Heart rate variability of dogs living in restricted spaces

Variabilidade da frequência cardíaca em cães com restrição de espaço

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Highlights

Heart rate variability did not differ in dogs with or without space restriction.
The autonomic nervous system likely adapts in restricted environments.
The five-domain model is essential to characterize failures in welfare.

Abstract

When animal welfare conditions are not met, stress can occur. Among other effects, this may lead to cardiovascular alterations related to sympathovagal imbalance. This study aimed to integrate heart rate variability (HRV) analysis with a welfare assessment of eight dogs allocated into groups with and without space restriction to determine the influence of space restriction stress using current methods. The study was conducted in the dogs' home environments, with 24-hour ambulatory electrocardiogram recordings. Welfare was assessed in both hospital and home settings using the five-domain model, behavioral analysis, and stress evaluation. Data were analyzed statistically, with significance set at $p < 0.05$. The results showed higher welfare scores particularly in the environmental ($p < 0.001$) and behavioral ($p = 0.0002$) domains for dogs without space restrictions. There was no difference in HRV indices over 24 hours. Continuous space restriction was found to be detrimental to various animal needs, and dogs in this group showed clear welfare shortcomings. The HRV findings may indicate that restricted dogs did not experience greater activation of the sympathetic autonomic nervous system due to their environment though this does not imply that their welfare was unaffected. In conclusion, HRV analysis did not differ between dogs with or without space restriction; however, the five-domain model and stress assessment revealed welfare deficiencies in restricted dogs.
Key words: Quality of life. Space restriction. Sympathovagal balance. Well-being.

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Resumo

Quando as condições de bem-estar animal não são cumpridas, ocorre o estresse. Assim, entre outros fatores, podem ocorrer alterações cardiovasculares relacionadas a um desequilíbrio simpatovagal. Portanto, o presente estudo teve como objetivo integrar a análise da variabilidade da frequência cardíaca (VFC) e o bem-estar de oito cães, alocados em grupos com e sem restrição de espaço, de forma a determinar a influência do estresse da restrição de espaço utilizando métodos atuais. O estudo foi realizado na residência dos tutores dos cães, através de um eletrocardiograma ambulatorial com registros de 24 horas. O bem-estar dos animais foi avaliado no ambiente hospitalar e doméstico, com base no modelo dos cinco domínios, análise comportamental e de estresse. Os dados obtidos foram submetidos à análise estatística considerando p<0,05. Os resultados mostraram melhores índices de bem-estar, principalmente nos domínios ambiente (p<0,001) e comportamento (p=0,0002) no grupo de cães sem restrição de espaço. Não houve diferença nos índices de VFC em 24 horas. Entende-se que a restrição contínua do espaço é prejudicial às diferentes necessidades dos animais, assim, os cães deste grupo possuem falhas em relação ao bem-estar. Os resultados da análise da VFC podem indicar que os cães do grupo com restrição de espaço não apresentam maior ativação do sistema nervoso autônomo simpático relacionado ao ambiente, o que não significa que o seu bem-estar não seja prejudicado. A partir deste estudo, pode-se concluir que a análise da VFC não diferiu em cães com ou sem restrição de espaço, no entanto, o modelo de cinco domínios e a avaliação do estresse caracterizaram falhas no bem-estar dos cães.

Palavras-chave: Bem-estar. Equilíbrio simpatovagal. Qualidade de vida. Restrição de espaço.

Introduction

According to Broom (1986), well-being is linked to an individual's ability to cope with the environment in which they live. The term well-being has been associated with various definitions, including the needs of animals (Broom & Molento, 2004). These needs are described in the five-domain model, which helps identify different situations related to well-being. The first three domains address physical needs (nutrition, environment, and health, respectively), the fourth relates to behavior, and the fifth concerns mental state, which results from the interaction of the other domains (Mellor, 2017).

From this model, animal welfare conditions can be defined, assigning positive and negative criteria to the experiences of the animal (Ceballos & Sant'Anna, 2018). Various needs influence welfare, and when these needs are no longer met, adaptation to the conditions fails (Dalla Villa et al., 2013). A failure to adapt can also lead to changes in behavioral patterns, which, when assessed together, serve as another indicator of stress (Silva et al., 2021).

Chronic stress results in a persistent homeostatic imbalance that, over time, is harmful to the body as a whole. Continuous stimulation of the hypothalamic-pituitary-adrenal axis and the sympathetic autonomic

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nervous system leads to increased release of cortisol and catecholamines. Prolonged stimulation causes damage to the cardiovascular system (Marques et al., 2010).

