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Behavioral and cardiac responses in horses (*Equus ferus caballus*) exposed to a novel object

낯선 물체에 대한 말의 행동 반응과 심박수 반응

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이 경 은

A Dissertation for the Degree of Master

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Graduate School of
Seoul National University
Veterinary Medicine Major

Kyung Eun Lee

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Advised by Professor Hang Lee

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Graduate School of
Seoul National University
Veterinary Medicine Major

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Abstract

Behavioral and cardiac responses in horses (*Equus ferus caballus*) exposed to a novel object

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This study aimed to investigate whether breed, sex, and age affected the temperament differently (more or less neophobic) in mature horses (*Equus ferus caballus*) when being surprised by a novel object. The study included Jeju crossbred (n = 12, age = 9.42 ± 4.57), Thoroughbred (n = 15, age = 10.73 ± 3.09) and Warmblood horses (n = 12, age = 13.08 ± 3.55) of the females (n = 22, age = 11.36 ± 4.24) and geldings (n = 17, age = 10.65 ± 3.66). A trained experimenter touched the right side of subject necks with a white plastic bag (novel object). The test ended when the escape response stopped and heart rate dropped down to basal levels. During the experiment, we recorded a reactivity score and escape duration as behavioral variables. We also examined multiple heart rate (HR) and heart rate variability (HRV) variables that were intended to represent emotional state: basal HR (BHR), maximum HR (MHR), and duration between timing of MHR and BHR (Tm−Tb); the standard deviation of N−N intervals (SDNN), root mean square of successive R−R intervals (RMSSD), and the ratio of low frequency to high frequency components of continuous series of beats (LF/HF). ANOVA revealed there were significant differences between breed in reactivity score and RMSSD. Post−hoc tests following ANOVA showed that Thoroughbreds had significantly higher reactivity scores (ANOVA: df = 2, P = 0.039) and lower RMSSD (ANOVA: df = 2, P = 0.031) values than Jeju crossbreds. In the breed within sex, significant differences in some parameters were detected. Post−hoc tests following ANOVA showed that reactivity score (df = 2, P = 0.049) and MHR (df = 2, P = 0.025) were significantly higher in female Thoroughbreds compared to female Jeju crossbreds, and RMSSD (df = 2, P = 0.001) was significantly higher in gelding Jeju crossbreds than in both of gelding Thoroughbreds and gelding Warmbloods. None of the behavioral, HR and HRV parameters exhibited sex−based differences, except for escape duration in sex within Jeju crossbreds. Age did not show significant correlations with any behavioral and HR indices but had significant negative correlations with SDNN and RMSSD among three HRV parameters. As previous studies showed, Pearson correlation analysis including all subject horses identified the positive relationship between behavioral reactivity score and MHR, and negative relationship between reactivity score and both of SDNN and RMSSD. When grouped by breed and sex for Pearson correlation analysis, the temperamental characteristics of each group were highlighted. Reactivity score had a significant positive correlation with MHR in all breed and sex groups and Thoroughbreds among breed showed significant negative correlations between reactivity score and SDNN. In addition, Thoroughbreds and females had significant negative correlations between escape duration and SDNN, RMSSD. Our results may contribute to improving the horse utility and safety, by understanding the temperament of a particular horse group and selecting appropriate individuals for certain purpose that requires different levels of reactivity.

Keyword: equine, temperament, behavior, heart rate, heart rate variability, breed, sex, age

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List of Abbreviation

ANOVA: analysis of variance

BHR: Basal heart rate

HR: Heart rate

HRV: Heart rate variability

IACUC: Institutional Animal Care and Use Committee

LF/HF: The ratio of low frequency to high frequency components of a continuous series of beats (power spectrum)

MHR: Maximum heart rate

RMSSD: Root mean square of successive R-R intervals

SDNN: The standard deviation of N-N intervals

Tb: Timing of BHR;

Tm: Timing of MHR

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1. Introduction

The horses have been attacked by the predators as prey animals for million years in the wild, so the susceptible response to the fear-eliciting stimulus was critical for their survival. As a result of the natural selection, the individuals who react agilely and sensitively to the potential danger were merit to survive (Shapira et al., 2008). Being this characteristic that contributes to determining horse temperament inherited, domestic horses respond to the fear-evoking novelty in much the same way as their ancestor horses in the wild (Christensen and Rundgren, 2008). It is known that the variable number of tandem repeat (VNTR) polymorphism of the dopamine D4 receptor (DRD4) gene have something to do with personality trait in human. Yukihide Momozawa (Momozawa et al., 2005) suggested that there was single nucleotide polymorphisms (SNPs) in the VNTR region in a horse which led to the different characteristics between individuals. Soyoung Song (Song et al., 2017) identified the SNPs related to aggressive or docile temperamental traits were in MAOA and AR genes by sequence analysis.

Temperament can be defined as the inborn properties of the nervous system (Sackman and Houpt, 2019). Monika et al. (2018) reported that the temperament of the horses is affected much by the genetic factors. Leiner and Fendt (2011) identified that the innately well−frightened horses have the further possibility to show flight response to another unfamiliar thing or new stimuli even after habituation. Because the fearfulness of horses can disturb their learning in training and can make the horse miss the aids of the riders, the horse' s inborn temperament is an important issue.

In a survey about the reason for the importance of horse temperament to the riders, breeders and leisure riders, the most commonly listed response were the simplification of daily work with the horses (47.9%) and the relationship between horse and human (44.9%) as well as the more comfortable and safer handling (31.5%) (Graf et al., 2013). With these results in mind, selecting the horse with a nice temperament is crucial to both handlers and riders. Therefore, it is necessary to know the temperament of each horse and based on the knowledge, the nervous and timid individuals have to be excluded for riding.

Over the half−century, the studies for evaluating the temperament and emotion of the horses through observing their behavioral and physiological change in a certain situation have been conducted (Visser et al., 2002, Christensen et al., 2005, Lansade et al., 2008, Kozak et al., 2018). In most of those papers, it was revealed that there were significant relations between behavior and physiology, which proved horses appearing highly startled behavior showed more physiological changes to the novel object as well.

