Physiological and behavioural responses of young horses to hot iron branding and microchip implantation

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ABSTRACT

Branding is the traditional and well-established method used to mark horses, but recently microchip transponders for implantation have become available. In this study, behaviour, physiological stress variables and skin temperature in foals were determined in response to hot-iron branding ($n = 7$) and microchip implantation ($n = 7$).

Salivary cortisol concentrations increased in response to branding (1.8 ± 0.2 ng/mL) and microchip implantation (1.4 ± 0.1 ng/mL), but cortisol release over time did not differ. In response to both manipulations there was a transient increase in heart rate ($P < 0.001$) and heart rate variability ($P < 0.01$). Branding and microchip implantation induced a comparable aversive behaviour (branding, score 3.86 ± 0.85; microchip, score 4.00 ± 0.82). Both techniques thus caused similar physiological and behavioural changes indicative of stress. Acutely, implantation of a microchip was as stressful as branding in foals. Branding caused a necrotising skin burn lasting at least 7 days. Moreover branding, but not microchip implantation ($P < 0.001$), was accompanied by a generalized increase in skin temperature which was comparable to low degree post-burn hypermetabolism in humans.

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Introduction

Identification of horses is necessary for breeding programs as well as to preclude substitution in competitions or sales and for disease control. Identification has traditionally been facilitated by hot iron branding with symbols specific for an owner or breed. As an alternative, microchip transponders are now available and, with few exceptions, have been made mandatory for horses in the European Union. Because microchip implantation is supposed to be less stressful for horses than branding, hot iron branding has been forbidden in several countries although it is still used in others.

In adult horses, branding was shown to provoke a stronger aversive reaction than microchip implantation (Lindegaard et al., 2009). Because adult horses are usually more used to humans than foals, these results may not be valid for the young animal. In a German report on foals (Pollmann, 1998), branding caused more acute discomfort than microchip implantation.

The objective of the present study was to compare the stress response and tissue reaction related to branding and microchip implantation in foals. By analysing behaviour and physiological stress variables, we tested the hypothesis that branding is more stressful than microchip implantation. In addition, we hypothesized that an inflammatory response at the branding site occurs.

Materials and methods

The experiment was approved by the competent authority in Brandenburg State, Germany (Landesamt für Ländliche Entwicklung, Landwirtschaft und Flurneuordnung, Licence 23-2347-A-5-2-2010).

Animals

Fourteen Warmblood foals of the Brandenburg State Stud were available for the study. The foals were all healthy and aged between 4 and 19 (10.4 ± 1.4) weeks; four were male and 10 female. All foals had been born at the Stud and kept under identical conditions since birth. Before and throughout the experiment, foals were kept in one herd with their dams on pasture with access to a straw-bedded group stable. From 18:30 to 08:00 h, horses were confined to the stable and mares were fed hay, oats and mineral supplements. Foals were fed oats via a creep system not accessible to mares. Water was available at all times. Before the experiment, foals were made familiar with saliva sampling and heart rate recordings.

Experimental design

The foals were sorted by gender, ranked by age, and allocated to two groups in alternating order. They were given either hot iron branding on the right thigh (group B, $n = 7$) or were implanted with a microchip on the left side of the neck

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(group M, n = 7). The animals were followed from 2 days before to 7 days after these manipulations. All procedures were made in the group stable which was part of the foals' familiar environment.

Branding and microchip implantation

Branding and microchip implantation were performed by the same experienced person and the mares were tied to the stable wall. The foals remained in the stable compartment but for branding or microchip implantation were moved individually into one corner. The foals were held firmly by two people, one winding the arm around the chest of the foal and the other holding the base of the foal's tail.

The branding site was clipped and the microchip implantation site disinfected but not clipped. Horses were branded with a plate iron measuring 9.5 × 5.5 cm with the branding symbol of the Brandenburg State Stud (a serpent winding around an arrow) with a double digit number below. The branding plate was placed on the thigh of the animal for approximately 1 s. Microchip transponders (13.8 × 2.1 mm; BackHome BioTec, Virbac) were implanted with a sterile needle 3 mm in diameter.

