Conditional symbolic analysis detects nonlinear influences of respiration on cardiovascular control in humans

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We propose a symbolic analysis framework for the quantitative characterization of complex dynamical systems. It allows the description of the time course of a single variable, the assessment of joint interactions and an analysis triggered by a conditioning input. The framework was applied to spontaneous variability of heart period (HP), systolic arterial pressure (SAP) and integrated muscle sympathetic nerve activity (MSNA) with the aim of characterizing cardiovascular control and nonlinear influences of respiration at rest in supine position, during orthostatic challenge induced by 80° head-up tilt (TILT) and about 3 min before evoked pre-syncope signs (PRESY). The approach detected (i) the exaggerated sympathetic modulation and vagal withdrawal from HP variability and the increased presence of fast MSNA variability components during PRESY compared with TILT; (ii) the increase of the SAP–HP coordination occurring at slow temporal scales and a decrease of that occurring at faster time
scales during PRESY compared with TILT; (iii) the reduction of the coordination between fast MSNA and SAP patterns during TILT and PRESY; (iv) the nonlinear influences of respiration leading to an increased likelihood to observe the abovementioned findings during expiration compared with inspiration one. The framework provided simple, quantitative indexes able to distinguish experimental conditions characterized by different states of the autonomic nervous system and to detect the early signs of a life threatening situation such as postural syncope.

1. Introduction

Respiration is one of the most relevant determinants of nonlinear dynamics in cardiovascular variability series. Evident nonlinear dynamics in muscle sympathetic nerve activity (MSNA) are the result of the activation of vagal afferents induced by lung inflation evoking, after an appropriate latency, a neural silence in MSNA during inspiration, whereas MSNA bursts occur more likely every cardiac beat during expiration [1–3]. The respiratory activity modulates membrane potentials of efferent vagal motoneurons [4] resulting in changes of heart period (HP) at the breathing rate (i.e. the respiratory sinus arrhythmia) [5]. The amplitude of respiratory-related HP variations and the importance of nonlinear HP dynamics induced by respiration depend on breathing rate and the level of stimulation of lung stretch receptors during inspiration [6,7]. Respiration is not only a powerful nonlinear modulator of the time course of a single cardiovascular variable, such as HP and MSNA, but it can also interfere with reflex mechanisms regulating the joint behaviour of two (or more) cardiovascular variables. Indeed, stimulation of carotid baroreceptors via brief neck suction pulses elicits a greater HP lengthening during late inspiratory and early expiratory phases [8], thus proving that respiration can modulate the baroreflex. The influence of respiration on baroreflex was also detected by the analysis of spontaneous variations of HP and systolic arterial pressure (SAP): indeed, a different distribution of spontaneous cardiac baroreflex sequences in expiratory and inspiratory phases was observed [9] with more frequent up baroreflex sequences with gain significantly different from 0 in expiration than inspiration, whereas the reverse situation holds for down baroreflex sequences. A brief neck pressure pulse compressing the carotid sinuses increases MSNA more remarkably during expiration than inspiration [10], thus demonstrating that respiration can modulate the baroreflex control of MSNA. Assessing the nonlinear influences of respiration on cardiovascular variables and reflex control mechanisms is a crucial issue not only to better understand human physiology, but also to derive more specific parameters helpful in clinics. For example, because it was observed that slow breathing counteracts sympathetic excitation by increasing tidal volume and stimulating lung stretch reflex in chronic obstructive pulmonary disease and heart failure patients [11,12], indexes describing the interactions between respiration and efferent sympathetic activity might be used to monitor the effectiveness of this countermeasure.

These nonlinear interferences of cardiovascular variability with respiration require specific tools to be revealed. These tools must be inherently nonlinear, be reliable over short data sequences, operate over a single series as well as over multivariate recordings, and allow the conditioning of the analysis according to a triggering signal (i.e. respiration). Symbolic analysis possesses all these features [13–20]. Indeed, because symbolic analysis is not exclusively limited to the description of first- and second-order statistical quantities (i.e. mean and autocorrelation function), it can account for nonlinearities. In addition, it is reliable over short data sequences owing to the exploitation of coarse graining procedures limiting the description of irrelevant details of the dynamics, it can deal with both univariate and multivariate recordings and allows the application of the strategies adopted for symbol coding and pattern classification separately during inspiratory and expiratory phases.

The aim of this study is to propose a framework for symbolic analysis of cardiovascular variability series. The framework is based on a previously published symbolic analysis approach [15] that was found helpful to infer autonomic profile from HP variability series [17,21]. This
methodology, originally devised to operate over a single series (univariate symbolic analysis), will be here extended to become a bivariate joint symbolic approach capable to describe the dynamical interactions between two series. Both univariate and bivariate joint techniques will be expanded further by conditioning the univariate or bivariate joint process in keeping with an additional signal disturbing the underlying dynamics, thus allowing the detection of nonlinear dynamics induced by the forcing input. Application is devised to describe the HP, SAP and MSNA variability series, the SAP–HP and MSNA–SAP dynamical interactions and the nonlinear dynamics induced by respiration during orthostatic challenge until symptoms of pre-syncpe occur.

2. Methods

(a) Univariate symbolic analysis

We followed the univariate symbolic approach previously described in reference [15]. The series \( x = \{x(i), i = 1, \ldots, N\} \), where \( i \) is the progressive counter and \( N \) is the series length, was transformed into a sequence of symbols, \( x_\xi = \{x_\xi(i), i = 1, \ldots, N\} \), via uniform quantization procedure. It consisted of coarse graining the full range of \( x \) with \( \xi \) equal-size bins and substituting \( x(i) \) inside each bin with the integer value coding the bin (i.e. \( 0 \leq x_\xi(i) \leq \xi - 1 \)). The symbolic series \( x_\xi \) was converted into a series of length \( L \) patterns, \( x_{\xi,L} = \{x_{\xi,L}(i), i = 1, \ldots, N - L + 1\} \), where \( x_{\xi,L}(i) = [x_\xi(i), x_\xi(i + 1), \ldots, x_\xi(i + L - 1)] \) is the ordered sequence of \( L \) consecutive symbols taken from \( x_\xi \). Given \( \xi \) and \( L \), the number of possible patterns is \( \xi^L \).