Among the physiological parameters, especially heart rate (HR) and heart rate variability (HRV) was identified to reflect the emotional state of the animal very well (Visser et al., 2002, Donzella et al., 2000, Sharpley et al., 2000, Frazier et al., 2004). And the researches figuring out the temperament and emotional state by HR responses have been reported many times in various animal species. (Von Borell et al., 2007, Rietmann et al., 2004, Sgoifo et al., 1997, Mohr et al., 2002, Palestrini et al., 2005, Désiré et al., 2004, Kuwahara et al., 2004). Especially in the horse, E.K. Visser (Visser et al., 2002) implemented the novel object test with an umbrella which was visual stimuli and handling test. The HR and HRV response during the tests were used to detect the traits of each young horse (9, 10, 21, 22 months). Christensen (Christensen et al., 2005) gave visual, auditory, olfactory stimuli to the twenty-four 2-year-old horses and examined the personality of the horses by their behavioral and HR-related actions. Emma Brunberga (Brunberg et al., 2013) performed a fear reaction test with Icelandic horses using a plastic bag.

Heart rate variability (HRV) is an appropriate, non-invasive method to evaluate autonomic nervous system (ANS) regulation of cardiac activity related to stress responses and welfare (von Borell et al., 2007). It reflects an ever changing psycho-physiological state of the animal that is controlled by the parasympathetic (vagal) and sympathetic activity of the ANS (Mohr et al., 2002). The most frequently used HRV parameters are SDNN, RMSSD and LF/HF (von Borell et al., 2007). The standard deviation of all inter-beats intervals (IBIs) (SDNN, ms), which is one of the parameters of HRV, is good predictors of overall variability present at the time of recording. As the total variance of HRV increases with the length of analyzed recording, in practice, it is inappropriate to compare SDNN measures obtained from the IBI data series of varying durations. This parameter reflects long- term variability of cardiac activity and is influenced by both sympathetic and parasympathetic activities. Significantly decreased SDNN was usually found with an increasing level of stress load (von Borell et al., 2007). The root mean square of successive differences (RMSSD) is the informative parameter of HRV time-domain measurement which is determined by calculating the difference between successive IBIs before squaring and summing them, the values are then averaged and the square root obtained. The RMSSD reflects changes in the autonomic nervous system (ANS) that are primarily vagally regulated. Decreased value of this parameter in reaction to external stress and even more to physiological load means a considerable decline of the vagal tone

(Kleiger et al., 1992). Many studies that have demonstrated the usefulness of the ratio of low frequency to high frequency components of continuous series of beats of the power spectrum (LF/HF ratio) as an indicator of sympathetic activity during a number of physical and psychological stresses with an increase in the LF/HF ratio being interpreted as a regulatory shift towards sympathetic dominance (Sloan et al., 1994).

Carlos (Gonzalez-De Cara et al., 2017) conducted five tests for judging the temperament of donkeys which is commonly used animal in assisted therapy and suggested the necessity of further studies in more various horse breed and wider age groups; sound test, tactile test, von Frey filament test, novel object test, fearfulness, and reactivity to human test.

Even though some studies exploring the nature of horse have been conducted so far, there was no study investigated the temperament with both of the behavioral, and HR and HRV-related physiological responses to the unfamiliar complex stimulating object in mature horses in terms of the breed, sex, and age. There have been several studies exploring the relationship between the temperament and gene of the Jeju crossbred horses, but no study existed which considered the relationship between the behavioral, physiological responses and temperament. Recently, as the numbers of people who are interested in horseback riding are increasing, the importance of breeding and training is highlighted in South Korea. Especially, Jeju crossbred horse is a horse breed which was crossbred between Jeju horses and Thoroughbred, and they account for approximately 30% of all horses in South Korea. The Jeju crossbred horses are known to be bold and not sensitive compared with Pony which is a small-sized horse, and is commonly used for youth horseback riding in South Korea. The Jeju crossbred horses deserve being studied considering the large proportion of horse population living in South Korea and their economic significance as a riding horse due to their strong hoof, high feed efficiency and endurance.

This study aims to explore if there are temperamental differences between breed, sex, and age during the novel object test. We analyzed behavioral and HR and HRV-related parameters to make them complement one another and improve the reliability. Three horse types were used that are mostly used for horseback riding in South Korea; Thoroughbred, Warmblood, and Jeju crossbred horses. From the result of this research, the temperamentally most appropriate horse type can be selected and used for targeted purposes to increase the horse utility and safety for both of the horses and humans. Additionally, it will contribute to horse welfare and the economy.

2. Materials and Methods

2.1. Animals and Management

The study included 12 Jeju crossbred horses (eight mares and four geldings), 15 Thoroughbred horses (eight mares and seven geldings), and 12 Warmblood horses (six mares and six geldings). Jeju crossbreds ages ranged 4-21 years (mean ± SD: 9.42 ± 4.57) (Figure 1); Thoroughbreds, 6-15 (mean ± SD: 10.73 ± 3.09); and Warmbloods, 5-18 (mean ± SD: 13.08 ± 3.55). Ages of the horses did not show significant differences within breed and sex. The horses were kept as part of the university teaching horses and used for practical riding classes. They were not ridden more than once a day. On non-riding days they were exercised on a horse-walker for an hour. All horses were housed under comparable conditions in individual box stalls. Water was provided ad libitum by automatic drinkers and horses were fed hay and concentrates three times a day. All of them were checked to ensure that they were in good condition and no obvious cardiac abnormalities were present, which could affect HRV. All procedures were approved by the Seoul National University IACUC (Institutional Animal Care and Use Committee, SNU-190121-3-1).

A

B

C

Figure 1. Pictures of subject horse breeds (A) Jeju crossbred, (B) Thoroughbred and (C) Warmblood horse

2.2. Experimental design

2.2.1. Test environment

Indoor horse−riding place with 30 m x 70 m size was used as a test arena. The test arena building was located right next to the stables. All horses were familiar with this area for more than one year since the horses exercise in the site at least five days a week.

