  Brainstem findings include persistent increases in dendritic spines (indicating neuronal maturational delay) and delayed maturation of synapses in medullary respiratory centers, decreased tyrosine hydroxylase immunoreactivity in catecholaminergic neurons [64].
- Long QT syndrome is associated with sodium- and potassium-channel polymorphisms. Overall, it is estimated that 5%–10% of SIDS cases are associated with a defective cardiac ion channel and hence an increased potential for a lethal arrhythmia [65], [66].
- Genetic studies have identified mutations in SIDS infants pertinent to early embryologic development of the autonomic nervous system [67].

1.3. Hypothesized Mechanisms Underlying SIDS

Given that the definition of SIDS is reliant on the elimination of other causes of death, it is not surprising that there are no conclusive findings on etiology. This has led to a vast number of theories on the mechanisms responsible for SIDS. The population of infants who die of SIDS is likely to represent a mixed population with various etiologies and disease entities contributing to one common endpoint (i.e. death) rather than all deaths being attributed to one single cause [68]. In the following paragraph, the main putative pathways will be described.

1.3.1. Respiratory Function

Respiratory failure has been proposed to contribute to SIDS, given that sleep increases the possibility of airway obstruction and apparent life-threatening events such as prolonged apneas. Furthermore, some studies have shown that SIDS infants present defects in respiratory control resulting in altered respiratory function, prolonged periods of “breath holding”, a failure of autoresuscitation, and defective arousal mechanisms, for example, in response to altered oxygen or carbon dioxide levels [69]. Figure 1-3 illustrates a hypothesized series of events involving respiratory failure leading to SIDS. Nonetheless, studies investigating alterations in breathing patterns or respiratory rates are inconclusive [70]–[73]. Other hypotheses involve peripheral sensory chemoreceptors, including the

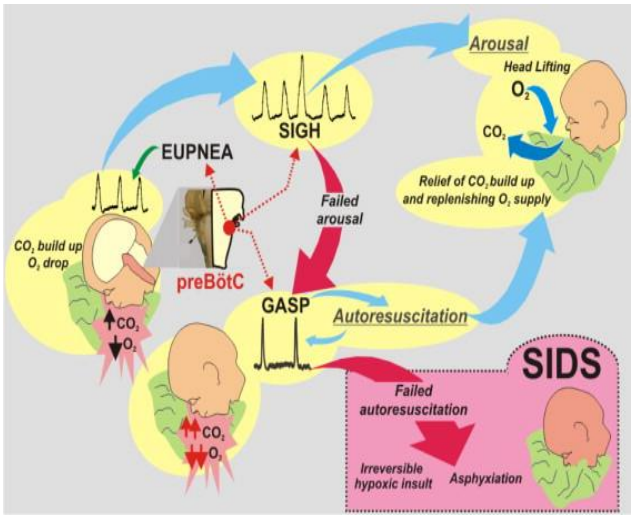

Figure 1-3: During prone sleeping, re-breathing exhaled air can increase CO_2 and decrease O_2 levels. The described mechanism should initiate the arousal response, beginning with sigh generation during continued eupneic breathing. If the arousal is successful, the infant will lift the head and reposition, relieving the build-up of CO2. If arousal fails, a more severe hypoxic state is reached and eupneic breathing will transition to gasping, thanks to the network reconfiguration of the pre-Bötzinger complex (preBötC). Brainstem abnormalities, however, may alter preBötC network and impair sigh and gasp generation which may increase SIDS vulnerability [69].

carotid body, or dysfunction or immaturity in centrally located brainstem networks controlling upper airway functions.

1.3.2. Cardiovascular Function

Extended evidence in SIDS infants including altered heart rate (HR) and heart rate variability (HRV), defects in centrally mediated cardiac control (primarily brainstem centers), autonomic imbalances, prolongation of the QT interval and severe bradycardia has led to the conclusion that arrhythmia and cardiovascular changes are involved in the death in SIDS infants [70], [74], [75]. Nonetheless, inconsistencies in findings make it difficult to confirm a cardiac cause for SIDS.

1.3.3. Nervous System Abnormalities

Nervous system dysfunction has been proposed as a major factor leading to SIDS, potentially due to abnormal development or to maturational delay in particular brainstem regions, given their direct influence over homeostatic processes including cardiorespiratory control, sleep regulation, and arousal. Moreover, the peak period for SIDS, i.e. 2 to 4 months of age, is a crucial time for changes in neural control. Nonetheless, until now the literature has been contradictory as the ability to apply

histological and molecular techniques to examine post-mortem specimens are limited due to rapid deterioration of tissues after death.

Studies over the last 30 years show that the brainstems of infants who died from SIDS exhibit abnormalities in many major neurotransmitters and receptor systems including: catecholamines, neuropeptides, acetylcholinergic, indoleamines (predominantly involving serotonin and its receptors), aminoacids, brain derived neurotrophic growth factors, and some cytokines. A pattern is emerging for particular brainstem and hypothalamus nuclei being consistently affected, including the dorsal motor nucleus of the vagus, nucleus of the solitary tract, arcuate nucleus and raphe [76]. These changes have been attributed to ischemic damage, but many pathological findings overlap with observations from controls [77], [78]. Furthermore, while all these abnormalities have the potential to alter brain function, it should be noted that most of these studies report findings only in a subset of SIDS infants [79]–[81]. Moreover, the reported differences in neurotransmitter or receptor expression between SIDS cases and control cases can further be influenced by factors such as maternal cigarette smoking during pregnancy, highlighting the importance of accounting for these factors when trying to interpret neurochemical changes [4], [82].

Changes in the peripheral nervous system may impact on SIDS as well. Studies have reported histological changes in the carotid bodies in SIDS cases, which may suggest exposure to sustained hypoxemia and have the potential to impact the ability of chemoreceptors to respond adequately to changes in oxygen levels [83]. However, conflicting outcomes regarding carotid body size, histological changes, and the number of neurosecretory granules and transmitter levels have been reported make interpretation of the results challenging.

Lastly, since peripheral and central networks are highly integrated, it is likely that a change in one system may subsequently affect the other, and thus that both processes could contribute to nervous system dysfunction in SIDS.

1.3.4. Immune responses and infectious agents

Presence of a mild cold or upper respiratory infection [84] close to the time of death and findings of markers of infection and inflammation in many SIDS infants have led to the hypothesis that they might be immunologically underdeveloped and that stressors on the immune system may contribute to death [85]. Increased levels of immunoglobulins have been reported in SIDS victims [86], and reports of the presence of viruses (e.g. rhinovirus, cytomegalovirus) and of bacteria in the pathology of SIDS

have strengthened the argument for immune-mediated responses in the pathology of SIDS. However, many of these are also present in control cases, suggesting that their presence may be more co-incidental than causative. Thus, the presence of one, or a combination of, infectious agents is likely to increase the vulnerability rather than being responsible for death in SIDS infants.

1.4. Risk Factors Interactions and Triple Risk Model

It has become evident that the mechanisms of death in infants classified as SIDS involve a complex interplay of individual vulnerabilities with developmental stages and environmental factors, rather than a convenient and simplistic "single cause". In 1970, Bergman was the first to hypothesize that SIDS did not depend on any single characteristic but on an interaction of risk factors with variable probabilities [87]. Not long after, Wedgwood expanded this concept and grouped these risk factors into the first "triple risk hypothesis" consisting of general, developmental and physiological factors [88]. These factors needed to overlap and their synergy to exceed the threshold for survival for an infant to succumb to death. This concept has evolved in time, with many contributions suggesting different definitions and varied emphasis on the various risk factors [89], [90]. These theories culminated in the currently most accepted model in the field, the triple risk model presented by Filiano and Kinney in 1994 [3]. They posit that SIDS results from the simultaneous occurrence of: (1) an underlying vulnerability (i.e. prematurity, genetic abnormalities, etc.) (2) a critical developmental period in homeostatic control (i.e. the first year of life), and (3) exposure to exogenous stressor(s) (i.e.

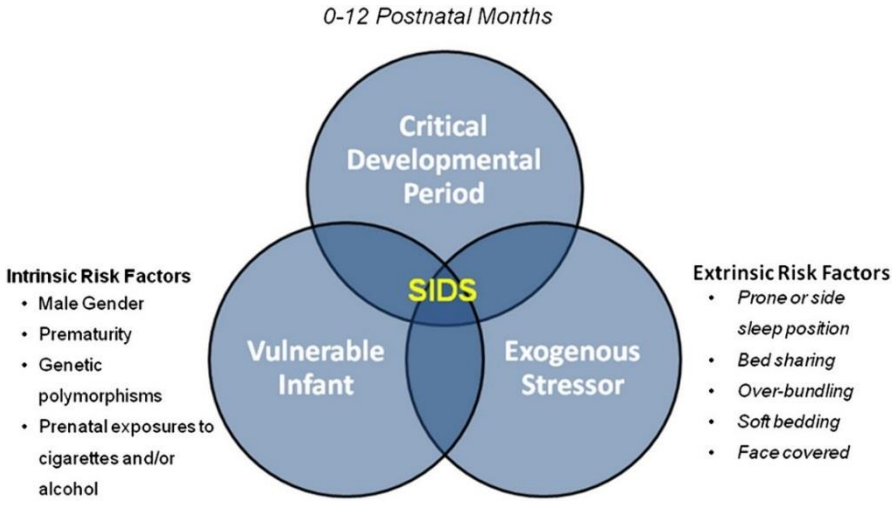

Figure 1-4: The triple risk model for SIDS, presenting the three elements that must be present: 1) baby's vulnerability, 2) critical developmental period, 3) exposure to one or more outside stressor

being placed in a prone position for sleep, overheating etc.). Figure 1-4 provides a schematic representation of this model.

Even if the factors involved may be multiple, the triple risk model does not exclude the possibility that most SIDS deaths might occur with a single common pathway upon which multiple stressors impinge to produce sudden death during the critical period.

1.5. Sleep, Autonomic Nervous System and Cardiorespiratory Control

Even if consensus on the mechanisms leading to SIDS has yet to be reached, a failure of autonomic control of the cardiorespiratory system and an impaired arousal from sleep have been identified as crucial players in the cascade leading to SIDS, and both these factors are heavily influenced by sleep patterns. For this reason, the next section will present an overview on the development of sleep patterns and arousals in newborns, and a summary of the autonomic nervous system structure and functions, with a focus on the regulation and interaction of HRV and breathing

1.5.1. Sleep Development

The maturation of sleep is one of the most important physiological processes occurring during the first year of life and is particularly rapid during the first six months after birth. A human infant shows prolonged and characteristic epochs of stable behavior, that have been called 'behavioral states'. Many physiological variables are inter-related and mutually influencing during the state cycles and change their properties at the transitions [91].

Sleep states architecture in infants is quite different from those in adults: in infants, sleep states are classified as active sleep (AS) and quiet sleep (QS), which can be seen as precursors of adult rapid eye movement sleep (REM sleep) and non-rapid eye movement sleep (NREM sleep), respectively. During QS, high voltage low amplitude electroencephalograph activity is observable, with absence of eye movements and regular HR and respiration. On the other hand, AS is characterized by low amplitude high frequency electroencephalograph activity, eye movements, and irregular HR and respiration. Additionally, a third state, indeterminate sleep (I), can be defined when criteria for AS and QS are not met fully [92].

Interestingly, cyclical sleep patterns can be observed already in the human fetus from 28 weeks of gestation. By this time, AS can be easily identified, while QS becomes clearly identifiable only around the last month of gestation. The percentage of time spent in QS increases with gestational age and by two months of age infants spends equal amounts of time in both states. From term to six months of

age, the proportion of AS decreases. While the specific functions of the different sleep stages are not yet well understood, many believe that deep sleep is essential for physical rest, while REM sleep is important for memory consolidation [93]–[95]. The major change in sleep-wake pattern occurs between six weeks and three months post-term age. During the first six months after term, consolidation and entrainment of sleep at night develops and sleep periods lengthen.

1.5.2. Autonomic Nervous System

The autonomic nervous system (ANS) is a component of the peripheral nervous system and comprises a set of nerves and nerve cells that innervates blood vessels and the airways, heart, intestines and urogenital organs. These nerves regulate and coordinate bodily functions based on secretory activity of glands, on contraction and relaxation of smooth muscle and cardiac muscle, and on sensations arising from deep viscera.

The ANS consists of a central and a peripheral component: it is controlled by centers located in the spinal cord, brain stem and hypothalamus. Nerves sprout from this central part, reaching autonomic ganglia from which other nerves connect with the peripheral tissues that are the target of this system, such as smooth muscles, cardiac muscle and secretory cells.

The autonomic nerves arrange in an intricate network with many branching and merging points. Along these nerves there are prominent swellings, the autonomic ganglia, which consist of large aggregates of ganglion neurons and efferent nerve fibers. Within a ganglion, the incoming fibers end many of terminal boutons forming synapses on ganglion neurons. New fibers emerge from these neurons, directed to a target in the peripheral organs, whereas other incoming fibers pass through one ganglion to terminate in another ganglion. In addition, there are autonomic afferent (sensory) fibers sprouting from neurons in the cranial and spinal sensory ganglia and are distributed to the peripheral organs. A schematic of the ANS structure is shown in Figure 1-5 [96].

The peripheral ANS is made of two branches, the sympathetic and parasympathetic. The sympathetic system typically releases norepinephrine in response to intrinsic and/or extrinsic stressors via afferent autonomic pathways, which transmit responses to visceral organs via efferent autonomy pathways. Conversely, the parasympathetic system typically releases acetylcholine to restore the body to restful conditions after stressful events [97].

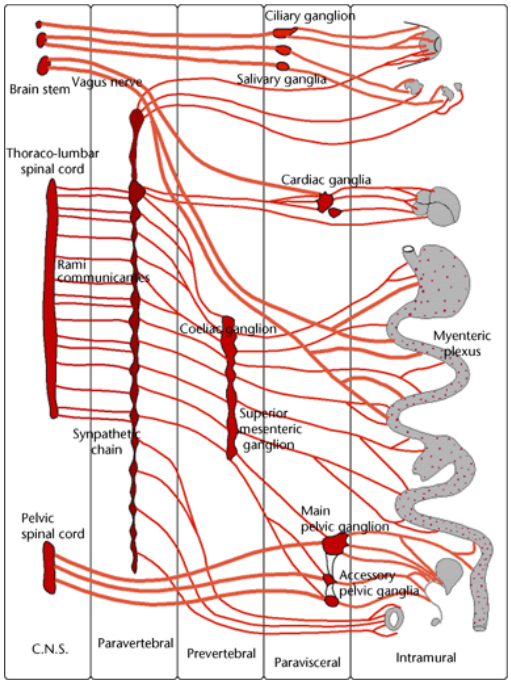

Figure 1-5: General plan of the distribution of autonomic nerves and ganglia, as they are spread between the spinal cord (left) and the peripheral organs. The central component is represented by some nuclei in the brainstem (top left) and a long column of neurons in the spinal cord .

Autonomic activity increases with gestational age in the fetus during pregnancy and postnatally in the infant. Sympathetic development occurs early on, while the parasympathetic activity matures mostly during the last trimester and the first months of life [98].

1.5.3. Heart Rate Variability and Cardiorespiratory Interaction

Core functions of ANS are the regulation of HR, blood pressure, breathing rate, thermoregulation, sweating, gastrointestinal motility and secretion, as well as other visceral activities that maintain homeostasis. One of the most interesting functions and a primary focus of this thesis is ANS control over the heart muscle: sympathetic fibers increase HR, atrioventricular conduction, and contractility of cardiac muscle, while they dilate the coronary arteries. In contrast, parasympathetic nerves slow down the HR and reduce heart contractility, favoring conservation of energy. These effects are integrated with several other factors, such as the spontaneous contractility of the heart itself, the peripheral resistance of the vascular tree, the effects of circulating hormones and the metabolic supply to the heart.

This complex interplay generates the so-called heart rate variability (HRV), the change in the time intervals between adjacent heartbeats. For this reason, HRV has been for long time considered a powerful tool, providing a window to observe non-invasively the interaction between the sympathetic and parasympathetic nervous systems and their capability to respond properly to internal and external challenges [9].

HR is also influenced by respiration; the neuronal control of breathing and HR are closely linked, functionally as well as anatomically. Several modes of interaction exist between these two subsystems: perhaps the most well-known example is respiratory sinus arrhythmia (RSA) that was firstly observed as early as 1733 [99]. It consists of a HR increase during inspiration and decrease during expiration [100], [101]. This phenomenon involves both central and reflex interactions, with the regulation of cardiac vagal outflow involving the same neuronal processes that generate the respiratory rhythm and reside within the brainstem, and a mechanical interaction of the two systems in the thoracic cavity [100], [102]. RSA amplitude is generally categorized as a linear interaction, but the cardiovascular and respiratory systems also show complex interplay with both linear and nonlinear interactions.

Other modes of interactions are related to the signal phase rather than the amplitude, such as the phase locking of HR and respiratory rate [27], [29], [103], [104]. Two interacting oscillators are *n:m* phase locked if marked events of one oscillator occur at fixed phases of the other oscillator. This phase synchronization condition implies also the frequency synchronization between the two rhythms, i.e. *n* periods of the first rhythm have exactly the same duration as *m* periods of the second one. Thus, period of cardiovascular phase locking can be described as short intermittent periods during which the phases of the R peaks and respiration maintain a stable relation with different integer ratios, known as phase locking ratios. The physiological mechanisms behind the phase synchronization are still not fully understood. Recent results have led to an hypothesized link to central nervous coupling factors; these physiological circuits might coordinate cardiovascular and respiratory rhythms in the brainstem through the control of phase synchronization between nerve discharges in order to improve energy efficiency [105], [106].

These different modes of interactions are not exclusive, rather they may simultaneously coexist, representing different aspects of neural regulation and acting on different time scales [31], [32]. Figure 1-6 shows three examples, one where RSA is present, another where phase locking occurs and one where they both occur simultaneously.

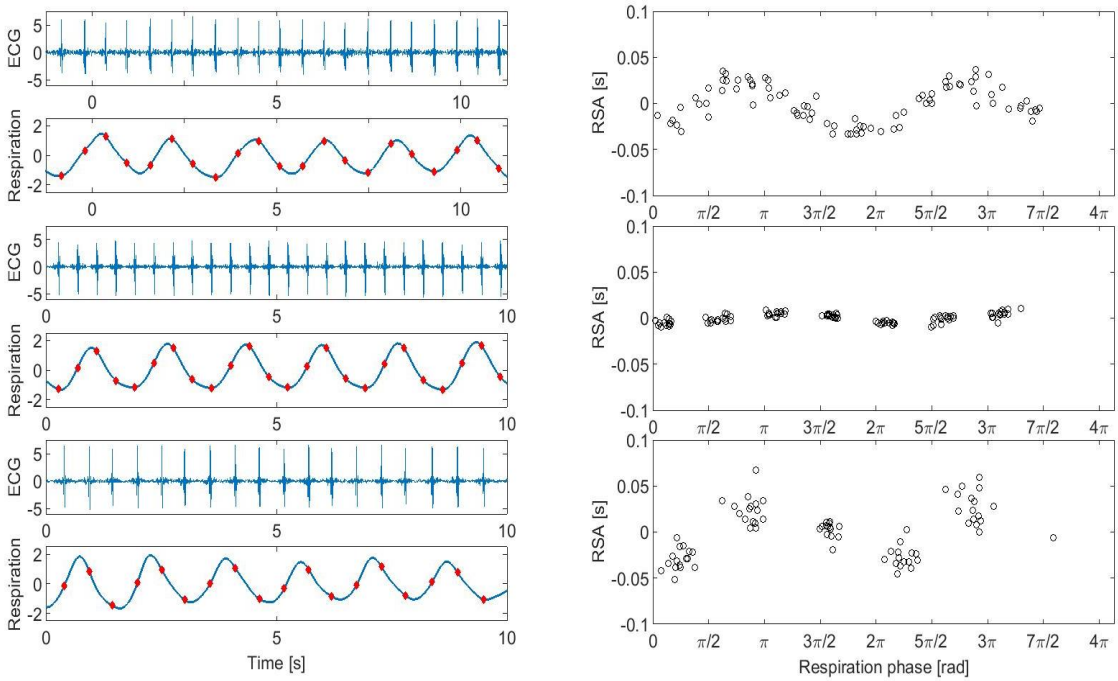

Figure 1-6: Example of presence of RSA and phase locking. On the left there are three 10 seconds segments of ECG and breathing, and on the right the corresponding values of RSA and distribution of R peaks occurrence with respect to respiratory phase. In the first row there is an example of RSA without phase locking. In the second row an example of phase locking (4:1) without RSA. In the third row an example of phase locking (3:1) concurrent with RSA.

RSA has been observed in full-term infants indicating the presence of cardiorespiratory coupling even at this early life stage [107]. This coupling increases in strength and consistency with GA at birth, reflecting the transition from sympathetic to parasympathetic dominance during the postnatal period with an increasing influence of breathing modulating HR [108]. Overall, immaturity in linear cardiorespiratory coupling is observed in preterm infants, mostly in the form of lower short-term HRV, an indirect measure of RSA [109].

The close interaction between cardiac and respiratory control is critical for survival. This synergy is crucial for the homeostatic regulation of blood gases, and essential for regulating central nervous functions, such as arousal [10], [11]. Arousal from sleep involves both physiological and behavioral responses and has long been considered a vital survival response for restoring homeostasis in reaction to various life-threatening situations, such as prolonged hypoxia or hypotension. There are two distinct

arousal types defined in infants, subcortical activation and full cortical arousal, which reflect the hierarchical activation from the brainstem (including heart rate, blood pressure, and ventilation changes) to the cortex.

