

Evaluation of pain and inflammation associated with hot iron branding and microchip transponder injection in horses

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Objective—To compare effects of hot iron branding and microchip transponder injection regarding aversive behavioral reactions indicative of pain and inflammation in horses.

Animals—7 adult horses.

Procedures—In a randomized controlled clinical crossover study, behavioral reactions to hot iron branding and microchip transponder injection were scored by 4 observers. Local and systemic inflammation including allodynia were assessed and compared by use of physiologic and biochemical responses obtained repeatedly for the 168-hour study period. Serum cortisol concentration was measured repeatedly throughout the first 24 hours of the study. Sham treatments were performed 1 day before and 7 days after treatments.

Results—Hot iron branding elicited a significantly stronger aversive reaction indicative of pain than did microchip transponder injection (odds ratio [OR], 12.83). Allodynia quantified by means of skin sensitivity to von Frey monofilaments was significantly greater after hot iron branding than after microchip transponder injection (OR, 2.59). Neither treatment induced signs of spontaneously occurring pain that were observed during the remaining study period, and neither treatment induced increased serum cortisol concentrations. Comparison with sham treatments indicated no memory of an unpleasant event. The hot iron branding areas had significantly increased skin temperature and swelling (OR, 14.6). Systemic inflammation as measured via serum amyloid A concentration was not detected after any of the treatments.

Conclusions and Clinical Relevance—Microchip transponder injection induced less signs of pain and inflammation and did not seem to pose a higher long-term risk than hot iron branding. Consequently, results indicated that hot iron branding does inflict more pain and should be abandoned where possible. (*Am J Vet Res* 2009;70:840–847)

According to legislation in Denmark and the European Union,^{1–3} individual identification of horses is required for animal health reasons and to ensure compliance with certain public health requirements because horses may be slaughtered for human consumption. Furthermore, unique identification is important to ensure correct identification at competitions and shows and when buying and selling horses.

Methods of identification of horses and foals born in several European countries as well as other parts of the world include hot iron branding and microchip transponder injection. Hot iron branding is still widely

ABBREVIATIONS

NRS	Numeric rating scale
OR	Odds ratio
SAA	Serum amyloid A
VFM	von Frey monofilaments
VAS	Visual analogue scale

used as a means of identification. The availability of microchip transponders has led to an ongoing discussion regarding whether hot iron branding or microchip transponder injection has different effects regarding the welfare of the horse. To the authors' knowledge, only 1 study⁴ comparing hot iron branding and microchip transponder injection of horses (or any other species) has been reported in the scientific literature. That study was conducted with Warmblood foals, and on the basis of heart rates and behavioral observations, it was concluded that hot iron branding caused more discomfort than microchip transponder injection or no treatment. The author also concluded that there was no evidence of prolonged consequences for the foals; however, this was based on behavioral observations lasting < 24 hours after treatment, and no other physical or biochemical analyses were performed. However sparse the scientific literature regarding this issue in horses might be, sev-

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eral studies⁵⁻¹¹ conducted on cattle have compared hot iron branding to freeze branding and sham branding, but not microchip transponder injection. The overall impression from these studies is that hot iron branding is substantially more painful and induces more inflammation than freeze branding or sham branding. Behavioral reactions were compared in 5 studies^{5-8,11} in cattle, and all 5 revealed that hot iron branding elicited significantly stronger escape-avoidance reactions indicative of pain than freeze branding or sham branding. Three of those studies compared heart rates and concluded that heart rates were significantly increased after hot iron branding, compared with freeze branding and sham branding⁷; significantly increased after hot iron branding and freeze branding, compared with sham branding⁵; or substantially increased after hot iron branding and freeze branding, compared with sham branding.⁶ Serum cortisol concentrations were measured in 4 studies, of which 2 revealed that hot iron branding and freeze branding resulted in significantly increased concentrations, compared with sham branding,^{5,9} and 2 revealed significantly increased serum cortisol concentrations after all treatments (hot iron, freeze, and sham branding), although concentrations did not differ among treatments.^{6,7} Some of these studies also deal with other important variables such as serum epinephrine concentration,^{6,7} skin temperature,^{6,10} and sensitivity to touch⁹ and subsequent handling ease indicative of memory of a bad experience.⁸ The only study dealing with hot iron branding of horses, reported by Pollmann,⁴ has several limitations because only heart rate and behavior were observed and observations were terminated < 24 hours after treatment. To conclude whether microchip transponder injection or hot iron branding should be preferred from an animal welfare perspective, observations on physical and biochemical indicators of inflammation, including skin reaction and skin sensitivity as well as behavioral observations, are needed. Furthermore, a study period lasting for > 24 hours is needed to follow the course of the inflammatory reaction.

