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Are indicators of stress and pain recognizable during lip twitch in horses? A behavioral investigation

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ABSTRACT

Lip twitching is a physical restraint method for horses that may function by inducing short-term stress and/or pain. The present study aimed to: 1) evaluate whether behavioral indicators of pain and stress could be recognized during the application of a lip twitch in horses across different experimental conditions involving electrical, mechanical and thermal stimulation; 2) explore the relationship between observed variables and individual personality traits. Twelve horses were initially included in a two-sessions twitch trial. This study focused on data collected during the first session, which was completed by ten horses out of twelve. Behavioral analysis was performed on 2-minute videos recorded during three-time intervals (Baseline, Twitch and Post-Twitch) applying an ethogram. Personality was evaluated using a validated questionnaire completed by the horses' caretakers. Any interval, condition and personality effect on the behavior was analyzed using a GLMM. Results showed that maintenance of a natural head position decreased significantly during Twitch and Post-Twitch ($p<0.001$), while unequal forelimb weight distribution increased during Twitch and Post-Twitch ($p<0.001$) compared to Baseline. A significant effect of interval was found for the duration of ears forward ($p = 0.026$), with shorter durations recorded during Twitch compared to both Baseline and Post-Twitch. Furthermore, ears movement decreased ($p<0.001$) and head movements increased ($p<0.001$) during Twitch. Horses' responses to twitch varied based on personality: neurotic horses displayed rather passive stress signs (prolonged ear-backward, $p=0.032$), while agreeable horses showed more active avoidance (moving away, $p=0.073$). These findings suggest that in horses tolerating the application of the twitch, the restraint induces mild stress, with individual coping mechanisms influenced by personality. Thus, it appears important to consider individual characteristics when applying the technique in a clinical context. This approach is essential to ensuring the safety of both horses and personnel during veterinary interventions, especially in the absence of pharmacological restraint or training.

Data availability statement: The data supporting the findings of this study are available upon reasonable request from the corresponding author.

Introduction

Lip twitching is a widely used physical restraint technique to facilitate equine handling and veterinary procedures. When applied correctly, it can help avoid or reduce the need for sedatives and tranquilizers, thereby eliminating their potential side effects. An additional advantage is the perceived immediate return to the horse's preexisting state as soon as the twitch is removed. Interestingly, despite its common use, the modulatory mechanisms behind its effects remain incompletely understood.

Twitching consists in applying a twisted rope to a horse's upper lip, often resulting in a calm or immobilized state, reducing the horse's apparent sensitivity to discomfort. Previous studies suggest that lip twitching may increase pain tolerance (Blum et al., 2024; Lagerweij et al., 1984) while others indicate that it enables horses to tolerate procedures, such as ear clipping, through a sedative-like effect, with

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reduced occurrence of defensive behavior (Ali et al., 2017; Lagerweij et al., 1984). This ambiguity raises questions about whether this response stems from pain reduction, stress-induced immobilizations, or a combination of both. Pain reduction might rely on mechanisms similar to those involved in Condition Pain Modulation (CPM) paradigms. They operate on a 'pain-inhibits-pain' principle and are extensively used in human pain research (Moont et al., 2010). Through activation of descending inhibitory pathways, the twitch might act as a conditioning noxious stimulus able to inhibit the response to further nociceptive stimuli (Blum et al., 2024).

Flakoll et al. (2017) describe the immobility caused by an ear twitch as a fear- and pain-induced freezing response observed in various species, including horses (Flakoll et al., 2017). Similarly, stress-induced immobilization has been observed as one of the possible mechanisms of lip twitch action (Schelp, 2000). In natural conditions, this immobilization might represent a coping response to stressors and predators (Koolhaas et al., 1999). It was observed in several species and is characterized by passive avoidance behaviors displayed when threats are perceived (Erhard and Mendl, 1999). The observation that horses appear immobilized or 'frozen' during twitch application might corroborate this hypothesis but would need further investigation to be confirmed.

Behavioral indicators such as facial expressions and anticipatory reactions are essential for assessing a horse's experience of discomfort, stress and pain. Facial expressions, specifically ear positions – such as asymmetrical alignment or backward-orientation - have been indicated as reliable markers of fear (Hötzel et al., 2019) and may also reflect discomfort or suboptimal handling practices (Dalla Costa et al., 2017; van Loon and Van Dierendonck, 2015). When analyzing equine behavior in response to noxious stimulation, personality should also be considered as it might influence the behavioral response characteristics (Ijichi et al., 2014). The study of personality traits, particularly through the taxonomy of The Big Five (TB5), provides a structured approach to understanding individual differences. TB5 outlines five broad domains - openness to experience, conscientiousness, extraversion, agreeableness, and neuroticism – that collectively capture the most significant dimensions of personality variability (McCrae and John, 1992). Kabigting (2021) describes The Big Five as an essential framework for investigating enduring personality's patterns, revealing how these traits interplay to shape behavior and adapt over time.

This paradigm offers a foundational perspective, particularly useful for exploring behavioral differences not only in humas but also in animals like horses (Jolivald et al., 2022). Their individual temperamental traits might influence the responses to stressors such as lip twitch. Based on previous reports and clinical experience, individual behavioral responses to lip twitching appear to differ profoundly. One study suggests that personality traits – such as extroversion and neuroticism – significantly affect stress reactivity, with extroverted horses often displaying stronger stress responses than their introverted peers (Ijichi et al., 2013). These temperament variations underscore the importance of including personality assessments when evaluating stress and pain responses.

