Investigating the relationship of cortical frequencies and blood biomarkers using surface electroencephalography for lameness detection in adult horses

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Abstract: Musculoskeletal injuries (MSI) critically impact equine welfare, causing over 70% of thoroughbred fatalities (Crawford, 2021) and resulting in nearly 468,000 lame horses annually (Manfredi, 2023). This study investigated the correlation between surface electroencephalography (sEEG) and Prostaglandin E2 (PGE2) biomarkers in mature horses. Eight Quarter Horses underwent sEEG recordings while blood samples were collected for PGE2 analysis. Correlation analyses revealed a moderate non-significant relationship between EEG and PGE2 levels in the serum for delta, beta, and gamma waves ($r \leq 0.55$, $P \geq 0.16$), and for theta and alpha waves ($r \leq 0.57$, $P \geq 0.14$). Theta frequency showed significant increased activity in non-lame horses ($P = 0.04$), No significant differences in PGE2 levels pre- and post-evaluation were found ($P > 0.67$). However, higher PGE2 concentrations in serum compared to plasma were observed ($P < 0.01$). These results suggest a potential link between sEEG wave frequency, especially Theta, and pain, in addition with the used of PGE2, indicating a pathway for early lameness detection.

Keywords: Equine electroencephalography, Inflammation, Lameness, Prostaglandin E2, Welfare

Abbreviations:
EEG: Electroencephalography
PGE2: Prostaglandin E 2
SP: Substance P
BCS: Body Condition Score
BW: Body Weight
LS: Lameness Score
MSI: Musculoskeletal injuries

Introduction

Horses with musculoskeletal injuries (MSI) have a major risk of suffering a fatality, and surface electroencephalography (sEEG) along with biomarkers provide a promising alternative to current methods for evaluating pain in a non-invasive manner. Although these methods are well-

established in other species such as castration procedures in calves (Coetzee, 2013) and acute pain in sheeps (Ong, 1997) the benefits of its usage in the equine industry remain unexplored. Examining the connection between blood biomarkers and sEEG in mature horses may help identify lameness earlier and more accurately, which would improve performance and animal welfare.

Equine welfare has become a central concern due to the prevalence of musculoskeletal injuries and lameness across various equine disciplines. Despite ongoing research efforts to address this problem, these injuries continue to have serious ethical, health, and economic implications, particularly due to the risk of severe injury or death to both horses and riders. In thoroughbred racing, MSI accounts for over 70% of equine fatalities, with studies showing that between 7% and 49% of race day injuries lead to the horse's death (Crawford, 2021). As a result, there has been a growing focus on establishing health and welfare programs, such as Equine Ethics and Wellbeing Commission (Luke, 2023) and Horseracing Integrity and Safe Authority (HISA) designed to reduce the incidence and impact of these injuries in recent years.

When a horse experiences pain, compensatory gait alterations can lead to overloading and damage to muscles, joints, and ligaments (Hafsa Zaneb, 2009). If left unaddressed, these compensations can exacerbate the issue, further contributing to the progression of lameness. Performance horses are particularly vulnerable due to repetitive stresses and extreme physical demands, making early detection and management crucial to maintaining musculoskeletal health (McGreevy, 2011). Additionally, premature foal training, improper hoof balance, exposure to uneven surfaces, trauma, and muscular exhaustion and inflammation are factors that contribute to lameness in horses (Stacey, 2011). Beyond performance, lameness has a substantial negative influence on horse welfare and causes large financial losses for the equine sector (Jeffcott, 1982; Lindner, 1993). It has been estimated that the equine industry faces $678 million in economic losses annually due lameness cases (Manfredi, 2023).

Lameness evaluation in horses typically involves visual assessment of the horse's movement to identify any points of sensitivity or discomfort. Advanced imaging techniques, computed tomography (CT) and magnetic resonance imaging (MRI) enhances tissue delineation in horses, expanding diagnostic capabilities (Nelson, 2017). Both imaging modalities are significantly more expensive than radiographs and performing them under general anesthesia further increases costs due to anesthesia, hospitalization, and prolonged procedure time (Manso-Díaz, 2021). While effective in pinpointing the cause of lameness, these methods are only employed once the source of pain is reasonably suspected, as scanning multiple anatomical regions is impractical (Puchalski, 2011).