The setting of \( \xi \) and \( L \) was guided by the following principles: (i) the avoidance of a too rough coarse graining; (ii) the preservation of a minimal complexity of the pattern; (iii) the necessity to warrant a reliable statistics by limiting the number of possible patterns (i.e. \( \xi^L \)) compared with their actual quantity (i.e. \( N - L + 1 \)). Because in short-term cardiovascular variability analysis, \( N = 300 \) is a traditional choice (in most applications corresponds to about 5 min recording), the fulfilment of the previously listed principles led to \( \xi = 6 \) and \( L = 3 \). The pattern \( x_{\xi,L=6,L=3}(i) \) was classified into four classes: (i) no variation (0V); (ii) one variation (1V); (iii) two like variations (2LV); and (iv) two unlike variations (2UV). The 0V pattern was characterized by the lowest level of complexity (all symbols are equal), whereas the 2UV pattern featured the highest level of complexity (all symbols are different compared with the previous one and variations between consecutive original values were of opposite sign). 1V and 2LV patterns exhibited an intermediate level of complexity given that in the 1V pattern only two consecutive symbols were equal and in the 2LV pattern all symbols were different, but variations between consecutive original values were of the same sign. The 2LV pattern was more variable than the 1V one owing to the higher variability of symbols. Because any pattern \( x_{\xi,L=6,L=3}(i) \) in \( x_{\xi,L=6,L=3} \) was univocally associated with one of the four classes, the sum of the number of 0V, 1V, 2LV and 2UV patterns was \( N - L + 1 \). The percentage of 0V, 1V, 2LV and 2UV patterns (i.e. 0V%, 1V%, 2LV% and 2UV%) was computed by dividing their number by \( N - L + 1 \) and, then, by multiplying the result by 100.

(b) Bivariate joint symbolic analysis

The contemporaneously recorded series \( x = \{x(i), i = 1, \ldots, N\} \) and \( y = \{y(i), i = 1, \ldots, N\} \) were first transformed into sequences of symbols, \( x_\xi = \{x_\xi(i), i = 1, \ldots, N\} \) and \( y_\xi = \{y_\xi(i), i = 1, \ldots, N\} \), by applying separately the uniform quantization procedure described in §2\( a \) and then, \( x_\xi \) and \( y_\xi \) were both converted into a series of length \( L \) patterns, \( x_{\xi,L} = \{x_{\xi,L}(i), i = 1, \ldots, N - L + 1\} \) and \( y_{\xi,L} = \{y_{\xi,L}(i), i = 1, \ldots, N - L + 1\} \), with \( x_{\xi,L}(i) = [x_\xi(i), x_\xi(i + 1), \ldots, x_\xi(i + L - 1)] \) and \( y_{\xi,L}(i) = [y_\xi(i), y_\xi(i + 1), \ldots, y_\xi(i + L - 1)] \). The interactions between \( x \) and \( y \) were studied by building the set \( x_{\xi,L}, y_{\xi,L} = \{[x_{\xi,L}(i), y_{\xi,L}(i + \tau)], i = 1, \ldots, N - L - \tau + 1\} \), where \( [x_{\xi,L}(i), y_{\xi,L}(i + \tau)] \) was the joint scheme associating a pattern of \( x \), \( x_{\xi,L}(i) \), to the corresponding pattern of \( y \), \( y_{\xi,L}(i + \tau) \), and \( \tau \) was the delay between \( x \) and \( y \) with values of \( x \) linked to \( \tau \)-step-ahead values.
of $y$. Forms of coordinated activity between $x$ and $y$ were investigated by considering the following two categories: (i) coordinated (C) joint scheme with $x_{\xi}=6, L=3(i)$ and $y_{\xi}=6, L=3(i+\tau)$ belonging to the same class (e.g. 2UV and 2UV); (ii) uncoordinated (UNC) joint scheme with $x_{\xi}=6, L=3(i)$ and $y_{\xi}=6, L=3(i+\tau)$ belonging to different classes (e.g. 1V and 2UV). The total amount of C and UNC schemes is equal to $N-L-\tau+1$. The percentage of C and UNC schemes was computed by dividing their number by $N-L-\tau+1$ and, then, by multiplying the result by 100. C patterns can be further divided into four categories with both $x_{\xi}=6, L=3(i)$ and $y_{\xi}=6, L=3(i+\tau)$ belonging to 0V, 1V, 2LV and 2UV classes, respectively, indicated as $0V\rightarrow0V$, $1V\rightarrow1V$, $2LV\rightarrow2LV$ and $2UV\rightarrow2UV$ in the following. The total amount of $0V\rightarrow0V$, $1V\rightarrow1V$, $2LV\rightarrow2LV$ and $2UV\rightarrow2UV$ patterns is equal to the number of C schemes. The percentage of $0V\rightarrow0V$, $1V\rightarrow1V$, $2LV\rightarrow2LV$ and $2UV\rightarrow2UV$ patterns (i.e. $0V\%$, $1V\%$, $2LV\%$ and $2UV\%$) was computed by dividing their amount by the number of C ones and, then, by multiplying the result by 100.