Because hot iron branding causes skin damage, it is reasonable to assume that it causes pain and elicits an inflammatory reaction. Therefore, the aim of the study reported here was to evaluate the relative degree of pain as determined by behavioral responses to hot iron branding and microchip transponder injection (referred to as treatments). Secondary aims of the study were to quantify the relative degree of stress as well as local and systemic inflammatory reactions associated with the 2 treatments by measuring serum cortisol concentration, SAA concentration, and the classical inflammatory indicators edema, skin temperature, and skin sensitivity. Our hypotheses were that hot iron branding would induce greater aversive reactions, higher serum concentrations of cortisol, and more marked local and systemic inflammatory reactions than microchip transponder injection as measured via SAA concentration, skin temperature, skin edema, and skin sensitivity.

Materials and Methods

Study design—The study was conducted as a randomized controlled clinical crossover study with 7 horses subjected to both treatments with a washout pe-

riod of 14 days. On day 0 at hour 0 in the first trial period, horses were subjected to the treatment randomly assigned by drawing lots and received the other treatment on day 0 at hour 0 in the second trial period. Four horses received hot iron branding in the first study period, and 3 horses received microchip transponder injection in the first study period; the situation was reversed in the second study period. The experimental protocol was approved by the Danish Animal Experimentation Inspectorate.

Horses and preexperimental procedure—Seven research horses of different breeds (4 Standardbred trotters, 1 pony, and 2 horses of a mixed riding-type breed), 6 to 18 years of age, owned by the University of Copenhagen were included in the study after passing a thorough clinical examination including serum biochemical and hematologic analyses with results within reference limits. All horses were stabled in 3 × 4-m box stalls with a constant temperature of 13 ± 1°C and fed a grain mixture twice daily as well as hay and water ad libitum. Horses were accustomed to frequent handling, including the entire experimental setup for 2 weeks prior to the beginning of the trial. At 3 days prior to the treatments, horses had bilateral hair clipping on the thighs for hot iron branding (approx 30 × 30 cm) or on the middle of the neck from the mane down for microchip transponder injection (approx 10 × 10 cm). Contact areas for the heart rate monitors were also clipped. After antiseptic preparation, an indwelling jugular vein catheter was inserted under local anesthesia at 24 hours prior to the treatments. One hour prior to the treatments, horses were fitted with a heart rate monitoring system^a set to record heart rate every 15 seconds for a period of 12 hours.

Treatments—All hot iron brands were applied on the left thigh of the horse by the same experienced person from the Danish federation of breeding associations.^b Horses were branded with a plate iron measuring 6 × 9 cm with the letters DK. After being heated to red heat over a propane flame, the iron was applied for approximately 1 second.

For microchip transponder injection, a veterinarian wearing sterile gloves used the 3-mm-diameter sterile needle provided by the manufacturer of the microchip transponder^c to inject the cylindrical microchip measuring 2 × 13 mm approximately 2.5 cm into the middle of the neck at the border of the crest fat and the rhomboid cervical muscle. This procedure, including standard surgical preparation of the clipped skin, was executed according to the standard operating procedure at the Department of Large Animal Sciences at the University of Copenhagen. All microchip transponder injections were performed by the same person. To minimize movement and for safety reasons, horses were placed at the same location outside the stable between 2 large bales of straw for both treatments.

Sham treatments—In each trial period, horses were subjected to a sham treatment 1 day prior to the treatment and again 7 days after treatment. Horses were handled and positioned in the exact same fashion as when intended for treatment, and the procedure was videographically recorded and scored in the same

way as the treatment procedures. In the trial period in which the horse was assigned to hot iron branding, the unheated branding iron was placed on the right thigh for approximately 1 second at both occasions.