The limitations of the aforementioned traditional approaches, which frequently miss early indicators of musculoskeletal issues, contribute to the need for novel techniques to assess lameness in horses. Current diagnostic methods are typically reactive, identifying lameness only after significant damage has occurred. A more proactive, non-invasive approach is necessary to improve equine welfare and performance outcomes. One promising method is the use of biomarkers, such as Prostaglandin E2 (PGE2), which is an important eicosanoid linked to joint diseases (Hoxha, 2018). PGE2 is an inflammatory mediator that plays a key role in joint disorders, and its early detection could help identify joint inflammation before clinical signs of lameness become evident (Wang, 2014). In addition to biomarkers, advanced technologies like electroencephalography (EEG) offer a novel way to assess pain in horses. Surface electroencephalography (sEEG)measures electrical brain activity, capturing changes in brainwave patterns that are linked to pain and behavioral states. For instance, low-frequency

brainwaves, such as delta (0-3 Hz), are linked to increased comfort and pain reduction, commonly used for alleviating headaches and muscle contractions (Mir, 2021), while theta waves (4-7 Hz) are associated with chronic pain conditions and behavioral state identification (Simis, 2022). Combining biomarkers like PGE2 with EEG could offer a comprehensive evaluation system for early lameness detection in horses, helping to prevent long-term injuries and enhance their overall welfare. This study aims to explore the relationship between surface electroencephalography (sEEG) brainwaves and blood biomarkers, specifically Prostaglandin E2, in mature horses diagnosed with lameness. It is hypothesized that lower-frequency sEEG brainwaves will correlate with elevated levels of PGE2, an established biomarker of inflammation, and align with observed lameness scores. The goal of the study is to provide a more thorough, non-invasive technique for evaluating pain and inflammation in horses by looking at this correlation.

Materials and Methods

Ethical approval

All materials and methods used for this study were performed under the approval and authority of Tarleton State University, College of Agriculture & Natural Resources and Institutional Animal Care & Use Committee (IACUC) under the protocol number: 10-031-2023

Subjects: Animals

In this study eight horses were selected from the Tarleton State University's Equine Center herd. The sample included 3 mares and 5 geldings, all Quarter Horse breed, with an average age of 16.5 ± 3.6 years (range: 13 to 22 years), 573 ± 54 kg of body weight (BW), and 7 ± 0.88 score of Body Condition Score (BCS) (range: 5.50 to 8.00 score) with normal overall health. All were kept under the same husbandry conditions throughout the study. The control group (non-lame horse) consisted of one individual horse, a 17-year-old mare with a BW of 1200 lbs and BCS of 6.5 score. The experimental group (lame horses) included 3 mares and 5 geldings with an average age of 16.13 ± 3.64 years (range: 13 to 22 years), 1262.13 ± 120. 21lbs (range: 1006 to 1370 lbs) of BW, and 7 ± 0.93 score of BCS (range: 5.50 to 8.00 score).

EEG Activity Collection

For the electroencephalogram (EEG), each horse was individually haltered and taken outside of the paddock for the cap placement. The horse's face was cleaned with alcohol wipes, gel was applied to the EEG cap's electrodes, and the cap's middle electrodes were positioned approximately 10 cm above the horse's eyelids. Additionally, the average length of all the horses from eye-to-eye was 19.56 ± 0.53cm (range: 19 to 20 centimeters). To ensure proper contact, more gel was added, and electrodes were moved in circular motions while being lightly pressed to the horse's face until all sensors indicated a frequency of 10 kOhm or less from the > 50 kOhm sampling rate. The EEG recording lasted 5 minutes.

Both control and experimental groups were collected two times (pre- and post-), before and after lameness evaluation. The collections were for EEG activity and blood biomarkers (PGE2 plasma and Substance P serum).

Blood sampling analysis

Blood samples were taken via jugular venipuncture (10mL) using 20 G needles (0.9 × 40 mm). Blood was collected into Clot activator for serum, allowed to clot for 45 minutes, and K2 ethylenediamine tetraacetic acid (EDTA) blood tubes (BD Vacutainer, Franklin Lakes, NJ, USA) for plasma, which were immediately placed on ice. Plasma samples were spun at 12,000 × g for 20 min, and all were collected and stored at −80 °C until biomarker analysis. The prostaglandin E2 (ADI-930–001) and Substance P (ADI-901-018A) concentrations were determined following the manufacturer's recommendation using an enzyme-linked immunosorbent assay (ELISA) kit (Enzo Life Science, Villeurbanne, France), previously validated by Kanai (2021), PGE2 analytical sensitivity was 8.3 pg/mL and inter- and intra-assay variation of 9.8% and 12.1%, respectively. Samples were diluted 1:25, 1:10, and 1:8 using the assay buffer provided from the manufacturer. For substance P, analytical sensitivity was 5.3 pg/mL and inter- and intra-assay variation of 7.9% and 4.1%, respectively. Samples were diluted 1:64, 1:40, and 1:20 using the assay buffer provided from the manufacturer. The yield of extractions was determined by the manufacturer to be approximately 90% to 100%, and the cross-reactivity of other substances was less than 0.1%.