2.2.2. Test objects

For the novel object test, the plastic bag which was first seen to the horses was used. The plastic bag was tied at the end of a slender, flexible stick and the length of it was about 1 m. The plastic bag is a complex stimulus including visual, auditory and tactile senses, and regularly used for such studies. (Christensen.J.W, 2008; Søndergaard, Eva, 2010; Brunberg, Emma, 2013; Hintze and Sara, 2017)

2.2.3. Test Procedures

The test was conducted between 09:00 and 11:00 AM when the test area was not used. For leading of the horses, a halter and chifney bit (for safety) with a 5 m lead rope was used. The horse was led to the testing arena by a previously known handler during the entire experiment. When it entered the area, the HR equipment was attached and 10 min was given for adaptation to the situation. The experimenter stood quietly next to the horse without touching it during the adaptation time. After 10 min, an assistant who is familiar with the test horses measured the HR as a baseline. Subsequently, the experimenter gently touched between the left side of the shoulder and neck of the horse body at a regular term of approximately 1 time/sec. If the horse showed flight responses or was willing to escape, the lead rope was released but the tester still held the end of the rope. When the horse showed no more evasion response with stable standing and the HR dropped back to the individual baseline level, the stimuli were stopped. Through the whole experiment, the second experimenter checked the HR and informed the experimenter when he should stop the touching action.

2.3. Data acquisition

2.3.1. Behavioral observations

An assistant recorded videos of the novel object test at approximately 4 m from the subject horse. Videos were later scored to calculate the duration of escape responses, beginning from stimulus presentation to the lack of an escape response (Table 1).

Table 1. Ethogram of recorded behaviors in the novel object test

Behavioral parameter	Definition	Remarks
Reactivity score	Rated by the experimenter during the test according to the behavioral reactivity scale	Represented as 1–5 scale
Escape duration (s)	The duration between the start and end of an escape response	

The end of an escape response was coded when the test horse stood still for over 15 s and did not exhibit physical signs of fearfulness (e.g., stiff neck muscles and head moving along with the stimulus). A trained experimenter assessed behavioral reactivity using the following scale: 1 (very fearless, no reaction) to 5 (very fearful, showing flight response) (Table 2).

Table 2. Behavioral reactivity score during the novel object test

Behavioral reactivity scale	Description
5	The horse suddenly jumps away from the novel object, typically followed by trotting, galloping, alertness, and possibly snorting; keeps far away from the novel object despite handler encouragement
4	The horse is alert and suddenly jumps away from the novel object, typically followed by trotting, galloping, further alertness, and possibly snorting
3	The horse escapes and exhibits vigilant behavior (elevated neck, head, and ears oriented toward the test stimulus), typically followed by trotting, galloping, alertness, and possibly snorting
2	The horse escapes but is easily restrained
1	The horse does not react to the novel object and approaches it without hesitation

2.3.2. HR and HRV monitoring and videotaping

HR was recorded continuously from 2 min before starting the stimuli to the end of the test with Polar V800 Equine heart rate sensors (Polar Electro OY, Kempele, Finland). The device consisted of an electrode belt with a built-in transmitter and a wristwatch receiver. Water and gel were used to improve the contact between electrode and skin. All horses had experienced this belt equipment several times beforehand for their health examination, so they accepted it immediately. Recordings in the novel object test were exactly 2 min for each horse. Data were analyzed using Kubios HRV standard software 3.2.0. (Biomedical Signal Analysis Group, Finland). HR and HRV measures were conducted after artifact correction based on visual inspection.

For each horse, the following HR and HRV parameters were obtained (Table 3). Base HR (BHR) during the 5 s period before plastic-bag introduction and Maximum HR (MHR) during the test were measured (Figure 2). Latency to reach MHR (Tm-Tb) was calculated as the difference between the timing of BHR (Tb) and MHR (Tm) (Figure 2). Of the HRV parameters, the standard deviation of the beat-to-beat intervals (SDNN), root mean square of the successive differences in ms (RMSSD) and low frequency:

high frequency ratio (LF/HF) were analyzed (Table 3).

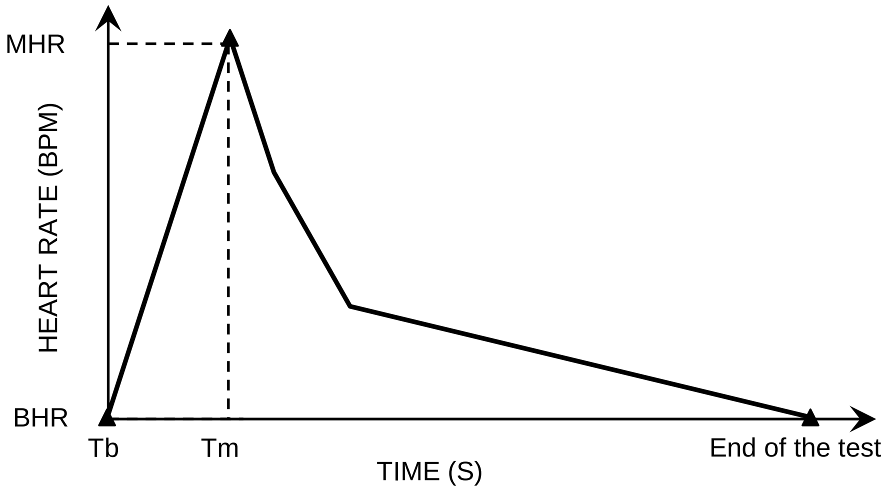

Figure 2. Graph of HR related variables; BHR, basal heart rate; MHR, maximum heart rate; Tb, the timing of BHR; Tm, the timing of MHR.

Table 3. HR and HRV parameters in response to the novel object

Cardiac parameters	Definition
BHR (bpm)	The reference point for further calculations reflecting HR changes upon presentation of novel object
MHR (bpm)	Peak HR during the novel object test
Tm−Tb (s)	Difference between Tb and Tm
SDNN (ms)	The standard deviation of the beat−to−beat intervals
RMSSD (ms)	The square root of the mean squared differences of successive beat−to−beat intervals
LF/HF	The ratio of low frequency to high frequency components of the continuous series of beats (power spectrum)

HR, heart rate; BHR, basal HR; MHR, maximum HR; Tm, timing of MHR; Tb, timing of BHR; SDNN, standard deviation of normal to normal interval; RMSSD, root mean square of the successive difference; LF, low frequency; HF, high frequency.