Physiological parameters can be used to quantify stress and reduce the subjectivity of assessments. When focusing on cardiovascular parameters, one option is the analysis of heart rate variability (HRV) (Kartashova et al., 2021). This method reflects oscillations in heart rate both immediately and over extended periods (Marques et al., 2010).

Through this method, it is possible to evaluate autonomic activity and balance, as well as the efficiency of the body's responses to various physiological stimuli aimed at maintaining homeostasis (Song et al., 2006; Marães, 2010). The indices serve as indicators of cardiovascular health, revealing the heart's ability to adapt to stimuli from the autonomic nervous system (Mohr et al., 2002; Pumprla et al., 2002).

HRV can be measured by performing a continuous electrocardiogram (Holter), which records the heart's electrical activity for 24 hours or more (Cavalcanti, 2012). Studies cited by Von Borell et al. (2007) show that HRV has been successfully used to assess autonomic balance in production animals and pets, including determining stress levels and the degree of well-being related to the environment in which these animals live (Vanderlei et al., 2009; Sá et al., 2013).

Therefore, the aim of this study was to determine the influence of space-restriction stress on cardiac autonomic parameters, as well as to evaluate the dog's home environment based on the five-domain concept and its behavior.

Material And Methods

After approval from the Ethics Committee for the Use of Animals at the Federal University of Fronteira Sul (CEUA – 5659080223), the research was conducted at the homes of dog owners living in the municipality of Realeza, Paraná, who agreed to participate after signing the free and informed consent form.

The sample size was calculated using an unpaired t-test for independent samples, with an a priori power analysis. The calculated effect was based on data described by Kuhne et al. (2014), with $\alpha = 0.05$ and statistical power set at 0.95, using the G*Power 3.1.9.7 program.

Eight dogs were analyzed and allocated into two groups: four dogs with space restrictions (SR) and four without space restrictions (WSR) at home, though without free access to the street. They were randomly selected according to their owners' consent to participate in the study.

Screening

The dogs were screened through anamnesis, physical examination, echocardiogram, and computerized electrocardiogram at the Superintendence of the University Veterinary Hospital Unit of the Federal University of Fronteira Sul, Realeza campus. The inclusion criteria were healthy adult dogs, 1–7 years old, of small or medium size. Brachycephalic dogs and those with any form of heart disease were excluded from the study.

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Welfare assessment

The criteria described in the Five Domains Model were used to assess the well-being of the selected dogs (Table 1). The quality-of-life assessment, as described by Mellor (2017), aimed to identify criteria indicating shortcomings in animal welfare, as well as to highlight how and where improvements could be made. A scoring system was applied, starting at "none" (0), representing the lowest degree of welfare offered, and going up to "+++," the highest welfare classification for the criteria analyzed. The scores for the two groups were then summed.

Table 1
Criteria observed for assessing and scoring animal welfare, based on the Five Domains model

Criteria	Evaluation of improved well-being and opportunities for improvement			
	None (0)	Low Level (+)	Middle Level (++)	High Level (+++)
Domain 1 - Nutrition				
Water access	No access to water.	Restricted access to water for long periods.	Free access to water of inadequate quality and/or quantity.	Free access to water of adequate quality and quantity.
Food access	No access to food.	Restricted access to food for long periods.	Inadequate access to food.	Access to adequate food.
Food quality	Unbalanced nutrition.	Balanced feed with lower digestibility; low variety in food supply.	Balanced feed with good digestibility; moderate variety in the food offered.	Highly digestible balanced feed; high variety of food offered.
Domain 2 - Environment				
Thermal comfort	Direct exposure to unsuitable temperatures and inclement weather.	Minimal protection against inadequate temperatures and inclement weather.	Partial shelter from unsuitable temperatures and inclement weather.	Efficient shelter from inadequate temperatures and inclement weather.
Stimuli the animal is exposed to	Monotonous environment, lack of routine, and unsuitable conditions for rest	Monotonous environment, presence of routine, and unsuitable conditions for rest.	Monotonous environment, presence of routine, and conditions conducive to rest.	Stimulating environment, presence of routine, and conditions conducive to rest.
Space the animal has access to	Confined at least 70% of the time on a chain <5 m from the residence, unable to walk.	Confined at least 70% of the time on a chain >5 m from the residence, unable to walk.	Free access to the residence but no possibility of walking.	Free access to the residence and the possibility of outings.