1.5.4. Effects of sleep states on autonomic cardiorespiratory control and arousal

There are marked differences in cardiorespiratory control between AS and QS. Assessment of cardiovascular control is commonly made by studies of HR and HRV and blood pressure and its variability. In infants, HR and overall HRV are higher in AS compared to QS, as a result of the predominance of sympathetic activity in AS. Sleep state also affects blood pressure, with higher values in AS and increased variability. Respiratory control is also affected by sleep state [94]. In infants, respiratory rate decreases from wake to sleep, and breathing is more variable in AS, with short apneas occurring more frequently in this state than in QS [93]. Voluntary control of ventilation is abolished at sleep onset and upper airway resistance is increased due to muscle hypotonia. It is nowadays recognized that the two sleep states have different effects on the control of the respiratory rhythm generator, respiratory muscles and lung volumes. During QS, when lying supine, due to tonic activity in the diaphragm and intercostal muscles, abdominal and thoracic respiratory movements are largely synchronous with each other. In contrast, during AS, there is a reduction in the tonic activity of the diaphragm and intercostal muscles that produces a paradoxical inward rib cage motion during inspiration while the abdominal wall moves outwards. In addition, the horizontal configuration of the diaphragm in neonates causes the lower ribcage to move inward during inspiration, thereby reducing diaphragmatic efficacy. Oxygen saturation levels are consistently lower and more variable in AS than QS due to the paradoxical inward rib cage motion and increased oxygen consumption in AS. Although end-tidal carbon dioxide levels do not change from one to six months of age in the term infant, they are consistently lower in AS than QS. The rate and depth of breathing during sleep oscillate in cycles of 7–13s in both AS and QS; minute ventilation is higher in AS than QS due to increases in rate while depth remains relatively constant [94].

Infant arousal responses are affected by postnatal age and these maturational effects are also sleep-state-dependent. Previous studies have demonstrated that in response to respiratory, tactile and auditory stimulation, total arousability is reduced with increasing age during QS, whilst remaining unchanged in AS [110], [111]. Nonetheless, there are conflicting findings, depending on what type of stimulus is used and what measure is employed to identify arousal.

2. HEART RATE VARIABILITY AND CARDIORESPIRATORY ANALYSIS

2.1. Introduction

In the previous chapter the definition of SIDS and the hypothesized mechanisms leading to it have been highlighted. Furthermore, the crucial role of autonomic activity and cardiorespiratory control was discussed.

The first part of this chapter will present methods proposed for heart rate variability analysis, as a marker of autonomic nervous system activity. No clinical standards are in place for the newborn population, thus adaptation of guidelines for adults and fetuses was necessary. We also evaluated nonlinear parameters which are not yet used in clinical work but can increase our understanding of the complex behavior of autonomic regulation.

In the second part of the chapter, methods that address cardiorespiratory interaction will be presented. Few studies in the past have been performed in populations at risk for SIDS (near-miss SIDS, SIDS siblings, premature infants etc.) regarding their respiratory behavior, for example investigating periodic breathing, apneic events etc. Even fewer studies have addressed the coupling between breathing and HRV dynamics. Given the hypothesized SIDS mechanisms presented in the previous chapter, the contribution of our work is extremely novel and relevant.

Furthermore, this comprehensive view of the cardiac and respiratory systems follows a more physiological perspective, since systems are not analyzed separately but are looked as a complex network, encompassing control cycles using either positive or negative feedback mechanisms and showing complex behavior. Our approach finds its root in a new field of study, called Network

Physiology, which views the human organism as an integrated network of interconnected and interacting organ systems, each representing a separate regulatory network. The behavior of one physiological system may affect the dynamics of all other systems in the network of physiologic networks. Due to this intertwining, failure of one system can trigger a cascade of failures throughout the entire network. Network Physiology offers a framework to address the question of how different physiological systems interact and behave together. It stresses the advantages of this holistic approach over the reductionist methods, which analyze every system separately. Recent publications have highlighted the new insights afforded by this novel approach [112]. Looking at different physiological systems as dynamically interacting could shine light on the process of horizontal integration at the level of organ to organ interaction required to maintain an optimal health status.

2.2. Monovariate analysis

Newborn HRV analysis encompasses techniques from various domains. There are still no standard norms developed for this particular population, thus parameters from adult and fetal HR analysis are usually adapted. The Task Force of 1996 suggests the evaluation of parameters on five-minute windows for adults' HR [33]. However, since neonatal HR is higher, a similar number of beats occurs in about three minutes allowing a shorter reference length.

2.1.1. Traditional HRV Analytic Approaches

Time domain

For the time domain analysis, measures adapted from adult studies' have a long history: SDNN, which is the standard deviation of normal to normal intervals (NN), defined as the RR distances excluding anomalous beats (e.g. ectopic beats), and root mean of successive differences, RMSSD. SDNN estimates overall HRV, RMSSD estimates short-term components of HRV [33]. In addition to these traditional parameters, standard measures from fetal HR analyses were computed: short term variability (STV), interval index (II), differential index (DI) and long term irregularity (LTI) [113], [114]. All these measures are calculated in seconds. Generally long-term variability parameters evaluate a combination of sympathetic and parasympathetic nervous systems. In contrast, measures related to the beat to beat variability are influenced largely by the parasympathetic nervous system, tied to rapid ANS reactivity.

Regarding breathing rate analysis, the inter breath interval series (IBI) can be analyzed calculating mean, standard deviation and the coefficient of variation (CV) defined as the standard deviation of the respiration rate divided by the mean respiration rate.

Frequency domain

Estimation of the power spectral density (PSD) can be performed with parametric (autoregressive) and nonparametric (FFT) approaches on RR series and respiration, usually resampled at 2 to 5 Hz [115]. The literature does not provide precise values for frequency bands ranges, thus they are commonly chosen based on prior experience and observation of the spectra, with consideration of the general breathing characteristics of infants [116]–[118]. Commonly used frequency bands are Low Frequency (LF), 0.05-0.2 Hz, and High Frequency (HF), 0.2-1.5 Hz. LF contributions can be associated with a combination of sympathetic and parasympathetic activity, while HF are mostly mediated by breathing and vagal activity. Frequency domain analysis is suited for a short time scale (3–5 min) as the HRV signal needs to maintain its stationarity [119].

These approaches have been used since the 1950s to characterize newborns development [118], reactivity to stressful events [116], [120], [121], to evaluate interventions efficacy [122]–[124] and to predict poor outcome [125], [126].

2.1.2. Nonlinear HRV Analytic Approaches

The introduction of nonlinear approaches to signal processing led to the consideration of a set of methods investigating geometric and dynamic properties of time series. Their statistical use can be of great importance, even in diagnostic field and in clinical knowledge related to different cardiovascular pathologies [127]. Various existing techniques aim at quantifying the degree of similarity and/or complexity in time series, which can be computed directly on the sequence of inter-beat intervals.

Entropy Measures

Approximate Entropy (ApEn) was proposed by Pincus to quantify regularity and complexity of a time series, defined as the presence of repetitive patterns within a certain tolerance *r* and at different lags [128]. ApEn is seen as a significant analytic advancement over previous entropy estimation approaches, which required very long and completely noise-free datasets to determine a precise value of entropy, since neither of these demands are typically met in biological datasets. Pincus advanced the field by positing that an 'approximate' estimate of entropy could be used to hierarchically rank sets

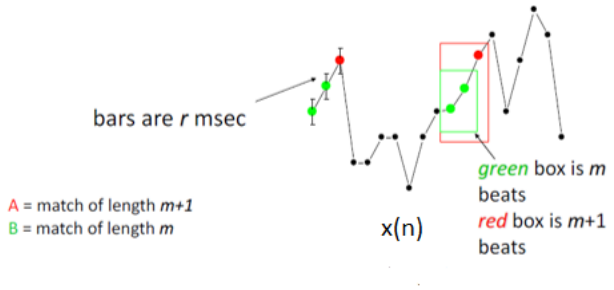

Figure 2-1: A graphic representation of the process of match finding used to calculate SampEn and ApEn

of time series. Improvements and corrections were introduced with the Sample Entropy (SampEn) [129].

The rationale is to quantify the degree of regularity or loss of regularity in a time series without a priori information on its structure. Given a discrete time series *x(n)* of length N, a threshold *r*, and an embedded dimension *m* (usually *m* = 1, 2, 3, *r*=0.1−0.25 std of the input data), *x(n)* is split into a series of subsequences $X_m(i)$ of length *m*. A distance based on proper definitions, such as Euclidean or Chebyshev, is calculated between two subsequences. The total count of subsequences of length *m* whose distance is less than *r* is denominated $B_i(r)$, whereas $A_i(r)$ accounts for subsequences of length *m*+1. ApEn (*m,r,*N) then averages the natural logarithms of $A_i(r)$ and $B_i(r)$ and calculates their difference. SampEn *(m, r*, N) instead is obtained as the natural logarithm of the ratio between the averages of $A_i(r)$ and $B_i(r)$. Figure 2-1 shows a graphical representation of the technique.

ApEn major limitation is a strong dependence on the length of the time series analyzed and the count of the self-matches of each pattern, which introduces a bias that is incompatible with the aim of measuring new information generated in the signal. SampEn does not count self-matches thus improving the estimation.

A challenging problem for entropy estimation is the choice of the tolerance *r*, to assess if two patterns could be defined similar. To address this issue, an improvement to SampEn was presented by Lake in 2006 [21]. He approached the problem considering the HRV signal sufficiently stochastic to be able to model it as a random process. This new entropy estimator can be seen as a measure of Gaussianity of the signal and the central limit theorem suggests that this reflects the physiological complexity of the underlying signal transduction processes. This is because this theorem establishes that when independent random variables are added, their properly normalized sum tends toward a normal

distribution even if the original variables themselves are not normally distributed. The old deterministic approach involved calculating probabilities while the new stochastic approach calculates probability densities. To convert the probabilities to densities it is necessary to divide by the volume of the matching region, which is $(2r)^m$, which is equivalent to add a factor of log(2r) to SampEn. The result was called Quadratic Sample Entropy (QSE) and it allows to compare estimates obtained with different *r* values.

Inaccuracy in the probability estimate may still arise because all these parameters are obtained from the ratio of B and A, which are the total number of subsequences of length *m* and *m*+1 respectively, whose distance is less than *r*. Thus, the reliability and stability of the estimate is dependent on the magnitude of the numerator A and denominator B. For this reason, it is important to maximize this value: min(A, B-A). Owing to the flexibility to vary *r* introduced by QSE, inaccurate probability estimates can often be avoided, for instance with the method of the Minimum Numerator Count [21], [130]. This approach calculates ratios with specified minimum values of the numerator and denominator, in the attempt to also minimize *r*.

Phase Rectified Signal Average (PRSA)

PRSA is a technique presented by Bauer, which aims at compressing a signal into a shorter sequence without losing any relevant quasi-periodicities, and eliminating at the same time non-stationarities, artifacts, and noise [131]. This technique consists of 3 simple steps: at first, anchor points (AP) are chosen based on a certain property of the signal, such as increases or decreases in the signal. Increases can be defined by comparing averages of T values of the time series. This averaging acts as a low pass filter and the parameter T sets an upper frequency limit for the periodicities that can be detected by PRSA. Typically, half of all points of the signal will be APs according to the chosen definition. Thanks to this process we can extract the phase information of the oscillations from the signal itself. Afterwards, windows of length 2L, are defined around each AP. It is important to underline the appropriate choice for the parameter L; it should be larger than the period of the slowest oscillation to be detected. Lastly, the windows are aligned at the AP and the PRSA curve is obtained by averaging the aligned windows. Thanks to this averaging procedure, components that are not phase synchronized with the AP, will

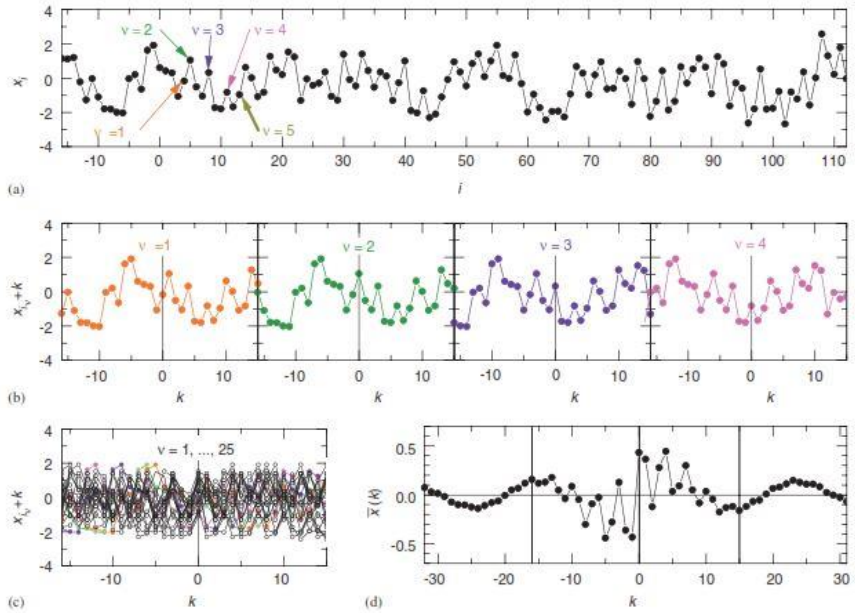

Figure 2-2: Illustration of the PRSA technique: (a) Anchor points (AP) are selected from the original time series x(n); in this example increase events are selected, with T=1. (b) Windows of length 2L are defined around each AP (c) all the windows are centered in the AP and overlapped and average. (d) The final PRSA curve is obtained [131]

have zero mean and thus they will cancel out; instead, the events that have a fixed phase relationship with the AP in all the 2L windows will have the same pattern and thus will be kept in the average. Figure 2-2 summarizes the steps of this procedure.

PRSA offers the ability to analyze separately HR accelerations and decelerations, by properly choosing the AP definition criterion. This affords the opportunity to investigate rapid parasympathetic influences as well later acting sympathetic/parasympathetic ANS mechanisms. Proposed parameters for these curves are the Δx, Δy, Acceleration Phase Rectified Slope (APRS) and the Deceleration Phase Rectified Slope (DPRS) [23].

These novel analytical techniques have been employed to characterize development of HR development with gestational age [132], [133], to compose risk scores for unfavorable outcome to predict death and morbidities [134], to detect atrial fibrillations in very short time series [135], and to predict mortality after myocardial infarction [136], [137].

2.3. Bivariate Analysis

In the last century, knowledge about relationships between respiratory and cardiovascular systems has gradually grown and several modes of interaction have been described, encompassing amplitude and phase modulation and both linear and nonlinear relationships.

In this section we will present several methods that have been proposed to quantify the interaction between subsystems, ranging from traditional to more complex signal-processing techniques. Some of the methods presented will also address the issue of directionality of the relationship, which is a crucial step to be able to infer causal relationships.

2.3.1. Traditional Cardiorespiratory Analytic Approaches

Cross-spectral Analysis

Cross-spectral analysis consists of estimating amplitude and phase cross-spectrum and coherence, towards the identification of significant relationship between two time series as a function of frequency and to determine their phase lag.

In the '90s, surrogate series were proposed as a method to test for nonlinearity in a time series [138]. Recently, they have been applied to identify an adaptive significance threshold for the coupling between two time series expressed in the coherence function [139], [140]. With this approach, N pairs of surrogate time series, with features of the original series but completely uncoupled, are generated as realizations of independent stochastic processes, such as the Iterative Amplitude Adjusted Fourier Transform (IAAFT) [141]. The coherence is then estimated between each pair of surrogate series and its empirical sampling distribution (frequency histogram) computed accordingly at each frequency of the signal bandwidth. The threshold for zero coherence is then defined at the 95th percentile of the obtained coherence sampling distribution. Figure 2-3 shows the steps of this procedure and Figure 2-4 represents an example where this technique is applied to identify the peak of coherence between HR and breathing of a newborn. In red is portrayed the adaptive threshold obtained with the surrogate method.

Crosspectral analysis has been widely used to quantify sympatho-vagal balance, with measures such as LF/HF. In particular, it was employed to quantify autonomic activity during tilt test and to characterze baroreflex sensitivity [142]–[144]. Results obtained provided useful insight into the physiology of autonomic control, nonetheless limitations have arised in the use and interpretation of this approach [145].

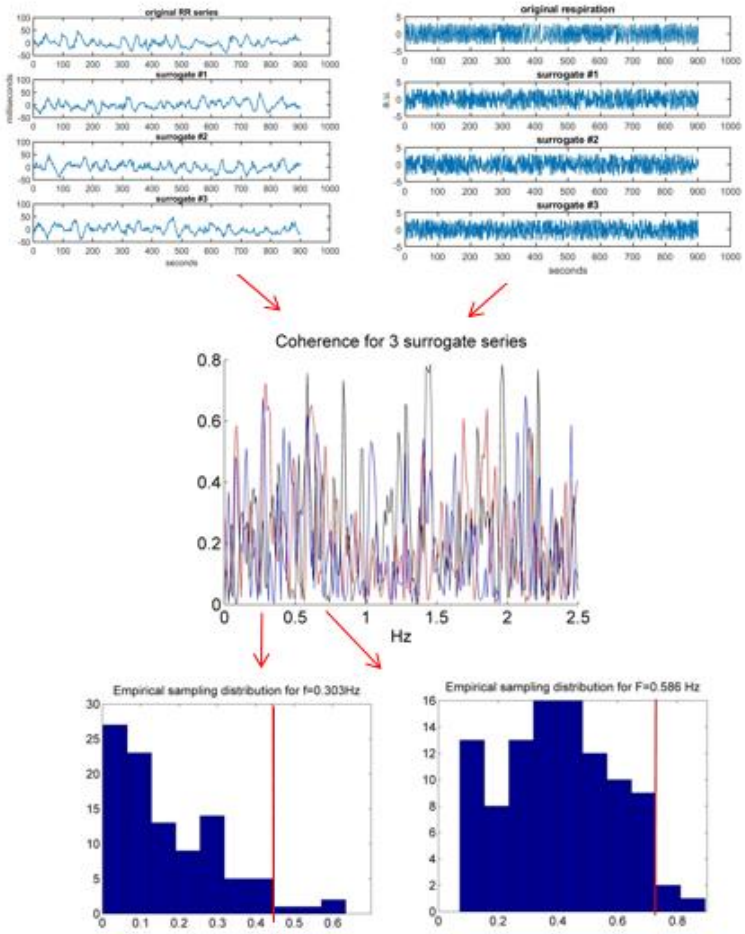

Figure 2-3: Steps involved in coherence analysis with surrogate data adaptive threshold. In the first panel surrogates for the RR series and the breathing are shown. In the middle panel the coherence values are shown in blue while the adaptive threshold in red. In the bottom panel two examples of empirical sample distributions are shown, at 0.303 Hz and at 0.586 with relative threshold shown in red.

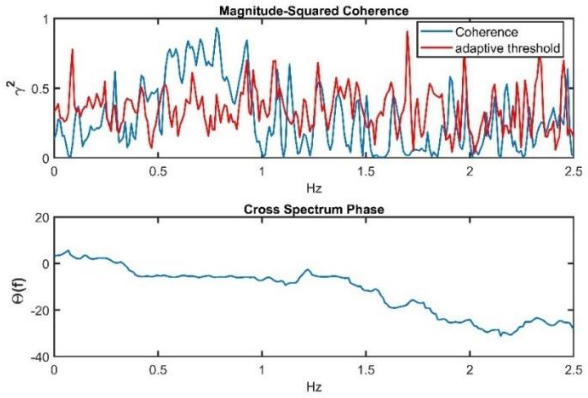

Figure 2-4: Example of coherence analysis with surrogate data adapting threshold. In the panel on top the coherence values are shown in blue

2.3.2. Novel Approaches

Cross-spectral analysis has proved to be a very powerful tool to unveil information about the cardiorespiratory interaction. Nonetheless, its application lies on the assumption of stationarity and linearity of the systems under consideration. As already explained, these assumptions are often too strict to capture the behavior of the cardiorespiratory system. Moreover, cross-spectral analysis cannot measure directionality of the relationships, and thus, it can only partially reveal the underlying interacting mechanisms responsible for the changes in complexity, especially when knowledge of the underlying physiology is limited. The methods exposed in this section address all these limitations.

BPRSA

The Bivariate PRSA (BPRSA) is an evolution of the PRSA, which highlights interrelationships between two signals supposed synchronous, defined as a trigger and a target signal [146]. The BPRSA algorithm consists in 3 major steps similar to the ones for PRSA, except for the fact that the anchor points detection is based on the trigger signal and then transferred on the target signal, where the rest of the analysis is carried on.

BPRSA transforms the target signal in a compressed version, maintaining only (quasi) periodicities that are coupled to the trigger signal, eliminating uncoupled periodicities, artifacts, or noise. Comparing the BPRSA transformation of the target signal with the PRSA transformation of the trigger signal is possible to infer potential trigger and target signal interaction. BPRSA was employed for early neonatal epileptic seizure detection and for the assessment of spontaneous baroreflex sensitivity [147], [148].

Transfer Entropy

Transfer Entropy is an evolution of monovariate estimates of entropy. It is an index which evaluates the information flow from a source system X to a destination system Y, considered as two interacting dynamical subsystems. Y_n, X_n are the stochastic variables obtained by sampling the stochastic processes, describing the state visited by the systems X and Y over time. Y_n^-, X_n^- are the vector variables representing the entire history of the processes.

