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Original Research

Pharmacopuncture Versus Acepromazine in Stress Responses of Horses During Road Transport

Tatianne Leme Oliveira Santos Godoi DVM, MSc [a], Julia Dias Villas-Boas DVM, MSc [a], Norma Aparecida dos Santos Almeida PhD [a], Pablo Ignacio Trigo DVM, PhD [b], Fernando Queiroz de Almeida DVM, PhD [b], Magda Alves de Medeiros DVM, PhD [a,*]

[a] *Department of Physiological Sciences, Universidade Federal Rural do Rio de Janeiro, Rio de Janeiro, Brazil*
[b] *Veterinary Institute, Universidade Federal Rural do Rio de Janeiro, Rio de Janeiro, Brazil*

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ABSTRACT

Acupuncture has been shown to have the beneficial effect of reducing stress responses in animals and humans. Pharmacopuncture is the injection of subclinical doses of drugs into acupoints to give therapeutic results without side effects. This study compared the effects of injecting the usual dose of acepromazine (ACP; 0.1 mg/kg, intramuscularly [I.M.]) with those of pharmacopuncture (1/10 ACP dose at the governing vessel 1 [GV 1] acupoint) on the stress responses of healthy horses undergoing road transport for 2.5 hours. Four different treatments were applied immediately before loading, with 8 animals/treatment: injection of saline or ACP (0.1 mg/kg, I.M.) at the base of the neck; and injection of saline or 1/10 ACP (0.01 mg/kg) at the GV 1 acupoint. The road transport increased heart rate (HR), respiratory rate, body temperature, and serum cortisol of the untreated horses (injected with saline at the base of the neck). Pharmacopuncture at GV 1 reduced the average HR and transport-induced increase in HR at unloading, without changing the other variables. On the other hand, ACP (0.1 mg/kg) produced significant sedation and reduced the transport-induced increase in respiratory rate but without preventing the stress-induced increase of cortisol. Other acupuncture points and drugs should be tested to verify the beneficial effect of this therapy to reduce stress in horses during road transport.

1. Introduction

Transport of horses for racing competitions, breeding, recreation, sale, and slaughter has increased significantly over the years. During transport, the animals are subjected to a wide range of stress factors including loss of balance, isolation from herd, forced proximity to unfamiliar or aggressive horses, novel or threatening surroundings, exposure to new pathogens, restraint, extremes in temperature, water and feed deprivation, and dust and particulate matter from the road [1]. As a result, transport can induce acute changes such as increases in heart rate (HR), respiratory rate (RR) [2-5], concentration of cortisol [2-4] and ACTH [6], and dehydration [7]. Transport can also increase the potential risk of infectious diseases [8] and is considered a contributing factor to the pathogenesis of the respiratory disease commonly known as shipping fever [9]. Therefore, strategies to minimize stress during transport should be considered in order to maintain the well-being of horses and to avoid any serious consequences to their health and welfare.

A common stress response will combine behavioral and physiological responses, and its magnitude will depend on several endogenous and exogenous factors, as well as the duration and intensity of the stressors. The physiological parameters used to measure stress responses include

* Corresponding author at: Magda Alves de Medeiros, DVM, PhD, Department of Physiological Sciences, Universidade Federal Rural do Rio de Janeiro, BR 465 KM 7, 23890 000 Seropédica–RJ, Brazil.
E-mail address: magda.medeiros@gmail.com (M.A. de Medeiros).

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hormone levels, white blood cell profiles, and changes in HR and respiratory rate (RR). The parameters most commonly used to study transport-induced stress are autonomic changes (HR and HR variability) and hypothalamus-pituitary-adrenal axis activation (mainly cortisol level) [4]. However, there is no one parameter able to quantify the stress response in all situations, suggesting that the physiological response to stress is the result of a complex interaction of many systems.

Acepromazine is a phenothiazine derivative commonly used in horses as a sedative during transportation and for clinical or surgical procedures. However, it might cause cardiorespiratory depression and severe hypotension [10], as well as prolonged paralysis of the penis retractor muscle [11]. Furthermore, acepromazine is classified as a controlled substance in many racing and equestrian jurisdictions [12].