The present study aimed to: 1) evaluate whether behavioral indicators of pain and stress could be recognized during the application of a lip twitch in horses across different experimental conditions involving electrical, mechanical and thermal stimulation; 2) explore the relationship between observed variables and individual personality traits.

We hypothesized that lip twitching would induce behavioral changes indicative of pain and/or stress – such as altered posture (e.g., changes in head position or weight distribution), facial expressions (e.g., ear position or movement) and activity level (e.g. increased head movement or avoidance behavior) - that could be detected through systematic behavioral analysis. Additionally, we anticipated that individual personality traits, particularly neuroticism and agreeableness, would significantly influence horses' responses to the procedure. Neurotic horses were anticipated to display more passive stress behaviors (e.g., prolonged backward ear position), while agreeable horses were expected to exhibit more active responses (e.g., increased movements or attempts to avoid the stimulus), reflecting different coping strategies.

Materials and methods

Ethics statement

Ethical approval was obtained from the Committee for Animal Experimentation of the Canton of Bern, Switzerland (license number BE81/2022).

Horses

Twelve healthy horses, 11 geldings (10 warmblood and 1 Freiberger) and 1 mare (Freiberger), belonging to the Swiss Armed Forces, were included in the study. The median age was 8 years (min 5.5 - max 16 years old) and the median weight was 570 kg (min 535 – max 607 kg). Body Condition Score, on a 5-point scale, ranged from 3.0 to 3.4. All horses were housed in single boxes in large stables at the National Equine Center in Bern, Switzerland and adhered to a consistent management adapted to their individual needs with *ad libitum* water available and fed with hay. Each horse fulfilled strict inclusion criteria, ensuring they were free from any detectable orthopedic, neurologic, or systemic disease, as well as evident pain, lameness, or mobility impairment. Detailed records of prior exposure to lip twitching were not available; however, no signs of distress or avoidance were observed during initial handling, and all horses were accustomed to routine management procedures. Shortly before starting the experimental procedure, a pair of horses were gathered from their own stall and transferred to a spacious, vacant stable they were familiar with. For minimizing social separation discomfort, while one horse underwent testing in the corridor, the order remained in a nearby box within visual range.

Data collection

This study was part of a larger a prospective, experimental, randomized trial investigating the lip twitch as a conditioning stimulus in the context of a conditioned pain modulation (CPM) paradigm in horses (Blum et al., 2024). Typically, CPM is assessed by calculating the differences in nociceptive thresholds determined in response to test stimuli before and after the application of a conditioning stimulus. The experiments took place from November 2022 to January 2023, with temperatures ranging from approximately 5.1 to 14.8°C. In the morning of the testing day, horses underwent a physical examination by a veterinarian to ensure their health and fitness for the study. Experimental procedures were conducted in the afternoon, at least one hour after feeding or training. Methodological details for the main trials have been previously reported (Blum et al., 2024). Briefly, two experimental sessions per horse were planned, two weeks apart. Different test stimuli were used in the two sessions. In one session, electrical stimulation and in the other thermal and mechanical stimulations were used. The order (which session first) was randomized by using a computer-generated randomization sequence. Thresholds were defined before, during and after twitching as following: electrical nociceptive thresholds were assessed by transcutaneous stimulation of the lateral digital nerve using an automated threshold tracking device (Paintracker V1, Dolosys GmbH, Germany), with intensity gradually increased until a consistent withdrawal reflex was observed; mechanical thresholds were measured using a pressure algometer (ProdPro, Top Cat Metrology Ltd, UK) applied to the right metacarpus, with stimulation stopped at behavioral signs such as limb lifting, stomping, weight shifting, or upon reaching a 25 N cut-off; thermal thresholds were determined on the medial antebrachium using a handheld purpose made thermal stimulator with a heating rate of 0.6°C/sec. The probe was removed at the first observable adverse reaction or upon reaching a maximum of 52°C.

The present investigation included only the first experimental session for each horse and focused on data collected in absence of manually applied test stimuli.

Experimental intervals

Data recording was carried out during four distinct time intervals: Baseline (before test stimuli were started and before twitching), Baseline Stimulation (during which nociceptive thresholds were assessed using test stimuli), Twitch (during twitching), and Post-Twitch (immediately after the twitch was removed). Initially horses were instrumented with the experimental equipment. They were then left undisturbed for approximately 10 minutes before baseline was recorded. During Baseline, video and physiological data were collected for 5 minutes in absence of any stimulation. This was followed by the Baseline Stimulation interval, during which nociceptive thresholds were assessed. Although this phase was crucial to the conditioned pain modulation experimental setup (Blum et al., 2024) it was not included in the behavioral analysis of the current study. In the Twitch interval a lip twitch was applied in a consistent manner always by the same person (SB). The twitch consisted of a wooden handle and a double rope to be twisted around the horses' upper lip. Twitching was maintained for a total of 5 minutes. Electrical stimulation occurred automatically throughout this interval at 30 seconds intervals, while thermal and mechanical stimulation were applied only during the last 3 minutes, as described by Blum et al. (2024). Immediately following removal of the twitch, the Post-Twitch interval began. Data in the Post-Twitch interval were collected for a total of 15 minutes. The timeline of the experiment is graphically represented in Supplementary Material (Supplementary Figure S1).

