Influence of Traumatic Brain Injury by Fluid Percussion on Heart Rate Variability in the Acute Phase of Damage in Rats

Raphael S. Nascimento, Fernando S. Fiorin, Caroline C. E. Santo, Luiz F. F. Royes, and Jefferson L. B. Marques

***Abstract*—Traumatic brain injury (TBI) is a condition that changes the autonomic system, modulating the heart rate variability (HRV) at all levels of brain lesions. Although fluid percussion injury (FPI) model can reproduce all degrees of severity of clinical TBI, there is still a lack of comprehensive analysis of linear and non-linear HRV metrics following FPI. The present study sought to assess the influence of the FPI model on time-domain (HR, mean NN, SD1, SD2, SDNN, RMSSD, and SD1/SD2) and frequency-domain (LF, HF, and LF/HF). A non-invasive electrocardiogram recording was used in anesthetized and awake male Wistar rats, both before and for three days after moderate FPI. Although a decrease in the SD2 occurred in the anesthetized state, an increase in HFnu led to a reduction in HR during baseline evaluations. Post-TBI analyses revealed that neither the sham nor the TBI groups exhibited HR alterations under the influence of isoflurane; however, both groups showed a decrease in parasympathetic activity (RMSSD, SD1, and HFnu). Under isoflurane anesthesia, only the TBI group exhibited changes in LFnu, HFnu, and LF/HF metrics for three days. In contrast, awake animals experienced an increase in HR for three days post-injury, with a critical period at 24 hours when SD2, LFnu, HFnu, and LF/HF were altered. With few exceptions, the sham group did not exhibit significant differences in the awake state. Therefore, the effects of isoflurane predominate over TBI effects in both time- and frequency-domain metrics, while FPI in awake animals indicates a critical period of altered specific metrics at 24 hours post-injury.**

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Index Terms*—Fluid percussion injury; Heart rate variability; Autonomic nervous system; Electrocardiogram; Isoflurane *anesthesia.

The associate editor coordinating the review of this manuscript and approving it for publication was Guadalupe Dorantes-Mendez (*Corresponding author: Raphael Santos do Nascimento*).

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I. Introduction

TRAUMATIC brain injury (TBI) consists of any traumatic aggression that causes anatomical injuries or functional impairment in the brain [1]. It is well known that TBI acts primarily on the physiological system by disturbing the central regulation associated with the hypothalamus-pituitary axis [2]. This central autonomic system regulation is comprised of a complex network in the central nervous system linked to the cerebral cortex areas, stria terminalis, brainstem centers, amygdala, and hypothalamus [3]. In this sense, numerous processes involving cardiovascular complications in the acute and long-term post-TBI period, especially cardiovascular autonomic function impairment or dysautonomia, have been described [1].

Cardiovascular autonomic dysfunction is associated with altered variability in the sympathetic and parasympathetic autonomic nervous system (ANS). In this sense, heart rate variability (HRV) has been suggested as a form of evaluating ANS conditions, and it has become instrumental in predicting all degrees of TBI injuries [4]. The HRV evaluation is a non-invasive electrocardiographic marker that reflects the activity of ANS structures (i.e., sympathetic and parasympathetic) on the sinus node of the heart, which are fluctuations between the intervals of consecutive heartbeats [5].

In moderate and severe TBI patients, evidence has revealed reduced HRV, reflected by sympathetic-vagal balance (sympathetic predominance) decreases of HR signals. These have been associated with increased mortality rates in these patients [6], [7].

Although the fluid percussion injury (FPI) model is one of the most used TBI animal models, to date no investigation about the effects of FPI on HRV parameters has been observed in detail. FPI can reproduce intracranial hemorrhage, cerebral edema, and progressive gray matter damage, which are key pathophysiological hallmarks of human TBI [8].

Moreover, in experimental models, the anesthetized methods of recording the electrocardiogram (ECG) impair a better translational approach to what occurs in TBI patients [9]. In this sense, the use of anesthetic solutions for ECG recording affects the cardiovascular system [10], making it difficult to develop therapeutic approaches. Despite isoflurane being standard anesthetic widely used in both clinical practice and experimental models, different anesthetics for cardiac

evaluation are still controversial as they influence the ANS [10], [11].

Thus, this study sought to investigate the principal metrics of the HRV following moderate FPI using a non-invasive method in comparison with isoflurane anesthesia in rats; the assessment was carried out pre- and post-TBI.

II. Experimental Procedure

A. Animals

Ten 180-day-old adult male Wistar rats weighing 420–500 g were used in this study. All animals were kept under a controlled light environment (12 h light/dark cycle, constant temperature of 23±2 °C, 55% relative humidity), with ad libitum access to water and food. The animals were housed in a ventilated rack (27 x 36 x 31 cm), which was covered by shavings. All animals used complied with the Committee in Care and Use of Experimental Animal Resources of the Federal University of Santa Catarina (protocol no. 27120803-17). All procedures with animals followed ARRIVE guidelines as well as the principles of the 3Rs, minimizing the number of animals used consistent with scientific aims.

B. Experimental Design for Signal Acquisition

For ECG recordings, a preliminary training period was required to conform to the study previously, with some modifications [12]. All animals were habituated every other day, for 30 days (60 min/day for each animal), in a suitable glass box with an open top (30 x 20 x 23 cm), jointly with a custom-made jacket. After the adaptation period, the animals were subjected to the ECG baseline recording. Firstly, in the morning (9 a.m.-12 p.m.), the animals were individually anesthetized with isoflurane (2.5% mixed with 100% O2) and subjected to the recording. After the assessments, the animals were returned to their home cage for a minimum resting period of 3 h. The animals were submitted to the evaluations in the afternoon (3 p.m.-6 p.m.) in the awake state. After that, the animals were randomly assigned to two groups: sham, and moderate FPI. Then, 24, 48, and 72 h after TBI, ECG recordings identical to baseline were performed on the anesthetized (morning period) and awake (afternoon period) rats (Fig. 1A). Although ECG recordings were performed at two different times, in healthy subjects HF and the LF/HF ratio exhibit a circadian pattern in the morning and afternoon [13]. This timing interval was important to minimize potential anesthesia effects in awake animals. Immediately after all ECG recordings were finished, the animals were euthanized by decapitation following isoflurane anesthesia according to the experimental protocol.

