# Heart rate variability in sleeping neonates

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Etude de la variabilité du rythme cardiaque pendant le sommeil de nouveau-nés

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**Abstract**

*To assess maturation of the Autonomic Nervous System and sleep states, Heart Rate Variability (HRV) was studied in 24 healthy sleeping newborns, aged from 31 to 41 weeks, conceptional age (CA).*

*Spectral analysis of the interbeat interval (RR) signal, was performed by Short-Time Fourier Transform, in three frequency bands: high (HF), mid (MF), and low (LF), providing amplitude signals which were averaged over epochs of 512 heartbeats.*

*A Principal Component Analysis using HF, MF, and LF was used to describe the population under study.*

*Discriminant analyses, using HF, MF, and LF, were performed between age groups, with better results in Active Sleep than in Quiet Sleep. In the same way, discriminant analyses between sleep states were performed in each age group, showing a quality of discrimination which grew regularly with age.*

*Hypothesis tests (Fisher's F-test, Student's t-test) showed that all HRV variables and RR increased significantly with age, especially HF at 37-38 weeks CA, and, to a lesser extent, LF at 39-41 weeks CA. These results suggest a steep increase of vagal tone at 37-38 weeks, and a much more regular increase of sympathetic tone from 31 to 41 weeks.*

*This method of investigation uses evolutionary spectral analysis, data analysis on small observation units, thus taking into account within-subject variance, on the one hand, but also classical statistics, performed on global within-subject means, in order to assume independence of observations, on the other hand. It seems appropriate for describing and comparing long duration time signals.*

*Keywords: Heart Rate Variability; Spectral Analysis; Data Analysis; Sleep; Newborn Infant; Premature.*

**Résumé**

*Nous avons étudié la maturation du Système Nerveux Autonome et de la différenciation en stades du sommeil, par le biais de la Variabilité du Rythme Cardiaque (VRC), dans une population constituée de 24 nouveau-nés sains, entre 31 et 41 semaines d'âge conceptionnel (AC), enregistrés pendant leur sommeil.*

*La méthode d'analyse spectrale utilisée pour l'étude du signal RR (temps écoulé entre deux battements cardiaques consécutifs, donné pour chaque battement) est la Transformation de Fourier à Court Terme, calculée dans trois bandes de fréquence: haute (HF), moyenne (MF), et basse (LF). Cette procédure fournit trois signaux en amplitude, dont on a calculé la moyenne par périodes de 512 battements.*

*Une Analyse en Composantes Principales utilisant les trois variables HF, MF, et LF permet tout d'abord de décrire la population étudiée.*

*Des analyses discriminantes, utlisant ces mêmes variables HF, MF, et LF, sont ensuite pratiquées, d'abord entre groupes d'âge, avec de meilleurs résultats en Sommeil Agité qu'en Sommeil Calme, puis entre stades du sommeil, montrant une qualité de discrimination qui croît régulièrement avec l'âge.*

*Des tests d'hypothèse (test F de Fisher, test t de Student) montrent que l'intervalle RR moyen et toutes les variables de VRC augmentent avec l'âge, surtout la HF à 37-38 semaines d'AC, et, dans une moindre mesure, la LF à 39-41 semaines d'AC. Ces résultats sont en faveur d'une importante augmentation du tonus vagal à 37-38 semaines d'AC, et d'une croissance beaucoup plus régulière du tonus sympathique de 31 à 41 semaines d'AC.*

*Cette méthode d'étude utilise des techniques d'analyse spectrale évolutive et d'analyse des données sur de petites fenêtres d'observation, de façon à prendre en compte la variance intra-individuelle, d'une part, mais aussi des classiques tests d'hypothèse sur les moyennes globales par individu, de façon à assurer l'indépendance des observations, d'autre part. Elle semble bien appropriée à la description et à la comparaison de signaux temporels de longue durée.*

*Mots-clés: Variabilité du Rythme Cardiaque; Analyse Spectrale; Analyse des Données; Sommeil; Nouveau-né; Prématuré.*

# Contents

# 1 Introduction

The variations of heart rate have long been used as a means to investigate the Autonomic Nervous System (ANS) [26]. It is now well established that short-term (or high frequency, HF) variations reflect only parasympathetic control of heart rate, whereas long-term (or low frequency, LF) variations reflect both sympathetic and parasympathetic control [2, 12, 25, 27].

HF variations are related to the respiratory cycle (period: 1 to 3 seconds, in newborn infants), and carry the part of Heart Rate Variability (HRV) which is referred to as Respiratory Sinus Arrhythmia (RSA) [17, 18, 25]. Various origins have been attributed to LF variations [2, 7, 12, 27], the period of which ranges from 4 to 30 seconds in infants [29] .

In infants from 1 week to 6 months, HF variations have been reported to be higher in quiet sleep (QS) than in active sleep (AS) [13, 14]; the contrary is true for LF variations [14]. According to Harper et al., sleep states in infants may be discriminated by HRV variables on these grounds, with a high degree of accuracy [14].

In full-term newborns and in older infants, Baldzer et al. [3], Harper et al.[13, 14], Hathorn [15, 16], Schechtman et al. [29] have studied RSA, or HRV in different frequency bands, in AS and in QS.

In premature compared to full-term newborns, Äärimaa et al. [1] for QS only, Katona et al. [19] in both AS and QS, also have studied total HRV.

However, to our knowledge, no study so far has analyzed, according to HRV, maturation of the ANS, on the one hand, and of sleep state organization, on the other hand, in healthy premature and full-term newborn infants.

Our aim in the present study was to analyze:

- the age-related modifications of variables quantifying HRV in different frequency bands (and thus parasympathetic and sympathetic tones), in each sleep state.
- the differentiation between sleep states, as measured by these same HRV variables.