In the trial period of microchip transponder injection, the horse was subjected to standard surgical preparation on the right side of the neck and the skin handled in the same way as for microchip injection. Instead of injecting a microchip, a short period of moderate pressure was applied to the skin by the tip of the index finger of the operator.

Outcome measures—The primary outcome measure was the behavioral score at the time of treatment. All treatments were recorded videographically as well as observed by the 4 observers. All observers scored each horse's reaction individually on the basis of the videographic recordings and according to definitions (**Appendix**). Scores were masked from the other observers. After scoring, the values were transformed into a dichotomous variable, where categories 0 and 1 were considered as no or insubstantial aversive reaction and therefore indicative of no pain (score = 0) and categories 2 and 3 were considered a true aversive reaction and thereby indicative of pain (score = 1).

A clinical examination (including general well-being, respiratory rate, heart rate, rectal temperature, color of oral mucosa, capillary refill time, skin turgor, and intestinal peristalsis) and the secondary outcome variables skin temperature, skin sensitivity, skin reaction, and scoring for signs of pain were measured at 24 hours and 30 minutes prior to treatments as well as 30 minutes, 1, 2, 4, 6, 8, 12, 24, 48, 72, 96, 120, 144, and 168 hours after treatment.

At each time point, skin temperature was measured in the treated area as well as in the corresponding contralateral control area by use of an infrared thermometer.^d The standard operating procedure and instructions specific for the infrared thermometer were followed. The emissivity on the infrared thermometer was set to 0.98 for skin, and each measurement was taken at the recommended distance of approximately 20 cm from the skin surface. Each measurement lasted for 15 seconds, and the infrared thermometer subsequently calculated a mean skin temperature.

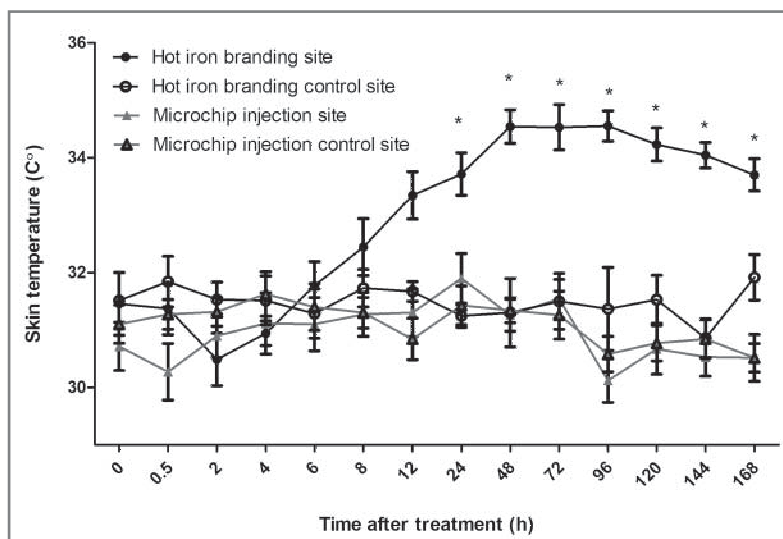
Skin sensitivity was evaluated by the use of VFM.^c At each time point, increasing mechanical stimuli were applied within the clipped areas of either the thigh or neck approximately 1 to 2 cm from the treated area as well as on the contralateral control area. Each stimulus lasted 1 second and was repeated 3 times at intervals of 3 seconds, each in a slightly different place. When the baseline threshold was being determined, the first stimulus applied was a VFM exerting a pressure of 4.0 g. If an avoidance response was observed, VFMs of decreasing force were applied until no response was made or the minimum diameter (equaling a pressure of 0.008 g) was applied. If no response was made, VFMs of increasing force were applied until an avoidance response was made

or the maximum pressure of 300 g was applied. An avoidance response was defined as tail flicking, movement of the ears or head, avoidance of the stimulus by shrugging of the skin musculature, kicking, or stepping to the side. A simple movement reflex on first touch of the VFM to the skin was not accepted as an avoidance response. During a measurement period, the first VFM force used for each time point was the threshold obtained from the previous measurement. The same operator made all VFM threshold measurements, and all measurements were made with the horses standing in a walkway with minimal restraint.

