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Quantitative sensory testing of the equine face

Kata O. Veres-Nyéki[1] | **József Nyéki**[2] | **Gábor Bodó**[3] | **Claudia Spadavecchia**[1]

[1]Anaesthesiology Division, Department of Clinical Veterinary Medicine, Vetsuisse Faculty, University of Bern, Bern, Switzerland

[2]KDV Hungária Ltd, Varbó, Hungary

[3]Equine Department and Clinic, University of Veterinary Medicine, Üllő, Hungary

Correspondence
Kata O. Veres-Nyéki, Department of Clinical Science and Services, The Royal Veterinary College, Hawkshead Lane, North Mymms, Hatfield, Hertfordshire AL9 7TA, UK.
Email: kveresnyeki@rvc.ac.uk

Present address
Kata O. Veres-Nyéki, Department of Clinical Science and Services, The Royal Veterinary College, Hawkshead Lane, North Mymms, Hatfield, Hertfordshire AL9 7TA, UK

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Abstract

Background: Quantitative sensory testing methods are now standard in the evaluation of sensory function in man, while few normal equine values have been reported.

Objectives: The aim of this experimental study was (a) to define the tactile sensory, mechanical nociceptive and thermal nociceptive thresholds of the equine face; (b) to assess the effect of age, sex, stimulation site and shaving; (c) to evaluate the reliability of the methods and (d) to provide reference facial quantitative sensory testing values.

Study design: Method description.

Methods: Thirty-four healthy Warmblood horses were used in the study. Six (tactile sensory threshold) and five (mechanical nociceptive and thermal nociceptive thresholds) areas of the left side of the face with clear anatomical landmarks were evaluated. Ten horses had two (mechanical nociceptive threshold) or three (tactile sensory and thermal nociceptive thresholds) of these areas shaved for another study. A linear Mixed model was used for data analysis.

Results: All thresholds increased with age (tactile sensory threshold: by 0.90 g/y (CI = [0.12 g; 0.36 g]) $P = .001$; mechanical nociceptive threshold: by 0.25 N/y (CI = [0.13-0.36 N]) $P = .000$; thermal nociceptive threshold: by 0.2°C/y (CI = [0.055-0.361]) $P = .008$). Sex had no effect on thresholds (tactile sensory threshold: $P = .1$; mechanical nociceptive threshold: $P = .09$; thermal nociceptive threshold: $P = .2$). Stimulation site affected tactile sensory and mechanical nociceptive thresholds ($P = .001$ and $P = .008$), but not thermal nociceptive threshold ($P = .9$). Shaving had no significant effect on any of the thresholds (tactile sensory threshold: $P = .06$; mechanical nociceptive threshold: $P = .08$; thermal nociceptive threshold: $P = .09$).

Main limitations: Only the left side was investigated and measurements were obtained on a single occasion.

Conclusions: Handheld quantitative sensory testing does not require shaving or clipping to provide reliable measurements. Stimulation over the nostril (tactile sensory threshold), temporomandibular joint (mechanical nociceptive threshold) and supraorbital foramen (thermal nociceptive threshold) resulted in the most consistent thresholds.

The abstract is available in Portuguese in the Supporting Information section of the online version of this article.

KEYWORDS
horse, quantitative sensory testing, trigeminal, tactile sensory threshold, mechanical nociceptive threshold, thermal nociceptive threshold

1 | INTRODUCTION

Quantitative sensory testing methods are now standard in the evaluation of sensory function in human subjects. By quantifying the response to well-defined thermal and mechanical stimuli it is possible to evaluate the sensitivity of skin areas innervated by specific nerve branches. Good reliability and high short- and long-term reproducibility have been reported for these noninvasive tests in man.[1] Von Frey filaments are typically applied to evaluate the function of large myelinated A-β sensory fibres to determine the tactile sensory threshold.[2] Pressure algometry and thermal stimulation are used to define mechanical and thermal nociceptive thresholds, for assessing the function of A-δ and C-fibres respectively.[1,3–5]

Currently, few normal equine quantitative sensory testing values based on the behavioural responses to mechanical and thermal stimuli have been reported in the literature.[6–9] Von Frey filaments were used in horses to evaluate skin sensitivity after branding or microchip placement[10] and to investigate the effects of epidural ketamine on wound sensitivity,[11] but there are no reported reference values for tactile sensory threshold. Mechanical and thermal nociceptive thresholds have been evaluated either with handheld algometers[12–14] or with wireless actuator systems[8,9] for the back, neck and specific regions of the limbs. Thermal threshold at the nostrils, determined using a wireless testing system, has also been reported.[9]