The identification was performed on 2 days, 1 week apart. On each day, seven foals were branded or implanted with a microchip in alternating order. Procedures started at 08:00 h with the first foal and the interval between identification of individual foals was 5 min. Foals and mares remained in the stable until 3 h after identification.

Behaviour and local alterations

The foals were observed from fixation through to branding or microchip implantation. In addition, behaviour was recorded by video camera. The occurrence of specific behavioural patterns was scored as outlined in Tables 1 and 2 by two independent observers and mean scores were obtained. For behaviour in response to branding and microchip implantation, subscores for the head and neck, body, and limbs and a total score were calculated based on the scheme by Lindegaard et al. (2009). To assess localized alterations, branding and microchip implantation sites were photographed 1 day before, immediately after and for 7 days after branding and microchip implantation. The photographs were scored by two independent veterinarians as outlined in Table 3 and mean scores were obtained.

Cortisol

For cortisol analysis, saliva was collected with a cotton-based swab (Salivette, Sarstedt). The Salivette was inserted at the angle of the lips into the mouth of the foal and placed gently onto the tongue for 1 min until it was well soaked. After centrifugation for 10 min at 1000 g, saliva was aspirated and frozen at −20 °C until analysis. Collection of saliva was well tolerated by all foals.

To obtain baseline values, saliva was collected on 2 days before identification of the foals at 07:00, 07:30, 13:00, 13:30, 18:00 and 18:30 h. On the day of identification, saliva was collected 60 and 30 min before branding or microchip implantation in the first foal (corresponding to 07:00 and 07:30 h) and at 30 min intervals from 30 min to 180 min after branding or microchip implantation.

Cortisol concentrations were determined by enzyme immunoassay (Schmidt et al., 2010d). The antiserum cross-reacts with several cortisol metabolites and values have to be interpreted as cortisol immunoreactivity. The intra-assay and inter-assay coefficients of variation (CV) were 5.0% and 6.7%, respectively, and the minimal detectable concentration was 0.3 pg/well.

Heart rate and heart rate variability

Heart rate (HR) and heart rate variability (HRV), were determined with a portable recording system (S810i, Polar) attached to the thorax of the foal (Schmidt et al., 2010d). Recordings were made for 3 h beginning at 07:00 h at 2 days before and on the day of branding or microchip implantation from 60 min before the identification to 180 min thereafter.

Data were retrieved to a computer via infrared transmission. HR and HRV were analysed for seven periods of 5 min at 30 min intervals 2 days before branding or microchip implantation. On the day of branding or microchip implantation, periods

Table 1
Scores used for behaviour of foals during fixation and during clipping of the branding site or disinfection of the microchip injection site.

Behavioural score	Definition
0	• No reaction
1	• Raising the head
	• Slight moving (forwards, backwards or to the side)
2	• Shaking the head
	• Vigorous moving (forwards, backwards or to the side)
	• Kicking or pounding movements with the limbs

Table 2
Scores for behaviour of foals during hot iron branding and during microchip injection.

Body region	Behavioural score	Definition
Head and neck	0	• No reaction
	1	• Nodding with the head
		• Sideways movement of neck
		• Raising of head or neck
	2	• Shaking the head
Body	0	• No reaction
	1	• Lowering the croup
		• Slight moving forwards, sideways or backwards
	2	• Vigorous moving forwards
	3	• Jumping forwards, sideways or backwards
		• Jumping upwards
	4	• Rearing
Limbs	0	• No reaction
	2	• Kicking with front or hind limbs
	3	• Lashing out with both hind legs
Other		• Vocalization[a]
		• Defecation/urination[a]
		• Sweating[a]

[a] Provided on recording sheet but not observed in any of the foals.

