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Nonlinear effects of respiration on the crosstalk between cardiovascular and cerebrovascular control systems

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Cardiovascular and cerebrovascular regulatory systems are vital control mechanisms responsible for guaranteeing homeostasis and are affected by respiration. This work proposes the investigation of cardiovascular and cerebrovascular control systems and the nonlinear influences of respiration on both regulations through joint symbolic analysis (JSA), conditioned or unconditioned on respiration. Interactions between cardiovascular and cerebrovascular regulatory systems were evaluated as well by performing correlation analysis between JSA indexes describing the two control

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systems. Heart period, systolic and mean arterial pressure, mean cerebral blood flow velocity and respiration were acquired on a beat-to-beat basis in 13 subjects experiencing recurrent syncope episodes (SYNC) and 13 healthy individuals (non-SYNC) in supine resting condition and during head-up tilt test at 60° (TILT). Results showed that JSA distinguished conditions and groups, whereas time domain parameters detected only the effect of TILT. Respiration affected cardiovascular and cerebrovascular regulatory systems in a nonlinear way and was able to modulate the interactions between the two control systems with different outcome in non-SYNC and SYNC groups, thus suggesting that the analysis of the impact of respiration on cardiovascular and cerebrovascular regulatory systems might improve our understanding of the mechanisms underpinning the development of postural-related syncope.

1. Introduction

Cardiovascular and cerebrovascular systems are regulated by several control mechanisms aiming at avoiding that physiological variables assume risky values [1]. Among these control mechanisms, cardiac baroreflex and cerebral autoregulation play a relevant role. Cardiac baroreflex is a short-term regulatory reflex that adjusts heart period (HP) in response to changes in arterial pressure (AP) [2]. The baroreflex sensitivity, an index quantifying the magnitude of HP changes driven by a unit variation of systolic AP (SAP), is a very important clinical marker to predict mortality in specific cohorts of patients, e.g. after myocardial infarction [3]. Cerebral autoregulation is a homeostatic mechanism responsible for maintaining mean cerebral blood flow relatively constant, despite the changes in mean AP (MAP) [4–7]. Cerebral autoregulation has been studied for decades but only the advent of transcranial Doppler ultrasound technique, providing a non-invasive estimation of the velocity of the blood in the middle cerebral arteries, allowed the assessment of a variable linked to the cerebral blood flow (CBF), i.e. the CBF velocity (CBFV), with a time resolution similar to that of continuous AP recordings [8,9]. The application of simple procedures leading to the evaluation of the CBFV response to a drop of AP through the inflation of large thigh cuffs [4,10] (i.e. thigh cuff manoeuvre) or a forced exhalation against a closed airway [11–13] (i.e. the Valsalva manoeuvre), and the direct assessment of spontaneous CBFV fluctuations [14–16] has enlarged the possibility of studying the relation between mean CBFV (MCBFV) and MAP series. Respiration is known to influence both baroreflex control and cerebral autoregulation, and this influence is nonlinear, because the effect of respiration on both regulatory systems depends on the respiratory phase [6,7,17–20]. Control breathing experiments proved the important role of respiration in modulating HP–SAP [7,17–19] and MCBFV–MAP interactions [7,17]. These studies pointed out that, on the one hand, respiration must be taken into account when investigating baroreflex control and cerebral autoregulation and, on the other hand, respiration might modulate their interactions.

Symbolic analysis (SA) is an emerging branch of signal processing allowing the classification of relevant patterns, while discarding insignificant details [21–33]. SA methods appear to be particularly suited for the assessment of the HP–SAP and MCBFV–MAP joint nonlinear interactions and the inherent nonlinear influences of respiration because they provide strategies for the construction of HP–SAP and MCBFV–MAP joint patterns without presuming linearity, assumed by, for example, cross-correlation or coherence function, and allowing a joint analysis [28–33] gated by respiration [33].

The aim of this study was to apply joint SA (JSA) and joint conditional SA (JCSA) to assess whether respiration influences cardiovascular and cerebrovascular control systems, as described by HP–SAP and MCBFV–MAP symbolic indexes, respectively, and can modulate the crosstalk between them. We hypothesize that, if respiration was able to affect cardiovascular and cerebrovascular controls in a nonlinear way, JCSA would provide different results from JSA. The HP–SAP and MCBFV–MAP variability interactions were assessed according to the JSA and JCSA approaches set in [33] in a population of individuals experiencing recurrent

episodes of postural syncope (SYNC) [34]. Syncope was evoked by a prolonged exposure to an orthostatic challenge (i.e. head-up tilt test at 60°). Results were compared with those derived from age- and gender-matched healthy subjects never experiencing postural syncope (non-SYNC) and undergoing the same postural challenge. The contemporaneous evaluation of indexes describing a cardiovascular systemic control and a cerebral homeostatic regulation allowed us to check whether heart–brain interactions were present, and this presence depended on the experimental condition and/or population.

