TITLE: The importance of measuring skin resistance for electrical nociceptive stimulation in standing horses

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Abstract:	Electrical stimulation is commonly used in antinociceptive studies in standing horses. With this correspondence, we would like to point out the importance of measuring and reducing the skin resistance between electrodes below 3 kΩ. Some studies did not include this measurement, which may lead to heterogeneous and less accurate data.

We are writing this letter in order to draw attention to some inappropriate methodology we used previously for electrical stimulation in antinociceptive studies in standing horses [1,2], in order to prevent others from making the same mistake. We would like to draw attention to the importance of measuring and minimising the skin resistance between electrodes to guarantee consistent and reproducible stimuli ~~when electrical stimulation is used in antinociceptive studies in standing horses~~. Electrical stimulation has been validated under experimental conditions for assessing antinociception in conscious horses [1]. However, an important variable 'skin resistance' was not taken into consideration in some of our studies [1,2]. Recording the data in volts (V) only, omitting skin resistance (kΩ) which influences the current intensity (mA), may lead to heterogeneous and less accurate data. Ohm´s law states that current intensity is equal to voltage divided by resistance; therefore increases in skin resistance will reduce the intensity of the electrical stimulus transmitted to the horse.

Lopes et al. (2016) reported very high and heterogeneous nociceptive electrical thresholds (ENT) for 45 minutes after a bolus of saline [2]. Mean voltage ranged from 7 to 20 V. The authors claimed that the electrical resistance was constant by maintaining the same distance of 7 cm between the electrodes in all cases. Preparation of the area was performed by 'shaving and washing with soap and water'. That study concluded that 'an electrical stimulus did not determine the degree of antinociception accurately'. However, it may be argued that the excessive voltages reported were due to high (unmeasured) skin resistance. In contrast, when skin resistance was maintained below 3 kΩ, mean ENT varied from 1.7 to 1.9 V for 45 minutes after a bolus of saline in another study using the same horses [3]. Lopes et al. (2016) considered that their results [2] concurred with the validation study using the same methodology [1]. Luna et al. (2015) reported that electrical stimuli produced the most false negatives of all the stimuli (thermal, mechanical and electrical) applied [1]. Failure to maintain appropriate skin resistance may have contributed to this poor performance.

This information~~ese report~~ demonstrates ~~indicate~~ that it is essential to measure the skin resistance between the electrodes and to maintain it below 3 kΩ [3,4,5]. Two main factors must be considered: the distance between electrodes, and a proper cleaning process. The distance is short (1 – 2 cm) when the electrodes are placed over the lateral palmar digital nerves [4,5], but distances up to 7 – 8 cm have been used when electrodes are placed in the skin immediately proximal to the coronary band [1,2,3]. A thorough

cleaning process is necessary, especially if the inter-electrode distance is high (7 – 8 cm). We have described a strict protocol elsewhere [3]. After electrode placement, resistance should be measured with a multimeter to confirm appropriate resistance [3,4,5]. The electrodes are then secured with adhesive bandages or wrap strips [3,5]. Througout the investigation, resistance beween electrodes should be measured before each electrical stimulus is applied.

In conclusion, arising from our experience with the same horses, equipment and experimental conditions, we would like to reiterate the importance of appropriate methodology when electrical antinociception is used for research in horses. Proper clipping and cleaning should be routine practice in order to maintain skin resistance values below 3 kΩ, measured by a multimeter.

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