The Transfer Entropy from X to Y is defined as:

$$TE_{X\to Y}=\sum p(Y_n, Y_n^-, X_n^-)\log\frac{p(Y_n|Y_n^-,X_n^-)}{p(Y_n|Y_n^-)} \qquad (1)$$

From definition (1), it emerges that TE can be also expressed as a difference of two conditional entropies (CE):

$$TE_{X\to Y}=H(Y_n|Y_n^-)\text{-}H(Y_n|Y_n^-, X_n^-) \qquad (2)$$

In other words, TE quantifies the information provided by the past of the process X about the present of the process Y, that is not already provided by the past of Y.

TE framework is a powerful tool to detect information transfer given that it does not require any model assumption describing the interactions regulating the system dynamics. Moreover, it can uncover purely nonlinear interactions and deal with a range of interaction delays.

Nevertheless, the TE method requires the approximation of the infinite-dimension variables representing the past of the processes. In the following paragraph, we will briefly clarify this issue. A reconstruction of the past of the system dynamics is represented by the processes X and Y with reference to the present state of the destination process Y. This provides a vector $V=[V_n^Y, V_n^X]$, which is a subset of the past states chose among Y_n^-, X_n^- and containing the most significant past variables to explain the present of the destination system.

Two approaches can be applied: uniform and non-uniform embedding schemes.

- Uniform: components to be included in the embedding vectors are selected a priori and separately for each time series. For example, the vector Y_n^- is approximated using the embedding vector $V_n^Y=[Y_{n-m}, Y_{n-2m} \ldots Y_{n-dm}]$, where d and m are the embedding dimension and embedding delay respectively. Following this approach, TE estimation consists of two steps: collection of past states of the process and estimation of entropy, with a chosen estimator. The main limitation in this case stems from arbitrariness and potential redundancy of the estimate, which may cause problems such as overfitting and detection of false influences. For the tests of significance employed with this approach, please refer to [149].
- Non-uniform: this technique consists of a progressive selection among the available variables describing the past of the observed processes X, Y, considered up to a maximum lag, and to identify the most informative variable for the destination variable Y_n. Thus, a criterion for maximum relevance and minimum redundancy is applied for candidate selection, and the resulting embedding vector V includes only the components of X_n^- and Y_n^-, which contribute most to the description of Y_n. Moreover, the variables included into the embedding vector are associated by definition with a statistically significant contribution to the description of Y. Thus, the statistical significance of the TE estimated with non-uniform embedding emerges from the selection of at least one past component of the source process. Otherwise, the estimated TE will be zero and nonsignificant.

Another crucial aspect of TE method is the choice of the appropriate method to estimate the joint probability distribution capable of fully-describing the interrelationship between X and Y, to estimate the two conditional entropies needed to obtain the TE value.

- LIN: The first approach adopts the linear estimator assuming that the overall process has a joint Gaussian distribution. Under this assumption, the two CE terms defining the TE can be quantified by means of linear regressions involving variables taken from the embedding vector, depending on which embedding method is used.
- BIN: The second estimator is based on a fixed state space partitioning, which consists of a uniform quantization of the time series. Then entropies are computed by approximating probability distributions with the frequencies of occurrence of the quantized states.
- KNN: The third estimator is based on K-Nearest Neighbor technique (KNN), a powerful non-parametric technique for classification, density and regression estimation. It estimates entropy terms through a neighbor search in the space defined by all the variables selected as most informative ones

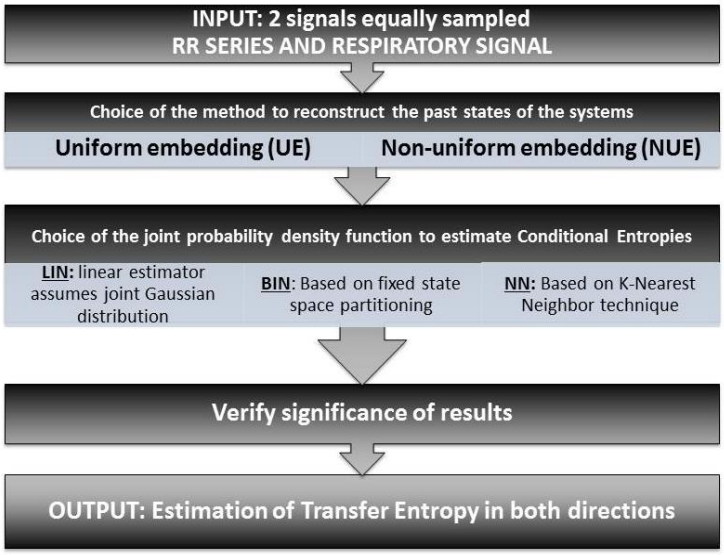

Figure 2-5: TE calculation steps: 1) selection of the 2 signals of interest equally spaced. TE will be calculated evaluating the directionality signal 1-> signal 2 and viceversa. 2) Choice of the method to approximate the infinite-dimension past states of the systems (UE vs. NUE). 3) Choice of Conditional Entropy estimator (LIN vs. BIN vs KNN) and TE estimation. 4) Verification of TE results significance

A potential problem with uniform and non-uniform embedding procedures relates to the issue of dimensionality. Adopting the non-uniform embedding could overcome this risk. As a matter of fact, this method reduces the candidates of significant past states, preventing the risk of probability density function to assume a constant value and computing the search in an extreme sparse hyperspace. Surrogate series obtained with time shifts, were employed to test results significance. In this thesis, N=100 surrogate series were employed and the threshold was set as higher than the 95th percentile. Moreover, we introduced a novel application to quantify the "memory" of the effect of a system on another. For instance, in the case of the influence of the RR series on the breathing we evaluated the Transfer Entropy parameter keeping the RR series fixed and shifting the breathing series, in order to

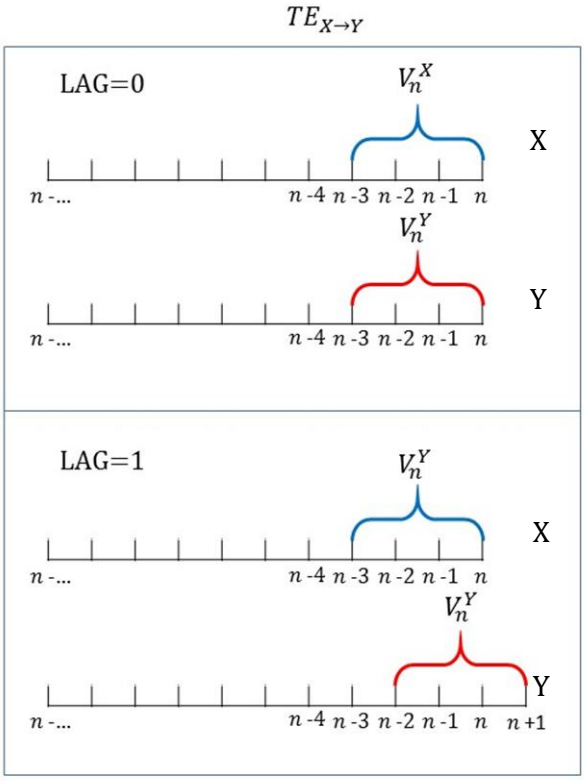

Figure 2-6: TE calculation at different lags. On the top panel, the vectors $[\mathbf{V_n^Y}, \mathbf{V_n^X}]$, which are a subset of the past states chosen among $\mathbf{Y_n^-}, \mathbf{X_n^-}$, are selected without any shift, hence Lag=0, and this corresponds to the traditional Transfer Entropy. On the bottom panel, $\mathbf{V_n^X}$ is calculated without shift, but $\mathbf{V_n^Y}$ is selected on the shifted Y series by a sample 1, hence Lag=1, and thus quantifies the information provided by the selected part of the process X on the shifted portion of the process Y, that is not already provided by the past of Y. This allows to quantify how long does the effect of X on Y lasts.

quantify the effect of the same RR series data points on the following breathing samples. Figure 2-6 shows an example of TE calculation at different lags.

The MuTE toolbox was employed to estimate Transfer Entropy values. A detailed description of the methods can be found in Montalto et al. [149]. The main steps for TE estimation procedure are summarized in Figure 2-5.

Transfer entropy has been utilized to test the effect of age and gender on cardiorespiratory interaction complexity [150], characterize tilt response [151], [152], and highlight the importance of information storage, transfer and modification in interacting systems [153].

Phase Locking

Synchronization can be defined as the adjustment of the rhythms of self-sustained oscillators due to their interaction. Given the phases of the two oscillators $\phi_1(t)$ and $\phi_2(t)$, a generalized *n:m* phase locking ratio fulfills the condition expressed in (3):

$$|n\phi_1(t) - m\phi_2(t) - \delta| < cost \qquad (3)$$

where *n* and *m* are integers and δ is an average phase shift. Thus, in the synchronized case, phase differences should present small fluctuations around a constant, while in the unsynchronized one, differences should randomly vary for every instant of time. ϕ_{RR} and ϕ_{RESP} were defined as the first and second oscillators respectively, *n* indicates the number of heartbeats with respect to *m* respiratory cycles. Different *n:m* synchronization ratios periods can be highlighted with a graphic tool called cardiorespiratory synchrogram, shown in Figure 7 panel c [27], [154].

Instantaneous phase of the ECG (ϕ_{RR}) is defined as linearly increasing between an R peak and the successive one and it is computed as in (4):

$$\phi_{RR}(t) = 2\pi k + 2\pi \frac{t - t_k}{t_{k+1} - t_k} \qquad (4)$$

where t_k are the times of appearance of the kth R peak. The respiratory signal was detrended and filtered with a Savitzky-Golay (S-G) filter. S-G smoothing is achieved with a process of convolution, by fitting successive windows of data with a polynomial with the method of linear least squares. We chose the order of the polynomial to be equal to 4, and the length of the moving window equal to 95 samples. The band pass was 0.05-3.5 Hz. Then, the instantaneous phase (ϕ_{RESP}) was computed by means of functions provided by the Data Analysis with Model of Coupled Oscillators (DAMOCO) Toolbox [30], [155], [156]. Respiration signal protophase was computed via the Hilbert transform and the phase was

derived with appropriate transformation of protophase [157]. All the instantaneous phases were resampled at 200 Hz in order to obtain two synchronous phase series.

Of the several approaches proposed to quantify the level of synchronization, the method of the synchronization index λ was chosen, given its proven reliability [158]. In order to estimate the degree of synchronization by means of the λ index, ϕ_1 and ϕ_2 will be considered as cyclic (mod 2π). The process starts fixing a value for the phase of the first oscillator, Θ, to observe the phase of the second oscillator $\phi_2|_{\phi_1=\theta}$ at each time t_i when ϕ_1= Θ. In the case of 1:1 phase locking, the values of ϕ_2 in the time points when ϕ_1= Θ are going to be scattered around a constant, due to weak noise. This distribution can be characterized computing the intensity of its first Fourier mode. In order to strengthen the statistic, this process can be repeated with a binning-like procedure for different values of Θ. The average of the obtained results provides a measure related to circular variance, that is the λ index. The resulting λ is bounded between 0 and 1, where 0 corresponds to absence of synchronization and 1 to synchronization in the free-noise case. This method can be then generalized in the case of n:m locking, simply rescaling the phases as $\phi_1 \rightarrow \phi_1/n$ and $\phi_2 \rightarrow \phi_2/m$.

The λ index is calculated on moving windows. The choice of the length of this window is crucial for the estimation of the index and it represents a tradeoff between the time resolution and the physiological accuracy of the estimate. In our case, we experimented with different values and then selected windows of 1000 samples (5 s) overlapping by 50 samples (250 ms), which seemed the best fit when compared to the visually perceived coupling.

Significance of the λ Index needs to be tested, since spurious period of phase locking can occur in any two time series. The index can be compared with an arbitrary selected threshold, or for more accuracy statistical testing can be performed using surrogate dataset.

From the λ Index, the percentage of time spent in each particular *n:m* ratio (2:1,3:1,4:1,5:1, 3:2,5:2 etc.) and average length of all the periods were calculated. Giving the assumption that in each instant of time the ratios of synchronization are mutually exclusive, we also estimated the percentage of time spent in a synchronized state adding together all the ratios with respect to a single breathing cycle, resulting in the so-called *n:*1 synchronization and the average duration of period in synchronized state. An example of this analysis is portrayed in Figure 2-7: in panels a) and b) 1-minute segments respectively of HR and respiration are shown, in panel c) the corresponding synchrogram and in panel d) the relative λ index, with a horizontal line showing the threshold at 0.7.

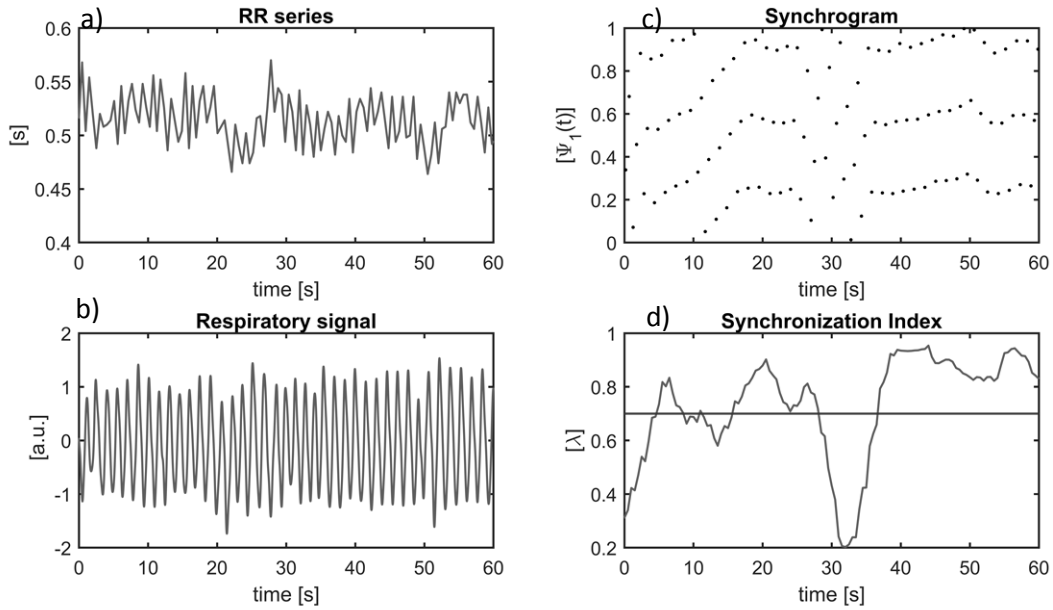

Figure 2-7: a): The RR series (extracted from the ECG signal) in a 60 seconds window. b): Respiratory signal for the same window described in (a). The signal shown in (a) and (b) are obtained from a full term subject in quiet sleep. c): The synchrogram extracted by the joined analysis of RR series and respiratory signal. d): The horizontal line represents the threshold for the computed synchronization index (λ). Synchronization index values above the threshold indicates epoch of synchronization between cardiac and respiratory systems.

Figure 2-8 shows a block diagram summarizing the multiple steps involved in phase locking (PL) estimation.

The phase of breathing can also be used to calculate the RSA amplitude parameter. It is computed based on the method reported by Bartsch [32]. Firstly, the phase of the respiratory signal ϕ_{RESP} is obtained, then for each 2 breath cycles, the mean RR value is calculated and is subtracted from all the RR distances, basically obtaining a normalized RR series. Such extracted series in then plotted over the period of 2 breathing cycles, $\phi_{RESP} \in [0, 4\pi]$. Then RR values are fitted with a least-square sinusoidal approach. The RSA amplitude is defined as the amplitude of the derived sinusoid.

Techniques quantifying periods of cardiorespiratory synchronization and phase locking have been employed to characterize the cardiorespiratory behavior during obstructive sleep apnea [104], [159], the effect of mental task and meditation [160]–[162], the effect of anesthesia [163], and the development of cardiorespiratory control in infants during first 6 months of life [158].

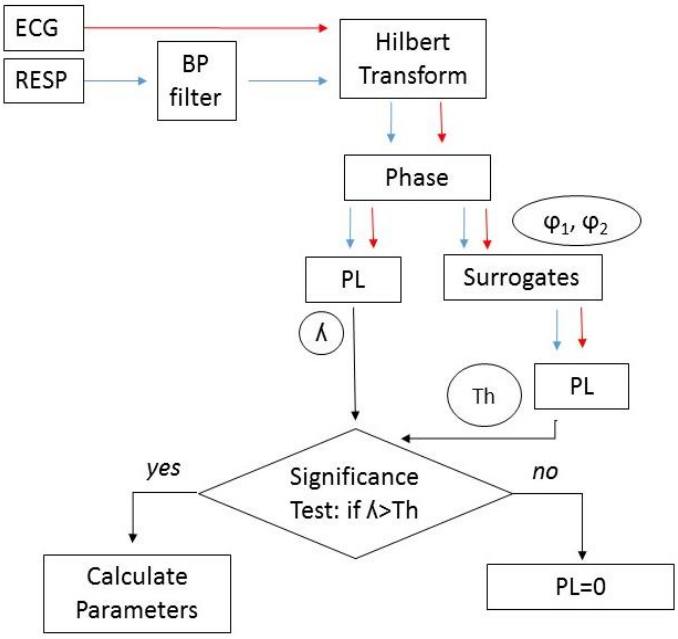

Figure 2-8: Presentation of phase locking (PL) procedure in a block scheme: breathing signals are band passed and then Hilbert transform is applied on both breathing and ECG signals to obtain phase functions. Then, PL is calculated using phase functions. In parallel, surrogates are generated and PL is calculated for each pair of surrogates in order to calculate an optimal threshold. This threshold is utilized to detect period of significant phase locking and to calculate relative parameters.

Directionality

To trace the changes in coupling degree and/or directionality imposed by slow drift of the coupling parameters in the system, the index of directionality $d^{RR,RESP}$ was computed from the time series of phase data: ϕ_{RR} and ϕ_{RESP}. In the following paragraphs we briefly summarize the model presented by Rosenblum et al. [164]. A simple model of two coupled phase oscillators is proposed, where each system can be represented by its own phase variable ϕ so that its time variation can be expressed as $\dot{\phi} = \omega$, with $\omega = 2\pi/T$ being the natural frequency of the considered oscillator and T the period of oscillation.

The phase space of the model can be expressed as:

$$\dot{\phi}_1 = \omega_1 + \varepsilon_1 \cdot f_1(\phi_2, \phi_1) + \zeta_1(t) \quad (5)$$

$$\dot{\phi}_2 = \omega_2 + \varepsilon_2 \cdot f_2(\phi_1, \phi_2) + \zeta_2(t)$$

The continuous phase variables ($\dot{\phi}_1, \dot{\phi}_2$) consider the natural angular frequency of the system (ω_1, ω_2) and the random terms (ζ_1, ζ_2), accounting for amplitude fluctuations and perturbations which are intrinsic characteristic of biological systems. The coupling terms consist of a 2π-periodic functions (f_1, f_2) and strength of interaction parameters ($\varepsilon_1, \varepsilon_2$).

Given the assumption that phase variables can be estimated directly from the measured time series, it is possible to obtain an approximated reconstruction of both cardiac and respiratory oscillators to understand the causal relationship between the subsystems. The Evolution Map Approach [164] algorithm was used to reveal asymmetric directionality strength from short noisy records and quantify which of the systems under analysis influenced its counterparts more strongly.

Both ϕ_1 and ϕ_2 are unwrapped phase variables, defined as continuous quantities represented on the whole real line, not limited from 0 to 2π. Eq. 6 shows that to reconstruct the real weakly coupled oscillators from a single recorded realization of the process. It is necessary to fit the dependencies of Δ_1 and Δ_2 over ϕ_1 and ϕ_2 upon considering phase variable increments generated by and unknown two dimensional noisy map.

$$\Delta_1(k) = \omega_1\tau + \mathcal{F}_1[\phi_2(t_k), \phi_1(t_k)] + \xi_1(t_k) \qquad (6)$$
$$\Delta_2(k) = \omega_2\tau + \mathcal{F}_2[\phi_1(t_k), \phi_2(t_k)] + \xi_2(t_k)$$

Δ_1 and Δ_2 represent the phase variable increments over time computed as differences over a specific temporal window of length τ. They can be computed from phase variables ϕ_1 and ϕ_2.

The deterministic part $\mathcal{F}_1$ and $\mathcal{F}_2$ of the map can be estimated as shown in (7), fitting the dependences of Δ_1 and Δ_2 over ϕ_1 and ϕ_2 with a least mean square approach. Giving the assumption that phase variables are cyclic, the most appropriate choice of a family function is the finite Fourier series:

$$\mathcal{F}_1 \approx F_1 = \sum_m A_m e^{ia\phi_1 + ib\phi_2} \quad (7)$$
$$\mathcal{F}_2 \approx F_2 = \sum_n A_n e^{ia\phi_1 + ib\phi_2}$$

In this analysis the maximum order of Fourier expansions is set to 3, in the following computation $|a| \leq 3$ and $|b| \leq 3$. F_1 and F_2 can describe the deterministic (ω and $\mathcal{F}$) and stochastic (ξ) link between phase variables and their increments. They can serve as smoothing functions by filtering out noise by means of the least square fitting approach.