SDNN measures the state of balance between sympathetic and parasympathetic activities of the heart. RMSSD is normally used to estimate the high frequency beat-to-beat variations that represent vagal regulatory activity. Decreased SDNN and RMSSD indicate a shift toward a sympathetic dominance, while increased values of these parameters reflect a shift towards parasympathetic dominance. LF/HF ratio indicates sympathetic activity during a number of physical and psychological stresses. An increase in the LF/HF ratio means a regulatory shift toward sympathetic dominance (Visser et al., 2002; von Borell et al., 2007).

2.4. Statistical analysis

The distributions of data were evaluated by visualization on QQ−plot and the Shapiro−Wilks normality test. If a normal distribution could not be assumed, data were log−transformed (escaping duration, Tm−Tb, SDNN, RMSSD, and LF/HF) and performed using parametric statistics. Significance was indicated by $P<0.05$.

All response variables were analyzed according to the three independent factors (breed, sex, age). The differences of behavior, HR and HRV parameters between the breeds and the breeds within the sex factor were tested for significance with a one−way analysis of variance (ANOVA). After the null hypothesis was rejected, a post−hoc comparison was performed using Tukey's HSD (honestly significant difference) test. Independent samples T−test was conducted to compare the response variables between the sex and the sex within the breed. Breed × sex interaction was verified using two−way ANOVA and a Tukey's HSD test. Age was analyzed with Pearson correlation analysis. Pearson correlation coefficients between temporal and other numerical parameters were calculated for all individuals, then within breed and within sex.

3. Results

The two-way ANOVA test also showed that the breed × sex interaction was not significant.

3.1. Behavioral responses in the novel object test

3.1.1. Differences among breed

We found that breed had a significant effect on reactivity scores, but not on escape durations ($P < 0.05$; Table 4). Thoroughbreds had the highest reactivity scores, followed by Warmblood and Jeju crossbred horses. Importantly, Thoroughbreds had significantly higher reactivity than Jeju crossbreds ($P < 0.05$), but not than Warmbloods. We also found that female Thoroughbreds were significantly more reactive than female Jeju crossbreds, whereas no breed differences were present in geldings ($P < 0.05$; Table 5). The significant difference of reactivity score between Thoroughbreds and Jeju crossbreds was stronger when comparing breed within females than in all subject horses. Escape duration did not differ among breed, whether overall or within sex.

Table 4. Means ± SD of behavioral parameters, calculated for breed and sex

Type		n	Behavior	
			Reactivity score	Escape duration (s)
Breed	Jeju crossbred	12	2.75 ± 1.01 **b**	1.94 ± 0.22
	Thoroughbred	15	3.73 ± 0.93 **a**	1.99 ± 0.27
	Warmblood	12	3.58 ± 0.95	2.03 ± 0.20
Sex	Female	22	3.45 ± 1.08	1.98 ±0.23
	Gelding	17	3.29 ± 1.02	2.00 ±0.25

Different letters within a single column indicate significance at P < 0.05.
Escape duration was log-transformed for normal distribution.
n, the number of horses used for a particular group.
SD, standard deviation.

Table 5. Means ± SD of behavioral parameters, calculated for breed within sex during the novel object test

Type	n	Behavior	
		Reactivity score	Escape duration (s)
Female	22		
Jeju crossbred	8	2.75 ± 1.09 **b**	1.86 ± 0.21 **x**
Thoroughbred	8	4.00 ± 0.87 **a**	2.07 ± 0.26
Warmblood	6	3.67 ± 0.75	2.01 ± 0.13
Gelding	17		
Jeju crossbred	4	2.75 ± 0.83	2.11 ± 0.13 **y**
Thoroughbred	7	3.43 ± 0.90	1.89 ± 0.26
Warmblood	6	3.5 ± 1.12	2.05 ± 0.25

Different letters within a single column indicate significance at $P < 0.05$.
Escape duration was log-transformed for normal distribution.
n, the number of horses used for a particular group.
SD, standard deviation.

3.1.2. Differences between sex

As for the sex, t−test revealed that there was not any significant difference between female and gelding in behavioral parameters (Table 4) and sex within breed also showed no evident differences, leaving only one exception for escape duration difference in sex within Jeju crossbred horses ($P < 0.05$; Table 5). Female Jeju crossbred horses showed significantly shorter escape duration compared to Gelding Jeju crossbred horses.

3.1.3. Differences among age

Pearson correlation analysis revealed that the age was not related to any behavioral reactions (Table 6).

Table 6. Correlations in parameters studied in the novel object test about the age of horse

	Behavior	
	Reactivity score	Escape duration (s)
age	0.165	0.118

Different letters within a single column indicate significance at P < 0.05.
Escape duration was log-transformed for normal distribution.

3.2. HR and HRV responses in the novel object test

3.2.1. Differences among breed

Among the HR and HRV parameters, RMSSD exhibited significant between−breed differences, with significantly higher values in Jeju crossbreds than in Thoroughbreds ($P < 0.05$; Table 7). Within females, we observed a significant between−breed difference in MHR ($P < 0.05$; Table 8). Specifically, female Jeju crossbreds scored significantly lower MHR than female Thoroughbreds ($P < 0.05$). Among the geldings, the difference between Jeju crossbred horses and other breeds was obvious in RMSSD ($P < 0.05$). Jeju crossbred horses showed significantly pronounced RMSSD figures, followed by Warmblood and Thoroughbred horses in order, and the difference between breed was larger in gelding compared to in all subjects. However, Thoroughbred and Warmblood horses did not have significantly different RMSSD values. Tm−Tb, SDNN and LF/HF did not vary between breed overall or within sex.