For man, region-specific age- and gender-matched reference values have been established and can be used to diagnose sensory dysfunction.[3] Different body areas are known to have different sensitivity to specific stimulation modalities, as, for example, lower thermal thresholds are found in the human subjects' face compared with other body regions.[1,3]

The aim of this experimental study was (a) to define the tactile sensory, mechanical nociceptive) and thermal nociceptive thresholds of the equine face; (b) to assess the effect of age, sex, stimulation site and shaving on the quantitative sensory testing values; (c) to evaluate the reliability of the selected sites and stimulation methods and (d) to provide reference quantitative sensory testing values to be later compared with those of patients affected by sensory alterations.

2 | MATERIALS AND METHODS

2.1 | Animals

Thirty-four Warmblood horses (15 mares, five stallions and 14 geldings), aged 1-23 years (mean: 10.5 years, SD: 6.5) were included in the study. At 3 years of age or less, the nervous system is likely to be immature: four were 1-year-olds (three colts, one filly) and two were 3-year-olds (two fillies). Experiments were performed in two different locations (10 horses in Switzerland and 24 horses in Hungary). All horses were clinically healthy without any known neurological disorders. Sensory testing was performed after daily exercise, between two feeds with the horses loosely restrained with halter and lead rope by a familiar person in the horses' usual stable environment. Only the left side of the face was evaluated. To assess the effect of shaving on threshold values, 10 horses had the following areas shaved (ca. 4 × 4 cm) the day before data collection: above the infraorbital and supraorbital foramen and lateral canthus of the eye. Ambient temperature was between 3 and 13°C during measurements.

2.2 | Behavioural assessment

The intensity of responses to stimuli was assessed using a visual analogue scale (VAS) by the same investigator (K.V.N.). The response after the application of the devices was judged to be positive if muscle twitch (similar to what a fly might provoke), blinking if areas close to the eye had been stimulated, movement of the lips when areas around the lips had been stimulated or if a horse moved its head slightly away from the stimulus. This latter behavioural reaction was typical to the mechanical and thermal nociceptive threshold testing, while the more gentle reactions were observed with von Frey filament use.

2.3 | Tactile sensory threshold

To detect sensory threshold, von Frey filaments were applied perpendicularly to the skin—bent during 1.5 seconds, kept in contact with the skin for another 1.5 seconds and released in 1.5 seconds[15] (in an increasing order of filaments' size (Table S1) until a muscle twitch was detected in response to stimulation. Due to their elastic nature, each size of von Frey filament can deliver a calibrated force, which is constant and independent of the level of bending. Therefore, the effect of subject's movement on the force is buffered.[2] The tactile sensory threshold was determined when a second application of a filament of the same size provoked a similar response.

2.4 | Mechanical nociceptive threshold

A purpose-built, handheld, calibrated pressure algometer with a silicon tip of 0.5 cm^2 was used to assess mechanical nociceptive thresholds. A force application rate of 5 N/s was targeted. The instrument automatically displayed the maximum force applied and was reset to zero before each measurement. The tip of the probe was pressed gradually and perpendicularly against the skin surface until horses showed clear aversive reaction (ie moved the head away from the stimulus) or the cut-off value (24.6 N, corresponding to a pressure of 492.3 kPa) was reached. At this point, pressure was immediately released, and the probe was lifted from the skin. The evaluator (K.V.N.) was unaware of the pressure until the stimulation was aborted. The threshold determination procedure was repeated twice for each site.

2.5 | Thermal nociceptive threshold

Thermal nociceptive threshold was evaluated using a handheld, contact-thermode based testing device. The stimulation probe had a diameter of 1 cm. The initial probe temperature was set at 30°C, with a rate of temperature increase of 0.4°C/s. The probe was held perpendicular to the skin surface and kept in light contact with the skin until the horse showed a clear aversive reaction (eg moved the head away the probe) or the cut-off value (55°C) was reached. The probe was lifted from the skin and the temperature at the point of reaction was recorded as thermal nociceptive threshold. The evaluator (K.V.N.) was unaware of the temperature until the stimulation was aborted. The probe was then cooled down to 30°C and the threshold was determined again. Care was taken not to position the probe exactly on the same spot to avoid sensitisation.

2.6 | Evaluated areas

Points were selected to evaluate the innervation area of all three sensory afferents of the trigeminal nerve (supraorbital, infraorbital and mental nerves) and chosen due to clear anatomical landmarks, lying above either bony structures or muscles (Figure 1). The numbers assigned to each area represent the order of testing.