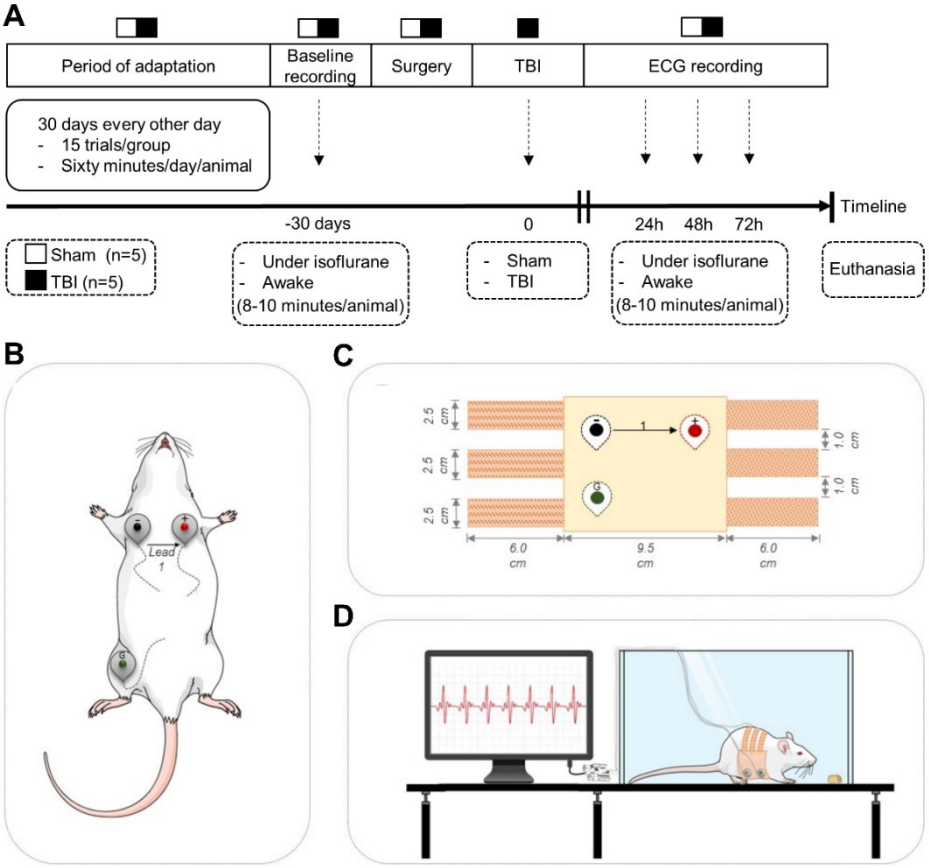

Fig. 1. (A) Timeline of the experimental protocol for electrocardiogram measurements after moderate severity TBI. During a period of 30 days, the animals were adapted to wear jackets with electrocardiographic recording wires. After, baseline recordings were performed in animals under anesthesia and awake. After surgery and FPI procedures, recordings were performed for three consecutive days in the same conditions for both sham and TBI groups, and the animals were euthanized. General functional schematic of ECG recordings in rats. (B) Lead positioning on the rat's body, (C) custom-made signal acquisition jacket, and (D) ECG recording general setup.

C. Electrocardiogram Recording

The ECG samples were carried out through three surface electrodes measured in Lead I (Fig. 1B) according to Nascimento et al. [15]. The animals were carefully shaved in the electrode insertion regions. To obtain the best fixation constant of the electrodes on the animal skin in the awake state, a custom-made jacket was also developed for the animals' average thoracic circumference; it was produced from brim fabric with elastane (97% cotton, 3% elastane) and three fabric velcro tapes (Fig. 1C). This was connected to an ECG amplifier using a hardware module developed in our laboratory, which exported data as a text file of electrocardiogram amplitude data points. The ECG signals were digitized using a 12-bit analog-to-digital converter (500 Hz sampling frequency), resampled at 500 Hz into the corresponding time series, and digitally filtered (0.1–40 Hz bandpass). Afterward, 8–10 min samples were recorded for all animals. These signals were posteriorly visually inspected, and we selected a 5-min sample for each one for analysis, devoid of the maximum movement artifacts, arrhythmic events, or ectopic beats [16], [18]. All HRV analysis were performed equally using a 5-min ECG segment obtained from the rats for both the sham (baseline) and TBI groups (24, 48, and 72h).

D. TBI Model

Briefly, the animals were anesthetized with ketamine (100 mg/Kg) and xylazine (10 mg/Kg) in a single intraperitoneal injection and placed in a rodent stereotaxic apparatus [14]. A 3-mm wide unilateral burr hole was drilled into the right convexity over the parietal cortex (2 mm posterior to bregma and 3 mm lateral to the midline); close attention was paid to maintain an intact dura mater. A plastic injury cannula was cemented into the craniectomy with dental cement. After 24 h, the animals were anesthetized with isoflurane (1% inhaled), and the injury cannula was attached to the fluid percussion device. A brief transient pressure fluid pulse impact was applied to the exposed dura conform described in [14]. A pressure pulse of 2.0 ± 0.2 atm was recorded, with an overall mortality of the animals of 16.67% indicating a moderate TBI. The sham group was submitted to the identical procedure except for the FPI. The cannula with dental cement was removed, and a surgical clamp was utilized at the location of the incision.