Behavioral assessment

Behavioral responses were assessed using a modified version of the ethogram originally developed by Sue McDonnell (Torcivia and McDonnell, 2021), incorporating validated indicators of discomfort and pain in horses. The modified ethogram included both state and event behaviors, they were grouped by anatomical region (e.g. ears, eyes, head, limbs, tail) and served as a standardized framework for video analysis across all experimental intervals. Behaviors potentially occurring during twitching were selected based on previous literature (Dyson, 2022; Gleerup et al., 2015; Mullard et al., 2017, Trindade et al., 2020) and grouped according to seven body parts (Table 1). A focal animal continuous recording method (Bateson and Martin, 2021) was used to monitor the behavioral reaction of the horse during the three experimental intervals (Baseline, Twitch and Post-Twitch). For each horse, two-minute video segments were analyzed from three intervals: the last 2 minutes of Baseline, the first 2 minutes of Twitch and the first 2 minutes of Post-Twitch. Videos were recorded using a camera (GoPro) positioned 2 m from the animals on a tripod. This arrangement gave the highest probability of capturing the behavior and face of the horse without interfering with the experimental procedures. Videos were analyzed by a trained observer (EDC) using Solomon Coder (copyright 2006–2008 by Andras Peter). Both, the frequency and duration of observed behaviors were considered.

To quantify stress-related responses, a composite stress score was calculated for each interval by summing the frequencies of behaviors identified in the literature as indicative of stress (Table 1, last column). This additive score reflects the cumulative expression of stress-related behaviors and aligns with methods used in previous studies the developed or applied behavioral indicators equine stress (e.g. Gleerup et al., 2015; Young et al., 2012).

Personality assessment

To assess personality traits in the participating horses, we used a previously validated caretaker-based questionnaire as described by Ijichi et al. (2013) and further supported by Jolivald et al. (2022). This instrument is widely used in equine behavioral research and is designed to identify five key personality traits: extroversion, agreeableness, neuroticism, and gregariousness towards horses and people. The questionnaire includes a series of items rated using a combination of visual analogue scales (VAS) and Likert-type scales, aimed at capturing a detailed profile of each horse's typical behavioral tendencies.

Table 1
Ethogram used for behavior evaluation during the three experimental intervals (Baseline, Twitch and Post-Twitch). Behaviors are grouped by body region and categorized as either state (S) and event (E) behaviors, recorded by duration or frequency. The "Stress" column indicates behaviors considered potentially stress-related as described by Kaiser et al. (2006).

Body part	Behavior	Description	S/E	Stress
Ears	Forward	Erect and parallel with pine facing forward	S	
	Backward	Both ears rotated back behind vertical or flat	S	x
	Asymmetrical	Divergent, one erected and one with pinnae outward, one ear forward and other sideward	S	x
	Movement	Changing the ear position	E	
Eyes	Open, sclera visible	Sclera always visible or easily noticeable at rest	S	x
	Open soft	Upper and lower eyelids are opened, no tension is visible	S	
	Closed	Both eyelids, upper and lower, touched each other to close the eye	S	x
	Partially closed	The opening of the eye is shortened, but 50% of the eyes are opened	S	x
	Blinking	Any movement of the upper eyelid towards the lower eyelid	E	
Head	Natural position	Upper neckline is in same height as the wither's line	S	
	Moving	Head is moved (e.g., hand tossed, twisted, moved away or towards the twitch)	E	x
Forelimbs	Pawing	Reaching a forelimb cranially and dragging the hoof along or above the substrate while sweeping caudally, often in rhythmic series	E	x
	Resting - two forelimbs equal	Equal distribution of weight into forelimbs	S	
	Unequal distribution of weight forelimbs	Weight is distributed differently between the two forelimbs (avoidance)	S	x
Body	Moving forward	Moves at least once with two limbs forward resulting in new position	S	x
	Moving backward	Moves at least once with two limbs backward resulting in new position	S	x
	Sidewards movement	Moves at least once with two limbs sideways resulting in new position	S	x
Hindlimbs	Weight shift	Shifting the weight	E	x
	Stomping	Act of flexing and then extending a limb abruptly, sharply striking the hoof against the floor	E	x
	Resting - two hindlimbs equal	Equal distribution of weight into hindlimbs	S	
	Unequal distribution of weight hindlimbs	Weight is distributed differently between the two hindlimbs limbs (avoidance)	S	x
Tail	Swish	Tail is flicked to one side and/or the other of the quarters	E	x

Questionnaires were completed by caretakers or riders with substantial daily contact and familiarity with the horses, to enhance the reliability of the assessments. The full questionnaire is available as Supplementary material in (Ijichi et al., 2013). Responses were processed to quantify the individual contribution of each personality profiles: Agreeableness is related to those horses who are easy to handle; Neuroticism is described by anxiety and fearfulness; Extroversion is characterized by energic and active behavior; Gregariousness towards people, and Gregariousness towards horses are both related to social behavior (Ijichi et al., 2013).

Statistical analysis

All data were inserted into a common dataset and then analyzed with IBM SPSS Statistic 28 (SPSS Inc., Chicago, USA). To answer the research question regarding whether lip twitching would induce behavioral changes indicative of discomfort and/or pain, a Generalized Linear Mixed Model (GLMM) was applied. The GLMM was used to evaluate the effects of intervals (Baseline, Twitch, Post-Twitch) and condition (type of nociceptive stimulation e.g., electrical or mechanical/thermal) on mean duration and frequency of recorded behaviors. The experimental intervals and the condition were included as the main fixed effect. Horse identity was included as a random effect in all models to account for repeated measures. The duration of behavior recorded as states (continuous data) were modeled using a Gamma distribution with a log link, while behaviors recorded as frequency (count data) was modeled using a Poisson distribution with a log link. Model selection was guided by biological relevance and comparison of model fit using the Akaike Information Criterion (AIC). Model stability and estimate reliability were assessed by inspecting standard errors and confidence intervals of the fixed effects. Results are reported as estimated marginal means ± standard error of the mean (SEM).