Lameness and BCS evaluations

The AAEP lameness scale evaluation was performed by a licensed DVM. Lameness severity was assigned a value from 0 to 5 (**Table 1**). Lameness evaluations consisted of horses walking in a straight line, figure eight or zig-zag motion, and trotting in a hard surface. Then they were evaluated while walking and trotting in circles (left and right) and backing up or moving forward with their head held high at a paddock with flat and solid ground. Additionally, the veterinarian performed palpations within the back, loin, croup and limbs, flexed the limbs and joints, and applied pressure to different points of the horse's body to seek sensitivity or discomfort. The horses underwent lameness testing while wearing the sEEG cap.

Table 1: Defining lameness scores.

Score	Meaning
0	Lameness not perceptible under any circumstances.
1	Lameness is difficult to observe and is not consistently apparent, regardless.
2	Lameness is consistently observable at a trot under all circumstances.
3	Lameness is consistently observable at a trot under all circumstances.
4	Lameness is obvious at a walk.
5	Lameness produces minimal weight bearing in motion and/or at rest or a complete inability to move.

Body condition scores by the veterinarian and principal investigator were subjectively appraised with the original description by Henneke et al. (1983) ranging from BCS 1 (very poor) to BCS 9 (extremely fat).

Post-Evaluation Procedures

After the lameness test, the EEG and blood draw collections were repeated. The EEG cap was removed, the horse's forehead was cleaned with alcohol wipes, and the cap was prepared for the next horse. The horse was returned to the catching paddock, and the process was repeated

with the next horse. This sequence was repeated until all horses in each group were evaluated. After all groups were assessed, the area was cleaned, and data were stored for analysis.

Statistical analysis

Data was analyzed using SAS software version 9.4 (SAS Institute, Cary, NC). All parameters were tested for normality and transformed if non-normal. Assigned LS ranged from 0-3, and data were assigned as non-lame (0-1) and lame (2-3). Spearman correlation procedure was used to measure the relationship between EEG frequencies, lameness score categories, substance P concentration, and PGE2 concentration in plasma and serum based in fixed effect of time. The GLIMMIX procedure of SAS was used to evaluate the interaction effect of each EEG brainwave frequency activity with lameness score categories at time 0 (pre lameness evaluation). Data are presented as means ± pooled SE. Data for delta was reduced by 10,000 units for analysis efficiency. Means were separated using the Least Square Mean Back-Transformed model statement. No statistical differences were considered significant if $P \geq 0.05$.

Results

Table 1. Delta frequency (EEG) correlations with Lameness Score (LS), Prostaglandin E_2 in Plasma (PGE2P), Prostaglandin E2 in Serum (PGE2S) and Substance P (SPS) at Pre and Post Lameness Evaluation.

	Pre			*P ≥ 0.115*	**Post**			*P ≥ 0.160*
	LS	**PGE2P**	**PGE2S**	**SPS**	**LS**	**PGE2P**	**PGE2S**	**SPS**
EEG	0.05006	0.14286	0.16667	0.40476	-0.025	0.2381	0.54762	0.42857
LS		0.12516	0.27534	-0.6008		0.25031	0.20025	0.02503
PGE2P			0.52381	-0.2857			0.28571	-0.2381
PGE2S				-0.0238				0.02381

Electroencephalography activity measurements were taken before lameness evaluation (Pre) and after Lameness Evaluation (Post), both for 5 minutes. Horses were assigned lameness Scores, based on veterinarian evaluation, ranged from 0-3. Data were assigned as non-lame (0-1) and lame (2-3). Blood sampling for biomarkers was draw based on time (Pre and Post). Differences in prostaglandin E_2 matrices (plasma and serum) was selected for further analysis. P values is presented as the lowest value within the effects values found. Data for delta was reduced by 10,000 units for analysis efficiency.

Table 2. Theta frequency (EEG) correlations with Lameness Score (LS), Prostaglandin E_2 in Plasma (PGE2P), Prostaglandin E2 in Serum (PGE2S) and Substance P (SPS) at Pre and Post Lameness Evaluation.