E. Heart Rate Variability

The HRV parameters in the rat model were analyzed using the HRVtool app (HRVtool, Greifswald, MV, Germany, version 1.03), which is a custom-made software using Matlab (MathWorks Inc., Natick, MA, U.S.A., version 8.5). For the extraction of parameters in the frequency domain, the RR intervals were interpolated by a cubic spline to generate an equidistant time series, using Hamming window and polynomial fitting to remove the trend.

Data analyses of linear HRV indices in the time and frequency domains and non-linear indices were conducted according to the recommendations of the task force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology [16]. For the analysis in the time domain, components derived from the value of NN intervals (or RR intervals) were calculated, including mean NN intervals (mean of all NN intervals), mean HR (mean heart rate), RMSSD (root mean square of successive NN interval differences), SDNN (standard deviation of all NN intervals), SD1 (standard deviation of instantaneous [short term] beat-to-beat interval variability), the SD2 (standard deviation of the long term NN intervals) and SD1/D2 (ratio between the short and long term of the NN intervals. The RMSSD reflects changes in parasympathetic activity in short-term recordings; the SDNN reflects information about the component degrees that contribute to the HRV in the period recording. The SDNN can only be compared in similar duration measurements for shorter recording evaluations because it can increase in longer measurements. The SD1 and SD2 represent non-linear parameters that reflect the parasympathetic and global vagal balance activities. The ratio between SD1/SD2 is indicated as sympathetic activity, although it is also associated with the global vagal balance [17].

For the analysis of the domain frequency, HRV measures using the fast Fourier transformation (FFT) algorithm of the following components in normalized units were derived: low-frequency power in normalized units (LFnu, 0.1–1 Hz) and high-frequency power in normalized units (HFnu, 1–3.5 Hz) [18]. Despite the controversies regarding LF influence, the LF and HF components reflect sympathetic and parasympathetic activities, respectively. The ratio between LF and HF (LF/HF) components reflects the global sympathetic-vagal balance [16], [18].

F. Statistical Analysis

The normality of the data was evaluated using D'Agostino & Pearson. Data were expressed as the mean ± standard error of the mean (SEM). Data analysis was conducted using repeated measures two-way analyses of variance (ANOVA) to verify the effects of TBI and Student's paired t-tests to evaluate the effects of isoflurane anesthesia. Sidak's multiple tests comparing the groups were applied to post hoc analysis. $p < 0.05$ was deemed statistically significant. All statistical analyses were performed using the GraphPad Prism statistical software (GraphPad Software, Inc., San Diego, CA, U.S.A., version 8.0). A post hoc power analysis (G*Power 3.1) indicated that, given the observed effect size (Cohen's $d = 0.91$), $\alpha = .05$, and total $n = 10$ (5 per group), the achieved statistical power was approximately 0.36.

III. Results

A. Anesthetic Effects on the HRV of Naive Rats

In order to identify the wave and rhythm profiles, we evaluated the ECG tracings (2-s segment) on the same animal anesthetized and awake state (Fig. 2A). The representative

image revealed an increase in signal amplitude (± 0.005 V) and a marked loss of rhythmicity during the anesthesia (Fig. 2A). Spectral power estimation (5-min time series) at baseline under anesthetized and awake conditions confirmed the frequency domain changes. Although the spectrum is predominantly sympathetic activity (LF), it was possible to observe high parasympathetic activity (HF) induced by the anesthetic (Fig. 2B) in comparison with awake animal (Fig. 2C).

Statistical analyses revealed that HRV parameters were significantly different among animals anesthetized and awake and variating on all-time series indexes. In the time domain, HR ($p < 0.01$), mean NN ($p < 0.01$), SD2 ($p < 0.05$), and SD1/SD2 ($p < 0.05$) parameters were statistically different between the anesthetized and awake states. The SD2 and SD1/SD2 indicated a decrease in global sympathetic-vagal balance in the anesthetized state, while HR and mean NN suggest a decrease in overall HRV. When evaluated in the frequency domain, all data showed a significant difference (LFnu [$p < 0.01$], HFnu [$p < 0.01$], and LF/HF [$p < 0.05$]) between the awake and anesthetized states. In contrast, SDNN, RMSSD, and SD1 did not statistically differ between the animal states. Although SDNN was not statistically different, its metric was reduced. It is important to note that the HRV indices obtained a more significant SEM between most of the measured parameters under the animals anesthetized except for the frequency domain metrics (LFnu, HFnu, and LF/HF) (Table I).

B. TBI Impact in the HRV on Rats in the Anesthetized and Awake States

The results obtained on the administration of anesthesia post-TBI did not reveal any differences under the parameter HR ($F[3,12] = 1.246$; $p = 0.3364$; Fig. 3A). However, the findings on the awake rats revealed a change of HRV global activity 24, 48, and 72 h after TBI, as evidenced by HR ($F[3,12] = 6.119$; $p = 0.0091$; Fig. 3D). Post hoc analysis demonstrated higher HR ($p < 0.05$) in the awake state compared to the baseline. The sham group was only statistically significant in the HR below 48 h compared to the baseline in the awake condition ($p = 0.045$).

The SDNN ($F[3,12] = 11.80$; $p = 0.0007$; Fig. 3B), as well as RMSSD ($F[3,12] = 12.93$; $p = 0.0005$; Fig. 3C) analysis was statistically significant, both in the anesthetized animals.

Nonetheless, statistical tests among the periods triggered differences in the two parameters after 24, 48, and 72 h in the TBI group ($p < 0.01$; Fig. 3B; 3C), whereas in the RMSSD index there was also statistical difference in 24 and 48 h in the sham group ($p < 0.05$; Fig. 2C).

In the awake rats, we did not find significant differences in SDNN ($F[3,12] = 2.210$; $p = 0.1397$; Fig. 3E) in the TBI group; although we noted a drop in the first 24 h post-injury. The RMSSD differed statistically ($F[3,12] = 8.399$; $p = 0.0028$; Fig. 3F) in the awake condition. Nevertheless, post hoc analysis did not show differences in both the parameters SDNN and RMSSD recorded after TBI ($p > 0.05$; Fig. 3E; 3F). The sham group only indicated differences in RMSSD in 72 h in the awake state ($p = 0.035$; Fig. 3F).