Table 3
Scores for description of localized changes at the branding and microchip implantation sites.

Response	Score	Definition
Open wound	0	Absent
	1	<20% of branding site
	2	20–40% of branding site
	3	>40% of branding site
Necrosis	0	Absent
	1	Just visible
	2	Well visible
	3	Pronounced and/or thick necrosis
Exudate	0	Absent
	1	Drips at 1 location
	2	Drips at >1 location
	3	Expanded plane with exudate
Tissue demarcation	0	Absent
	1	<20% of branding site
	2	20–40% of branding site
	3	>40% of branding site

of 5 min were analysed, starting at 60 and 30 min before the identification and at 0, 5, 10, 30, 60, 120 and 180 min thereafter. Heart rate variability was analysed for 5 min intervals using the Kubios HRV software (Department of Applied Physics, University of Kuopio). Artefact corrections were made following established procedures (Tarvainen and Niskanen, 2008).

The HRV variables standard deviation of beat-to-beat (RR) interval (SDRR) and root mean square of successive RR differences (RMSSD) were calculated (Schmidt et al., 2010b,d). In addition, HR was analysed for 30 s intervals, starting at 60 and 30 min before fixation, immediately after fixation, during fixation, from 0 to 30, 30 to 60, 60 to 90 and 90 to 120 s after branding and microchip implantation and for 30 s starting 5, 10 and 15 min thereafter.

Thermography

Superficial body temperature was determined by infrared thermography with an uncooled micro-bolometer thermal imaging camera (ThermoPro TP 8, Wuhan). Images were taken of the left and right thigh and the left and right side of the neck in foals of group B while in foals of group M images of the right thigh and the right and left side of the neck were obtained. Thermographic patterns were analysed 1 day before branding or microchip implantation as well as 60 min thereafter, and from 1 to 7 days after the respective procedures.

Images were taken from a distance of 1.5 m with the foal standing. In order to exclude effects of direct sunlight, wind or rain on skin temperature, thermography was always done in the stable in the morning before foals were released onto

pasture. Images were assigned to thermographic software (Exam 5.6, Inframedic) and were analysed for average, minimum and maximum temperature in the region of interest. An elliptic figure from the software was centered on the branding site and extended to the cranial and caudal brim of the thigh or centred on the microchip implantation location. Treated and corresponding contralateral areas were analysed. Results are shown for average temperature only.

Statistical analysis

Statistical analysis was performed with the PASW 17.0 statistics package (SPSS). For parametric data, changes over time and differences between groups were analysed by ANOVA using a general linear model for repeated measures with group as between subject factor. Scored data were compared between groups by Mann Whitney U Test. Data given are means ± SEM. $P < 0.05$ was considered significant.

Results

Behaviour and localized alterations

Scored behaviour of the foals during fixation and preparation was 1.6 ± 0.5 for group B and 1.7 ± 0.7 for group M ($P = 0.81$). During branding or microchip implantation, scores did not differ between groups (Table 4). Necrosis at the branding site increased until day 3 after branding and persisted for the entire study period. No changes were seen on the right thigh of foals implanted with a microchip ($P < 0.001$ vs. group B at all times after treatment). A low degree of demarcation was present from day 2 after branding onwards, but differences between groups were significant only on day 7 ($P < 0.05$). Open wounds and exudate were rarely observed (Fig. 1). No changes were seen at the microchip implantation sites.

Cortisol

Cortisol concentrations on the days before identification decreased throughout the day ($P < 0.001$) in both groups (Fig. 2). Cortisol concentrations at 60 and 30 min before branding or microchip implantation were similar to the morning values on the two previous days. Cortisol concentrations changed in response to both branding and microchip implantation. Maximal concentrations were reached at 60 min after microchip implantation (1.4 ± 0.1 ng/mL) and 30 min after branding (1.8 ± 0.2 ng/mL; Fig. 2). Changes over time were significant ($P < 0.01$), but values did not differ significantly between groups ($P = 0.36$ for cortisol curves, $P = 0.15$ for maximal values).