2.7 | Data analysis

Descriptive statistics was used to report the mean age of the horses, the rate of application of mechanical and thermal stimuli and the VAS scores. The Shapiro-Wilk test was used to evaluate normality and parametric data are reported as mean and standard deviation, while nonparametric data are reported as median and range.

To evaluate test-retest reliability of nociceptive thresholds, intra-class correlation (ICC) coefficient was calculated for all measurements. The Wilcoxon Signed Rank Test was used to evaluate the difference between the two threshold values obtained for mechanical nociceptive threshold and thermal nociceptive threshold.

A linear mixed model was used to analyse sensory thresholds and to examine the effects of age, sex, stimulation site and shaving on the thresholds and consistency of thresholds at the different areas. From the two measurements performed to define the nociceptive thresholds, the higher value was selected for analysis by the model (not applicable to tactile sensory threshold). If no response was evoked, the cut-off values were used for statistical analysis and number of nonresponders are reported. Contingency tables were used to evaluate the percentage of nonresponders after stimulation of shaved/nonshaved areas. The SPSS Statistics (24) software (IBM) was used for data analysis.

3 | RESULTS

Sensory testing of the facial area was well tolerated by all horses. No adverse events were observed during or after stimulation.

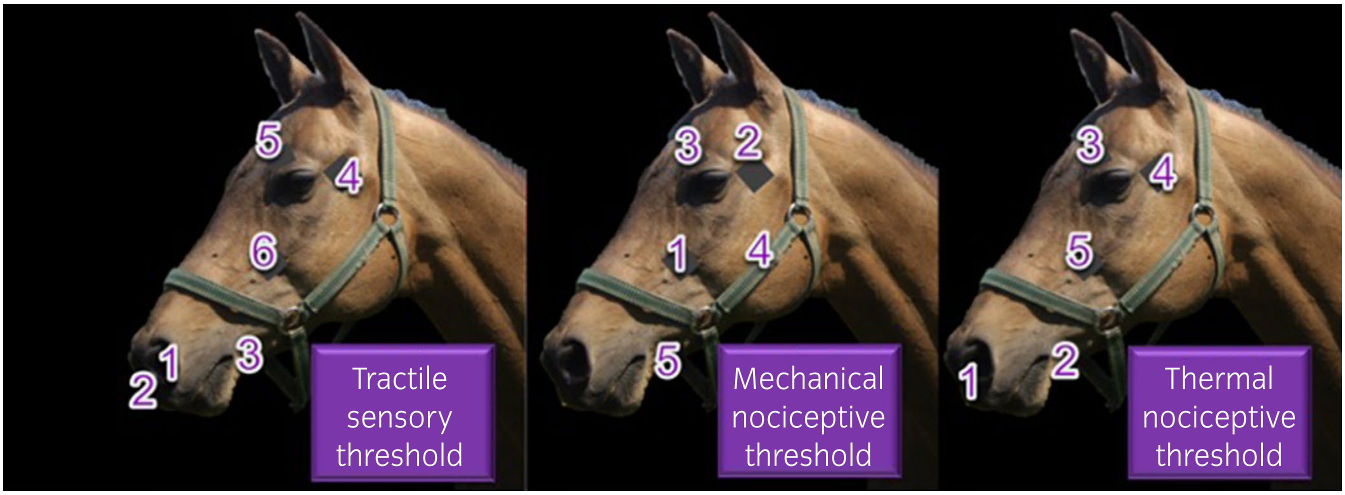

FIGURE 1 Evaluated areas for each quantitative sensory testing methods, in the order of assessment. Tactile sensory threshold, mechanical nociceptive threshold and thermal nociceptive threshold

3.1 | Tactile sensory threshold

Stimulation provoked no response (Figure 2) in 5.9% of the tests (n = 204). Age had a significant effect on tactile sensory threshold (*P* = .001) and every year of increase in age increased the threshold by 0.90 g (CI = [0.12 g; 0.36 g]), while sex and shaving had no significant effects (*P* = .1 and *P* = .06 respectively) (Figure 2). Although the thresholds were not significantly different, subjectively, shaved areas had lower sensitivity which might be clinically relevant. Stimulation site had a significant effect on tactile sensory threshold (*P* < .01) (Table 1), with nostril (site 1) thresholds being the most consistent (Table 2). The median VAS score for behavioural response to stimulation was 7 mm (SD = 7 mm, range = 0-46 mm of maximum 100 mm).