The cross-dependency coefficients of phase dynamics of the two systems can be extracted from F_1 and F_2 as:

$$c_1^2 = \iint_0^{2\pi} \left(\frac{\partial F_1}{\partial \phi_2}\right)^2 d\phi_1 d\phi_2 \qquad (8)$$

$$c_2^2 = \iint_0^{2\pi} \left(\frac{\partial F_2}{\partial \phi_1}\right)^2 d\phi_1 d\phi_2$$

The directionality index can be expressed as:

$$d^{(1,2)} = \frac{(c_2 - c_1)}{(c_2 + c_1)} \qquad (9)$$

The EMA algorithm computes a normalized directionality index *d*. Index *d* varies from 1 to -1. In the case of unidirectional coupling from S1 to S2, *d* is 1, in the opposite case when the unidirectional coupling is from S2 to S1, *d* is -1. Positive intermediate values of d express a stronger or weaker S1 to S2 coupling strength, the negative intermediate values a coupling strength in the opposite direction (S2 to S1). In the case of absence of interaction when $c_2 = c_1$, d is zero.

3. HEART RATE ANALYSIS RESULTS

3.1. Introduction

The first chapters have presented the clinical question and the methods chosen to address it.

This third chapter presents the results of our investigation and it is organized in four main points: firstly, we will review the effect of sleep states on the various methodologies proposed to quantify autonomic control and cardiorespiratory coupling. In addition, we will present the investigation of the effect of sleep states on the novel methodologies proposed, namely transfer entropy and phase locking.

Second, a study evaluating the effect of one of the major extrinsic factor for SIDS, the prone position during sleep, will be presented. Next, results regarding the effect of an intrinsic factor, prematurity, will be discussed. Lastly, the investigation of the autonomic parameters in a population exposed to a combination of intrinsic (alcohol and smoking exposure during pregnancy) and an extrinsic autonomic challenge (head up tilt) will be outlined.

Each part will have a subsection highlighting relevant methodological aspects investigated, such as the optimal tuning of hyper parameters or relationship between different metrics.

3.2. Sleep States Effects on Cardiorespiratory Regulation

Sleep is a central activity in humans: it is characterized by the activation of numerous cortical, subcortical and medullar neural circuits, and is regulated by hormonal changes, cardiovascular challenges, circadian variations and other factors. Autonomic activity is a key factor in sleep physiology, modulating HRV and breathing. Previous newborn studies employing traditional time and frequency domain analysis have shown HRV characteristics to be dependent on the behavioral state of the infant. HR was faster in active sleep (AS) than quiet sleep (QS), global HRV (SDNN or LTV), VLF and LF power were reported to be higher in AS, while HF power was found to be increased in QS. In some studies, STV or RMSSD have shown no state difference, while in another studies, were predominant in QS. Thus, long term HRV is generally increased during AS while short-term HRV is increased during QS. These findings have been attributed to a major vagal activity and fall of sympathetic activity during QS, and the inverse behavior combined with more variable respiratory patterns and increased body movement in AS [117], [165], [166].

In addition to these traditional linear parameters, entropy measures have provided further information about the complexity of HRV behavior. Interestingly, QS in infants is characterized by an increase in Sample Entropy [167]–[169]. Thus, almost unexpectedly, a more complex dynamic of the human sympatho-vagal balance can emerge in a resting or relatively inactive state. This can be understood in light of the fact that decrease in SampEn has been shown in situation of reduction of vagal influence on the heart paired with sympathetic activation, such as following a positional change from supine to standing in human adults [170], [171]. This relationship between entropy values and the sympatho-vagal balance is also supported by animal studies [172], [173].

Shifting from a single signal to a multi-signal perspective, evaluating the mechanisms underlying cardiorespiratory interactions in different sleep states is huge challenge given the transient and nonlinear characteristics of the two signals. Both systems are under neuroautonomic control that regulates their complex dynamics and further influences their coupling through intrinsic feedback mechanisms at different time scales.

Analytic techniques to address this topic have ranged from cross-spectral analysis to nonlinear methods, considering linear and nonlinear relations between HR and respiration signal [174], [175]. Cross-spectral and coherence analyses have shown significant coupling in the newborn population in

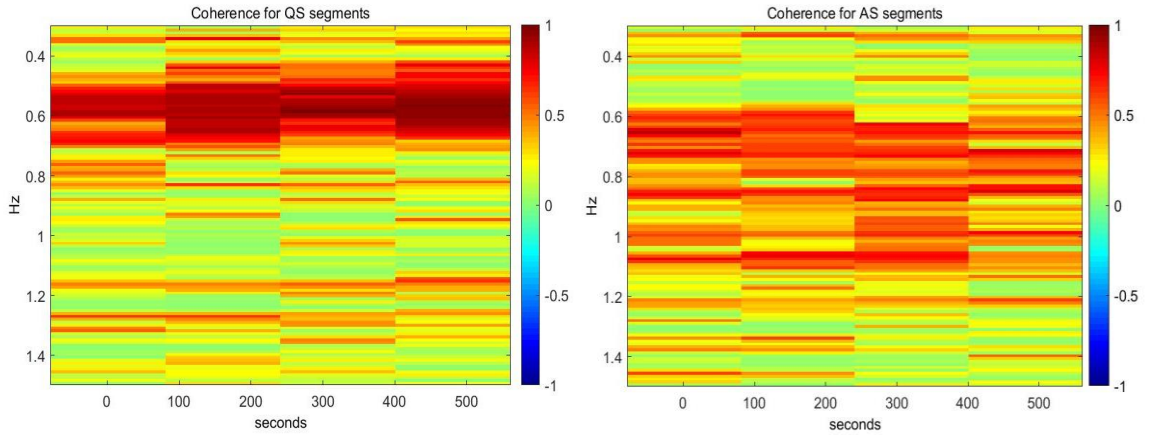

Figure 3-1: Heatmaps of 2 coherence values for 10 minutes recordings of 2 subjects: on the left the subject was in quiet sleep on the right the subject was in active sleep.

the HF, mostly in QS. As an example, Figure 3-1 shows coherence values for 10 minutes of active and quiet sleep in two newborns, with clear significant HF peaks in QS (left) and weak and inconsistent coupling for AS(right). This coupling is also influenced by gestational age at birth. Furthermore, the linear phase relationship between the two signals was not found to be stable [140], [176].

From the information presented above, the extent to which sleep states influence HRV and cardiorespiratory measures is evident. For this reason, we decided to investigate sleep states effect also on the more novel approaches proposed in this thesis, Transfer Entropy (TE) and Phase locking (PL), and compare the results with traditional techniques [34], [35].

3.2.1. Experimental Protocol and Dataset

The analyses presented were performed on data from 151 newborns (gestational age (GA) at birth 38-40 weeks), selected based on their GA from a larger cohort of 326 infants (GA at birth 35-41 weeks). Out of the selected infants, 33 returned for a one-month follow-up. None of the infants enrolled had been admitted to the Neonatal Intensive Care Unit (NICU) nor had any major illness, congenital abnormalities or known genetic disorders. Mothers were at least 18 years of age and displayed no evidence of major illness or psychiatric disorders during the pregnancy. These signals were acquired at Columbia University Medical Center (CUMC), upon mothers' consent and Institutional Review Board (IRB) approval.

ECG and respiratory activity were recorded non-invasively at a sampling rate of 500 Hz and 200 Hz, respectively, by means of three leads on the chest in standard positions (RA, RL, LL, DATAQ

Instruments) and by a respiratory inductance belt around the infant abdomen (Ambulatory Monitoring Inc., Ardsley, NY, USA). During the acquisition, infants were sleeping and lying supine. Sleep states were classified into AS and QS [177].

For time and frequency domain analysis and entropy analysis segments of 300 consecutive beats in the same sleep state were identified and then analyzed: the total number of segments was 525 (304 AS, 221 QS) for newborns and 247 (108 AS, 139 QS) for one-month infants. The average length of the 300 beats segments was 148.30 ± 14.01 s for newborns in AS, 151.63 ± 12.29 s for newborns in QS, 124.02 ± 8.55 s for one-month infants in AS and 129.87 ± 10.04 s one-month infants in QS. The length

Table 3-1: Parameter values (mean ± SD) for 300-beat segments, for newborn and one-month-old infants in AS and QS. *p*-values indicate statistical comparison between AS and QS for newborn and one-month-old infants, and between newborn and 1-month-old infants in AS and QS.

Parameter	Parameter Values				*p*-Values			
	NB		OM		AS vs. QS		NB vs. OM	
	AS	QS	AS	QS	NB	OM	AS	QS
Time domain								
RR mean [s]	0.496 ± 0.047	0.509 ± 0.039	0.415 ± 0.029	0.425 ± 0.022	n.s.	n.s.	<0.01	<0.01
SDNN [ms]	34.028 ±13.940	25.982 ± 10.140	23.168 ± 6.567	15.334 ± 6.008	<0.01	<0.01	<0.01	<0.01
RMSSD [ms]	17.382 ± 8.869	18.165 ± 7.815	11.730 ± 3.896	10.050 ± 4.924	n.s.	n.s.	<0.01	<0.01
IBI mean [s]	1.240 ± 0.260	1.483 ± 0.264	1.315 ± 0.240	1.630 ± 0.340	<0.01	<0.01	n.s.	<0.05
IBI IQR [s]	0.440 ± 0.146	0.258 ± 0.088	0.404 ± 0.144	0.278 ± 0.102	<0.01	<0.01	n.s.	n.s.
Frequency domain [%]								
LF/(LF+HF)	0.891 ± 0.065	0.770 ± 0.125	0.873 ± 0.102	0.7745± 0.177	<0.01	<0.05	n.s	n.s.
HF/(LF+HF)	0.101 ± 0.056	0.230 ± 0.125	0.116 ± 0.091	0.195 ± 0.143	<0.01	n.s.	n.s	n.s.
Conventional entropies [bits]								
SampEn1	1.76 ± 0.26	1.98 ± 0.14	1.69 ± 0.28	1.94 ± 0.15	<0.01	<0.01	n.s.	n.s.
SampEn2	1.63 ± 0.31	1.84 ± 0.19	1.58 ± 0.30	1.86 ± 0.16	<0.01	<0.01	n.s.	n.s.
SampEn3	1.52 ± 0.37	1.69 ± 0.25	1.49 ± 0.35	1.74 ± 0.24	<0.01	<0.01	n.s.	n.s.
QSE1	7.89 ± 0.21	8.05 ± 0.16	7.82 ± 0.25	8.04 ± 0.18	<0.01	<0.05	n.s.	n.s.
QSE2	7.92 ± 0.21	8.07 ± 0.15	7.87 ± 0.25	8.09 ± 0.14	<0.01	<0.01	n.s.	n.s.
QSE3	7.97 ± 0.21	8.11 ± 0.14	7.91 ± 0.26	8.12 ± 0.15	<0.01	<0.01	n.s.	n.s.
Transfer entropy [bits]								
RR→RESP	0.03 ± 0.02	0.04 ± 0.02	0.04 ± 0.02	0.06 ± 0.02	< 0.05	< 0.01	n.s.	< 0.01
RESP→RR	0.04 ± 0.02	0.09 ± 0.06	0.03 ± 0.02	0.10 ± 0.06	< 0.01	< 0.01	n.s.	< 0.05
Phase locking [%]								
Synch 3:1	0.02±0.02	0.10±0.10	0.01±0.012	0.04±0.05	<0.01	<0.01	n.s.	< 0.01
Synch 4:1	0.01±0.01	0.01±0.03	0.01±0.02	0.07±0.06	n.s	<0.01	n.s.	< 0.01
Synch n:1	0.06±0.05	0.23±0.14	0.07±0.05	0.30±0.17	<0.01	<0.01	n.s.	< 0.01

of the segments was chosen based on previous studies, which showed that 300 beats was an appropriate number for TE estimation [149]. For PL analysis, segments of three minutes length in a continuous sleep state were analyzed: 514 three-minute epochs were considered for newborns (275 AS, 239 QS), while 247 epochs were considered for one-month infants (103 AS, 144 QS).

3.2.2. Results and Discussion

In the comparison between sleep states, time and frequency domain parameters showed that overall RR variability (SDNN) and mean RR were lower in AS, combined with lower mean IBI and higher IBI variability. No difference was found in beat-to-beat variability (RMSSD) both at birth and at 1 month of age. SampEn, QSE and HF power % were significantly higher in QS. At lag=0, TE values were higher in QS and comparing the two directionalities, there was no clear difference in AS, while in QS RESP → RR directionality was clearly dominant with respect to RR → RESP, both for newborn and one-month infants. TE values at lag=0 are shown in Figure 3-2. When looking at the values obtained with

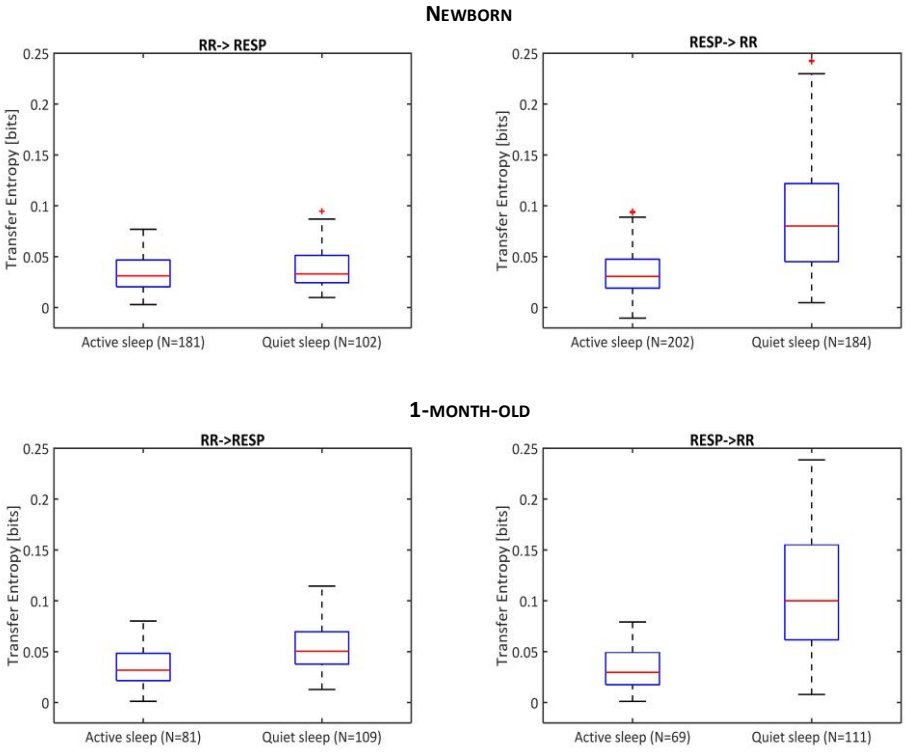

Figure 3-2: Boxplot of TE values at lag=0. In the first row are shown values for newborn infants in active and quiet sleep with directionality RR →RESP on the left and RESP →RR on the right. In the second row, the same information is presented for one-month infants.

lags, different regulatory dynamics behind the two directionalities were unveiled. As portrayed in Figure 3-3, TE from RR → RESP reports a significant between-subjects effect for both sleep state and lag ($p<0.001$ and $p<0.001$), but no interaction effect between the two. The information flow from RR to RESP is slow but steady, acting within 2 beats and lasting around 10 beats. The amount of significant TE estimates, based on surrogate testing, progressively decreases as a function of increasing

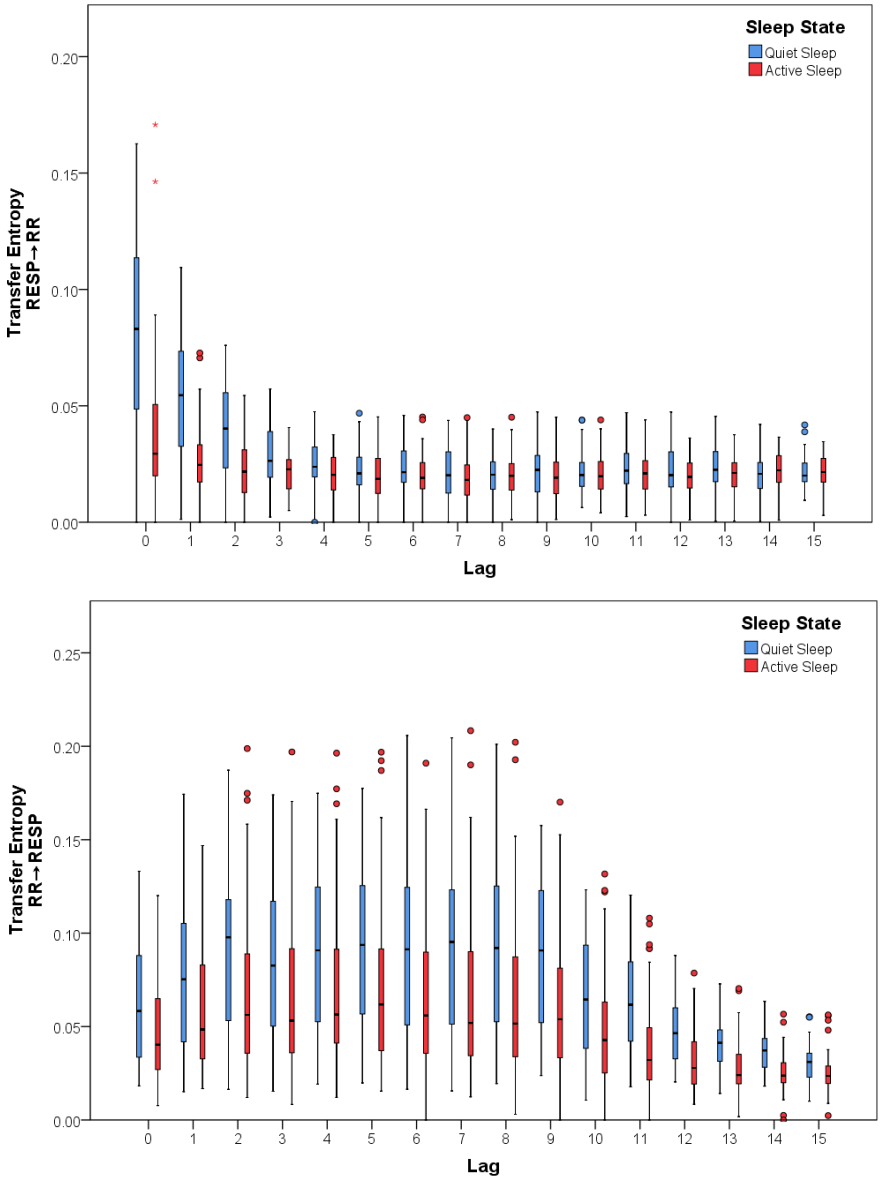

Figure 3-3: In the upper panel, TE RR-> RESP is portrayed at different lags, while on the lower panel, TE RESP-> RR is shown. In both panels, quiet sleep values are shown in green and active sleep values in red.

lag. The non-significant estimates are on average 15 over 174 in the lag interval 0-12 and they further increase up to an average of 62 from lag 12 to 15. Post hoc tests showed that values at lag=0 were significantly different from the successive 8 lags. On the other hand, TE RESP→RR shows a fast but not lasting effect of breathing on RR, with significant between-subjects effects for both sleep state and lag ($p<0.001$ and $p<0.001$) as well as an interaction effect ($p<0.001$). Surrogate testing procedure excluded an average of 66 TE values from lag 4 to lag 15 but only around 30 from 0 to 3. No differences in TE distributions were found comparing each pairs of lags from lag 3 to lag 15.

These two dynamics might reflect that different information transfer directionalities are in fact driven by different autonomic branches of the ANS [178]. As a matter of fact, the sympathetic branch intervenes on a slower time scale, but its effect last longer in the target system, whereas the parasympathetic has a punctual yet rapidly vanishing action. Given these considerations, it is possible to speculate that that the vagal system is possibly more implicated in the directionality from RESP to RR while the sympathetic in the opposite directionality.

Regarding the cardiorespiratory synchronization, our findings showed a significant higher value both in percentage of time spent in coupling and in duration of the coupled epochs in QS, both at the newborn and one-month stage, as in Figure 3-4.

We applied BPRSA technique to a subset of the infants (N=4), using the respiratory phase as the trigger signal and the RR series as the target. Moreover, anchor points (APs) were identified both with the increment and decrement definition in the respiratory phase. Univariate PRSA was also estimated for the respiratory phase. Comparing the BPRSA transformation of the target signal with the monovariate PRSA transformation of the trigger signal is possible to observe potential trigger and target signal interaction. Figure 3-6 illustrates two examples: in the left panels a clear entrainment is visible whereas the panels on the right show a weak coupling. In the first case, the median frequency of cardiorespiratory coupling had been estimated with coherence method at 0.62 Hz, in the second case was 0.89 Hz. This example shows how BPRSA is sensitive to CRC, extracting periodic interrelated behaviors, often masked by resetting due to internal and external perturbations.