For this purpose, we elaborated a method of analysis of heart rate based on spectral analysis of total tracing duration by Short-Time Fourier Transform, data analysis (Principal Component Analysis, Discriminant Analyses) of the resulting signals in 3 frequency bands, averaged by epochs of 512 heartbeats, and hypothesis tests on the differences between age groups and sleep states.

# 2 Materials and methods

## 2.1 Subjects

24 neonates, all clinically and neurologically healthy [1], were studied between the 2nd and 11th day of extrauterine life by polygraphic sleep recordings.

They had been divided according to conceptional age (CA) at the time of the recording in 3 different age groups:

- 8 full-term ('term'): 39 w$\leq$CA$\leq$41 w
- 8 intermediate ('intm'): 37 w$\leq$CA$\leq$38 w
- 8 premature ('prem'): 31 w$\leq$CA$\leq$36 w

Data on age groups are given in Table I.

Table I
*Age and birthweight of the population under study*

| Age | GA (w+d) | LA (w+d) | CA (w+d) | BW (g) |
|---|---|---|---|---|
| full-term (n=8) | 40w1d±2d | 3d±1d | 40w4d±6d | 3400±390 |
| intermediate (n=8) | 37w3d±3d | 4d±1d | 38w±4d | 2900±320 |
| premature (n=8) | 33w5d±2w | 6d±3d | 34w4d±1w6d | 2110±140 |

(GA = Gestational age; LA = Legal Age; CA = Conceptional Age = GA +LA; BW = Birth Weight; w = weeks; d = days; g = grams. Numbers in the table represent mean ± 1 standard deviation.)

## 2.2 Recordings

Recordings were performed during a sleep between two feedings. All neonates were lying supine, either in their beds, at ambient temperature (25° to 26°C) for full-term newborns, or in incubator (30° to 36°C) for prematures.

Data were collected in 2 different ways:

- Paper tracings of EEG (4 channels), Rapid Eye Movements (REMs), and body movements (detected by piezoelectric transducers), ECG, and a binary clock. In addition, behaviour was continuously observed and noted.
- Analog tape recordings of the ECG and binary clock channels, which were later digitized at 282 Hz.

Sleep states were coded visually on the paper tracings, using REM and EEG patterns, characteristic for the given conceptional age [9, 10], in 3 different

[1] In particular, Apgar score was at least 8 at 1', and always 10 at 5'.

states: Active Sleep (AS = REMS), Intermediate Sleep (IS), and Quiet Sleep (QS = nREMS). Correspondence between paper tracings and tape recordings was ensured by the binary clock. Recordings were performed until awakening of the subject. All recordings contained at least one complete sleep cycle, yielding at least one 512 beat epoch of AS and one of QS.

## 2.3 ECG signal processing

ECG digital tape recordings were then processed by using a signal analysis procedure originally introduced by one of us (F. Kauffmann) [20]:

QRS detection by using a Signal to Noise Ratio increase algorithm, producing the *RR signal*, which associates to each heartbeat number the time in ms from the preceding R wave to the current one; cutting out of the sequential RR signal in epochs of 512 heartbeats, which were the observation units on which HRV was quantified and subsequently processed by statistical analysis. Only epochs consisting of either pure AS or pure QS were analyzed. An artifact correction program enabled us to replace each nondetection or false alarm by the value of the baseline. Every epoch containing more than 10% (corrected) artifacts was rejected. In this way, we selected 338 epochs, 225 in AS and 113 in QS of 512 beats each.

Every raw RR series (not resampled) was processed by Short-Time Fourier Transform, a non-stationary spectral analysis procedure which uses complex demodulation [24, 30], and provides a dynamic evaluation of spectral amplitude in given frequency bands.

The estimation of spectral amplitude $A_{f_0}$ of the RR signal in the frequency band $[\ f_0 - \varepsilon\ ,\ f_0 + \varepsilon\ ]$ is given by the formula:

$$A_{f_0}(n) = \left| \sum_{k=0}^{n} RR(k) w_\varepsilon(n-k) exp(-2\pi i f_0 k) \right|$$

where $(w_\varepsilon)$ is a low-pass filter with $[\ 0\ ,\ \varepsilon\ ]$ passband, the terms of which are rapidly decaying to zero (this ensures a relative straightness of the effective evaluation window). If RR(n) is in milliseconds, so is $A_{f_0}$(n).

In our case, frequency was measured by its inverse: period in heartbeats, not constant in seconds, since sampling was performed at each beat; but conversion in 'equivalent seconds' is possible within one epoch by multiplying number of beats by mean RR in seconds (or, equivalently, in 'equivalent hertz', by dividing frequency measured in cycles per beat by mean RR in seconds [4]).

In the present study, we focused our attention on three types of HRV: High Frequency (HF) variations (with period in heartbeats: 3 to 8, ranging from 1 to 4 equivalent seconds), and two sub-types of low frequency variations: Mid

Frequency (MF) variations (10 to 25, ranging from 4 to 12 equivalent seconds), and (very) Low Frequency (LF) variations (30 to 100, ranging from 12 to 50 equivalent seconds). An illustration of these signals is presented on Fig. 1.