Acupuncture has been used to reduce stress responses in humans and animals [13-15]. Pharmacopuncture, the injection of subclinical doses of drugs or small amounts of extracts of medicinal materials at acupoints, is an alternative method to stimulate acupoints. According to traditional Chinese medicine, this technique combines the efficacies of acupuncture and drugs by enhancing the mechanical stimulus at acupoints and producing effects similar to those with conventional doses [16]. In large animals, it reduces the undesirable side effects of drugs, residues in animal products and treatment costs and duration of restraint. Another advantage of pharmacopuncture is the use of ordinary materials like hypodermic needles [17,18]. The administration of a microdose of prostaglandin F2α in BaiHui (lumbosacral space) induces luteolysis, while it significantly decreases the side effects associated with conventional dosing [19]. A subclinical dose of acepromazine (Acepran 1%; Vetnil, Brazil) at the Ho Hai or Chang Qiangacu point (governing vessel 1 [GV 1]) produces a mild sedation (reduced excitement and reactivity to different stimuli) compared with the conventional dose of acepromazine [17].

Based on the hypothesis that stimulation of acupuncture points may reduce transport-induced stress, this study compared the effects of pharmacopuncture at the GV 1 acupoint, aquapuncture (injection of saline) at the GV 1, and acepromazine on transport-induced stress responses in horses.

2. Materials and Methods

2.1. Animals

A total of 16 stallions, 26-53 months old (average, 39.1 $\pm$ 10.7 months old), Brazilian Manga larga Marchador breed, with good body condition scores were selected from the horse herd kept by the Federal Rural University of Rio de Janeiro (UFRRJ). The animals were kept in stalls overnight and released into pastures during the day and did not have any work-related activities to perform during the experiment. The horses were familiar with each other and had known each other for at least 2 years before this study. The present experiment was approved by the committee for the use of human and animal subjects in teaching and research ethics of the Federal Rural University of Rio de Janeiro (COMEP-UFRRJ number 23083.003626/2011-61).

2.2. Experimental Design and Horse Transport

The transport was carried out on four different days (one trip/day) in the back of an open truck (2.46 $\times$ 5.76 m) (Fig. 1). The 16 stallions were divided into two sets of 8 animals each. Each set was transported twice with a week interval between trips. For each trip, each set of 8 animals was divided into four experimental treatments (see 2.3. *Experimental groups and treatments*), giving 2 animals per treatment per trip. The horses were independently tied to the sides of the truck by halters, and each horse was allotted a different experimental group and position on the truck (order of loading) for the second trip (Fig. 1A). Although the horses had been adapted to loading, they had had no recent transport experience. All experimental procedures were performed in the morning between 8:30 AM and 12:00 PM to avoid the diurnal rhythm of cortisol release.

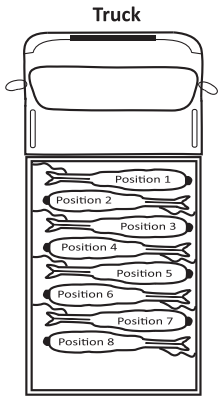
On the day of the experiment, the first action was to fit all the horses with a heart monitor (model RS 800 G3; Polar, Kempele, Finland), each with a different codification signal to prevent interference. Subsequently, RR (thoracic observation) and rectal temperature were taken, and blood samples (S1) were collected (Fig. 1B). Afterward, each animal received the treatment it had been assigned to and was immediately loaded onto the truck calmly and without the use of force. The loading did not last more than 90 seconds per horse. They stood transversally to the vehicle axis, and each animal was faced alternately in the truck. Transport was started immediately after loading on a two-way national road in Seropedica, Rio de Janeiro state, Brazil (highway BR 465) in the region around the Federal Rural University of Rio de Janeiro for 2.5 hours at speeds of up to 60 Km/h. The region is predominantly flat, with small towns and moderate traffic. All trips took place on sunny days, and the temperature was between 27°C and 30°C and 75%-80% relative humidity.

At the end of each trip, blood samples were collected immediately after unloading second blood sample [S2] and again 30 minutes later third blood sample [S3]. The rectal temperature and RR (thoracic observation) were also taken at 30 minutes after unloading. During and after transport, the horses' degrees of activity/wakefulness were recorded. After all blood samples had been collected, the HR monitor was removed, and the horses were returned to their stalls. All blood samples were collected by jugular venipuncture.