Edema of the treated area was evaluated via direct subjective observation and graded on a scale from 0 to 5, where 0 was equal to no reaction and 5 was equal to a strong reaction. The same observer evaluated the edema at all times, and edema was recorded by use of digital photographs.

Evidence of pain was evaluated repeatedly throughout the study period by 1 observer who used an NRS modified after Pritchett et al¹² and by use of a VAS. The NRS is a combination of behavioral, postural, and socialization measures that give indications of the well-being of the horse. These variables include the horse's position in the box, the position of the head and neck, position and movement of the ears, reaction to an opened box door, reaction to the approach of the observer, reaction to feed, and gross behaviors indicative of pain (eg, pawing, sweating, flehmen, continuously taking a foot off the ground, or standing up and lying down repeatedly). Possible outcomes of each variable were described and associated with a score from 0 to 4, and scores were subsequently summed to yield a pain score. The VAS is a more subjective type of pain score where the observer assigns a score between 0 and 100 on the basis of his or her interpretation of the horse's well-being. With the VAS, 0 is considered no evidence of pain, and 100 is considered evidence of the worst imaginable pain.

The order of data collection was: signs of pain evaluated by use of NRS and VAS, clinical examination, skin temperature, skin sensitivity, edema, and blood



sampling. Regarding skin temperature, skin sensitivity, and edema, the control side was always scored first.

Blood sampling procedures—Blood samples were collected from the indwelling jugular vein catheter 30 minutes before treatments to obtain a baseline and at 30 minutes and 1, 2, 4, 6, 8, 12, 24, 48, 72, and 168 hours after treatments. Ten milliliters of blood was collected and discarded before collecting 20 mL into serum tubes. The jugular vein catheter was flushed with heparinized saline (0.9% NaCl) solution after each sampling. Blood was stored at 5°C and analyzed within 24 hours for SAA concentration by use of a commercially available automated turbidometric immunoassay,¹ and serum cortisol concentration was measured by use of chemiluminescence.⁸ The assays were subjected to daily internal quality control, and only results from accepted runs were used.

Statistical analysis—Skin temperature was analyzed by use of a generalized linear model where the temperature was modeled as a function of time and the interaction between treatment and side, such that repeated measurements over time on each horse could be positively correlated.

The primary variable of interest of this part of the experiment was the interaction between side and treatment because this determined whether the temperature changes between the treatment side and the contralateral control side differed by treatment. A test for trend for time was performed, and the time effect was fitted as a continuous second-degree polynomial.