For the personality data, responses from the questionnaire were compiled into a matrix with questionnaire items as variables (columns) and horses as cases (rows). Scores were standardized (z-scores) to account for scale differences and ensure equal weighting across traits. The standardized dataset was then subjected to Principal Component Analysis (PCA) to identify clusters of related traits and group horses based on shared personality characteristics. These PCA-derived personality groups were used to explore behavioral differences during the experiment and answer the question whether horse personality can affect behavioral reaction during lip twitch application. A GLMM was run to explore the effects of the interaction between personality (Neurotic, Agreeable) and intervals on mean duration and frequency of recorded behaviors.

Results

Two horses did not complete the session, therefore, they were not included in the statistical analysis. Specifically, Horse 2 was excluded because the lip twitch could not be applied due to excessive aversion; Horse 12 was excluded because of reaching the cut off set for the stress score during the baseline and following the application of the twitch (Blum et al., 2024). Of the remaining 10 horses, 6 were exposed to electrical and 4 to mechanical and thermal stimuli as test stimuli in the first experimental session taken into account in the current study.

Behavior across the three time intervals

Duration (sec) of behaviors showed by horses over the three intervals are reported in Table 2; horses never showed the following behaviors: open eyes with sclera visible, eyes closed or partially closed, therefore they were not included in the statistical analysis. Frequencies of behaviors are reported in Table 3; horses did not show blinking, weight shift in hindlimbs and stomping, while tail swish was shown only by three horses, therefore all these behaviors were not included in the statistical analysis.

"Interval" had a significant effect on the duration of the following behaviors: ears forward ($p=0.026$), ears backward ($p=0.034$), head being maintained in a natural position ($p<0.001$), resting - two forelimbs equal ($p<0.001$) and unequal distribution of weight forelimbs ($p<0.001$). Compared to Baseline and Post-Twitch, during Twitch there was a significant decrease in the ears forward ($p=0.058$) and head being maintained in a natural position ($p<0.001$). In Baseline horses had a longer resting two forelimbs equal compared to Twitch and Post-Twitch ($p<0.001$). During Twitch, compared to Baseline and Post-Twitch intervals, there was a significant increase in the ears backward ($p=0.013$) (Figure 1a). The "condition" (which test stimulus was applied) did not significantly impact these behaviors ($p>0.050$). In Baseline horses had a shorter unequal distribution of weight forelimbs compared to Twitch and Post-Twitch ($p<0.001$), additionally, "condition" had a significant effect ($p=0.031$) on this behavior, with horses exposed to electrical stimuli showing longer duration of unequal distribution of weight forelimbs (87.6±9.9 s) than those exposed to thermal/mechanical stimuli (53.5±11.1 s).

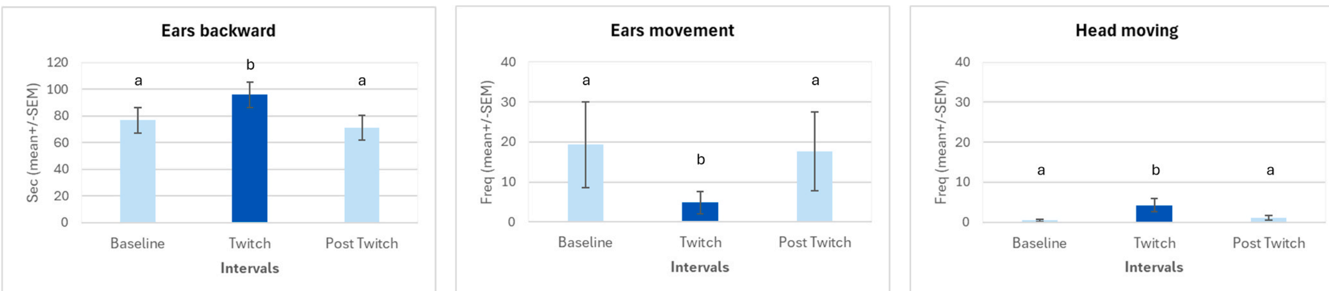
The factor "Interval" had also a significant effect on the frequency of ears movements ($p<0.001$), head movements ($p<0.001$) and pawing ($p=0.040$). Horses exhibited fewer ear movements ($p<0.001$) (Figure 1b) and pawing ($p=0.023$) during Twitch compared to Baseline and Post-Twitch, while they moved their head more frequently during Twitch ($p<0.001$) compared to the other two intervals (Figure 1c). The factor "condition" had no significant effects on these behavioral expressions ($p>0.050$).

Table 2
Table reporting the estimated marginal means and standard error (SEM) of the duration (in seconds) of behaviors classified as states, recorded during the three intervals: Baseline, Twitch and Post-Twitch. Behaviors are grouped by body part. For each behavior, p-values of the GLMM of "interval" and "condition" effects are reported in the last two columns. "Condition" refers to the nociceptive stimulation applied (electrical or mechanical/thermal).