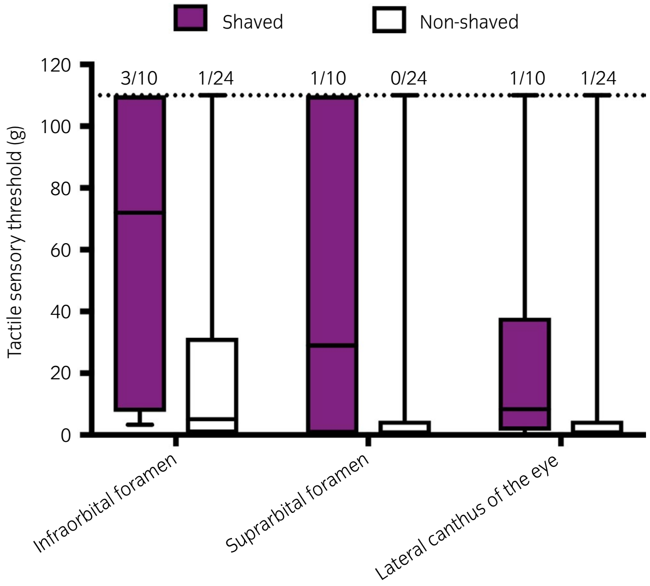

FIGURE 2 Comparison of the median tactile sensory threshold per stimulation sites. Data were collected from 10 shaved and 24 nonshaved horses. Numbers above box-plots representing the nonresponder horses

3.2 | Mechanical nociceptive threshold

Stimulation provoked no response in 27.4% of the tests (n = 340) (Tables S2 and S3). The mean force application rate was 6.21 N/s (SD = 0.98 N/s). The median VAS score for behavioural response to stimulation was 13 mm (range = 0-53 mm).

Age had a significant effect on threshold values (*P* < .01 and every year of increase in age increased the threshold by 0.25 N (CI = [0.13-0.36 N]). Sex and shaving had no significant effect on threshold (*P* = .09 and *P* = .08, respectively, Figure 3). Stimulation site has a significant effect on the mechanical nociceptive threshold (*P* = .008) (Table 1). Thresholds over the temporomandibular joint (site 2) were the most consistent (Table 2).

The reliability of the test was good with an ICC coefficient of 0.829 (CI: 0.768-0.874). There was no significant difference between the first and the second measurements (*P* = .3, Figure 4).

3.3 | Thermal nociceptive threshold

Stimulation provoked no response in 7.1% of the tests (n = 340) (Tables S2 and S3). Cut-off temperatures were never reached at site 3, 4 and 5 for shaved horses. The mean rate of temperature increase was 0.37°C/s (SD = 0.04°C/s). The mean VAS score for behavioural response to stimulation was 15.56 mm (SD = 8.2 mm, range was 0-58 mm).

Age had a significant effect on thermal nociceptive threshold (*P* = .008) and every year of increase in age increased the threshold by 0.2°C (CI = [0.055-0.361]). Sex and shaving had no significant effect on threshold (*P* = .2 and .09, respectively, Figure 5), but the range of thresholds in the shaved areas was smaller.

Stimulation site had no significant effect on the thermal nociceptive threshold (*P* = .9). Thresholds over the supraorbital foramen (site 3) were the most consistent (Table 2).

The reliability of the test was good, with an ICC coefficient of 0.809 (CI: 0.741-0.858). There was no significant difference between the first and the second measurements (*P* = .3, Figure 6).

TABLE 1 Mean tactile sensory thresholds, mechanical nociceptive thresholds and thermal nociceptive thresholds of the equine face, and their confidence intervals (CI)

	Tactile sensory threshold			Mechanical nociceptive threshold			Thermal nociceptive threshold		
Site	Mean (g)	CI		Mean (N)	CI		Mean (°C)	CI	
1	6.1^{a,b,c}	1.7	10.4	23.0^{f,g}	21.5	24.5	46.9	44.8	49.0
2	27.3^{a,d}	12.7	42.0	21.0	19.6	22.4	47.8	46.0	49.6
3	16.7	4.8	29.1	19.5^{f,h}	17.7	21.2	47.2	45.7	48.8
4	12.2^{e}	2.7	21.6	22.4^{h}	20.9	23.8	47.1	45.2	48.9
5	22.3^{b}	9.4	35.2	20.4^{g}	18.9	21.8	46.4	44.5	48.3
6	33.2^{c,d,e}	19.5	47.0						

Note: Significant difference: [a] (*P* = .006), [b](*P* = .02), [c](*P* = .001), [d](*P* = .006), [e](*P* = .01), [f](*P* = .002), [g](*P* = .02), [h](*P* = .02).

