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Characterization of cardiorespiratory phase synchronization and directionality in late premature and full term infants

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Abstract

Objective: Though the mutual influence of cardiovascular and respiratory rhythms in healthy newborns has been documented, its full characterization is still pending. In general, the activity of many physiological subsystems has a well-expressed rhythmic character, and often an interdependency between physiological rhythms emerges early in development. Traditional methods of data analysis only address the quantification of the strength of subsystem interactions. In this work, we will investigate system interrelationships in terms of the possible presence of causal or directional interplays. *Approach*: In this paper, we propose a methodological application that quantifies phase coupling and its directionality in a population of newborn infants born between 35 and 40 weeks of gestational age (GA). The aim is to assess whether GA at birth significantly influences the development of phase synchronization and the directionality of the coupling between the cardiovascular and respiratory system activity. 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. *Main results*: Results from our investigation show a different directionality profile as a function of GA and sleep state. *Significance*: These findings are a contribution to the understanding of higher risk for the documented negative outcomes in the late preterm population. Moreover, these parameters could provide a tool for the development of early markers of cardiorespiratory dysregulation in infants.

1. Introduction

In the last few decades, knowledge about relationships between respiratory and cardiovascular systems has gradually grown with intermittent reports of investigations on associations of biological rhythms and central nervous system (CNS) activity (Loewy and Spyer 1990, Rosenblum *et al* 1998, 2002). Biological rhythms associated with peripheral autonomic system activity, on the other hand, have been thoroughly investigated (Porges and Byrne 1992, Bernston *et al* 1993, Penzel *et al* 2007). Neural systems not only control cardiovascular signals, such as arterial blood pressure (ABP) and heart rate (HR). Respiratory signals, such as inter-breath interval (IBI) series, are modulated by neural control as well.

The human cardiorespiratory system is highly rhythmic with oscillations in the HR tied to those in blood pressure and blood flow, but also influenced by inspiratory and expiratory rhythmic phases in respiration. Rhythmic functioning is a peculiar and ubiquitous characteristic of many physiological systems (Schulz *et al* 2013, Dick *et al* 2014). Understanding the nature of the interaction between respiration and HR has informed the etiology of many adult pathologies, but many aspects of these complex interactions are still unexplained. Investigating the emergence of these interactions in infants represents an even more challenging field of study. At birth, the activation of the cardiorespiratory system is initiated but it is not fully developed either anatomically or functionally.

Several modes of interaction exist between these two subsystems: the most well-known is the HR amplitude modulation driven by respiration, referred to as respiratory sinus arrhythmia (RSA). It consists of a HR increase during inspiration and decrease during expiration (Angelone and Coulter 1964, Yasuma and Hayano 2004). This

modulation can be categorized as a linear interaction, but nonlinear relationships have also been identified, such as phase synchronization between heartbeats and breathing cycles (Schäfer *et al* 1999, Lotrič and Stefanovska 2000, Hoyer *et al* 2001, Kabir *et al* 2010). Many studies describe the cardiorespiratory coupling as an interaction between two subsystems which can be modeled as two weakly coupled chaotic oscillators (Rosenblum *et al* 1998, Schäfer *et al* 1999, Pikovsky *et al* 2000). The basic idea is that given two weakly coupled systems, the amplitude of their oscillations may remain uncorrelated whereas their phases do mutually perturb. With this assumption it becomes possible to investigate cardiorespiratory synchronization by means of a phase analysis of RR series and respiratory signal rather than applying a classical amplitude analysis. The behavior of the cardiorespiratory system can be seen as synergetic, meaning a multi-stable system switching between several phase attractive maps, with a preference for a specific set of phase relations, which can be seen as attracting frequency ratios (Hoyer *et al* 2001). 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 (Bartsch *et al* 2012, 2014).

RSA generation is associated mainly with direct brainstem modulation of the cardiac vagal preganglionic neurons and by inhibition of cardiac vagal efferent activity by lung inflation. RSA is also thought to improve pulmonary gas exchange (Yasuma and Hayano 2004). On the other hand, the physiological mechanisms behind the phase synchronization are still not fully understood. Recent results have led to hypothesize a link with the 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, thus improving energy efficiency (Langhorst *et al* 1983, Schäfer *et al* 1998).

RSA is observed in full term infants, indicating the presence of cardiorespiratory coupling even at this early life stage (Hathorn 1987). This coupling increases in strength and consistency with GA at birth, reflecting the transition from sympathetic to parasympathetic dominance during the postnatal period (Van Ravenswaaij-Arts *et al* 1994, Lucchini *et al* n.d.) with an increasing influence of respiration modulating HR. Overall, immaturity in linear cardiorespiratory coupling is observed in preterm infants, mostly in the form of lower HRV in the high frequency range which can be seen as an indirect measure of RSA (Longin *et al* 2006). This developmental difference between term and preterm infants highlights that the latter are at higher risk for impaired responses to cardiorespiratory stress.