			Pre	*P ≥ 0.115*			**Post**	*P ≥ 0.139*
	LS	**PGE2P**	**PGE2S**	**SPS**	**LS**	**PGE2P**	**PGE2S**	**SPS**
EEG	0.17522	0.2381	0.33333	0.28571	-0.1001	-0.1905	0.57413	0.54762
LS		0.12516	0.27534	-0.6008		0.25031	0.20025	0.02503
PGE2P			0.52381	-0.2857			0.28571	-0.2381
PGE2S				-0.0238				0.02381

Electroencephalography activity measurements were collected before lameness evaluation (Pre) and after Lameness Evaluation (Post), both for 5 minutes. Horses were assigned lameness Scores, based on veterinarian evaluation, ranged from 0-3. Data were assigned as non-lame (0-1) and lame (2-3). Blood sampling for biomarkers was collected based on time (Pre and Post). Differences in prostaglandin E_2 matrices (plasma and serum) was selected for further analysis. P values is presented as the lowest value within the effects values found.

Table 3. Alpha frequency (EEG) correlations with Lameness Score (LS), Prostaglandin E_2 in Plasma (PGE2P), Prostaglandin E2 in Serum (PGE2S) and Substance P (SPS) at Pre and Post Lameness Evaluation.

	Pre			*P ≥ 0.115*	**Post**			*P ≥ 0.160*
	LS	**PGE2P**	**PGE2S**	**SPS**	**LS**	**PGE2P**	**PGE2S**	**SPS**
EEG	-0.1001	0.40476	-0.0714	0	-0.1001	-0.1905	0.57143	0.54762
LS		0.12516	0.27534	-0.6008		0.25031	0.20025	0.02503
PGE2P			0.52381	-0.2857			0.28571	-0.2381
PGE2S				-0.0238				0.02381

Electroencephalography activity measurements were collected before lameness evaluation (Pre) and after Lameness Evaluation (Post), both for 5 minutes. Horses were assigned lameness Scores, based on veterinarian evaluation, ranged from 0-3. Data were assigned as non-lame (0-1) and lame (2-3). Blood sampling for biomarkers was collected based on time (Pre and Post). Differences in prostaglandin E_2 matrices (plasma and serum) was selected for further analysis. P values is presented as the lowest value within the effects values found.

Table 4. Beta frequency (EEG) correlations with Lameness Score (LS), Prostaglandin E_2 in Plasma (PGE2P), Prostaglandin E2 in Serum (PGE2S) and Substance P (SPS) at Pre and Post Lameness Evaluation.

	Pre			*P ≥ 0.115*	**Post**			*P ≥ 0.160*
	LS	**PGE2P**	**PGE2S**	**SPS**	**LS**	**PGE2P**	**PGE2S**	**SPS**
EEG	0.35044	0.45238	0.30952	0.04762	-0.025	-0.2381	0.54762	0.42857
LS		0.12516	0.27534	-0.6008		0.25031	0.20025	0.02503
PGE2P			0.52381	-0.2857			0.28571	-0.2381
PGE2S				-0.0238				0.02381

Electroencephalography activity measurements were collected before lameness evaluation (Pre) and after Lameness Evaluation (Post), both for 5 minutes. Horses were assigned lameness Scores, based on veterinarian evaluation, ranged from 0-3. Data were assigned as non-lame (0-1) and lame (2-3). Blood sampling for biomarkers was collected based on time (Pre and Post). Differences in prostaglandin E_2 matrices (plasma and serum) was selected for further analysis. P values is presented as the lowest value within the effects values found.

Table 5. Gamma frequency (EEG) correlations with Lameness Score (LS), Prostaglandin E_2 in Plasma (PGE2P), Prostaglandin E2 in Serum (PGE2S) and Substance P (SPS) at Pre and Post Lameness Evaluation.

	Pre			*P ≥ 0.0856*	Post			*P ≥ 0.160*
	LS	**PGE2P**	**PGE2S**	**SPS**	**LS**	**PGE2P**	**PGE2S**	**SPS**
EEG	0.55069	0.35714	0.64286	-0.0238	-0.025	-0.2381	0.54762	0.42857
LS		0.12516	0.27534	-0.6008		0.25031	0.20025	0.02503
PGE2P			0.52381	-0.2857			0.28571	-0.2381
PGE2S				-0.0238				0.02381

Electroencephalography activity measurements were collected before lameness evaluation (Pre) and after Lameness Evaluation (Post), both for 5 minutes. Horses were assigned lameness Scores, based on veterinarian evaluation, ranged from 0-3. Data were assigned as non-lame (0-1) and lame (2-3). Blood sampling for biomarkers was collected based on time (Pre and Post). Differences in prostaglandin E_2 matrices (plasma and serum) was selected for further analysis. P values is presented as the lowest value within the effects values found.