2. Methods

(a) Joint symbolic analysis

We exploited the JSA approach described in [33]. Briefly, given two synchronously recorded time series $x = \{x(i),\ i = 1, \ldots, N\}$ and $y = \{y(i),\ i = 1, \ldots, N\}$, where i is the sample counter and N is the series length, they are first transformed via uniform binning procedure over $\xi = 6$ bins into a sequence of integers from 0 to $\xi - 1$. Then, $L = 3$ consecutive symbols are grouped together to form patterns. Each pattern shares two symbols with the adjacent one. Thus, the number of patterns in each series is $N - L + 1$. Patterns were classified into four classes [25] according to the shape of the pattern: (i) 0 variations (0V), i.e. all the symbols are equal, (ii) 1 variation (1V), i.e. two consecutive symbols are equal and the third one has a different value, (iii) 2 like variations (2LV), i.e. the pattern looks like an ascending or descending ramp, and (iv) 2 unlike variations (2UV), i.e. the pattern looks like a peak or a valley. From the two series of patterns, we build a series of joint patterns formed by associating one pattern of x and one of y. The two patterns are separated in time by a latency τ, thus the number of joint patterns is $N - L - \tau + 1$. Among all possible combinations between patterns of x and y (i.e. 16 families), we consider only joint schemes where the pattern family built over x is equal to that over y. These patterns, referred to as coordinated patterns [33], can be subdivided into four classes labelled as 0V–0V, 1V–1V, 2LV–2LV and 2UV–2UV and their percentage inside the coordinated family can be evaluated (i.e. 0V–0V%, 1V–1V%, 2LV–2LV% and 2UV–2UV%). We remark that the 0V–0V patterns describe coordinated behaviours at the slowest time scale, whereas the 2UV–2UV family those at the fastest time scale. The 1V–1V and 2LV–2LV patterns typify the association among the two series at time scales faster than the 0V–0V ones, but slower than the 2UV–2UV patterns with the interactions described by the 1V–1V class occurring at time scales slower than those illustrated by the 2LV–2LV patterns. In addition, because the direction of the changes does not matter, both in-phase and out-of-phase matched behaviours are accounted for.

(b) Joint conditional symbolic analysis

We exploited the JCSA approach described in [33]. Briefly, given the coordinated patterns defined in §2*a* (i.e. 0V–0V, 1V–1V, 2LV–2LV and 2UV–2UV), they can be conditioned on the respiratory phase. 0V–0V, 1V–1V, 2LV–2LV and 2UV–2UV patterns whose symbols are associated with events all occurring in the inspiratory (INSP) phase are classified as 0V–0V$|_{\mathrm{INSP}}$, 1V–1V$|_{\mathrm{INSP}}$, 2LV–2LV$|_{\mathrm{INSP}}$ and 2UV–2UV$|_{\mathrm{INSP}}$. Analogously, we define as 0V–0V$|_{\mathrm{EXP}}$, 1V–1V$|_{\mathrm{EXP}}$, 2LV–2LV$|_{\mathrm{EXP}}$ and 2UV–2UV$|_{\mathrm{EXP}}$ the joint patterns whose values are all linkable to the expiratory (EXP) phase. The percentages of the patterns belonging to each class inside the family of the coordinated patterns in the INSP phase are labelled as 0V–0V%$|_{\mathrm{INSP}}$, 1V–1V%$|_{\mathrm{INSP}}$, 2LV–2LV%$|_{\mathrm{INSP}}$ and 2UV–2UV%$|_{\mathrm{INSP}}$ and those in the EXP phase as 0V–0V%$|_{\mathrm{EXP}}$, 1V–1V%$|_{\mathrm{EXP}}$, 2LV–2LV%$|_{\mathrm{EXP}}$ and 2UV–2UV%$|_{\mathrm{EXP}}$.

3. Experimental protocol and data analysis

(a) Population and experimental protocol

Thirteen SYNC subjects (age: 28 ± 9 years, min. = 18 years, max. = 44 years; five males) with previous history of unexplained syncope (more than three events during the foregoing year) were

enrolled in this study together with 13 non-SYNC healthy subjects (age: 27 ± 8 years, min. $= 18$ years, max. $= 44$ years; five males) [34]. The two groups had similar age and gender composition. The study took place at the Neurology Division of Sacro Cuore Hospital, Negrar, Italy, adhered to the principles of the Declaration of Helsinki for medical research involving humans and was approved by the local ethical committee. Subjects avoided the intake of caffeine or alcohol containing beverage for 24 h before the experiment. All of them signed a written informed consent before performing the experiment. The protocol consisted of 10 min of recording at rest in supine position (REST) followed by head-up tilt test (TILT). TILT was performed in a controlled environment, with subjects laying on the tilt table supported by two belts at the level of thigh and waist and with both feet touching the footrest of the table. The tilt table inclination was 60°. The maximum duration of the TILT session was 40 min. All SYNC subjects experienced presyncope signs before the end of the TILT session and exhibited spontaneous recovery after returning to the supine position. Subjects returned to the supine condition as soon as presyncope signs were observed. None of the non-SYNC subjects experienced presyncope symptoms before the end of the TILT session. Data are available through the corresponding author's ResearchGate profile (https://www.researchgate.net/profile/Alberto_Porta).

(b) Signal acquisition and variability series extraction

Electrocardiogram (lead II) was acquired together with AP measured at the level of middle finger through a photopletysmographic device (Finapres Medical Systems, Ohmenda, The Netherlands). CBFV and respiration were measured at the level of the middle cerebral artery by means of a transcranial Doppler ultrasonographic device (Multi-Dop T2, Dwl, San Juan Capistrano, CA) and through a thoracic impedance belt, respectively. Signals were synchronously acquired at a sampling rate of 1000 Hz and stored in a personal computer for off-line analysis. CBFV and respiratory signals were low-pass filtered with a sixth-order Butterworth filter with cut-off frequency of 10 Hz. Attention was paid to avoid phase distortion.

From the raw signals, cardiovascular and cerebrovascular variability series were extracted. HP was approximated as the time distance between the ith and the $(i+1)$th R-wave peaks on the electrocardiogram, where i is the cardiac beat counter. The application of the parabolic interpolation over the R-wave peak allowed the minimization of the jitters in the R-wave apex location. The ith SAP (i.e. SAP(i)) was measured as the maximum of AP signal inside the ith HP (i.e. HP(i)). The ith diastolic AP value (i.e. DAP(i)) was taken as the minimum of AP between the occurrences of SAP(i) and SAP($i+1$). We computed MAP values by integrating AP between the occurrences of DAP($i-1$) and DAP(i) and, then, by dividing the result by the duration of the ith diastolic interval (i.e. the time distance between the occurrences of DAP($i-1$) and DAP(i)). We calculated the MCBFV values by integrating CBFV between the diastolic values (i.e. the minima of the CBFV close to the occurrences of DAP($i-1$) and DAP(i)) and, then, by dividing the result by the time distance between the two diastolic values. 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.