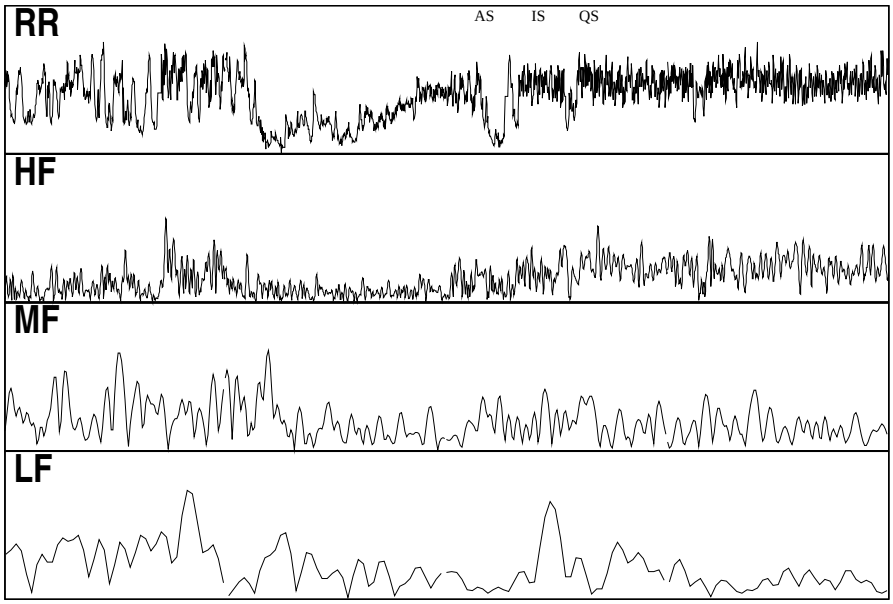


Figure 1. *RR signal and Short-Time Fourier Transform in the three frequency bands HF, MF, and LF (from top to bottom), in a series of 2048 consecutive heartbeats of a full-term newborn, during about 16 minutes, and showing a typical transition from Active Sleep (AS) to Quiet Sleep (QS); y-scales are from 350 to 650 ms for RR and from 0 to 100 ms for the other signals. Notice the changes in HF (↗ in QS) and in LF (↘ in QS).*

These frequency bands are comparable to the ones used by Harper, Schechtman et al.[14, 28, 29].

We measured in each epoch of 512 beats the mean amplitude of the raw RR and of the 3 extracted (HF, MF, LF) time signals. As a matter of fact, on our data, these mean amplitudes, obtained by Short-Time Fourier Transform, are highly correlated to Fast Fourier Transform (FFT) amplitudes in the same frequency bands (r=.99, .97 and .91, respectively), and one may think of the HRV variables HF, MF and LF as plain FFT amplitudes.

### 2.4 Statistical processing

It was conducted on the statistical software SICLA [5], developed at INRIA, in three ways:

- A Principal Component Analysis, using HRV variables HF, MF, and LF, was performed on all 338 epochs.
- Discriminant Analyses (Linear Discriminant Analysis [5, 6], Regularized Discriminant Analysis [11]) between the two extreme age groups: full-term (39-41 w. CA) and pre-term (31-36 w. CA), using the same HRV variables, were performed on 512 beat epochs in each sleep state. Similarly, discriminant analyses between sleep states were performed on epochs divided in three age groups. These analyses yielded percentages of well-classified epochs, and Mahalanobis distances (a measure of the quality of discrimination).
- Hypothesis tests (F-tests in analysis of variance, paired and unpaired 2-tailed t-tests for the comparison of means) between age groups or sleep states were performed on the variables RR, HF, MF, and LF, averaged within one individual and/or one sleep state, yielding F- or t-scores for each test, allowing us to assess the influence of age or sleep state on each variable under study.

## 3 Results

### 3.1 Principal Component Analysis

It was performed, using all 3 HRV variables, on all 338 epochs of 512 heartbeats, without any sleep state or age group distinction.

Fig. 2 shows the projection onto the principal factor plane of all 338 epochs.

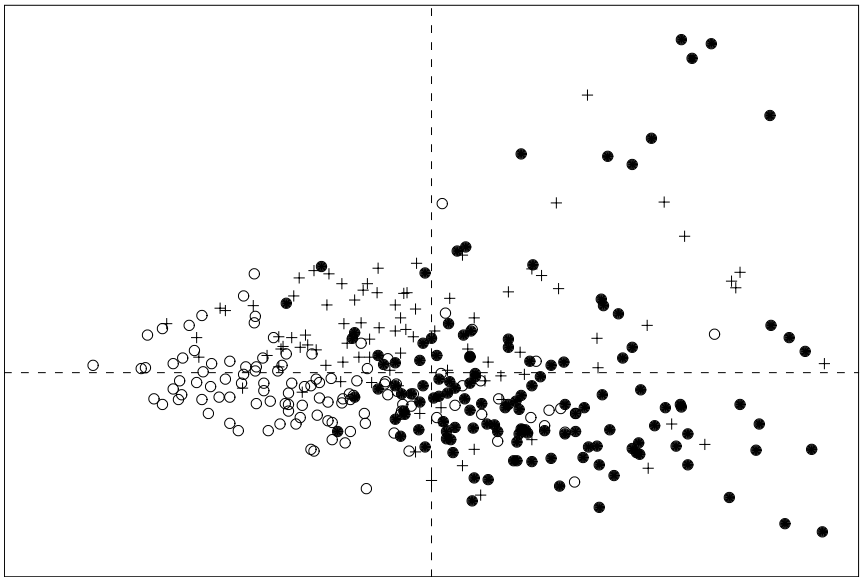

Figure 2. *Projection of all epochs onto the principal factor plane ($x = 0.69HF + 0.93MF + 0.82LF$, $y = 0.71HF - 0.07MF - 0.51LF$); full dots ($\bullet$) represent full-term neonate epochs, stars ($*$) intermediate ones, and empty dots ($\circ$) premature ones.*

On this figure, it is clear that full-term neonate (39-41 w. CA) epochs occupy the right part of the principal factor plane, and pre-term neonate (31-36 w. CA) epochs the left part of it. Intermediate neonate (37-38 w. CA) epochs are homogeneously displayed along the x-axis. One can therefore see the first factor, $x = 0.69HF + 0.93MF + 0.82LF$ (67% of total variance of the statistical cloud), representing total HRV, as a 'maturity factor'.

