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Preliminary study of heart rate variability in Criollo horses for the elucidation of their neurophysiological characteristics of autonomic nerve function

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The Criollo is an Argentine horse breed with a calm temperament. Although its temperament is considered to be related to its neurophysiological characteristics, the details of this are unknown. Therefore, we analyzed the heart rate variability in Criollos as a preliminary study to deepen the neurophysiological understanding of their autonomic function. Electrocardiograms were recorded from Criollos and Thoroughbreds, and the power spectrum of heart rate variability was analyzed. Compared with Thoroughbreds, Criollos showed (i) a significantly higher high-frequency component, which is an index of parasympathetic nerve activity, and (ii) tendency toward a lower ratio of low- to high-frequency power, which is an index of the autonomic balance. These results revealed that parasympathetic nerves might be more active in Criollos compared with Thoroughbreds.

Key words: *autonomic nerve, Criollo, heart rate variability, horse*

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The Criollo (in Spanish), or Crioulo (in Portuguese), is a breed of horses from Argentina and is defined as such in this manuscript. The population of Criollos was established from rewilded horses that were brought to the South America from Spain [18]. The Andalusian from Spain, Arab from the Iberian Peninsula, and Barb from North Africa are the breeds that influenced the characteristics of the Criollo. With a height at the withers of about 140 cm [13], Criollos are shorter than Thoroughbreds and other riding horse breeds, and their chests and backs are relatively broad [4]. They are also mild-mannered, resistant to diseases, and have good physical strength. These characteristics make it easy even for a relatively small person to control Criollos and provide more stability for riders. Therefore, Criollos are suitable for riding and are known to have been used by gauchos (South American cowboys) as stock horses. Today, Criollos have many other uses, such as for recreational horseback riding and for horse trekking. They have also been used in horse-assisted therapy [16] because of their calm temperament. Meanwhile, there have been few reports of physiological studies on Criollos, and the neurophysiological mechanisms underlying their mild temperament, as well as the physiological indicators that may be able to explain their calm temperament, have not been investigated much.

Riding horses are generally evaluated by pedigree information and equestrian competition results. However, no available technology can objectively and quantitatively evaluate horse temperament. In recent years, a mathematical model called Best Linear Unbiased Prediction (BLUP) has been used to improve horse breeding [7]. BLUP methods require quantitative data to assess equine performance. If individuals with excellent temperament types that are best suited to work with humans can be selected quantitatively, the indices used in this process may be applied to effectively breed horses with favored traits.

In general, the sympathetic nervous system predominates when an animal is in an excited state, while the parasympathetic nervous system predominates when an animal is in a

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calm state. This suggests that the calm temperament of the Criollo may be related to autonomic nerve functions. Heart rate variability analysis is known to be useful for evaluating autonomic nerve functions [1, 12, 14]. Beat-to-beat intervals of the heart, that is, RR intervals in an ECG, are not constant but change all the time. One method that mathematically analyzes the frequency component of the heart rate variability spectrum is called power spectral analysis. Heart rate variability is caused by autonomic nervous system activity, and a power spectral analysis of heart rate variability is believed to be able to provide quantitative indices of sympathetic and parasympathetic functions [1, 10, 14, 15]. In an earlier study, significant correlation was observed between a number of behavioral responses and parameters of heart rate variability in Thoroughbreds [19]. Moreover, attempts have been made to establish assessment criteria for horses with physical/behavioral characteristics suitable for horse-assisted therapy by using heart rate variability [5]. It is possible that the heart rate variability of Criollos reflects their aptitude as therapy horses. Therefore, in this study, we performed a power spectral analysis of heart rate variability as a preliminary study to deepen the neurophysiological understanding of the autonomic function of Criollos.

Three Argentine Criollos (age, 13.7 ± 7.1 years; body weight, 431 ± 2 kg [mean ± SE]; mares) and five Thoroughbreds (age, 12.0 ± 5.3 years; body weight, 531 ± 23 kg; 2 mares and 3 geldings) were used. All horses were pastured in approximately 1 to 3 hectares of field for 3 to 7 hr during the day. All experiments using horses were conducted in accordance with the Animal Experimentation Guidelines of The University of Tokyo and were approved by the Institutional Animal Care and Use Committee of the Graduate School of Agricultural and Life Sciences at The University of Tokyo. All horses received blood biochemistry tests to analyze myocardial injury markers, including lactate dehydrogenase-11 (LDH-11), creatine kinase-myocardial band (CK-MB), and cardiac troponin I; heart failure markers, including atrial natriuretic peptides (ANPs); and electrolytes, including sodium, chloride, potassium, magnesium and calcium. It was also confirmed that none of the animals had myocardial issues that affect heart rate variability.

