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Behavioural, endocrine and cardiac autonomic responses to a model of startle in horses

Julia Dias Villas-Boas[a], Daniel Penteado Martins Dias[b], Pablo Ignacio Trigo[c], Norma Aparecida dos Santos Almeida[a], Fernando Queiroz de Almeida[c], Magda Alves de Medeiros[a,*]

[a] Department of Physiological Sciences, Federal Rural University of Rio de Janeiro, BR 465 KM 7, 23890 000, Rio de Janeiro, Brazil
[b] Department of Physiology, School of Medicine of Ribeirão Preto, University of São Paulo, 14049-900, Ribeirão Preto, SP, Brazil
[c] Veterinary Institute, Federal Rural University of Rio de Janeiro, BR 465 KM 7, 23890 000, Rio de Janeiro, Brazil

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ABSTRACT

Startle is a fast response elicited by sudden acoustic, tactile or visual stimuli in a variety of animal species and in humans. The magnitude of startle response can be modulated by external and internal variables and can be a useful tool to study the sensory-motor integration in animals. Different stimuli have been used to induce startle in horses, which makes it difficult to compare the responses to these different approaches. The present study uses ultra-short-term heart rate variability (HRV) analysis to characterize the cardiac autonomic modulation, reactivity assessment and blood cortisol measurements to describe the behavioural and endocrine responses to a simple, easy to replicate, effective and safe method of startle (an umbrella is abruptly opened near the horse). The ultra-short-term (64 s) heart rate (HR) series were interpolated (4 Hz) and divided into 256 points segments then the spectra calculated (Fast Fourier Transform). The spectra were then integrated into low (LF; 0.01–0.07 Hz; Index of Cardiac Sympathetic Modulation) and high (HF; 0.07–0.50 Hz; Index of Cardiac Parasympathetic Modulation) frequency bands. Following the startle test, the HR ($p = 0.0101$), the power of the LF band of the cardiac interval spectrum ($p = 0.0002$) and the LF/HF ratio ($p = 0.0066$) were found to be higher, whereas the power of the HF band of the cardiac interval spectrum was found to be lower ($p = 0.0002$). Also, the horses showed a noticeable escape response, with latency of reaction varying from 0.28 to 1.28 s, duration of reaction ranging from 1.52 to 7.92 s and escape distance covered varying from 3.43 to 9.97 m. However, the endocrine measurements failed to reveal significant changes in the cortisol levels after the startle test. We conclude that the startle test used in the current study was effective to produce changes in behavioural parameters and cardiac autonomic modulation of the horses and can therefore be an appropriate tool for neurobiological studies. Furthermore, the use of ultra-short segments (64 s) for HRV analysis appears to be effective and promising for the detection of mental stress in horses.

1. Introduction

Startle responses are defensive reflexes induced by unexpected and intense stimuli, which are characterized by coordinated eyelid closure and contraction of face, neck, foreleg and hind leg. Startle is also associated with increases in heart rate (HR) and arrest of other on-going behaviours. Although several kinds of stimuli (acoustic, tactile or visual) can induce startle in animals and humans, the acoustic startle response (ASR) has been investigated the most (Koch, 1999). The ASR is a proven, reliable and accurate approach to investigate the brain mechanisms of learning, memory, emotions and movement control since the magnitude

Abbreviations: ACTH, adrenocorticotropic hormone; ASR, acoustic startle response; FFT, Fast Fourier Transform; HF, high frequency; HR, heart rate; HRV, heart rate variability; LF, low frequency; PSD, power spectral density; VLF, very low frequency.

* Corresponding author. Tel./fax: +55 2126823222.
E-mail addresses: juliavillasboas@yahoo.com.br (J.D. Villas-Boas), danielpenteado@gmail.com (D.P.M. Dias), pablotrigo@hotmail.com (P.I. Trigo), normaalmeida@gmail.com (N.A.d.S. Almeida), almeidafq@yahoo.com.br (F.Q.d. Almeida), magda.medeiros@gmail.com (M.A.d. Medeiros).

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of ASR can be increased or decreased by a variety of pathological conditions and experimental manipulations (Davis, 1990; Koch, 1999). For example, changes in emotional and perceptual homeostasis, i.e. conditioned and unconditioned aversive events, can enhance the magnitude of ASR (Bradley et al., 1990; Lang et al., 1990). Alternatively, the repeated application of startling stimuli, prior to the presentation of a prepulse (prepulse inhibition) or a pleasant emotional context (Lang et al., 1990; Schmid et al., 1995) may lead to attenuated startle responses (Koch et al., 1996).

Besides the behavioural response, startle induces autonomic and endocrine changes. Literature shows that the transitory increase (<60 s) in HR induced by startle is consistently observed in different experimental animals and is mediated by the sympathetic and parasympathetic divisions of the autonomic nervous system (Baudrie et al., 1997; Vila et al., 2007). On the other hand, the startle-induced increase in corticosterone levels is not observed in all strains of rats (Glowa et al., 1992). A combined study of these responses is important for a better understanding of the physiological effects of startle tests on horses since the magnitude of autonomic, endocrine and behavioural responses to a stimulus cannot always be correlated.