Fig. 3 shows the same projection onto the principal factor plane, but emphasizes sleep state distinction.

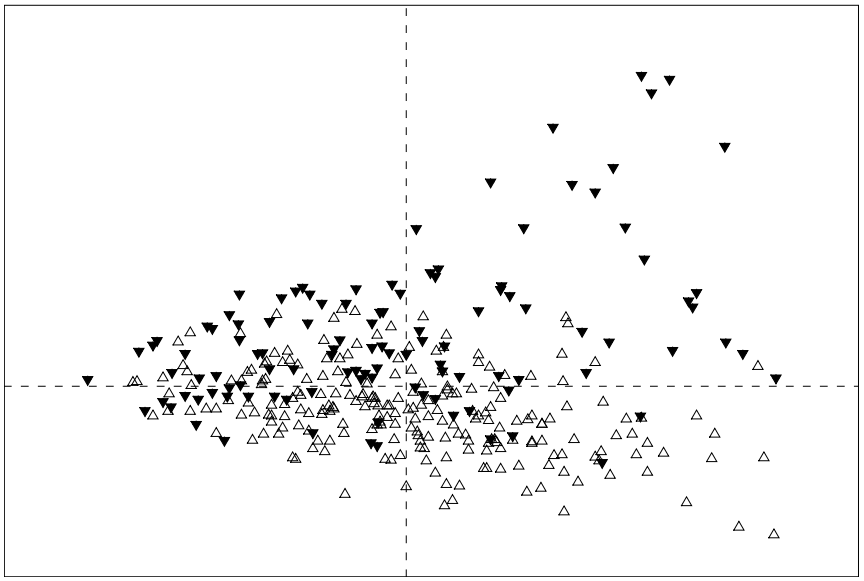

Figure 3. *The same as Fig. 2, but for sleep state discrimination; Active Sleep epochs are indicated by empty* $\Delta$*'s, Quiet Sleep epochs by full* $\blacktriangledown$*'s.*

AS epochs are predominantly found under the x-axis, and QS epochs over it. The second factor, $y = 0.71HF - 0.07MF - 0.51LF$ (26% of total variance of the statistical cloud), representing mainly an opposition HF/LF, thus might be seen as a 'sleep state factor'; but the same strong opposition HF/LF for the second factor, with a negligible coefficient for MF, was found when performing the same analyses in each sleep state, so that, to our meaning, this factor is not directly related to sleep state.

A synthetic view of all 24 subjects (averages of epochs within one given subject) and centres of gravity of age and sleep state groups is displayed on Fig. 4, where the x-axis may be seen as a 'maturity axis', since premature newborns are clearly predominant on the left part of this axis, and full-term newborns on the right part of it, whereas intermediate neonates are displayed in a rather uniform way along this same axis.

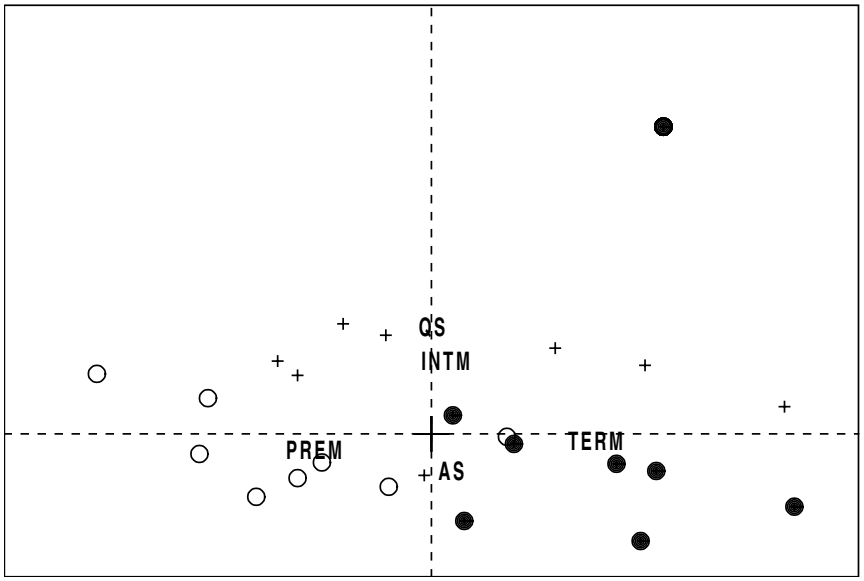


Figure 4. *Projection of all 24 subjects and centres of gravity of each age (TERM, INTM, PREM) and sleep state (AS, QS) group onto the principal factor plane ($x = 0.69HF + 0.93MF + 0.82LF$, $y = 0.71HF - 0.07MF - 0.51LF$); full-term neonates are indicated by full dots (•), intermediate ones by stars (∗), and prematures by empty dots (○). The x-axis may be seen as a 'maturity axis'.*

## 3.2 Discriminant analyses

First, to discriminate full-term from pre-term epochs (intermediate ones were temporarily discarded), globally, and in each sleep state separately, Linear Discriminant Analysis [5, 6] and Regularized Discriminant Analysis [11], both of them after cross-validation [23], were performed on the set of 247 non-intermediate epochs.

