Exploring surface electroencephalography and Substance P for the identification of lameness in mature horses

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Introduction: Lameness is a clinical sign that affects all horses (Crecan et al., 2022; Kaneene et al., 1997; Kane et al., 2000), and it can often be caused by pain from structural or functional disorders of the locomotor system (Swor et al., 2019). Early, non-invasive detection of lameness is essential for advancing equine health and welfare. The application of precision animal health technologies, including surface electroencephalography (sEEG), has been previously evaluated in horses and other species for the assessment of stress (De Camp et al., 2020), pain (Stomp et al., 2020), and identifying the various stages of sleep (Williams et al., 2008), but there is limited information involving the utility of sEEG to detect lameness in mature horses.

Objectives: Therefore, the objective of this study was to determine the effect of conducting a lameness evaluation on a systemic biomarker of pain (substance P; SubP) and sEEG recordings in mature horses.

Methods: Eight Quarter Horses (3 mares and 5 geldings, 16.5 ± 3.6 yr, 573 ± 54 kg BW) were used to test the hypothesis that performing a lameness evaluation would increase the amplitude of sEEG recordings but not affect the concentration of a systemic neuropeptide related to pain. An 8-channel sEEG cap was secured between the parietal and frontal regions, with electrodes 3, 4, and 7 positioned 10 cm above the upper eyelid (Figure 1). A ground electrode was placed above the nose. Electrode gel was used to improve conductivity. Resting state sEEG (Mentalab; Munich, Germany) data were recorded at a minimum 250 Hz sampling rate for 5 min. EEG data were processed in Matlab/EEGlab (filtered at 0.2Hz - 50 Hz and artifact rejected) and Fast fourier transforms were computed using Notepad++ and Cartool into bands: delta (0-3 Hz), theta (4-7 Hz), alpha (8-12 Hz), beta (13-30 Hz), and gamma (31-50 Hz). Blood was also collected via jugular venipuncture into sterile, non-additive tubes. Samples and sEEG recordings were obtained immediately prior to and following a lameness exam was performed and lameness scores (LS) assigned by a licensed veterinarian (Table 1). Blood was allowed to clot and serum was harvested and stored at -80°C for later analyses of SubP by commercial ELISA. Serum data were analyzed using the mixed procedure in SAS with the main effect of time, and the CORR procedure was used to determine the relationship between age, LS, and SubP. Frequency bands from the sEEG recordings were log transformed and analyzed individually using the GLIMMIX procedure.

Results:

Assigned LS ranged from 0 to 3, and there was no relationship ($r = 0.02$; $P = 0.94$) observed between SubP and LS, but there was a moderate to strong relationship between age and LS ($r = 0.56$; $P = 0.02$) (Table 1). There was no effect of time on SubP concentrations ($P = 0.07$) (Graph 1). For sEEG frequency bands, there was no effect of time by electrode interaction ($0.84 \leq P \leq 0.98$) or time main effect ($0.53 \leq P \leq 0.85$) for any of the or any of the sEEG frequency bands (Graph 2-6). Electrode was found to be significant for Gamma ($P < 0.01$) and a tendency ($P = 0.05$) for Beta sEEG frequency bands, whereas frequencies were not significant ($0.12 \leq P \leq 0.21$).

Conclusion:

These data indicate that lameness evaluation does not incite changes in sEEG data or systemic levels of SubP. However, the negative correlation between Age and SubP (Table 2) aligns with Barbariga et al. (2018), who reported decreasing SubP levels with Age in both mice ($P \leq 0.005$) and human corneas ($P \leq 0.04$).

Conversely, the moderate to strong relationship between Age and lameness ($r = 0.56$, $P = 0.02$) is consistent with Murray et al. (2010), who observed that the likelihood of lameness increased by 1.06 times with each additional year of Age.

Lastly, Caron et al. (1992) reported that synovia from healthy horses had significantly lower SubP concentrations than those with joint diseases ($P \leq 0.05$), while Guber et al. (2024) found SubP to be an unreliable marker for reflecting pain or predicting outcomes in equine colic, which may explain our findings of no significant relationship between SubP and LS ($r = 0.02$, $P = 0.94$). For no time effect on the sEEG frequency bands, Stomp et al. (2020) found that horses have highly consistent individual EEG profiles over time. De Camp et al. (2020) found EEG patterns differed between resting and stress conditions in horses, suggesting potential for further research on using EEG to assess welfare.