TABLE I
COMPARISON OF HRV PARAMETERS

HRV measurements n = 10	Baseline		Mean diff/CI 95%	*p*-value
	Anesthetized	Awake		
Time-domain parameters:				
HR (BPM)	309(15.05)	427.75(10.05)	118(88.74, 148.8)	**
Mean NN (ms)	197.88(10.55)	140.88(3.42)	-57(-77.65, -36.35)	**
SDNN (ms)	7.49(1.71)	11.01(1.53)	3.53(-1.76, 8.81)	ns
RMSSD (ms)	9.58(2.05)	9.85(1.49)	0.27(-6.56, 7.10)	ns
SD1 (ms)	6.76(1.44)	6.96(1.06)	0.2(-4.64, 5.04)	ns
SD2 (ms)	6.84(1.22)	11.25(1.13)	4.41(1.27, 7.55)	*
SD1/SD2	0.98(0.12)	0.62(0.07)	-0.36(0.70, -0.02)	*
Frequency-domain parameters:				
LFnu	28.89(2.12)	36.73(2.18)	7.84(2.58, 13.11)	**
HFnu	71.11(2.12)	63.27(2.18)	-7.84(-13.11, -2.58)	**
LF/HF	0.41(0.04)	0.59(0.06)	0.18(0.04, 0.32)	*

The data are given as mean ± SEM unless otherwise indicated. Mean/diff, mean paired difference; CI, confidence interval with 95%; HR, mean heart rate; mean NN, mean of all NN intervals; SDNN, standard derivation of all NN intervals; RMSSD, root mean square of successive NN interval differences; SD1, standard deviation of instantaneous (short term) beat-to-beat interval variability; SD2, standard deviation of the long term NN intervals; SD1/SD2, the ratio between short- and long-term NN intervals; LFnu, low-frequency power in normalized units; HFnu, high-frequency power in normalized units; LF/HF, ratio absolute between LF and HF; BPM, beats per minute; ms, milliseconds. Data analysis was conducted using the Student's paired t-tests. *$p < 0.05$, and **$p < 0.01$ were considered statistically significant; ns, not significant ($p > 0.05$).

Statistical analysis of non-linear HRV indices in the time domain indicated that there were differences in all metrics SD1 ($F[3,12] = 12.93$; $p = 0.0005$; Fig. 4A), SD2 ($F[3,12] = 5.361$; $p = 0.0142$; Fig. 4B), and SD1/SD2 ($F[3,12] = 28.59$; $p < 0.0001$.; Fig. 4C) in anesthetized rats, respectively. Additionally, the analysis among the periods recorded indicated a decrease in SD1 and SD1/SD2 after 24, 48, and 72 h ($p < 0.0000001$; Fig. 4A; 4C) of TBI, as well as in 72 h by SD2 ($p = 0.031$; Fig. 4B) compared to the baseline. We also observed that the sham group was significant in SD1 in 24 h ($p = 0.020$; Fig. 4A) and 48 h ($p = 0.027$; Fig. 4A), as well as in SD1/SD2 in the first 24 h ($p = 0.013$; Fig. 4C) and 48 h ($p = 0.012$; Fig. 4C) post-TBI compared with baseline.