(c) Time domain analysis and computation of joint symbolic analysis and joint conditional symbolic analysis parameters

The series HP $= \{\mathrm{HP}(i),\ i = 1, \ldots, N\}$, SAP $= \{\mathrm{SAP}(i),\ i = 1, \ldots, N\}$, DAP $= \{\mathrm{DAP}(i),\ i = 1 \ldots, N\}$, MAP $= \{\mathrm{MAP}(i),\ i = 1, \ldots, N\}$ and MCBFV $= \{\mathrm{MCBFV}(i),\ i = 1, \ldots, N\}$, where N is the total series length, were computed. Sequences of $N = 250$ consecutive synchronous values were selected from each HP, SAP, MAP and MCBFV series. The length of the series allows one to focus on short-term regulatory mechanisms [35]. The beginning of the TILT epoch started 5 min after the onset of the head-up tilt manoeuvre. The rationale of this choice is to avoid the initial transient adjustment of the cardiac variables, thus limiting the influence of non-stationarities over the analysis, and to explore cardiovascular and cerebrovascular response to the manoeuvre before the occurrence

of presyncope signs. Selection of the sequences was made at random at REST and in the first 10 min of TILT. Attention was paid to select periods of analysis in which the power spectrum of the respiratory signal featured a dominant peak, thus facilitating the automatic detection of INSP and EXP phases. The detected onset and offset of the INSP and EXP phases were verified by an operator and eventually adjusted. The HP, SAP and DAP series were manually checked for values coming from ectopic beats or misdetections and these values were eventually corrected through cubic spline interpolation. Corrections did not exceed the 5% of the overall length of the sequence considered for analysis. If evident non-stationarities of the mean and the variance were present, the random selection was carried out again. Stationarity of the selected sequences was finally checked according to [36]. Mean and variance of HP, SAP, MAP and MCBFV variability series were extracted, indicated as μ_{HP}, μ_{SAP}, μ_{MAP}, μ_{MCBFV} and σ^2_{HP}, σ^2_{SAP}, σ^2_{MAP}, σ^2_{MCBFV} and expressed in ms, mmHg, mmHg, cm s^{-1}, ms^2, mmHg^2, mmHg^2, cm^2 s^{-2}, respectively. The latency, τ, between the two interacting signals was fixed before applying JSA and JCSA. The latency, τ, between HP and SAP samples was set to 1 beat with SAP lagging behind HP, whereas the latency, τ, between MCBFV and MAP samples was set to 0. The rationale of this choice is that, whereas MCBFV and MAP could interact with each other within the time resolution of the analysis (i.e. the current diastolic interval), a minimal delay must be hypothesized between HP and SAP, because HP(i) cannot affect SAP(i) owing to the measurement conventions. JSA led to the calculation of 0V–0V%, 1V–1V%, 2LV–2LV% and 2UV–2UV% patterns relevant to the HP–SAP and MCBV–MAP variability interactions, whereas JCSA led to the computation of the same parameters in the INSP and EXP phases (i.e. 0V–0V%$|_{INSP}$, 1V–1V%$|_{INSP}$, 2LV–2LV%$|_{INSP}$, 2UV–2UV%$|_{INSP}$ and 0V–0V%$|_{EXP}$, 1V–1V%$|_{EXP}$, 2LV–2LV%$|_{EXP}$, 2UV–2UV%$|_{EXP}$, respectively).

(d) Statistical analysis

The null hypothesis of Gaussianity of the distribution of the parameters was tested according to Kolmogorov–Smirnov test. If it was rejected, then the values of the indexes plus 1 were log-transformed before applying any additional statistical test. The addition of 1 allowed us to map 0 again to 0 after the log-transformation. Two-way repeated measures analysis of variance was used to check the significance of the differences between non-SYNC and SYNC groups within the same experimental condition and between experimental conditions (i.e. REST and TILT) within the same group (one factor repetition, Holm–Sidak test for multiple comparisons). Correlation analysis was computed to test the association between symbolic indexes derived from HP–SAP and MCBFV–MAP analyses in non-SYNC and SYNC subjects at REST and during TILT. The Spearman rank correlation coefficient, ρ, and the probability of type I error, p, were computed. Statistical analysis was carried out using a commercial statistical program (SigmaPlot, v. 11.0, Systat Software, Inc., Chicago, IL). A value of $p < 0.05$ was always considered statistically significant.

4. Results

(a) Time domain analysis of heart period, systolic arterial pressure, mean arterial pressure and mean cerebral blood flow velocity

Table 1 shows results relevant to time domain parameters in terms of mean and variance extracted from the considered variability series (i.e. HP, SAP, MAP and MCBFV) at REST and during TILT in non-SYNC and SYNC subjects. As to the mean, regardless of the group (i.e. SYNC or non-SYNC), μ_{HP} and μ_{MCBFV} significantly decreased during TILT, whereas μ_{SAP} and μ_{MAP} remained stable. As to the variance, regardless of the group (i.e. SYNC or non-SYNC), σ^2_{MAP} and σ^2_{MCBFV} were not affected by TILT and σ^2_{SAP} increased significantly. The σ^2_{HP} decreased significantly during TILT only in SYNC subjects. Remarkably, the between-group differences in time-domain parameters were not statistically significant.