(c) Univariate conditional symbolic analysis

We followed the approach described in §2a to build the series of patterns, $x_{\xi}=6, L=3$, from the original series $x$ and classify each pattern according to the $0V$, $1V$, $2LV$ and $2UV$ categories. Univariate conditional symbolic analysis necessitates an additional conditioning signal $s = \{s(i), i = 1, \ldots, N\}$, and the definition of a set of conditions imposing a partition over $s$ (i.e. the set of conditions divides $s$ into subsets the intersection of which is the empty set and their reunion is $s$). The most trivial partition over $s$ is defined by the two conditions $s(i) \leq \bar{s}$ and $s(i) > \bar{s}$, where $\bar{s}$ is a value assigned according to a given criterion. In alternative, the conditioning signal might be the first derivative of $s$, $\dot{s}$. In this case, the two conditions $\dot{s}(i) \leq 0$ and $\dot{s}(i) > 0$ can trigger the analysis based on the ascending and descending portions of $s$. If the set of conditions imposes a partition over $s$, then $x_{\xi}=6, L=3$ is also partitioned into subsets. For example, the subset of $x_{\xi}=6, L=3$ associated with the condition $s(i) \leq \bar{s}$, $x_{\xi}=6, L=3|s(i) \leq \bar{s}$ contains $x_{\xi}=6, L=3(i)$ if and only if the associated value of $s$, $s(i)$, fulfills the condition $s(i) \leq \bar{s}$. In any subset of $x_{\xi}=6, L=3$ defined according to a given condition over $s$, a classification of the patterns into the $0V$, $1V$, $2LV$ and $2UV$ classes can be performed. For example, given the condition $s(i) \leq \bar{s}$, the $0V|s(i) \leq \bar{s}$, $1V|s(i) \leq \bar{s}$, $2LV|s(i) \leq \bar{s}$ and $2UV|s(i) \leq \bar{s}$ patterns represent the $0V$, $1V$, $2LV$ and $2UV$ schemes found in $x_{\xi}=6, L=3|s(i) \leq \bar{s}$. The percentage of $0V|s(i) \leq \bar{s}$, $1V|s(i) \leq \bar{s}$, $2LV|s(i) \leq \bar{s}$ and $2UV|s(i) \leq \bar{s}$ (i.e. $0V\%|s(i) \leq \bar{s}$, $1V\%|s(i) \leq \bar{s}$, $2LV\%|s(i) \leq \bar{s}$ and $2UV\%|s(i) \leq \bar{s}$) was computed by dividing their number by the cardinality of $x_{\xi}=6, L=3|s(i) \leq \bar{s}$ and, then, by multiplying the result by 100. The cardinality of $x_{\xi}=6, L=3|s(i) \leq \bar{s}$ was smaller than $N-L+1$ (it is equal to $N-L+1$ only if $\bar{s}$ is the maximum of $s$) and it tended to decrease as a function of the number of conditions set over $s$. Because the reliability of the percentage of patterns associated with a given condition decreases with the number of conditions and $N$ cannot be enlarged given the constraint set by short-term cardiovascular variability analysis, the number of conditions was here limited to 2. In this study, the conditioning signal was characterized by a strong periodical dynamics alternating sequence of peaks and troughs (i.e. the respiratory signal). Given the peculiarity of the adopted conditioning signal, we set the two conditions $\dot{s}(i) > 0$ and $\dot{s}(i) \leq 0$, thus delineating inspiration (INS) and expiration (EXP) phases over $s$ and providing symbolic analysis given INS and EXP phases. After delineating INS and EXP phases, the set $x_{\xi}=6, L=3$ can be partitioned into four subsets associated with the INS and EXP phases and to the transitions from INS to EXP (INS–EXP) and vice versa (EXP–INS). More specifically, the subsets $x_{\xi}=6, L=3|\text{INS}$ and $x_{\xi}=6, L=3|\text{EXP}$ collected patterns of $x_{\xi}=6, L=3$ formed by samples of $x$ whose corresponding values of $s$ belonged to the INS and EXP phases, respectively, whereas patterns of $x_{\xi}=6, L=3$ formed by values of $x$ whose corresponding values of $s$ initially belonged to the INS phase (i.e. $\dot{s}(i) > 0$) and finally to the EXP phase (i.e. $\dot{s}(i+2) \leq 0$) were collected in $x_{\xi}=6, L=3|\text{INS–EXP}$ or, if the reverse transition was detected, in $x_{\xi}=6, L=3|\text{EXP–INS}$. We considered only the subsets $x_{\xi}=6, L=3|\text{INS}$ and $x_{\xi}=6, L=3|\text{EXP}$, because all values of $x$ were associated with values of $s$ belonging to the same phase of $s$. The percentage of the $0V$, $1V$, $2LV$ and $2UV$ patterns occurring in the INS phase (i.e. $0V\%|\text{INS}$, $1V\%|\text{INS}$, $2LV\%|\text{INS}$ and $2UV\%|\text{INS}$) was computed...
by dividing the number of the 0V, 1V, 2LV and 2UV patterns occurring in the INSP phase by the total number of patterns occurring in the INSP phase (i.e. the cardinality of \( x_\xi = 6, L = 3 \mid \text{INSP} \)) and, then, by multiplying the results by 100. Analogously, \( 0V\% \mid \text{EXP}, 1V\% \mid \text{EXP}, 2LV\% \mid \text{EXP} \) and \( 2UV\% \mid \text{EXP} \) were calculated.

(d) Bivariate joint conditional symbolic analysis

We followed the approach described in §2b to build the series of joint patterns, \( x_\xi = 6, L = 3 \mid y_\xi = 6, L = 3 \) from the original series \( x \) and \( y \). As in the case of the univariate conditional symbolic approach described in §2c, the bivariate joint conditional symbolic approach needs an additional conditioning signal \( s \) and the definition of a set of conditions imposing a partition over \( s \). Analogous to §2c, the assignment of the respiratory signal as a conditioning input and the imposition of a partition over its first derivative led to the separation between INSP from EXP phases and to the partition of \( x_\xi = 6, L = 3 \mid y_\xi = 6, L = 3 \mid \text{INSPEXP} \) into four subsets associated with the INSP and EXP phases and to the transitions from INSP to EXP (INSPEXP) and vice versa (EXPINSPEXP). More specifically, the subsets \( x_\xi = 6, L = 3 \mid y_\xi = 6, L = 3 \mid \text{INSPEXP} \) collected patterns of \( x_\xi = 6, L = 3 \mid y_\xi = 6, L = 3 \) formed by samples of \( x \) whose corresponding values of \( s \) belong to the INSP and EXP phases, respectively, whereas \( x_\xi = 6, L = 3 \mid y_\xi = 6, L = 3 \mid \text{INSPExp} \) and \( x_\xi = 6, L = 3 \mid y_\xi = 6, L = 3 \mid \text{EXPINSPEXP} \) brought together patterns of \( x_\xi = 6, L = 3 \mid y_\xi = 6, L = 3 \) formed by values of \( x \) whose corresponding values of \( s \) initially belonged to the INSP phase (i.e. \( s(i) > 0 \)) and finally to the EXP phase (i.e. \( s(i + 2) \leq 0 \)) and vice versa. In keeping with the approach set in §2b, the joint patterns belonging to the subsets \( x_\xi = 6, L = 3 \mid y_\xi = 6, L = 3 \mid \text{INSPEXP} \), \( x_\xi = 6, L = 3 \mid y_\xi = 6, L = 3 \mid \text{EXPINSPEXP} \) and \( x_\xi = 6, L = 3 \mid y_\xi = 6, L = 3 \mid \text{EXPINSPEXP} \) could be subdivided further into the C and UNC categories and those labelled as C patterns could be distinguished into the 0V–0V, 1V–1V, 2LV–2LV, and 2UV–2UV categories. As set in §2c, classification was performed only over the subsets \( x_\xi = 6, L = 3 \mid y_\xi = 6, L = 3 \mid \text{INSPEXP} \) and \( x_\xi = 6, L = 3 \mid y_\xi = 6, L = 3 \mid \text{EXP} \). Therefore, we calculated the percentage of C and UNC patterns occurring in the INSP phase (i.e. \( C\% \mid \text{INSPEXP} \) and \( UNC\% \mid \text{INSPEXP} \)) by normalizing the number of C and UNC patterns in the INSP phase by the cardinality of \( x_\xi = 6, L = 3 \mid y_\xi = 6, L = 3 \mid \text{INSPEXP} \) and, then, by multiplying the results by 100. We computed the percentage of the 0V–0V, 1V–1V, 2LV–2LV and 2UV–2UV patterns occurring in the INSP phase (i.e. 0V–0V\% \mid \text{INSPEXP}, 1V–1V\% \mid \text{INSPEXP}, 2LV–2LV\% \mid \text{INSPEXP} and 2UV–2UV\% \mid \text{INSPEXP}) by dividing the number of the 0V–0V, 1V–1V, 2LV–2LV and 2UV–2UV patterns occurring in the INSP phase by the number of C patterns occurring in the INSP phase and, then, by multiplying the results by 100. Analogously, \( C\% \mid \text{EXP}, UNC\% \mid \text{EXP}, 0V–0V\% \mid \text{EXP}, 1V–1V\% \mid \text{EXP}, 2LV–2LV\% \mid \text{EXP} \) and \( 2UV–2UV\% \mid \text{EXP} \) were calculated.