Body part	Behavior	Baseline		Twitch		Post Twitch		GLMM	
		Mean	SEM	Mean	SEM	Mean	SEM	Interval	Condition
Ears	Forward	19.8	4.7	6.4	4.9	15.7	4.7	**0.026**	0.381
	Backward	76.8	9.4	96.0	9.7	71.2	9.4	**0.034**	0.932
	Asymmetrical	23.5	7.2	15.6	7.5	23.3	7.2	0.635	0.682
Eyes	Open soft	116.9	4.2	118.4	4.4	110.8	4.2	0.346	0.421
Head	Natural position	120.1	7.1	59.4	7.1	112.2	7.1	**<0.001**	0.542
Body	Moving forward	0.5	0.9	2.5	0.9	1.5	0.9	0.306	0.115
	Moving backward	2.4	1.3	3.6	1.3	3.0	1.3	0.799	0.612
	Sidewards movement	1.2	0.8	0.6	0.8	0.5	0.8	0.746	0.486
Forelimbs	Resting - two forelimbs equal	83.6	11.2	32.9	11.2	6.5	11.2	**<0.001**	0.090
	Unequal distribution of weight forelimbs	28.4	11.9	85.2	11.9	98.0	11.9	**<0.001**	**0.031**
Hindlimbs	Resting – two hindlimbs equal	70.2	18.7	48.2	16.9	31.3	17.8	0.131	0.590
	Unequal distribution of weight hindlimbs	35.7	17.3	67.3	15.5	75.9	16.4	0.091	0.275

Table 3
Table reporting the estimated marginal means and standard error (SEM) of the frequency of behaviors recorded as events during the three experimental intervals: Baseline, Twitch and Post-Twitch. Behaviors are grouped by body parts. For each behavior, p-values of the GLMM of "interval" and "condition" effects are reported in the last two columns. "Condition" refers to the nociceptive stimulation applied (electrical or mechanical/thermal).

Body part	Behavior	Baseline		Twitch		Post Twitch		GLMM	
		Mean	SE	Mean	SE	Mean	SE	Interval	Condition
Ears	Movement	19.3	10.7	4.8	2.8	17.7	9.9	**<0.001**	0.722
Head	Moving	0.5	0.3	4.3	1.6	1.1	0.5	**<0.001**	0.549
Forelimbs	Pawing	0.3	0.2	0.2	0.1	0.8	0.4	**0.040**	0.610



a b c

Figure 1. Estimated marginal means and standard error (SEM) of ears backward (a), ears movements (b) and head movements (c) across the three intervals: Baseline, Twitch and Post Twitch. Non-capital letters "a" and "b" above the bars indicate statistical differences (GLMM, p<0.05).

Considering the composite stress score, compared to Baseline (6±4) and Post-Twitch (11±7), there was a significant increase of the score during the Twitch interval (16±10) (p=0.004), also in this case the "condition" was not significant (p=0.889).

Personality assessment and its influence on behavioral responses

The PCA of the five personality traits for the 12 horses identified two main factors with eigenvalue greater than 1. The first two components explained 76.5% of variance of personality traits among horses. Most of the personality traits (extroversion, agreeableness, gregariousness towards people and gregariousness towards horses) loaded strongly on the first Principal Component accounting for 54.7% of the total variance and range from agreeableness/gregariousness towards horses to extroversion, suggesting that this Component is important in the description of the personality traits linked with sociability and cooperation. The second Principal Component counts for 21.7% of variance and seems to be more related to the level of anxiety ranging from neuroticism to gregariousness towards people and horses. The analysis grouped the horses into three main personality groups: Group Extroverted (N=2), Group Neurotic (N=4), and Group Agreeable (N= 6). The distribution of personality traits across the different horses is also shown in Figure 2.

Due to the small sample size of Group Extroverted (only one horse of this group completed the experimental session), only Groups Neurotic and Agreeable were included in further statistical analysis to ensure sufficient statistical power and reliability. The interaction between personality and intervals had a significant effect on the duration of ears backward (p=0.038) and moving away (p=0.035) that was the sum of the movements of the body forward, backward and sideways. Neurotic horses kept their ears backward longer across all three intervals when compared to agreeable horses, with the difference being significant during Twitch (p=0.032) (Figure 3a). Although not significantly,

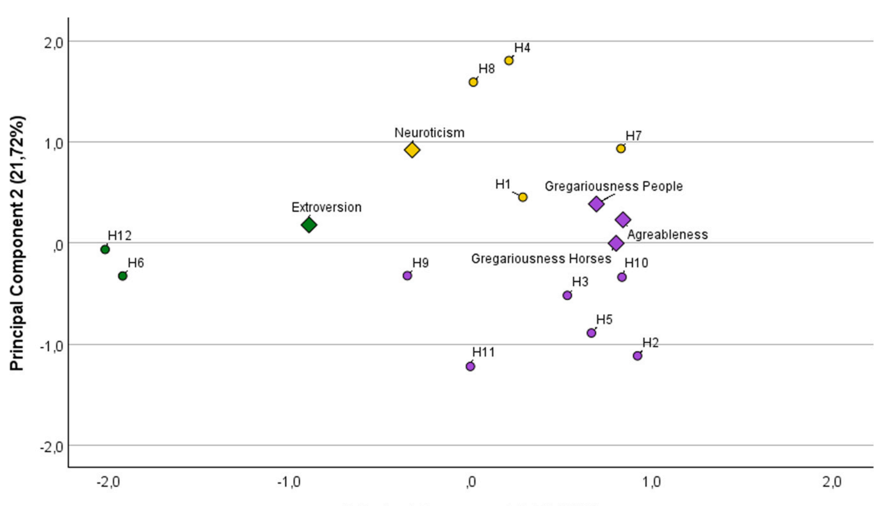


Figure 2. Loading plot of the descriptors of personality traits and single horses on the first and second Principal Components. Personality traits are represented by rhombus and horses clustered by the same personality characteristics, indicated by colors.