Table 7. Means ± SD of HR and HRV parameters, calculated for breed and sex

	Type	n	Heart rate		Heart rate variability		
			MHR (bpm)	Tm-Tb (s)	SDNN (ms)	RMSSD (ms)	LF/HF
Breed	Jeju crossbred	12	132.42 ± 13.31	1.54 ± 0.18	1.90 ± 0.18	1.81 ± 0.14 **a**	0.21 ± 0.19
	Thoroughbred	15	146.07 ± 23.74	1.56 ± 0.22	1.80 ± 0.16	1.67 ± 0.15 **b**	0.38 ± 0.51
	Warmblood	12	137.58 ± 15.54	1.70 ± 0.22	1.81 ± 0.13	1.69 ± 0.13	0.44 ± 0.34
Sex	Female	22	141.50 ± 16.62	1.57 ±0.22	1.80 ± 0.19	1.69 ± 0.17	0.36 ± 0.44
	Gelding	17	136.35 ± 22.28	1.62 ±0.22	1.87 ± 0.12	1.76 ± 0.12	0.33 ± 0.32

Different letters within a single column indicate significance at $P < 0.05$.
Tm-Tb, SDNN, and RMSSD were log-transformed for normal distribution.
n, the number of horses used for a particular group.
SD, standard deviation; BHR, basal heart rate; MHR, maximum heart rate; Tb, timing of BHR; Tm, timing of MHR; SDNN, the standard deviation of N-N intervals; RMSSD, root mean square of successive R-R intervals; LF/HF, the ratio of low frequency to high frequency components of continuous series of beats (power spectrum).

Table 8. Means ± SD of HR and HRV parameters, calculated for breed within sex during the novel object test

		Heart rate		Heart rate variability		
Type	n	MHR (bpm)	Tm-Tb (s)	SDNN (ms)	RMSSD (ms)	LF/HF
Female	22					
Jeju crossbred	8	133.13 ± 10.93 **b**	1.51 ± 0.20	1.86 ± 0.19	1.76 ± 0.14	0.27 ± 0.19
Thoroughbred	8	153.88 ± 16.03 **a**	1.58 ± 0.25	1.77 ± 0.21	1.67 ± 0.19	0.29 ± 0.58
Warmblood	6	136.17 ± 13.58	1.65 ± 0.16	1.77 ± 0.14	1.61 ± 0.13	0.57 ± 0.40
Gelding	17					
Jeju crossbred	4	131 ± 17.03	1.58 ± 0.10	2.00 ± 0.10	1.92 ± 0.08 **a**	0.08 ± 0.09
Thoroughbred	7	137.14 ± 27.65	1.53 ± 0.19	1.83 ± 0.08	1.66 ± 0.08 **b**	0.50 ± 0.38
Warmblood	6	139 ± 17.16	1.76 ± 0.25	1.84 ± 0.12	1.76 ± 0.07 **b**	0.32 ± 0.19

Different letters within a single column indicate significance at $P < 0.05$.
Tm-Tb, SDNN, RMSSD, and LF/HF were log-transformed for normal distribution.
n, the number of horses used for a particular group.
SD, standard deviation; BHR, basal heart rate; MHR, maximum heart rate; Tb, timing of BHR; Tm, timing of MHR; SDNN, the standard deviation of N-N intervals; RMSSD, root mean square of successive R-R intervals; LF/HF, the ratio of low frequency to high frequency components of continuous series of beats (power spectrum).

3.2.2. Differences between sex

No distinct differences existed with regard to cardiac parameters between sex and sex within the breed (Table 9).

Table 9. Means ± SD of HR and HRV parameters, calculated for sex within breed during the novel object test

		Heart rate		Heart rate variability		
Type	n	MHR (bpm)	Tm-Tb (s)	SDNN (ms)	RMSSD (ms)	LF/HF
Jeju crossbred	12					
Female	8	133.13 ± 10.93	1.51 ± 0.20	1.86 ± 0.19	1.76 ± 0.14	0.27 ± 0.19
Gelding	4	131.00 ± 17.03	1.58 ± 0.10	2.00 ± 0.10	1.92 ± 0.08	0.08 ± 0.09
Thoroughbred	15					
Female	8	153.88 ± 16.03	1.58 ± 0.25	1.77 ± 0.21	1.67 ± 0.19	0.29 ± 0.58
Gelding	7	137.14 ± 27.65	1.53 ± 0.19	1.83 ± 0.08	1.66 ± 0.08	0.50 ± 0.38
Warmblood	12					
Female	6	136.17 ± 13.58	1.65 ± 0.16	1.77 ± 0.14	1.61 ± 0.13	0.57 ± 0.40
Gelding	6	139.00 ± 17.16	1.76 ± 0.25	1.84 ± 0.12	1.76 ± 0.07	0.32 ± 0.19

Different letters within a single column indicate significance at $P < 0.05$.
Tm-Tb, SDNN, RMSSD, and LF/HF were log-transformed for normal distribution.
n, the number of horses used for a particular group.
SD, standard deviation; BHR, basal heart rate; MHR, maximum heart rate; Tb, timing of BHR; Tm, timing of MHR; SDNN, the standard deviation of N-N intervals; RMSSD, root mean square of successive R-R intervals; LF/HF, the ratio of low frequency to high frequency components of continuous series of beats (power spectrum).

3.2.3. Differences among age

Age did not affect HR responses but had an impact on some of the HRV indices (Table 10). As a result of correlation tests, when horses got older, the SDNN and RMSSD values decreased significantly ($P < 0.05$). In contrast, there was no effect of age on the LF/HF ratio.

Table 10. Correlations in HR and HRV parameters studied in the novel object test about the age of horse

	Heart rate		Heart rate variability		
	MHR (bpm)	Tm-Tb (s)	SDNN (ms)	RMSSD (ms)	LF/HF
age	0.187	0.267	**-0.488**	**-0.416**	0.073

Different letters within a single column indicate significance at P < 0.05.
Tm-Tb, SDNN, RMSSD, and LF/HF were log-transformed for normal distribution.
BHR, basal heart rate; MHR, maximum heart rate; Tb, timing of BHR; Tm, timing of MHR; SDNN, the standard deviation of N-N intervals; RMSSD, root mean square of successive R-R intervals; LF/HF, the ratio of low frequency to high frequency components of continuous series of beats (power spectrum).