Table 6. Main effect interactions of Electroencephalography (EEG) frequencies and Lameness Score (LS) at Pre-Lameness Evaluation.

Effect	**NumDF**	**DenDF**	**Fvalue**	**Pr>F**
EEG	4	22	579.31	<0.0001*
LS	1	6	0.34	0.58
EEG*LS	4	22	3.06	0.04*

Electroencephalography activity measurements were taken before lameness evaluation (Pre) for 5 minutes. Horses were assigned lameness Scores, based on veterinarian evaluation, ranged from 0-3. Data were assigned as non-lame (0-1) and lame (2-3). EEG frequencies effect contain the five brainwaves' measurements (delta, theta, alpha, beta and gamma). Asterisks (*) represent significant differences ($P \leq 0.05$) within main effect or interaction.

Table 7. Main effect interactions of Electroencephalography (EEG) brainwave frequencies and Lameness Score (LS) at Pre-Lameness Evaluation.

	Effect	**NumDF**	**DenDF**	**Fvalue**	**Pr>F**
Delta	LS	1	5	0.39	0.5611
	EGG	1	5	1.18	0.3264
Theta	LS	1	5	1.05	0.3524
	EGG	1	5	0.48	0.5185
Alpha	LS	1	5	0.78	0.4163
	EGG	1	5	0.9	0.3862
Beta	LS	1	5	0.2	0.4163

	EGG	1	5	0.66	0.4545
Gamma	LS	1	5	0.33	0.5917
	EGG	1	5	0	0.9724

Electroencephalography activity measurements were taken before lameness evaluation (Pre) for 5 minutes. Horses were assigned lameness Scores, based on veterinarian evaluation, ranged from 0-3. Data were assigned as non-lame (0-1) and lame (2-3). EEG frequencies effect contain the five brainwaves' measurements (delta, theta, alpha, beta and gamma). Data for delta was reduced by 10,000 units for analysis efficiency.

Table 8. Main effect interactions of Electroencephalography (EEG) brainwave frequencies by Lameness Score categories: (L and NL) at Pre-Lameness Evaluation.

	LCat	**Estimate**	**SE**	**LS Mean**	**SE Mean**
Delta	L	4.5609	1.058	1674.24	240.463
Delta	NL	5.4934	1.058	4254.1	610.997
Theta	L	8.4265	0.8071	6324.45	6060.07
Theta	NL	9.6115	0.8071	20684.63	19819.95
Alpha	L	7.3705	0.8633	2305.67	2425.84
Alpha	NL	8.4746	0.8633	6954.86	7317.35
Betha	L	6.425	0.854	888.68	920.9
Betha	NL	6.9623	0.854	1520.87	1576.02
Gamma	L	5.3807	0.7574	289.334	254.688
Gamma	NL	6.0042	0.7574	539.749	475.117

Electroencephalography activity measurements were taken before lameness evaluation (Pre) for 5 minutes. Horses were assigned lameness Scores, based on veterinarian evaluation, ranged from 0-3. Data were assigned as non-lame (0-1) and lame (2-3). EEG frequencies effect contain the five brainwaves' measurements (delta, theta, alpha, beta and gamma). Data for delta was reduced by 10,000 units for analysis efficiency. Data for brainwave frequencies interaction by lameness score categories: (L and NL) was back-transformed for analysis efficiency.