An apex-base (AB) lead ECG was recorded using a Holter monitor (SER-102, Softron, Tokyo, Japan) for 90 min during the day, avoiding immediately before feeding to avoid excitement but allowing the horses to move around freely in the stable. The sampling rate was 250 Hz. All ECG waveform components (RR interval, PR interval, QRS duration and QT interval) were continuously analyzed using software (SBP2000, Softron). For each horse, the computer program first detected R waves and obtained the RR interval tachogram as the raw heart rate variability. From this tachogram, a data set of 512 points was resampled at 2.5 Hz, and the frequency component of the RR interval was analyzed based on the Cooley-Tukey Fast Fourier Transform algorithm [3]. Two major spectral components, low-frequency (LF: 0.01–0.07 Hz) power and high-frequency (HF: 0.07–0.60 Hz) power, were detected using software (SRV, Softron). The HF power was used as an index of parasympathetic nerve activity, and the LF/HF ratio was used as an index that reflects the balance between sympathetic and parasympathetic nerve activities [8]. The differences in electrocardiogram and heart rate variability parameters were compared between the Criollos and Thoroughbreds. Permutation p-values were calculated using EZR (Saitama Medical Center, Jichi Medical University, Omiya, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria). Statistical significance was defined as $P<0.05$.

Slight differences were observed between Criollos and Thoroughbreds in P-wave height and S-wave depth. However, there was no difference in other waveform components, such as the R-wave peak, which is important in heart rate variability analysis (Fig. 1).

The RR interval, PR interval, QRS duration, and QT interval values of the Criollos were similar to those in an earlier paper [17]. No significant difference in mean RR interval was observed between Criollos and Thoroughbreds. On the other hand, the PR interval, QRS duration, and QT interval were significantly shorter for Criollos compared with Thoroughbreds (Fig. 2).

The results from the power spectral analysis showed that the HF power tended to be greater for Criollos than Thoroughbreds, although the difference was statistically insignificant (Fig. 3). On the other hand, the LF power was similar for both breeds, and the LF/HF ratio tended to be lower for Criollos than Thoroughbreds (Fig. 3).

Our analysis of ECG waveforms revealed significant differences in PR interval, QRS duration, and QT interval but no difference in RR interval between Criollos and Thoroughbreds. In this study, electrocardiograms were recorded

Fig. 1. Representative ECG waveforms from Criollos and Thoroughbreds.

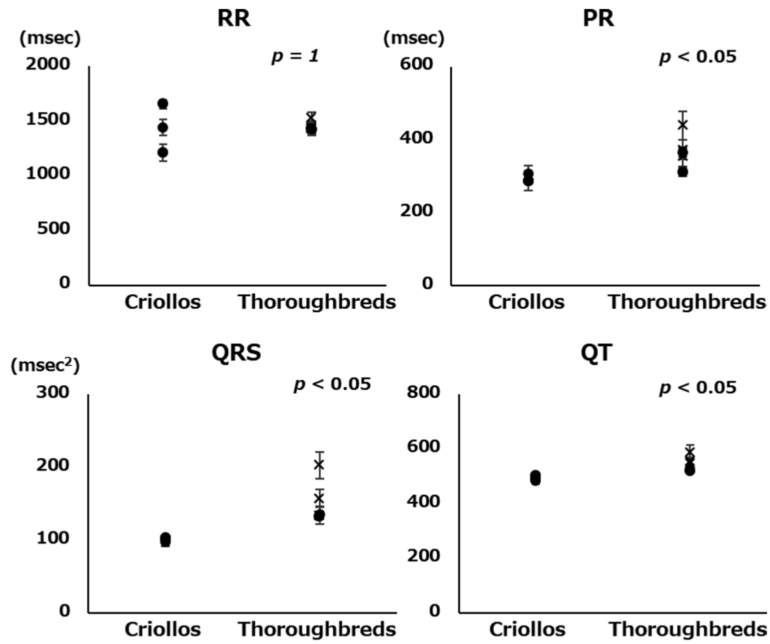

Fig. 2. RR interval, PR interval, QRS duration, and QT interval of the Criollo horses and Thoroughbreds. Each data point and error bar represent the mean and standard deviation of all waveform analyses in each individual animal. Permutation *P*-values between the Criollos and Thoroughbreds are indicated. Symbols: ●, mares; ×, geldings.