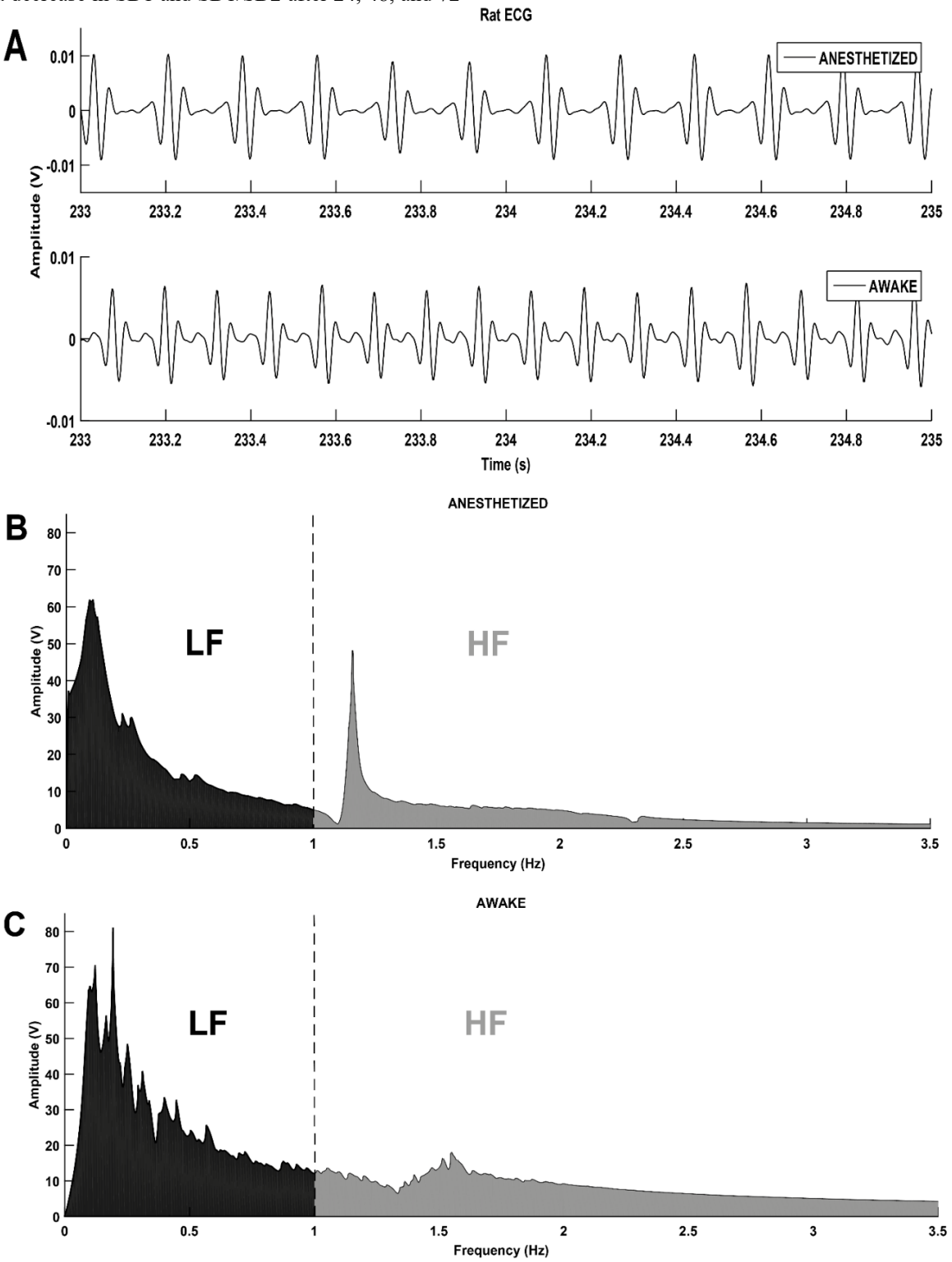

Fig. 2. Representation of the ECG signal in the time and frequency domain on the same rat baseline. (A) A segment of 2 s in the time domain in the anesthetized and awake states. (B-C) Power spectral estimation using the FFT algorithm with LF and HF bands. (B) Anesthetized and (C) awake states are represented.

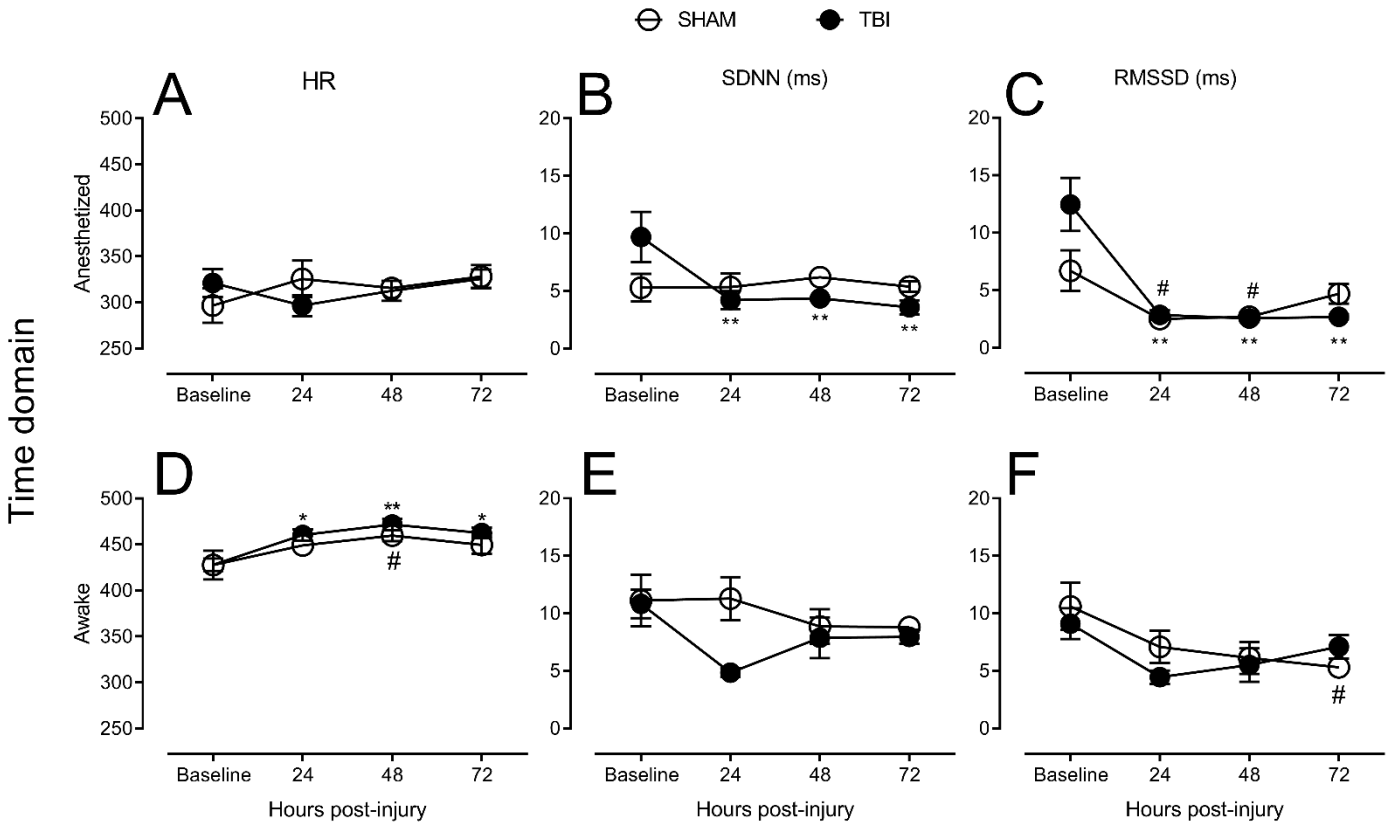

Fig. 3. The effects of surgical procedure and TBI on the HRV linear parameters in the time domain on the animals in the anesthetized and awake states. (A-D) HR, heart rate, (B-E) SDNN, standard derivation of all NN intervals, and (C-F) RMSSD, root mean square of successive NN interval differences. Data analyses were performed using two-way ANOVA. Sidak's multiple tests comparing the groups were applied when appropriate. Data are presented as mean ± SEM (n = 5 per group). *p < 0.05 compared to the baseline (TBI group), **p < 0.01 compared to the baseline (TBI group), and #p < 0.05 compared to the baseline (SHAM group).