Figure 1

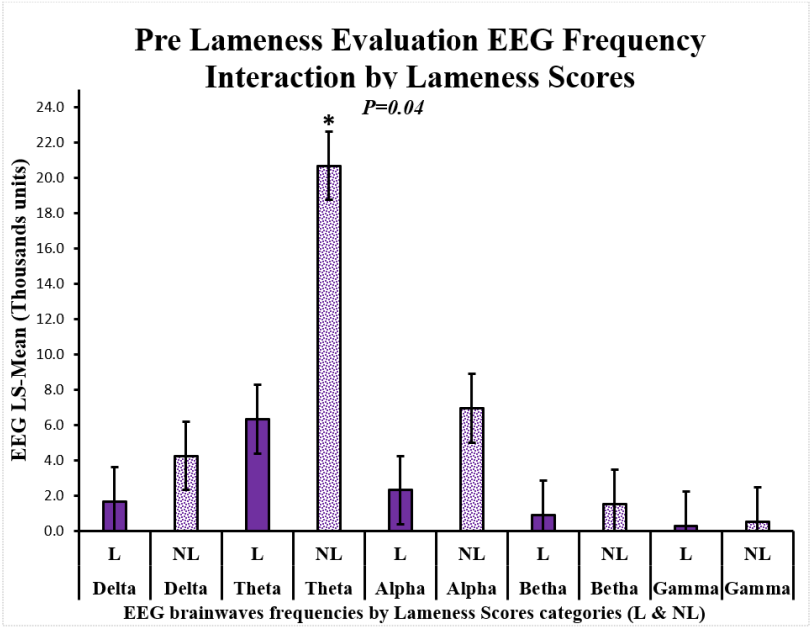

Figure 1. Main effect interactions of Electroencephalography (EEG) brainwave frequencies by Lameness Score categories: (L and NL) at Pre-Lameness Evaluation. Electroencephalography activity measurements were taken before lameness evaluation (Pre) for 5 minutes. Horses were assigned lameness Scores, based on veterinarian evaluation, ranged from 0-3. Data were assigned as non-lame (0-1) and lame (2-3). EEG frequencies effect contain the five brainwaves' measurements (delta, theta, alpha, beta and gamma). Data for delta was reduced by 10,000 units for analysis efficiency. Data for brainwave frequencies interaction by lameness score categories: (L and NL) was back-transformed for analysis efficiency. Least squares mean with asterisks (*) represent significant differences ($P \leq 0.05$) between EEG brainwave interaction with lameness score category.

Figure 2

Concentration differences of Prostaglandin E2 matrices by time

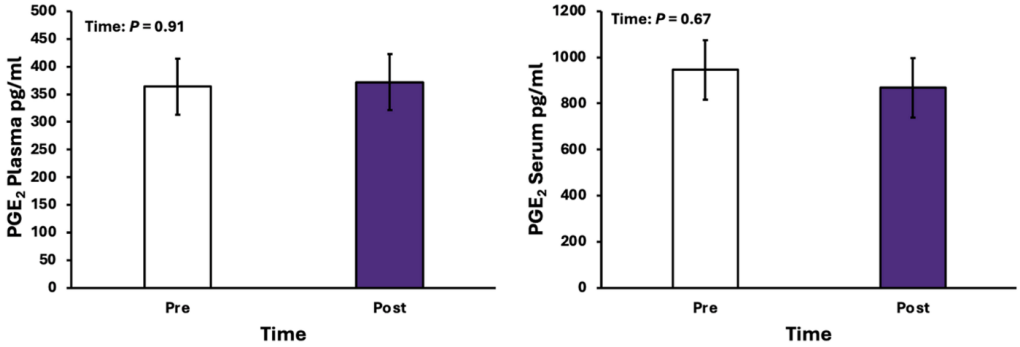

Figure 2. Concentration differences of Prostaglandin E_2 matrices by time. Blood samples were draw before lameness evaluation (Pre) and after (Post). This samples were aliquoted as plasma and serum. Means with different letter subscripts represent significant differences in PGE2 matrices by time ($P \leq 0.05$).

Figure 3

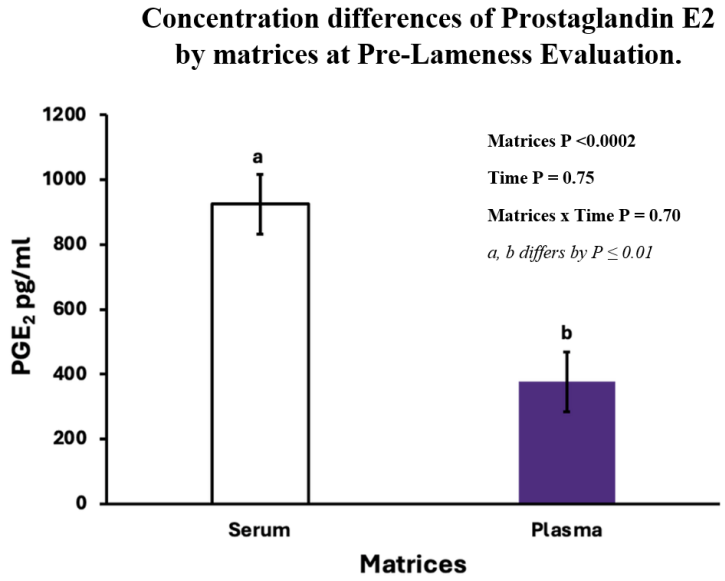

Figure 3. Concentration differences of Prostaglandin E_2 by matrices at Pre-Lameness Evaluation. Blood samples were draw before lameness evaluation (Pre) and after (Post). This samples were aliquoted as plasma and serum. Means with different letter subscripts represent significant differences in PGE2 concentration by matrices ($P \leq 0.05$).