3.3. Correlations between behavioral and HR, HRV parameters in the novel object test

3.3.1. Correlation test with total horses

We found there were some significant correlations between behavioral and HR, HRV responses in all horses and within each breed and sex (Table 11). Reactivity score was positively correlated with MHR among HR parameters ($P < 0.05$), and negatively correlated with both of SDNN and RMSSD among HRV parameters when including all tested horses ($P < 0.05$). However, escape duration was distinctly correlated with none of the cardiac variables.

Table 11. Correlations between behavioral and cardiac parameters in the novel object test, including all subject horses grouped by breed and sex

Behavior			n	Heart rate		Heart rate variability		
				MHR (bpm)	Tm-Tb (s)	SDNN (ms)	RMSSD (ms)	LF/HF
Reactivity score	Total		39	**0.697**	0.232	**-0.321**	**-0.323**	0.220
	Breed	Jeju crossbred	12	**0.807**	0.311	0.126	0.070	-0.462
		Thoroughbreds	15	**0.690**	0.160	**-0.644**	-0.429	0.129
		Warmbloods	12	**0.646**	0.168	-0.140	-0.146	0.569
	Sex	Female	22	**0.638**	0.145	-0.248	-0.298	0.186
		Gelding	17	**0.775**	0.374	-0.470	-0.355	0.286
Escape duration (s)	Total		39	0.126	0.235	-0.278	-0.203	0.166
	Breed	Jeju crossbred	12	-0.096	0.075	0.468	0.500	-0.261
		Thoroughbreds	15	0.076	0.089	**-0.699**	**-0.614**	0.270
		Warmbloods	12	0.434	0.537	-0.430	-0.152	0.064
	Sex	Female	22	0.319	0.026	**-0.451**	**-0.516**	0.220
		Gelding	17	-0.032	0.472	-0.029	0.271	0.087

Correlations significant at $P < 0.05$ are marked in bold.
Escape duration, Tm-Tb, SDNN, RMSSD and LF/HF were log-transformed for normal distribution.
n, total number of horses used for a particular group.
BHR, basal heart rate; MHR, maximum heart rate; Tb, timing of BHR; Tm, timing of MHR; SDNN, the standard deviation of N-N intervals; RMSSD, root mean square of successive R-R intervals; LF/HF, the ratio of low frequency to high frequency components of continuous series of beats (power spectrum).

3.3.2. Correlation test within each breed and sex group

For all breed, the reactivity score was significantly correlated with MHR ($P < 0.05$), showing the highest positive correlation in Jeju crossbreds (Table 11). However, other significant correlations between behavioral and cardiac indices were only detected in Thoroughbreds and not in the other two breeds. In detail, the reactivity score had a significant negative correlation with SDNN ($P < 0.05$) and escape duration also had significant negative correlations with both SDNN and RMSSD in Thoroughbreds ($P < 0.05$).

For the most part, the reactivity score had significant positive correlations with MHR in both sexes ($P < 0.05$), while identifying no correlation with other HR and HRV parameters (Table 11). Escape duration was not significantly correlated with HR variables. However, the negative correlation coefficients were detected between escape duration and both of SDNN and RMSSD in females ($P < 0.05$).

4. Discussion

4.1. Behavioral responses in the novel object test

In the present study, of the behavioral parameters, the reactivity score had significant differences between the breeds but not escaping duration. The significant difference was only between Thoroughbred and Jeju crossbred horses in the reactivity score among the three species. Thoroughbred horses showed significantly higher reactivity scores than Jeju crossbred horses. Jeju crossbred horses showed the lowest reactivity score compared with other breeds, thus it is expected that the Jeju crossbred horses could be used for youth horse riding with relatively higher level of safety and might contribute to the popularization of the horseback riding. As for the breed within sex, the female Thoroughbred showed significantly higher value only in reactivity score than the female Jeju crossbred horses and there was no difference in breed within gelding horses. The P−value was higher in the latter one, the difference in the reactivity score between the female Thoroughbred and the female Jeju crossbred horses, than the former one, the difference in the reactivity score between breeds within females. Oki et al. (2007) revealed that the ratio of the calm horses in males was generally larger than in the females. By inference, females might have more restless characteristics and show active reactivity, thus the difference in females would be more distinct compared with that in male. Regarding the escape duration, the results revealed that there were no significant differences between either breeds or breeds within sex. Going on the test time, all horses finally stopped their escaping responses regardless of breed, but there were significant differences between breeds and breeds within sex on how much they showed initial flight response to the novel object, suggesting that the horse can be highly surprised at the moment when they encounter an unexpected objects−eliciting situation even while riding. For this reason, when riding the Thoroughbred horses, especially the female one, the rider is recommended to check the surroundings and careful attention is needed.

Graf et al. (2014) conducted the novel object test with Thoroughbred, Warmblood, draft horses, heavy warmblood, Ponies, other breeds and found that there were significant differences between some species in behavioral reactivity score. Distinct differences were revealed between Warmblood and Pony, Warmblood and draft horse, but there were no significant differences between Warmblood and Thoroughbred. Warmblood horses showed lower reactivity scores to overall test parameters than other species. Takeda et al. (2017) revealed that as the weight of calves increased, the calmer they were. The animals in this study and the previous one differed in weight and feeding environment, which could lead to a different result. Besides, even though both Ponies and Jeju crossbred horses were small-sized horses, they had different temperaments. Ponies were identified to be restless and nervous (Napolitano et al., 2008) whereas Jeju crossbred horses were generally known to be less fearful and bold characteristics.

As for the sex factor, there were no significant differences between sex and sex within the breeds in behavioral parameters of the test. These results were in agreement with the findings by Von Borstel et al. (2011) who revealed there were no clear differences between sex (mare vs gelding) in a horse temperament test which evaluated behavioral and physiological response of the riding, led and released free horses. In horse performance test conducted by Graf et al. (2014), which compared the temperament of horses by their behavior, the significant differences between stallion & mare, and stallion & gelding were confirmed, but no difference was found between mare & gelding. By inference, because of castration, the geldings cannot produce or produce less testosterone which largely affects the behavioral sexual traits, and it might be the reason for the little temperamental differences in mare and gelding. In addition, there were no behavioral differences between sex in the breed.