SD1 (F[1,4] = 0.1759; p = 0.6965; Fig. 4D) and SD1/SD2 (F[1,4] = 0.0891; p = 0.7802; Fig. 4F) did not differ statistically in the awake animals. On the other hand, there were differences in SD2 (F[3,12] = 4.238; p = 0.0293; Fig. 4E), implicating changes of HRV in vagal global activity on the awake animals post-TBI. In the awake animals, the comparison between the periods recorded was only significant in SD2 (24 h, p = 0.046; Fig. 4E) after TBI compared to the baseline.

Data analysis of linear HRV indices in the frequency domain revealed that there were differences in all measured parameters LFnu, Hfnu, and LF/HF (F[3,12] = 18.83; p < 0.0001; Fig. 5A), (F[3,12] = 18.80; p < 0.0001; Fig. 5B), and (F[3,12] = 17.18; p = 0.0001; Fig. 5C), respectively, in the anesthetized rats. In the same way, post hoc analysis was significant in LF and HF after 24 (p = 0.009 to both; Fig. 5A; 4B), 48 (p = 0.003 to both; 5A; 5B), and 72 h (p = 0.02 to both; 5A; 5B) of injury in the anesthetized state, whereas the LF/HF ratio was triggered in 24 (p = 0.026; Fig. 5C), 48 (p = 0.006; Fig. 5C), and 72 (p = 0.039; Fig. 5C) h post-TBI. In addition, the sham group was altered differently in the LFnu component in 24 h (p = 0.049; Fig. 5A) under anesthesia.

Regarding the awake state, our data demonstrated changes in all components, LFnu, Hfnu, and LF/HF (F[3,12] = 7.096; p = 0.0053; Fig. 5D), (F[3,12] = 6.978; p = 0.0057; Fig. 5E), and (F[3,12] = 8.123; p = 0.0032; Fig. 5F), respectively. Analysis among the periods demonstrated higher sympathetic activity (LFnu, p = 0.004; Fig. 5D) and global sympathetic-vagal balance (LF/HF, p = 0.001; Fig. 5F) and lower parasympathetic activity (HFnu, p = 0.006; Fig. 5E) in the first 24 h compared to the baseline of the animals in the awake condition. It is important to evidence that all parameters returned to the baseline values 48 and 72 h after TBI (p > 0.05). The results found in the sham group did not differ in any parameter in the awake rats and all recorded periods (p > 0.05).

IV. Discussion

In the present study, experimental data confirmed the presence of cardiovascular autonomic dysfunction following the FPI model in awake animals. Furthermore, the use of isoflurane to evaluate the HRV parameters in experimental designs influences ANS evaluation. Importantly, to the best of our knowledge, this study is a pioneer in evaluating linear and non-linear HRV parameters in the FPI model in awake rats.

The time-domain analysis of HRV has contributed to a better understanding of ANS [20], [21]. Although the frequency domain analysis may also assess HRV global activity, it enables ANS parameters (sympathetic and parasympathetic) to be separately evaluated [21].