Over the years, many methods have been proposed to quantify the interaction between subsystems, ranging from traditional to more complex signal-processing techniques, e.g. cross-spectra, mutual information, phase-locking analysis, and time delay stability (Lin *et al* 2016, Penzel *et al* 2016). Nonetheless, such linear and nonlinear approaches provide measures of symmetric interaction and are limited in their ability to evaluate possible different causal mechanisms that may underlie cardiorespiratory behavior.

Rosenblum *et al* proposed a method which exploits the notion of phase synchronization of irregular oscillators: the attempt was to reveal whether the interaction is bi- or uni-directional and to quantify the degree of asymmetry in the systems' coupling (Rosenblum and Pikovsky 2001). The method assumes that the system under study can be modeled by coupled self-sustained systems. This approach starts from the fact that a weak coupling firstly affects the phases of the oscillators, not their amplitudes. Thus, to quantify the coupling strength of interaction, one has to analyze relation between the phases of the systems only.

These novel methodologies stem from the experience of a new field of study, called network physiology (NP). NP offers a frame of study to address the question of how different physiological systems interact and behave together. Moreover, 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 (Bartsch *et al* 2015).

In this study, we are interested in quantifying this type of cardiorespiratory interaction in a population of late-preterm infants, born between $35^{0/7}$ and $36^{6/7}$ weeks of gestation. This population is known to be at higher risk of morbidity and mortality than term infants. Even more important, in the normal clinical path, they are treated the same as term infants both in Europe and the US, unless they show signs of distress (Engle *et al* 2007, Kugelman and Colin 2013).

Investigation into the cardiorespiratory coupling in this population is warranted given many studies documenting an impaired cardiorespiratory coupling in many pathologies, such as apnea of prematurity, and potentially involved in the pathophysiology of sudden infant death syndrome (SIDS) (Garcia *et al* 2013a, 2013b). Therefore, a set of methods focused on cardiorespiratory phase coupling may provide quantitative indices of underlying mechanisms and early identification of possible risk states.

2. Materials and methods

2.1. Population

The Institutional Review Boards of the New York State Psychiatric Institute and of the Columbia University Medical Center (CUMC) approved all consent and data acquisition procedures used to collect the data. Mothers

signed informed consent forms prior to enrollment in the study. The newborn dataset includes 273 infants born at the Morgan Stanley Children's Hospital of New York between $35^{0/7}$ and $40^{6/7}$ weeks of GA. None of the infants were admitted to the Neonatal Intensive Care Unit and review of the maternal medical chart revealed no evidence of major illness, genetic disorders, or past/present medicated/non-medicated psychiatric complaints. All infants had a minimum Apgar score of 8 after 5 min of life.

The subjects who met inclusion criteria were recruited and tested 12–84 h after birth. Infants were grouped based on GA: Late Preterm (LPT, $35^{0/7}$ to $36^{6/7}$ weeks GA at birth, $n = 66$), Early Term (ET, $37^{0/1}$ to $38^{6/7}$ weeks GA at birth, $n = 93$) and Full Term infants (FT, $39^{0/1}$ to $40^{6/7}$ weeks GA at birth, $n = 114$).

2.2. Signals acquisition and preprocessing

The electrocardiograph (ECG) was recorded with three leads, placed on the infant's chest (left abdomen, left and right scapula) and the signal was amplified and collected using the DATAQ Instruments ECG system (Medelex, NY, NY). A respiratory inductance belt (Ambulatory Monitoring Inc., Ardsley, NY) was placed around the infant's abdomen to measure the respiration signal. ECG and respiration signals were acquired at 500 Hz and 200 Hz respectively.

Acquisition started within ~30 min after feeding and lasted 10 min, with infants sleeping in supine position. Sleep states were classified into active sleep (AS), quiet sleep (QS), indeterminate (I) and awake (W). The minimum length for a segment to be classified either as AS or QS was 120 s, I and W segments were discarded from the analysis. The sleep state coding assessment was based on respiratory variability (Isler *et al* 2016) and confirmed by behavioral codes entered throughout the study to determine when infants were awake, crying, or fussy.

The R peaks were detected on the ECG with a proprietary software (Gmark, Ledano Solutions) based on the Pan–Tompkins algorithm (Pan and Tompkins 1985) and then checked with visual inspection. The respiration signal was filtered with a bandpass filter (0.05–3.5 Hz), peaks of inspiration were detected with automated marking software and each record was modified for incorrect marks manually. The thresholds of acceptance for RR interval were set as 0.3–0.667 s, with an absolute variation between consecutive RR intervals of 10%, while for respiration thresholds were 0.5–2.5 s and an absolute change of 40%. Segments of 3 min length in a continuous sleep state were analyzed, providing 279 QS segments and 419 AS segments.