Discussion

Using data from channel 8 (superior and centrally located over the midline), frequency (EEG) correlations with lameness scores (LS), Prostaglandin E_2 in Plasma (PGE2P), Prostaglandin E_2 in Serum (PGE2S) and Substance P (SPS) at pre and post lameness evaluation fixed effect of time were analyzed. Correlation analyses revealed a moderate non-significant relationship between EEG and PGE2 levels in the serum for delta (Table 1), beta (Table 4), and gamma (Table 5) waves ($r \leq 0.55$, $P \geq 0.16$), and for theta (Table 2) and alpha (Table 3) waves ($r \leq 0.57$, $P \geq 0.14$). Additionally, Theta presented a moderate non-significant correlation relationship between EEG and SP levels in the serum ($r \leq 0.55$, $P \geq 0.14$). Significant differences in interactions were presented in Table 6 by EEG frequencies (EEG) with lameness scores categories (LS) ($P = 0.04$) In Figure 1, regarding the significant interaction of EEG frequencies (EEG) with lameness scores categories (LS) are presented a highest interaction activity by Theta frequency as non-lame category. Stomp et al. (2020) concluded that EEG activity was characterized by more theta waves activity, implying that lowered theta power may reveal the subjective perception of spontaneous chronic pain by horses.

No differences in PGE2 levels were observed pre- and post-lameness evaluation for both plasma ($P = 0.91$) and serum ($P = 0.67$) in Figure 2. However, higher concentrations were found in PGE2 serum (Figure 3) compared to plasma ($P < 0.01$). This significance of concentrations is consistent with the findings of Yu et al. (2011), where they reported that metabolite profiles from plasma and serum were distinct, with 104 metabolites showing significantly higher concentrations in serum.

The significance of advanced equine wellbeing lies in its ability to close a crucial gap in non-invasive lameness detection techniques, which significantly impact a horse's quality of life. Population-based welfare assessments target specific groups, such as racehorses or leisure

horses, to evaluate broader welfare issues rather than individual animals (Hockenhull, 2014). These assessments are vital for identifying widespread welfare problems, implementing preventive measures, and improving overall care practices across equine discipline. Musculoskeletal injuries (MSI) pose a significant challenge in various equine disciplines globally, affecting not only horses but also riders, who face increased risks of serious injury or fatality when a horse experience an MSI. Performance horses frequently encounter orthopedic issues due to the rigorous physical demands of their disciplines. Accurate diagnosis, treatment, and prognosis of sports-related injuries depend on veterinarians' technical knowledge and competence in the specific discipline. Moreover, owners report that their satisfaction with their veterinarian is influenced by the clinician's understanding of the horse's performance discipline (Johnson, 2020). Based on this pilot data, there may be a relationship between sEEG wave frequency and pain presence, particularly in the lower frequency bands (delta and theta). Correlations for biomarkers PGE2 advance the aim to identify and differentiate a correlation for early detection of lameness.