When looking at the behavior of parameters as a function of age (at birth vs one month), time domain parameters showed an increase in HR and a decrease in variability. No entropy differences were found across ages. In TE lag=0 the major differences occurred in QS, with an increase in information flow in

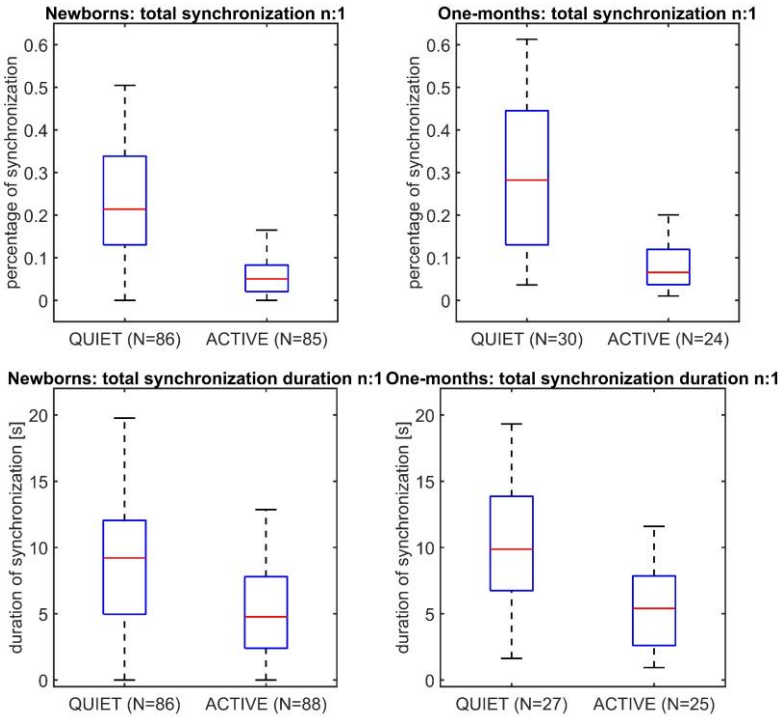

Figure 3-4: Total percentage of time spent in coupling and length of the coupled epochs in active and quiet sleep. Boxplot represent newborns on the left and one month infants on the right, N is the number of subjects

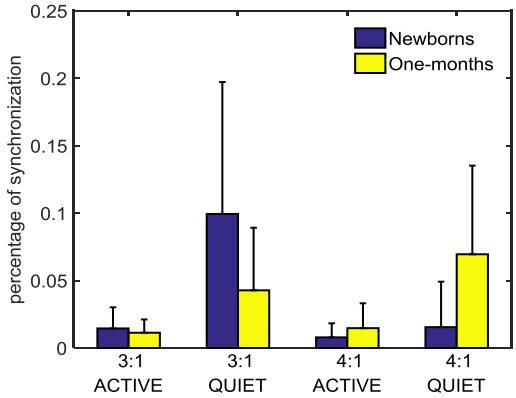

Figure 3-5: Bargraph of the percentage of synchronization on different *n:m* ratios for newborns (blue) and one month infants (yellow)

both directions with age, while in AS values remained unchanged. An increasing trend with age in terms of synchronization was observed, even though significance was not achieved. Moreover, the ratio of the dominant synchronization shifted from a majority of 3:1 with newborns, to 4:1 with one-month infants, as visible in Figure 3-5. All parameters values are reported in Table 3-1.

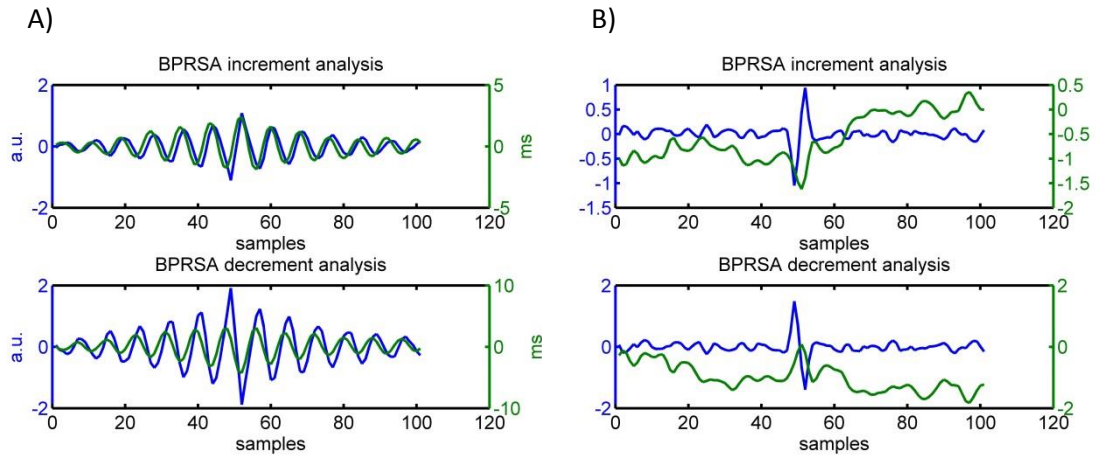

Figure 3-6: PRSA curves of respiratory phase signal are shown in blue, BPRSA curve in green. The upper panel refers to increment APs, the lower panel to decrement APs. (A) Subject with a clear and synchronous coupling in QS (B) Subject with a weak coupling in AS.

These findings are convergent with previous studies, which suggested increased parasympathetic activation in QS and in AS a simplification of HR dynamics, and thus a lowering in entropy values, potentially following parasympathetic withdrawal and sympathetic activation [170], [172]. Our results also converge with previous results by Pincus et al., who found higher values of approximated entropy (ApEn) in QS with respect to AS [18], and with another measure of complexity based on Mutual Information, AIF [175].

Our focus comparing newborns and one-month infants is related to the fact that epidemiological studies have shown that the incidence of SIDS peaks between 2-4 month of age. Thus, it is of particular importance to note a shift in the typical cardiorespiratory synchronization ratio between a low risk period and a high risk one.

Additionally, TE results illustrate that QS is a state in which information flow between HR and respiration is higher than in AS. Given that SampEn and QSE should provide a measure of complexity of a signal, higher HR entropy values in QS could be associated with an increased interaction with other physiological systems. These findings suggest that cardiorespiratory interactions in QS transfer more information than in AS in healthy infants, as also suggested by Frasch et al [175] .

In QS, coupling is stronger for both information flow directions. Moreover, the main direction of the information flow is RESP → RR in QS, while in AS this not evident in both newborn and 1-month-old infants.

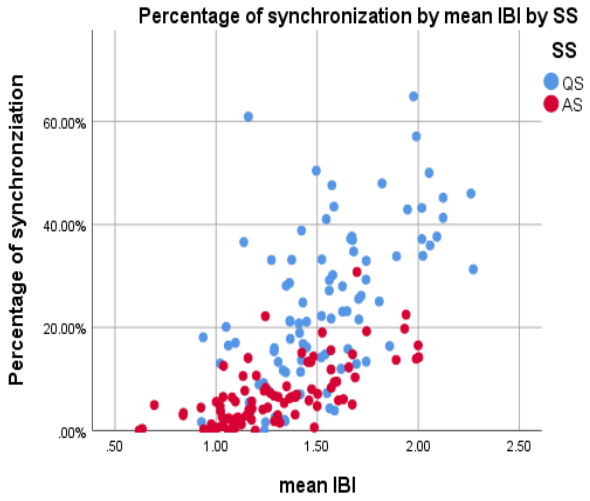

Figure 3-7: Scatterplot of percentage of synchronization and mean IBI in quiet (blue) and active (red) sleep, showing a positive correlation between the two variables

As highlighted in previous infant report [179], the directionality of cardiorespiratory interaction is not as consistent as in adults, with phenomena like respiratory sinus arrhythmia. Nonetheless, our results seem to indicate a difference in directionality balance based on sleep state. This could be driven by differences in the average breathing frequency. That is, when breathing frequency is higher there is less opportunity for respiration to dynamically modulate HR. Higher breathing frequency occurs more often in AS, and this could account for an absence of a dominant directionality.

In addition to this, we found a correlation between phase synchronization and mean IBI, as shown in figure 3-7, which had not been previously identified in adults [31], [32]. Even in this case, given the very different breathing frequency ranges between infants and adults, we might infer that very short IBI could have an impact on synchronization. Nonetheless, this hypothesis needs further investigation. Lastly, an age-dependent change in information flow happens only in QS. AS is per se a state of lower coupling between HR and respiration and this does not dramatically change with age.

METHODOLOGICAL REMARKS:

Entropy measures tend to be influenced by the choice of the parameters *r*, *m*, *N*, as previously reported [180]. In our study, the consistency of entropy measures can be observed independently of the parameter choice. In fact, our results were not affected by parameter *m*. In preliminary studies, we also tested the same measures on segments with *N* equal to 100 and 200 beats and

used 3 minutes epochs, and obtained comparable results. This strongly suggests that the difference between groups remains stable.

For the PRSA technique, the choice of T can be optimized depending on the frequency of interest, since PRSA is most sensitive for the detection of oscillations with frequency f=1/(2.5T) [146]. In this particular case, given our interest in the cardiorespiratory interaction, we tuned the parameter T using the mean breathing frequency of each segment, obtained as the coherence value above threshold in the HF band.

Lastly, the investigation of different modes of cardiorespiratory interaction in the same population shows how complementary forms of coupling occur with an intermittent behavior, with different strength and directionality patterns across physiological states.

3.3. Effect of External Stressor: Supine vs Prone Position

In this next study, we employed a multi-parametric approach for the analysis of HR in the at-risk population of prematurely born infants. Premature birth precludes full in-utero development of the ANS and may thereby increase the probability of SIDS occurrence. As sleep position has emerged as the primary SIDS postnatal risk factor we investigated the effects of prone versus supine position on a range of HRV parameters. Specifically, we incorporated a wide array of mathematical techniques to analyze HR signals collected prior to hospital discharge and then again two months later. This novel and comprehensive approach combined multiple indices utilizing time domain, frequency domain and nonlinear methods. Furthermore, as premature infants share physiological characteristics of the fetus and the infant, we employed a set of parameters typically employed in fetal HR analysis, as well as in standard adult HR studies.

The approach we utilized in this study also allowed investigation of several aspects of premature autonomic control that occur at different time scales, and involve different control mechanisms, known to be influenced by position and sleep state in the newborn. Moreover, the availability of a follow-up study at two months of age allowed the evaluation of the development of ANS control during the postnatal period [36], [181].

3.3.1. Experimental protocol and data set

The dataset was acquired in the Infant Physiology Laboratory at CUMC, New York. It was approved by the IRB of the CUMC and mothers provided informed consent at enrolment. This study included data collected at two time points: the first performed on 35 healthy premature infants immediately prior

to discharge from hospital (GA at birth 28.7 ±2 weeks and post menstrual age at time of study 37.7 ±2.4 weeks) and repeated on 24 of these infants at a follow-up study two months after discharge (GA at birth 27.9 ±1.6 weeks and post menstrual age at time of study 49.2±3.2 weeks).

The duration of the first study protocol was 6 hours. During the first 3 hours, infants slept in supine position and were then moved to prone for the rest of the study. Infants were fed right before the start of the study and again after 3 hours. The follow up study at 2 months was designed to last one hour, due to difficulties in obtaining periods of uninterrupted sleep with older infants. Thus, infants spent only 30 minutes in each position. Only AS presented enough data for each baby, thus all results are summarized for AS only.

ECG was acquired at 500 Hz and the RR series in seconds were obtained using a customized peak detection algorithm followed by visual inspection. In this dataset breathing was not available so the analysis focused only on the HRV parameters. In the first study, only patients with at least three 3 minutes' segments of good quality RR series with continuous sleep state were accepted. In the follow up, due to the reduced length of the study, patients with at least one 3 minutes' segment of good quality RR series with continuous sleep state were included. This requirement reduced the number of infants available for the analysis: for the first study 20 infants met the criteria, while for the follow up 10 infants met these criteria.

3.3.2. Results

Analysis by sleep position in the first study showed clear differences both in the long-term variability parameters (LTI and PRSA) and in the short-term variability parameters (SampEn and QSE). Supine position was characterized by higher variability but lower complexity. In the follow-up study, the most robust state differences were observed only for the parameters quantifying short-term variability, specifically, RMSSD and STV, and entropies. Results are shown in Table 3-2.

From a physiological standpoint, these results support the hypothesis that prone position is a strong autonomic challenge for the infants and thus can be a major risk factor for SIDS, if combined with an intrinsic vulnerability. Interestingly, the response to this autonomic challenge is different at discharge and at 2 months of age, which corresponds to the peak of SIDS events. The very premature condition of the infants analyzed is an intrinsic vulnerability since the early birth is a strong stressor for the infant's physiological development since their systems were ill prepared for the relatively early transition to extra-uterine life.

Table 3-2: Parameters' results for the first and follow up study. P-values were obtained with Student's t-test for the first study and with the Wilcoxon Signed Rank test for the follow up.

	Prone	Supine	P-val
First study - newborn			
LTI	0.040 ± 0.018	0.049±0.23	<0.001
SDNN	0.0212 ± 0.008	0.0237 ± 0.009	<0.05
PRSA ΔY	15.66 ± 10.18	18.71 ± 10.68	<0.01
SampEn	0.554 ± 0.183	0.443 ± 0.133	<0.001
QSE m=1	-4.201 ± 0.415	-4.042 ± 0.304	<0.001
Second study- 2 months			
Mean RR	0.414± 0.048	0.447 ± 0.0727	<0.05
RMSSD	0.014 ± 0.009	0.015 ± 0.011	<0.05
STV	0.01 ± 0.006	0.011 ± 0.008	<0.05
QSE m=2	-3.27 ± 0.848	-3.15± 0.783	<0.05

In the follow-up study, at two months of postnatal age, the parameters most strongly associated with the effects of sleeping position, were measures of short-term variability, generally associated with parasympathetic regulation. With increasing postnatal age, infant parasympathetic system should take the lead in HR autonomic regulation. The fact that at 2 months of age measures of vagal activity are altered in prone position might suggest suppressive effect of this position altering the correct sympato-vagal balance. Lastly, the behavior of more variability and less complexity in supine sleep vs prone, mirrors the results obtained in quiet sleep and once more contributes to the understanding that variability and complexity characterize different aspects of a signal.

METHODOLOGICAL REMARKS:

Different values of T were tested to calculate PRSA curves, and only values above 30 samples could discern the two positions, as portrayed in Figure 3-8. These values correspond to about 10 seconds and thus are related to long term variability. Moreover, the introduction of QSE calculated with the Minimum Count of Matches (MCM) approach, proved to be a significant improvement. As shown in Figure 3-9, QSE is less dependent from the length of the segment analyzed: SampEn values collapse when N approaches 0 because not enough matches are found. With the adjustment of *r* introduced by the MCM procedure and the log(2r) correction, QSE is more stable and consistent. This is relevant since in real applications often only short segments are available for the analysis and QSE can provide a reliable entropy estimate even in these circumstances.

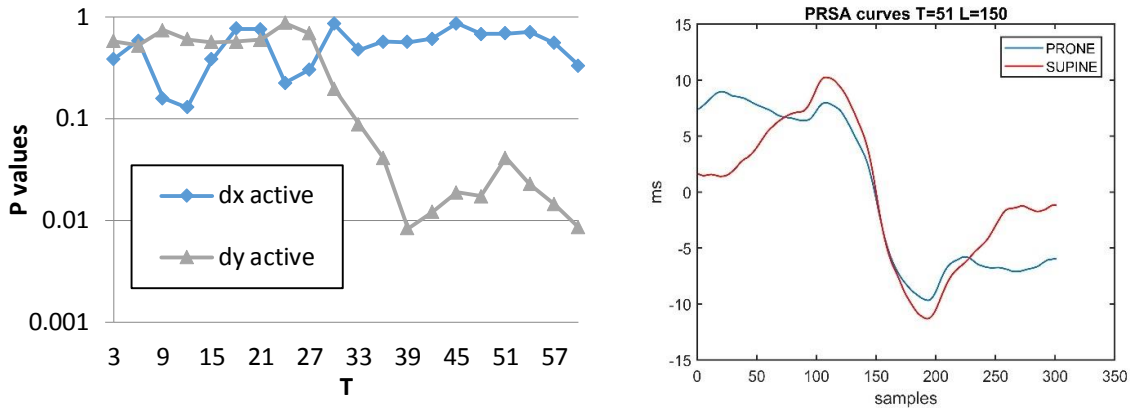

Figure 3-8: On the left a representation of p-values for dx and dy in AS as a function of the parameter T, showing dy reaching significance only after T>33. On the right a comparision of 2 PRSA curves obtained with T=51 and L=150 with one subject in prone position (blue) and in supine position (red).

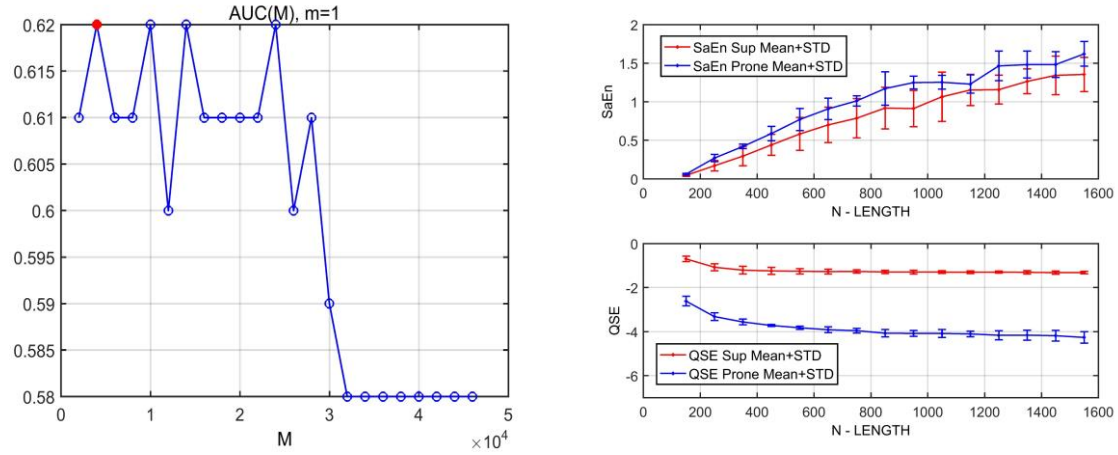

Figure 3-9: On the left a graph showing the area under the Roc curve for the QSE parameter as a function of the minimum count of matches. The red dot indicates the number of matches selected to perform the analysis. On the right SampEn and QSE for a subject in supine position (red) and prone (blue) position as a function of N, the length of the segment analyzed.

In this application, the choice of the MNC value was optimized with three steps: the MCM for each segment was computed as $M_{max} = (N_x - m)(N_x - m+1)/2$ where N_x is the number of samples of each time series and *m* the embedded dimension. Among these, the smallest M_{max} was chosen and set as M_{max} for all the series. Since 3 minutes segments have around N = 300, this translated in $M_{max} \approx$ 300^2 = 90'000. The QSE was then computed with MNC varying from 1 to M_{max} with a step of 2'000 (M = 1:2'000:M_{max}). For each M, *r* was initialized to zero and increased of successive steps of: $r_{t+1} = r_t + 0{:}015$

*std(sig) until M matches were found. Lastly, the optimal M is chosen as the one obtaining QSE values more capable of discriminating the supine and prone condition, thus maximizing the area under the ROC curve.

3.4. Effect of Intrinsic Vulnerability: Prematurity

Infants born at 35-37 weeks of GA are at higher risk for a range of pathological conditions and poorer neurodevelopmental outcomes, however, mechanisms responsible are not fully understood [182]. The purpose of the analyses presented in this section was to assess newborn autonomic development as a function of GA at birth, with traditional and novel signal processing technique, focusing on cardiorespiratory regulation. Several studies indicating irregular cardiorespiratory coupling as a leading cause of several pathologies underscore the need to investigate this phenomenon in this at-risk population [69]. In particular, novel techniques will allow to investigate linear and nonlinear coupling and infer on possible directionality of the interaction between the cardiac and the respiratory system.

3.4.1. Experimental Protocol and Dataset

This study included 329 newborns from $35^{0/7}$- $40^{6/7}$ weeks GA, including both singleton and multiple births, born at the Morgan Stanley Children's Hospital of New York. None of the infants enrolled had been admitted to the NICU or had any major illness, congenital abnormalities or known genetic disorders. Mothers were at least eighteen years of age and displayed no evidence of major illness, or psychiatric disorders during the pregnancy. The IRB of the New York State Psychiatric Institute and of CUMC approved the study and mothers signed informed consent forms prior to enrollment in the study.

Newborn infants were tested 12-84 hours after delivery (avg. 39±15 h). Infant recordings were collected in a quiet room near the nursery approximately 30 minutes after a day time feeding. The study protocol designed to include assessment of sleep dependent behavior and learning, required a 10-minute baseline recording of ECG and respiration. Respiration and ECG were acquired as outlined in paragraph 3.2.1. The respiration signal was filtered with a bandpass filter (0.05 - 3.5 Hz). Breath inspiration intervals and ECG R waves were marked with a customized peak detection algorithm, followed by visual inspection of the signals to remove marks due to erroneous automated identification. Thresholds of acceptance for RR interval were set as 0.3-0.667 seconds, with an absolute variation between consecutive RR intervals of 10%, while for respiration thresholds were 0.5-2.5 seconds and an absolute change of 40%.

Sleep states were classified into AS, QS, Indeterminate (I) and Awake (W). The minimum length for a segment to be classified either as AS or QS was 120 seconds, I and W segments were discarded from the analysis. The sleep state coding assessment was based on respiratory variability [183] and confirmed by behavioral codes entered throughout the study to determine when infants were awake, crying, or fussy. Of 329 infants enrolled, for 281 it was possible to perform sleep state coding. Following the guidelines of the American College of Obstetricians and Gynecologists, infants were divided into three groups: newborns whose GA was (1) 35-36 weeks, called Late Preterm (LPT); (2) GA 37-38 weeks, categorized as Early Term (ET) and (3) GA 39-40 weeks, Full Term (FT).

Segments of three minutes length in a continuous sleep state were analyzed providing with a total of 114 infants of 281 in QS (19 LPT, 40 ET, 55 FT) and a total of 171 infants of 281 in AS included in the final analysis (51 LPT, 60 ET, 60 FT). Parameters computed for this analysis were time and frequency domain, QSE and transfer entropy, and lastly phase locking and directionality.