TABLE 2 Order of consistency of thresholds evaluating tactile sensory threshold, mechanical nociceptive threshold and thermal nociceptive threshold of the equine face

Order of threshold consistency	Stimulation site	Estimates of covariance	Standard error (SE)
Tactile sensory threshold			
1	Site 1 (nostril)	116.86	31.14
2	Site 4 (lateral canthus of the eye)	689.3	175
3	Site 3 (mental foramen)	1171.44	289.37
4	Site 5 (supraorbital foramen)	1325.34	327.33
5	Site 6 (infraorbital foramen)	1505.89	376.02
6	Site 2 (upper muzzle)	1724.25	429.11
Mechanical nociceptive threshold			
1	Site 2 (temporomandibular joint)	15.43	3.81
2	Site 1 (infraorbital foramen)	16.50	4.11
3	Site 4 (masseter muscle)	16.64	4.17
4	Site 5 (mental foramen)	17.55	4.34
5	Site 3 (supraorbital foramen)	25.02	5.96
Thermal nociceptive threshold			
1	Site 3 (supraorbital foramen)	18.74	4.66
2	Site 2 (mental foramen)	24.06	7.39
3	Site 4 (lateral canthus of the eye)	27.27	6.78
4	Site 5 (infraorbital foramen)	29.61	7.39
5	Site 1 (upper muzzle)	35.1	8.69

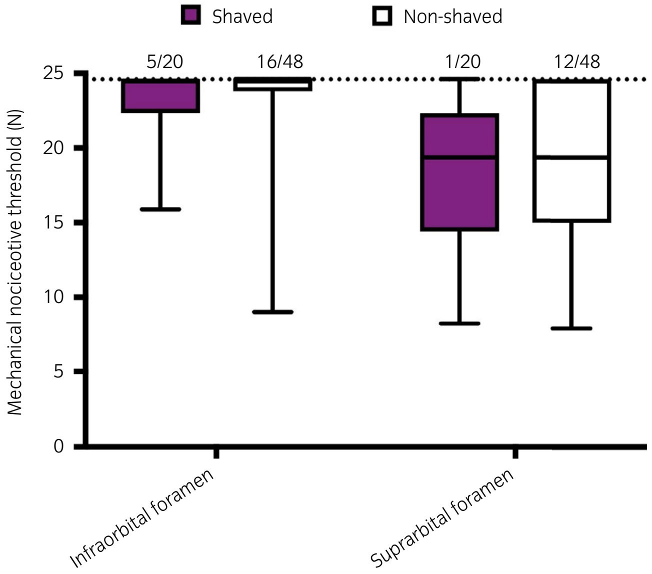

FIGURE 3 The effect of shaving on mechanical nociceptive threshold. Data were collected from 10 shaved and 24 nonshaved horses. Numbers above box-plots representing the nonresponder horses per stimulations

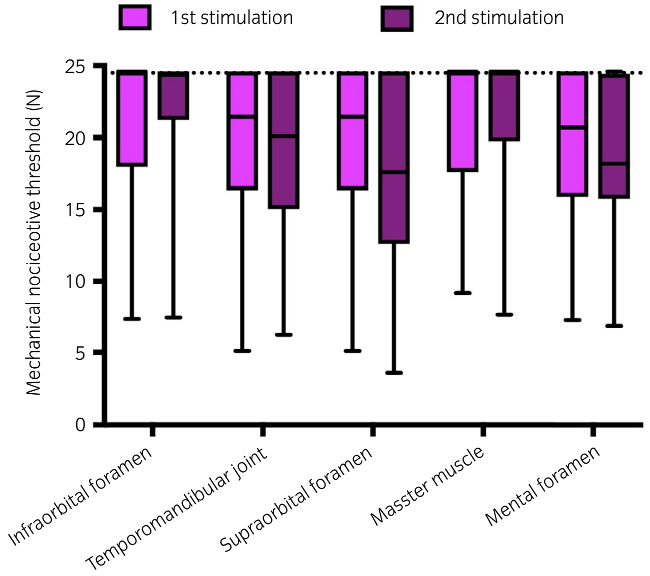

FIGURE 4 Median and interquartile range of thresholds after the first and second stimulation at each site to assess test-retest reliability of mechanical nociceptive threshold