Table II

*Discrimination, between full-term and pre-term epochs: discriminant analyses, using HF, MF, and LF, performed on 247 epochs of 512 heartbeats.*

| Sleep state | L D A | | | M D | R D A |
|---|---|---|---|---|---|
| All epochs | 85% | | | 3.41 | 85% |
| | o.g.\a.g. | t. | p. | | |
| (247 =131 t. +116 p.) | t. | 110 | 21 | | |
| | p. | 20 | 96 | | |
| Active Sleep | 87% | | | 3.56 | 87% |
| | o.g.\a.g. | t. | p. | | |
| (173 =96 t. +77 p.) | t. | 85 | 11 | | |
| | p. | 12 | 65 | | |
| Quiet Sleep | 77% | | | 3.46 | 80% |
| | o.g\a.g. | t. | p. | | |
| (74 =35 t. +39 p.) | t. | 24 | 11 | | |
| | p. | 6 | 33 | | |

(LDA = Linear Discriminant Analysis; o.g. = origin group; a.g. = affectation group; t. = full-term newborn (39-41 w. CA) epochs; p. = pre-term newborn (31-36 w. CA) epochs; MD = Mahalanobis Distance; RDA = Regularized Discriminant Analysis; '%' indicate percentages of well-classified epochs, globally, or within one given sleep state.)

As may be seen on Table II, these analyses yield rather satisfying percentages of well-classified epochs, especially in AS. Mahalanobis distance between the centres of gravity of groups, which evaluates the quality of (linear) discrimination [5, 6], is higher in each sleep state than globally. A previous attempt to discriminate all 338 epochs in 3 age groups ('term', 'intm' and 'prem') had given very poor results (less than 50% of well-classified epochs), as might be suspected from Fig. 4.

Secondly, in order to discriminate sleep states in the whole population and in the different age groups, similar discriminant analyses were performed on the set of all 338 epochs.

Table III

*Evolution of between-state discrimination: discriminant analyses, using HF, MF, and LF, performed on all 338 epochs of 512 heartbeats.*

| Age group | L D A | | | M D | R D A |
|---|---|---|---|---|---|
| All epochs | 77% | | | 1.90 | 79% |
| | o.g.\a.g. | AS | QS | | |
| (338 =225 AS +113 QS) | AS | 209 | 16 | | |
| | QS | 61 | 52 | | |
| full-term | 82% | | | 3.32 | 88% |
| | o.g.\a.g. | AS | QS | | |
| (131 =96 AS +35 QS) | AS | 94 | 2 | | |
| | QS | 21 | 14 | | |
| intermediate | 76% | | | 2.73 | 79% |
| | o.g.\a.g. | AS | QS | | |
| (91 =52 AS +39 QS) | AS | 43 | 9 | | |
| | QS | 13 | 26 | | |
| premature | 71% | | | .84 | 72% |
| | o.g\a.g. | AS | QS | | |
| (116 =77 AS +39 QS) | AS | 71 | 6 | | |
| | QS | 28 | 11 | | |

(LDA = Linear Discriminant Analysis; o.g. = origin group; a.g. = affectation group; AS = Active Sleep; QS = Quiet Sleep; MD = Mahalanobis Distance; RDA = Regularized Discriminant Analysis; '%' indicate percentages of well-classified epochs, globally or within one given age group.)

As may be seen on Table III, these analyses yield satisfying percentages of well-classified epochs in the 'term' group, but much poorer ones in the other groups. AS is much better recognized than QS by using these methods. The evolution of Mahalanobis distance between groups shows a constant progression from the 'prem' group to the 'term' group.

The analysis of the correlation coefficient r of the Discriminant Linear Form (which provides the decision rule for linear discrimination between age groups and sleep states) with HRV variables is presented on Table IV.

Table IV
*Correlation of Discriminant Linear Form (DLF) with HRV variables:*

Table IVa *between age groups*

| r(DLF,..) | HF | MF | LF |
|---|---|---|---|
| All epochs | .75 | .89 | .82 |
| Active Sleep | .87 | .94 | .89 |
| Quiet Sleep | .90 | .79 | .67 |

Table IVb *between sleep states*

| r(DLF,..) | HF | MF | LF |
|---|---|---|---|
| All epochs | -.66 | .16 | .57 |
| full-term | -.80 | .14 | .68 |
| intermediate | -.86 | -.05 | .26 |
| premature | -.33 | .24 | .76 |

Table IVa shows that all HRV variables are relevant and contribute in the same way to age group discrimination. Table IVb confirms that an opposition between HF and LF is an important feature in sleep state discrimination. As a matter of fact, we also performed the same analyses for sleep state discrimination with only HF and LF (as was suggested by Fig. 2 and its legend, which shows that the contribution of MF to the 2nd factor is negligible). The results proved slightly better, which suggests that MF, which is important in age group discrimination, brings no contribution to sleep state discrimination, except statistical noise.

### 3.3 Hypothesis tests

The HRV variables and RR, measured in 512 beat epochs, were then averaged, yielding for each variable 48 within-subject-and-sleep-state means, or 24 within-subject means after conditioning by sleep state (24 in AS and 24 in QS).

The trends between age groups, for mean RR and each HRV variable, are displayed on Fig. 5. A focus on the evolution of HF and LF is also shown on Fig. 6.