Table 1. Time domain parameters. Non-SYNC, group without history of recurrent postural syncope; SYNC, group with history of recurrent postural syncope; REST, supine resting condition; TILT, head-up tilt at 60°; μ_{HP}, HP mean; σ^2_{HP}, HP variance; μ_{SAP}, SAP mean; σ^2_{SAP}, SAP variance; μ_{MAP}, MAP mean; σ^2_{MAP}, MAP variance; μ_{MCBFV}, MCBFV mean; σ^2_{MCBFV}, MCBFV variance. Results are reported as mean ± standard deviation. Asterisk indicates $p < 0.05$ compared with REST.

	non-SYNC		SYNC	
parameter	REST	TILT	REST	TILT
μ_{HP} (ms)	848.13 ± 188.76	674.07 ± 107.25*	910.17 ± 142.79	745.58 ± 111.91*
σ^2_{HP} (ms^2)	2492.08 ± 2496.00	1749.15 ± 1173.13	4051.92 ± 3726.97	1962.85 ± 1896.86*
μ_{SAP} (mmHg)	134.57 ± 39.05	129.38 ± 32.56	125.19 ± 21.12	138.53 ± 23.04
σ^2_{SAP} (mmHg^2)	35.48 ± 22.86	45.77 ± 26.82*	24.48 ± 20.75	35.68 ± 17.17*
μ_{MAP} (mmHg)	15.93 ± 11.03	15.25 ± 6.88	17.30 ± 4.48	15.57 ± 3.65
σ^2_{MAP} (mmHg^2)	4.70 ± 8.34	2.56 ± 3.19	3.27 ± 6.54	2.60 ± 3.75
μ_{MCBFV} (cm s^{-1})	42.21 ± 38.24	31.78 ± 28.86*	58.99 ± 69.94	45.00 ± 48.78*
σ^2_{MCBFV} (cm^2 s^{-2})	29.61 ± 50.13	20.38 ± 36.31	50.80 ± 93.86	39.87 ± 77.00

(b) Heart period–systolic arterial pressure and mean cerebral blood flow velocity–mean arterial pressure symbolic indexes

The grouped bar graphs of figure 1 show the results relevant to the rate of occurrence of the 0V–0V joint pattern family (i.e. 0V–0V%) as derived from JSA and JCSA computed over HP and SAP series in figure 1*a–c* and over MAP and MCBFV series in figure 1*d–f*. Findings relevant to JSA are shown in figure 1*a*,*d*, whereas those relevant to JCSA are depicted in figure 1*b*,*c*,*e*,*f* divided into those conditioned on INSP (figure 1*b*,*e*) and EXP (figure 1*c*,*f*) phases. The 0V–0V% index is reported as mean plus standard deviation as a function of the experimental condition (i.e. REST and TILT) in both non-SYNC (black bars) and SYNC (white bars) subjects. When 0V–0V% was assessed over HP and SAP series, regardless of the respiratory phase (figure 1*a*), 0V–0V% was able to detect differences between conditions but unable to find differences between groups. Indeed, 0V–0V% increased during TILT in non-SYNC subjects, whereas SYNC and non-SYNC groups could not be distinguished both at REST and during TILT (figure 1*a*). When 0V–0V% was assessed over MCBFV and MAP series interactions and regardless of the respiratory phase (figure 1*d*), 0V–0V% was unable to detect differences between either conditions or groups. Similar conclusions could be drawn when the HP–SAP variability interactions were conditioned on the respiratory phase (figure 1*b*,*c*). Conversely, MCBFV–MAP analysis carried out after conditioning on the respiratory phase detected differences between experimental conditions with the same group: indeed, 0V–0V% in the EXP phase significantly increased during TILT in SYNC individuals (figure 1*f*), whereas it remained stable in the INSP phase (figure 1*e*).

Figure 2 has the same structure as figure 1 but it is relevant to the rate of occurrence of the 1V–1V joint pattern family (i.e. 1V–1V%). When 1V–1V% was evaluated over HP and SAP series regardless of the respiratory phase (figure 2*a*), 1V–1V% was able to detect differences between conditions and groups. Indeed, 1V–1V% decreased during TILT in non-SYNC and it was larger in SYNC group than in non-SYNC one during TILT (figure 2*a*). When 1V–1V% was assessed over MCBFV and MAP series and regardless of the respiratory phase (figure 2*d*), 1V–1V% was unable to detect differences between either conditions or groups. Similar conclusions could be drawn when 1V–1V% was assessed over HP and SAP series after conditioning on the EXP phase (figure 2*c*) and over MCBFV and MAP series after conditioning to both INSP and EXP phases (figure 2*e*,*f*). Conversely, when the 1V–1V% was computed over HP and SAP series during the

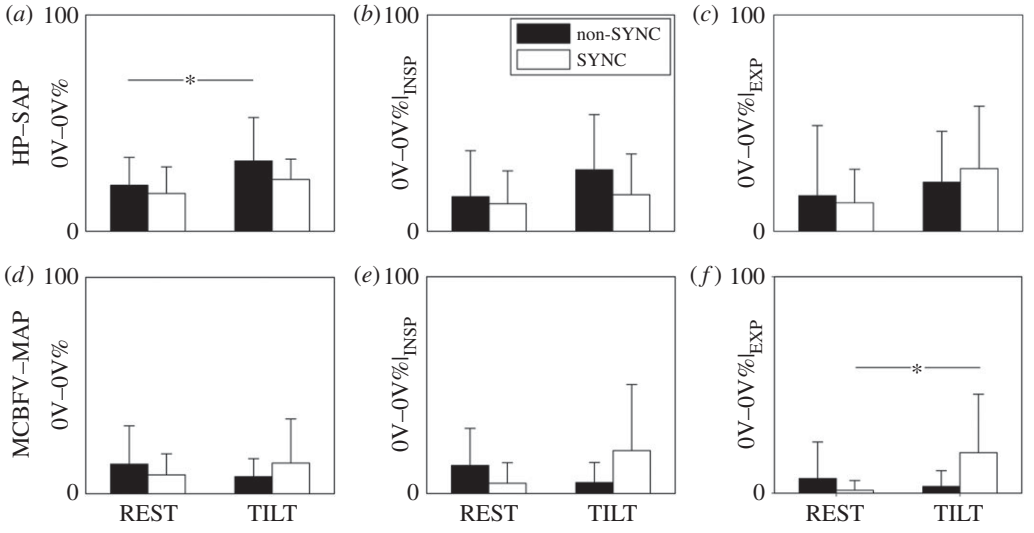

Figure 1. Grouped bar graphs report 0V–0V% assessed over HP–SAP (*a–c*) and MCBFV–MAP (*d–f*) patterns as a function of the experimental condition (i.e. REST and TILT) in both non-SYNC (black bars) and SYNC (white bars) subjects. The analysis was unconditioned on respiration (*a,d*) and conditioned on respiratory INSP (*b,e*) and EXP (*c,f*) phases. Results are reported as mean plus standard deviation. Asterisk indicates $p < 0.05$ between experimental conditions within the same group.