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Domain 3 - Health			
Physical health	Presence of diseases/ alterations that could lead to physiological impairment.	-	-	Absence of diseases/alterations that could lead to physiological impairment.
Domain 4 - Behavior			
Interaction with the environment	Highly restricted ability to explore the environment and move freely.	Moderately restricted ability to explore the environment and move freely.	Slight restrictions on the ability to explore the environment and move freely.	No restrictions on the ability to explore the environment and move freely.
Interaction with other animals/ humans	Little interactive activity; lack of company and participation in pleasant routine activities.	Moderately restricted interactive activity; little company and participation in pleasant routine activities.	Interactive activity with few restrictions; possibility of companionship and participation in pleasant routine activities.	Unrestricted interactive activity; presence of company and good participation in pleasant routine activities.

Adapted from Mellor (2017).

The assessment of domains one and three (nutrition and health) was based on information obtained from the anamnesis and physical examination during screening. For domains two and four (environment and behavior), observations were made of the animals' living conditions, as well as their behavior during screening and at home. Animals with SR were defined as those spending ≥70% of the day restrained by chains shorter than 5 meters. The chain length was measured for these cases.

The animals' behavior was further described using a scale for assessing and recognizing stress in dogs, adapted to veterinary practice, with levels ranging from 0 to 5 (Table 2). According to this scale, dogs displaying friendly, calm behaviors relaxed and cooperative even when alert were classified as having no or low stress. By contrast, dogs showing signs such as tension, panting, reluctance or ease of handling, anxiety, trembling, barking, or hiding, among other behaviors indicating fear or stress, were scored accordingly (Overall, 2013).

Table 2
Scale for assessing and recognizing stress in dogs, based on Overall (2013)

Stress value	Dog's behavior and appearance
0	Calm, very friendly, helpful, easily managed, outgoing
1	Calm dog, appears relaxed
2	Alert but cooperative
3	Appears alert but cooperative; not very comfortable or relaxed, but still easily led; may need encouragement
4	Visibly tense, anxious; may be panting, trembling, whining; may be difficult to handle; uncooperative
5	Extremely stressed; barking, howling, tries to hide

Adapted from Overall (2013).

Holter monitoring in the home

A Cardios-brand continuous electrocardiogram (Holter) with a four-lead, three-channel electrode system was used to assess HRV over 24-hour recordings. Before electrode placement, the sites were clipped and aseptically cleaned. The electrodes were positioned as follows: green on the right hemithorax between the fifth and sixth intercostal spaces, black on the left hemithorax between the fifth and sixth intercostal spaces, white on the sternal manubrium, and red on the xiphoid cartilage.

To secure the electrodes and the Holter, bandages and adhesive plaster were applied to protect the device components, fixing it along the dogs' thoracic spine. After installation, the owners were instructed to maintain their normal routines. The device remained in place for 24 hours. A free-form log sheet was provided so owners could record the dogs' routines and the time spent on different activities. The device was retrieved by the researchers at the owners' homes.

Data from the Holter were analyzed using CardioManager S540 software, which provided parameters such as the number of QRS complexes, as well as the maximum, average, and minimum heart rates over 24 hours, possible arrhythmias, and HRV.

HRV was evaluated through statistical calculations and time-domain analysis in milliseconds (Table 3).

Table 3
HRV variables in the time domain

Variables	Definition
NNMED	Average of all normal RR intervals (or NN intervals).
SDNN	Standard deviation of NN intervals.
SDANN	Standard deviation of the average NN interval every 5 minutes over a period of time.
SDNNI	Average of the standard deviations of the NN intervals every 5 minutes.
RMSSD	Square root of the mean of the square of the differences between adjacent normal NN intervals over a period of time.
pNN50	Percentage of adjacent NN intervals with a duration difference greater than 50 ms.

Statistical analysis

The data obtained were tabulated and analyzed using descriptive statistics and statistical software (GraphPad Prism 5.0). Normality was assessed using the Shapiro–Wilk test. Parametric variables were expressed as mean ± standard deviation, and non-parametric variables as median (interquartile range). Parametric variables were analyzed using the unpaired t-test, and non-parametric variables using the Mann–Whitney test, with $p < 0.05$ considered statistically significant.

Results

The dogs in this study were divided into two groups. The WSR group included three males and one female, aged between 1 and 7 years. The SR group consisted of four females, aged between 3 and 6 years. The animal welfare assessments for dogs in the groups with and without space restrictions are presented in Table 4.