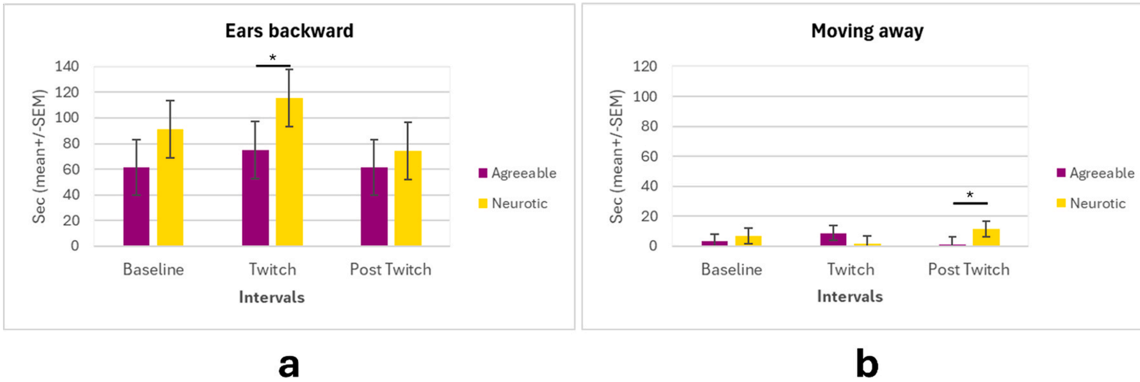


Figure 3. Estimated marginal means and standard error (SEM) of ears backward (a) and moving away (b) behaviors across three intervals (Baseline, Twitch, Post Twitch) for two personality groups: Neurotic and Agreeable. Significant differences (GLMM, $p<0.05$) between personality groups for each interval are indicated by asterisk (*).

agreeable horses moved away longer during the twitch compared to neurotic ones ($p=0.073$), while the opposite was observed in the Post-Twitch interval ($p=0.013$) (Figure 3b).

While not significant at Baseline, agreeable horses showed more ear movements (26±14) compared to neurotic horses (14±7). A general reduction in ear movement was observed during Twitch in both groups (3±2 in neurotic group and 6±3 in agreeable group); while in Post-Twitch both groups increased the number of ear movements to 19±10.

The interaction between personality and intervals had an effect on the composite stress score ($p<0.001$): with neurotic horses having a higher score (11±5) compared to agreeable (4±2) during Baseline. The frequency of these behaviors further increased in the Post-Twitch, 19±6 for neurotic compared to 9±3 for agreeable horses (Figure 4).

Discussion

As initially hypothesized, the present study showed that the lip twitch induced observable behavioral changes consistent with mild stress in the horses (ten out of twelve) that completed the procedure. In contrast, a high level of stress and aversion to the twitch application was observed in the two horses that were excluded. Before applying a lip twitch, specific behavioral signs can indicate a horse's intolerance, aiding in assessment and stress prevention. In the two horses that were excluded from the study, distinct behavioral signs indicated marked aversion to the twitch. These included sustained backward ear positioning, elevated head posture, restlessness and pronounced avoidance behaviors in response to the lip contact. Although these animals were not included in the final statistical analysis, these observations suggest that such behaviors may serve as early indicators of intolerance to twitch application and could help guide ethical decisions during clinical handling or experimental procedures.

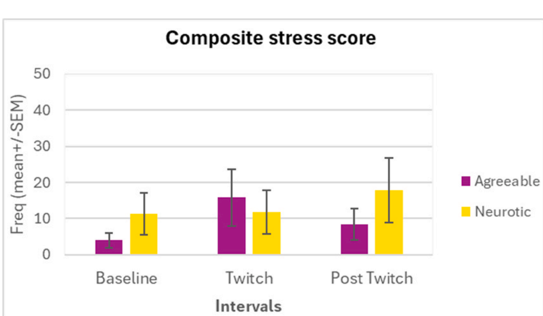


Figure 4. Estimated marginal means and standard error (SEM) of stress behavior score across the three intervals (Baseline, Twitch and Post Twitch) for two personality groups: neurotic and agreeable.

In the current study, we observed that during twitching, the horses' ear position changed significantly: there was a decrease in the duration of ears forward and an increase in the time spent with ears backward compared to both Baseline and Post-Twitch intervals. Ear position is a well-established marker of emotional valence in horses, with backward or asymmetrical positioning often linked to negative emotions including acute pain (Gleerup et al., 2015), discomfort or stress, in general, including those caused by inappropriate handling or environmental factors (Lundblad et al., 2021). The simultaneous reduction of forward ear positioning during twitching suggests a withdrawal of attention from the environment and a shift towards a negative emotional state, and a certain degree of discomfort could reasonably be assumed to occur during the procedure. In addition, our study revealed reductions in voluntary behaviors, such as ear movements and natural head position during twitching. We also observed a significant decrease in resting of the forelimbs during and after twitching, further suggesting a disruption of relaxed postures typically associated with low arousal states. Similarly, pawing behavior, typically associated with moderate agitation or frustration, was significantly reduced during the Twitch interval, indicating a generalized suppression of active behaviors during restraint.

Reduced voluntary behaviors such as decreased ear movements, natural head position and pawing during twitching may be indicative of either temporary coping mechanisms or behavioral inhibitions in response to the restraint. Such responses are common across species when animals are confronted with novel or uncontrollable situations and when they perceive threats. For example, pigs display a phenomenon known as tonic immobility – a marked inhibition of movement that is often interpreted as a fear-related adaptive strategy to minimize perceived danger (Erhard and Mendl, 1997). Boissy (1995) elaborated on this concept identifying tonic immobility as one of several strategies through which fear can manifest, alongside with active avoidance and defensive aggression. Similarly, chickens exhibit tonic immobility as a fear-driven response to stressors (Boissy, 1995), further supporting the notion that immobility can serve as a widespread behavioral strategy to cope with threats they cannot escape. Extrapolating to equine species, these findings suggest that horses may adopt these strategies when faced with the temporary application of the lip twitch.