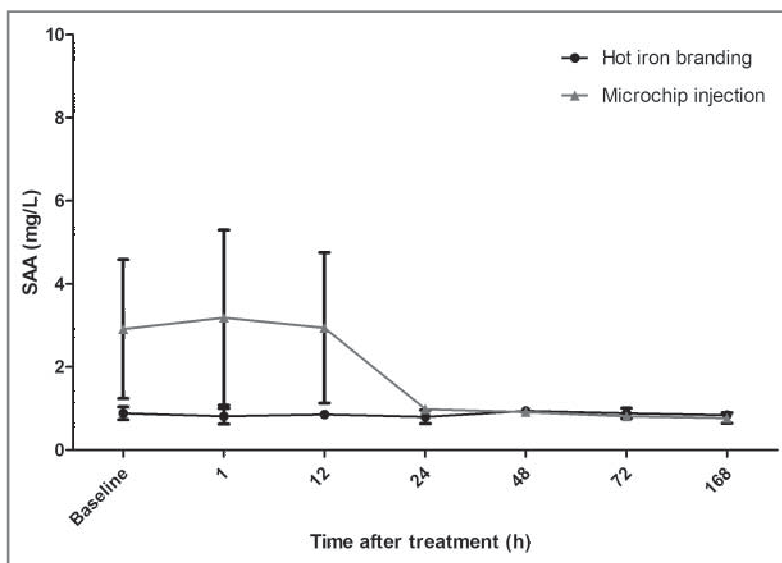
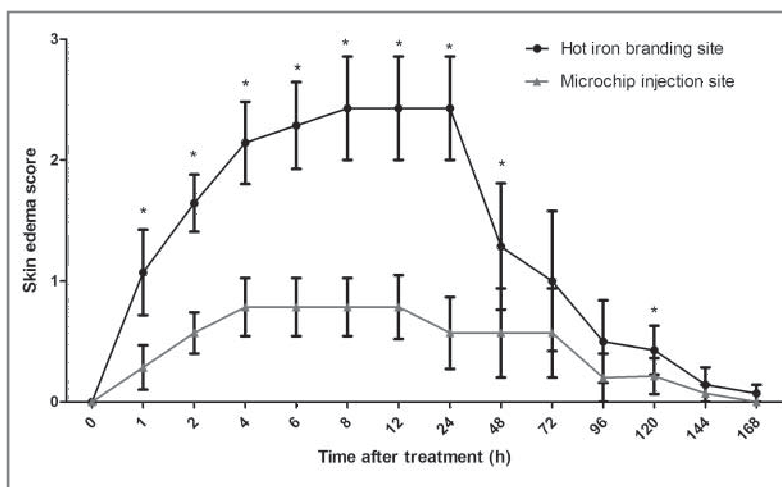
The number of VFM sizes was too large to analyze with the present data set. Instead, an increased skin sensitivity index was generated; this was defined as 1 if the skin sensitivity was increased on the treated side relative to the control side and 0 otherwise. Skin sensitivity index and behavior score were both analyzed by use of a logistic mixed effect model with horse as a random effect. The mean effect of skin sensitivity index was a function of treatment and time whereas behavior score was a function of treatment and observer. The primary hypothesis for both of these response variables was the difference between the 2 treatments as it related to the different effects of hot iron branding versus microchip transponder injection. Regarding observer agreement, this cannot be estimated properly with scores from 4 observers on 7 horses. However, in the logistic regression model, it can be tested whether there is any effect of observer (ie, whether the 4 observers typically agreed).

The edema score was modeled by use of a polynomial regression model with fixed effects of time, horse, and treatment.

The polynomial regression model is an extension of a classical logistic regression model, in which multiple ordered response categories are allowed. Values of $P < 0.05$ were considered significant.

Results

Behavioral score—Both hot iron branding (OR, 17.93; $P < 0.001$) and microchip transponder injection (OR, 14.85; $P < 0.001$) had an effect on behavioral scores, compared with their respective pretreatment sham procedures. However, the type of identification method had a significant ($P < 0.001$) effect on behavioral scores as well. The final model had an OR of 12.83, meaning that with 95% confidence, the odds for observing a reaction was between 3.10 and 53.10 times as great in horses being hot iron branded as in horses being injected with microchip transponders. There was no difference between first and second sham treatments for any of the treatments. Observer had no effect on behavioral score ($P = 0.81$).



Skin temperature—Treatment method had an effect on skin temperature. The difference in skin temperature between horses that were hot iron branded and microchip transponder injected appeared 24 hours after treatment and lasted throughout the study period (Figure 1). Horses in the hot iron-branded group had significantly ($P < 0.001$) higher skin temperature (1.43°C higher) in the treated area, compared with the nontreated contralateral control area. Horses in the microchip transponder-injected group had a nonsignificantly ($P = 0.27$) lower skin temperature (0.16°C) in the treated area, compared with the nontreated contralateral control area.

Skin sensitivity—There was a continuous significant ($P = 0.01$) difference between the 2 treatments resulting in an OR of 2.59 for increased skin sensitivity after hot iron branding, compared with microchip transponder injection, indicating that with 95% confidence, the odds for observing increased skin sensitivity were between 1.39 and 4.82 as great after hot iron branding, compared with microchip transponder injection.