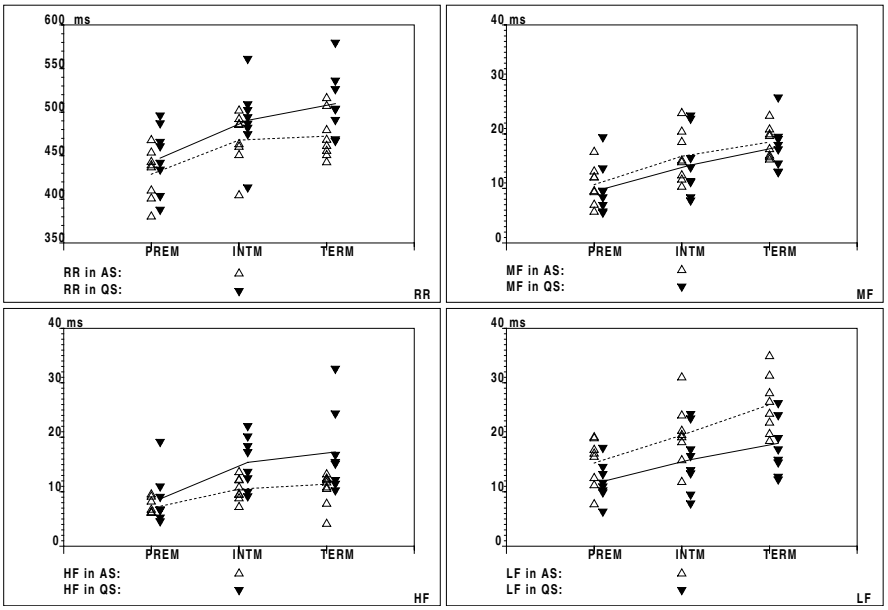


Figure 5. *Evolution of within-individual-and-sleep-state means, for RR, HF, MF, and LF, in AS and QS. Means are indicated by △'s for AS and by ▼'s for QS. Interrupted lines stand for AS, and continuous ones for QS.*

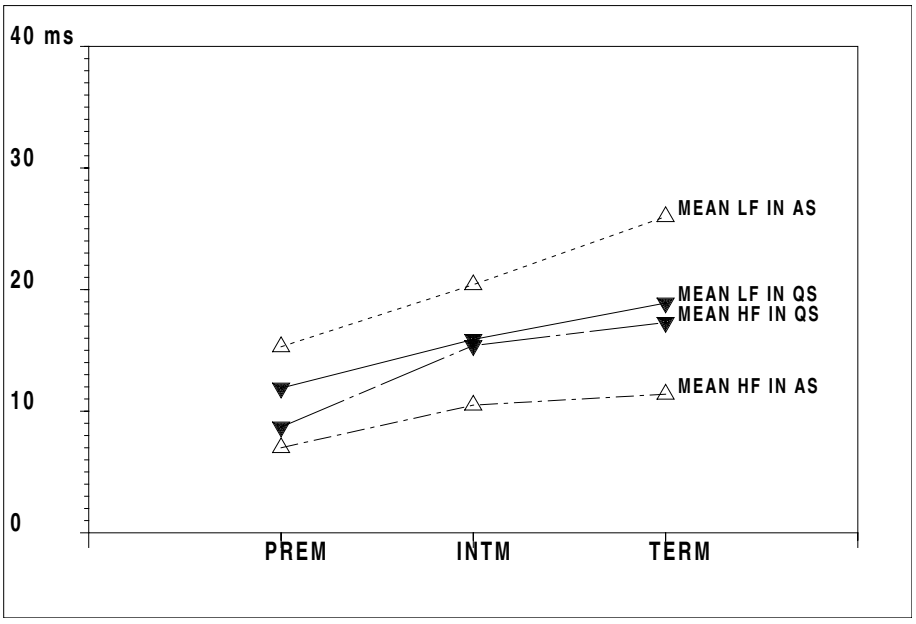


Figure 6. *The same, focused on HF and LF means.*

Hypothesis tests consisted in a (2 factor) ANOVA test, t-tests for the comparison of means and F-tests for the comparison of variances between age groups and sleep states.

First,the results of a two-way analysis of variance performed on the 48 within-subject-and-sleep-state means are presented on Table V.

Table V
*2-way analysis of variance (3 ages, 2 sleep states) on RR and HRV*

| n = 48 | $F^2_{42}$ | (age group) | $F^1_{42}$ | (sleep state) | $F^2_{42}$ | (interaction) |
|---|---|---|---|---|---|---|
| HF | 9.92 | $\star\star\star$ | 11.26 | $\star\star$ | 1.08 | NS |
| MF | 12.37 | $\star\star\star\star$ | .67 | NS | .04 | NS |
| LF | 11.13 | $\star\star\star$ | 13.08 | $\star\star\star$ | .87 | NS |
| RR | 10.56 | $\star\star\star$ | 6.97 | $\star\star$ | .33 | NS |

($\star\star$: p$\leq$.01; $\star\star\star$: p$\leq$.001; $\star\star\star\star$: p$\leq$.0001; NS: non significant)

It may be seen on this table that mean heart rate (RR) and all HRV variables: HF, MF and LF are relevant for age discrimination. On the contrary, only HF, LF, and, to a lesser extent, RR provide significant between-sleep-state differences. No interaction between age and sleep state has been found.

In more details, we studied separately, on the population of within-individual averages, calculated either globally, or within one given sleep state:

- Evolution of HRV with age:

  All HRV variables and RR increased with age, but not all with the same levels of significance, nor with the same regularity. The evolution of this increase of RR, HF, MF, and LF with age in the 24 neonates, first without consideration of sleep state (top), and then in AS (middle), and in QS (bottom), studied by a 1-way analysis of variance on age groups (1st column), and by Student's t-tests for the comparison of means between couples of age groups (other 3 columns), is presented on Table VI.