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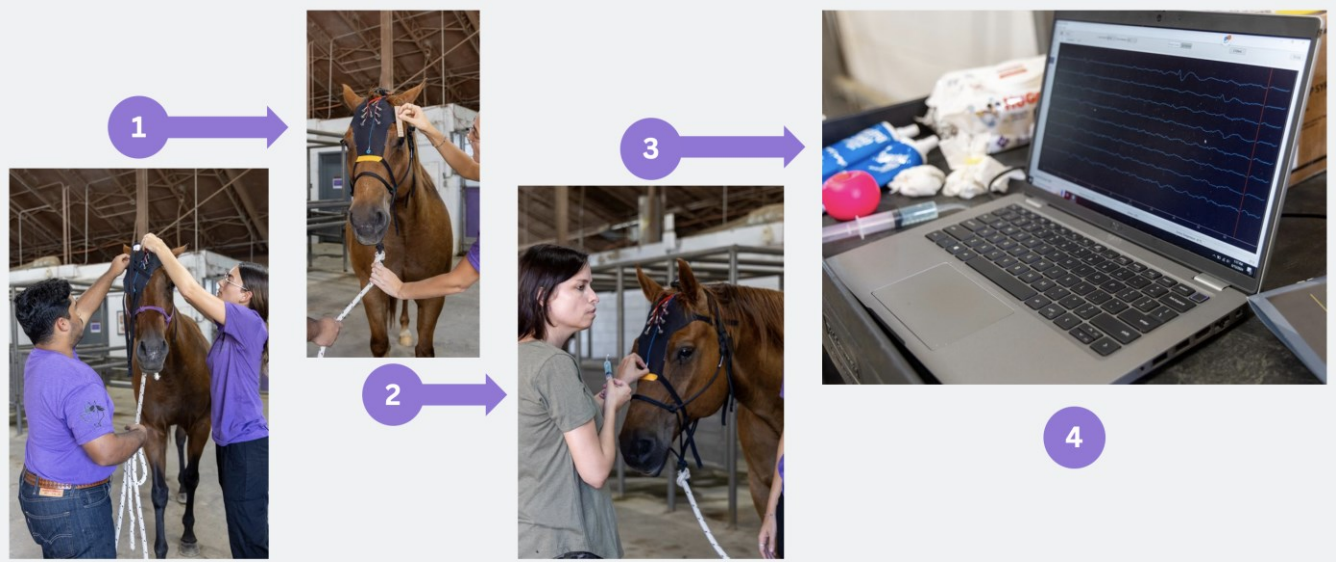
Attachments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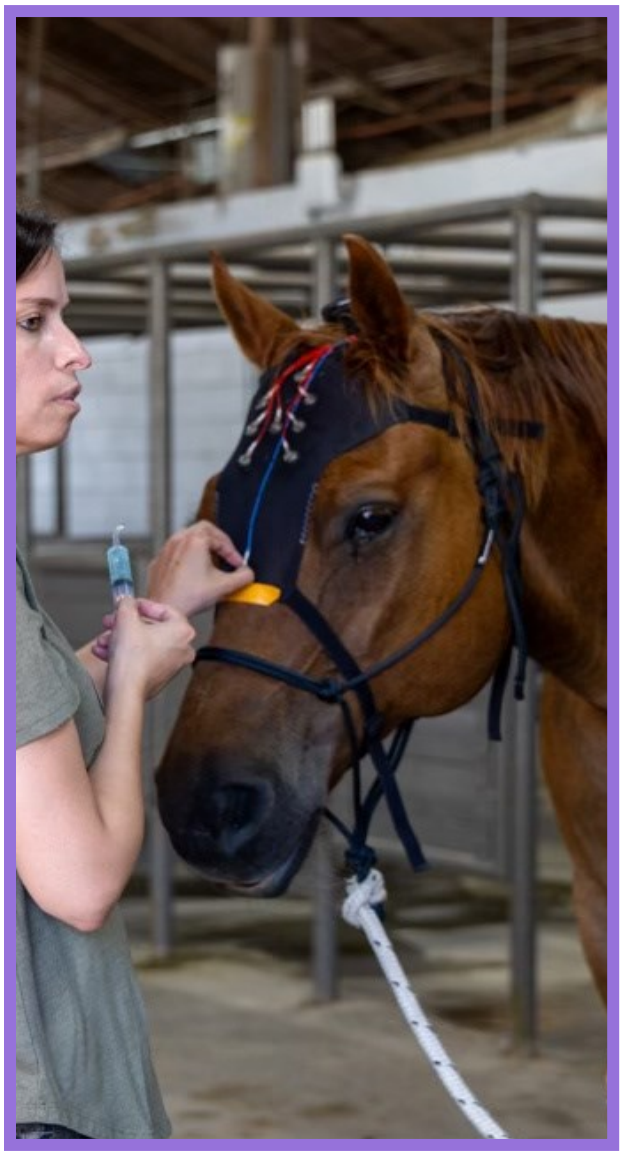
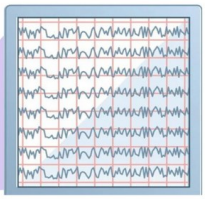
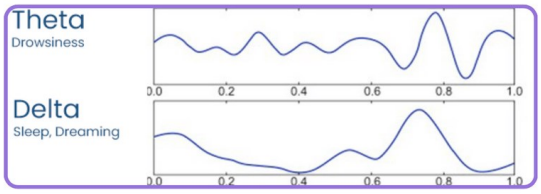


Methodology

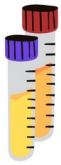


EEG Reading for 5 min Pre & Post LE



Resting state sEEG (Mentalab) data were recorded at a minimum 250 Hz sampling

Lameness Evaluation by D.V.M



Plasma & Serum Tubes prior and post LE

Serum and Plasma harvested & aliquoted for storage at -80 °C

PGE2 high sensitivity ELISA kit for Plasma & Serum matrices

Concentrations analyzed with BioTek CYTATION|5 imaging reader