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RESEARCH PAPER

AUTONOMIC MEASURES IN DIFFERENTIATING DEPRESSIVE DISORDERS: A POTENTIAL AID

Camilla Guccione, Keri Heilman, Stephen W. Porges, Simonetta Gentile, Vincenzo Caretti, Angelos Halaris

Abstract

Objective: The present study aimed at exploring the potential utility of autonomic regulation as a useful marker in the diagnostic differentiation between unipolar and bipolar depression.

Method: Respiratory sinus arrhythmia (RSA), low-frequency (LF) of heart rate variability, and systolic blood pressure (SBP) were assessed in patients with bipolar depression (31) and major depressive disorder (MDD=32), and in healthy controls (HCs=32). Since bipolar depressed subjects were maintained on specific medications to manage manic/hypomanic symptoms, we explored whether mood stabilizers (atypical antipsychotics and anticonvulsants or their combinations) could independently affect the physiological parameters.

Results: When the autonomic measures were analyzed by a multivariate analysis of variance (MANCOVA), after controlling for BMI, the combination of variables (RSA, LF, SBP) discriminated patients with bipolar depression and MDD from HC ($F_{(6, 178)}$=3.036, p=0.007, Λ=0.823, partial η^2=0.093). In any case, we cannot exclude that mood stabilizers might have affected SBP values in the bipolar group. To deconstruct this multivariate effect, pairwise ANOVAs and discriminant analyses contrasted groups and documented that RSA was the primary variable distinguishing the groups. Discriminant function analyses showed that RSA had a significant discriminating weight between bipolar depressed patients and HC subjects (p<0.0005). By contrast, RSA showed a trend towards the statistical significance in discriminating between bipolar depression and MDD patients (p=0.06).

Conclusions: The assessment of RSA and SBP in outpatient settings might be helpful in the differential diagnosis of affective disorders.

Key words: depressive disorders, polyvagal theory, heart rate variability, respiratory sinus arrhythmia, low frequency, cardiac vagal tone, systolic blood pressure

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Introduction

According to the polyvagal theory, both the high frequency (HF, also known as respiratory sinus arrhythmia, RSA) and the low frequency (LF) component of heart rate variability (HRV) might contribute to cardiac vagal tone (CVT) (Porges, 2007). HRV is operationalized as the fluctuation in the time intervals between adjacent heartbeats (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). Components of HRV are considered to represent non-invasive measures of autonomic nervous system (ANS) function enabling the detection of physiological and psychological stress responses (Kim et al., 2018). RSA is the HF component of HRV observed in the beat-to-beat heart rate time series as sequences of lengthening and shortening R-R intervals at the frequency of spontaneous breathing (Yasuma & Hayano, 2004). LF-HRV is defined by patterns of HRV occurring slower than RSA and reflecting the neural feedback through the vagus involved in blood pressure and vasomotor regulation (Porges, 2007). Chronic and prolonged stress has been defined as a maladaptive state frequently associated with sympathetic hyperactivation, which causes acute or chronic physical and psychological deficits (Kim et al., 2018).

Further clarifications that CVT reflects the influence of two vagal pathways on the heart, one myelinated and one unmyelinated are also supposed to possibly deepen our understanding of how RSA and HRV might reflect ANS dysregulation in different psychopathological disorders. The myelinated vagal pathway is the most phylogenetically evolved (Porges, 1995), originates in

the nucleus ambiguus (NA), actively inhibits sympathetic influences on the heart, and regulates hypothalamic-pituitary-adrenal axis (HPA) activity. This pathway, while interacting with the superior emotional circuits (Canli et al., 1998; Fox, 1991; Noesselt et al., 2005; Simon-Thomas et al., 2005), is strongly associated with emotion and social engagement behaviors (Porges, 2007). From a neurophysiological perspective, the myelinated vagal efferent pathways (B-fibers) lead to rapid, instantaneous changes in heart rate (HR) via nicotinic preganglionic receptors on the sinoatrial node, and they correspond to the RSA or HF domain (Cheng & Powley, 2000). The unmyelinated vagal pathway is evolutionally the most ancient, regulates metabolic demands, and originates in the dorsal motor nucleus. The unmyelinated vagal efferent pathways (C-fibers) produce slow and inhibitory changes in HR via muscarinic preganglionic receptors on the sinoatrial node. It has been proposed that the influence of these pathways on HRV is reflected in the LF domain (Porges, 2007).