An increase in head movements observed during twitching in our study may reflect attempts to adjust to the procedure or minor frustration at being unable to escape the procedure. This finding aligns with Young et al. (2012) who reported that repetitive head movements often indicate low-to-moderate intensity stress levels. Such behaviors, which are typically associated with avoiding the procedure due to discomfort or frustration highlight the complex nature of the horse's response to temporary stimuli. Taken together, these dual behavioral responses – behavioral inhibition and active expressions of frustration highlight the complexity and multifaceted nature of stress-related behaviors in horses.

These observations suggest that stress responses in horses can vary depending on both the intensity and nature of the stressor, potentially reflecting a dynamic interaction between emotional regulation and coping mechanisms.

Prior research suggested that the lip twitch can induce a sedative effect on horses, likely mediated by endorphin release, which reduces pain perception and promotes calmness (Flakoll et al., 2017). This potential analgesic effect is typically associated with a reduction in stress behaviors, with horses exhibiting decreased head movements and an overall calmer demeanor.

In our study we observed increased head movements, a decrease in the duration of time horses maintained a natural head position and an increase in unequal forelimb weight distribution during the Twitch interval. These responses could reflect the horse's adjustment to the presence of the twitch rather than evident discomfort. The changes in weight distribution observed during twitching for forelimbs seem to align with prior research linking such behaviors to pain, discomfort or stress, as seen in conditions like laminitis or tiredness (Rietmann et al., 2004; Trindade et al., 2020).

Changes in forelimb weight distribution were significantly influenced by the condition, involving the application of different nociceptive stimuli. Horses exposed to electrical stimuli showed longer durations of weight shifting than those exposed to thermal and mechanical stimuli. This result was expected, as electrical stimulation of the lateral digital nerve was specifically designed to evoke a response in the stimulated limb. In contrast, no mechanical or thermal stimuli were applied during the first two minutes of the Twitch and Post-Twitch intervals, while the automated device continued to deliver electrical stimuli throughout all observation periods. Therefore, the increased weight shifting observed in horses exposed to electrical stimulation likely reflects a direct response to ongoing peripheral nerve activation rather than a generalized stress response. These behavioral changes were accompanied by a significant increase in the composite stress score during the Twitch interval compared to Baseline and Post-Twitch, supporting the interpretation that the lip twitch induced a mild but measurable stress response in horses.

Including the role of personality traits in shaping behavioral responses to twitching was a central focus of this study, emphasizing the influence of individual temperament on coping strategies and behavioral outcomes. The results revealed that personality traits like neuroticism and agreeableness (and gregariousness) play an important role in determining how horses respond to the experimental situation. Neurotic horses, characterized by emotional instability and heightened sensitivity, exhibited more pronounced stress-related behaviors and alterations in weight distribution during the baseline and post-twitch intervals. These observations align with previous research linking neuroticism to increased stress reactivity and emotional sensitivity (Ijichi et al., 2014). Furthermore, neurotic horses demonstrated a significantly reduced tendency to move away from the nociceptive stimulus during twitching, suggesting a stronger immobilization/freezing response. The interaction between personality and interval significantly affected the duration of backward ear positioning, with neurotic horses maintaining ears backward for longer periods across all intervals, and particularly during Twitch. During the Twitch interval, agreeable horses tended to move away from the nociceptive stimulus more than neurotic horses, showing a more proactive response, indicating heightened expressivity. In the Post-Twitch interval, neurotic horses exhibited significantly more avoidance movements than agreeable horses.

The increased backward ear position observed in neurotic horses in our study aligns with previous research (Dalla Costa et al., 2014; Lundblad et al., 2021) which identified this behavior as a critical marker for stress and pain and we can highlight a potential link between neurotic personality traits and heightened sensitivity to acute stress and discomfort. These behaviors were notably exacerbated during the application of the twitch suggesting a compounded effect of neurotic temperament combined with increased sensitivity, potentially leading to lower pain thresholds and higher levels of frustration.

The interaction between personality and interval also had a significant effect on the composite stress score, with neurotic horses displaying higher stress scores than agreeable horses during Baseline and the difference became even more pronounced during the Post-Twitch interval.

Comparable findings have been reported in previous studies, including (Ijichi et al., 2014) which highlighted the similar relationship between neuroticisms and increased pain sensitivity. The relationship between neuroticism and heightened pain perception is not unique to horses. Studies in human medicine have consistently found that individuals with higher levels of neuroticism experience greater pain sensitivity to adverse stimuli (Ijichi et al., 2014; Jones et al., 2003). This parallel between humans and equine studies underscores the universal influence of personality traits on enhanced stress or pain responses, reinforcing the importance of temperament as a determinant of behavioral and physiological outcomes across species.

In our study, agreeable or gregarious horses – characterized by empathy and cooperative tendencies exhibited fewer baseline stress behaviors and adopted active responses during the twitch interval such as increased movements away from the stimulus. These proactive coping strategy contrasts with the immobilization or inhibited behavior seen in neurotic horses. While such active reactions suggest resilience, they also pose challenges for handlers due to the increased risk of flight responses. These findings align with prior studies showing that temperament potentially influences stress responses and coping strategies (Masko et al., 2020).