4 | DISCUSSION

This study was designed to evaluate the feasibility of performing quantitative sensory testing of the equine face and to investigate the impact of age, sex, stimulation site and shaving on sensory thresholds. Similar to reports in man,[3] we found that all quantitative sensory testing thresholds increased with age. This might be due to a decreased innervation density,[16] but results from studies in dogs and horses provide contradictory evidence in this respect. While young and geriatric dogs had the highest thermal nociceptive thresholds compared with adult dogs,[16] mechanical nociceptive thresholds decreased with age in dogs.[17] Young horses had higher mechanical

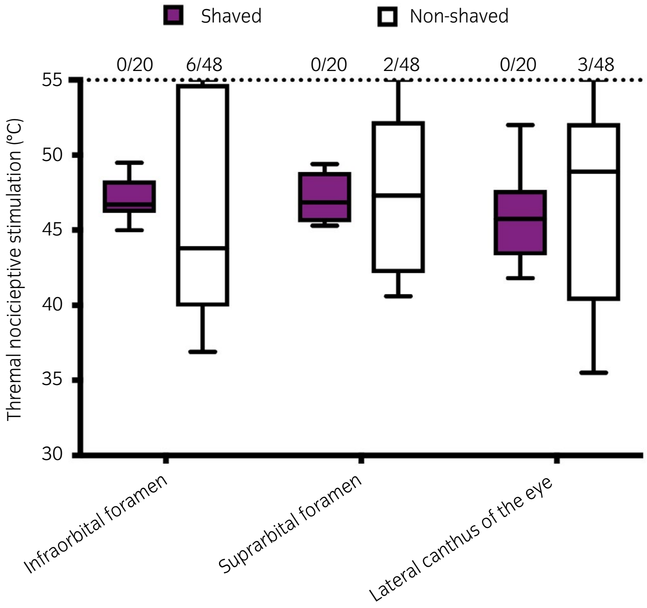

FIGURE 5 The effect of shaving on thermal nociceptive threshold. Data were collected from 10 shaved and 24 nonshaved horses. Numbers above box-plots representing the nonresponder horses per stimulations

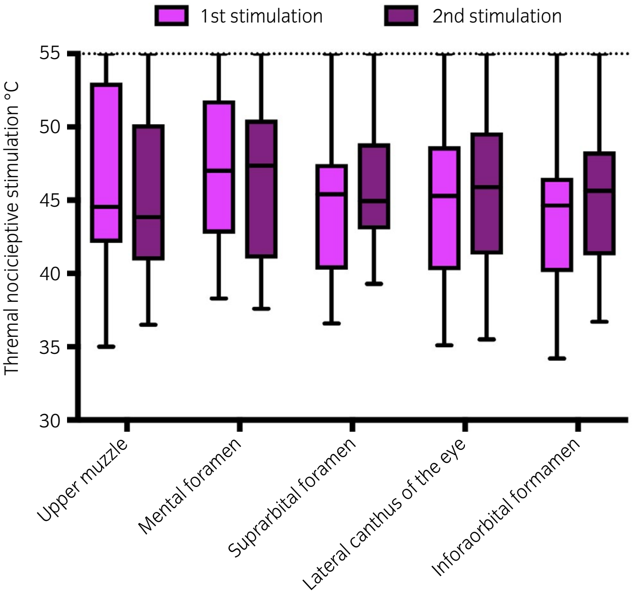

FIGURE 6 Median and interquartile range of thresholds after the first and second stimulation at each site to assess test-retest reliability of thermal nociceptive threshold

nociceptive threshold than adult horses when sites over the trunk were evaluated.[12]

We have found that sex does not affect the quantitative sensory testing thresholds in the equine face. In man, mechanical nociceptive thresholds are higher in males,[3,15,18] while in dogs sex affects tactile sensory threshold, mechanical nociceptive threshold and thermal nociceptive threshold.[16,19]

Hair covering may interfere with quantitative sensory testing thresholds, especially thermal nociceptive threshold, acting as an insulation layer.[20] In previous studies test areas were shaved.[20,21] However, horse-owners might be reluctant to have their horses clipped or shaved for quantitative sensory testing. We have found that shaving did not significantly affect tactile sensory thresholds, mechanical nociceptive thresholds and thermal nociceptive thresholds on the equine face, which may facilitate the use of this technique in clinical cases. Grint et al[22] also found that clipping the stimulation site did not result in significant difference in mechanical nociceptive threshold of the limbs in donkeys, but the effect of clipping on tactile sensory threshold and thermal nociceptive threshold has not been evaluated before.