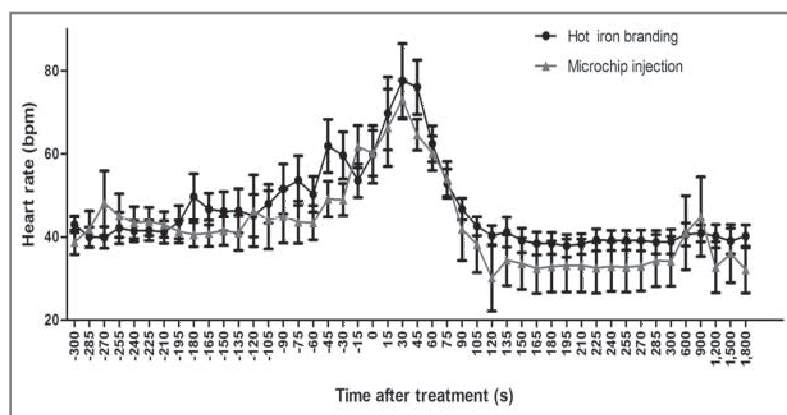
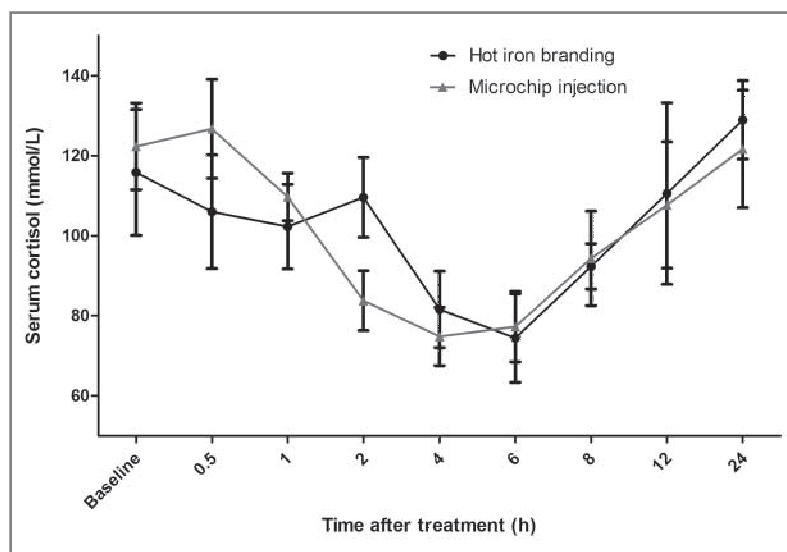
Edema—Method of identification had a significant ($P < 0.001$) effect on the degree of edema with an OR

of 14.6, indicating that with 95% confidence, the odds for observing a skin reaction in the form of swelling or edema were from 6.97 to 30.83 as great after hot iron branding, compared with microchip transponder injection. These differences were significant at 1, 2, 4, 6, 8, 12, 24, 48, and 120 hours after treatment (Figure 2).

Serum amyloid A and serum cortisol concentrations—Neither hot iron branding nor microchip transponder injection elicited an increase in SAA concentration (Figure 3). Serum cortisol concentrations did not differ between the 2 treatments. Values were within reference limits and followed the typical circadian pattern (Figure 4).

NRS and VAS pain scoring—No signs of pain were recognized in the subsequent study period. The NRS pain scores were between 0 and 1 of a maximum of 22, and VAS pain scores were 0 at all time points after both treatments.

Heart rate—All horses had an increased heart rate in association with both treatments, and although the heart rate appeared slightly more increased after hot iron branding than after microchip transponder injection, this difference was not significant (Figure 5; $P = 0.12$).



Discussion

Hot iron branding caused significantly greater aversive reactions indicative of pain than did microchip transponder injection. Because it was concluded to be impossible to mask the treatments (simply because of the different nature of the 2 treatments), the study was not masked. To compensate for this, a rigid guide regarding the scoring of behavioral reactions was made to make the reaction scores as objective as possible, and scoring was conducted by 4 individuals; their scores were based on videographic sequences of the treatment procedures without knowing the scores of the 3 other observers. Because no differences existed among observers ($P = 0.81$), we concluded that results of the study should be considered valid and pertinent regarding the discussion of hot iron branding of horses.