2.3. Phase estimation

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 equation (1):

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

where t_k are the times of appearance of the kth R peak. The respiratory signal was detrended and filtered with a Savitzky–Golay filter and then its instantaneous phase (ϕ_{RESP}) was computed by means of functions provided by the data analysis with model of coupled oscillators (DAMOCO) Toolbox (Rosenblum and Pikovsky 2001, Kralemann *et al* 2007, 2008). Respiration signal protophase is computed via the Hilbert transform and the phase is derived with appropriate transformation of protophase (Kralemann *et al* 2011). Instantaneous phases of ECG and respiration were both resampled at 200 Hz to obtain two synchronous phase series.

2.4. Phase synchronization

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 ϕ_1 and ϕ_2, a generalized n:m phase locking ratio fulfills the condition expressed in equation (2):

$$|n\phi_1(t) - m\phi_2(t) - \delta| < \mathrm{const} \tag{2}$$

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, and n indicates the number of heartbeats with respect to m respiratory cycles. Different n:m synchronization periods can be highlighted with a graphic tool called a cardiorespiratory synchrogram, shown in figure 1 panel (c) (Schäfer *et al* 1999, Cysarz *et al* 2004). Defining R_i ($i = 1, \ldots, n_R$) as the series of R peaks and I_j ($j = 1, \ldots, n_I$) as the series of inspiratory onsets, it is possible to compute the relative distance ($\Psi(t_i)$) between the current respiratory onset and the successive R peaks as

$$\Psi(t_i) = \left(\frac{R_i - I_j}{I_{j+1} - I_j} + j \right) \bmod b. \tag{3}$$

Panel (c) of figure 1 shows relative distances φ_i (plotted as dots) with respect to single breathing cycles ($b = 1$).

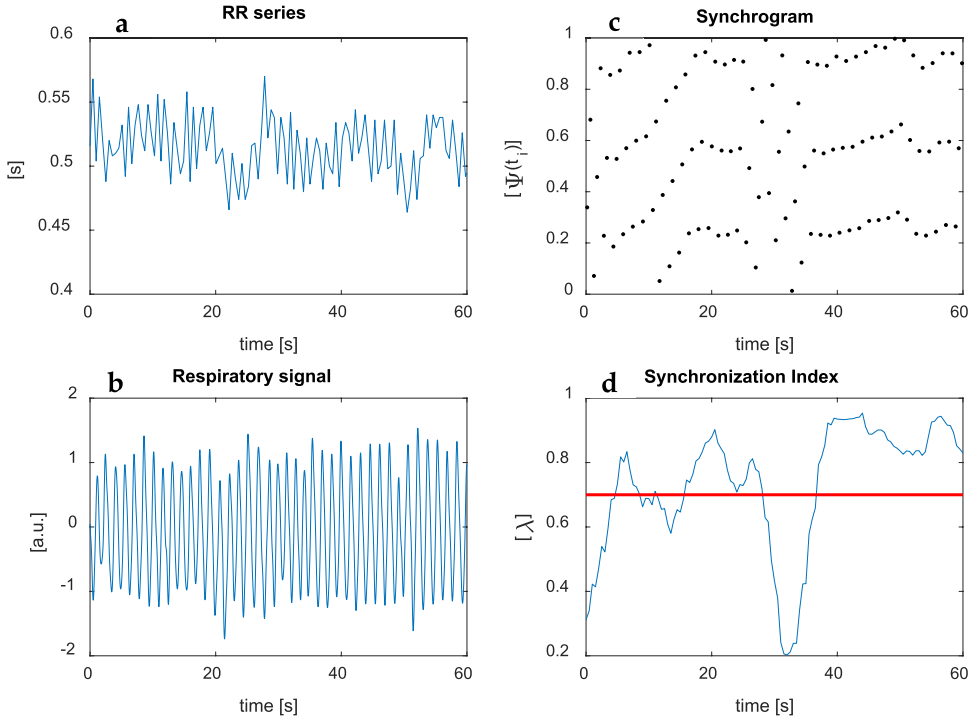

Figure 1. (a) The RR series (extracted from the ECG signal) in a 60 s window. (b) Respiratory signal for the same window described in (a). The signals shown in (a) and (b) are obtained from a full term subject in quiet sleep. (c) The synchrogram extracted by the joined analysis of RR series and respiratory signal. The dot graph represents the relative distance between R peaks and respiratory onsets. (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.