3. Experimental protocol and data analysis

(a) Experimental protocol

As part of the European Space Agency Medium-Term-Bedrest Whey Protein study identified in the ClinicalTrials.gov by NCT01655979, nine healthy male volunteers (age: from 23 to 42 years, median = 32; body mass index: from 22 to 26 kg m\(^{-2}\), median = 24 kg m\(^{-2}\)) were studied. The study, conducted in compliance with the protocol (and its subsequent amendments), was approved by the independent ethics committee Aerztekamer Nordrhein, Duesseldorf, Germany (approval no. 2010426/11-151). The study adhered to the principles of the Declaration of Helsinki for medical research involving human subjects, and all subjects gave their written informed consent. Full access to this database is available free of charge by contacting the corresponding author.

Electrocardiogram (lead II), non-invasive finger blood pressure (Finometer MIDI, Finapres Medical Systems, The Netherlands), respiratory activity through a thoracic impedance device, and MSNA were acquired using analogue-to-digital board AT-MIO-16E2 and LABVIEW software (National Instruments, Austin, TX). Raw MSNA was obtained by microneurography using a tungsten electrode inserted into the muscle nerve fascicles of the right peroneal nerve in

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the popliteal space [22]. The raw neural signal was amplified, band pass filtered (bandwidth between 700 and 2000 Hz), rectified and integrated with a time constant of 0.1 s, thus obtaining the integrated MSNA. This signal fulfilled traditional criteria previously reported [23]. All analyses were performed over the integrated MSNA signal. Signals were sampled at 500 Hz. Each experimental session consisted of 10 min of baseline recording at rest in supine position (REST) followed by 15 min of recording during 80° head-up tilt (TILT). Given that none of the subjects exhibited signs of orthostatic intolerance at the end of TILT, the pre-syncpe condition was evoked by the application of stepwise lower body negative pressure for 3 min [24]. The lower body negative pressure was decreased from −10 to −40 mmHg with steps of −10 mmHg. All subjects experienced pre-syncpe signs ranging from 1 to 11 min after the onset of the lower body negative pressure protocol. The recording period before the occurrence of pre-syncpe symptoms was labelled as PRESY and lasted less than 3 min (the average duration was 2 min). The duration of the PRESY period was kept shorter than that of REST and TILT periods to operate in the quasi-stationary conditions imposed by the stepwise protocol and fulfil the prerequisite of stationarity required by symbolic analysis.

(b) Variability series extraction and data analysis

After detecting the ventricular depolarization on the electrocardiogram (QRS complex) and locating its apex using parabolic interpolation, the temporal distance between the ith and the (i+1)th QRS parabolic apex was computed and used as an approximation of the ith HP, HP(i). The maximum of arterial pressure inside HP(i) was taken as the ith SAP, SAP(i). The occurrences of QRS and SAP peaks were carefully checked to avoid erroneous detections or missed beats. If isolated ectopic beats affected HP and SAP values, these measures were linearly interpolated using the HP and SAP values immediately following and preceding the HP and SAP measures affected by ectopic beats. The average value of MSNA computed during HP(i) was taken as ith MSNA, MSNA(i) [25]. HP = {HP(i), i = 1, ..., N}, SAP = {SAP(i), i = 1, ..., N} and MSNA = {MSNA(i), i = 1, ..., N} were extracted on a beat-to-beat basis, where i was the progressive cardiac beat number and N was the series length. All series were linearly detrended before converting them into symbolic sequences with ξ = 6 and L = 3. Owing to the measure convention HP(i) could not influence SAP(i), because SAP(i) occurred before that HP(i) could be measured [26], whereas SAP(i) can influence HP(i) through the fast vagal arm of the cardiac baroreflex [26]. Therefore, when assessing the SAP–HP joint interactions, we assumed a delay τ of the HP–SAP closed loop equal to one beat. Owing to the latency of the sympathetic response at the perineal nerve to changes in arterial pressure [27], a delay τ of one beat at REST or two beats during TILT and PRESY was assumed when the MSNA–SAP joint interactions were evaluated. The respiratory signal was used as a conditioning input. The peaks and troughs of the respiratory signal were automatically detected, thus defining the INSP and EXP phases as the trough-to-peak and peak-to-trough periods, respectively. HP series drove the process of association of a pattern to a respiratory phase. HP(i) belonged to the INSP or EXP phase if the first QRS complex fell within the INSP or EXP phase, respectively. A pattern HPξ=6,L=3(i) belonged to the INSP or EXP phase if all HP measures fell in the same respiratory phase. If HPξ=6,L=3(i) belonged to a given respiratory phase also the associated pattern of SAP, SAPξ=6,L=3(i+τ), with τ = 1, was linked to the same phase. If SAPξ=6,L=3(i) belonged to a given respiratory phase also MSNAξ=6,L=3(i+τ), with τ = 1 at REST or τ = 2 during TILT and PRESY, was linked to the same respiratory phase. Sequences of 256 consecutive measures were randomly selected inside REST and TILT periods. Sequences could be shorter during PRESY with a minimal length of 200 cardiac beats. If evident non-stationarities, such as very slow drifting of the mean or sudden changes of the variance, were present despite the linear detrending, the random selection was carried out again. The mean and the variance of HP and SAP and the MSNA burst frequency were computed, indicated as μHP, μSAP, σ2HP and σ2SAP, b0MSNA and expressed in ms, mmHg, ms2, mmHg2 and bursts min−1.
Table 1. Time domain indexes. $\mu_{HP}$, HP mean; $\sigma^2_{HP}$, HP variance; $\mu_{SAP}$, SAP mean; $\sigma^2_{SAP}$, SAP variance; $bf_{MSNA}$, MSNA burst frequency; REST, resting condition in supine position; TILT, 80° head-up tilt; PRESY, period just before observing the symptoms of pre-syncope. Results are reported as mean ± s.d. The symbols # and § indicate $p < 0.05$ versus REST and versus TILT, respectively.