Although there is no specific measure of the sympathetic nervous system (SNS) amongst the short-term HRV measurements (Shaffer & Ginsberg, 2017; Skyschally et al., 1996), however, it has been suggested that blood pressure can be a reliable parameter of sympathetic activity (Brown et al., 1994). Several studies addressed the relationship between blood pressure (BP) and some parameters of HRV (Mori et al., 2014; Hemingway et al., 2005; Singh et al., 1998). These findings highlighted an inverse association between BP and LF and RSA/HF (Mori et al., 2014; Hemingway et al., 2005; Singh et al., 1998), and a positive association between BP and sympathetic activity, determined by baroreceptor system function (Heusser et al., 2010; Bristow et al., 1969).

The close connection between physical and mental health underlines the robust heart-brain interactions that is clearly illustrated through the concept of *Psychocardiology* (Halaris, 2013; Halaris, 2018a; 2018b), and *Neurocardiology* (Porges & Kolacz, 2018). The high comorbidity between psychiatric disorders and cardiovascular diseases should require multidisciplinary collaboration in the study and effective management of these disorders. Patients diagnosed with Bipolar Disorders (BDs) show twice the risk of developing hypertension than the general population (McIntyre et al., 2010). Further generalized anxiety disorder (GAD) and major depressive disorder (MDD) are often positively associated with hypertension (Carroll et al., 2010).

Reduced RSA/HF has been proposed as a diagnostic differentiation marker between unipolar and bipolar depression (Hage et al., 2017; Moon et al., 2013; Chang et al., 2015), with patients with bipolar depression showing a lower LF than MDD patients and healthy control subjects (HCs) (Hage et al., 2017; Moon et al., 2013; Chang et al., 2015).

In this cross-sectional clinical design study, we investigated RSA, LF, and systolic blood pressure (SBP) in patients with MDD or bipolar depression and in HCs, to possibly assess their utility as objective markers in the diagnostic differentiation between these two conditions. We consider both RSA and LF as measures of vagal activity, as we recorded HRV during a resting condition and in a supine position, and BP as an index of sympathetic activity. We hypothesized that the three measures of autonomic regulation might be significantly different amongst the three groups of subjects, after controlling for confounding variables, such as the BMI and the drug regimens in bipolar depressed patients.

Materials and Methods

Participants

Ninety-five adult participants between 20 and 67 years of age (mean±SD: 43.5±12.5 were included in the study: 31 suffered from bipolar depression, 32 from MDD, and 32 were HCs. Demographic characteristics, namely, age, sex (40 men and 55 women), body mass index (BMI), and ethnicity were recorded for all participants (**table 1**).

Patients were diagnosed according to the Diagnostic and Statistical Manual for Mental Disorders, Text Revision Fourth (DSM-IV-TR, American Psychiatric Association, 2000) criteria. Prospective study patients were subjected to two preliminary screening visits to determine eligibility by assessing inclusion and exclusion criteria. Their depressive episode had to be of at least 1-month duration. A minimum score of 18 on the 17-item Hamilton Rating Scale for Depression (HAMD-17) (Miller et al., 1985) was required for study admission. Other Axis I diagnoses, active suicidality, uncontrolled hypertension, dyslipidemia or diabetes mellitus, history of smoking or substance abuse in the previous six months, and history of heart disease were exclusion criteria.

If the patients were receiving antidepressant medications, they had to undergo a 2-week washout (4-week washout for fluoxetine).

Only bipolar depressed patients with manic/hypomanic symptoms controlled by mood stabilizers (valproic acid, lamotrigine, topiramate) or second-generation antipsychotics (SGAs, quetiapine, aripiprazole), or a combination of the two, were included in the study. All these drugs show a low anticholinergic effect (Hage et al., 2017; Birkhofer et al., 2013) (**table 2**).

Lastly, to qualify as a HC subject, the candidate did not have psychiatric disorders or critical medical conditions and was not taking any medications.

Blood pressure measurement

Both the SBP and diastolic blood pressure (DBP) were measured in millimeters of mercury (mmHg) at the experimental study visit, before the ECG recording, by using *Welch Allyn Spot Vital Signs* device (Alpert, 2007).

Recording of HRV measurements

In this study, RSA/HF and LF components of HRV were measured at the experimental visit using the *SphygmoCor CPVH* system. The test was carried out between 08:00h and 11:00h in all subjects sitting in the same appropriate room to minimize environmental factors and diurnal fluctuations in ANS function. Participants were asked to recline comfortably on the examination table, a three-lead ECG was attached to their chest, and they had to rest for 15 min before the ECG recording was started. The ECG data were collected over a 10-min period.