Heart rate and heart rate variability

In response to both methods of identification there was an immediate but transient increase in HR ($P < 0.001$), but neither differences between groups ($P = 0.82$), nor interactions of group - time were significant ($P = 0.93$; Fig. 3a). Mean HR increased from 72 ± 4 and 70 ± 3 beats/min 60 min before branding or microchip implantation, respectively, to 88 ± 6 and 90 ± 8 beats/min directly thereafter. For the HRV variable SDRR, a transient increase after branding and microchip implantation occurred ($P < 0.01$), but did not differ between groups ($P = 0.90$; Fig. 3b). The RMSSD did not change over time ($P = 0.53$) and did not differ between groups ($P = 0.95$; data not shown).

Table 4
Behaviour score of foals in response to hot-iron branding or microchip implantation ($n = 7$ per group, values are mean ± SEM).

Group	Score head and neck	Score body	Score limbs	Total score
Microchip	1.6 ± 0.3	1.7 ± 0.3	0.14 ± 0.14	3.4 ± 0.5
Branding	1.1 ± 0.3	2.5 ± 0.5	0.00 ± 0.00	3.6 ± 0.7
	$P = 0.62$	$P = 0.26$	$P = 0.71$	$P = 0.90$
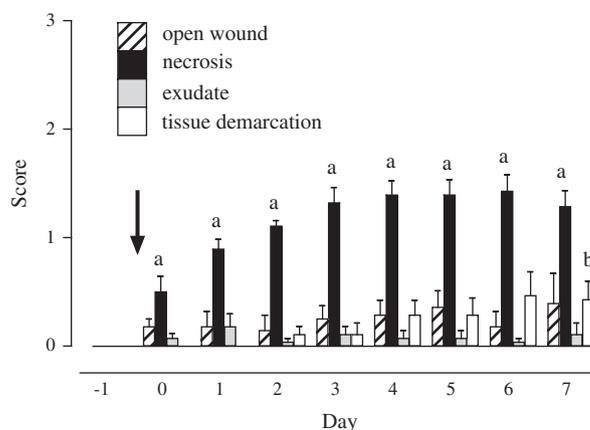
Fig. 1. Degree of localized clinical alterations at the branding site in foals of group B (hot iron branding) from one day before to seven days after branding (arrow, $n = 7$ per group). Scores for the right thigh of group M foals (microchip implantation) were 0 at all times and data are not shown in the figure. Superscript letters indicate significant difference of group B from 0 values in group M for respective variable (a, $P < 0.001$; b, $P < 0.05$).
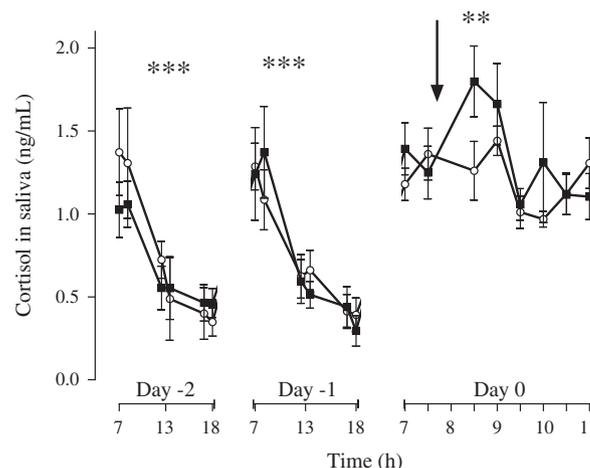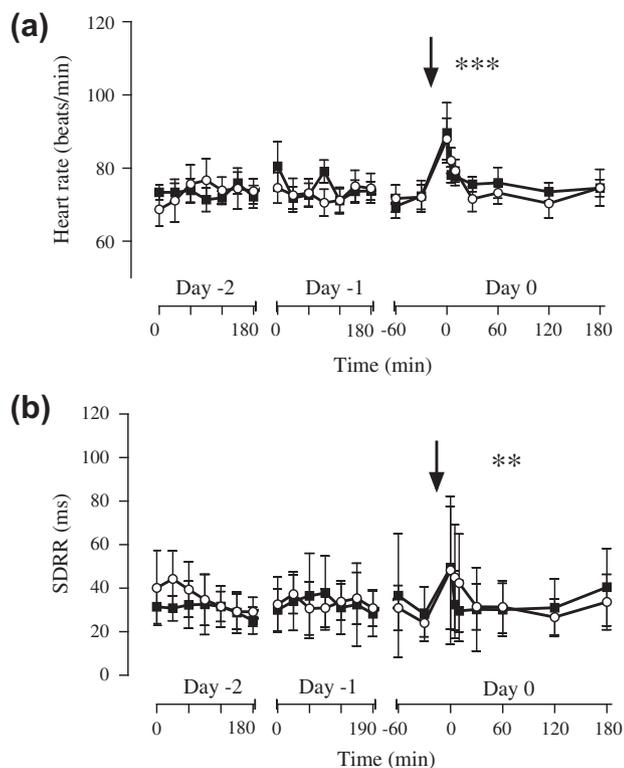