Table VI

*Evolution of HRV and RR with age: Fisher's F and Student's t. From top to bottom: Globally (All), in Active Sleep (AS), and in Quiet Sleep (QS).*

| All | $F^2_{21}$ | (all 24) | t | (8t./8p.) | t | (8t./8i.) | t | (8i./8p.) |
|---|---|---|---|---|---|---|---|---|
| HF | 7.96 | $\star\star$ | 3.41 | $\star\star$ | .27 | NS | 4.69 | $\star\star\star$ |
| MF | 6.28 | $\star\star$ | 4.02 | $\star\star$ | 1.71 | NS | 1.66 | NS |
| LF | 8.19 | $\star\star$ | 4.47 | $\star\star\star$ | 2.32 | $\star$ | 1.50 | NS |
| RR | 5.01 | $\star$ | 3.04 | $\star\star$ | .35 | NS | 2.54 | $\star$ |

| AS | $F^2_{21}$ | (all 24) | t | (8t./8p.) | t | (8t./8i.) | t | (8i./8p.) |
|---|---|---|---|---|---|---|---|---|
| HF | 11.97 | $\star\star\star$ | 4.98 | $\star\star$ | .94 | NS | 3.51 | $\star\star$ |
| MF | 8.91 | $\star\star$ | 4.86 | $\star\star\star$ | 1.35 | NS | 2.51 | $\star$ |
| LF | 8.59 | $\star\star$ | 4.34 | $\star\star\star$ | 2.02 | (p=.06) | 2.03 | (p=.06) |
| RR | 5.48 | $\star$ | 3.13 | $\star\star$ | .31 | NS | 2.59 | $\star$ |

| QS | $F^2_{21}$ | (all 24) | t | (8t./8p.) | t | (8t./8i.) | t | (8i./8p.) |
|---|---|---|---|---|---|---|---|---|
| HF | 4.84 | $\star$ | 2.74 | $\star$ | .60 | NS | 2.85 | $\star$ |
| MF | 4.71 | $\star$ | 3.41 | $\star\star$ | 1.26 | NS | 1.65 | NS |
| LF | 3.18 | (p=.06) | 2.82 | $\star$ | .79 | NS | 1.63 | NS |
| RR | 5.42 | $\star$ | 3.31 | $\star\star$ | .97 | NS | 2.19 | $\star$ |

(t. = full-term newborns (39-41 w. CA); i. = intermediate newborns (37-38 w. CA); p. = premature newborns (31-36 w. CA); $\star$: p$\leq$.05; $\star\star$: p$\leq$.01; $\star\star\star$: p$\leq$.001; NS: non significant)

All variables show significant between-age-group differences at Fisher's F-test, and these differences are most emphasized in AS.
To distinguish further between frequency bands, in contiguous age groups: For HF, the strongest changes (on the increase with CA) occur between the 'prem' and 'intm' groups; for MF this remains true, but only in AS; as regards LF, differences are nearly significant between the 'term' and 'intm' groups, and between the 'intm' and 'prem' groups, equally in each case, but only in AS.

- Evolution of sleep state distinction by HRV variables with age:

  Modifications of scores obtained at paired t-test for the comparison of means between sleep states on all 24 subjects (1st column), and within each age group (other 3 columns) are shown on Table VII.

Table VII
*Evolution of between-state distinction. Means of differences between Active Sleep (AS) and Quiet Sleep (QS): paired t-test for the comparison of means of values in AS* minus *values in QS.*

| t | all | (24/24) | 'term' | (8/8) | 'intm' | (8/8) | 'prem' | (8/8) |
|---|---|---|---|---|---|---|---|---|
| HF | -3.46 | $\star\star$ | -2.44 | $\star$ | -2.96 | $\star$ | -.80 | NS |
| MF | 1.12 | NS | .94 | NS | .79 | NS | .39 | NS |
| LF | 6.42 | $\star\star\star$ | 6.15 | $\star\star\star$ | 2.84 | $\star$ | 3.63 | $\star\star$ |
| RR | -5.69 | $\star\star\star$ | -4.19 | $\star\star$ | -3.00 | $\star$ | -2.78 | $\star$ |

($\star$: p$\leq$.05; $\star\star$: p$\leq$.01; $\star\star\star$: p$\leq$.001; $\star\star\star\star$: p$\leq$.0001; NS: non significant)

It is confirmed on this table that MF is not relevant for sleep state discrimination, whatever the age. HF, and most of all LF, show significant between-state differences among full-term newborns ('term'). In the 'intm' group, HF is still significant, and so is LF. In the 'prem' group, only LF shows significant between-state differences.

# 4 Discussion

## 4.1 Physiological implications

Data obtained by the analysis presented here demonstrate two main points, from the physiological point of view:

First, HRV parameters are highly discriminant for age, between premature and full-term newborns. All HRV variables are on the increase from the pre-term group (31-36 w. CA) to the full-term group (39-41 w. CA), but HF endures a rather steep increase from the pre-term to the intermediate group, remaining relatively stable afterwards, whereas LF grows much more regularly from 31 to 41 weeks CA. This is in favour of an important increase of vagal tone at 37 and 38 weeks, with a much slower growth afterwards, and of a much more constant increase of sympathetic tone from 31 to 41 weeks.

In the second place, concerning sleep states, we found that according to HRV parameters, sleep states may be discriminated better and better as CA increases, to reach satisfying levels of discrimination (over 80%, globally) only in the full-term group. AS is much better recognized than QS; the fact that QS is rather poorly recognized by Linear Discriminant Analysis has no physiological interpretation, but is merely a consequence of the choice made for the decision rule (see below statistical considerations on discriminant analyses). Between-age differences at hypothesis tests are higher in AS than in QS. This suggests an earlier differentiation of AS, as compared to QS, which is in agreement with

previous findings [8]. But since significant differences between sleep states in prematures are found only in LF, (higher in AS than in QS), this also suggests earlier maturation of sympathetic heart rate control during AS, HF (lower in AS than in QS) becoming discriminant later, from 37 weeks on.