HRV editing and analysis

Heart rate data were visually inspected and edited off-line with *CardioEdit* software (Brain-Body Center, 2007). Editing consisted of integer arithmetic (i.e., dividing intervals when detections were missed and

adding intervals when spuriously invalid detections occurred) or manual insertion/deletion of missing/spurious detections based on the ECG recording. RSA was calculated using *CardioBatch Plus* software (Brain-Body Center for Psychophysiology and Bioengineering, 2016) consistent with the procedures developed by Porges (Porges, 1985). *CardioBatch Plus* quantifies the amplitude of RSA using age-specific parameters that are sensitive to the maturational shifts in the frequency of spontaneous breathing. The method, when applied to adult participants, includes the following steps: timing sequential R-R intervals to the nearest millisecond; producing time-base data by resampling the sequential R-R intervals into 500 ms intervals; detrending the time-based series with a 21-point cubic moving polynomial (Porges & Bohrer, 1990) that is stepped through the data to create a smoothed template and the template is subtracted from the original time-based series to generate a detrended residual series; bandpass filtering the detrended time series to extract the variance in the heart period pattern associated with spontaneous breathing in adults (0.12–0.40Hz); and transforming the variance estimates with a natural logarithm to normalize the distribution of RSA estimates (Riniolo & Porges, 1997). These procedures are statistically equivalent to frequency domain methods (i.e., spectral analysis) for the calculation of the amplitude of RSA when heart period data are stationary (Porges & Byrne, 1992). Each cardiac variable was quantified during sequential 30-s epochs within each condition and averages of the within condition epochs were used in the data analyses. LF-HRV was quantified by applying different parameters to the steps used to quantify RSA. To calculate LF-HRV, the data were detrended with a 51-point cubic moving polynomial and bandpass filtered to accumulate the component of heart rate variation occurring within a frequency range of 0.04-0.10Hz. The data were then transformed with a natural logarithm.

Statistical analysis

All data were analyzed using the Statistical Package for Social Science (SPSS Version 20, IBM, 2010) and the level of significance for the analyses was set at $p<0.05$. One-way analyses of variance (ANOVA) were used to compare age, BMI, and HAMD-17 scores between the three groups (**table 1**). Subsequently, Tukey's HDS test was carried out to determine which specific group's means (compared with each other) were different. Chi-square analyses were used to compare sex and ethnicity between the three groups (**table 1**). A Pearson's correlation was run to assess the relationships among RSA, LF, SBP, and DBP within each group (**table 3**). An initial ANOVA was run to determine whether there were any statistically significant differences between the means of the three independent groups on the variables which measure autonomic regulation.

The statistically significant variables identified in the above analyses were used as combined dependent variables in the one-way multivariate analysis of covariance (MANCOVA) (**table 4**). The MANCOVA was run to determine whether the three groups of the independent variable differed statistically significantly based on the combined dependent variables after accounting for the covariate. The combined dependent variables were LF, RSA, and SBP, which measure autonomic regulation. The continuous covariate was BMI because it can strongly influence the HRV parameters. BMI was linearly related to the dependent variables, and its inclusion into the analysis can increase the ability to detect differences between groups of a categorical independent variable. Pairwise comparisons with a Bonferroni-adjusted p-value analysis were made for the three autonomic variables (**table 5**). Furthermore, Discriminant Function Analyses (DFA) were used to identify the underlying dimensionality of multivariate data and to identify the contribution of each autonomic variable (**table 6**, **7**).

Finally, during the clinical trial, the bipolar depression group was divided according to two criteria: if patients had received a pharmacological treatment with one (monotherapy: SGA or anticonvulsant), or with two drugs (combination therapy: SGA plus anticonvulsant). An independent-samples t-test was run to determine if there were differences in BMI, RSA, LF, and SBP parameters between these two groups of patients (**table 8**). ANOVA was used to compare the means of the three independent groups on BMI, RSA, LF, and SBP (**table 9**). The aim was to verify whether the type of medication used had a significant impact on those dependent variables.

Results

Participants: demographic and clinical characteristics

The three groups did not differ in sex ($\chi^2_{(2)}=0.474$, $p=0.789$), age ($F_{(2,92)}=2.003$, $p=0.141$), or ethnicity ($\chi^2_{(6)}=8.667$, $p=0.193$).