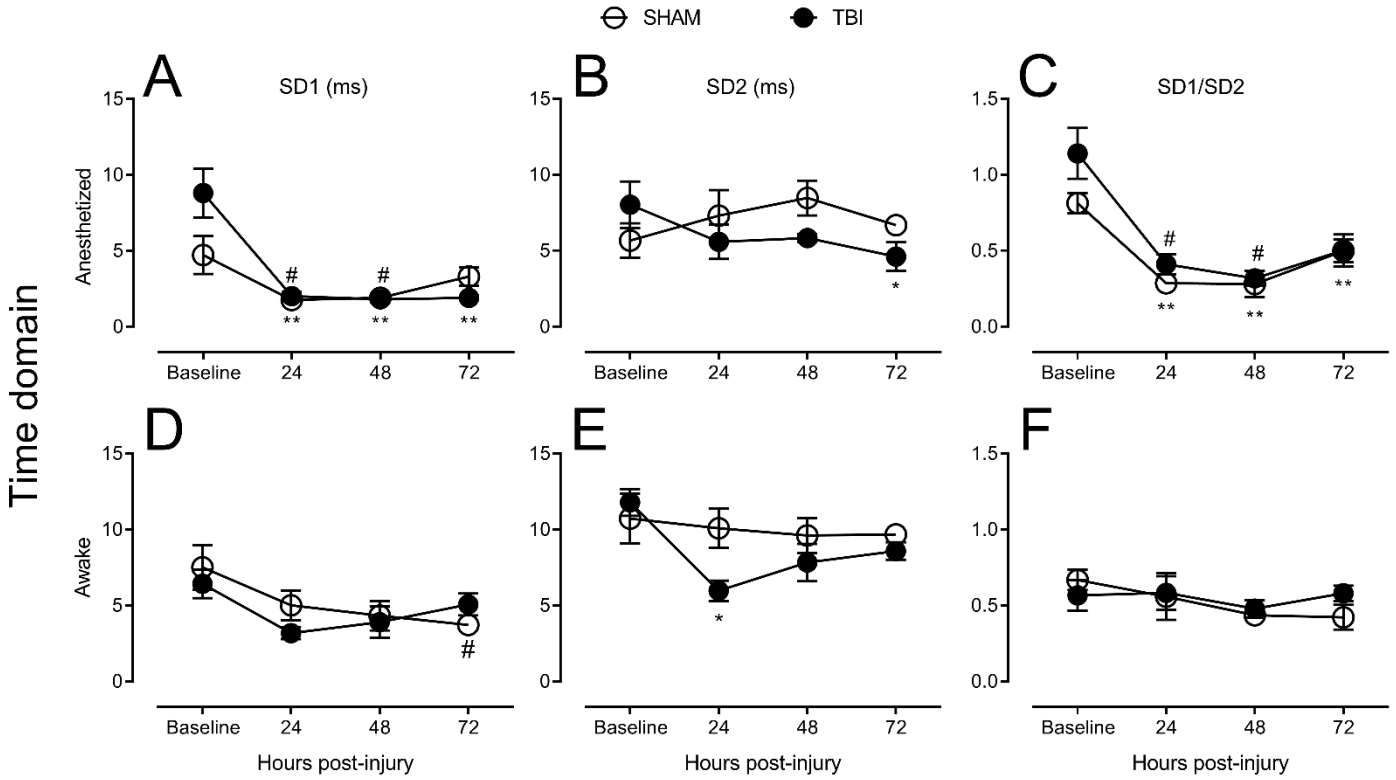

Fig. 4. The effects of surgical procedure and TBI on the HRV non-linear parameters in the time domain on the animals in the (A, B, and C) anesthetized and (D, E, and F) awake states. (A-D) SD1, the standard deviation of instantaneous (short term) beat-to-beat interval variability; (B-E) SD2, the standard deviation of the long term NN intervals; (C-F) SD1/SD2, the ratio between the short- and long-term NN intervals. Data analyses were conducted using two-way ANOVA. Sidak's multiple tests comparing the groups were applied when appropriate. Data are presented as mean ± SEM (n = 5 per group). *$p < 0.05$ compared to the baseline (TBI group), **$p < 0.01$ compared to the baseline (TBI group), and #$p < 0.05$ compared to the baseline (SHAM group).

In addition, HRV measurement abnormalities have been associated with various markers of cardiac autonomic disorders. For example, low HRV global activity metrics (indexed by HR, mean NN, SDNN, and SD2) are linked as important variables of anxiety evoked by stress and anesthesia conditions [22].

Although literature is scarce, in non-linear index evaluated in mild TBI, lower SD1 has been linked to a higher risk of arrhythmias, sudden cardiac complications, and difficulties in recovering from physical exercise post-TBI. In the other reduced metrics, these patients presented less flexibility of the cardiovascular system, contributing to greater fatigue in SD2, and autonomic imbalance, with predominance of sympathetic activity, indicating an increased risk of cardiovascular complications in stressful situations in SD1/SD2 [23].

In our study, in general measurements under anesthesia (e.g., HR, mean NN, SDNN, and SD2), we observed a significant change compared to the awake animals, suggesting low HRV global activity during anesthesia maintenance. Similarly, recent reports have disclosed lower HR after using this anesthetic in rats and mice compared to the awake condition or other anesthetic types [24], [25].

In linear (RMSSD and HF) and non-linear (SD1) parasympathetic measurements, research has shown that low HF levels may be associated with an increasing anxiety state, thereby becoming a pivotal component of clinical interest [22], [26]. Regarding the sympathetic (LF) and sympathetic-vagal balance (indexed by the LF/HF and SD1/SD2) activities, the literature has speculated that the LF/HF ratio increased may significantly contribute to the progression of a stressful and depressive state [27].

In the present study, the results obtained in the awake animals demonstrated a significant decrease in HRV global activity by mean NN and SD2 and an expressive HR increase after 24 h of TBI compared to the baseline, reinforcing the notion that these animals possibly suffered post-TBI stress. Thus, these findings confirmed the hypothesis above (i.e., the increased HR decreases the HRV overall parameters after injury).

It is important to note that the use of Poincaré plots as an index of nonlinearity should be interpreted with caution, especially when applied to short-term HR recordings. Previous studies have shown that the reliability of Poincaré-derived measures in capturing nonlinear dynamics depends strongly on the duration of the time series [28], [29], [30]. Short recordings, such as those used in the present study, may not fully reflect the contribution of nonlinear components to HR dynamics, particularly under stressful or pathological conditions.

Nevertheless, short-term Poincaré analysis has been widely employed as a complementary tool to describe the instantaneous beat-to-beat variability and autonomic modulation of HR, providing useful insights even when nonlinear interactions are not fully captured [29], [30].