Startle responses are frequently observed in horses; furthermore, the analysis of startle reactions in equines is important as it can be a useful tool to assess stress and welfare. Startle tests have been combined with other measurements to predict temperament in horses. The literature suggests that a horse's reaction to novelty, suddenness and to social isolation might be associated with a general trait of "fearfulness" (Lansade et al., 2008). Excessive reactions of fear can hamper the use of horses, and can even pose a risk to the animals themselves and people. Furthermore, exaggerated fearful reactions have also been associated with an impaired learning ability of horses (Heird et al., 1986).

Different types of stimuli have been used to produce suddenness or startle in horses. The umbrella opening, a relatively common method, has been used in different ways. To study the existence of a "fearfulness" trait in horses and the effect of social isolation on the emotional reactivity, Lansade and colleagues used the umbrella opening when horses were eating (Lansade et al., 2008; Lansade et al., 2012). Other authors have induced the startle reaction by opening a coloured umbrella while the horses were walking to evaluate the influence of soy lecithin and corn oil diet on the behaviour (Holland et al., 1996). To study the effect of habituation and active human handling, an umbrella was manually opened when horses were released in an arena or when held on a lead rope by the handler (Górecka et al., 2007). HR and the heart rate variability (HRV) were analysed in young horses using a Novel Object test, in which an umbrella was lowered from the ceiling (Visser et al., 2002). Furthermore Anderson and colleagues with the aim to find appropriate methods of selecting horses for therapeutic riding programs used the umbrella opening between a series of other stimuli (a walking and vocalizing toy pig and a balloon popping near the horse). In this case the umbrella was opened by a handler standing in front of the animal (Anderson et al., 1999).

The variation in the methods used to produce startle makes the comparisons between the parameters studied difficult. Therefore the present study proposes the use of ultra-short-term HRV analysis to assess the cardiac autonomic responses to a simple, easy to replicate and effective method of startle - an umbrella is abruptly opened near the horse. Our hypothesis is this method of startle produces well-defined behavioural and autonomic responses in equines, and the ultra-short-term HRV analysis can be used to characterize this autonomic response.

2. Methods

2.1. Animals

Six Brazilian Sport horses (3 males and 3 females; 6–8 years old; 450–550 kg in weight), with appropriate body condition scores (between 5.0 and 5.5) from the Brazilian Army Riding School were used in the experimental protocols. The sample size used was based on previous studies and on the variability of the parameters studied. These horses had been undergoing eventing training since they were 5 years old and followed a 6-day-week training routine including galloping, jumping and dressage exercises. They were housed in 4×4 m individual masonry box stalls, with water dispenser, feeder and wood shavings bedding. The stall doors allow visual contact between horses. The horses were fed with concentrated coast-cross hay and had free access to tap water.

All experimental procedures were approved by the Committee on Animal and Human Research and Ethics of the Federal Rural University of Rio de Janeiro/COMEP-UFRRJ/Brazil (protocol #230833.002064/2012-10).

2.2. Experimental design

Early in the morning (0600–0700 h) a heart monitor (RS 800 G3, Polar, Kempele, Finland) was strapped to the chest of the horses to record the HR, beat-by-beat and then the baseline blood samples (S1) were collected. The animals were then left to rest quietly for 20 min in their stalls. Next, each horse was taken individually to a covered arena (70×30 m, known by the animals and often used for dressage exercises) and subjected to the startle test, the abrupt opening of an umbrella. Briefly, the horse was led by a known handler and positioned at a predetermined location, with its back to a low wall (70 cm high) that surrounds the arena and held loosely by its lead rope. The horse was left undisturbed until signs of quietness and inattention were seen (no attempts to escape or other significant movements). Then, a rainbow coloured umbrella (diameter of 70 cm) was suddenly opened and spun for 2 min by a person that was hidden behind the wall at a distance of approximately 1.5 m from the rump of the animal. The umbrella was positioned clearly in the visual field of the animal (an angle of approximately 45 degrees to the tail of the horse, Fig. 1A). Following the test, the horse was kept in the arena, by the lead rope for an additional 3 min in order to record the behavioural responses on a videotape for analysis. After which the horse was returned to its stall and blood samples were collected at 30 and 60 min following the startle test (Fig. 1B).

2.3. Behavioural analysis of reactivity

The horses were videotaped with a camera (SDR H20, Panasonic, Tokyo, Japan) positioned on a tripod in the arena at a distance of about 20 meters. The images were later processed and analysed by computer (ImageJ, U.S. National Institute of Health, http://rsb.info.nih.gov/nih-image). The behavioural analysis was done according to (Redondo et al., 2009); three parameters were assessed: (1) Latency of reaction: time between the beginning of the test and the first reaction of the animal; (2) Duration: total time spent in the motor response to the stimulus; and (3) Covered distance: displacement of the animal in response to the stimulus.

2.4. Cortisol analysis

Blood samples from the jugular vein were collected in SST Vacutainer® tubes. Following the collection, the blood was centrifuged for 10 min at 3200 rpm. The serum (~3 mL) was collected in plastic tubes and kept at −20 °C. Serum cortisol concentrations were determined, in duplicate, by a double antibody

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