Fig. 2. Cortisol concentrations in saliva of foals on two days before and in response to hot iron branding (group B, ■) or microchip implantation (group M, ○) on day 0 (arrow; $n = 7$ per group). ***$P < 0.001$ and **$P < 0.01$ over time for respective day, no significant differences between groups and no significant interactions group × time.

When analysed for intervals of 30 s, HR increased when the foals were caught, decreased with ongoing fixation, and increased again after branding and microchip implantation (differences over time, $P < 0.001$; differences between groups, $P = 0.91$; interactions group × time, $P = 0.81$; Fig. 4).

Superficial body temperature

Average skin temperature on the right (branding) and left thigh (control) increased in response to branding ($P < 0.001$ over time), but did not differ between these locations in foals of group B. Skin temperature remained unchanged on the right thigh of foals implanted with a microchip in the neck (group M) and thus was significantly lower ($P < 0.01$) than in foals after branding (group B). This difference between groups lasted for at least seven days (Fig. 5a). Interactions group × time were significant ($P < 0.001$). Thermography revealed an increase in skin temperature on both sides of the neck in foals of group B ($P < 0.001$ over time), but not in foals implanted with a microchip (group M; $P < 0.05$ between groups; interactions group × time, $P < 0.001$; Fig. 5b).

Fig. 3. (a) Heart rate (HR) and (b) SDRR (standard deviation of RR interval) determined for 5 min intervals in foals on two days before and in response to hot iron branding (group B, ■) or microchip implantation (group M, ○) on day 0 (arrow; $n = 7$ per group). ***$P < 0.001$ and **$P < 0.01$ over time for respective day, no significant differences between groups.
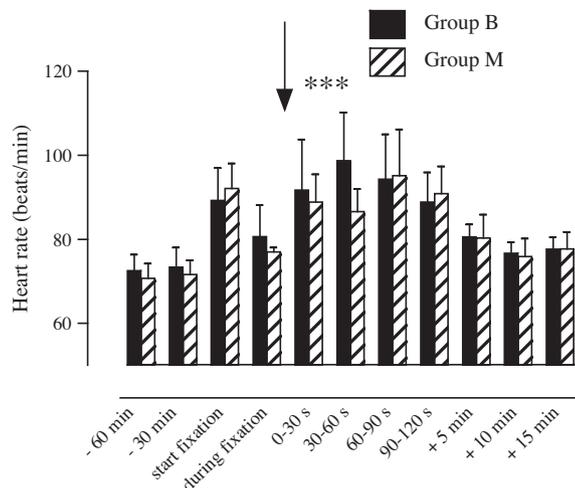
Fig. 4. Heart rate (HR) determined for intervals of 30 s in foals before, during and after hot iron branding (group B, $n = 7$) or microchip implantation (group M, $n = 7$; arrow). ***$P < 0.001$ over time, no significant differences between groups.