Results of the present study were in complete agreement with several studies^{5-8,11} in cattle (hot iron branding was compared with freeze branding or sham treatment) and to the results obtained by Pollmann⁴ in a study comparing hot iron branding and microchip transponder injection of foals, although there were certain important differences. There has been a lack of investigation of other reactions and longer-term reactions than behavioral, heart rate, and hormonal changes (serum cortisol and epinephrine concentrations) immediately after the treatment, and Pollmann⁴ concluded that hot iron

branding did not cause any long-term consequences to the foals. However, this conclusion was based solely on behavioral observations of < 24 hours after treatment. The present study, which lasted 168 hours after treatment, revealed that there were several important long-term consequences to horses subjected to hot iron branding.

The present study revealed significantly increased skin sensitivity around the treatment site after hot iron branding, compared with microchip transponder injection. This was in contrast to a study⁹ in heifers in which increased skin sensitivity, tested by simply placing a hand on the branded site and on the contralateral control site, was not observed 1 and 7 days after branding. The reason for this difference might be that the stimulus applied in that study was too irregular. More likely, as suggested by the authors themselves, this might be the result of not accustoming heifers to this so-called touch test and thereby observing similar escape-avoidance reactions to the branded site and the unbranded contralateral control site. In the present study, skin sensitivity was assessed by use of VFM, which has been used to quantify skin sensitivity after tissue damage in horses.¹³ Additionally, the horses were thoroughly accustomed to the procedure for 2 weeks prior to treatment. The hypersensitivity observed is termed hyperalgesia or allodynia, depending on whether the applied stimulus is considered noxious or not.¹⁴ Furthermore, it might be defined as primary or secondary hyperalgesia or allodynia depending on whether it is caused by local or central sensitization caused by the inflammatory reaction.^{15,16} In the present study, mechanical stimuli were applied by the use of VFM, which is generally believed to be a non-noxious stimulus¹⁷; the increased reaction is therefore considered allodynia. The stimulus was applied close to the injured area and not directly on it, so the reaction observed in the present study was considered secondary allodynia. However, whether allodynia is primary or secondary was not important in this setting because both are the results of sensitization caused by a local inflammatory reaction.¹⁵

This local inflammatory reaction was also indicated by increased skin temperature and edema after hot iron branding, compared with microchip transponder injection. Increased skin temperature at the hot iron-branding site was observed from 8 hours after treatment and throughout the study period (168 hours), which was in accordance with 2 studies in cattle in which increased skin temperature was observed 4 days⁶ and 2 to 168 hours¹⁰ after hot iron branding.

Heart rate was increased immediately after hot iron branding in several studies in cattle⁵⁻⁷ and in foals,⁴ which is in accordance with results in our study. Although an increase of a similar magnitude was detected after microchip transponder injection, heart rates remained consistently higher after hot iron branding than after microchip transponder injection for the first 5 minutes after treatment.

Plasma cortisol concentration has been identified as a potential indicator of pain in horses¹⁸ but is also increased after stressful nonpainful situations such as

transportation^{19,20} and exercise.²¹ To avoid bias of the serum cortisol concentrations and behavioral scorings, the present study used adult horses thoroughly accustomed to handling and to all procedures included in the experimental setup prior to the actual treatment. Serum cortisol concentrations after hot iron branding and microchip transponder injection were compared, but not with a control group or the sham treatments. Serum cortisol concentrations did not differ between the 2 treatments. Because values were within reference limits at all sampling points and followed the typical circadian pattern (Figure 4), it was concluded that neither hot iron branding nor microchip transponder injection elicited a meaningful change in serum cortisol concentrations. This differed from results of studies^{5,7,9} in cattle where serum cortisol concentrations were significantly increased between 5 and 40 minutes after hot iron branding or freeze branding. In 2 studies,^{6,7} however, serum cortisol concentrations were also increased after sham treatments, so it is suggested that serum cortisol concentration is a less sensitive indicator of pain than it is of stress, which we attempted to eliminate in our study. Other reasonable explanations to account for these differences might be that the pain response elicited by the 2 treatments was either too small or too short-lived to induce an increase in this hormone, or the sampling intervals of 30 minutes might have been too long, although a significant increase in serum concentration of this hormone would have been expected at 30 minutes after treatment.