<table>
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<th></th>
<th>REST</th>
<th>TILT</th>
<th>PRESY</th>
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<tbody>
<tr>
<td>$\mu_{HP}$ (ms)</td>
<td>881.17 ± 92.69</td>
<td>631.81 ± 96.17#</td>
<td>451.06 ± 89.65§</td>
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<td>$\sigma^2_{HP}$ (ms²)</td>
<td>2377.20 ± 870.01</td>
<td>1955.89 ± 1148.86</td>
<td>126.33 ± 254.94#</td>
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<tr>
<td>$\mu_{SAP}$ (mmHg)</td>
<td>125.59 ± 8.13</td>
<td>131.60 ± 8.74#</td>
<td>114.65 ± 7.72§</td>
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<tr>
<td>$\sigma^2_{SAP}$ (mmHg²)</td>
<td>9.98 ± 4.73</td>
<td>40.74 ± 21.11</td>
<td>54.66 ± 42.16#</td>
</tr>
<tr>
<td>$bf_{MSNA}$ (bursts min$^{-1}$)</td>
<td>22.11 ± 5.75</td>
<td>28.22 ± 4.92</td>
<td>32.22 ± 8.73§</td>
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(c) Statistical analysis

One-way repeated-measures analysis of variance (Holm–Sidak test for multiple comparisons) was applied to check the significance of the changes of the parameters given any pair of experimental conditions (i.e. REST versus TILT, TILT versus PRESY and REST versus PRESY). If the normality test (Kolmogorov–Smirnov test) was not fulfilled, the Friedman repeated-measures analysis of variance on ranks (Dunn’s test for multiple comparisons) was applied. After pooling together all experimental conditions, the paired $t$-test was applied to check the difference between C% and UNC%. The Wilcoxon signed ranked test was used if normality test was not passed. Statistical analysis was carried out using a commercial statistical program (SIGMASTAT, SPSS, v. 3.0.1). A $p < 0.05$ was considered significant.

4. Results

(a) Time domain parameters

Time domain parameters are reported in table 1. HP mean, $\mu_{HP}$, significantly decreased during TILT, and an additional reduction was detected during PRESY. SAP mean, $\mu_{SAP}$, significantly increased during TILT but dropped during PRESY. The HP variance, $\sigma^2_{HP}$, was unmodified during TILT, but decreased dramatically during PRESY compared with REST and TILT. Also the SAP variance, $\sigma^2_{SAP}$, was unaffected by TILT, but increased remarkably during PRESY only compared with REST. MSNA burst frequency, $bf_{MSNA}$, augmented significantly only during PRESY.

(b) Results of the univariate symbolic analysis

Figure 1 depicts the results of the univariate symbolic analysis. The indexes 0V% (figure 1a–c), 1V% (figure 1d,e,f), 2LV% (figure 1g,h,i) and 2UV% (figure 1j,k,l) are shown as a function of the experimental condition (i.e. REST, TILT and PRESY). When computed over the HP series, 0V% progressively increased (figure 1a), 1V% gradually decreased (figure 1d), 2LV% diminished during PRESY both compared with REST and TILT (figure 1g), and 2UV% was smaller during both TILT and PRESY compared with REST (figure 1j). When evaluated over the SAP series, 0V%, 1V% and 2LV% did not show any remarkable difference during the protocol (figure 1b,c,h,i). Only 2UV% differentiated the experimental conditions with values during TILT and PRESY smaller than those during REST (figure 1k). As in the case of indexes derived from SAP series, 0V%, 1V% and 2LV% obtained from MSNA series did not detect any significant difference between experimental conditions (figure 1c,f,i). Conversely, 2UV% was found to be larger during PRESY than during TILT (figure 1l).
Figure 1. Bar graphs report (a–c) 0V%, (d–f) 1V%, (g–i) 2LV% and (j–l) 2UV% as a function of the experimental condition (i.e. REST, TILT and PRESY). The parameters were computed over HP (a,d,g,j) SAP (b,e,h,k) and MSNA series (c,f,i,l) via univariate symbolic analysis. Values are reported as mean ± standard deviation. The symbols # and § indicate p < 0.05 versus REST and TILT, respectively.

(c) Results of bivariate joint symbolic analysis

After pooling together, all experimental conditions UNC% assessed over the SAP–HP joint patterns were significantly larger than C% (61.22 ± 6.43 versus 38.78 ± 6.43). When the MSNA–SAP joint patterns were analysed, the dominance of UNC% was confirmed (70.93 ± 4.97 versus 29.07 ± 4.97).
Figure 2 depicts the results of the bivariate joint symbolic analysis. The indexes C% (figure 2a,b), 0V–0V% (figure 2c,d), 1V–1V% (figure 2e,f), 2LV–2LV% (figure 2g,h) and 2UV–2UV% (figure 2i,j) are shown as a function of the experimental condition (i.e. REST, TILT and PRESY). When computed over the SAP–HP joint patterns C% increased during PRESY compared with REST (figure 2a), 0V–0V% augmented during PRESY compared with both REST and TILT (figure 2c) and 1V–1V% decreased during PRESY compared with REST and TILT (figure 2e). Conversely, 2LV–2LV% and 2UV–2UV% were not influenced by the experimental conditions (figure 2g,i). When calculated over the MSNA–SAP joint patterns the majority of the indexes remained unmodified (i.e. C%, 0V–0V%, 1V–1V% and 2LV–2LV%, figure 2b,d,f,l). Only 2UV–2UV% was reduced during TILT and PRESY compared with REST (figure 2j).

(d) Results of univariate conditional symbolic analysis

Figure 3 shows the results of the univariate symbolic analysis conditioned to the INSP phase. The indexes 0V%|INS (figure 3a–c), 1V%|INS (figure 3d–f), 2LV%|INS (figure 3g–i) and 2UV%|INS (figure 3j–l) are shown as a function of the experimental condition (i.e. REST, TILT and PRESY). When computed over the HP series 0V%|INS increased during TILT and PRESY compared with REST (figure 3a), 2LV%|INS diminished during PRESY compared with REST (figure 3g), and both 1V%|INS (figure 3d) and 2UV%|INS (figure 3j) were unaffected by the experimental conditions. When computed over the SAP and MSNA, series neither TILT nor PRESY modified the indexes (figure 3b,e,h,k and figure 3c,f,i,l, respectively).

Figure 4 shows the results of the univariate symbolic analysis conditioned to the EXP phase. The indexes 0V%|EXP (figure 4a–c), 1V%|EXP (figure 4d–f), 2LV%|EXP (figure 4g–i) and 2UV%|EXP (figure 4j–l) are shown as a function of the experimental condition (i.e. REST, TILT and PRESY). When computed over the HP series 0V%|EXP increased and 1V%|EXP diminished during PRESY compared with REST and TILT (figure 4a,d), 2LV%|EXP diminished during PRESY compared with TILT (figure 4g) and 2UV%|EXP was unaffected by the experimental conditions (figure 4j). When computed over the SAP, series 2UV%|EXP decreased during both TILT and PRESY compared with REST (figure 4k), whereas 0V%|EXP, 1V%|EXP, and 2LV%|EXP remained unvaried during the protocol (figure 4b,e,l). In addition, when computed over the MSNA, series 0V%|EXP, 1V%|EXP and 2LV%|EXP were unmodified by the protocol (figure 4c,f,i), whereas 2UV%|EXP increased during PRESY compared with TILT (figure 4l).