Discussion

The objective of this study was to compare the stress in foals induced by hot iron branding and microchip implantation and to analyse the degree of inflammation and tissue destruction at the marking site. Thus, no non-treated control group was included. Our hypothesis was to find a pronounced response to branding and/or microchip implantation and no major response to handling.
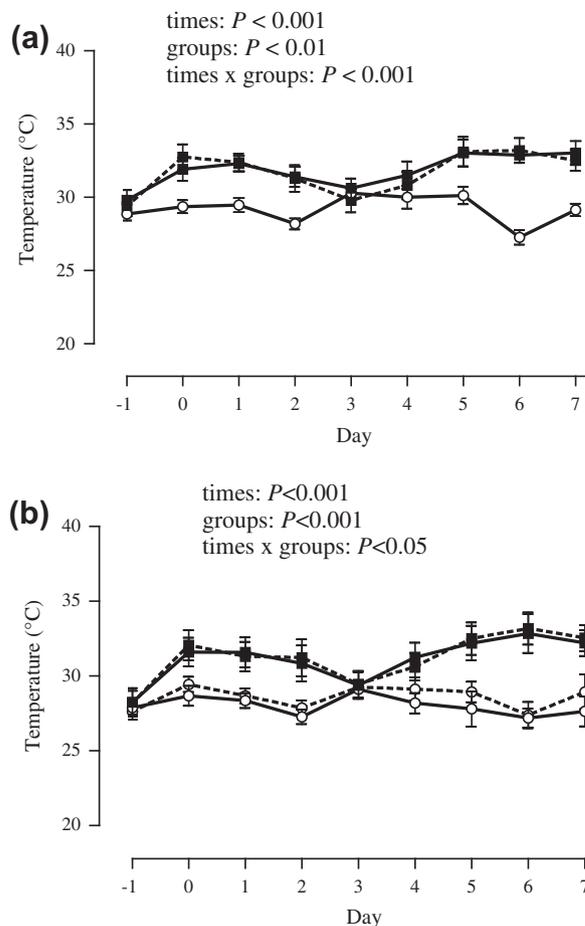
Fig. 5. (a) Average temperature on the right (—) and left (- - - -) thigh and (b) average temperature on the right (- - - -) and left (—) side of the neck in foals either branded on the right thigh (group B, ■; $n = 7$) or implanted with a microchip on the left side of the neck (group M, ○; $n = 7$). Significant differences are indicated in the figures.

With an evident response of the foals to fixation, differentiation between effects of firmly holding the animals and the identification procedure is difficult. This would have required additional groups submitted to fixation only and not handled at all.

Branding induced a localized necrosis, but no alterations occurred after microchip implantation. Both branding and microchip implantation induced an increase in cortisol release and a transient increase in HR. Neither cortisol release, nor HR or behaviour scores differed between groups. Pronounced aversive behaviour such as rearing or lashing out with the hindlegs was not shown. Thus, in combination with fixation of the foals, implantation of a microchip acutely is as stressful as branding. The increase in salivary cortisol concentrations at first did not appear to be pronounced, but this has to be compared to the marked decrease throughout the day before the experiment. This diurnal cortisol rhythm in horses is well known (Hoffsis et al., 1970). Cortisol release in response to the short-term stimulus of branding and microchip implantation, albeit evident, was less pronounced than in horses during transport (Schmidt et al., 2010b–d), training (Schmidt et al., 2010a) or at weaning (Erber et al., 2011). Nevertheless, the increase in cortisol release indicates that branding and microchip implantation were perceived as stressful by the foals.