Hot iron branding results in a hairless scar because of the inflammatory reaction instigated by the second- or third-degree burn that it causes.²² Serum amyloid A is a major acute phase protein that is useful for evaluating inflammatory reactions in horses and other species.²³ A variety of inflammatory as well as infectious conditions including aseptic arthritis,²⁴ castration,²⁵ streptococcal lymphadenitis,²⁶ and IM injection of turpentine oil²⁷ cause increased SAA concentrations. In the present study, SAA concentrations were not affected by either of the 2 treatments. This was interesting because hot iron branding, but not microchip transponder injection, resulted in substantial classic local signs of inflammation including heat, edema, and pain. However, this local reaction may have been too small to induce a systemic inflammatory response within the 168-hour study period. A reasonable explanation might be that the damaged area constituted too small a fraction of the entire skin area of each horse. Alternatively, damage to the skin may not elicit an SAA response.

Infections and chronic hyperplastic and neoplastic cutaneous lesions at the brand site have been reported in cattle subjected to hot iron branding,^{28,29} and infection and neoplastic growth at the microchip injection site have been reported in dogs and a cat.³⁰⁻³² Consequently, these adverse reactions should also be considered potential sequelae to microchip transponder injection and hot iron branding in horses. To the authors' knowledge, other sequelae or long-term sequelae (> 168 hours) from hot iron branding or microchip transponder injection in horses have not been reported in the scientific literature and have not been observed by the authors at the Large Animal Hospital of the Univer-

sity of Copenhagen. Furthermore, there are no reports on the proportion of adverse reactions induced by either treatment in any species. However, Hooven²⁸ stated that hot iron branding in cattle is often followed by openwound infections and parasite infestations, and the British Small Animal Veterinary Association considers that microchip transponder injection represents a safe and reliable form of companion animal identification. Only 3 cases of infection after microchip transponder injection were reported to the Federation of European Companion Animal Veterinary Associations and the British Small Animal Veterinary Association from members throughout Europe in 2000 and 2001.³⁰ It is difficult to make an objective evaluation of the long-term safety of either identification method, but it seems reasonable to conclude that microchip transponder injection does not seem to pose a higher risk for long-term sequelae than hot iron branding. Therefore, recommendations regarding permanent identification of horses are based on short-term animal welfare aspects in combination with an evaluation of the usefulness of the identification method.

Originally, hot iron branding was used as a method of identifying animals by applying the specific brand of the owner. Nowadays, brands may include a number (typically 3 digits) for better identification. However, a non-peer-reviewed publication of the German Equestrian Association³³ indicated that it was impossible to read all 3 digits in 30% to 50% of the studied horses. In contrast, Sorenson et al³⁴ were able to read all microchip transponders 1 year after implantation in 49 dogs and cats.

The present study revealed that hot iron branding elicited a substantial aversive reaction indicative of pain followed by a week-long local inflammation including allodynia of the skin. Because use of a microchip is superior to hot iron branding regarding correct identification and microchip transponder injection does not seem to pose a higher risk than hot iron branding for long-term complications, we conclude that applying a hot iron brand to a horse does inflict more pain and should be abandoned where possible.

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- a. Polar Equine Heart Rate Monitor S720i or S810i, Polar Electro Danmark Aps, Holte, Denmark.
 - b. Dansk Landbrugsrådgivning, Landscentret/Heste, Århus, Denmark.
 - c. RID Microtransponder, Datamars SA, Bedano-Lugano, Switzerland.
 - d. Raytek Raynger MX4, Raytek, Santa Cruz, Calif.
 - e. Touch Test Sensory Evaluator, North Coast Medical Inc, Morgan Hill, Calif.
 - f. LZ-SAA R1/R2, EIKEN Chemical Co Ltd, Tokyo, Japan.
 - g. Immulite 2000 Advanced Immunoassay System, Siemens Healthcare Diagnostics, Tarrytown, NY.
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Appendix

Behavioral scores used in a study of hot iron branding and microchip transponder injection in horses.

Behavioral score	Definition
0	No reaction, ear twitching, shrug, or shiver of skin musculature
1	Raises the neck or moves it to the side, steps to the side, looks back
2	Pins down the ears, restless, snorts, tail flick, escape behavior (jumps to the side, forward, or back)
3	Kicks, rears, stomps, excessive movement