(e) Results of bivariate joint conditional symbolic analysis

After pooling together, all experimental conditions UNC%|INS and UNC%|EXP assessed over SAP–HP joint patterns were significantly larger than C%|INS and C%|EXP, respectively (66.07 ± 14.97 versus 33.93 ± 14.97 and 58.83 ± 9.01 versus 41.17 ± 9.01). If respiratory phase was taken into account, the dominance of the UNC% patterns over the C% ones was observed even when the MSNA–SAP joint patterns were analysed (71.85 ± 18.23 versus 28.15 ± 18.23 in the INSP phase and 69.62 ± 8.01 versus 30.38 ± 8.01 in the EXP phase).

Figure 5 depicts the results of the bivariate joint conditional symbolic analysis in the INSP phase. The indexes C%|INS (figure 5a,b), 0V–0V%|INS (figure 5c,d), 1V–1V%|INS (figure 5e,f), 2LV–2LV%|INS (figure 5g,h) and 2UV–2UV%|INS (figure 5i,j) are shown as a function of the experimental condition (i.e. REST, TILT and PRESY). When computed over both the SAP–HP and MSNA–SAP joint patterns assessed in the INSP phase, all indexes did not vary during the protocol.

Figure 6 depicts the results of the bivariate joint conditional symbolic analysis in the EXP phase. The indexes C%|EXP (figure 6a,b), 0V–0V%|EXP (figure 6c,d), 1V–1V%|EXP (figure 6e,f), 2LV–2LV%|EXP (figure 6g,h) and 2UV–2UV%|EXP (figure 6i,j) are shown as a function of the experimental condition (i.e. REST, TILT and PRESY). When computed over the SAP–HP joint patterns C%|EXP augmented during PRESY compared with REST (figure 6a), 0V–0V%|EXP and 1V–1V%|EXP, respectively, increased and decreased during PRESY compared with REST and...
Figure 2. Bar graphs report (a,b) C%, (c,d) 0V–0V%, (e,f) 1V–1V%, (g,h) 2LV–2LV% and (i,j) 2UV–2UV% as a function of the experimental condition (i.e. REST, TILT and PRESY). The parameters were computed over the SAP and HP series pair (a,c,e,g,i) and over the MSNA and SAP series pair (b,d,f,h,i) via bivariate joint symbolic analysis. Values are reported as mean ± standard deviation. The symbols # and § indicate p < 0.05 versus REST and TILT, respectively.
Figure 3. Bar graphs report (a–c) 0 V%|INSPI, (d–f) 1 V%|INSPI, (g–i) 2LV%|INSPI and (j–l) 2UV%|INSPI as a function of the experimental condition (i.e. REST, TILT and PRESY). The parameters were computed over HP (a,d,g,j), SAP (b,e,h,k) and MSNA series (c,f,i,l) via univariate symbolic analysis conditioned to the INSP phase. Values are reported as mean + standard deviation. The symbols # indicates $p < 0.05$ versus REST.

TILT (figure 6c,e), 2LV–2LV%|EXP and 2UV–2UV%|EXP remained unmodified during the protocol (figure 6g,i). When computed over the MSNA–SAP joint patterns, only 2UV–2UV%|EXP decreased during TILT and PRESY compared with REST (figure 6j), whereas all remaining indexes did not exhibit any significant difference between experimental conditions (figure 6b,d,f,h).

5. Discussion

The study proposes a compact framework based on symbolization capable to derive quantitative indexes describing the time course of a single variable, the joint interactions between time series
and interferences of an exogenous, strongly periodical, signal over the dynamics of a single variable and the joint behaviour of a time series pair.

The main experimental findings of this study can be summarized as follows: (i) the classification of HP variability patterns during TILT was compatible with an increased sympathetic modulation and vagal withdrawal compared with REST; (ii) during PRESY, indexes derived from HP dynamics suggested an exaggerated sympathetic modulation and vagal withdrawal; (iii) during PRESY, indexes derived from MSNA series indicated the presence of fast MSNA variability components hardly in keeping with the resonance frequency of microvasculature and baroreflex control; (iv) coordinated SAP–HP patterns increased during ...

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**Figure 4.** Bar graphs report (a–c) 0V%\(_{\text{EXP}}\), (d–f) 1V%\(_{\text{EXP}}\), (g–i) 2LV%\(_{\text{EXP}}\) and (j–l) 2UV%\(_{\text{EXP}}\) as a function of the experimental condition (i.e. REST, TILT and PRESY). The parameters were computed over HP (a,d,g,j), SAP (b,e,h,k) and MSNA series (c,f,i,l) via univariate symbolic analysis conditioned to the EXP phase. Values are reported as mean ± standard deviation. The symbols # and § indicate \(p < 0.05\) versus REST and TILT, respectively.
Figure 5. Bar graphs report (a,b) C%\textsubscript{INSP}, (c,d) 0V–0V\%\textsubscript{INSP}, (e,f) 1V–1V\%\textsubscript{INSP}, (g,h) 2LV–2LV\%\textsubscript{INSP} and (i,j) 2UV–2UV\%\textsubscript{INSP} as a function of the experimental condition (i.e. REST, TILT and PRESY). The parameters were computed over the SAP and HP series pair (a,c,e,g,i) and the MSNA and SAP series pair (b,d,f,h,j) via bivariate joint symbolic analysis conditioned to the INSP phase.
Figure 6. Bar graphs report (a,b) $C_{\%|\text{EXP}}$, (c,d) $0V–0V_{\%|\text{EXP}}$, (e,f) $1V–1V_{\%|\text{EXP}}$, (g,h) $2LV–2LV_{\%|\text{EXP}}$ and (i,j) $2UV–2UV_{\%|\text{EXP}}$ as a function of the experimental condition (i.e. REST, TILT and PRESY). The parameters were computed over the SAP and HP series pair (a,c,e,g,i) and the MSNA and SAP series pair (b,d,f,h,j) via bivariate joint symbolic analysis conditioned to the EXP phase. Values are reported as mean $\pm$ standard deviation. The symbols # and § indicate $p < 0.05$ versus REST and TILT, respectively.
PRESY, thus suggesting that an exaggerated coordination between HP and SAP series can play a role in the development of syncope; (v) SAP–HP coordination occurring at slow temporal scales progressively increased during TILT and PRESY, while that occurring at faster time scales decreased; (vi) at REST, a significant contribution to the MSNA–SAP coupling was given by the coordination between fast MSNA and SAP patterns and this contribution was reduced during TILT and PRESY; (vii) nonlinear interferences of respiration with HP and MSNA variability were detected as suggested by the more evident differentiation of PRESY from TILT and REST in the EXP phase than in the INSP one; (viii) respiration disturbed the SAP–HP and MSNA–SAP coordination in a nonlinear fashion as suggested by the different statistical power of bivariate joint symbolic indexes computed during the EXP phase compared with the INSP one.