Of the several approaches employed to quantify the level of synchronization, the method of the synchronization index λ was chosen for its proven reliability (Mrowka *et al* 2000). 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 and compute the distribution η_i defined as $\eta_i = \phi_2|_{\phi_1=\theta}$ at each time t_i when $\phi_1 = \theta$.

In the case of 1:1 phase locking, the values of ϕ_2 in the time points when $\phi_1 = \theta$ are going to be scattered around a constant, due to weak noise. The distribution η_i can be characterized computing the intensity of its first Fourier mode. To strengthen the statistic, this process can be repeated with a binning-like procedure for different values of θ.

In case of N points binning, the index Λ_l computed for the l-th bin can be expressed as

$$\Lambda_l^2 = M_l^{-2}\left(\sum_{i=1}^{M_l}\cos\eta_i\right)^2 + M_l^{-2}\left(\sum_{i=1}^{M_l}\sin\eta_i\right)^2 \tag{4}$$

where M_l is the number of points in the l-th bin. In this work, the cyclic phase of the first oscillator was divided in 10 equally spaced bins.

The average of the obtained Λ_l over all N bins provides a measure related to circular variance that is indeed the λ index:

$$\lambda = N^{-1}\sum_{i=1}^{N}\Lambda_l. \tag{5}$$

The resulting λ in equation (5) 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 $\phi_1 \to \phi_1/n$ and $\phi_2 \to \phi_2/m$.

In this study, the λ index was calculated on windows of 1000 samples (5 s) overlapping by 50 samples (250 ms). λ values above a threshold of 0.7 were considered significantly coupled. This value was empirically identified from visual inspection of the synchrograms of the available recordings. 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 the average length of all the periods were calculated. Given 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. It results in the so-called n:1 synchronization percentage and n:1 synchronization duration, the average time duration of a period in synchronized state. An example of this analysis is portrayed in figure 1: panels (a) and (b) show 1 min segments respectively of HR and respiration, panel (c) reports the corresponding synchrogram and panel (d) the relative λ index, with a horizontal line showing the 0.7 threshold.

2.5. Directionality index

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 $d^{\mathrm{RR,RESP}}$ 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* (2002). 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 $\dot{\phi}$ 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

$$\begin{aligned} \dot{\phi}_1 &= \omega_1 + \varepsilon_1 \cdot f_1(\phi_2, \phi_1) + \zeta_1(t) \\ \dot{\phi}_2 &= \omega_2 + \varepsilon_2 \cdot f_2(\phi_1, \phi_2) + \zeta_2(t). \end{aligned} \tag{6}$$

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 characteristics of biological systems. The coupling terms consist of 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. In this work, the evolution map approach (EMA) (Rosenblum *et al* 2002) algorithm has been used: it is capable of revealing asymmetric directionality strength from short noisy records and quantifying which of the systems under analysis influences 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. In this work the phase variable increments are computed using a fourth-order Savitzky–Golay filter. Equation (7) shows that to reconstruct the real weakly coupled oscillators from a single recorded realization of the process, it is necessary to fit the dependences of Δ_1 and Δ_2 over ϕ_1 and ϕ_2 upon considering phase variable increments generated by an unknown two-dimensional noisy map:

$$\begin{aligned} \Delta_1(k) &= \omega_1\tau + \mathcal{F}_1[\phi_2(t_k), \phi_1(t_k)] + \xi_1(t_k) \\ \Delta_2(k) &= \omega_2\tau + \mathcal{F}_2[\phi_1(t_k), \phi_2(t_k)] + \xi_2(t_k). \end{aligned} \tag{7}$$

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 equation (8) shows, 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:

$$\begin{aligned} \mathcal{F}_1 \approx F_1 &= \sum_m A_m e^{ia\phi_1 + ib\phi_2} \\ \mathcal{F}_2 \approx F_2 &= \sum_n A_n e^{ia\phi_1 + ib\phi_2}. \end{aligned} \tag{8}$$

In this analysis the maximum order of Fourier expansions is set to 3, in the following computation $|a| \leqslant 3$ and $|b| \leqslant 3$. 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 be also seen as smoothing functions because they are able to filter out noise by means of the least square fitting.