3.4.2. Results and Discussions.

Time domain and frequency domain results presented in Table 3-3, show increasing RR mean interval, short-term HRV (RMSSD), HR complexity (QSE) and linear cardiorespiratory coupling (HF RR, HF biv) with GA, indicating an increasing autonomic and cardiorespiratory control as a function of GA at birth (respectively, $p=0.001$, $p<0.001$, $p=0.007$ $p<0.001$, $p=0.008$).

TE presented significant changes by GA only in the directionality RESP-> RR, with increasing values as a function of GA ($p<0.05$). Post-hoc analysis shows that LPT were significantly different from FT and ET. TE in both directions was significantly influenced by sleep states (RR->RESP $p<0.001$ and RESP->RR $p<0.001$), but no interaction occurred between GA and sleep states (RR->RESP $p=0.0257$ and RESP->RR $p=0.0258$).

Measures of cardiorespiratory phase locking indicate no relationship between time spent in phase-synchronized state and GA group. Differences were instead detected with sleep state in all GA groups, with more frequent and longer synchronization in QS. No interaction effect was found between the two independent variables, GA and sleep state (n:1 synchro % $p=0.874$ and n:1 synchro duration $p=0.740$). Results are shown in Table 3-4 and 3-5.

Previous work highlighted a change in the quantity of synchronization during the first 6 months of life [35], [158]. Thus, it was surprising to discover in our study that the quantity of synchronized time did not change significantly within the last weeks of GA. This might be because this aspect of

Table 3-3: Descriptive statistics (mean ± std) of time and frequency domain parameters by gestational age (GA) in quiet sleep (QS) and active sleep (AS). The groups are: late preterm (LPT), early term (ET), and full term (FT).

state	GA	RR mean	SDNN	RMSSD	IBI IQR	HF_RR	QSE	HF_biv
QS	LPT	0.48 ± 0.03	21.32 ± 11.88	10.20 ± 6.83	0.27±0.10	0.38 ± 0.19	3.16±0.69	0.556 ± 0.19
	ET	0.49 ± 0.04	22.87 ± 10.85	13.23 ± 7.06	0.28±0.08	0.46 ± 0.18	3.45±0.69	0.597 ± 0.19
	FT	0.51 ± 0.04	24.59 ± 11.47	15.72 ± 7.67	0.26± 0.11	0.50 ± 0.17	3.74±0.64	0.663 ± 0.19
AS	LPT	0.48 ± 0.04	28.80 ± 12.19	11.60 ± 5.40	0.40±0.15	0.29 ± 0.12	3.37±0.58	0.379 ± 0.13
	ET	0.49 ± 0.0	30.93 ± 12.90	12.29 ± 6.09	0.42±0.13	0.31 ± 0.14	3.43±0.67	0.395 ± 0.13
	FT	0.50 ± 0.05	31.83 ± 13.87	13.40 ± 6.51	0.39± 0.13	0.32 ± 0.13	3.63±0.67	0.413 ± 0.13

Table 3-4: Descriptive statistics (mean ± std) of the transfer entropy from RR to breathing (RR->RESP) and from breathing to RR (RESP->RR), of the square root of the percentage of time in synchronized state and the average synchronization duration and number of subjects by gestational age (GA) in quiet sleep (QS) and active sleep (AS)

state	GA	RR→RESP	RESP→RR	n:1 synchro %	n:1 synchro duration	N Subjects
QS	LPT	0.065±0.033	0.054±0.029	0.436 ± 0.209	2.99 ± 1.02	19
	ET	0.066±0.039	0.083±0.039	0.454 ± 0.184	3.03 ± 1.01	41
	FT	0.064±0.029	0.084±0.042	0.473 ± 0.170	3.00 ± 0.90	58
AS	LPT	0.040±0.023	0.032±0.018	0.185 ± 0.124	1.82 ± 1.04	54
	ET	0.042±0.021	0.039±0.023	0.218 ± 0.127	2.07 ± 0.84	62
	FT	0.052±0.028	0.042±0.032	0.221 ± 0.139	1.92 ± 0.97	68

Table 3-5: 2-way Anova p-values of the square root of the percentage of time in synchronized state and the average synchronization duration by gestational age (GA) and sleep state along with their interaction effect

Parameter		p-val	Partial η^2
n:1 synchro %	state	0.000	0.357
	GA group	0.342	0.007
	state * GA group	0.874	0.001
n:1 synchro duration	state	0.000	0.214
	GA_group	0.559	0.004
	state * GA group	0.740	0.002

cardiorespiratory regulation mainly develops in the months following birth. Thus, differences between previous and current findings might be related to differential influences of intra-uterine versus extra-uterine life, rather than of GA at birth.

The multivariate test with d and breathing frequency as dependent variable and GA and sleep state as independent, showed significant differences by GA and state (respectively, p = 0.003; p = 0.001). No significant interaction was detected between GA and state (p = 0.972). The test of between-subjects'

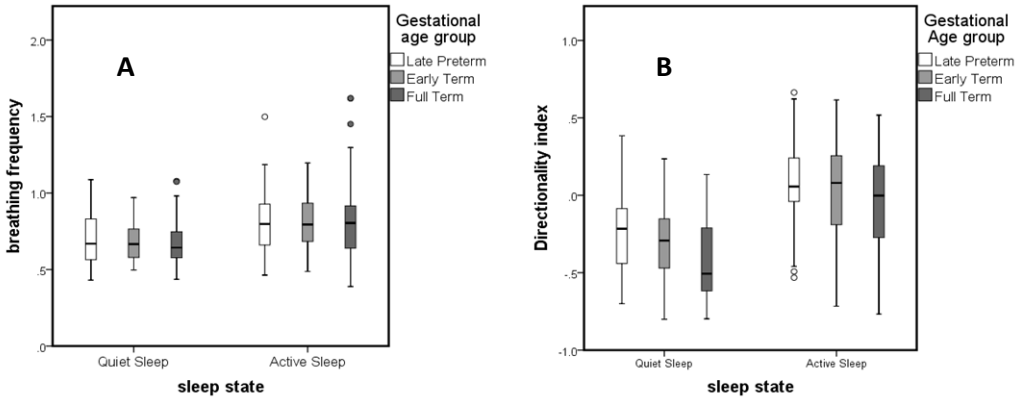

Figure 3-10: a): Boxplots show breathing frequency by gestational age (GA) and sleep state. Breathing frequency is not significantly different by GA at birth but is significantly different by sleep state. b): Boxplots show directionality index by GA and sleep state. Directionality index is statistically different both by GA at birth and by sleep state. Neither breathing frequency nor directionality index show any interaction effect between GA and sleep state.

effects highlighted how each dependent variable differed based on the independent variables. To account for multiple ANOVAs, we used a Bonferroni correction accepting statistical significance at $p < 0.025$. Results are reported in Table 3-6 and 3-7 and shown in Figure 3-10. The test shows that sleep state significantly influences both *d* and respiratory frequency, while GA at birth influences significantly only *d*. Directionality index *d* decreases with GA at birth. In QS all the three GA groups show dominant influence of breathing on HR ($d<0$), but this influence grows with GA (*d* becomes more negative). On the other hand, in AS a balanced relationship is present in LPT ($d\approx0$) and it moves toward a dominant relationship from breathing to HR in FT ($d<0$). Post-hoc tests of the significant ANOVAs showed that among the GA groups, LPT were significantly different from ET which were also significantly different from FT.

Previously, another research group reported a similar directionality shift but in full term newborns monitored during the first months of life, suggesting that the maturation of this aspect of the cardiorespiratory interaction occurring in the immature preterm infant continues in the first months of life until a final set point is reached in later childhood [184].

In the current study, in QS lower values of *d* were observed in all GA groups signifying a stronger influence of breathing on HR, as previously demonstrated with other coupling measures [185].

Mrowka et al. had hypothesized that directionality depends on respiratory frequency. In particular, respiration rate would act as a lowpass filter, i.e. below a set respiratory frequency directionality is

Table 3-6: Descriptive statistics (mean ± std) for directionality index and respiration frequency by gestational age (GA) and sleep state and number of subjects

State	GA group	Directionality index	Respiration frequency	N SUBJECT
QS	**LPT**	-0.240 ± 0.292	0.699 ± 0.181	19
	ET	-0.307 ± 0.266	0.676 ± 0.126	41
	FT	-0.417± 0.259	0.668 ± 0.149	59
AS	**LPT**	0.083 ±0 .262	0.815 ± 0.200	55
	ET	0.044 ± 0.306	0.808 ± 0.180	62
	FT	-0.028 ± 0.284	0.818 ± 0.237	68

Table 3-7: Multivariate model analysis of directionality index and respiration frequency by gestational age (GA) and sleep state along with their interaction effect and post hoc analysis

Parameters		p-val	Partial η^2	Post hoc
Directionality index	**GA group**	0.001	0.049	LPT>ET>FT
	state	<0.001	0.273	
	GA group * state	0.816	0.001	
Respiration frequency	**GA group**	0.881	0.001	LPT>ET>FT
	state	<0.001	0.097	
	GA group * state	0.857	0.001	

mainly from respiration to HR, whereas above a certain respiratory frequency the interaction becomes bidirectional. The underlying mechanisms for this lowpass filter effect could be related to lower information transmission to the cardiac oscillator caused by reduced information from the vagal nerve to the atrial pace-maker cells when respiration frequency is above a certain threshold, proposed at 0.6 Hz [184]. Thus, the cardiac influence on respiration would be weak and frequency independent, while the coupling from the respiration to HR for low respiratory frequencies would be strong compared with the strength of the cardiac influence, and of similar entity for higher frequencies [184]. Figure 3-11 shows the directionality index histograms of the three GA groups at breathing frequencies ≥0.6 Hz (in each panel on the right) and at breathing frequencies <0.6 Hz (in each panel on the left). This figure illustrates the occurrence of this bimodal influence: concurrently with breathing frequencies <0.6 Hz, an increased polarization toward negative values of *d* occurs, especially in ET and FT. Nonetheless, the mean breathing frequency does not change significantly in this specific GA window (35-40 weeks) (p=0.881); thus, the significant change in directionality with GA at birth (p=0.001) cannot be explained solely because of breathing frequency. One explanation for this phenomenon could be that the

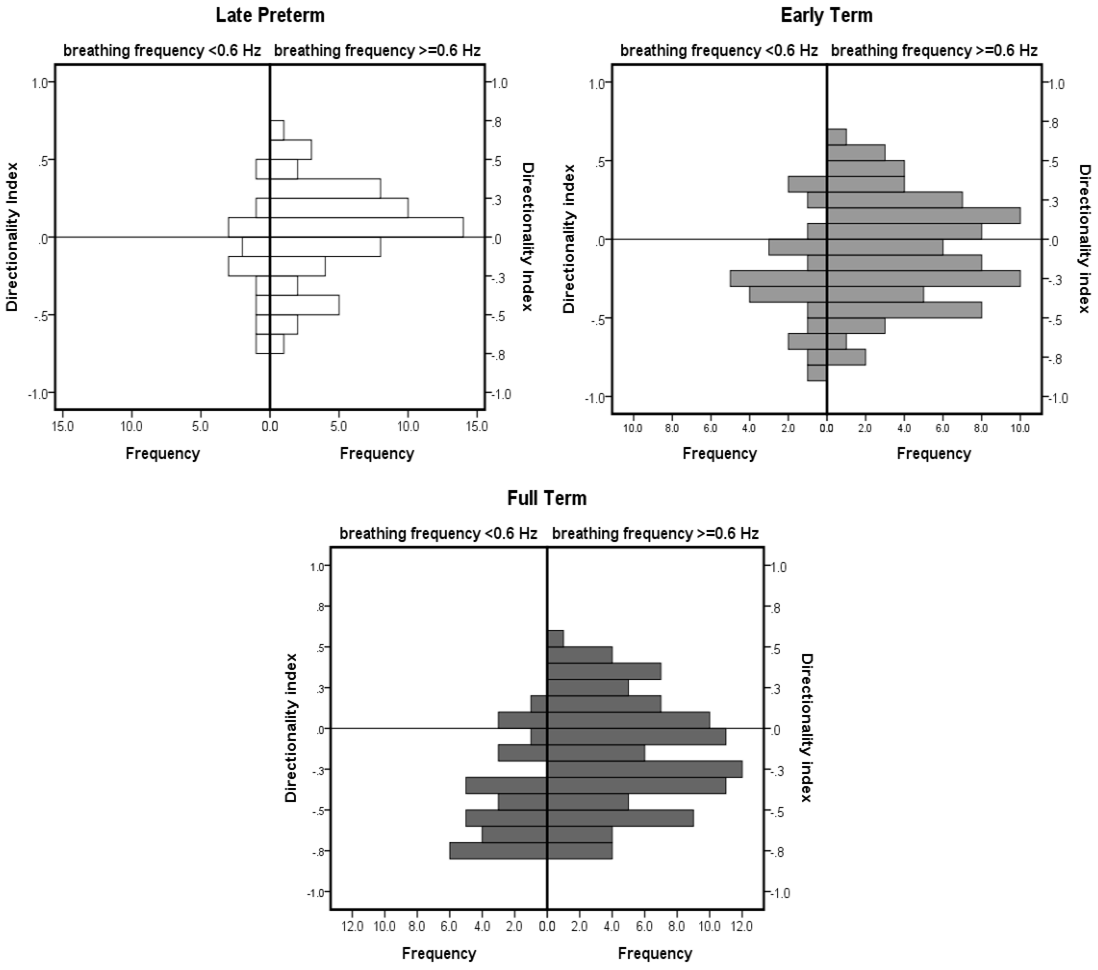

Figure 3-11: In each panel, histograms of directionality index distribution are plotted, on the left for breathing frequencies < 0.6 Hz, and on the right for breathing frequencies ≥ 0.6 Hz in order for LPT, ET and FT

threshold for the low pass filter effect is still adapting between 35-40 weeks GA. At a younger GA, for instance in the LPT group, the value for the cutoff frequency might be lower when compared to more mature conditions, such as FT.

Given that this cutoff frequency is potentially related to vagal nerve regulation, these findings would be consistent with previous studies showing immature vagal function in premature infants. Morphological studies also demonstrate a rapid developmental increase in number of myelinated vagal fibers with postconceptional age (PCA), and by 40 weeks after conception, total fiber counts were comparable to those of adolescents. Interestingly, the number of total myelinated vagus fibers in

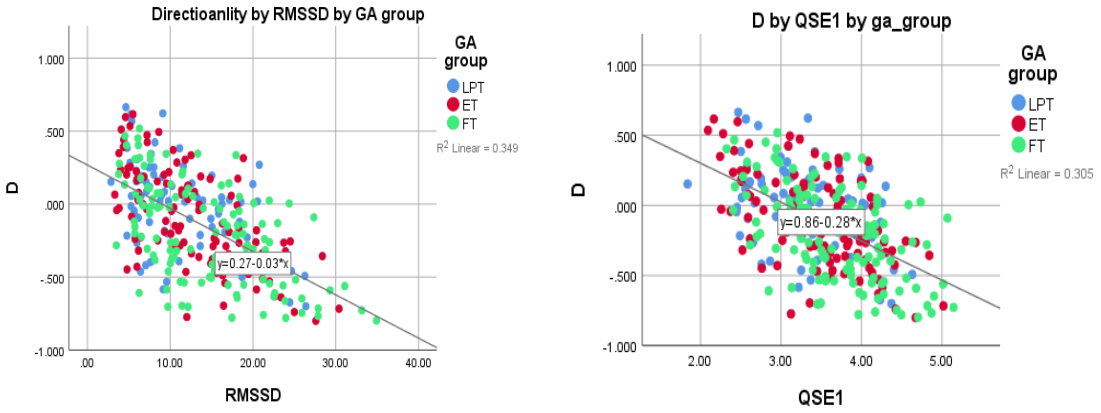

Figure 3-12: On the left, the scatterplot of directionality index and RMSSD and on the right the scatterplot of the directionality index and QSE. In blue are reported values for LPT, in red for ET and in green for FT.

preterm infants (≤38 weeks PCA) was found to be significantly smaller than for the term or adolescent age groups [186].

This hypothesis is supported even further by the fact that the directionality index *d* was found to be negatively correlated at every age group with QSE and RMSSD, parameters associated with parasympathetic activity, as shown in Figure 3-12. Thus, a decreasing value of *d* with GA might be driven by a complex interplay of parasympathetic and respiratory regulatory effects.

The question remains as to physiological meaning behind this type of nonlinear coupling. One possible explanation could be that this type of synchronization involves an energy consumption benefit, namely, the reduction in intrathoracic pressure during inspiration increases cardiac filling and consequently cardiac output. However, this hypothesis needs further investigation.

METHODOLOGICAL REMARKS:

From a methodological perspective, the detrimental impact of even minor data contamination with artefact was evaluated. The widespread practice of visual inspection of data for extra or missing RR intervals remains problematic both in terms of reliability and practicality. Furthermore, any fixed threshold value may give rise to classification errors, especially when inter-individual physiological differences in baseline HR are not accounted for [187].

QSE is a novel nonlinear measure mostly correlated with parasympathetic activity as other more traditional measures, such as RMSSD [34], [171], [173]. Nonetheless, the application of this novel measures has the advantage of not requiring signal stationarity [188].

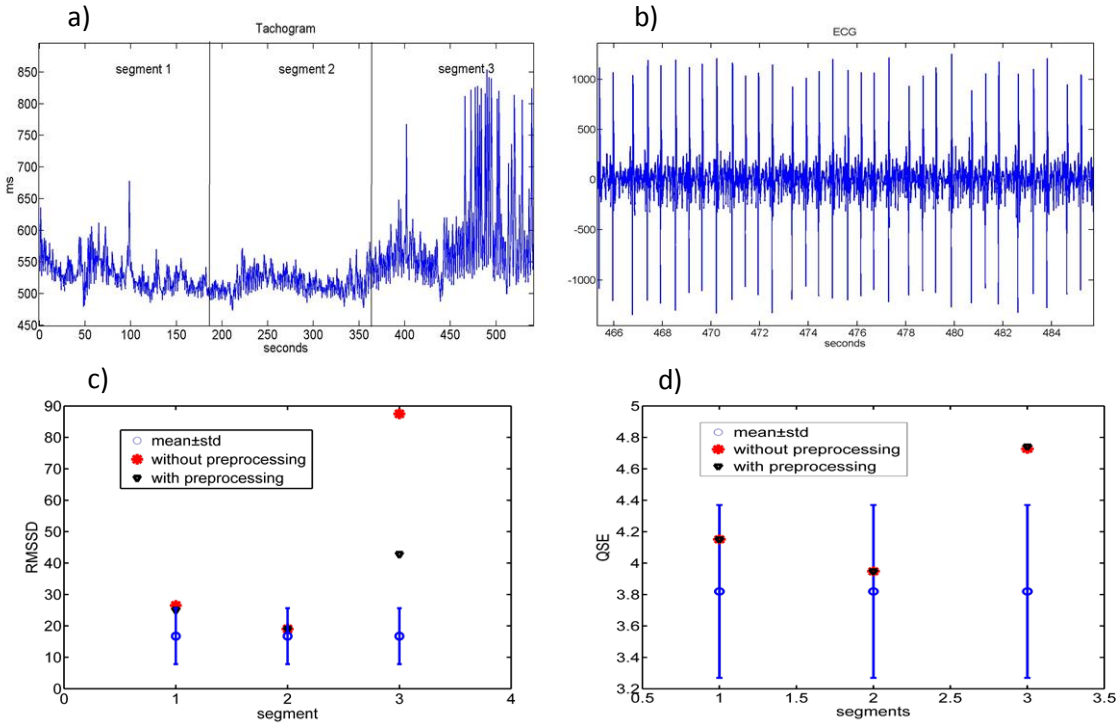

Figure 3-13: a) RR interval series for a three 3-minute segment. b) ECG tracing corresponding to RR series in a) from 466 to 485 s showing transient bradycardia. c) RMSSD values for the three segments in red when no preprocessing is applied on the RR interval series, and in black after the RR artifact removal is applied. d) QSE m=1 values with the same logic as panel c). In panel c) and d), averages and standard deviations for the FT group are shown in blue. Preprocessing was performed excluding all RR intervals out of the range of 266 and 667 ms. In this case 6.6% of the last segment was excluded during the preprocessing.

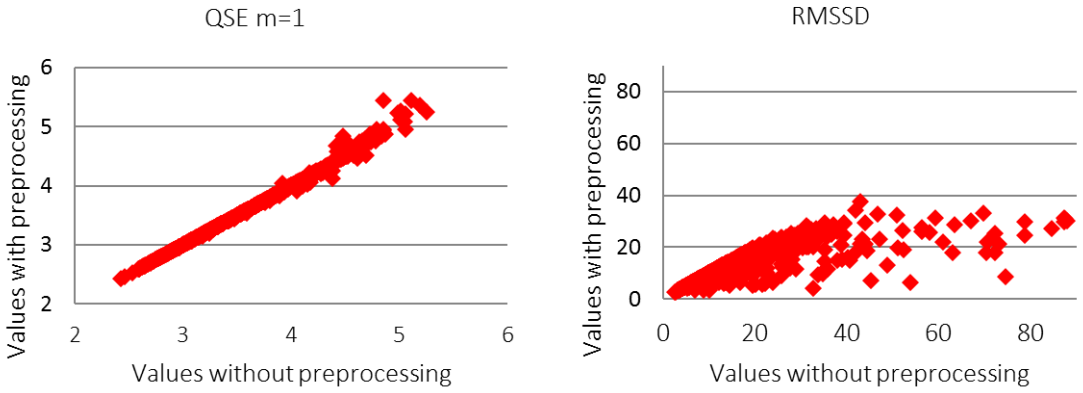

Figure 3-14: Values of RMSSD and QSE of the three populations before and after RR intervals series preprocessing are plotted against one another.