2.3. Experimental Groups and Treatments

The animals were randomly assigned to four different treatments performed immediately before loading, as follows: (1) control (CTL-SAL): 0.01 ml/kg of isotonic saline solution, injected intramuscularly (I.M) at the base of the neck (n = 8); (2) acepromazine (CTL-ACP): 0.1 mg/kg acepromazine, I.M., injected at the base of the neck (n = 8); (3) aquapuncture (GV 1-SAL): 1 ml per 100 kg of isotonic saline solution injected at the acupoint GV 1 (n = 8); and (4) pharmacopuncture (GV 1–ACP 1/10): 0.01 mg/kg of acepromazine injected at the acupoint GV 1(1/10 of the recommended dose) (n = 8).

A Position of each horse and experimental group for each trip on the truck



Set A
8 horses

Trip 1

Position on the truck	Horse	Treatment
1	A1	CTL-SAL
2	A2	GV1-SAL
3	A3	GV1-ACP 1/10
4	A4	CTL-ACP
5	A5	CTL-SAL
6	A6	GV1-SAL
7	A7	GV1-ACP 1/10
8	A8	CTL-ACP

Trip 2

Position on the truck	Horse	Treatment
1	A6	CTL-ACP
2	A5	GV1-ACP 1/10
3	A8	CTL-SAL
4	A7	GV1-SAL
5	A2	CTL-ACP
6	A1	GV1-ACP 1/10
7	A4	CTL-SAL
8	A3	GV1-SAL

Set B
8 horses

Trip 1

Position on the truck	Horse	Treatment
1	B1	GV1-ACP 1/10
2	B2	CTL-ACP
3	B3	GV1-SAL
4	B4	CTL-SAL
5	B5	GV1-ACP 1/10
6	B6	CTL-ACP
7	B7	GV1-SAL
8	B8	CTL-SAL

Trip 2

Position on the truck	Horse	Treatment
1	A6	GV1-SAL
2	A5	CTL-SAL
3	A8	CTL-ACP
4	A7	GV1-ACP 1/10
5	A2	CTL-SAL
6	A1	GV1-SAL
7	A4	GV1-ACP 1/10
8	A3	CTL-ACP

B Experimental timeline

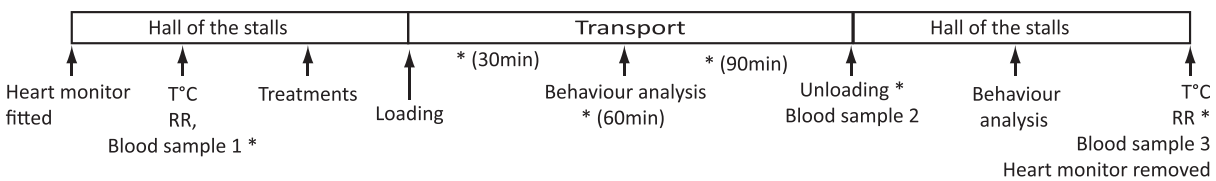


Fig. 1. Experimental design. (**A**) The positions of each horse and experimental group for each trip on the truck are shown. Drawing at left represents the truck with the animals loaded. (**B**) Experimental timeline. *When HRs were analyzed.

In all groups, the injected volume was 1 ml per 100 kg, saline solution was the treatment vehicle, and a 25 × 7 mm hypodermic needle was used. The GV 1 acupoint, also known as Ho Hai or Chang Qiang, is located at a depression between the anus and the ventral tail base (transpositional equine acupuncture atlas) [20].

2.4. Heart Rate

Heart rate was recorded with an HR monitor (Polar) attached to the thorax of the horse with an elastic girth. The girth has two electrodes, one in the precordial region and the other near the withers in the thoracic region. The signals collected by the electrodes were sent to a transmitter and then to the heart monitor. Data stored in the monitors were transmitted to a computer by infrared interface and analyzed by Pro Trainer 5 software (Polar). Heart rate was recorded throughout the experiment; however only 1-minute periods at different stages of the experiment were selected for analysis, as follows: basal, first blood sample (S1), loading; 30, 60, and 90 minutes after the beginning of transportation; unloading; and second and third blood collection (S2 and S3).

2.5. Cortisol Analysis

Blood samples were centrifuged, and sera were collected in plastic tubes and kept at −20°C. Serum cortisol concentrations were determined in duplicate by a double-antibody radioimmunoassay method using a commercial kit (RD coated tube cortisol I125; RIA, Costa Mesa, CA, USA). The sensitivity of the assay was 0.17 μg/dL. The intra- and interassay coefficients of variation were 3.31% and 4.47%, respectively.