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Table 4

Description of the animal welfare assessment, based on the Five Domains model adapted from Mellor (2017) in dogs without space restriction (WSR) and with space restriction (SR)

Criteria	WSR					SR				
	Dog 1	Dog 2	Dog 3	Dog 4	Group sum (+)	Dog 5	Dog 6	Dog 7	Dog 8	Group sum (+)
Domain 1 - Nutrition										
Water access	+++	+++	+++	+++	12	++	++	+++	+++	10
Food access	+++	+++	+++	+++	12	+++	+++	+++	+++	12
Food quality	+++	++	++	++	9	0	0	++	++	4
Total					33					26
Domain 2 - Environment										
Thermal comfort	+++	+++	+++	+++	12	++	+	++	+++	8
Stimuli the animal is exposed to	+++	+++	+++	+++	12	+	+	+	++	5
Space the animal has access to	+++	+++	+++	+++	12	0	0	0	0	0
Total					36					13
Domain 3 - Health										
Physical health	+++	+++	+++	+++	12	+++	+++	+++	+++	12
Total					12					12
Domain 4 - Behavior										
Interaction with the environment	+++	+++	+++	++	11	0	0	0	0	0
Interaction with other animals/ humans	+++	+++	+++	+++	12	0	0	+	+	2
Total					23					2

Adapted from Mellor (2017).
Note: 0 = None; + = Low level; ++ = Medium level; +++ = High level.

Overall, the WSR group showed better results than the SR group, particularly in the domains of environment and behavior (Table 5). The most notable differences in these criteria were for environment ($p < 0.001$) and behavior ($p = 0.0002$), as the WSR dogs had free access to the entire home and the opportunity for daily walks. Additionally, interaction with the environment and human–animal interaction were significantly better in the WSR group, and the use of environmental enrichment was also significantly greater.

Table 5
Median sums of the animal welfare assessment of the groups of dogs without space restriction (WSR) and with space restriction (SR)

Domains	WSR	SR	p
Nutrition	3[a]	2.5[a]	0.16665
Environment	3[a]	1[b]	<0.001
Health	3[a]	3[a]	1
Behavior	3[a]	0[b]	0.0002

Note: Medians in the same row followed by different lowercase letters are considered statistically different.

By contrast, the SR group dogs were kept on chains (three on chains of 1.5 m and one on a chain of approximately 2.0 m), continuously outside their homes. This limited their ability to move, explore their surroundings, and maintain thermal comfort. Environmental enrichment was generally scarce, and human–animal interaction was reduced; in three of the four cases, the guardians' contact with the dogs was largely limited to feeding times.

In domain 1 (nutrition), access to water and food was similar between groups, but food quality differed. Half of the SR dogs were fed unbalanced diets, which was not the case for the WSR dogs. Furthermore, the WSR group also had access to other food types such as wet food and snacks providing additional food enrichment.

Behavior-based stress assessments, according to Overall (2013), also differed between groups. In the WSR group, three of the four dogs were calm, friendly, relaxed, and cooperative, remaining alert only briefly, mainly during screening and physical restraint. These dogs were classified at stress levels 0 to 2. The remaining WSR dog the youngest and most energetic stayed tense during evaluations, appeared somewhat uncomfortable but was easily handled, and was classified as level 3.

In the SR group, although the dogs could be handled with some ease, three of the four were anxious and alert throughout the evaluations. One dog displayed fear, panting, and trembling. Overall, SR dogs showed higher stress levels during consultations, with classifications ranging from level 3 to 4.

Regarding the ambulatory electrocardiogram (Holter) recordings, there were no differences between the two groups in minimum, average, and maximum heart rates over 24 hours, nor in HRV indices in the time domain (Table 6).

Table 6
Mean values ± standard deviations or median (minimum–maximum) of the minimum, mean, and maximum heart rates and HRV indices in the time domain obtained from the 24-hour Holter of dogs without space restriction (WSR) and with space restriction (SR)

Parameter	WSR	SR	p
HR MIN (bpm)	37 ± 2.9	38.7 ± 6	0.6202
HR MED (bpm)	77.7 ± 16.3	89.5 ± 18	0.3673
HR MAX (bpm)	249.5 (220.5–250.0)	250 (249.3–250)	0.7143
NNMED (ms)	897.3 ± 195.1	756.5 ± 186.7	0.3373
SDNN (ms)	339.5 ± 99.7	278.0 ± 50.7	0.3138
SDANN (ms)	228.8 ± 40	198.0 ± 40	0.3188
SDNNi (ms)	232 ± 63.4	208.3 ± 37.4	0.5427
RMSSD (ms)	212.3 ± 104.3	177.3 ± 62	0.5849
pNN50 (ms)	66.6 ± 11.2	61.7 ± 7.5	0.4941

Because higher nighttime heart rates were noted in the SR group, an additional statistical analysis was performed on minimum, average, and maximum heart rates for morning (06:00–12:00), afternoon (12:00–18:00), evening (18:00–00:00), and early morning (00:00–06:00) periods, based on Holter data. Although absolute values differed, no statistically significant differences were found between the groups (Table 7).