(a) A framework based on symbolic analysis for the quantitative characterization of complex dynamical systems

We propose a framework based on symbolic analysis for the quantitative characterization of complex dynamical systems. The main feature of this approach is its versatility in providing tools capable to face the typical problems posed by complex dynamical system analysis. For example, this framework can be fruitfully exploited when it is needed: (i) a data-driven approach; (ii) a dynamical description of a variable accounting for its past values and its relation with other system variables; (iii) a characterization of the coordination among several, contemporaneously recorded series; (iv) an assessment of the effects, even nonlinear, of a forcing, exogenous, signal over the time course of a variable and/or over the dynamical interactions among a set of variables; (v) an approach independent of the number of system variables and/or constituents, and (vi) a conditional analysis triggered by a flexible set of situations that can be rendered as complex as required. Besides versatility, the proposed framework has other remarkable properties typical of the symbolic analysis tools (i.e. easiness of implementation, rapidity of index computation, reliability over short data sequences, filtering properties over noise superposed to data).

We applied the proposed framework to face the issue of the description of the cardiovascular control from spontaneous beat-to-beat fluctuations of cardiovascular variables. This problem poses the abovementioned challenges owing to the presence of multiple, contemporaneously active, regulatory mechanisms and the lack of a strict formalization of the causal relations among them [28,29]. The proposed indexes distinguished experimental conditions, identified early signs of syncope, detected nonlinear interactions and allowed sound interpretations and the formulation of hypotheses.

(b) Univariate symbolic analysis of heart period, systolic arterial pressure and muscle sympathetic nerve activity variability during orthostatic challenge

We confirm that the percentage of stable patterns (i.e. 0V%) assessed from HP variability increased during TILT [15,30]. Because 0V% derived from HP variability augmented gradually with tilt table inclination [21], it was suggested that 0V% can track the gradual increase of sympathetic modulation with the magnitude of the gravitational stimulus [31–33]. In keeping with this interpretation, the significant increase of 0V% computed from HP variability observed during PRESY compared with TILT suggested an exaggerated sympathetic modulation just before the appearance of pre-syncpe symptoms. This observation was corroborated by the significant increase of SAP variance and MSNA burst frequency reported in table 1. The decreased percentage of the HP patterns more variable than the 0V ones (i.e. 1V, 2LV and 2UV) during PRESY also suggested an important vagal withdrawal [21]. This observation was corroborated by the dramatic drop of the HP variance reported in table 1. This pattern of response to the orthostatic challenge is frequently reported before syncope episodes induced by modification of posture [34]. Given that in the HP variability an increased presence of 0V patterns and a decreased incidence of more variable ones, such that observed during graded head-up tilt [21], is associated with a loss
of complexity of the cardiac control [35], we suggest that the evoked syncope is characterized by a dramatic reduction of complexity of the cardiac control. This relevant reduction of complexity might be again a consequence of the exaggerated sympathetic activation limiting considerably the possibility to respond to stimulatory inputs and the range of temporal scales involved in cardiovascular regulation [35].

When the univariate symbolic analysis was applied to SAP variability, 0V% remained stable during TILT and PRESY, thus suggesting a steady complexity of the vascular control. This result is not surprising, because we found that the sympathetic activation induced by TILT did not produce any additional reduction of complexity of the vascular control compared with REST [35]. The unique symbolic parameter derived from SAP series that exhibited a change during TILT and PRESY was the percentage of the most variable patterns (i.e. 2UV%). The observed decrease of 2UV% in SAP series might be the consequence of a reduced impact of the intrathoracic pressure on stroke volume and cardiac output owing to the diminished venous return, the reduced respiratory sinus arrhythmia mediated by the vagal withdrawal and the increased ventricular stiffness during sympathetic activation.

Univariate symbolic analysis emphasized that MSNA variability behaved very differently from HP and SAP series. Indeed, while in both HP and SAP series, 2UV% significantly decreased during PRESY, thus suggesting a reduced importance of fast changes in HP and SAP variability, in MSNA series, 2UV% significantly increased during PRESY compared with TILT. This result indicated a more dominant presence of fast components in MSNA variability just before the occurrence of pre-syncope symptoms. These rapid variations might contribute to the occurrence of syncope, because patterns of MSNA variability can be no more able to match with the intrinsic slow resonance frequency of the microvasculature and baroreflex control [36] and, thus, they can be of little help in producing synchronized vasomotor activity and SAP variations in spite of a high MSNA burst frequency.

(c) Bivariate joint symbolic analysis of SAP–HP and MSNA–SAP variability interactions during orthostatic challenge

It is not surprising to find out that the SAP–HP coordinated activity was significantly smaller than the uncoordinated one. Indeed, several mechanisms contribute to produce HP and SAP changes independently of those described by the SAP–HP joint analysis (e.g. peripheral resistance can modify SAP without involving HP). The same situation holds even for the pair formed by MSNA and SAP series. The mechanical feed-forward pathway from HP to SAP might contribute to the MSNA–SAP decoupling by inducing SAP variations largely independent of the sympathetic vascular control. Also the noisy nature of MSNA series compared with the SAP one might be responsible for a certain degree of MSNA–SAP uncoordination. It is remarkable that the rate of coordinated SAP–HP patterns increased during PRESY, thus suggesting that an exaggerated coordination between HP and SAP series and a loss of HP–SAP complexity can play a role in the development of syncope.

The proposed analysis allowed the decomposition of the coordinated activity according to the different complexity of the patterns that contributed to it. It is worth observing that at REST the 1V–1V patterns were mainly responsible for the SAP–HP coordination, thus suggesting that neither fast nor slow rhythms, but a mix of the two provides the basis of the SAP–HP coordination. However, we found that 1V–1V% decreased during PRESY, whereas 0V–0V% increased. Taken all these findings together, we suggested that both slow and fast rhythms present in HP and SAP series contribute to the SAP–HP coordination at REST, but, whereas coordination between slow HP and SAP rhythms was strengthened during PRESY, the one between faster HP and SAP fluctuations was weakened. A direct involvement of autonomic control via sympathetic activation and vagal withdrawal can be hypothesized to explain the increase of 0V–0V% and the decrease of 1V–1V% during PRESY.
When indexes of coordinated behaviour derived from SAP and HP series were compared with those derived from MSNA and SAP at REST, the most evident difference was that 2UV−2UV% was larger in MSNA–SAP interactions (figure 2j) than in SAP–HP ones (figure 2i). This finding suggested that coupling between fast features of MSNA and SAP series, quantified by 2UV−2UV%, plays a significant role in the MSNA–SAP coordination. Remarkably, orthostatic challenge significantly reduced 2UV−2UV%, thus suggesting that sympathetic overactivity and/or the increase of sympathetic modulation was able to reduce this type of coordinated MSNA–SAP behaviour probably by augmenting the importance of slower rhythms [32].