2.6. Degree of Activity/Wakefulness

Behavioral assessment was performed during and after transport (after the second blood collection) by two trained and experienced researchers using a scale with six levels of activity/wakefulness (Table I). Data are averages of the scores assigned by the two researchers.

Table I
IActivity/wakefulness scores

Degree of Activity	Behavior
Score 1 (sedation +++)	Not reactive and intense head lowering
Score 2 (sedation ++)	Slightly reactive with considerable head lowering
Score 3 (sedation +)	Slightly reactive with little head lowering
Score 4 (normal/quiet)	Alert with no signs of agitation
Score 5 (unrest +)	Agitated (resistant to manipulation)
Score 6 (unrest ++)	Very agitated and aggressive

2.7. Statistical Analysis

Data are means ± standard error. HR, RR, temperature, and cortisol data were analyzed by repeated measures analysis of variance (ANOVA), followed by the Student-Newman-Keuls test for all pair-wise multiple comparisons. To analyze the degree of activity/wakefulness, we analyzed the semi-quantitative data by using the Kruskal-Wallis test followed by the Dunn test. Statistical analyses were performed using SigmaStat version 3.5 software, and the graphs were built using Graph Pad Prism 4 Project software.

3. Results

3.1. Physiological and Behavioral Changes During Transport

Analysis of HR revealed a significant effect of time ($F_{[8,\ 224]} = 8.261$, $P < .001$) and a tendency of effect of treatment ($F_{[3,\ 224]} = 2.812$, $P = .057$) but no effect of interaction. The loading and unloading times had the highest HR values, where loading was different for all other time points (except unloading) and unloading time was different from all other time points except loading (Fig. 2A). Considering the average HR (when all time points were considered together), the GV 1-ACP 1/10 treatment showed significantly lower HRs than the CTL-SAL treatment ($P = .035$) (Fig. 2B). At unloading, the GV 1-ACP 1/10 group had significantly lower HRs than the CTL-SAL group, with no differences between the other treatments ($P = .003$) (Fig. 2C).

Body temperature significantly increased from 37.4°C before transport to 37.96°C immediately after transport ($F_{[1,\ 28]} = 23.101$, $P = .001$) (Fig. 3A), but there was not a significant treatment effect or interaction ($P > .05$). Likewise, the transport also produced a significant increase in the RR, with an average RR before transport equal to 25.55 breaths per minute (BPM) and average RR after transport equal to 36.5 BPM ($F_{[1,\ 28]} = 47.97$, $P = .0001$). Additionally, the CTL-ACP treatment had significantly lower RRs than those of CTL-SAL and GV 1-SAL after transport ($P < .01$) (Fig. 3B). Analysis of serum cortisol showed the effect of the time factor ($F_{[2,\ 56]} = 30.12$, $P = .0001$) (Fig. 4) but no effect of the treatment and interaction factors. Immediately after unloading (S2) the cortisol levels were significantly higher than before (S1) and 30 minutes after unloading (S3), with no significant differences between the cortisol levels at S1 and S3 ($P < .001$). No differences were found among the groups at any time point studied.