The general conclusion that both branding and microchip implantation are stressful in foals is in agreement with results obtained in adult horses (Lindegaard et al., 2009), but results of the two studies differ in detail. Horses are usually registered and

marked as suckling foals. Because habituation to humans affects an animal's stress response, experiments in adult horses may only partially be comparable to studies in foals. In adult horses, branding induced more pronounced behavioural changes than microchip implantation, but cortisol release did not increase in both groups (Lindegaard et al., 2009).

In foals, fixation for branding or microchip implantation induced an immediate behavioural response and a transient increase in HR which did not occur in adult horses. Although familiar with humans, the foals were less accustomed to handling than adult horses. The response to fixation might therefore have masked differences in the response to branding and microchip implantation, but in this case the response to the actual identification would be less pronounced than the response to fixation alone. Handling also affects the stress response in cattle. In beef cattle with little contact to humans, hot iron branding and cold branding evoked a comparable response (Lay et al., 1992a), while dairy cows accustomed to handling showed a more pronounced escape-avoidance response to hot iron than to freeze branding (Lay et al., 1992b). Thus, it cannot be excluded that when handling effects are subtracted, branding is more stressful and/or painful than microchip implantation in horses.

Branding and microchip injection caused an increase in HR. Analysis of HR for intervals of 30 s revealed that this increase was of short duration and consisted of two peaks, one each at the beginning of fixation and one after identification. The two increases were clearly distinguishable. Since increases in HR are triggered by the sympathetic nervous system, these short-term responses are in agreement with a short-term epinephrine release in calves after branding (Lay et al., 1992b,c).

Neither branding nor microchip implantation was associated with a decrease in HRV, but the increase in HR alone provided evidence of increased sympathetic activity. We recently found a decrease in HRV in foals at weaning (Erber et al., 2011). The difference to the current study may indicate that, in contrast to a prolonged stress at weaning, the short-term stimuli of branding and microchip injection are not sufficient to cause detectable changes in HRV. Because HRV for short recording intervals can be biased by artefacts, HRV was calculated for 5 min intervals only and not for 30 s intervals.

While the acute stress response of foals did not differ between groups, thermography revealed a systemic response to branding, but not microchip implantation. In foals marked by branding, a prolonged increase in skin temperature was not only found at the branding site, but also on the contralateral thigh and on both sides of the neck. This is different from adult horses (Lindegaard et al., 2009), where branding induced only a localized increase in skin temperature.

Because the same branding irons are used in foals and adult horses, the size of the branding lesion related to body size is greater in foals, potentially explaining a different response. Not surprisingly, branding caused necrotic lesions. However, our results demonstrated that branding-induced burn injuries in foals are not restricted to a local response, but are associated with a reset in thermoregulation.

In humans, burn injury leads to a hypermetabolic state (Wilmore et al., 1974), which is already pronounced with burns covering 20% of body surface (Kelemen et al., 1996). Although relative size of branding-induced lesion in foals was smaller, the increase in temperature may be caused by a reset in regulatory pathways as in post-burn hypermetabolism. Thus, studies on branding and microchip implantation should not be restricted to stress, but need also to focus on tissue destruction and inflammatory alterations.

Like in lambs submitted to castration and tail docking, painful insults experienced early in life may enhance future pain perception (McCracken et al., 2010). It is axiomatic that painful procedures should be avoided where possible, especially in young animals.

Conclusions

Branding and microchip implantation induce a transient stress response in foals. Although this response is less pronounced than the reaction of horses to other anthropogenic stressors, both procedures are perceived as stressful. The stress response in the present study was caused in part by human fixation of the foals and did not differ between treatments. Branding, but not microchip implantation, caused a necrotising burn wound and a generalized increase in superficial body temperature, which together are indicative of significant tissue damage. Branding thus induces more prolonged alterations in foals than implantation of a microchip.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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