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

$$\begin{aligned} c_1^2 &= \int\int_0^{2\pi} \left(\frac{\partial F_1}{\partial \phi_2}\right)^2 d\phi_1 d\phi_2 \\ c_2^2 &= \int\int_0^{2\pi} \left(\frac{\partial F_2}{\partial \phi_1}\right)^2 d\phi_1 d\phi_2. \end{aligned} \tag{9}$$

The directionality index can be expressed as

$$d^{(1,2)} = \frac{c_2 - c_1}{c_1 + c_2}. \tag{10}$$

The EMA algorithm computes a normalized directionality index d. Index d varies from 1 to -1. In the case of unidirectional coupling from S_1 to S_2, d is 1, in the opposite case when the unidirectional coupling is from S_2 to S_1, d is -1. Positive intermediate values of d express a stronger or weaker S_1 to S_2 coupling strength, the negative

Table 1. 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 group	RR_mean	SDNN	RMSSD	HF_RR	HF_biv
QS	LPT	0.476 ± 0.034	21.32 ± 11.88	10.20 ± 6.83	0.377 ± 0.190	0.556 ± 0.193
	ET	0.492 ± 0.042	22.87 ± 10.85	13.23 ± 7.06	0.464 ± 0.178	0.597 ± 0.192
	FT	0.507 ± 0.040	24.59 ± 11.47	15.72 ± 7.67	0.497 ± 0.175	0.663 ± 0.196
AS	LPT	0.482 ± 0.040	28.80 ± 12.19	11.60 ± 5.40	0.292 ± 0.122	0.379 ± 0.127
	ET	0.486 ± 0.044	30.93 ± 12.90	12.29 ± 6.09	0.307 ± 0.137	0.395 ± 0.133
	FT	0.497 ± 0.050	31.83 ± 13.87	13.40 ± 6.51	0.321 ± 0.135	0.413 ± 0.131

intermediate values a coupling strength in the opposite direction (S_2 to S_1). In the case of absence of interaction when $c_2 = c_1$, d is zero.

2.6. Time and frequency domain

Traditional time domain and frequency domain parameters have been computed along with the novel approaches based on phase analysis. Time domain parameters are computed in a univariate fashion for HRV (Malik 1996). Frequency domain analysis used a parametric approach. The autoregressive model order is set to 10. The univariate frequency analysis is performed on RR series, while the bivariate approach consisted of the cross-spectrum of RR series and respiration. The frequency bands are grouped as very low frequency (VLF) 0.01–0.04 Hz, low frequency (LF) 0.04–0.2 Hz, high frequency (HF) 0.35–1.5 Hz.

2.7. Statistical analysis

Parameters were checked for normal distribution. High frequency parameters for RR series (HF_RR) and bivariate analysis (HF_biv), together with measures of phase locking (n:1 synchro %, n:1 synchro duration) did not pass the test and were transformed by means of square root operation. Hours of life at time of testing (HoL), infant sex and mode of delivery (MoD) were set as covariates for all analyses.

The effect of sleep state and GA and their interaction on time domain, frequency domain and phase locking parameters was tested with multiple 2-way Ancovas, and post hoc analyses were performed to identify differences within specific GA groups. A 2-way multivariate Ancova was performed to test the effect of sleep state and GA and their interaction on the directionality index d and the respiration frequency. Follow-up univariate analyses were also performed to test which parameter was driving any significant differences. All statistical analyses were performed with IBM SPSS software.

3. Results

The time domain and frequency domain results, presented in table 1, show increasing mean RR interval (RR_mean) and short-term HRV (RMSSD) and linear cardiorespiratory coupling (HF_RR, HF_biv) with GA, indicating an increasing cardiorespiratory coupling and autonomic control as a function of GA at birth (respectively, $p = 0.001$, $p < 0.001$, $p = 0.007$, $p = 0.008$). Subsequently, cardiorespiratory coupling was analyzed in term of phase coupling (n:1 synchro %, n:1 synchro duration) and directionality (d). The main results 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 (respectively $p = 0.874$ and $p = 0.740$). The results from statistical analysis are reported in tables 2 and 3. (Descriptive statistics for d and breathing frequency are reported in table 4 and corresponding distributions are shown in figure 2.)

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: $F(4.588) = 4.151$, $p = 0.003$; Wilks' $\Lambda = 0.946$ and $F(2.294) = 56.582$, $p = 0.000$; Wilks' $\Lambda = 0.722$). No significant interaction was detected between GA and state ($F(4.588) = 0.130$, $p = 0.972$; Wilks' $\Lambda = 0.998$). The test of between-subjects' effects highlighted how each dependent variable differed based on the independent variables. Results are reported in table 5. To account for multiple ANOVAs, we used a Bonferroni correction accepting statistical significance at $p < 0.025$. This test showed that sleep state significantly influenced both d and respiratory frequency, while GA at birth influenced significantly only d. Directionality index d decreased 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 ($d \approx 0$) 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 are also significantly different from FT.