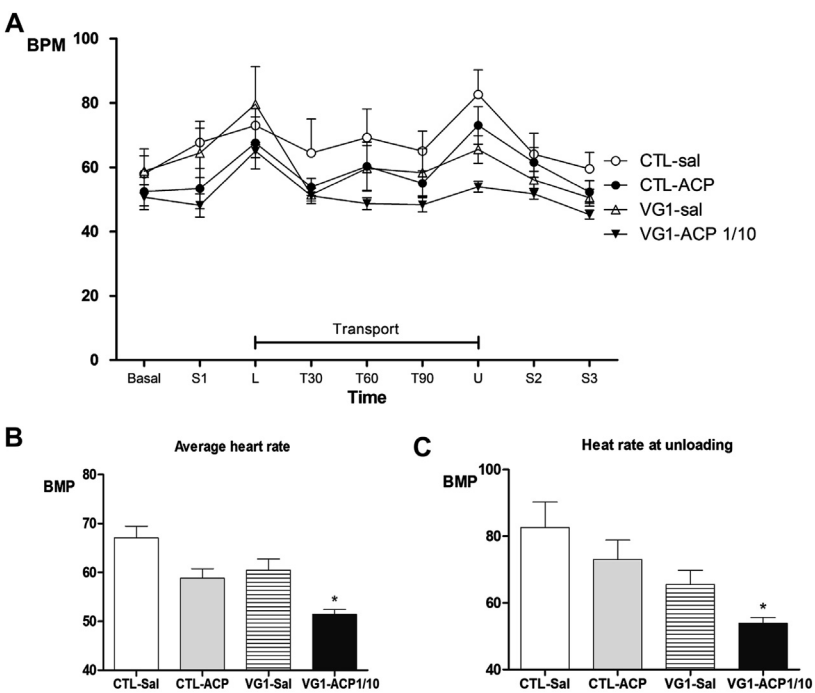


Fig. 2. Effect of pharmacopuncture at the GV 1 point on the HR of horses undergoing road transport stress. Data are BPM ± SEM. (**A**) HR at basal, first blood sample (S1), loading (L); at 30, 60, and 90 minutes after the beginning of transportation; and at unloading (UL) and second and third blood collection (S2, immediately after unloading and S3, 30 min after unloading). (**B**) HR for all time points together; (**C**) HR for only the unloading moment. *Significant differences were found from the CTL-SAL group (two-way ANOVA for repeated measures followed by the Student-Newman-Keuls test for all pairwise multiple comparisons, $P < .05$). ANOVA, analysis of variance; BPM, beats per minute; HR, heart rate.

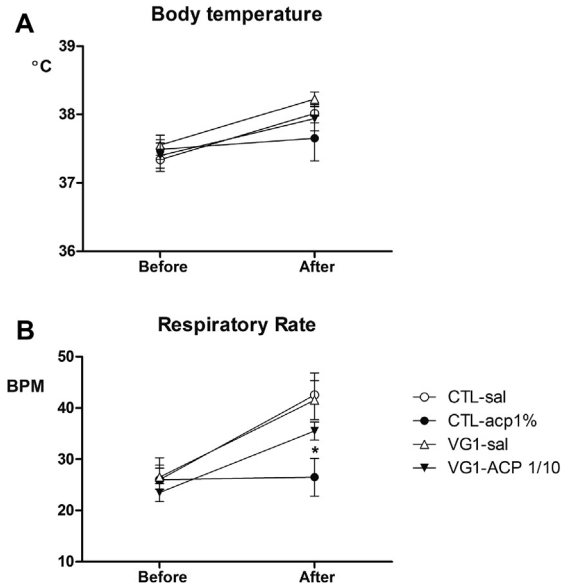


Fig. 3. Effect of pharmacopuncture at GV 1 point on the body temperature (**A**) and respiratory rate (**B**) in horses undergoing road transport stress. Data are averages (°C and breaths per minute) ± SEM. *Significant differences from CTL-SAL and GV 1-SAL (two-way ANOVA for repeated measures followed by the Student-Newman-Keuls test for all pairwise multiple comparisons, $P < .05$). ANOVA, analysis of variance.

All loading and unloading were carried without problems (fights or falls). In the analysis of degree of activity during transport, the CTL-ACP treatment group had a significantly lower activity score than the CTL-SAL and GV 1-SAL groups, with no differences among all the other treatments (Kruskal-Wallis test followed by the Dunn test, $P = .0019$) (Fig. 5). No differences were found among the treatments for the degree of activity after transport.

The animals in all the experimental treatment groups were equally distributed in the first and second trips for the purpose of checking whether the repetition of the transport (first versus second trip) could change the parameters studied. Two-way ANOVA for repeated measures was performed with the factors trip (first and second) and time

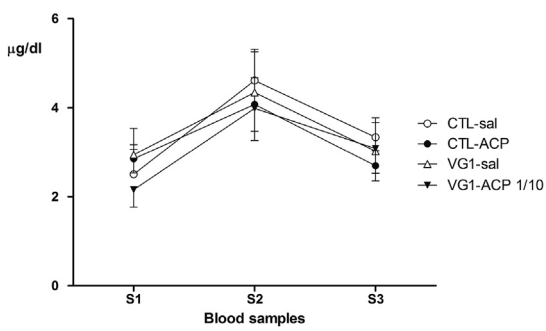


Fig. 4. Effect of pharmacopuncture at GV 1 point on the serum cortisol levels of horses undergoing road transport stress. Data are averages of serum cortisol concentration ± SEM. S2 had higher cortisol levels than S1 and S3. (Two-way ANOVA for repeated measures followed by the Student-Newman-Keuls test for all pairwise multiple comparisons, $P < .05$.). ANOVA, analysis of variance.