(d) Univariate conditional symbolic analysis can detect the nonlinear influences of respiration on heart period, systolic arterial pressure and muscle sympathetic nerve activity variability

If a powerful nonlinear dynamics was evoked by respiration, we would expect that the bar graphs reporting the result obtained from the univariate symbolic analysis, regardless of the respiratory phase (figure 1) would be very different from those reporting the findings obtained from the univariate symbolic analysis conditioned by INSP (figure 3) and EXP (figure 4) phases. For example, a modification of the percentage of a specific pattern during the protocol might be evident in a specific respiratory phase (e.g. EXP), whereas it would be blurred in the other one (i.e. INSP) and, thus, less easily detectable in the correspondent bar graph reporting the results, regardless of the respiratory phase. Conversely, in our protocol, the panels in figure 1 report trends that can be found in figures 3 and 4. This remark suggests that nonlinear interactions of HP, SAP and MSNA variability with respiration are weak. This is not surprising because, when the identification of nonlinear dynamics was carried out through a surrogate data approach [37], the rate of detection of the nonlinear dynamics in HP and SAP series was limited, especially during TILT [6]. In addition, a reduction of the statistical power of all indexes in figures 3 and 4 compared with figure 1 is expected as a consequence of the reduced number of detected patterns in the case of conditional analysis compared with the unconditional one. However, a closer look allows us to detect some systematic differences between figure 3 and figure 4, suggesting that a nonlinear interference, even though weak, of respiration with HP and MSNA variability occurred. Indeed, symbolic analysis gated by respiration revealed a typical nonlinear property of HP variability referred to as time asymmetry and defined as the difference between the proportion of positive and negative HP variations [38]. Indeed, 2LV% assessed over HP variability was different in INSP and EXP phases (figure 3g versus figure 4g) and indicated that the respiratory activity is one of the possible determinants of HP variability time asymmetry even when respiratory sinus arrhythmia is dramatically reduced, as during TILT. Therefore, even though it was recently shown that indexes describing HP variability time asymmetry provide non-redundant information compared with symbolic indexes [39], a certain degree of correlation can be expected between time asymmetry and symbolic indexes when symbolic analysis is gated by respiration. In addition, during PRESY, the presence of 0V patterns in HP variability increased and that of patterns more variable than the 0V ones decreased more evidently during EXP period (figure 4a,d,g) than during INSP one (figure 3a,d,g), thus suggesting that the increase of sympathetic modulation and the inhibition of vagal activity was facilitated during the EXP phase [1–3]. Similarly, during PRESY, the increase of 2UV% in MSNA variability was significant only during the EXP phase (figure 4l versus figure 3l).

(e) Bivariate joint conditional symbolic analysis can detect the nonlinear influences of respiration on the SAP–HP and MSNA–SAP variability interactions

As in the case of the univariate analysis, the trends of indexes also computed according to bivariate joint analysis of the SAP–HP and MSNA–SAP interactions, regardless of the respiratory
phase shown in figure 2, were similar to those obtained when the bivariate joint analysis was conditioned by the respiratory phase (figures 5 and 6). This result stresses that the nonlinear actions of respiration over the joint SAP–HP and MSNA–SAP behaviours were also weak. However, compared with the findings of the univariate analysis, the nonlinear interferences of respiration over bivariate joint patterns appear to be stronger. Indeed, when the bivariate joint analysis was conditioned by the INSP phase (figure 5), none of the joint indexes was affected by the experimental condition, whereas significant changes were found when the bivariate joint analysis was triggered by the EXP phase (figure 6). Remarkably, these changes did not involve mainly PRESY, as it was observed in the case of the univariate analysis, but also TILT. Respiratory-related nonlinear influences were detected over both SAP–HP and MSNA–SAP joint behaviours. The nonlinear effect of respiration on the SAP–HP interactions might be the result of the nonlinear effect of respiration on cardiac baroreflex [8,9] and on venous return leading to modulations of the relation from HP to SAP [26,40]. The nonlinear effect of respiration on the MSNA–SAP interactions might be the result of the nonlinear effect of respiration on the baroreflex control of MSNA [41]. Results are in accordance to studies suggesting that the stimulation of carotid baroreceptors via brief neck pulses is more effective during the EXP phase than during the INSP one [8,10].

(f) Limitations of the study and future developments

Because the EXP period is usually longer than the INSP one, symbolic patterns are less numerous in the INSP phase than in the EXP one. This difference might increase the statistical power of symbolic parameters in the EXP period compared with the INSP one, thus favouring the detection of significant differences in the EXP phase. Because the frame length of the analysis cannot be increased given the focus on short-term regulatory mechanisms [42], just larger databases would assure the increase of the statistical power of the study. Therefore, future trials designed to test the ability of respiratory-gated symbolic analysis should account for the previous observation to compute the sample size. The smallness of the sample size of this study was emphasized also by the inability to detect the decrease of the HP variance and the increase of the SAP variance during TILT [31–33,35]. The non-significant trends observed in this study during TILT might turn into significant differences if the sample size was augmented. Another issue that deserves a specific assessment is the choice of the delays in the SAP–HP and MSNA–SAP variability interactions. In this study, they were a priori set. Because it was recently suggested that the delay might play a role in the description of the coupling among cardiovascular variables [16], future studies should propose some strategies for its optimization on a case-by-case basis and according to the beat-to-beat dynamics of the interacting variables. In addition, future studies should optimize the delineation of the INSP onset accounting for the typical apneic phase (i.e. no INSP and no EXP) occurring before the genuine INSP beginning. This optimization should lead to the substitution of the threshold equal to 0 over the first derivative of the respiratory signal with a value computed on a case-by-case basis and indicating a more physiological onset of the INSP phase.

6. Conclusion

This study demonstrates that the proposed framework based on symbolic analysis can typify a single time series, describe joint interactions and detect nonlinear actions of an exogenous signal (here respiration) over a single time course and/or over any joint association. The framework provided simple, quantitative indexes able to distinguish experimental conditions characterized by different states of the autonomic nervous system (i.e. REST and TILT) and to detect the early signs of a life-threatening situation such as postural syncope. In addition, the framework can be generalized further by making more complex the joint schemes describing association among variables (e.g. joint patterns might be formed by more than two variables) and the circumstances triggering the conditional analysis (e.g. conditions can be defined based on
combination of situations and signals). Given its versatility, generality and potential in describing multivariate, eventually nonlinear, interactions the proposed framework might be fruitfully exploited to detect changes associated with pathological conditions and deserves to be tested in clinical settings.

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