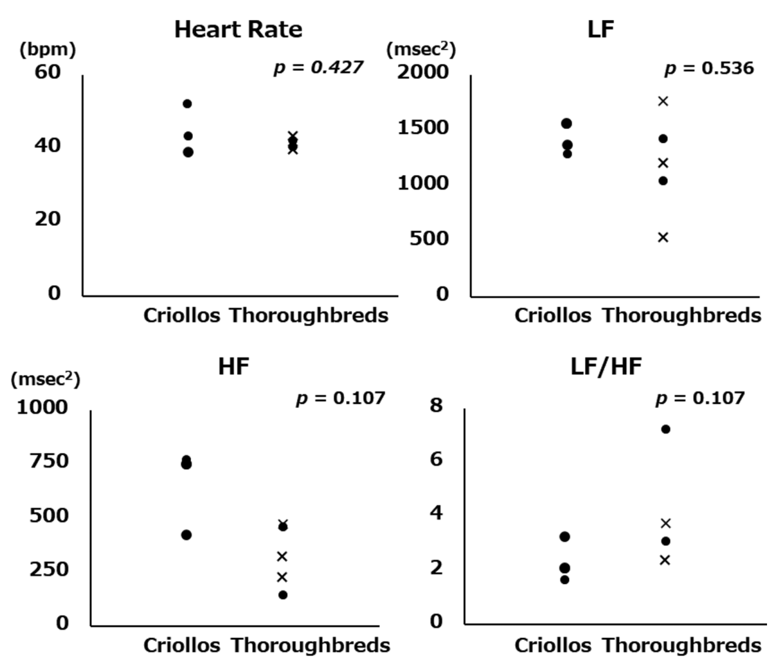

Fig. 3. Heart rate (HR), low-frequency (LF) power, high-frequency (HF) power, and LF/HF ratio of the Criollos and Thoroughbreds. HF power is an index of parasympathetic nerve activity, and the LF/HF ratio reflects the balance between sympathetic and parasympathetic nerve activities. Permutation *P*-values between the Criollos and Thoroughbreds are indicated. Symbols: ●, mares; ×, geldings.

in the AB leads, and it is possible that the magnitudes of the P and S waves were affected by displacement of the electrodes on the apical side. The limb leads are considered better for detailed examination of the waveforms of these parts. On the other hand, it is inferred that slight misalignment of the apical electrode does not affect the analysis results for the RR interval, PR interval, QRS duration, or QT interval. An earlier study reported that Colombian Criollos, which are closely related to Argentine Criollos, have smaller hearts than Thoroughbreds [2]. In our study, the body weights of the Criollos were lighter than those of the Thoroughbreds. This suggests that the differences in ECG parameter readings were presumably due to this difference in heart size rather than differences in autonomic nervous functions.

Furthermore, the results from the power spectral analysis of heart rate variability revealed that the HF power component tended to be higher in the Criollos than in the Thoroughbreds, indicating that parasympathetic nerve activity may be more dominant in the autonomic nervous system in Criollos. The Thoroughbreds used in this study were a mixture of mares and geldings. However, no consistent differences were found between mares and geldings. In addition, heart rate variability is strongly affected by fitness and the respiratory cycle, so in order to interpret the results, it is necessary to consider the exercise habits of the animals and resulting changes in respiratory and cardiovascular functions. The daily exercise habits of the Criollos and Thoroughbreds were almost the same, suggesting that the difference in heart rate variability between the two was not caused by their exercise habits.

Psychological stress induces physiological responses via the activation of the sympathetic nervous system. Kataoka *et al*. reported that the cerebral cortex and limbic system, which process emotions, and the hypothalamus, which regulates the autonomic nervous system, are connected via the dorsal peduncular cortex and the dorsal tenia tecta in the medial prefrontal cortex [6]. This neurotransmission pathway connects the mind and body. They also reported that rats that had this neurotransmission pathway inhibited by photoinhibition showed positive interaction behaviors to stress sources and did not move away from them, whereas normal rats usually exhibited escape behaviors [6]. These earlier findings indicate that temperament and autonomic nervous functions are directly related to physiological phenomena. Therefore, it can be hypothesized that heart rate variability, which reflects autonomic nervous functions, can be an index of temperament and that the dominance of parasympathetic nerve activity in Criollos may be one of the physiological characteristics that underlies their calm temperament. Our preliminary experimental data are consistent with this hypothesis. The results of heart rate variability analysis can be affected the animal's fear of an unfamiliar environment. In this experiment, the Holter electrocardiograph was a novel object for the animal. Therefore, it is thought that the results this time also reflect the reaction to a novel object or environment. The tendency for the HF power to be higher and the LF/HF ratio to be lower in Criollos may suggest that the Criollos were able to better control their emotions in response to a novel object or environment than the Thoroughbreds. However, the heart rate variability analysis was performed only under stress-free conditions in this study, and a statistical difference was not detected because the analysis results were just preliminary data obtained from a small number of samples. In order to better clarify the relationship between animal temperament and heart rate variability, it is necessary to analyze more heart rate variability data samples under stress conditions and look at stress indices such as stress hormone levels in the bloodstream. Moreover, it is known that the equine autonomic nervous system exhibits diurnal variations [9], as well as an alteration of balance with age [11]. A long-term analysis covering different times of the day and stress conditions using horses of different ages might be necessary to confirm that our current findings are indeed breed specific. In this context, our findings could be a step forward in the development of techniques to evaluate equine temperament objectively and quantitatively.

Potential Conflicts of Interest

The authors declare no conflicts of interest.

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