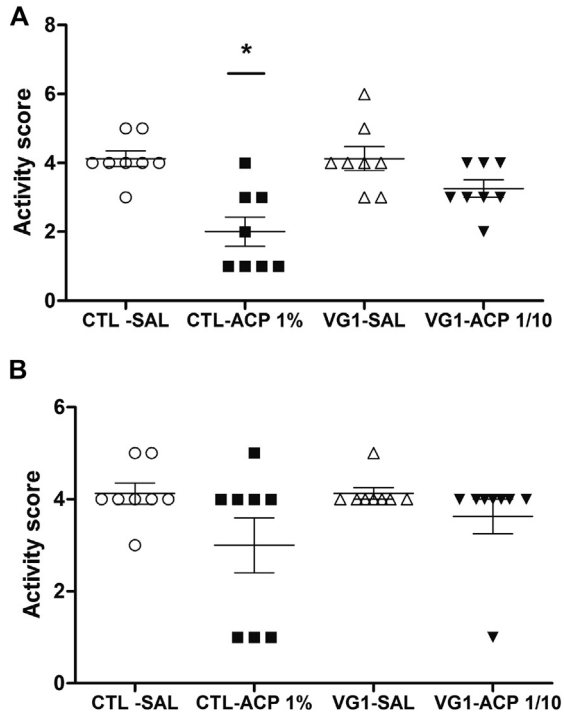


Fig. 5. Effect of pharmacopuncture at GV 1 point on the activity degree of horses undergoing road transport stress. The activity score of each animal per group is shown during (**A**) and after (**B**) transport. *Significant differences from CTL-SAL and GV 1-SAL groups (Kruskal-Wallis test followed by the Dunn test, $P = .002$).

(different time points of each variable). No effects of trip repetition were detected in variables of body temperature, HR, and degree of activity. However the second trip produced significantly lower values of RR (average RR in the first trip was 34.9 BPM and average RR in the second trip was 27.1 BPM, $F_{[2, 30]} = 8.323$, $P = .007$). However there were higher values of cortisol concentration in the second trip (average cortisol concentration in the first trip = 2.621 µL/dL and average cortisol concentration in the second trip = 3.986 µL/dL, $F_{[2, 60]} = 8.963$, $P = .005$).

4. Discussion

Road transport for 2.5 hours in the back of an open truck induced stress responses in horses. All stress markers such as HR, RR, body temperature, and serum cortisol level increased. Loading and unloading times had the highest HR values and were regarded as the moments of major physical activity (horses walk during loading and unloading) and/or moments of greatest stress. These results are in agreement with those of various other studies that have shown increases in the HR of horses during road transport [2-4,21,22]. Schmidt and colleagues (2010) demonstrated a significant increase in HR, especially at the beginning of transport, with sustained elevation throughout the trip compared to baseline [3,4]. On the other hand, other authors found high HR only at the beginning of transport [21]. These elevations in HR indicate that the loading may be the most stressful time of transport for horses [23]. In

our study, the unloading was also a critical moment for the horses.

An increase in serum cortisol levels immediately after transport has been observed by authors who confirmed it to be a stressor for horses [1,4,6,24,25]. The significant increases in body temperature and respiratory rate seen in the present study were probably the results of an increase in metabolic rate induced by cortisol [26] and thyroid hormones [24], an increase in muscle activity in order to maintain balance, and a decrease in convective heat loss. Furthermore, the ambient conditions could also contribute to an increase in body temperature and consequently an increase in RR, as the ambient temperature was 27°C-30°C and the horses were exposed to the sun during the experimental part of this study.

A comparison of the first and second trips showed that the animals had higher cortisol levels in the second trip, suggesting the second trip produced higher levels of stress. On the other hand, RRs were lower in the second trip. These results corroborate the idea that physiological stress responses are not linear but are the result of a complex interaction of several systems. Therefore, the repetition effect on the transportation cortisol levels and RR does not limit the analysis of our results, as all animals were transported twice and all experimental groups had the same number of animals in the first and second trips. In this way, our experimental design has some limitations, as for statistical purposes, the Latin square design (each 8-animal group was transported four times using four different treatments for a total of 32 samples) could be more advantageous than a randomized design (each 16-animal group divided into 2 groups was transported twice and received two different treatments, also giving 32 samples). However the randomized design was used here because our purpose was to use animals with no or very little experience with being transported (two trips versus four trips in the Latin square design). Furthermore, we did not transport 16 animals every trip because the loading takes 90 seconds per animal, which would require at least 24 minutes of difference between the first and last animal to be loaded. This difference would have interfered in the effect of the pharmacopuncture and acepromazine, and so it was avoided here. However, the position in the truck or the order of loading was randomized between the experimental treatments (all experimental treatments had an animal in all different positions).