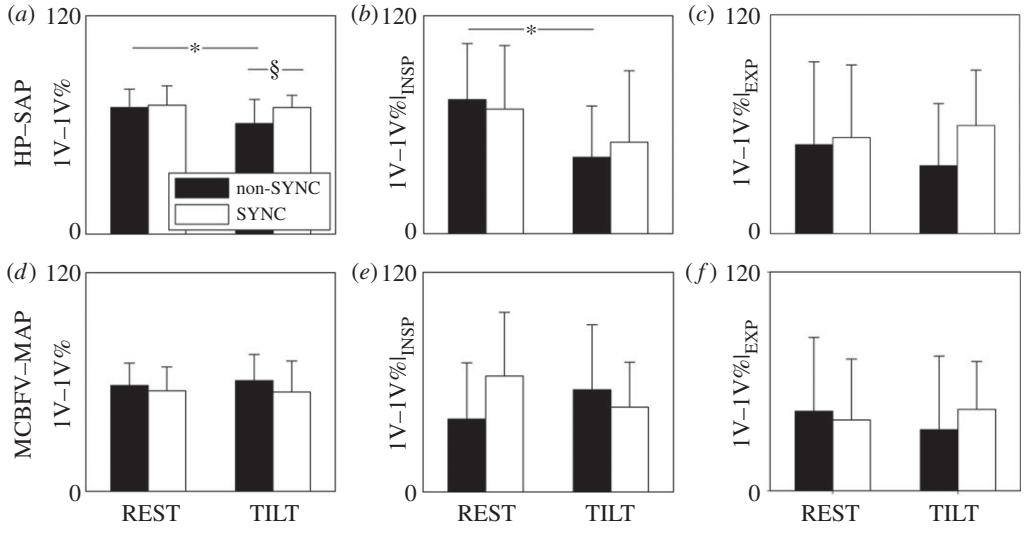

Figure 2. Grouped bar graphs report 1V–1V% assessed over HP–SAP (*a–c*) and MCBFV–MAP (*d–f*) patterns as a function of the experimental condition (i.e. REST and TILT) in both non-SYNC (black bars) and SYNC (white bars) subjects. The analysis was unconditioned on respiration (*a,d*) and conditioned on respiratory INSP (*b,e*) and EXP (*c,f*) phases. Results are reported as mean plus standard deviation. Asterisk and section symbol indicate $p < 0.05$ between experimental conditions within the same group and between groups within the same experimental condition, respectively.

INP phase a significant difference between conditions was detected in non-SYNC with 1V–1V% dropping during TILT (figure 2*b*).

Figure 3 has the same structure as figures 1 and 2 but it is relevant to the rate of occurrence of the 2LV–2LV joint pattern family (i.e. 2LV–2LV%). The absolute value of 2LV–2LV% was quite small, thus suggesting that this family was unlikely. No difference between conditions or groups

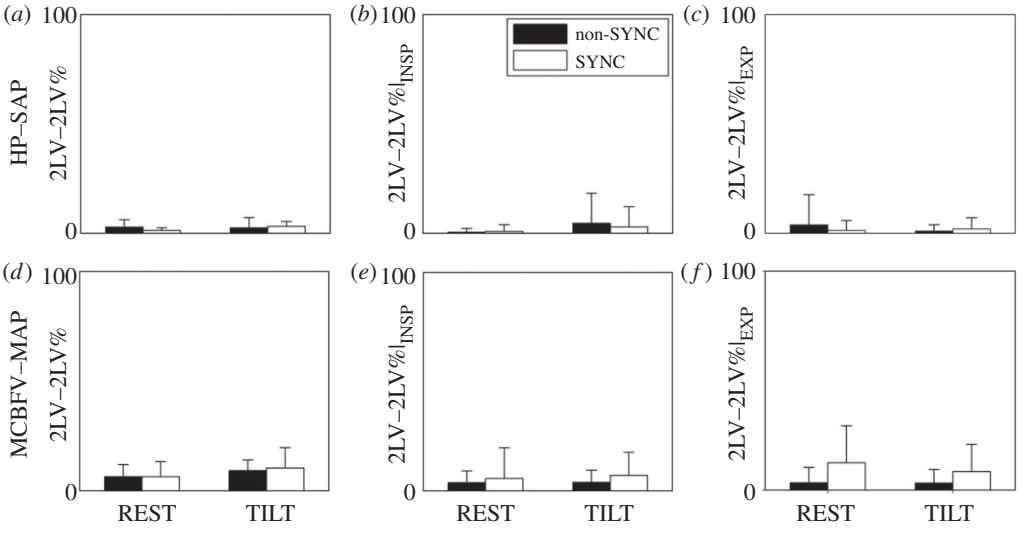

Figure 3. Grouped bar graphs report 2LV–2LV% assessed over HP–SAP (*a–c*) and MCBFV–MAP (*d–f*) patterns as a function of the experimental condition (i.e. REST and TILT) in both non-SYNC (black bars) and SYNC (white bars) subjects. The analysis was unconditioned on respiration (*a,d*) and conditioned on respiratory INSP (*b,e*) and EXP (*c,f*) phases. Results are reported as mean plus standard deviation.