The BMI values were significantly different between the three groups ($F_{(2,92)}=8.229$, $p=0.001$). BMI was significantly higher in the bipolar depression group (30.5±5.54), compared with the HC group (26.5±5.14) [3.91, 95% CI (0.70 to 7.20), $p=0.013$], and in the MDD group (31.8±5.68) compared to the HC group (26.5±5.14) [5.29, 95% CI (2.06 to 8.50), $p=0.001$].

Depression scores were different between the three groups ($F_{(2,92)}=240.904$, $p<0.0005$), as they resulted to be significantly higher in the bipolar depression (22.98±5.19) than in the HC group (0.88±1.16) [22.09, 95%CI (19.26 to 24.92), $p<0.0005$], and in the MDD (23.70±6.21) than the HC group (0.88±1.16) [22.81, 95% CI (20 to 25.62), $p<0.0005$].

Within Group Correlation Analyses

Within each group the autonomic variables were similar and correlated. In the bipolar depression group, there was a significantly moderate positive correlation between RSA and LF ($r_{(29)}=0.493$, $p=0.005$), with RSA explaining 24% of the variation in LF. There was a significantly strong positive correlation between SBP and DBP ($r_{(29)}= 0.639$, $p<0.0005$), with SBP explaining 41% of the variation in DBP.

The MDD group showed a significantly moderate positive correlation between RSA and LF ($r_{(30)}=0.529$, $p=0.002$), with RSA explaining 28% of the variation in LF. There were statistically significant, moderate negative correlations between RSA and SBP ($r_{(30)}=-0.412$, $p=0.019$), with RSA explaining 17% of the variation in SBP. There was a statistically significant, strong positive correlation between SBP and DBP ($r_{(30)}= 0.705$, $p<0.0005$), with SBP explaining 50% of the variation in DBP.

In the HC group, there was a statistically significant moderate positive correlation between RSA and LF ($r_{(30)}=0.692$, $p<0.0005$), with RSA explaining 48% of

Table 1. *Demographic and clinical characteristics*

	Bipolar depression	MDD	HC	P/x3 value
Participants	31	32	32	N=95
Age	44±12.17	45±10.99	40±13.8	0.141
BMI	30.5±5.54	31.8±5.68	26.5±5.14	0.001*
Female	34.5%	30.9%	34.5%	0.789
Male	30%	37.5%	32.5%	0.789
Ethnicity				0.193
Caucasian	71%	65.6%	81.3%	
African-American	12.9%	18.8%	6.3%	
Hispanic	16.1%	12.5%	3.1%	
Asian	0.0%	3.1%	9.4%	
HAMD-17	22.97 ± 5.19	23.70± 6.21	0.88± 1.16	<0.0005*

MDD: Major Depressive Disorder; HC: Healthy Control; BMI: Body Mass Index; HAMD-17: Hamilton Depression Scale-17 items.

Table 2. *Pharmacological therapy in bipolar depression group at the experimental study visit*

Types of Drugs	N	%
Quetiapine	4	13.3
Aripiprazole	2	6.7
Topiramate	2	6.7
Valproic Acid	3	10
Lamotrigine	10	33.3
Quetiapine+Lamotrigine	2	6.7
Ziprasidone+Lamotrigine	1	3.3
Quetiapine+Oxcarzopene	1	3.3
Aripiprazole+Lamotrigine	2	6.7
Aripiprazole+Valproic Acid	1	3.3
Asenapine+Lamotrigine	1	3.3
Aripiprazole+Valproic Acid	1	3.3
Total	**30**	**100**

the variation in LF. There were statistically significant moderate negative correlations between RSA and SBP ($r_{(30)}$=-0.355, p=0.046) with RSA explaining 13% of the variation in SBP. There was a statistically significant, strong positive correlation between SBP and DBP ($r_{(30)}$=0.603, p<0.0005), with SBP explaining 37% of the variation in DBP.