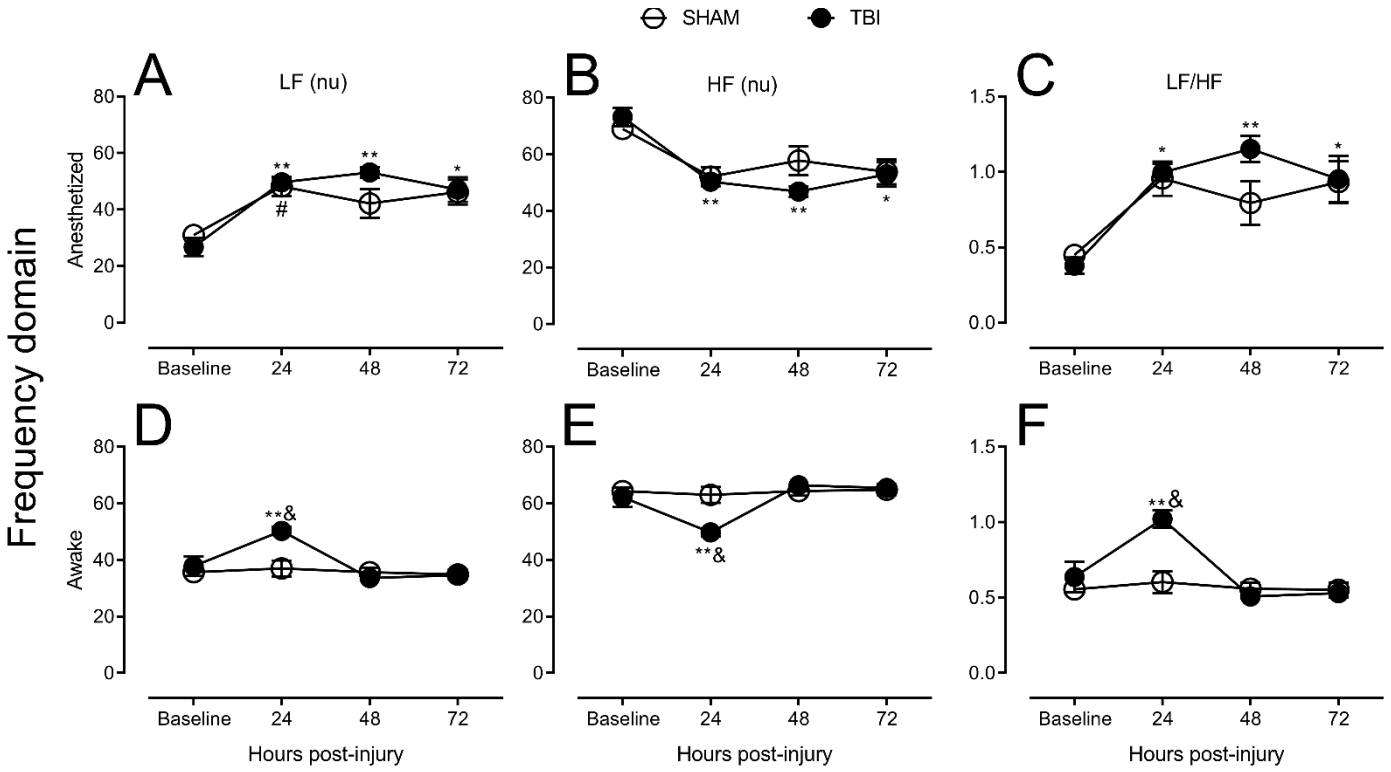

Fig. 5. The effects of surgical procedure and TBI on the HRV linear parameters in the frequency domain on the animals in the (A, B, and C) anesthetized and (D, E, and F) awake states. (A-D) LFnu, low-frequency power in normalized units; (B-E) HFnu, high-frequency power in normalized units; (C-F) LF/HF, ratio absolute between LF and HF. Data analyses were conducted using a two-way ANOVA. Sidak's multiple tests comparing the groups were applied when appropriate. Data are presented as mean ± SEM (n = 5 per group). *p < 0.05 compared to the baseline (TBI group), **p < 0.01 compared to the baseline (TBI group), #p < 0.05 compared to the baseline (SHAM group), and & < 0.01 compared to 48 and 72 h (TBI group).

Therefore, in this context, the Poincaré indices (SD1 and SD2) were interpreted primarily as geometric representations of short-term HRV patterns, reflecting alterations in autonomic balance rather than as comprehensive measures of nonlinear behavior.

Future studies using longer HR recordings or additional nonlinear analyses (e.g., entropy- or fractal-based methods) could help to further characterize the complexity of HR regulation after TBI and strengthen the interpretation of these findings.

The present study also indicated lower parasympathetic activity by the HFnu and RMSSD components in the first 24 h after injury in the awake rats, although RMSSD was not significant between the periods. These data establish that the autonomic function is predominantly controlled by sympathetic activity after a brain lesion. All the parasympathetic linear and non-linear HRV indices decreased 24 h after neurological injury in the anesthetized animals. Notably, these ECG parameters did not tend to return to the baseline values, as evidenced in awake animals, thereby suggesting a strong influence of anesthesia. In addition, HRV measurements performed under isoflurane resulted in similar values in some parameters in the sham and TBI groups. It is plausible to suggest that the isoflurane anesthesia effect predominates over trauma, masking the effect of brain injury and causing no alteration in HR after damage by the influence of both domains. However, it is important to note that the time domain is statistically predominant for three days after injury.

The sympathetic and sympathovagal balance activities reflected by LFnu and the LF/HF ratios, respectively, were increased in the first 24 h in the awake condition, returning posteriorly to the baseline values in 48 and 72 h. Our experimental discovery corroborates the study of Su et al. [21], who reported higher LF and LF/HF ratio prevalence in TBI patients with Glasgow coma scale scores of 4–15. The sympathetic hyperactivity also gradually increases the blood pressure after brain injury for the physiological system survival, given that early hypotension (low blood pressure) is a risk factor for mortality [31]. Differently, in an anesthetized state, data for both LF, HF, and the LF/HF ratios were activated in all recorded periods after injury.

It is important to note that data recording of TBI began 24 hours following, a period during which systemic inflammation may already be established. In this context, the inflammatory responses triggered by TBI may also partially contribute to modulating the ANS [32].

V. Study Limitations

This study presents limitations that should be considered. The small sample size was established in compliance with the 3Rs ethical principles, considering in particular the rats' welfare and the statistical analysis. In addition, the sampling frequency of 500 Hz for recording, although lower than other studies, complies with the international standards of the HRV analysis, such as the Task Force (1996).

Another factor is that the recordings were performed at different times of the day. Corroborating this fact, the animals

strictly followed the established schedule and protocol, discarding any circadian influence reflected in the groups. Finally, other types of monitoring, such as EEG were not applied for this study, since the aim was exclusively to analyze the cardiac autonomic system.

VI. Conclusion

This study showed that 24 hours is the critical period for global HRV modulation following moderate FPI in rats, with predominant alterations of sympathetic over parasympathetic modulation. This predominance occurs specifically in the SD2 time-domain, as well as in the LF, HF, and LF/HF ratio in the frequency-domain. Therefore, linear HRV indices in the frequency domain are more significantly impaired after FPI and contribute to global HRV alterations in the acute phase of this TBI model.

Acknowledgments

Sadly, while this study was being finalized, Professor Adair Roberto Soares Santos passed away. We and all the scientific academy involved deeply mourn his loss. The authors gratefully acknowledge the financial support provided by the Brazilian funding agencies Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPQ) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). RSN is the recipient of CNPQ fellowship and FSF is the recipient of CAPES fellowship. LFFR, and JLBM are the recipients of a Research Productivity Fellowship from CNPQ.

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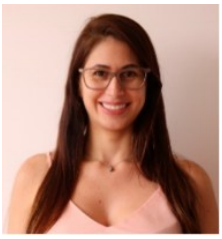

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