In this study, QSE proved to be less affected by noise and sporadic arrhythmias than RMSSD. To portray how signal nonstationary episodes can affect the parameter estimation, we report in Figure 3-13 an example of how ECG signal, RR series and parameters were extracted. For the infant whose data are shown as reference, there were episodes of alterations in the physiological heart rhythm. Panel a) shows the RR interval series for the three 3-minute segments, panel b) shows an example of the corresponding ECG tracing during transient bradycardia, which gave rise to the long RR intervals shown in the third segment of panel a). In panel c), values of RMSSD for the three segments are plotted. They are marked in red if no preprocessing was applied on the RR interval series, and in black if the measures were computed after the RR artefact removal is applied. Finally, panel d) shows QSE values with the same logic as panel c). In panel c) and d), averages and standard deviations for the FT group are depicted in grey.

In Figure 3-14, values of RMSSD and QSE before and after RR intervals series preprocessing are plotted. These two figures depict the extent to which preprocessing of data affects values of RMSSD. As portrayed in panel c) of Figure 3-13, the RMSSD value is decreased by almost 50% after preprocessing, whereas QSE compensated for the presence of artefacts.

This example illustrates that adding the QSE parameter to the analysis provided significantly more consistent results, over and above from RR intervals series preprocessing and thus QSE can be considered an important parameter to be added to the repertoire of measures used for ANS characterization.

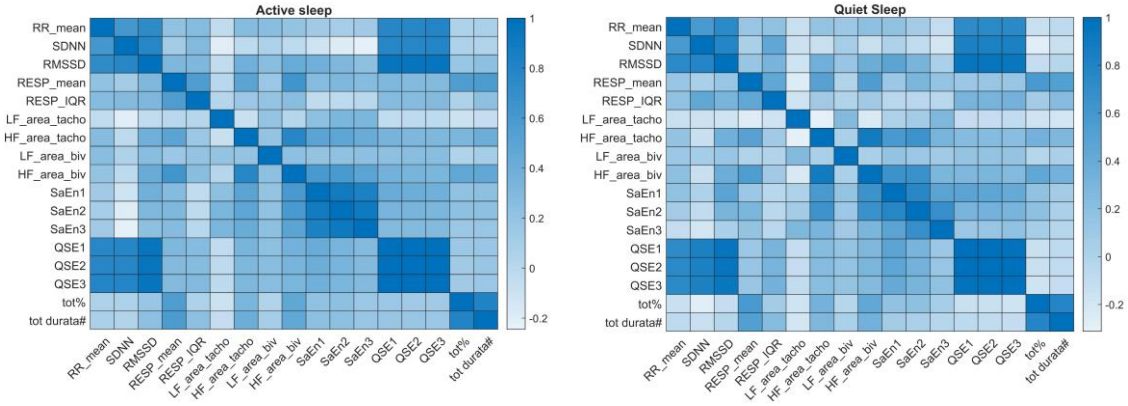

Figure 3-15: Correlation matrices for all the indices calculated in this section, on the left in active sleep and on the right in quiet

Figure 3-15 portrays the pairwise correlations of all features calculated in this section, for quiet and active sleep state. These plots reveal interesting characteristics: first, the correlation structure is similar in both stages, second, some features are uncorrelated independently of stage, like % of synchronization and synchronization duration. These preliminary considerations are now under further investigation, with feature selection and reduction analyses.

Lastly, especially when parameters are equally informative, issues like preprocessing time and robustness to artefacts should be taken into consideration.

3.5. Combination of Intrinsic Vulnerability and External Stressor: Alcohol and Smoking Exposure during Pregnancy and Head-up Tilt

This study investigates the effects of prenatal alcohol and smoke exposure on tilt response in newborn infants, since associations between exposure and increased 2- to 5-fold risk of SIDS have been reported [189]. The underlying hypothesis describes abnormalities in the control of homeostatic functions by the developing brainstem [190].

Graded head-up tilt has been widely used as a test to observe and quantify autonomic control. The tilt should elicit a shift of the sympathovagal balance toward a sympathetic activation and parasympathetic withdrawal [191]. Several indices have been proposed to quantify sympathetic and parasympathetic activity. However, there is no current consensus for standardized measures of vagal activation/withdrawal in infants.

This investigation entails a novel application of parameters of autonomic function control to characterize the immature neonatal ANS, whose cardiorespiratory interactions are not yet stable. The analysis was performed on two groups of infants, newborns unexposed and exposed in utero to both alcohol and smoking. Multiple measures of vagal withdrawal were investigated and compared: traditional time and frequency domain estimates along with novel complexity measures. The combined use of these indexes suggested different regulatory dynamics in the two groups and opens to novel perspective in physiological and clinical SIDS risk assessment.

3.5.1. Experimental protocol and data set

The subjects analyzed were enrolled in the Safe Passage study [39] and the IRB approved all experimental procedures involving human. Subjects were recruited at Tygerberg Hospital and affiliated clinics in Bellville, Cape Town, South Africa. Infants were routinely discharged from the hospital less

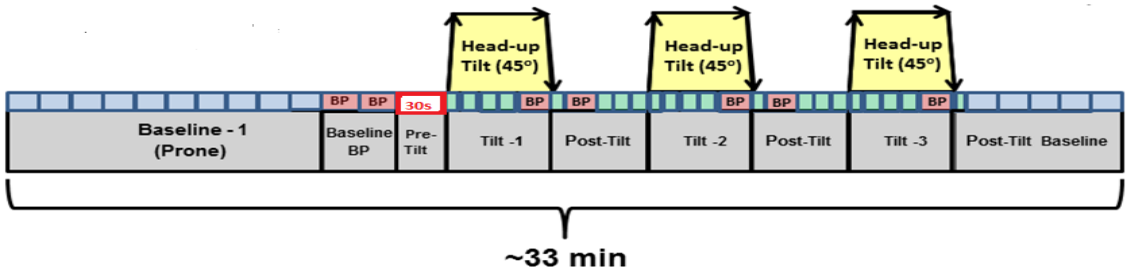

Figure 3-16: Schematic of cardiorespiratory assessments. Baseline epochs are 60 seconds in duration. The epochs immediately after head-up or tilts back to flat were 15 seconds in duration. All other epochs were 30 seconds in duration.

than 24 hours after delivery, they returned within 48-96 hours for assessment. At this visit, nurses recorded ECG, respiration, blood pressure (BP), and cortical brain activity (EEG) during sleep.

Data recordings were approximately half an hour long and occurred approximately 30 minutes after eating. The standardized procedure consisted of a 10-minutes baseline period and three rapid (~3–5 seconds) 45° head-up tilts, while the infant was in the prone position as it may accentuate the response evoked by tilting. Each of three tilts session was subdivided into the following blocks of time: 30-sec just prior to tilt (B); 15-seconds block right after the infant reached head-up position (I); three 30-seconds blocks (T1, T2, T3) before returning to flat position, as shown in Figure 3-16. This study analyzed data from the first tilt session out of the three. The first block of 15 seconds right after tilt was not considered due to its lack of stationarity.

ECG was recorded at 500 Hz and respiratory tracings were simultaneously collected at 20 Hz using a respiratory inductance belt, which was placed around each infant's chest. A modification of the Timeline Follow-Back Interview was employed by nurses to guide mothers' self-reported estimation of their tobacco and alcohol consumption during pregnancy [39]. Based on the assessment, subjects were divided in: i) control group (CG): no prenatal exposure to alcohol or tobacco smoke; ii) exposed group (EG): heavy prenatal exposure to both substances during the first, second or third trimester of pregnancy. Mothers in the EG had on average more than 7 drinks per trimester and smoked consistently during the whole duration of their pregnancy. Inclusion criteria for the cohorts in this study were: full-term infants (GA at birth ≥37 weeks), birthweight ≥2500 g, South African mixed ancestry, no resuscitation at birth or admission to the NICU, no prenatal exposures to drugs of abuse, no evidence of diabetes, hypertension, or pre-eclampsia during pregnancy in maternal medical records.

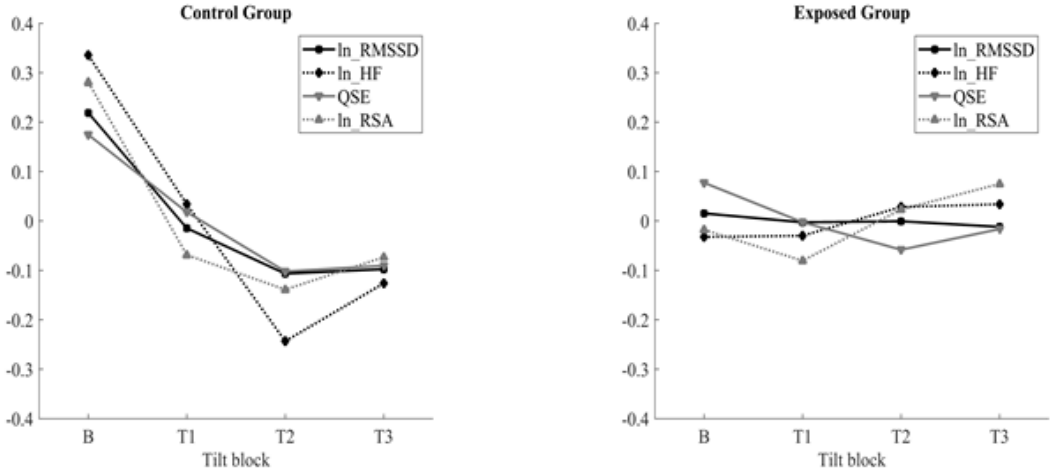

Figure 3-17: Left and right panel show the mean values of the four extracted parameters and their trends when plotted based upon the tilt blocks. On the left, CG show a consistent behavior across all indexes, characterized by a decrease from B to T3. On the right, group means in EG do exhibit a flatter tendency. The mean value for a given index (obtain by grouping means in B, T1, T2, T3) has been subtracted from mean values reported in Table 3-8 to provide a comparable scale across parameters

The total number of infants in the CG was 15 (3 males – 12 females), GA 39.7 ± 1.07 weeks while in the EG the total number was 13 (6 males – 7 females), GA is 39.2 ± 0.83 weeks. Infants sleep states were classified into active (AS) and quiet sleep (QS), awake (A) or indeterminate (I) by a Matlab automated algorithm [183]. In this study, the analyses were performed exclusively in periods classified as QS.

3.5.2. Results

The mean and standard deviation of parameters for each tilt block, F and p-value of Greenhouse-Geisser tests are reported in Table 3-8. Each parameter was analyzed having tilt block as within-subjects factor. Statistical analysis shows a significant within-subjects effect of tilt block on all extracted parameters in the CG. Left panel of Figure**Error! Reference source not found.** 3-17 shows the mean t rend of the extracted parameters for CG. The computed parameters show a consistent decrease in markers of vagal activation when comparing B vs T1 and B vs T2. On the other hand, the vagal activation pattern after segment T2 appears to exhibit an absent response.

To investigate the statistical significance of the reported differences between pairs of tilt blocks a series of post-hoc tests were performed. The pairwise comparisons showed a significant difference when comparing B vs T2 for all parameters (RMSSD *p-value*=0.01*,* ln_HF *p*-value=not significant (n.s.), QSE p-value<0.01, ln_RSA p-value<0.05).

Table 3-8: Mean ± std of parameter for each tilt block and within-subjects results of repeated measures ANOVA for CG and EG.

Control Group (N=15)				**Exposed Group (N=13)**			
B	T1	T2	T3	B	T1	T2	T3
ln_RMSSD							
-4.27 ± 0.69	-4.50 ± 0.62	-4.59 ± 0.68	-4.58 ± 0.67	-4.17 ± 0.53	-4.19 ± 0.60	-4.18 ± 0.49	-4.20 ± 0.57
p-value=0.001		F(1.83, 25.55)=9.29		*p-value*=n.s.		F(2.11, 25.30)=0.05	
ln_HF							
-10.46 ±1.45	-10.76 ±1.27	-11.04 ± 1.48	-10.92 ± 1.41	-10.26 ± 1.03	-10.26 ± 1.16	-10.20 ± 0.99	-10.19 ± 1.15
p-value<0.05		F(2.40, 33.52)=3.22		*p-value*=n.s.		F(2.10, 25.19)=0.07	
QSE							
3.72 ± 0.74	3.57 ± 0.58	3.44 ± 0.66	3.46 ± 0.73	3.89 ± 0.53	3.80 ± 0.58	3.74 ± 0.50	3.78 ± 0.52
p-value<0.001		F(2.58, 36.09)=7.66		*p-value*=n.s.		F(1.86, 22.37)=0.71	
ln_RSA							
-3.93 ± 0.77	-4.28 ± 0.74	-4.35 ± 0.84	-4.29 ± 0.77	-3.99 ± 0.61	-4.06 ± 0.72	-3.95 ± 0.61	-3.90 ± 0.67
p-value<0.05		F(1.69, 23.66)=4.24		*p-value*=n.s.		F(1.80, 21.53)=1.02	

The right panel of Figure 3-17, depicts a substantially different behavior for the EG group, with vagal tone estimators staying stable for every tilt point. The absence of any difference is confirmed by the results of repeated measures ANOVA. Results reported in Table 3-8 show lack of significance when performing the within-subject's analysis testing each variable of interest separately.

The direct comparison between CG and EG did not show any main effect associated with groups when performing repeated measures ANOVA with group as a factor, nonetheless with such small sample size the between subjects' variability was extremely high.

The reported results for RMSSD in CG are consistent with findings by Myers et al [192], which showed a significant decrease in beat-to-beat variability as consequence of tilt. The presented results for HF power are in accordance with previous studies showing a vagal withdrawal in a population of adults in response to tilt [191]. Complexity analysis performed in this study shows, for unexposed infants, a significant within-subjects decrease of QSE. This trend is similar to previous findings reporting Approximate Entropy (ApEn) decreasing as a consequence of vagal blockade [193]. The decrease in complexity on such short-term scale (m=1) may indicate a shift towards increased regularity of RR series due to vagal withdrawal and increased sympathetic activation.

As highlighted by Karemaker [194], the quantification of vagal tone is an open issue as it is its relationship with respiration and in particular to RSA. RSA definitely represents a respiration-related modulation in parasympathetic outflow to the sinus node, but the exact mechanisms involved in its

Table 3-9: Pearson correlation coefficient for RMSSD, RSA,HF and QSE

	RMSSD	***RSA***	***HF***	***QSE***
RMSSD	1	-	-	-
RSA	0.909**	1	-	-
HF	0.906**	0.884	1	-
QSE	0.902**	0.740	0.764	1

Table 3-10: Skewness and Kurtosis for the parameters proposed and for their log transformation when the original parameters did not pass the requirement for normality distribution.

	Skewness		Kurtosis	
	Statistic	Std. Error	Statistic	Kurtosis Std. Error
RMSSD	1.422	.361	2.173	.709
RSA	2.186	.361	6.814	.709
HF	2.453	.361	6.808	.709
QSE	.127	.361	-.489	.709
ln_RSA	-.330	.361	.612	.709
ln_HF	.023	.361	-.618	.709
ln_RMSSD	.102	.361	-.299	.709

generation are still the subject of discussion. Most likely, RSA is the results of an interaction of mechanical, central and baroreflex mechanisms [195]–[197]. For this reason, an exact equivalence between RSA and vagal tone cannot be attained. Nonetheless, RSA and other measures of variability from the time and frequency domain have proved to still be sensitive to vagal activity, in particular in cases of changes from rest to vagally stimulated conditions, such as tilt.

The results for EG show consistent lower vagal withdrawal in response to tilt challenge for every extracted parameter. In the literature, prenatal exposure to smoke has been reported to effectively impair receptors in the medullary 5-HT system, as well as alterations in cardiorespiratory control mechanisms and abnormalities in the pathogenesis of the parasympathetic systems [189]. It is possible to speculate that illustrated differences in vagal tone comparing CG and EG may effectively arise as a consequence of prenatal exposure and may contribute to the decreased infant's ANS capability to maintain homeostatic control when exposed to a direct physiological challenge. The reported findings seems to confirm the altered HR response to tilt as described for other groups of infants at high risk for SIDS [198].

METHODOLOGICAL REMARKS:

To compare the performances of the four measures of vagal activity proposed, we decided to address three main questions: 1) are these measures correlated? 2) are they reliable when estimated on short time periods? 3) do they conform to the assumption of normality required for parametric testing?

As illustrated in Table 3-9, the four metrics were highly and significantly correlated during baseline conditions. Moreover, when we evaluated the relation between estimates in short baseline segments (30 seconds) as used in this analysis and longer baseline segments (3 minutes), which were used for all our previous analyses, we showed that all measures were highly correlated, with HF having the lowest value. Pearson correlation for RMSSD was 0.833, for RSA 0.800, for HF 0.619, and for QSE 0.858.

Lastly, we verified if the measures complied with the requirement of normality (i.e. skewness and kurtosis $<\pm 2$). In Table 3-10 measures of skewness and kurtosis are reported. RMSSD, RSA and HF did not have values within the bounds and were thus transformed with a log transform.

In conclusion, although the four metrics were highly correlated, they should not be considered interchangeable. Addressing these three questions allowed us to highlight that these measures differ in terms of statistical features and are differently influenced by segment length. Thus, we suggest that when choosing a parameter to assess a pathophysiological condition, we should not only consider its ability to discriminate different conditions but the compliance with requirements for statistical analysis and the consistency with different segment lengths.

4. DISCUSSION AND CONCLUSIONS

The main purpose of this Ph.D. thesis was to characterize autonomic and cardiorespiratory regulation in newborn populations at risk for Sudden Infant Death Syndrome (SIDS). The final objective was to provide a pool of parameters capable of highlighting the emergence of alterations in these crucial pathways for the maintenance of homeostasis. The employed tools were comprised of signal processing techniques to analyze heart rate and breathing tracings, acquired from the period right after birth to 1-2 months later. Though heart rate variability and breathing had been previously investigated in studies with similar populations, no final consensus had emerged on this topic. The novel aspect of this Ph.D. Thesis lies in the analytic approach: instead of looking the cardiac and the respiratory systems separately with traditional linear techniques, we have incorporated nonlinear techniques and introduced state of the art methodologies capable of characterizing the interactions between the two systems.

The biggest challenge in SIDS research lies in its almost complete unpredictability. Until now, no one has been able to explain the mechanisms that lead to this death nor to propose reliable markers of risk. Most of what we know comes from epidemiological studies, which have highlighted several risk factors, such as sleep practices related to the type of bedding or the position during sleep, or infant vulnerabilities (prematurity, IUGR etc) or maternal exposure (cigarette smoking, less prenatal care, etc.) [64].

More information has come from post mortem examinations, such as brain autopsies or genetic testing, and animal models which have pointed to abnormalities related to the brainstem and genetic

alteration in promoter regions of the serotonin transporter (5-HTT) in infants who died of SIDS. The brainstem and the serotonin pathways play a crucial role in the regulation of many vital functions, including cardiorespiratory regulation, sleep cycling and arousal [5].

These evidences all together have led the research community to propose the triple risk model, which is now the most widely accepted model to describe the possible mechanism leading to SIDS. It states that SIDS results from the simultaneous occurrence of: an underlying vulnerability, a critical developmental period in homeostatic control and exposure to exogenous stressor(s). The simultaneous presence of these three factors triggers a cascade of events most likely involving the autonomic nervous system, mechanisms of arousal and cardiorespiratory response [3]. Nevertheless, none of these factors has been properly characterized, limiting the usefulness of this approach.

A more detailed description about risk factors, mechanisms and models of SIDS can be found in Chapter 1.

The intuition behind the work presented in this Ph.D. thesis, is the fact that novel signal processing techniques could be capable of extracting relevant information from physiological signals, such as HR and breathing, which have been for long time used as a probe to investigate cardiac autonomic modulation. These signals have the peculiarity of being easily and noninvasively obtainable from infants. Several attempts had been done in the past to retrospectively look at data from infants subsequently died of SIDS and analyze their HR tracings but no univocal conclusion had been reached [16], [199], [200]. Our approach instead focuses on populations exposed to at least one of the risk factors mentioned in the triple risk model and characterizes them, in order identify and describe common tracts in this “abnormal” autonomic profile. Moreover, we introduced novel techniques which go beyond the linear interpretation and consider the complex origin of the physiological signals. Lastly, instead of the classical reductionist approach where each physiological signal is characterized separately, we have explored techniques to view them as a whole and evaluate their interactions. We also investigated different modes of cardiorespiratory interaction, ranging from linear amplitude, to nonlinear complexity modulation and phase relationships.

Thus, in this work novel methods to analyze the HR and breathing signals were proposed, including entropies, phase rectified signal averaging (PRSA), phase locking and directionality [131], [149], [164]. A thorough explanation of all these methods can be found in Chapter 2.

Chapter 3 is divided in four main parts: firstly, we present the results from investigation of the effect of sleep states on the parameters of interest. We highlight that not only sleep states affect mean HR and traditional measures of HRV, with quiet sleep (QS) having lower HR and less variability, but also entropy measures inform us that in QS there is a higher level of complexity. This finding, which at first might have seemed counterintuitive, becomes more interpretable looking at the results from Transfer Entropy, which indicate a stronger flow of information in QS, with a main direction from breathing to HR, possibly indicating a more complex network of interaction in this state [34]. A newly proposed technique evaluating Transfer Entropy at various lags also highlighted different temporal profiles of information exchange between the cardiorespiratory systems. The information of timing would complete the information previously obtained regarding amplitude and directionality and could assist us in discerning the relative role of the sympathetic and parasympathetic branches, which are known to work with different activation times. This is relevant because growing literature is suggesting that a state of sympathetic hyperactivity could lead to an increase in cardiovascular morbidity and mortality [201], [202]. Nonetheless, while some HRV parameters are considered to assess parasympathetic activity, there is no widely accepted HRV index used as a marker of sympathetic nervous system activity only.