4.1. Pharmacopuncture Effect

A single injection of 1/10th dose of acepromazine in the GV 1 acupoint was able to reduce the HR compared to those in the CTL-SAL and GV 1-SAL groups, when all time points were taken together (average HR). This treatment also significantly reduced HR at unloading compared to that in the CTL-SAL group. On the other hand, pharmacopuncture at GV 1 did not change any other parameter studied. Adding these data to the previously described data from the literature (see following discussion), we can speculate that pharmacopuncture can modulate the autonomic nervous system and consequently reduce the HR.

Studies in humans [13,15] and rodents [14,27] have shown that stimulation of acupoints can prevent stress-induced changes in cardiac function. These studies pointed out that acupuncture reduced the stress-induced elevation of the HR and the low-frequency component of HR variability (which indicates an increase in sympathetic tone) and increased the high-frequency component (which indicates an increase in parasympathetic tone). Taking these data together suggests that acupuncture can stimulate parasympathetic activity and inhibit sympathetic activity in subjects/animals under stress. This effect may be related directly to the action of acupuncture on the autonomic nervous system and consequently on the cardiovascular system or indirectly on the brain structures related to the activation of the sympathetic-adrenal system. The direct effect of acupuncture on the sympathetic system was suggested in studies with healthy subjects, in whom the effect of acupuncture at acupoint E36 promoted a significant reduction in the stress-induced elevation of blood pressure and was related to sympathetic inhibition [28]. Moreover, modulation of the cardiovascular system by acupuncture has also been related to the activation of a long-loop neural pathway involving the hypothalamus arcuate nucleus, the ventrolateral periaqueductal gray, and the rostral ventrolateral medulla [29].

Luna and colleagues [17], using the same experimental protocol as this experiment, did not observe any effect of the treatments on HR [17]. However, that study was carried out in animals at rest (unstressed) and HR was measured using a stethoscope, a less precise method. In the present study, HR was measured using an HR monitor (Polar) in animals subjected to transport stress.

Previous studies have shown contradictory results concerning the effect of acupuncture on cortisol levels. While some studies indicate that acupuncture can promote a reduction of increased stress-induced cortisol [30,31], as in our study, other studies failed to show this reduction [32]. The acupuncture-induced reduction of cortisol levels could be expected as the stimulation of acupoints was able to reduce stress-induced c-fos expression in the parvocellular portion of the paraventricular nucleus of the hypothalamus [33,34], and its activation is related to the release of ACTH and consequently the release of cortisol. Furthermore, although the effect of opioids on the release of cortisol can be species-dependent, generally opioids inhibit the hypothalamus-pituitary-adrenal axis (for a review see [35]). It is well known that acupuncture stimuli induce the release of opioids [36]. On the other hand, the effect of acupuncture on increased stress-induced cortisol levels can be changed by important issues such as species, types of stimulation, combination of acupoint, and type and intensity of stress.

Luna et al. [17], measuring the sedation score (sum of parameters such as ptosis and labial response to different stimuli) and the lowering of the head (measured by the distance from the animal's muzzle to the ground), observed that the pharmacopuncture at GV 1 produced sedation [17]. In the present study, the behavioral analysis was limited to assigning a score of activity/wakefulness with 6 degrees of activity while the animals were in the truck and after unloading. Thus, the behavioral analysis

allowed observations only of large variations in the degree of activity. In this sense, behavioral analysis is always a challenge. Stressor events commonly produce significant effects in the same direction for physiological and behavioral responses; however, the same behavioral responses have many different expressions, making quantification of the stress-induced behavioral response complicated [37].