Recommendations: Sample size must be acknowledged, not all ranges of LS were present, and there was only one sampling day due to limited time. Further research could be done focused on lameness cases with surgically induced osteoarthritis to see if there is a relationship between x-rays and the different sEEG bands over time.

References:

Barbariga, M., Rabiolo, A., Fonteyne, P., Bignami, F., Rama, P., & Ferrari, G. (2018). The effect of aging on nerve morphology and substance P expression in mouse and human corneas. *Investigative Ophthalmology & Visual Science*, *59*(13), 5329-5335.

Caron, J. P., Bowker, R. M., Abhold, R. H., Toppin, D. S., Sonea, I. M., & Vex, K. B. (1992). Substance P in the synovial membrane and fluid of the equine middle carpal joint. *Equine Veterinary Journal*, *24*(5), 364-366.

Crecan, C. M., Morar, I. A., Lupsan, A. F., Repciuc, C. C., Rus, M. A., & Pestean, C. P. (2022). Development of a Novel Approach for Detection of Equine Lameness Based on Inertial Sensors: A Preliminary Study. *Sensors*, *22*(18), 7082.

De Camp, N. V., Ladwig-Wiegard, M., Geitner, C. I., Bergeler, J., & Thöne-Reineke, C. (2020). EEG based assessment of stress in horses: a pilot study. *PeerJ*, *8*, e8629.

Kane, A. J., Traub-Dargatz, J., Losinger, W. C., & Garber, L. P. (2000, November). The occurrence and causes of lameness and laminitis in the US horse population. In *AAEP Proceedings* (Vol. 46, pp. 277-280).

Kaneene, J. B., Ross, W. A., & Miller, R. (1997). The Michigan equine monitoring system. II. Frequencies and impact of selected health problems. *Preventive veterinary medicine*, *29*(4), 277-292.

Murray, R. C., Walters, J. M., Snart, H., Dyson, S. J., & Parkin, T. D. (2010). Identification of risk factors for lameness in dressage horses. *The Veterinary Journal*, *184*(1), 27-36.

Stomp, M., d'Ingeo, S., Henry, S., Lesimple, C., Cousillas, H., & Hausberger, M. (2020). EEG individual power profiles correlate with tension along spine in horses. *Plos one*, *15*(12), e0243970.

Swor, T. M., Dabareiner, R. M., Honnas, C. M., Cohen, N. D., & Black, J. B. (2019). Musculoskeletal problems associated with lameness and poor performance in cutting horses: 200 cases (2007–2015). *Journal of the American Veterinary Medical Association*, *254*(5), 619-625.

Williams, D. C., Aleman, M., Holliday, T. A., Fletcher, D. J., Tharp, B., Kass, P. H., ... & LeCouteur, R. A. (2008). Qualitative and quantitative characteristics of the electroencephalogram in normal horses during spontaneous drowsiness and sleep. *Journal of veterinary internal medicine*, *22*(3), 630-638.

Attachments

Figure 1: Measuring the EEG cap for correct placement and standard measurement.

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.

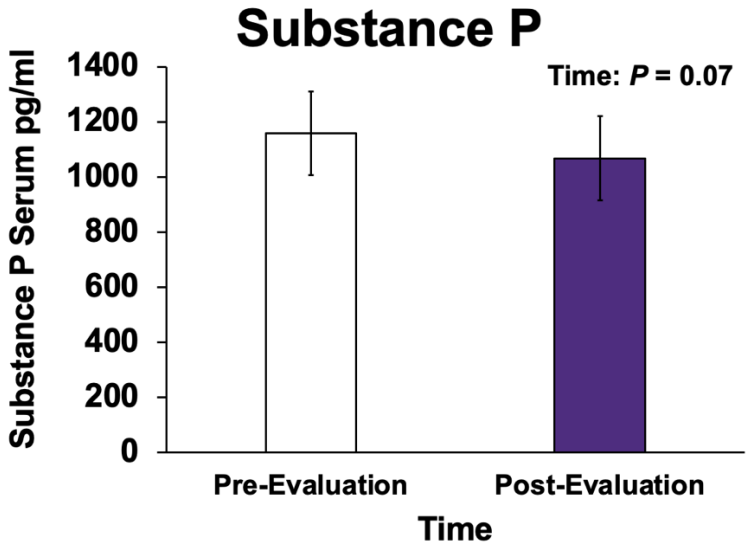
Body condition scores by the veterinarian and principal investigator were subjectively appraised ranging from BCS 1 (very poor) to BCS 9 (extremely fat).