Among the handheld quantitative sensory testing methods, the application of von Frey monofilaments is considered to be one of the most reliable and reproducible as it provokes a consistent stimulation if performed correctly—the force produced is independent from the degree of bending[2]—and it eliminates the effect of hand vibration.[15] The innocuous mechanical stimulation provoked by the monofilament activates the slowly adapting low threshold cutaneous mechanoreceptors (Merkel cell-neurite complexes) on the Aβ fibres.[1,3,23] The application of von Frey filaments normally does not provoke pain sensation in man.[24] However, in patients with allodynia,[3,23,24] pain can be provoked by von Frey filaments. It is also linked to stronger behavioural reactions in affected animals.[25]

Stimulation at the nostrils (site 1) gave the most consistent results, favouring this location for the assessment of trigeminal sensory function. Also, all horses responded to stimulation at that site within the range of monofilaments' sizes used.

Threshold differences within areas may be due to the variation of underlying soft tissue at each site[20,26] or differences in mechanoreceptor density at the area.[27] A thicker soft tissue layer disseminates the stimulus force more,[20,26] but we found that areas where skin lies directly over bony surfaces had the highest tactile sensory threshold. Therefore, we speculate that the lips and nose of the horse might have higher mechanoreceptor density than the other evaluated areas.

The tactile sensitivity of the equine face is lower than in man as the 0.07 g filament (similar to our size 5 filament 0.064 g) is considered to provide a supra-threshold stimulation on the human subjects' face[15] while horses responded to size 7-8 (7.7 mean, 0.145-0.320 g) monofilaments when stimulated at the nostrils. The higher sensitivity in man might be due to different methods adopted to define the threshold. As people can confirm the tactile sensory threshold verbally, one response out of three stimulations performed with the same filament is usually considered as threshold, while in this equine study, a behavioural reaction to two consecutive applications of the same filament was defined as setting the threshold in an effort to reduce the occurrence of false positive responses. Unfortunately, within the veterinary field there is no consensus about the optimal method to define the tactile sensory threshold[11,16,28,29] and method standardisation would facilitate data comparison.[2]

Despite the lack of statistically significant differences for tactile sensory thresholds between shaved and unshaved skin areas, thresholds of the shaved areas were subjectively higher which might be clinically relevant. Our findings support the importance of hair coverage in tactile sensory function in equines and underline the potential disadvantages of clipping the facial areas for husbandry or cosmetic purposes.[30]

Mechanical nociceptive threshold is usually quantified by applying gradually increasing pressure to the skin through a flat probe until a pain response is elicited.[23] In horses, pressure algometry has been found to be an objective, noninvasive tool to assess mechanical nociceptive thresholds[12] in several areas of the body,[20] but it has not been investigated for the face previously. When comparing threshold values reported in different studies, it is fundamental to consider the diameter of the stimulating tip. Taylor et al[31] found a nonlinear relationship between probe diameter and threshold when evaluating four different probe configurations. Duan et al[32] recommended the use of small diameter probe (0.01-0.1 cm^2) over 1 cm^2 probe as thresholds were found to be more consistent. Our probe size was smaller than 1 cm^2, but larger than those recommended by Duan et al, which might explain the high rate of failure to evoke responses in our subjects.

Similarly to Haussler, we found that an area where skin was lying directly over a bony surface had the highest mechanical nociceptive threshold compared with sites covered with soft tissue.[13] On the other hand, De Heus found lower mechanical nociceptive thresholds over bony surfaces compared with muscles in horses.[33] These threshold differences may be due to variation of underlying soft tissue at each site, as a thicker soft tissue layer will disseminate the stimulus force more,[20,26] or due to differences in mechanoreceptor density between the evaluated areas.[27] Similar to man, where the forehead is the most sensitive area to pressure algometry,[18] the area around the supraorbital foramen had the lowest mechanical nociceptive threshold in our horses. As stimulation over the temporomandibular joint resulted in the most consistent results and the response rate to stimulation was one of the highest, we concluded that this is the best area to evaluate mechanical nociceptive threshold in horses. Although there were no significant differences in mechanical nociceptive thresholds for shaved and unshaved areas, it seems that shaved horses are less likely to have reached the cut-off value without response.

