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The full details of the published version of the article are as follows:

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

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JOURNAL: Equine Veterinary Journal

PUBLISHER: Wiley

PUBLICATION DATE: November 2017

DOI: 10.1111/evj.12700



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

Journal:	<i>Equine Veterinary Journal</i>
Manuscript ID	EVJ-C-17-091.R1
Wiley - Manuscript type:	Correspondence
Discipline:	Anaesthesiology
Body System/Disorder:	Not applicable
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 k Ω . Some studies did not include this measurement, which may lead to heterogeneous and less accurate data.

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

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

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

34 cleaning process is necessary, especially if the inter-electrode distance is high (7 – 8
35 cm). We have described a strict protocol elsewhere [3]. After electrode placement,
36 resistance should be measured with a multimeter to confirm appropriate resistance
37 [3,4,5]. The electrodes are then secured with adhesive bandages or wrap strips [3,5].
38 Throughout the investigation, resistance between electrodes should be measured before
39 each electrical stimulus is applied.

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

45

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