This contrast in stress responses between personality traits highlights the role of coping strategies. While such active reactions suggest resilience, they also pose challenges for handlers due to the increased risk of flight responses. Coping styles provide additional context for these findings. Individuals who are exposed to a stressor or a predator tend to adopt either an active strategy, characterized by 'fight or flight' responses, or a passive strategy, involving behavioral inhibition or 'freezing' (Koolhaas et al., 2010). Reduced movements during the application of a lip twitch, also called immobilization response that are observed in neurotic horse aligns with a so-called passive coping style that is often linked to heightened anxiety and increased vulnerability to stress (Flakoll et al., 2017). This strategy mirrors patterns observed in humans, where fear of movement and re-injury contribute to a behavioral inhibition (Goubert et al., 2004; Ijichi et al., 2014). These findings emphasize the need to consider both personality traits and coping strategies in the field of horse handling and especially management of neurotic horses to reduce stress and improve welfare.

Practical implications and recommendations

These findings have several implications for equine veterinary practice: the lip twitch, when applied appropriately and for short durations, may serve as a practical alternative to sedation, particularly for minor procedures. However, the stress responses noticed in this study emphasize the importance of understanding individual personality traits and tailoring handling techniques accordingly. Neurotic horses with heightened sensitivity and predisposition to stress responses, may benefit from additional preparation such as habituation and desensitization training to the procedure, or from the use of sedative drugs. Agreeable horses, on the other hand, may require strategies to manage their active coping responses to prevent flight reactions and potential harm to themselves or their handlers. Building a positive association between lip twitch and a food reward can possibly reduce their active responses. Recognizing behavioral indicators such as backward ear position, head movements and weight distribution can help practitioners assess the level of discomfort experienced by the horse and make timely adjustments to handling techniques. It is important to underscore the value of using a multimodal approach for assessing emotional valence and welfare in horses during potentially aversive procedures. Given the high degree of individual variability, establishing behavior-based cut-off criteria is essential when applying physical restraint methods. This not only ensures equine welfare but also reduced the risk of injury to the personnel involved.

Limitations and future directions

Despite the valuable insights gained, the study has several limitations. The small sample size (12 horses) and the predominance of geldings (11 out of 12) limit the statistical power and generalizability of the findings. Previous studies already reported significant sex differences when assessing equine personality, with mares often scoring higher on measures of anxiety and reactivity compared to geldings and stallions (Duberstein and Gilkeson, 2010; Rankins and Wickens, 2020). The underrepresentation of mares potentially restricts the ability to evaluate sex-based variations in stress and as well responses when exposed to discomfort since mares can have several temperaments and influences of hormones.

Additionally, the exclusion of the extroverted horses from the sample narrowed the scope of the findings. Extroverted horses, characterized by sociability and assertiveness are considered to be more likely to exhibit resistance and restraint behaviors during aversive procedures as seen in a previous study (Ijichi et al., 2013). Increasing the number of extroverted horses in future research could provide a more comprehensive understanding of personality-linked responses, particularly in relation to behaviors such as rearing or escape attempts as Ijichi et al. (2014) observed. This suggests that extroversion plays a critical role in shaping how horses perceive and respond to stressors. Another important consideration is the validity of the personality assessment method. Although the questionnaire used has been previously validated for use in horses, it remains a subjective tool reliant on caretaker or handler impressions, which may introduce observer bias or variability in scoring. This potential subjectivity should be considered when interpreting personality-related results.

The focus on the initial two minutes of twitch application, during which no additional handling was performed, may limit the study's findings to immediate reactions. Longer observational periods could capture the progression of stress responses or adaptation over time. Furthermore, the reliance on subjective behavioral observations introduces potential observer bias. Incorporating objective physiological markers, such as heart rate variability and cortisol levels alongside behavioral and personality assessments could enhance the reliability and depth of future studies. Finally, the exclusive use of horses belonging to the Swiss Armed Forces ensured consistent handling and minimized transport-related stress to the location of testing but limited the diversity of the sample and thus the generalizability of the findings.

Future research should aim to expand the sample size, have a balanced sex representation and include a broader range of personality types to enhance the generalizability of findings. Incorporating objective physiological measures alongside behavioral observations would provide a more comprehensive understanding of the observed responses. Exploring the effects of handling methods across different personality types could identify welfare-optimized practices tailored to individual horses.

Conclusion

This study identified mild stress-related behavioral changes in horses undergoing lip twitching, including reductions in ear movements and natural head position, increased head movements, and weight shifting. Additionally, the findings highlighted the fact that individual behavioral response to twitching are influenced by personality traits. Tailored handlings strategies taking into consideration personality can reduce stress and improve welfare during veterinary procedures.

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Ethical statement

This research adhered to all relevant ethical guidelines for animal studies, including the ARRIVE guidelines 2.0 (Percie du Sert et al., 2020). Approval for the study was granted by the Cantonal Committee for Animal Experiments in Canton Bern, Switzerland (National Permission number 34973), ensuring compliance with local regulations and institutional standards for animal welfare.

CRediT authorship contribution statement

Emanuela Dalla Costa: Writing – review & editing, Visualization, Supervision, Methodology, Formal analysis, Data curation. **Claudia Spadavecchia:** Writing – review & editing, Supervision, Methodology, Investigation. **Stéphane Montavon:** Writing – review & editing, Supervision, Resources. **Agassi de Sales Nathali Adrielli:** Writing – review & editing, Data curation. **Jil Jessy Kellershohn:** Writing – review & editing, Writing – original draft, Data curation. **Severin Blum:** Writing – review & editing, Investigation, Data curation.

Declaration of Competing Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jveb.2025.08.003.

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