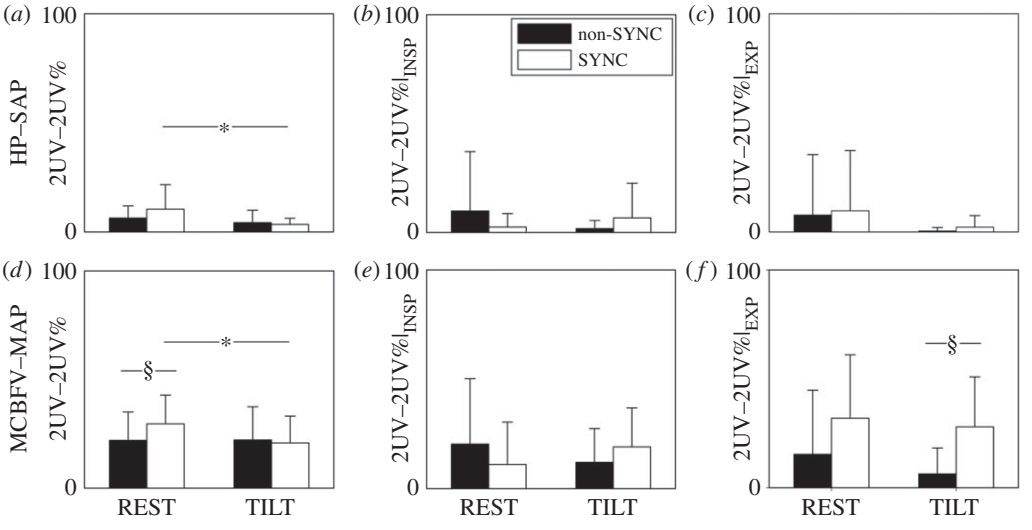

Figure 4. Grouped bar graphs report 2UV–2UV% assessed over HP–SAP (*a–c*) and MCBFV–MAP (*d–f*) patterns as a function of the experimental condition (i.e. REST and TILT) in both non-SYNC (black bars) and SYNC (white bars) subjects. The analysis was unconditioned on respiration (*a,d*) and conditioned on respiratory INSP (*b,e*) and EXP (*c,f*) phases. Results are reported as mean plus standard deviation. Asterisk and section symbol indicate $p < 0.05$ between experimental conditions within the same group and between groups within the same experimental condition, respectively.

was detected, regardless of the type of analysis (i.e. JSA or JCSA) and variability interactions (i.e. HP–SAP or MCBFV–MAP analysis).

Figure 4 has the same structure as figures 1–3 but it is relevant to the rate of occurrence of the 2UV–2UV joint pattern family (i.e. 2UV–2UV%). When 2UV–2UV% was assessed over HP and SAP series, regardless of the respiratory phase (figure 4*a*), 2UV–2UV% was able to detect

Table 2. Results of the correlation between JSA and JCSA parameters derived from HP–SAP and MCBFV–MAP patterns. Non-SYNC, group without history of recurrent postural syncope; SYNC, group with history of recurrent postural syncope; REST, supine resting condition; TILT, head-up tilt at 60°; ρ, Spearman correlation coefficient; p, type I error probability; 0V–0V%, percentage of 0V–0 V joint symbolic pattern; 1V–1V%, percentage of 1V–1V joint symbolic pattern; 2LV–2LV%, percentage of 2LV–2LV joint symbolic pattern; 2UV–2UV%, percentage of 2UV–2UV joint symbolic pattern; INSP, inspiratory phase; EXP, expiratory phase. Asterisk indicates a significant correlations with $p < 0.05$.

	non-SYNC				SYNC			
	REST		TILT		REST		TILT	
parameter	ρ	p	ρ	p	ρ	p	ρ	p
0V–0V%	0.407	0.168	0.484	0.094	0.226	0.457	0.049	0.873
1V–1V%	0.451	0.122	0.456	0.117	−0.286	0.344	−0.231	0.448
2LV–2LV%	0.198	0.517	0.006	0.985	−0.441	0.131	−0.239	0.431
2UV–2UV%	0.181	0.553	0.449	0.124	0.074	0.809	0.165	0.590
$0V–0V\%\|_{INSP}$	0.330	0.270	0.641*	$1.8{\cdot}10^{-2}$*	0.458	0.115	0.366	0.219
$1V–1V\%\|_{INSP}$	0.569*	$4.2{\cdot}10^{-2}$*	0.804*	$1.0{\cdot}10^{-3}$*	0.814*	$7.04{\cdot}10^{-4}$*	−0.063	0.838
$2LV–2LV\%\|_{INSP}$	−0.220	0.470	−0.064	0.836	−0.157	0.610	0.005	0.987
$2UV–2UV\%\|_{INSP}$	0.013	0.966	0.448	0.125	0.125	0.685	−0.051	0.869
$0V–0V\%\|_{EXP}$	−0.027	0.929	0.473	0.103	0.000	1.000	−0.508	0.076
$1V–1V\%\|_{EXP}$	0.743*	$4.0{\cdot}10^{-3}$*	0.393	0.184	0.447	0.126	−0.169	0.582
$2LV–2LV\%\|_{EXP}$	−0.189	0.537	0.565*	$4.4{\cdot}10^{-2}$*	−0.252	0.406	−0.419	0.154
$2UV–2UV\%\|_{EXP}$	0.528	0.064	0.377	0.204	0.057	0.852	0.364	0.221

differences between conditions but it was unable to find differences between groups. Indeed, 2UV–2UV% decreased during TILT in SYNC subjects, whereas SYNC and non-SYNC individuals could not be distinguished both at REST and during TILT (figure 4*a*). When 2UV–2UV% was assessed over MCBFV and MAP series and regardless of the respiratory phase (figure 4*d*), 2UV–2UV% was able to detect differences between both conditions and groups. Indeed, at REST 2UV–2UV% was larger in SYNC group than in non-SYNC one and it decreased significantly during TILT in SYNC individuals (figure 4*d*). When the 2UV–2UV% indexes assessing the HP–SAP variability interactions were conditioned on the respiratory phase, they were not able to detect either differences between conditions or groups (figure 4*b*,*c*). Conversely, 2UV–2UV% assessing the MCBFV–MAP variability interactions in the EXP phase showed that SYNC subjects were significantly different from non-SYNC individuals during TILT (figure 4*f*) with 2UV–2UV% significantly higher in SYNC subjects.