Group Differences

The three groups were statistically significantly different in RSA ($F_{(2,92)}$=12.483, p<0.0005), LF ($F_{(2,92)}$=4.100, p=0.020), and SBP ($F_{(2,92)}$=3.565, p=0.032). The one-way MANCOVA showed that there was a statistically significant difference between the three groups after controlling for BMI, $F_{(6, 178)}$ = 3.036, p=0.007, Wilks' Λ = 0.823, partial η^2= 0.093. Follow up univariate one-way ANCOVAs were performed. Due to the number of statistical contrasts the significance level was adjusted by a Bonferroni correction to p <0.0167. There were statistically significant differences in the adjusted mean for RSA ($F_{(2, 91)}$=9.297, p= 0.000, partial η^2 = 0.170), but not for SBP ($F_{(2, 91)}$=1.766, p= 0.177, partial η^2= 0.037) or LF ($F_{(2, 91)}$=2.206, p=0.116, partial η^2=0.046). RSA was statistically significantly lower in the bipolar depression group (4.27±0.18) compared to the HC group (5.36±0.18), a mean difference of -1.10 (95% CI, -1.73 to -0.47), p<0.0005, and compared to

Table 3. *Correlations in bipolar depression, MDD, HC groups*

Bipolar depression group	LF	SBP	DBP
RSA	0.493**	-0.25	-0.26
LF		-0.005	0.06
SBP			0.639**
MDD group	LF	SBP	DBP
RSA	0.529**	-0.412*	-0.141
LF		-0.23	-0.091
SBP			0.705**
HC group	LF	SBP	DBP
RSA	0.692**	-0.355*	-0.296
LF		-0.301	-0.331
SBP			0.603**

** Correlation is significant at the 0.01 level (2-tailed); * Correlation is significant at the 0.05 level (2-tailed); MDD, Major Depressive Disorder; HC, Healthy Control; RSA, - Respiratory Sinus Arrhythmia; LF, Low-Frequency Heart Rate Variability, SBP, Systolic Blood Pressure.

MDD group (4.90 ± 0.18), a mean difference of -0.63 (95% CI, -1.24 to 0.02), p=0.039 (**table 4**).

Discriminant Function Analyses

Two DFA's were carried out to determine the combination and weights in which specific variables (RSA, LF, SBP) best discriminate between groups: bipolar depression and HC; bipolar depression and MDD.

The Bipolar Depression and HC groups showed equality of covariance (Box's M=5.573, p=0.509), and the test of equality of group means showed that all the independent variables had significant discriminating power in differentiating the two groups: RSA (Wilks' Lambda=0.750, p<0.0005), LF (Wilks' Lambda=0.885, p=0.007), and SBP (Wilks' Lambda= 0.888, p= 0.007).

Analysis of the DFA model showed that one significant function emerged (Function 1: $\chi^2_{(3)}$=18.659, p<0.0005) (**table 6**). The structure matrix showed that the correlation coefficients were highest for RSA (r=0.951), and lower for LF (r=0.594) and SBP (r=-0.586), highlighting that RSA is the variable that best discriminates the bipolar depression group from the HC group (**table 7**). Canonical discriminant function coefficients and their standardized equivalents suggest that RSA was more relevant to function 1, and that this measure can discriminate between the two groups better than LF and SBP (**table 7**).

The bipolar depression and MDD groups showed an equality of covariance (Box's M=7.535, p=0.309), and the test of equality of group means assessed that RSA (Wilks'Lambda=0.891, p=0.008) was the most

Table 4. *Means, adjusted means, standard deviations, and standard errors for the three autonomic regulation measures for each group*

Autonomic Regulation Measures	**SBP**		**RSA**		**LF**	
Groups	M (SD)	Madj (SE)	M (SD)	Madj (SE)	M (SD)	Madj (SE)
Bipolar depression	121 (9.9)	120 (1.82)	4.23 (0.9)	4.27 (0.18)	3.73 (0.7)	3.77 (0.17)
MDD	119 (12)	118 (1.83)	4.80 (0.75)	4.90 (0.18)	3.89 (0.9)	3.97 (0.17)
HC	114 (10)	115 (1.88)	5.49 (1.25)	5.36 (0.18)	4.39 (0.05)	4.28 (0.17)

MDD, Major Depressive Disorder; HC, Healthy Control; SBP, Systolic Blood Pressure; RSA, Respiratory Sinus Arrhythmia; LF, Low-Frequency Heart Rate Variability.