Table 2: Spearman Correlation Coefficients

	PGE_2 Plasma	PGE_2 Serum	Substance P	LS
Age	0.3248	-0.0804	**-0.5454***	**0.5591***
PGE_2 Plasma	/	0.3799	-0.2235	0.0662
PGE_2 Serum		/	0.0000	0.0807
Substance P			/	**0.0192**

Reflects the correlations between variables using Spearman Correlation Coefficients. There was no relationship (r = 0.02; *P* = 0.94) observed between SubP and LS, but there was a moderate to strong relationship between age and LS (r = 0.56; *P* = 0.02) (Table 1).

Graph 1: Substance P Serum concentrations over time



Substance P serum concentrations were evaluated over time. There was no effect of time on SubP concentrations (*P* = 0.07).

Graph 2: Gamma frequency waves' (31-50 Hz) power spectral tendency over time.

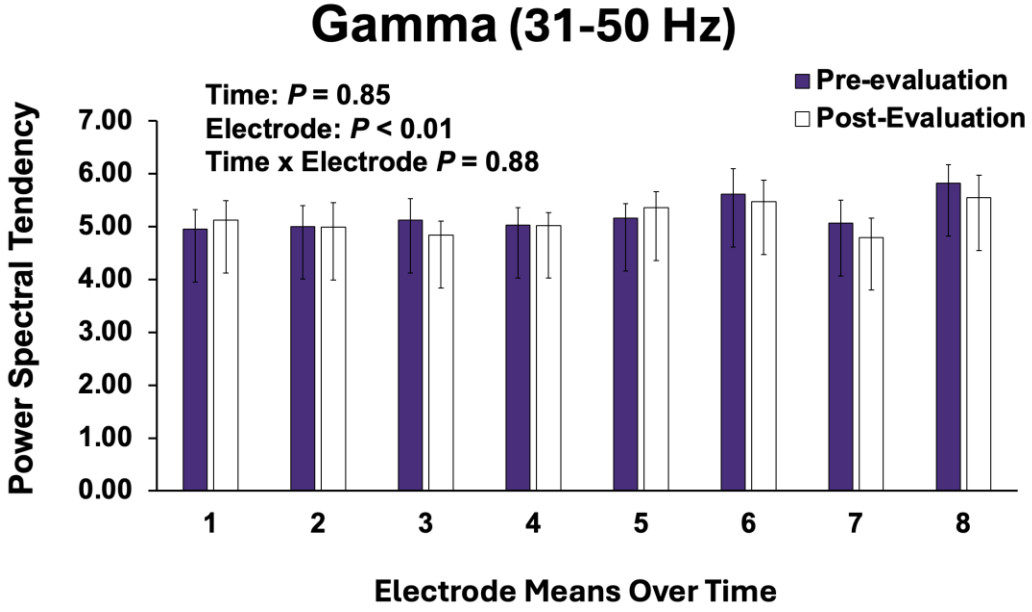


Figure represents the power spectral tendency for Gamma (31-50 Hz) of each electrode over time. There was no significance over time ($P = 0.85$). Gamma electrode was found to be significant ($P < 0.01$). Time by electrode was not significant ($P = 0.88$).

Graph 3: Beta frequency waves' (13-30 Hz) power spectral tendency over time.