(c) Correlation between heart period–systolic arterial pressure and mean cerebral blood flow velocity–mean arterial pressure symbolic indexes

Table 2 shows results relevant to the correlation analysis between corresponding JSA indexes derived from HP–SAP and MCBFV–MAP analyses as a function of the experimental condition (i.e. REST and TILT) in both groups (i.e. non-SYNC and SYNC). The results of correlation analysis over JCSA parameters are also given. Table 2 reports the Spearman rank correlation coefficient, ρ, and the type I error probability, p. A significant correlation with $p < 0.05$ is marked with an asterisk. It can be observed that the JSA parameters were not significantly correlated.

The result held, regardless of condition and group. Remarkably, when JSA was conditioned on the respiratory phase a significant correlation was detected in both non-SYNC and SYNC groups, but the scenario was completely different in the two populations. Indeed, in non-SYNC subjects, several JCSA indexes were significantly correlated and this situation occurred in both respiratory phases and in both experimental conditions. Conversely, in SYNC individuals, solely the percentages of the 1V–1V patterns derived from HP–SAP and MCBFV–MAP analyses were significantly correlated and this situation occurred exclusively at REST and during the INSP phase. It is worth noting that, when significant, the correlation coefficient is always positive.

5. Discussion

This study investigates the effect of respiration on cardiovascular and cerebrovascular control systems and the ability of respiration to modulate the interactions between them in a population experiencing recurrent postural syncope. The main findings can be summarized as follows: (i) time domain analysis of cardiovascular and cerebrovascular parameters was not able to differentiate healthy subjects from pathological individuals, whereas JSA could, (ii) a nonlinear influence of respiration on cardiovascular and cerebrovascular control systems was detectable, and (iii) respiration modulated the degree of association between cardiovascular and cerebrovascular control systems and this modulation depended on the experimental condition and population.

(a) Detecting nonlinear effects of respiration on cardiovascular and cerebrovascular control systems and their interactions

Under the hypothesis of no interactions or linear interactions of respiration, we expect that (i) the results of JCSA in the INSP and EXP phases would be comparable and similar to those derived from JSA unconditioned on respiration and (ii) the degree of coordination between cardiovascular and cerebrovascular control systems, as measured from the correlation coefficient between HP–SAP and MCBFV–MAP markers, computed in the INSP and EXP phases would be comparable and similar to that calculated regardless of the respiratory phase. The violation of the above-mentioned first condition allows us to detect nonlinear effects of respiration on cardiovascular and cerebrovascular control systems and the infringement of the above-mentioned second condition indicates nonlinear influences of respiration on the interaction between cardiovascular and cerebrovascular control systems. The proposed approach allows one to test both these conditions.

(b) Joint symbolic analysis distinguishes SYNC subjects from non-SYNC individuals, whereas time domain parameters do not

TILT provoked the expected changes of time domain parameters. More specifically, given that TILT leads to a sympathetic activation mainly driven by the drop of central blood volume [27,37–39], HP significantly decreased and SAP variance significantly increased in both non-SYNC and SYNC groups. In addition, the reduction of MCBFV during TILT in both populations is in agreement with the literature [40,41], being the likely consequence of the cerebral vasoconstriction associated with the challenge in both groups. The reduction of HP variance during TILT, observed exclusively in SYNC individuals, suggests an accentuated sympathetic activation and/or vagal withdrawal in this group compared with non-SYNC subjects. Unfortunately, time domain analysis based on the computation of mean and variance of cardiovascular and cerebrovascular variables did not allow the direct distinction of the two groups. Conversely, JSA unconditioned on respiration separated not only experimental conditions within the same group, like the time domain parameters, but also groups within the same experimental condition. For example, we confirmed that the percentage of 0V–0V joint

pattern describing the HP–SAP variability interactions increased in non-SYNC subjects during TILT [33] (figure 1*a*), whereas that of 1V–1V family decreased [33] (figure 2*a*), thus suggesting that sympathetic activation induced by the postural challenge increased the strength of the HP–SAP coupling at slow time scales but provoked the HP–SAP uncoupling at faster ones probably in relation to the sympathetic activation and vagal withdrawal associated with the stressor. Remarkably, the reduction of the percentage of 1V–1V patterns describing the HP–SAP variability interactions during TILT was less marked in SYNC subjects, leading to the separation between the two groups during TILT (figure 2*a*). This finding, in addition to the negligible increase of the percentage of 0V–0V patterns in SYNC subjects (figure 1*a*), allows us to speculate that individuals who will undergo postural syncope at the end of the head-up tilt test might fail to modulate the coordination of the HP and SAP dynamics in response to a postural challenge especially at slower time scales. Conversely, this ability is over-expressed at fastest time scales: indeed, solely in SYNC subjects, the percentage of 2UV–2UV patterns describing the HP–SAP variability interactions significantly decreased during TILT (figure 4*a*). In addition, when JSA was carried out over MCBFV and MAP series, indexes derived from classification of the joint patterns were able to distinguish both experimental conditions and groups. Indeed, the percentage of 2UV–2UV patterns derived from the MCBFV–MAP analysis decreased during TILT in SYNC individuals, and at REST it separated SYNC from non-SYNC subjects (figure 4*d*). We speculate that sympathetic activation and vagal withdrawal associated with TILT were more effective in decoupling MCBFV and MAP series at fastest time scales in SYNC subjects, mainly because the degree of the MCBFV–MAP coupling at these time scales at REST was stronger in this group than in the non-SYNC one.