The impact of age on temperament has still been in controversy. A previous study consisted that the older horses showed a significant higher behavioral reactivity to the novel stimuli than the younger ones (Graf et al., 2014). On the contrary, another study (Visser et al., 2002) reported that the frequency in the behavioral parameters reduced with increasing age when conducted novel object test with horses under 22 months. The present study could not find significant correlations between age and behavioral parameters. The result was somewhat in line with the study by Wolff et al. (1997) who reported that age did not influence the horse temperament.

It was reported that mature 'schooled' horses did not always exhibit typical behavioral responses, because they were trained not to run away without human order. However, younger horses who had not learned to control their flight instinct tended to run away when they were surprised (Munsters et al., 2012). Therefore, some level of correlations between age and behavioral responses could be found in premature horses, but as they grew, the training effect was able to shadow their reaction by nature to the unfamiliar stimuli. Therefore, analyzing physiological parameters with behavioral responses seemed to give more accurate information.

4.2. HR and HRV responses in the novel object test

All results about cardiac activity brought to the conclusion that Jeju crossbreds were more sensitive than Thoroughbreds not only in breed overall but also within both sexes. HRV is often used with HR parameters to get more reliable data on the stress degree of horses. Individuals exposed to a stressful situation have a lower vagal tone which means more vulnerable to stress. Normally, increased HR goes with vagal withdrawal (Sato et al., 2004) and this could be shown in the present study by the lower RMSSD in Jeju crossbred horses, compared to Thoroughbred. The lower RMSSD value of Jeju crossbred was more detective when making a comparison among breeds in the gelding. In addition, an increased HR but not in LF/HF ratio to stress-related test was reported by Visser et al. (2002), which was in line with the present study. Considering the changes in the sympathetic system represented the arousal dimension of effect (Yeates and Main, 2008), we propose that Jeju crossbreds are less astonished by the novel object compared with other breeds. Among breed within sex, the frequency of significant difference between breed in behavioral, HR and HRV parameters was greater within females compared to within gelding, meaning the temperamental difference was slightly more distinct in breed within a female.

We did not detect significant sex differences in HR and HRV responses, either overall or within a breed. A previous mare vs. gelding temperament tests evaluated using behavioral and cardiac responses when they were ridden, led, or roaming free, did not find clear differences between sex (König von Borstel et al., 2011). And another study examining sex differences in novel–object response among young horses (9-22 months old) (Visser et al., 2002), revealed that sex also did not affect HR variation. Considering the geldings are similar to premature male horses in terms of testosterone production, the lack of an observable sex difference in HR and HRV responses of our study confirms these previous findings. Whereas age was not significantly correlated with behavioral and HR–related parameters in this study, it had negative correlations with some HRV related values. A low degree of SDNN and RMSSD usually appeared in sympathetic activation associated with stressful circumstances. Our results share some similarities with Paolo’ s findings (Baragli et al., 2014). In the study, the younger horses showed a significantly higher frequency of avoidance to the stressful stimulus while the older subjects were less reactive and showed a shift toward control by the sympathetic nervous system, as indicated by lower heart rate variability.

With the previous study in mind (Munsters et al., 2012), our result could be explained that even if the older horses did not reveal or showed lower behavioral and heart rate related responses because of being educated compared to younger horses, they seemed to be more nervous to the unfamiliar thing than young horses.

4.3. Correlations between behavioral and HR, HRV parameters in the novel object test

4.3.1. Correlation test with total horses

Many studies reported that there were significant correlations between behavioral and HR, HRV variables during novel object test in horses (Christensen et al., 2005; Dai et al., 2015; Jezierski and Górecka, 1999; Wilk et al., 2016). Our study was in good agreement with the previous researches in that reactivity score and MHR were highly correlated in all horses grouped regardless of breed or sex. Given previous evidence that HR represented fear in the novel object test (Visser et al., 2002; von Borell et al., 2007), our results suggested that the stronger the behavioral response, the more fear horses felt. The previous finding (König von Borstel et al., 2011) proved the best correlations between RMSSD and both reactivity and the time to calm down during the suddenly moving stimulus. In our study, the significant correlation between the reactivity score and SDNN, RMSSD was consistent with previous research, but escape duration did not show any significant correlation with cardiac parameters.

4.3.2. Correlation test within each breed and sex group

When the correlations between behavioral and cardiac parameters were analyzed in terms of breed and sex, the results highlighted the characteristics of each group. As numerous studies mentioned before (Dai et al., 2015; Wilk et al., 2016) and the result with all individuals in our study showed, the significant positive correlation also was identified between reactivity score and MHR in all breed and sex groups.

Among the breeds, Thoroughbreds showed the highest frequency in a significant correlation between behavioral and cardiac parameters whereas other breeds did not have significant correlations apart from reactivity score and MHR. The decreased HRV indices, SDNN and RMSSD, means a shift of the autonomic balance toward sympathetic component dominance, which is from the lack of a sufficient parasympathetic counteraction to sympathetic activation (von Borell et al., 2007). Horse with a low degree of parasympathetic tone has been interpreted as a stressful context in the previous studies (Janczarek et al., 2018; Visser et al., 2002). Hence, individuals showing flight responses and longer escape duration could be considered as getting higher stress to the novel stimuli in Thoroughbreds.

We observed significant positive correlations between reactivity score and MHR in both sexes, which supported the previous finding (Christensen et al., 2005) and no other significant correlations existed between reactivity score and cardiac indices. With escape duration, the significant negative correlations with SDNN and RMSSD pointed out the temperamental traits of the female group. Decreased SDNN and RMSSD values mean a shift toward sympathetic component dominance and lower parasympathetic nerve activity. A high parasympathetic control function enhances the responsivity to environmental change and a low degree of parasympathetic tone is connected to panic. Our findings suggested that females showing the longer escaping duration from novel stimuli were thought to be more stressed. This could be explained by the evolutionary basis that the female's need to protect her foal in the wild. They ran far away from the potential danger and a cautious temperament was inherited by natural selection.