For thermal stimulation a cut-off of 55°C is used to avoid burn injuries, and no skin damage was observed immediately after stimulation or 1 day later in our horses. Other authors reported slightly different cut-off values, varying between 45 and 56°C.[9,20,34] When higher cut-off values were used, up to one-fifth of the horses suffered from mild burn injury on the nostrils.[21] However, these previous studies were not performed using handheld devices, therefore immediate removal of the hot probe was not possible. If a low cut-off value is selected, thermal nociceptive thresholds are often above the limit and stimulation is not successful.[22] A possible way to circumvent this limit could be to use thermodes of larger size, as it has been seen that there is a size-related decrease in thermal nociceptive threshold[1] probably due to a summation phenomenon.[23]

In our study, the rate of increase in temperature was set at approximately 0.4°C/s as higher (>0.8°C/s) rates were associated with burn injuries in horses[9,20] due to decreased heat-transfer.[35] Lower stimulation rates result in prolonged stimulation time[20] and burn injuries, probably due to increased contact time.[9] In donkeys, the rate of increase in temperature (0.4°C/s vs 0.8°C/s) had no influence on thermal nociceptive threshold.[22,34] Our selected 0.4°C/s rate of increase in temperature is considered to be associated with C fibre activation.[20]

We found that site of the stimulation has no effect on thermal nociceptive threshold, but stimulation at the immediate proximity of the supraorbital foramen (site 3) gave the most consistent results, with the least number of nonresponding horses. Therefore site 3 should be favoured for the assessment of trigeminal thermal sensory function in horses. Furthermore, shaving might decrease the sensitivity of the skin to heat; however, cut-off values were never reached in shaved areas.

In this study, all horses tolerated well quantitative sensory testing of the face, independently from age and experience. The described handheld devices could be used in clinical practice to assess sensory dysfunction of the face and response to treatment.

4.1 | Limitations

The number of horses included in this study is too low to provide reference values for equine face quantitative sensory testing. Nevertheless, as a quite narrow range of threshold values was found, we believe that the set of data we present could at least be considered a preliminary reference for comparison with future data sets.

Only areas on the left side of the face were evaluated, while it is known that there could be significant differences between the two sides.[15] However, no significant differences were found in horses and donkeys comparing the mechanical nociceptive threshold or thermal nociceptive threshold for the two sides of the body,[6,7,22,34] in healthy dogs between left and right side of the body using these quantitative sensory testing methods[16] and in humans when comparing the two sides of the face.[3,36] Distraction, boredom and fatigue are known to influence results[1] therefore our aim was to reduce the total experimental time.

Tests were always performed at the same order, which might have altered the threshold of the tests performed later at the same site. However, in human medicine this approach allows standardisation of the test procedure[3] and does not affect the thresholds while reducing between-subjects variability.[12]

Skin surface temperature was not measured in this study. Other authors reported the percent thermal excursion—(% TE = 100 × [threshold temperature−skin temperature]/[cut-out temperature−skin temperature]−beside threshold temperature[9] to eliminate the effect of the different skin temperature (and consequently, the effect of ambient temperature) on thermal nociceptive threshold. Skin temperatures are significantly lower, while thermal nociceptive thresholds are higher in low environmental temperature (<10°C).[9] Therefore, our thermal nociceptive threshold might be slightly lower if ambient temperature had been above 20°C.

Tests were performed during a single occasion and other studies have found no differences in values when retesting animals at different times.[1,14,16,36–39] The same investigator performed all the measurements to avoid inter-examiner variability and other studies have shown good to excellent inter-examiner reliability with handheld algometry in man and horses.[14,18,40]

5 | CONCLUSION

In this study, threshold values for tactile sensory, mechanical and thermal nociceptive stimulations were generated for the face in healthy adult horses. Handheld devices did not result in lasting tissue damage. Their application is simple and does not require shaving or clipping to provide reliable measurements in healthy horses. The combined application of all three modalities enables comprehensive evaluation of the equine trigeminal sensory function with good reliability. Most consistent results were acquired by stimulation over the nostril (tactile sensory threshold), temporomandibular joint (mechanical nociceptive threshold) and supraorbital foramen (thermal nociceptive threshold).

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CONFLICT OF INTEREST

No competing interests have been declared.

AUTHOR CONTRIBUTIONS

All authors contributed to interpretation of the data and preparation of the manuscript; K.O. Veres-Nyéki, G. Bodó and C. Spadavecchia also contributed to the study design; K.O. Veres-Nyéki and J. Nyéki contributed to the data collection, while K.O. Veres-Nyéki analysed the data.

ETHICAL ANIMAL RESEARCH

The experiments were performed with the approval of the Bernese Committee for Animal Experimentation, Switzerland (Tierversuche/Bewilligung 92/08) and the Ethics Committee of Scientific Animal Research, Pest County, Hungary (Állatkísérleti Engedély PE/EA/44-2/2019).

OWNER INFORMED CONSENT

Privately owned horses participated in the study with written consent of the owner.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Kata O. Veres-Nyéki https://orcid.org/0000-0003-0004-2529

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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