Table 2. Descriptive statistics (mean ± std) 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 group	*n*:1 synchro %	*n*:1 synchro duration	*N* subjects
QS	LPT	0.436 ± 0.209	2.99 ± 1.02	19
	ET	0.454 ± 0.184	3.03 ± 1.01	41
	FT	0.473 ± 0.170	3.00 ± 0.90	58
AS	LPT	0.185 ± 0.124	1.82 ± 1.04	54
	ET	0.218 ± 0.127	2.07 ± 0.84	62
	FT	0.221 ± 0.139	1.92 ± 0.97	68

Table 3. 2-way ANOVA 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*-value	Partial η^2
n:1 synchro %	State	<0.001	0.357
	GA group	0.342	0.007
	State ∗ GA group	0.874	0.001
n:1 synchro duration	State	<0.001	0.214
	GA_group	0.559	0.004
	State ∗ GA group	0.740	0.002

Table 4. Descriptive statistics (mean ± std) for directionality index and frequency of respiration by gestational age (GA) and sleep state and number of subjects.

State	GA group	Directionality index	Resp. freq.	*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 5. Multivariate model analysis of directionality index and frequency of respiration by gestational age (GA) and sleep state along with their interaction effect and post hoc analysis.

Parameters		*p*-value	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	
Resp. freq.	GA group	0.881	0.001	LPT > ET > FT
	State	<0.001	0.097	
	GA group ∗ state	0.857	0.001	

Previous studies suggested a dependence between d and breathing frequency, arguing that the cardiac influence on respiration is weak and frequency independent, while the coupling from the respiration to HR is strong compared with the strength of the cardiac influence for low respiratory frequencies, and of similar entity for higher frequencies (Mrowka *et al* 2003). The same group proposed a threshold of 0.6 Hz to investigate this mechanism in infants. Figure 3 shows the directionality index histograms of the three GA groups at breathing frequency ⩾0.6 Hz (in each panel on the right) and at breathing frequency <0.6 Hz (in each panel on the left). This figure illustrates the occurrence of this bimodal influence: concurrent with breathing frequency <0.6 Hz, an increased polarization toward negative values of d occurs, especially in ET and FT. Nonetheless, the significant evolution of d with GA ($p = 0.001$) is not accounted for by this mechanism, given that respiratory frequency does not change significantly with GA at birth ($p = 0.881$).

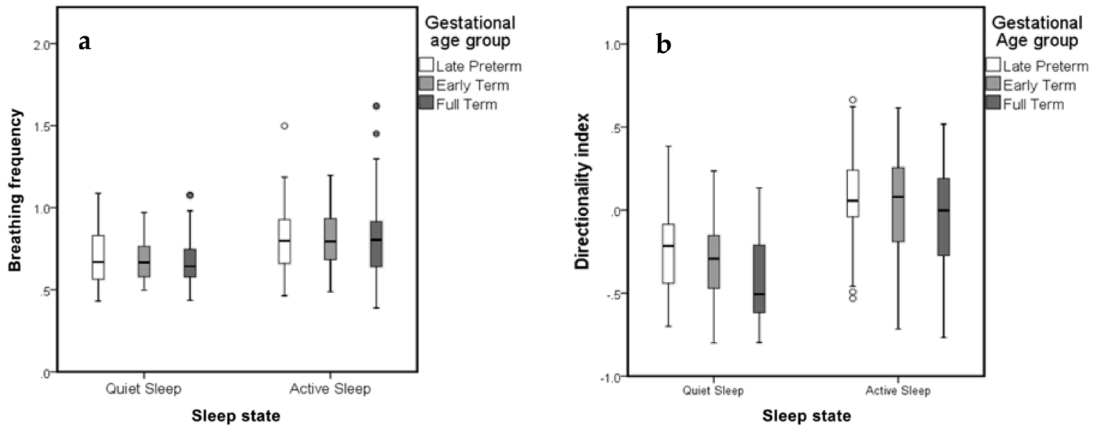

Figure 2. (a) Boxplots show breathing frequency by gestational age (GA) and sleep state. Breathing frequency is not significantly different by GA at birth but is significantly different by sleep state (p-value < 0.001). (b) Boxplots show directionality index by gestational age and sleep state. Directionality index is statistically different both by gestational age at birth (p-value $= 0.001$) and by sleep state (p-value < 0.001). Neither breathing frequency nor directionality index show any interaction effect between gestational age and sleep state.

4. Discussions

The goal of our study was to address the emergence of directionality in cardiorespiratory phase synchronization within the last weeks of pregnancy. In particular, we aimed at evaluating whether infants born between the 35th to 40th week of gestation exhibit preferential directionality interactions between the cardiac and the respiratory system.