(c) Nonlinear effects of respiration on cardiovascular and cerebrovascular control systems

Because indexes derived from JCSA exhibited the same trends as those derived from JSA, it might appear that nonlinear influences of respiration over HP–SAP and MCBFV–MAP variability interactions are irrelevant. For example, the grouped bar graphs relevant to the percentage of 0V–0V patterns derived from the HP–SAP analysis in INSP (figure 1*b*) and EXP (figure 1*c*) phases are comparable and similar to that showing the same index, regardless of the respiratory phase (figure 1*a*). The comparison of the results of JSA and JCSA relevant to 1V–1V, 2LV–2LV and 2UV–2UV patterns confirmed this impression (figures 2–4). However, a more careful observation of the grouped bar graphs suggests that nonlinear influences of respiration over HP–SAP and MCBFV–MAP variability interactions are present. For example, the percentage of 0V–0V patterns assessed over the MCBFV and MAP series in the EXP phase increased during TILT in SYNC individuals (figure 1*f*), whereas the same parameter remained steady in the INSP phase (figure 1*e*) or regardless of the respiratory phase (figure 1*d*). Another example of the nonlinear effect of respiration was provided by the percentage of 1V–1V patterns derived from the HP–SAP analysis: indeed, the drop in non-SYNC subjects during TILT was more marked in the INSP phase (figure 2*b*) than in the EXP one (figure 2*c*) or regardless of the respiratory phase (figure 2*a*).

(d) Respiration modulates the degree of association between cardiovascular and cerebrovascular control systems

The correlation analysis between a marker describing the HP–SAP variability interactions and the same index assessing the MCBFV–MAP ones is used to check whether cardiovascular and cerebrovascular control systems interact with each other. The correlation coefficient is taken as an indicator of the degree of coordination between them. When correlation analysis was performed over JSA indexes unconditioned on respiration, cardiovascular and cerebrovascular control systems appeared to work independently. This result held, regardless of the experimental condition and group. Conversely, when JCSA indexes were considered, the opposite conclusion was drawn, thus suggesting JSA might smear influences of respiration by mixing INSP and

EXP phases. This finding suggests a possible role of respiration in modulating the crosstalk between different physiological systems. Even more importantly, this modulating capability of respiration depends on the experimental condition and population. Indeed, while in non-SYNC subjects the degree of coordination between cardiovascular and cerebrovascular control systems was significant both at REST and during TILT, a completely different scenario was detected in the SYNC group. Indeed, in SYNC individuals, cardiovascular and cerebrovascular regulatory systems appeared to be coupled only at REST. This finding stresses that in SYNC subjects respiration can modulate the degree of interactions between the two control systems but its ability appeared to be impaired during TILT, thus possibly contributing to the development of postural syncope. Future studies should check whether countermeasures focused on the respiratory drive might be helpful in reversing this trend and preventing postural syncope. Interestingly, coordination between cardiovascular and cerebrovascular control systems in non-SYNC individuals occurred more likely between patterns characterized by slow time scales (i.e. the 0V–0V and 1V–1V schemes) than between those more directly influenced by respiration (i.e. the 2LV–2LV and 2UV–2UV schemes). Therefore, it seems that in both non-SYNC and SYNC subjects respiration might have the possibility to modulate the crosstalk between different control systems at time scales completely different from the dominant time scale of its action, thus stressing again the nonlinear nature of the phenomenon.

(e) Limitations of the study and future developments

The study is based on a respiratory signal recorded with a thoracic belt and a min–max procedure delineating the onset and the offset of the respiratory phases. We advocate, on the one hand, the contemporaneous recording of the respiratory activity according to different modalities directly assessing respiratory flow and/or volume to check whether conclusions of this study might depend on the type of signal transduction, and, on the other hand, the test of alternative methods for the delineation of the respiratory phases excluding the typical apnoeic phase at the end of the EXP phase. We also promote studies testing systematically respiratory patterns, alternative to spontaneous breathing, with the final aim to classify them according to their influence on cardiovascular and cerebrovascular control systems. A more systematic approach could improve our knowledge of the ability of the respiratory drive to interfere with physiological control mechanisms and modify key regulatory parameters. In addition, because some overlap exists between the information contained in the symbolic categories, we suggest also to perform specific studies aiming at quantifying possible relations among the percentages of symbolic categories. Because, in principle, a possible link between heart rate asymmetry (i.e. heart rate decelerates more rapidly than it accelerates) [42] and the observed differences between JCSA indexes might exist, we encourage future studies correlating results obtained from JCSA with markers describing heart rate asymmetry [42–44].

6. Conclusion

The study stresses that different regulatory systems involving systemic cardiovascular and cerebral homeostatic variables can interact with each other and it suggests that the degree of their interaction can be modulated by respiration. Because the detected influences of respiration vary according to the experimental condition and group, the findings support a more systematic use of a physiological input, in large part under voluntary control, such as respiration, to adjust the degree of coordination between cardiovascular and cerebrovascular regulatory systems with the final aim to favour specific control behaviours selected among others to improve the quality of life in pathological subjects and flexibility in coping with stressors in healthy individuals. In this context, the proposed analysis framework could be a viable tool to quantify the outcome of the application of countermeasures, focused on the respiratory function and specifically designed to interfere with cardiovascular and cerebrovascular regulatory systems, in both pathological and healthy individuals. In addition, the study encourages the joint monitoring of indexes derived

from different regulatory systems along the brain–heart axis to achieve a more integrated view on the state of the organism.

Data accessibility. Data are available through the corresponding author's ResearchGate profile (https://www.researchgate.net/profile/Alberto_Porta).

Authors' contributions. V.B.: analysis of the data, interpretation of the data, drafting the manuscript, critical revision of the work, final approval of the version to be published; A.M., B.D.M.: analysis of the data, final approval of the version to be published; G.R.: acquisition of the data, critical revision of the work, final approval of the version to be published; G.N., L.F.: critical revision of the work, final approval of the version to be published; A.P.: conception and design of the work, interpretation of the data, drafting the manuscript, critical revision of the work, final approval of the version to be published.

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