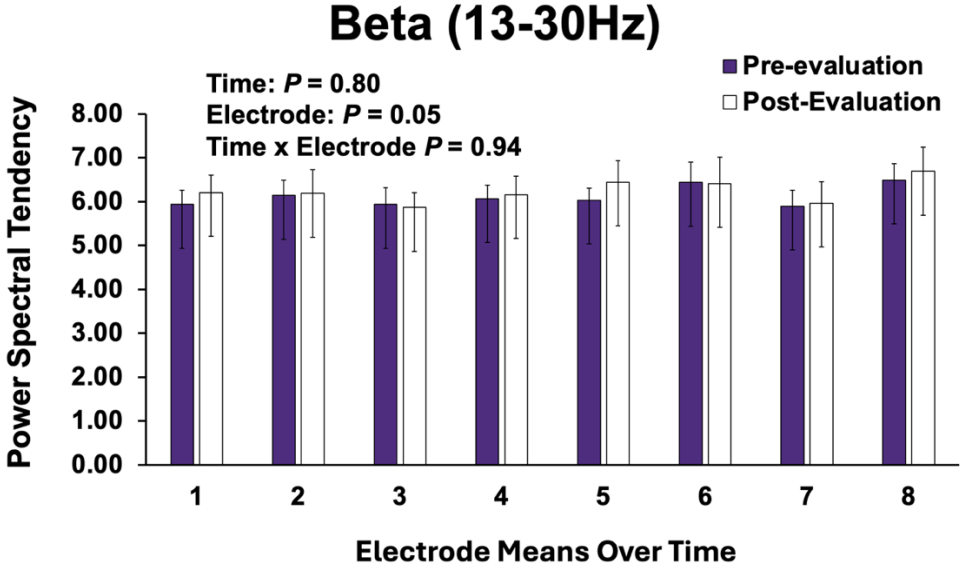


Figure represents the power spectral tendency for Beta (13-30 Hz) of each electrode over time. There was no significance over time ($P = 0.80$) or between time by electrode ($P = 0.94$). Beta electrode was found to be significant ($P = 0.05$).

Graph 4: Alpha frequency waves' (8-12 Hz) power spectral tendency over time.

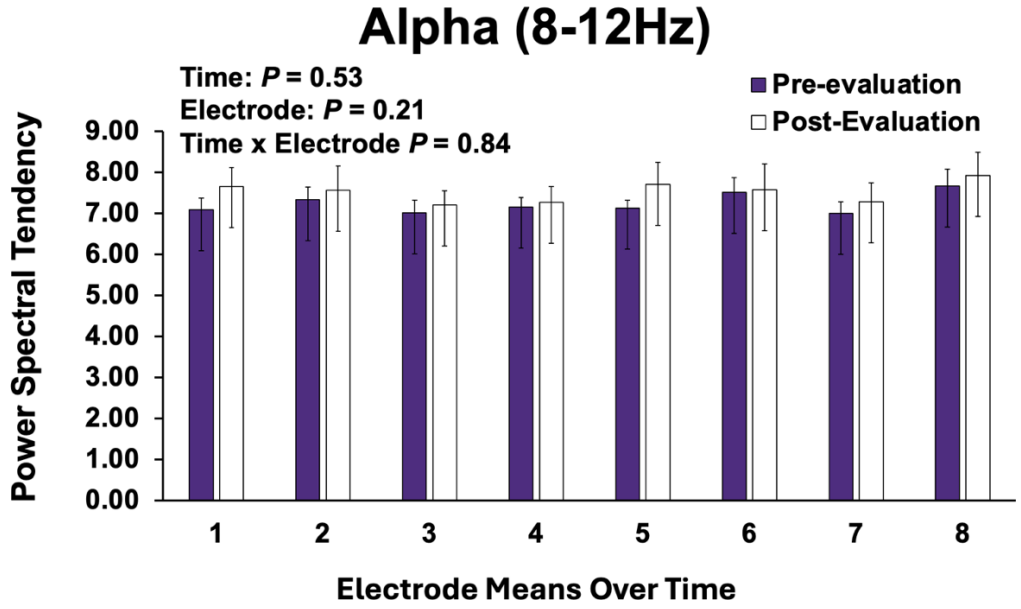


Figure represents the power spectral tendency for Alpha (8-12 Hz) of each electrode over time. There was no significance over time ($P = 0.53$), electrode ($P = 0.21$), or between time by electrode ($P = 0.84$).

Graph 5: Theta frequency waves' (4-7 Hz) power spectral tendency over time.