Table 5. *Pairwise contrasts for adjusted means for three autonomic measures for each group*

Difference in adjusted means (95% CI)			
	Bipolar depression vs HC	**Bipolar depression vs MDD**	**MDD vs HC**
SBP	4.95 (-1.52, 11.42)	2.70 (-3.53, 8.93)	2.25 (-4.38, 8.89)
RSA	-1.10 (-1.73, -0.47)*	-0.63 (-1.24, 0.02)*	-0.47 (-1.12, 0.18)
LF	-0.51 (-1.10, 0.08)	-0.20 (-0.77, 0.37)	-0.31 (-0.92, 0.30)

* Statistically significant difference (p<0.0167) based on Bonferroni Correction, 95% confidence interval (CI) is simultaneous confidence interval based on Bonferroni Correction; SBP measured in mmHg; RSA and LF measured in their natural Logarithm; MDD, Major Depressive Disorder; HC, Healthy Control.

important independent variable in the discriminant function. LF, and SBP were less important in the discriminant function (LF: Wilks'Lambda=0.992, p=0.483; SBP: Wilks'Lambda=0.990, p=0.444). Analysis of the DFA model permitted to detect that one function emerged (Function 1: $\chi^2_{(3)}$=7.338, p=0.06), albeit slightly below the statistical significance (**table 6**). The structure matrix showed that the correlation coefficients were highest for RSA (r=0.964), and lower for LF (r=-0.249) and SBP (r=-0.272), highlighting that RSA *is* the variable that could best discriminate the bipolar depression from the MDD group (**table 7**). Canonical discriminant function coefficients and their standardized equivalents suggest that RSA was more relevant to function 1, and that this measure could discriminate between the two groups better than LF and SBP (**table 7**).

Differences in the bipolar depression group based on drug treatment

The bipolar depressed patients were maintained on a mood stabilizer and/or antipsychotic to manage manic/hypomanic symptoms (**table 8**, **9**). One, out of the total 31 patients was eliminated from the analysis, as he was the only chlorpromazine, a first-generation antipsychotic (FGA) that is known to show stronger anticholinergic properties than SGAs (Muench & Hamer, 2010).

There was a statistically significant difference in SBP and DBP levels between patients on monotherapy versus patients on combination treatment, with blood pressure levels being higher in patients of the first (SBP: 124.33±8.99 mm/Hg; DBP: 81.19±6.53 mm/Hg) than in those of the second group (SBP: 113±7.33 mm/Hg; DBP: 74.78±6.32 mm/Hg) [SBP: $t_{(28)}$=3.329, p=0.002; DBP: $t_{(28)}$=2.487, p=0.019] (**table 8**).

Patients on anticonvulsants had higher SBP values than patients on SGAs or on drug combinations ($F_{(2,27)}$=7.053, p=0.003, ω^2=0.005) (**table 9**). The SBP increased in patients on anticonvulsants compared to patients on drug combinations [13.13, 95% CI (4.42 to 21.84), p=0.002] (**table 8**).

Discussion

The present study focused on RSA, LF, and SBP in patients with MDD or bipolar depression and in HC subjects to identify potential diagnostic biomarkers to distinguish between them.

Our results showed that bipolar depression, MDD, and HC subjects exhibited different autonomic profiles. Specifically, bipolar depressed subjects had a higher SBP than HC and MDD subjects, with the latter showing a higher SBP than HC subjects. The bipolar depressed patients also had a lower RSA and LF than both HC and MDD subjects, while these had lower RSA and LF than HC subjects. These data suggest that vagal activation is reduced, and sympathetic activation is increased in bipolar depression, as compared with both HCs and MDD. Further, MDD subjects resulted more similar to HCs, although they also tended to have less vagal activation and more sympathetic activation than HCs. However, RSA showed a significant discriminating weight between bipolar depressed patients and HC subjects. By contrast, RSA was close to statistical significance in discriminating between bipolar depressed and MDD patients (p=0.06). This finding is potentially important from the clinical point of view, as it suggests that there might be a continuum of autonomic dysregulation in affective disorders with depression representing a continuum between the two disorders. Since the p-value approached statistical significance, we encourage future studies to subtyping

Table 6. *Discriminant Function Analysis results*

	Discriminant Function	Percent Variance	Canonical Correlation	Significance of Discriminant	
				χ^2	p
Bipolar depression, HC	1	37	0.519	18.659	<0.0005
Bipolar depression, MDD	1	13	0.341	7.338	0.06

MDD: Major Depressive Disorder; HC: Healthy Control.