In addition, QS is also associated with stronger and longer periods of phase locking. Very interestingly, the profile of phase locking changes with age, with different predominant ratios when comparing newborn and one month old infants. This is particularly significant given that one month is the beginning of the critical period for SIDS and a reorganization in the type of coupling hints toward a change in the cardiorespiratory profile at this specific time [35].

Lastly, we noted that profiles of directionality by sleep state and relationships between measures of cardiorespiratory coupling and breathing frequency show peculiar characteristics, very different from the one described in adults.

All of these findings encouraged us to consider sleep state in further analysis as a significant factor when looking at cardiorespiratory interactions.

In the second part of Chapter 3, a study focused on the effect of sleeping position is presented. The prone sleeping position has long been considered the major risk factor for SIDS and early studies had observed decreased variation in behavior and respiratory pattern, increased HR, and increased

peripheral skin temperature during prone, compared with supine sleep, suggesting that infants are less capable to maintain adequate respiratory and metabolic homeostasis when sleeping prone [203]. Our results confirmed a different autonomic control depending on position, with lower HRV and higher values of entropy in prone position. In particular, at the newborn stage all variability parameters were altered by sleep position, while at 2 months of age, the main differences by position were only in the short-term variability and complexity, indicating that at this stage differences were mainly relative to parasympathetic development.

The capability to differentiate variability by time scales was granted by the introduction of novel nonlinear measures. As a matter of fact, techniques such as PRSA can deepen the information obtained from traditional analyses having parameters that can be adjusted, such as the parameter T. This value functions as a high pass filter, allowing the procedure to capture only oscillations pertaining to selected frequencies. In this case, parameters extracted from the PRSA curves were different as a function of position only in the case of curves obtained with T>30 sample, corresponding to 10 seconds and thus in the long term range. With respect to traditional time domain parameters for long term variability, the PRSA technique can take into account phase resetting, nonetheless it is not suitable for applications on short recordings.

In this study we also tested the performance of an improvement in entropy calculation, using the QSE with minimum count of matches, showing better performances and more consistency [36]. This is particularly relevant because the consistency of estimates on segments of different length is crucial for the application on real clinical data, which are often relatively short. Moreover, we have shown methodological ways to optimally choose the values of minimal count of matches.

Given that this is the only data set with longer recordings (1 to 6 hours depending on age), we intend to extend the characterization of signal features on multiple time scales with methods such as multiscale entropy [204]. This approach could expand our analysis addressing complexity on various scale factors. Other time scale could be more informative, but also the distribution along different time scales could contain information on the system. As a matter of fact, physiologic complexity is fundamentally related to the adaptive capacity of the organism to work on multiple scales and the identification of the loss of this capability might provide interesting information on system integrity and multiscale functionality.

In the third part of Chapter 3, the effect of prematurity on cardiorespiratory control is investigated. Results show how even few weeks of prematurity dramatically impact the capability of the infant to control these vital systems. Late preterm (LPT, GA at birth 35-36 weeks) and early term (ET, GA at birth 37-38 weeks) infants show diminished HRV and but no difference in the amount of cardiorespiratory phase locking. Previous works highlighted a change in the quantity of synchronization during the first months of life [35], [158]. Thus, it was surprising to discover in our study that the quantity of synchronized time did not change significantly within the last weeks of GA. This might be because this aspect of the cardiorespiratory modulation mainly develops in the months following birth and thus the differences between previous and current findings might be related to differential influences of intra-uterine versus extra-uterine life, rather than to GA at birth.

On the other hand, a different profile of cardiorespiratory directionality by GA at birth was found, with increasing predominance of respiratory drive on HR with GA at birth. Moreover, we have shown how this behavior is not driven by breathing frequency only, as previously proposed, since breathing frequency does not change in the GA time window we investigated [38]. In addition, the directionality index was found to be negatively correlated with measures of parasympathetic activity, supporting the conclusion that a development of parasympathetic control with gestational age can contribute to the shift toward respiratory drive over HR.

From a technical perspective, we showed that novel measures such QSE could offer useful alternative to traditional parameters, such as RMSSD, because are less influenced by noise and artifacts.

On the other hand, other approaches, such as phase locking estimation, do require a lengthy preprocessing. In the future, techniques for preprocessing optimization will be explored, for instance an optimal selection of SG filter parameter. Another parameter which could be optimized is the length of the window for the calculation of lambda parameter. In our case we selected a window of 1000 samples (5 s) overlapping by 50 samples (250 ms), but no testing was performed to search for an optimal choice.

These findings are relevant because prematurity is a condition that affects a high percentage of born live newborns. According to the March of Dimes, in 2016 the preterm birth rate was 9.8%, marking the second consecutive year of increase after steady declines over the previous 7 years. LPT infants have higher incidence of respiratory distress syndrome, temperature instability, hypoglycemia, hyperbilirubinemia, apnea, feeding problems, as well as higher rates of re-hospitalization and a two-fold increase in SIDS [206]–[208]. Moreover, LPT and ET have been found to be at higher risk for

impaired long-term neurodevelopment outcomes [209], [210]. Though the absolute risk is comparatively low, the relative risk is clinically relevant, given the high incidence of LPT and ET birth. Importantly, infants born after the 35th week of GA are commonly receiving care in standard newborn nurseries, just like full term infants, both in Europe and the US, unless they show signs of distress.

The last section of Chapter 3 tests the hypothesis of autonomic vulnerability as a result of smoking and alcohol exposure during pregnancy. To test the autonomic reactivity, infants were subjected to a head-up tilt test. The unexposed group showed a withdrawal in parasympathetic activity following the tilt, confirmed by a decrease in short term variability and complexity, but the same behavior was not found in the exposed group. These results reflect the effects on physiological tracings of alterations of exposure on the cardiorespiratory regulation, as previously suggested by autopsies and animal studies. Reports of impairment of cardiorespiratory function and/or arousal in infants exposed to cigarette smoke during gestation suggest that such exposure harms the fetal development of cardiorespiratory and arousal pathways, the majority of which are in the brainstem [111], [211], [212]. Brainstem neurotransmitter abnormalities are found in SIDS infants, particularly in the serotonergic system in the medulla oblongata which is involved in the modulation of cardiorespiratory interactions and arousal under homeostatic stress [5], [213]–[215]. SIDS infants exposed to cigarette smoke during fetal development also demonstrate abnormal binding to nicotinic receptors (nAChRs) in mesopontine regions related to arousal in combination with the medullary 5-HT abnormalities [216]. Moreover, maternal tobacco smoking significantly impairs both stimulus induced and spontaneous arousal from quiet sleep when infants sleep in the supine position, at the age when the incidence of SIDS is highest [217]. Less is known about the effect of alcohol, but the few studies available indicate an increased risk of SIDS with exposure especially in the first trimester, with similar findings of altered nicotinic receptor binding significantly correlated to number of drinks during pregnancy [216], [218].

Lastly, assessing the effect of smoking and alcohol is extremely challenging, due to the difficulty of quantifying them in terms of amount and length of exposure and because they are often associated with a life style presenting many confounder factors (drugs, inadequate prenatal care, postnatal smoking exposure etc).

A comparison between different measures of vagal activation showed how parameters which were highly correlated were not interchangeable due to different statistical properties and dependency on signal length. This is extremely important especially when comparing results from different studies.

Table 4-1 Summary of the methods and the findings of all the studies performed for the PhD thesis

Risk factors	Methods	Results
Sleep state	✓ Time & frequency domain ✓ Transfer Entropy with lags ✓ Phase Locking	✓ In QS lower HRV, higher QSE and TE, with major directionality from RESP to RR ✓ In QS longer Phase Locking periods ✓ Change of Phase Locking ratios with age
Sleep positions	✓ Time & frequency domain ✓ SampEn, QSE ✓ PRSA	✓ In prone position, lower short and long term HRV higher entropies at newborn age, at 2 months of age differences in short term HRV and entropies ✓ Better performance of QSE with minimum count of matches with respect to SampEn
Prematurity	✓ Time & frequency domain ✓ QSE ✓ Phase Locking ✓ Directionality index	✓ Late preterm and early term have reduced HRV ✓ No difference in amount of phase locking with GA, but differences in directionality profile. ✓ Directionality index changes with GA are only partially driven by breathing frequency
Smoking and Alcohol exposure	✓ Time & frequency domain ✓ QSE ✓ RSA amplitude	✓ The control group showed the expected vagal withdrawal pattern as a response to tilt, but the exposed group did not show any change.

Some limitations at the current state of this research are that firstly, the physiological interpretations of some of the novel parameters is still a topic of debate. For instance, it is not completely clear yet the role of synchronization in terms of efficient cardiovascular and respiratory control. A hypothesis is that a lack of synchronization might indicate reduced feedback mechanisms or interconnections in pathological conditions or in individuals at risk and that synchronization has a role in maintaining cardiorespiratory oscillations within physiological ranges. This is supported by the literature which shows an increase in synchronization from infancy to adulthood but a decrease with old age [32]. Nonetheless, previous studies reported contrasting information about the effects of apnea on synchronization [104], [219].

Moreover, some of the techniques proposed require extensive data pre-processing which is difficult to automatize due to the large intra subjects' variability in the newborn population and due to the high level of noise in the data as a consequence of the impossibility to cooperate with the subject. Some techniques could be explored in the future to address this limitation.

Results from each section of Chapter 3 are summarized in Table 1.

Clinical impact

Since 1990s, the SIDS rate in the U.S. has declined by more than 50 percent, but in recent years the rate of SIDS has plateaued, and SIDS remains the leading cause of death for infants one year and younger, with more than 2,000 SIDS victims each year. Public campaigns, such as the Back to Sleep campaign, have definitely raised awareness on the importance of minimizing external risk factors, however as clearly stated by the triple risk model, SIDS is much more than the results of unsafe sleep practices.

As the focus of public health should remain that of diminishing both intrinsic and extrinsic risk factors [220], the role of our community of researchers should be that of clarifying the mechanisms triggered by these risks, in order to be able to prevent them or intervene.

The contribution of this Ph.D. thesis in this context has been that of introducing a shift from a qualitative assessment of a "different autonomic profile" in population at risk for SIDS, to a quantitative one. The definition of a model such as the triple risk model is a great advancement in the understanding of this syndrome, nonetheless it still lacks the specificity of addressing the relative importance of each factor in relationship to the others.

The parameters proposed could characterize the differences in autonomic regulation in populations at risk for SIDS and highlight how external autonomic challenges such as sleep position or alcohol exposure might impose a stress from which an infant cannot recover. Results obtained show how traditional indices routinely employed in clinical studies often only open a window on a more complex picture. This reinforces our opinion on the value of the introduction of nonlinear advanced parameters to obtain additional physiological and clinical meaning, as suggested by Sassi et al. [127]. In their joint position statement regarding novel HR analysis techniques, they suggest applying them in conjunction with traditional methods. This approach should lead to a greater understanding of the complex mechanisms underlying HRV and could bridge the gap between unanswered clinical questions and analytical innovations. Our results show how nonlinear methods can replicate and extend findings first observed employing linear approaches. At the same time, our data suggest that these novel methods might be more advantageous in dealing with data collected under standard clinical conditions.

In addition, our experimental observations of complex regulating systems highlight how SIDS can be viewed as a "dynamical disease", which is a concept introduced by Glass to indicate a disease that

occurs in a control system operating in a range of control parameters that leads to abnormal dynamics [221]. These abnormal dynamics would correspond to bifurcations in the relevant equations describing the physiological system. Physiological regulations have been explained using the concept of homeostasis, maintaining constancy of a vital variable by constantly adjusting its deviation from a set point. The process of achieving this stability is called allostasis and it accounts for the ability to adapt successfully to the external demands. In this allostatic state, the spatiotemporal complexity of the brain's control systems may give rise to multiscale complexity in the state variables. While this dynamical adaptation is essential to make physiological regulations, hyper or hypo activation of this state may bring a system to the critical transition point (transition state in Figure 4-1) where the emergence of deviations could be early signs of disease onset and/or exacerbation (disease state in Figure 4-1). In complex diseases identification of this transition point between a normal and a disease state is often non trivial since the transition state may show little apparent change and also the path to condition deterioration may vary greatly between individuals.

Having showed that novel methods better capture the cardiorespiratory characteristics of the newborn population and how risk factors for SIDS alter them, we believe it would be a natural progression to incorporate them in a mathematical model. Structural cardiovascular and/or cardiorespiratory modeling has a long history. However, most of the proposed models only consider respiratory influences on heartbeat, not the opposite effect where the heartbeat affects respiration.

We would also like to propose a step further from a single system perspective to a multi system perspective. Thus, in this case the bifurcation theory would be applied rather than a single parameter,

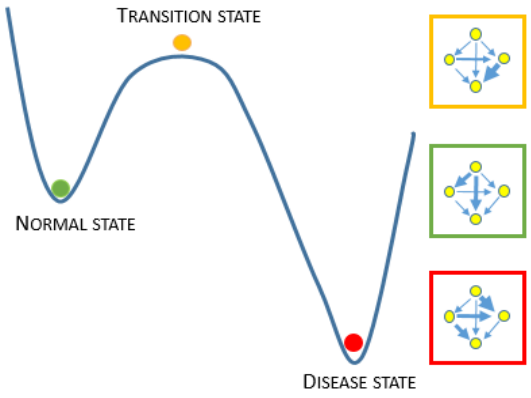

Figure 4-1: Schematic of dynamical disease

on the network of interaction of the systems, as suggested by the approaches of Network physiology. This approach could bring to the definition of a dynamical network marker for early warning of imminent bifurcation.

We feel that a tight collaboration between clinicians and technical experts could help in the proposal of alternative approaches to better identify markers of risk, and with this Ph.D. thesis with have made a first move toward this direction.

Ongoing developments

Application to PASS dataset

We are currently applying the pool of techniques proposed to a dataset of 10,000 infants, containing 18 cases of SIDS infants. The Safe Passage Study, started in 2003 and has recruited approximately 12,000 women from the United States and South Africa. These women were visited throughout their pregnancy as well as the child's first year of life. Physiological signals from baby and mother were acquired at multiple time points and information about health, housing, smoking, alcohol use, drug use, current medications and stressors collected. This dataset constitutes a unique opportunity to test the developed parameters and investigate their capability of risk stratification.

After calculating the parameters, we foresee to perform a process of feature selection or transformation. This will allow us to simplify the following steps of classification, but also will provide insight on physiological interpretation. We will start from the simplest methods, such as logistic regression with stepwise feature selection, and we will then test other techniques such as classification trees, random forest, and sparse support vector machine, to see which one deal better with the curse of dimensionality worsened by the large class imbalance. The total dataset will be divided in a training and a test set and parameters estimation will be performed with a cross validation. Evaluation of models' performance will be expressed in terms of classification accuracy, sensitivity and specificity. Our research team has previous experience in classification due to many studies related to IUGR classification.

A further step will be that of introducing in the model other information regarding clinical history (such as IUGR, maternal diabetes, hypertension etc.), socio-economic status and environmental information. Methods such as random forests are particularly suited to deal with mixed-data, encompassing both continuous and categorical variables.

Lastly, an intense effort is ongoing to address the issue on missing data. In particular, we are currently experimenting techniques to best tackle missing data in the exposure to alcohol and smoking. These variables are particularly challenging because they need to provide information both on amount and timing of exposure and they also need an optimal tradeoff between precision and number of classes

Extension of the Network Physiology framework

During this Ph.D. thesis, we have addressed the autonomic regulation and interaction of the cardiac and the respiratory systems during sleep. Nonetheless, we know that the factors analyzed, such as sleep states, exposure, and gestational age at birth, impact greatly other physiological measures, such as electroencephalogram (EEG). The chosen analytical approaches, exploiting the emerging fields of information dynamics and network physiology, will allow us to encompass EEG in our analysis in order to characterize the synchronous variation in fluctuations occurring during sleep in infants.

We have already collected high density scalp EEG both for LPT study and the Safe Passage study. We are currently working on the preprocessing of the EEG tracing for the LPT population and testing of the new algorithm. The LPT group was chosen as a start to test this novel approach, which will be then implemented in the larger PASS dataset, particularly addressing the question of how maternal substance use and psychiatric symptoms can interact and affect infant brain development and neurobehavioral outcome.

Point process application

All methods we have proposed so far had to deal with the intrinsic unevenly sample nature of heart beat events. In some applications raw data was used and in other interpolated and regularly re-sampled time series were considered. Nonetheless, both choices have limitations such as the necessity to convert number of beats into seconds or the possible spurious contributions of interpolation to the results. For this reason, we are now collaborating to encompass in our research a point process approach: with this method it is possible to characterize the probabilistic generative mechanism of each beat, even with short recordings under nonstationary conditions. The RR series is modeled using probability density functions (PDF) describing the probability of the time the next heartbeat will occur. This allows to obtain instantaneous HR and HRV measures without using any interpolation method

We are currently working on testing the proper PDF for infant population and afterward we are planning on applying the novel formulation of Transfer entropy in the context of point process, called

instantaneous point-process TE (ipTE). This new formulation is capable to finely track the non-stationary information transfer from one system to another, with a high-resolution in time.

Application to Sleep Quality assessment

An interesting aspect of the approaches proposed in this thesis is that, although parameters were tuned for the infant population, the approaches could be easily applied in other contexts where the evaluation of cardiorespiratory dynamics could be beneficial for diagnosis and prognosis.

For this reason, we are currently working on the acquisition of overnight recording, integrating sleep and physiological information in the natural home environment from late pregnancy through early childhood (1,3,5 years old). The focus of this project is to asses sleep quality, which is not only a marker for positive health, but has a major influence on multiple critical developmental domains.

In conclusion, this Ph.D. thesis proposes a multi-parametric approach encompassing linear and nonlinear parameters to characterize the cardiorespiratory system in infants at risk for SIDS. This quantitative approach provides useful information which could converge in novel monitoring solutions for risk assessment in the neonatal field, in order to predict points of bifurcation in the health status of the infant and guarantee timely interventions.

LIST OF PUBLICATIONS

Journal articles

- Lucchini, Maristella, et al. "Novel heart rate parameters for the assessment of autonomic nervous system function in premature infants." *Physiological measurement* 37.9 (2016): 1436.
- Lucchini, Maristella, et al. "Entropy Information of Cardiorespiratory Dynamics in Neonates during Sleep." *Entropy* 19.5 (2017): 225.
- Lucchini, Maristella, et al. "Characterization of cardiorespiratory phase synchronization and directionality in late premature and full term infants." *Physiological measurement* (2018).
- Lucchini, Maristella et al. "Multi-parametric Cardiorespiratory Analysis in Late Preterm, Early Term, and Full Term Infants at Birth." *Medical & Biological Engineering & Computing* (2018).

Book chapters

- Signorini, Maria G., et al. "Complex and Nonlinear Analysis of Heart Rate Variability in the Assessment of Fetal and Neonatal Wellbeing." *Complexity and Nonlinearity in Cardiovascular Signals*. Springer, Cham, 2017. 427-450.
- Myers, Michael M., et al. "Neonatal Monitoring: Prediction of Autonomic Regulation at 1 month from Newborn Assessments." *Sudden infant and early childhood death: The past, the present and the future.* University of Adelaide Press. 2018. 431-448

Conference proceedings

- Lucchini, Maristella et al. "Multi-parametric heart rate analysis in premature babies exposed to sudden infant death syndrome." *Engineering in Medicine and Biology Society (EMBC), 2014 36th Annual International Conference of the IEEE*. IEEE, 2014.
- Lucchini, Maristella et al. "Influence of sleep state and position on cardio-respiratory regulation in newborn babies." *Engineering in Medicine and Biology Society (EMBC), 2015 37th Annual International Conference of the IEEE.* IEEE, 2015.
- Lucchini, Maristella et al.. "Short and Long-Term Heart-Rate Parameters in Newborns with Different Post-menstrual Ages and Sleep Position". *XIV Mediterranean Conference on Medical and Biological Engineering and Computing 2016. IFMBE Proceedings,* 2016

- Lucchini, Maristella et al. "Feasibility study for the assessment of cardio-respiratory coupling in newborn infants." *Engineering in Medicine and Biology Society (EMBC), 2016 IEEE 38th Annual International Conference of the*. IEEE, 2016.
- Lucchini, Maristella et al. "Cardio-respiratory phase locking in newborn and one month infants as a function of sleep state." *EMBEC & NBC 2017*. Springer, Singapore, 2017. 791-794.
- Pini, Nicolò et al. "Influence of prenatal alcohol and smoke exposure on neonatal vagal tone in response to head-up tilt." *Engineering in Medicine and Biology Society (EMBC), 2018 IEEE 40th Annual International Conference of the*. IEEE, 2018.
- Pini, Nicolò et al. "Lagged Transfer Entropy Analysis to Investigate Cardiorespiratory Regulation in Newborns during Sleep" submitted to *12th International Conference on bio-inspired systems and signal processing*

Awards

- Rotary global grant del Distretto 2041: prevenzione e cural delle malattie (riguardo a malattie cardiovascolari)
- International Society for the Study and Prevention of Perinatal and Infant Death travel award for ISPID-ISA 2018
- International Society for Developmental Psychobiology Travel award for ISDP 2017

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