To this end, we have analyzed HR and respiration of healthy newborns as a function of GA at birth. Previous analyses on our study population explored the use of traditional time and frequency domain and entropy analyses (Lucchini *et al* n.d.), which suggested that LPT and ET autonomic control of HR is significantly less mature and their cardiorespiratory regulation less developed when compared to FT. However, previous approaches solely investigated linear coupling between HR and breathing, and, due to the type of analyses used in those previous studies, no inferences were possible regarding the directionality of the interaction between the cardiac and the respiratory system.

In the current study, time domain and frequency domain analyses confirmed previous findings, with increasing HRV and linear coupling with increases of GA. We then investigated cardiorespiratory coupling from a phase relationship perspective. This concept is based on the finding that cardiorespiratory systems are intimately linked with one another. Consequently, illnesses or distress may disrupt this complex connection. Our results confirmed the dependence of phase-locking with sleep state, as already found in previous studies in infants and adults (Bartsch *et al* 2007, Lucchini *et al* 2018). Moreover, results indicated that GA at birth differences are not apparent in the duration of phase locking, but rather in the directionality of the relationship between the cardiac and the respiratory system. Previous work highlighted a change in the quantity of synchronization during the first months of life (Mrowka *et al* 2000, Lucchini *et al* 2018). 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 due to the fact that the cardiorespiratory system mainly develops in the months following birth. In summary, these 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).

Regarding directionality, in both sleep states a GA-related shift occurs toward a stronger driving influence of respiration on HR. Previously, another research group reported a similar developmental shift but in full term newborns followed during the first months of life, suggesting that the maturation occurring in the last stage of pregnancy continues in the first months of life until a final set point is reached in later childhood (Mrowka *et al* 2003). 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 (Lucchini *et al* 2017).

Mrowka *et al* have hypothesized that directionality depends on respiratory frequency. In particular, respiration rate would act as a low pass filter, i.e. below a set respiratory frequency directionality is mainly from respiration to HR, whereas above a certain respiratory frequency threshold the interaction becomes bidirectional. The underlying mechanisms for this low pass 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, such as 0.6 Hz (Mrowka *et al* 2003).