On the other hand, it must be considered whether the effect of pharmacopuncture was just a dose-dependent effect of ACP, as the doses were 0.1 mg/kg of the acepromazine control group (CTL-ACP) and 0.01 mg/kg of the pharmacopuncture group (VG1-1/10 ACP). The recommended dose of acepromazine for horses may range, depending on the route of administration, from 0.01 to 0.04 mg/kg for intravenous injections and 0.08 mg/kg for I.M. injections [38]. The manufacturer suggests a dose of ACP from 0.05 to 0.1 mg/kg without addressing the route. The routes used in our study were I.M. for the CTL-ACP and CTL-SAL groups and I.M. and subcutaneous for the GV 1-SAL and VG1-ACP1/10 groups (as the acupoints were composed of muscular and subcutaneous tissue). Therefore, a low dose of ACP by the subcutaneous and I.M. route would not be expected to produce a significant effect. However pharmacopuncture at GV 1 produced a greater reduction in the HR than the usual dose of ACP without changing any other studied variables, suggesting a specific effect of pharmacopuncture.

The injection of saline in the GV 1 acupoint (aquapuncture) did not change any parameter studied, suggesting that this procedure is not effective in reducing transport-induced responses in horses. The ineffectiveness of aquapuncture may suggest a stronger stimulus or a specific substance is required in the acupoint to induce reduction in HR. This hypothesis agrees with the general theory of pharmacopuncture, where the application of a substance at acupoints can potentiate the effect of the drug. However this theory needs to be validated by scientific studies.

4.2. Acepromazine Effect

Acepromazine (0.1 mg/kg, I.M.) produced a significant decrease in the degree of activity during stress, suggesting sedation, blocked the increase in RR induced by stress and reduced the average HR compared to those in the CTL-SAL group. However, this dose of acepromazine did not prevent the stress-induced increase in cortisol levels and body temperatures.

Studies of acepromazine effects on the stress-induced increase in cortisol levels are contradictory. Whereas Bergeron and colleagues [39] found that acepromazine does not change the increase in cortisol induced by air transport in dogs [39], Lopez-Olivera and colleagues [40] demonstrated that acepromazine may reduce the increased cortisol levels induced by capture stress in mountain goats (*Rupicapra pyrenaica*). In this context, we must consider the type of stress to which the animal is being subjected, the degree of domestication of the animals, and the dose of acepromazine. Capture, for a wild animal, possibly represents more intense stress than transport for a domesticated animal and promotes more significant elevations of cortisol. So, for wild animals, sedation is critical in reducing the levels of cortisol. In domesticated animals transport-induced elevations of cortisol are milder, and the reducing action of acepromazine would tend to be smaller.

Although acepromazine can reduce body temperature [41], this was not observed in our study. In this case, the acepromazine was probably not enough to prevent the elevation of body temperature induced by transport-induced stress combined with the heat on the day of the experiment. On the other hand, acepromazine blocked the elevation in RR induced by transport. This result agrees with that of Luna et al [17], who observed a reduction in RRs induced by acepromazine, whereas aquapuncture and pharmacopuncture in the GV 1 caused no change in RRs. Cardiorespiratory depression is a side effect of phenothiazine in general and of acepromazine in horses in particular [10], and its use can be dangerous in animals with respiratory problems and in poorly ventilated areas.

The lack of effects of acepromazine in reducing stress responses during the transport of domestic animals must be taken into account when deciding whether to use this drug. On one hand there is the possible beneficial effect of this drug on aggressive animals primarily in terms of preventing accidents and injuries, and on the other hand, for “normal” animals, adequate manipulation can produce a reasonably safe management; and this drug may be, in this case, completely unnecessary and even harmful. We must remember that sedation can lead to a greater number of falls during transport and that respiratory depression can worsen respiratory conditions. Furthermore, if the drug does not prevent the increase in cortisol levels, it does not reduce the possible effects of long-term and repetitive increase of cortisol levels in the body, in other words, the consequences of stress to the animal. Furthermore, in most competitions and equestrian jurisdictions, acepromazine is classed as a controlled substance [12]. Based on these considerations, the use of acepromazine in horses during transport should be considered with caution.

5. Conclusions

A single application of pharmacopuncture at GV 1 reduced the stress-induced increase in the HR of horses, suggesting a possible autonomic effect. However, the pharmacopuncture was not able to change other variables, such as transport-induced increases in cortisol, body temperature, and respiratory rate. On the other hand, acepromazine produced significant sedation and reduced transport-induced increase in respiratory rate without changing the stress-induced increase in cortisol. Furthermore, the use of acepromazine in horses during transport should be considered with caution because it did not prevent the deleterious effect of stress and can cause respiratory depression. Other types of stimulation and acupuncture points should be tested to verify the beneficial effect of this therapy to reduce stress in horses.

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