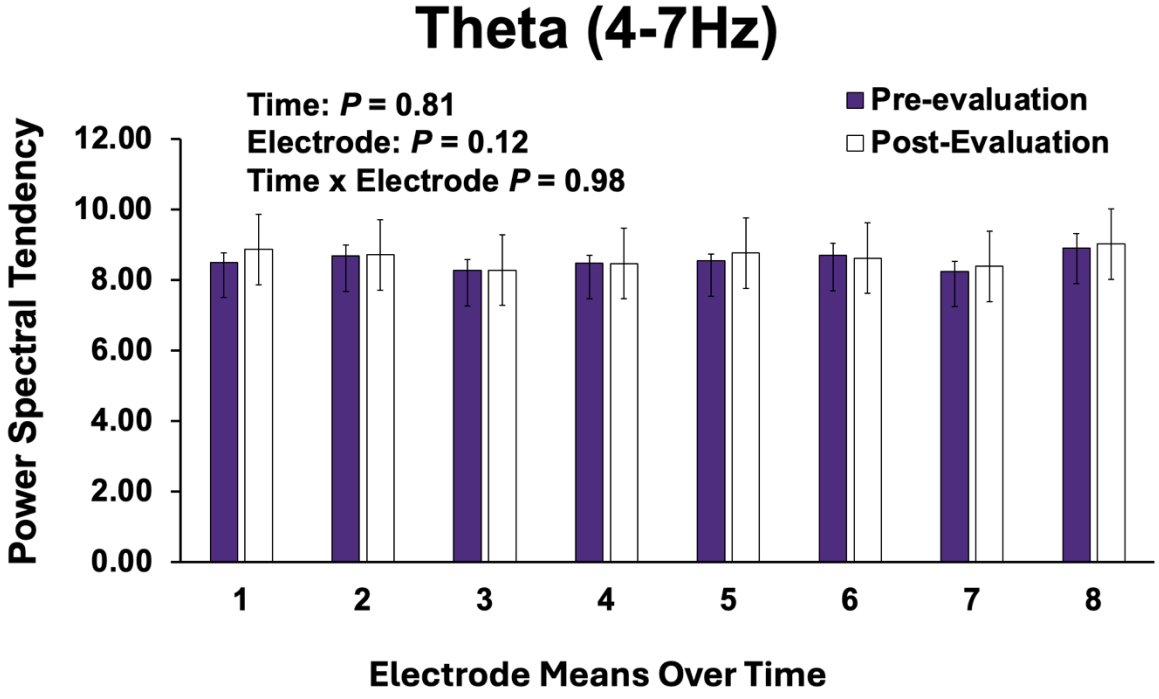


Figure represents the power spectral tendency for Theta (4-7 Hz) of each electrode over time. There was no significance over time ($P = 0.81$), electrode ($P = 0.12$), or between time by electrode ($P = 0.98$).

Graph 6: Delta frequency waves (0-3 Hz) power spectral tendency over time.

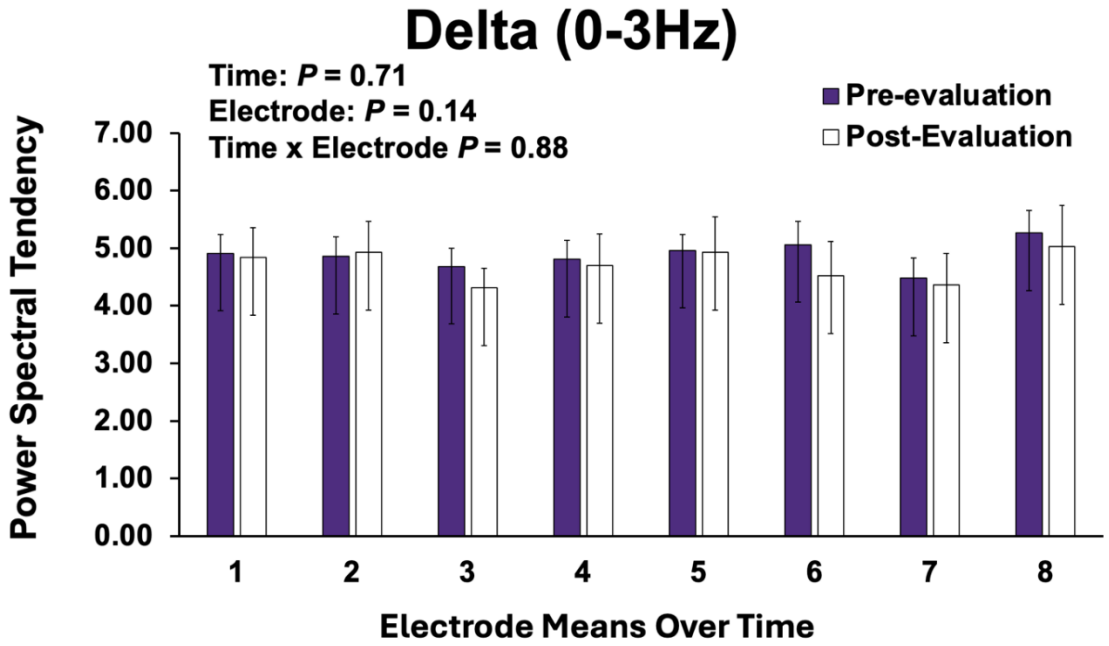


Figure represents the power spectral tendency for Theta (4-7 Hz) of each electrode over time. There was no significance over time ($P = 0.71$), electrode ($P = 0.14$), or between time by electrode ($P = 0.88$).