Table 7
Mean values ± standard deviations or median (minimum–maximum) of the minimum, mean, and maximum heart rates in the time domain in the morning, afternoon, evening, and dawn periods for dogs without space restriction (WSR) and with space restriction (SR)

Period of the day	HR MIN (bpm)			HR MED (bpm)			HR MAX (bpm)		
	WSR	SR	p	WSR	SR	p	WSR	SR	p
6:00–12:00 h	45 ± 5	49 ± 8	0.435	75 ± 10	88 ± 20	0.296	184 ± 19	198 ± 20	0.348
12:00–18:00 h	52 ± 15	58 ± 10	0.545	81 ± 24	92 ± 26	0.505	185 ± 37	177 ± 36	0.761
18:00–00:00 h	51 ± 14	55 ± 6	0.613	82 ± 19	92 ± 15	0.443	212 (163–224)	207 (179–209)	0.885
00:00–6:00 h	43 (38–55)	48 (39–49)	0.885	67 (54–8)	86 (59–90)	0.228	173 ± 23	202 ± 39	0.236

Discussion

In relation to the dogs' well-being, several aspects were assessed to compare quality of life between the groups, including space restriction. The literature clearly indicates that when environments differ in the degree of space restriction, welfare is lower for animals with less freedom of movement (Broom & Molento, 2004).

The conditions observed in the SR group's environment in this study are not uncommon in the municipality where the research was conducted. Other studies, such as Arruda et al. (2020), have used different models to assess the welfare of shelter dogs in Paraná. In their study, the authors reported similar scenarios in some shelters, where dogs lived in isolation, were chained, and housed in inadequately sized spaces highlighting compromised welfare.

Because the five domains described in Mellor's (2017) model are often interconnected, these cases illustrate how significantly restricting the space available to animals harms multiple needs beyond the environment itself. This includes limiting opportunities for species-specific behaviors, reducing human–animal interaction, and often affecting thermal comfort, as was observed in the present study.

During the study, differences between the groups emerged in behavioral indicators of stress levels: in general, SR group dogs displayed more stress-related behaviors. We also observed differences in owner–dog interaction, based on the activity sheets provided with the Holter to record the animals' routines. In the WSR group, notes were more varied, including wake-up times, bedtimes, feeding times, walks, and other records indicating greater owner involvement and interaction with the animal. By contrast, notes from SR group owners were mostly limited to feeding times, suggesting fewer opportunities for human–animal interaction.

Unlike what was observed in the SR group, dogs in the WSR group had more opportunities to explore their environment and interact with other animals and humans. Historically, dogs have lived in packs, possess a diverse behavioral repertoire, and have long been adapted to human interaction (Banerjee & Bhadra, 2022).

Providing environments where dogs can be in the presence of other animals or humans enriches their surroundings by enabling socialization, which in turn supports emotional well-being (Newbury et al., 2018; Arruda et al., 2020). Based on the results of this study, the SR group lacked essential elements for canine welfare, resulting in stress and influencing behavior, as evidenced in both the behavioral and welfare assessments.

Another link observed in the welfare assessment concerned nutrition. While there was no difference in access to food and water between the groups, half of the SR dogs were fed unbalanced natural diets, which can lead to health issues such as obesity, malnutrition, or diseases associated with poor nutrition. Proper nutritional management is essential for a better quality of life. Natural diets can be a viable option, but they must be carefully planned to offer nutritional benefits (Saad & França, 2010; Bragança & Queiroz, 2021).

As HRV values did not differ between WSR and SR dogs, the findings may suggest that restricted dogs do not experience greater activation of the sympathetic

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autonomic nervous system related to the stress of their living conditions. HRV can serve as an indicator of stress depending on the individual's perception of potential threats, which are necessary for survival instincts. It is possible that restricted dogs were not in environments that triggered the "fight or flight" responses associated with sympathetic autonomic nervous system activation (Thayer et al., 2012).