Table 7. *Canonical Discriminant Function coefficients and structure matrix*

	Standardized Coefficients		Unstandardized Coefficients		Structure Matrix	
	Bipolar depression, HC	Bipolar depression, MDD	Bipolar depression, HC	Bipolar depression, MDD	Bipolar depression, HC	Bipolar depression, MDD
	Function 1	Function 1	Function 1	Function 1	Function 1	Function 1
RSA	0.846	1.131	0.765	1.345	0.951	0.964
LF	0.009	-0.304	0.010	-0.347	0.594	0.249
SBP	-0.325	0.050	-0.304	0.005	-0.586	-0.272
Costant			0.173	-5.322		

RSA, Respiratory Sinus Arrhythmia; LF, Low-Frequency Heart Rate Variability; SBP, Systolic Blood Pressure; MDD: Major Depressive Disorder; HC: Healthy Control; Structure Matrix: pooled within-groups correlations between discriminating variables and standardized canonical discriminant functions.

Table 8. *Differences in the bipolar depression group based on monotherapy vs. combination therapy*

	Group	n	Mean	SD	t	df	Sig. (2-tailed)
RSA	Mono-Therapy	21	4.204	1.011	0.001	28	0.999
	Combination	9	4.203	0.773			
LF	Mono-Therapy	21	3.833	0.808	1.430	28	0.164
	Combination	9	3.422	0.438			
SBP	Mono-Therapy	21	124.33	8.985	3.329	28	0.002*
	Combination	9	113.00	7.331			
DBP	Mono-Therapy	21	81.19	6.532	2.487	28	0.019*
	Combination	9	74.78	6.320			
BMI	Mono-Therapy	21	30.76	5.612	0.493	28	0.626
	Combination	9	29.67	5.477			

*Statistically significant difference (p<0.05); CVT, Cardiac Vagal Tone, RSA, Respiratory Sinus Arrhythmia; LF, Low-Frequency Heart Rate Variability; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; BMI, Body Mass Index.

Table 9. *Differences in the BDD group based on treatment with atypical antipsychotics, anticonvulsants, or combination*

	Groups	n	Mean	SD	F	Sig.	Welch
RSA	Atypicals	6	4.0323	1.105	0.134	0.875	0.904
	Anticonvulsants	15	4.2729	1.003			
	Combination	9	4.2038	0.773			
LF	Atypicals	6	3.6617	0.574	1.235	0.307	0.247
	Anticonvulsants	15	3.9021	0.893			
	Combination	9	3.4220	0.438			
SBP	Atypicals	6	119.83	11.754	7.053	0.003*	0.005*
	Anticonvulsants	15	126.13	7.337			
	Combination	9	113.00	7.331			
DBP	Atypicals	6	80.67	11.483	3.015	0.066	0.060
	Anticonvulsants	15	81.40	3.699			
	Combination	9	74.78	6.320			
BMI	Atypicals	6	32.83	3.764	0.706	0.503	0.341
	Anticonvulsants	15	29.93	6.112			
	Combination	9	29.67	5.477			

*Statistically significant difference (p<0.05); Welch (ω^2): Robust Tests of Equality of Means; CVT, Cardiac Vagal Tone, RSA, Respiratory Sinus Arrhythmia; LF, Low-Frequency Heart Rate Variability; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; BMI, Body Mass Index.

bipolar depression and MDD on the basis of depression severity and duration, and to examine the potential discriminative weight of autonomic variables on these clinical parameters to confirm or not differences these two conditions.

Since our bipolar depressed patients were maintained on mood stabilizers and/or antipsychotics to manage manic/hypomanic symptoms, we investigated whether these drugs or their combinations could independently affect the physiological parameters. Our findings showed that patients on monotherapy showed higher sympathetic activation than those on combination treatment (>SBP and >DBP). Specifically, patients taking anticonvulsants had higher sympathetic activation than both patients on SGAs and patients on combination treatment (>SBP). Anticonvulsants may affect BP (Katsiki et al. 2014), and consequently, they may have an impact on the sympathetic nervous system (SNN).

The SGAs we prescribed show no significant anticholinergic activity (Hage et al., 2017; Birkhofer et al., 2013). Amongst anticonvulsants, valproic acid and lamotrigine were the most widely used in our study, and they do not possess a high anticholinergic activity; and, as such, they do not block significantly parasympathetic efferents (Moon et al., 2013). This equivalent ratio between the two classes of medications suggests that anticonvulsants may be effective in activating the sympathetic system, exemplified by SBP and DBP augmentation, compared to SGAs alone. Furthermore, according to previous research (Alvares et al., 2016;

Sajadieh et al., 2004), the fact that monotherapy had a greater impact on ANS functioning than combination treatment would suggest that psychotropic medications are a contributory factor and not a cause of autonomic deficits in bipolar depressed patients.