Considering that HRV is a valid method to assess autonomic nervous system (ANS) regulation of cardiac activity related to stress responses and welfare, we could identify the longer escape duration to the unfamiliar object meant the higher stress, even though it was not expressed by behavioral or HR responses, especially in Thoroughbreds and female. Therefore, when riding the Thoroughbred or female one, the rider needs to be more vigilant and cautious. Additionally, if the horse shows escaping or denying gestures from an object or situations continuously, the rider should stop pushing it and check the surrounding environment. The findings of this study will help clarify which horse responded less or more sensitively to the novel object according to breed, sex, age and infer the temperament of each horse group. It may contribute to not only horse welfare and safety but also the economy. In addition, the risk involved in horseback riding would be diminished by selecting appropriate individuals depending on the rider’s level and predicting the temperamental traits of the horse.

5. Conclusion

In conclusion, our study identified obvious differences between Thoroughbred and Jeju crossbred horses in behavioral, HR and HRV parameters when responding to novelty. All differences supported the significant bigger response and stress degree in Jeju crossbreds compared with Thoroughbreds, in overall and within sex. Warmbloods were intermediate in their responses and did not show striking differences compared to the other two breeds. Concerning sex, there was no an obvious difference between sex and sex within a breed. Age was not significantly correlated with behavioral and HR parameters but negatively correlated with two HRV indices, SDNN and RMSSD. It is thought that training effect shadows the natural responses to the stimuli as the horse gets to age. In the correlation analysis, the reactivity score had a significant positive correlation with MHR in all groups. Thoroughbred among breed and female between sex were identified that their behavioral responses to the novel object were closely related to panic and stress. These findings may contribute to not only horse welfare and safety but also the economy.

6. Implication

Like many other traits, temperament has both environmental and genetic components. In this study, we minimized environmental factors as much as possible through testing horses that had resided on a single farm for at least 1 year by the time of the experiment or were born there. However, differences in the recent living background among some of the subject horses before the experiment could lead to environmental effects overshadowing hereditary effects in the temperament test. Therefore, studies focusing on detailed environmental factors are necessary for a better understanding of horse temperament.

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국문 초록

본 연구는 성숙한 말(*Equus ferus caballus*)에게 놀람을 일으키는 낯선 자극을 가했을 때 행동, 심박수 그리고 심박수 변이의 변화가 품종, 성별, 나이에 따라 차이가 있는지 알아보는 것을 목표로 하였다. 실험한 말은 한라마 12마리(나이 = 9.42 ± 4.57), 더러브렛 15마리(나이 = 10.73 ± 3.09), 웜블러드 12마리(나이 = 13.08 ± 3.55)고, 이 중 암말은 22마리(나이 = 11.36 ± 4.24), 거세마는 17마리(나이 = 10.65 ± 3.66)였다. 본 실험에서는 시각, 청각, 촉각적으로 복합적인 새로운 자극원이 될 수 있는 하얀색 비닐봉지를 실험 대상마의 오른쪽 목 부위를 1회/1초 속도로 가볍게 두드리듯이 접촉시켰다. 실험 종료 시점은 말의 도피반응이 완전히 멈추고 심박수 또한 안정기 수준으로 도달한 때로 하였다. 행동반응 지표로는 반응점수(reactivity score)와 도피반응지속시간(escape duration)을 기록하였고, 심박수 지표로는 안정기 심박수(BHR), 최대심박수(MHR), 안정기 심박수(BHR)와 최대 심박수(MHR)일 때의 시차(Tm−Tb)를 기록하였으며, 심박수 변이 지표로는 NN간격 표준편차(SDNN), 인접한 RR간격 차이를 제곱한 값의 평균의 제곱근(RMSSD), 저주파/고주파 비율(LF/HF ratio)을 계산하여 기록하였다. ANOVA에 따른 사후검정 결과, 더러브렛은 한라마에 비해 반응점수(df=2, P=0.039)가 유의하게 높고 RMSSD (ANOVA: df=2, P=0.031)는 유의하게 낮은 값을 보였다. ANOVA로 성별 내 품종을 봤을 때, 각각 암말과 거세마 내에서 더러브렛과

한라마가 몇 가지 변수에 대해 뚜렷한 차이를 보였다. 더러브렛 암말(ANOVA: df=2, P=0.049) 이 한라마 암말보다 유의하게 높은 반응점수(ANOVA: df=2, P=0.049)와 MHR(ANOVA: df=2, P=0.025)을 보였고 거세마 내에서는 한라마 거세마가 더러브렛 거세마와 웜블러드 거세마 모두에 비해 현저하게 높은 RMSSD (df=2, P =0.001)를 나타냈다. 성별의 경우 한라마 거세마가 한라마 암말보다 유의하게 긴 도피반응 지속시간을 보이는 것(t-test: df=8.9461, P=0.043) 빼고는 전체 개체 및 각 품종내 성별에 있어 차이가 없었다. 나이는 SDNN, RMSSD와 유의한 음의 상관관계를 보였는데 저하된 SDNN과 RMSSD는 스트레스에 의한 것이므로 나이가 많을 수록 낮선 물체에 대해 더 많은 스트레스를 받음을 의미한다. 행동과 심박수 관련 변수들 간의 Pearson 상관분석 결과, 전체 개체에 대해서는 반응점수와 MHR 사이에 유의한 양의 상관관계가 있었고 반응점수와 SDNN, RMSSD 간에는 유의한 음의 상관관계가 있었다. 품종과 성별에 따라 집단을 나누어 상관분석을 했을 때 집단 내 성향과 각 집단 간 성격 차이가 뚜렷하게 보였다. 반응점수와 MHR은 모든 품종과 성별 집단에서 유의한 양의 상관관계를 보였고 특히 품종 중 더러브렛과 두 성별 중 암말의 경우 도피반응지속시간이 길수록 SDNN, RMSSD 수치가 유의하게 낮은 것으로 나타나 스트레스를 많이 받는다는 결과가 밝혀졌다. 본 연구 결과는 특정 집단 별 마필의 성격을 좀 더 잘 이해하고, 필요로 하는 말의 반응정도 등 목적에 따라 적절한 개체를 선별함으로써 말의 용도나 승마시의 안전성, 말의 복지를 높이는데 기여할 수 있다고 생각된다.

주요어: 말, 성격, 행동, 심박수, 심박수변이, 품종, 성별, 나이

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