This second point is coherent with the first one, developed above, and also with previous findings by Baldzer et al. [3], who used the opposition between LF and Respiratory Sinus Arrhythmia (which is known to be carried by HF) as a parameter for separation in two groups of healthy neonates, and speculated on a later postnatal development of the parasympathetic system as compared to the sympathetic system. From this point of view, we can also suggest an interpretation for the 2nd factor of our Principal Component Analysis, roughly speaking 'HF–LF', higher in full-term newborns than in prematures, and higher in QS than in AS: it may be closely related to vagal tone.

Our data should be compared with great precautions with previous studies on prematures, since our subjects are healthy premature and full-term neonates recorded in the first days of life, whereas in previous studies, infants were investigated at different postnatal ages [3], QS only being taken into account [1, 3], or total HRV only studied [19]. Some of our personal unpublished data point out the influence of postnatal age on HRV parameters. Especially, comparison with interesting data of Harper et al. [13], and Schechtman et al. [29] in infants between 1 week and 6 months of age may be inadequate because of the well-known rapid modifications of autonomic control during the first weeks of postnatal age.

## 4.2 About our methodology

Our methods of measurement and statistical analysis require some comment:

Given the length of the recordings (2 hours or more, up to 25000 heartbeats in some cases), 512 beats —about 4 minutes— appeared to us as a good duration for an observation unit to take within-subject variance into account. This variance is erased when we give means within one subject in a given sleep state. Conversely, within-subject means certainly are rather coarse evaluations of the activity in a given frequency band for one recording, but correspond to really independent observations, which is not the case with 512 beat epochs. The statistical procedure chosen here, working on 512 beat epochs for data analysis (thus taking into account within-subject variance), and on individuals for hypothesis tests (thus assuming independence of observations), enables us to take the best from each point of view. We avoided practicing data analysis on (too few) individuals, or hypothesis tests on non-independent observations (epochs). One may also think of within-subject-and-sleep-state means as primary subjects ('active/quiet babies'), and of the set of epochs in a given subject and in a given sleep state as a 'blowing-up' of such a subject. In this way, discriminant anal-

yses *a posteriori* may be seen as a robustness trial for the results of hypothesis tests.

Another question about parametric tests is the normality assumption. To settle this point, we simply confirmed the validity of our results by performing non- parametric tests on the same data: Mann-Whitney and Wilcoxon tests for unpaired and paired t-tests, Kruskal-Wallis test for F-test. The levels of significance were strictly the same. This does not show that our variables are normal, but plainly that t-test and F-test are very robust to deviances from normality. We chose to present these results in t-test and F-test scores only to set them in a well-known form.

Principal Component Analysis is a purely descriptive method, which makes no assumption on the statistical properties of the variables under study. Figures 2 and 3 show interesting qualitative properties of the statistical cloud of the 338 epochs studied here, which *a posteriori* confirm quantitative results obtained by discriminant analyses and hypothesis testing. Particularly, the 2nd factor ('HF–LF') is low in prematures, and grows as the 1st factor (total HRV) increases; this may be seen on the triangular shape of this 'cloud'.

Discriminant analyses theoretically assume normality, but are robust enough to tolerate violations of this normality assumption (see for example [21, 22]), and Regularized Discriminant Analysis [11], which may be seen, partially, as a generalization of Linear Discriminant Analysis, increases still this robustness. Detailed results of discrimination (analysis of sizes of origin and affectation groups) were available only for Linear Discriminant Analysis at the time of this study, so we presented both types of discrimination.

We stated above that the better identification of AS, as compared to QS, has no physiological interpretation. As a matter of fact, the decision rules assume a choice for the *a priori* probability of each group: in our case, we merely chose the 'natural' probabilities, proportional to group size. Since AS is twice as frequent as QS, this overcharges AS. If it was very important to recognize QS, and not so much AS, for instance, it would be possible to overcharge the probability of the QS group by a 'cost' coefficient, with a global percentage of well-classified epochs approximately constant, but better recognizing of QS.

### 4.3 Future prospects

Heart Rate Variability, as measured by the *RR signal* and its spectral components (HF, MF, and LF time signals), is by now a well-documented means to investigate the Autonomic Nervous System and its maturation. This study aimed to assess it by spectral analysis, data analysis and hypothesis tests in a rather classical way, which was intended to settle a background for further work on the same subject.

Particularly, one may notice that the statistical procedures we performed in this study used only the mean values (within one epoch) of the variables under study, which may be considered as rather poor, since we have (by Short-

Time Fourier Transform) a dynamic evaluation of the power in each chosen frequency band. Our motivation in using Short-Time Fourier Transform is that we intend to use the time signals (HF, MF, LF) provided by this spectral analysis procedure to calculate distance measures between them (and not only between their means over epochs of 512 points). Factor analysis by distance tables may then be aplied to obtain a model-independent description of the population.

In a less descriptive register, modelizations of the *RR signal* and its extracted spectral components, by a 'hidden Markov chain' underlying a linear model, on the one hand, and by non-linear models, such as bilinear models, or deterministic models with sensitive dependence upon initial conditions ('chaotic time series'), on the other hand, have been undertaken. These methods are still under assessment.

# 5 Conclusion

The present study suggests that a combination of purely descriptive data analysis on a set of all-subject short observation epochs, which takes into account within-subject variance, and of classical test analysis on the population of subjects, leaving out within-subject variance, but performing measures on independent samples (the subjects), is appropriate for describing HRV on long recordings accurately. As for HRV variables chosen for description, HF and LF are the most useful and are sufficient to describe maturation of the ANS and sleep state differentiation, at least in normal neonates.

Other methods intended for describing and comparing *RR signals* of different subjects, using distance measures between signals, are expected to give more information about the Autonomic Nervous System, on the background settled here.

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