In summary, when the autonomic regulation measures were analyzed as combined variables, they discriminated bipolar depression and MDD from HC, although mood stabilizers might have affected the SBP values of the bipolar depression group.

When the autonomic variables were analyzed individually, the RSA seems to differentiate among the three groups, in agreement with previous studies (Hage et al., 2017; Moon et al., 2013; Chang et al., 2015). Although the DFA's did not confirm that RSA could statistically differentiate between bipolar depressed and MDD patients, given the observed trend and our acknowledgement of within diagnostic category variability, we consider this observation as potentially important, although this finding requires to be deepened in future studies. As our metric of ventral vagal regulation is robust, future research should expand the metrics for dorsal vagal and sympathetic regulation. While dorsal vagal tone is observed in clinical crises, such as vasovagal syncope and clinical bradycardia, currently, there is no accepted validated index of tonic dorsal vagal cardioinhibitory tone. Our inference of dorsal vagal tone is based on the analytic contrasts extracting the unique sources of variance related the two vagal metrics (RSA and LF) to diagnostic category.

Lastly, we would like to highlight the regulatory influence the ANS exerts on the immune system. For example, it has been shown that inflammation can increase symptomatology in affective disorders, and its modulation can reverse resistance to drug treatment (Halaris et al., 2020).

A number of studies have shown a relationship between vagus nerve regulation and the reduction of the inflammatory response via the Cholinergic Anti-Inflammatory Pathway (CAIP) (Pavlov & Tracey, 2012; Cooper et al., 2015; Woody et al., 2017). A key regulatory mechanism is the CAIP that utilizes nicotinic acetylcholine receptors (nAChRs), specifically the α7 nAChR subtype. A decrease in the vagal tone has been demonstrated to lead to disinhibition of the inflammatory response as has been demonstrated in several studies. Central muscarinic cholinergic regulation of systemic inflammatory response and its reflection in HRV measures have been convincingly demonstrated (Pavlov et al., 2006). The HRV is inversely related to C-reactive protein levels and white blood cell counts in middle-aged and elderly subjects with no apparent heart disease, while suggesting that an autonomic imbalance might interact with an inflammatory process leading to atherosclerosis (Sajadieh et al., 2004). Previously, we had measured inflammation biomarkers in some of our studies and confirmed the presence of a pro-inflammatory status in the majority of our subjects suffering from bipolar depression and MDD (Halaris et al., 2015; Halaris et al., 2020), while confirming the report of Chamberlain et al. (2019).

Limitations

The first limitation of this study is the small sample size. However, it reveals that the measurements of autonomic regulation we investigated are closely related to each other.

The second limitation is the absence of data on the severity and duration of depression that might both influence autonomic dysregulation severity.

The last limitation is that bipolar depressed patients had to be maintained on psychotropic medications to control manic/hypomanic states, as we aimed to study bipolar and unipolar depression, excluding mood hyperactivation phases. Although some psychotropic drugs can affect autonomic regulation, most of our patients were taking medications that do not block significantly parasympathetic efferents (Chang et al., 2015). Therefore, the reduction in parasympathetic activity in our bipolar depressed patients could not be attributed to the medication they were receiving.

A final limitation of the study is the lack of evaluation of confounding factors, such as occasional use of tobacco or alcohol, that can influence autonomic regulation. Future studies on HRV should also take into consideration these variables.

Conclusion

Our preliminary results suggest that patients with bipolar depression might show a greater sympathetic tone reflected in higher blood pressure and lower ventral vagal tone than patients with MDD or HCs. Hence, we considered the autonomic measures used as indices for assessing parasympathetic-sympathetic coordination. Alteration of these autonomic measures has been found both in stress and several psychiatric disorders, including affective disorders (e.g., Moon et al., 2013; Quintana et al., 2015; Hage et al., 2017; Chang et al., 2015). It is generally well known that there is an increased psychophysiological stress in psychiatric disorders that could negatively affect the outcome and prognosis (Kim et al., 2018; Beauchaine, 2015; Beauchaine & Thayer, 2015; Chambers & Allen, 2007).

In conclusion, physiological parameters (e.g., RSA and SBP) can be easily assessed in outpatient settings, thus facilitating the differential diagnosis of affective disorders. In addition to other clinical tools, such as pharmacogenomic testing, history, and questionnaires, HRV analyses and BP measurement might add relevant physiological parameters to reach the final diagnosis.

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