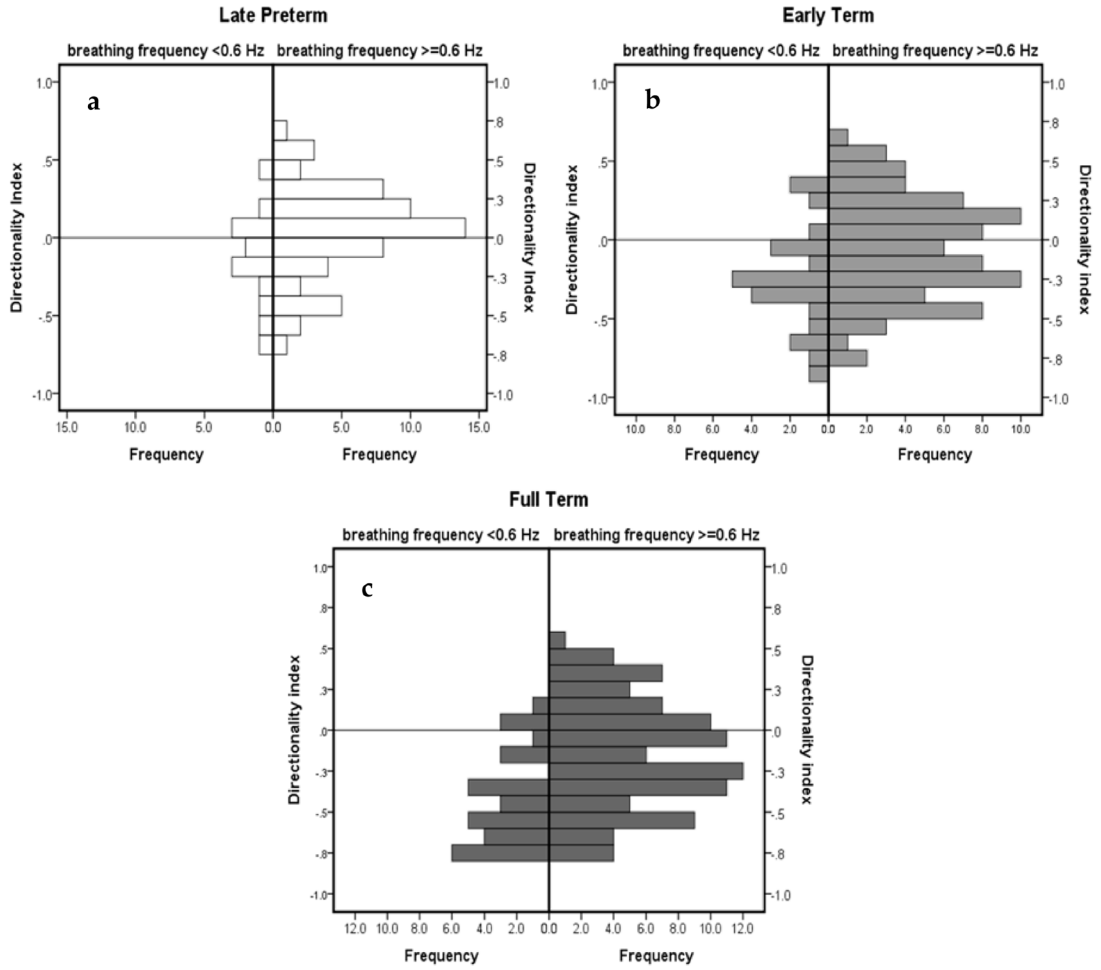

Figure 3. (a) Histograms of directionality index distribution are plotted, on the left for late preterm subjects with a breathing frequency <0.6 Hz, and on the right for late preterm subjects with a breathing frequency $\geqslant 0.6$ Hz. (b) Analogous histograms for early term. (c) Analogous histograms for full term. Histograms for subjects with a breathing frequency <0.6 Hz in all three panels show a progressive shift of directionality index towards negative values, indicating a growing unidirectional interaction characterized by a driving effect of respiration over heart rate.

In this study, breathing rate does not change significantly in this specific GA window (35–40 weeks); thus, the change in directionality with GA at birth cannot be explained solely because of breathing frequency. One explanation for this phenomenon could be 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. Morphological studies 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 preterm infants ($\leqslant$38 weeks PCA) was found to be significantly smaller than for the term or adolescent age groups (Sachis *et al* 1982).

The question remains as to the physiological meaning behind this type of nonlinear coupling. One possible explanation is 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.

In conclusion, it is well known that the last weeks of gestation are crucial regarding the development of CNS interconnections that are responsible for cardiorespiratory regulation. Thus, we hypothesize that the directionality imbalance shown by FT is an important step in the process of autonomic maturation, given that it evolves toward the unidirectional interaction observed later in childhood and adulthood (Mrowka *et al* 2003). The differential directionality of the cardiorespiratory profile in LPT and ET infants, as compared to FT, might provide insight into mechanisms underlying the increased risk of biological and developmental pathology in these GA groups.

Clark *et al*, using similar measures of cardiorespiratory interaction, found that coupling increases with chronological age postnatally, but the rate of increase was not affected by GA at birth (Clark *et al* 2012). This indi-

cates that the development of cardiorespiratory autonomic control is a postnatal age-dependent phenomenon. The increased occurrence of apparent life-threatening apneic events (ALTE) and SIDS in premature infants during the first months of life, suggests a longer window of adaptation associated with several high risk conditions.

It has been shown that the increased incidence of SIDS in premature infants is related to an underlying cardiorespiratory vulnerability. Defining factors underlying this increased vulnerability in preterm infants remains an important public health concern (Shapiro-Mendoza *et al* 2008). Previous studies have investigated regulatory systems associated with infants' responses to threatening stimuli, such as hypoxia, asphyxia or hyperthermia. Those studies have shown that infants who die of SIDS have fewer spontaneous arousals from sleep as compared to controls. Moreover, many SIDS victims had evidence of abnormalities in the brainstem networks responsible for cardiorespiratory control. This phenotype can increase risk vulnerability to exogenous stressors, such as the prone sleeping position.

This paper proposes a novel approach to address the clinical question of late preterm higher morbidity and mortality in the context of the new discipline of NP. Looking at different physiological systems as dynamically interacting could shed light on the process of horizontal integration at the level of organ to organ interaction required to maintain an optimal health status. This work is convergent with the goals proposed by Ivanov *et al* to develop building blocks for the creation of an atlas of dynamic organs interactions (Ivanov *et al* 2016). Moreover, the unique contribution of this study is the early developmental aspect in the newborn period given the dynamic physiologic relationship within rapidly developing organs.

One major focus in our future investigations will be testing the use of different types of surrogates to optimally define a threshold of significance for λ, the quantitative measure of phase coupling, rather than using a fixed value. Further studies will extend the focus on the interactions at different time scales and the investigation of the evolving relationships among the heart, lungs and the brain (Bartsch *et al* 2015).

Our major conclusion is that infants born even only 1–4 weeks early show irregular cardiorespiratory characteristics with respect to full term, suggesting a crucial role for last weeks of pregnancy in the maturation of the interaction between the cardiovascular and the respiratory systems. Moreover, the proposed measures of cardiorespiratory coupling provide a tool to assess maturity of cardiorespiratory regulation, thus serving as a potential biomarker for risk stratification.

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