The question also arises as to whether, after a certain period in the same environment, the body adapts to the stressors. This possibility aligns with the findings of Protopopova (2016), who measured cortisol levels to assess stress in shelter dogs and observed a decrease over time spent in the shelter. However, this does not mean that well-being is unaffected, as the absence of a physiological stress response does not imply the absence of harm exposure to stress is never beneficial for the individual (Broom, 2001).

Protopopova (2016) also examined the welfare of shelter dogs, noting that restricted space and limited social interaction reduce the diversity of their behavioral repertoire. While the author acknowledged the detrimental effects such environments can have on welfare, she did not determine their specific impacts on dogs' physiology or behavior. A similar outcome occurred in the present study: although the five-domain model revealed welfare issues in the dogs, it was not possible to detect related changes in the physiological parameters measured.

Furthermore, current studies linking HRV to emotional state (Katayama et al., 2016; Wormald et al., 2017; McGowan et al., 2018) typically use short-term measurements during controlled behavioral tests. This differs from the methodology applied in the present study, which analyzed HRV over a 24-hour period in the dogs' home environments during their usual daily activities.

According to Shaffer and Ginsberg (2017), several factors influence HRV results, including the duration of electrocardiographic recording. They note that short-term and long-term HRV measurements are affected by different regulatory mechanisms. Therefore, the methodology used here is not directly comparable to studies employing short-term recordings as physiological indicators of animal welfare because this study relied on 24-hour recordings.

A short electrocardiographic recording is most influenced by breathing, as respiratory sinus arrhythmia fluctuations in heart rate within the respiratory cycle may be present. The baroreflex, which is affected by blood pressure and mediates sympathovagal modulation, also plays a role (Shaffer et al., 2014; Shaffer & Ginsberg, 2017). By contrast, HRV obtained over a 24-hour period is subject to additional influencing factors, particularly slower regulatory mechanisms such as metabolism, sleep, and wakefulness. This was the case for the dogs in the present study, meaning that different recording durations require different analytical approaches and are not directly comparable (Shaffer & Ginsberg, 2017).

Thus, the duration of the test is an important consideration for the analysis and interpretation of HRV data, depending on the purpose. The recordings used in this study differ from those in which correlations have been found between HRV and animals' emotional state.

According to Ernst (2017), another relevant consideration when studying HRV is individual variation, as most current research examines this parameter only at the group level. For example, one dog in the WSR group (dog number 2), being the youngest and most energetic, had HRV values similar to those of dogs in the SR group, despite living in a different environment.

It is also known that dogs' close association with humans results in activity patterns similar to those of their owners. This finding supports the present study's HRV stratification by time period, which showed no differences in heart rates between groups at night the species' longest rest period (Banerjee & Bhadra, 2022). Therefore, although levels of human–animal interaction differed between the groups, no corresponding differences were observed in the physiological parameters assessed.

Furthermore, because the autonomic nervous system is integrated with various other physiological systems, interpreting HRV is inherently complex (Ernst, 2017). A deeper understanding of all factors involved is necessary when analyzing HRV in relation to stress and behavior.

As demonstrated in this study, the absence of changes in cardiovascular parameters does not imply an absence of differences in well-being, as shown by the five-domain model and stress level assessments. The model also helps identify areas where welfare can be improved, highlighting key priorities for animal care. Ultimately, the role of guardians is essential to animal welfare, as they directly influence health, environment, behavior, and the potential for human–animal interaction, which in turn can lead to higher or lower levels of well-being (Mellor, 2017; Mellor et al., 2020).

Conclusions

The analysis of heart rate and HRV from 24-hour recordings did not differ between the groups of dogs living with or without space restriction in this study, suggesting that chronic stress did not alter the autonomic nervous system as assessed by cardiac parameters. However, the application of the five-domain model and the behavioral stress assessment was essential for identifying situations in which the dogs' well-being was suboptimal, particularly in the environmental and behavioral domains.

Further research is recommended with animals not adapted to such living conditions. A deeper understanding is needed of the mechanisms whether external or internal that influence the parameters used, as well as the individual differences among animals, so that these measures can be integrated effectively into overall welfare assessments.

Ethical Statement

All procedures were approved by the Ethics Committee for the Use of Animals of the Federal University of Fronteira Sul, under protocol CEUA – 5659080223.

Conflict of Interest Statement

The authors declare no conflict of interest.

Acknowledgments

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