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**Effects of hot-iron disbudding, using regional anaesthesia with and without analgesia, on cortisol and behaviour of calves**

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**Abstract**

The objective of this study was to assess cortisol and behaviour changes in calves hot-iron disbudded after different analgesic protocols with carprofen. We assessed the response of calves (n=27) at 1, 3, 6 and 24 hours after disbudding with local anaesthesia (DA), local anaesthesia plus carprofen (DAC), disbudded only (D) or sham-disbudded (ND).

Immediately after the procedure, pain-related behaviours were more frequent in D than in any other group. At 1 h cortisol was higher in D compared with all other groups. At 3 h ND showed lower cortisol than all other groups but these did not differ from their own baseline levels. At 1 h D showed more head-shakes, ear-flicks and head-rubs than all other groups.
Groups D (3 h) and DA (3 and 6 h) showed more ear-flicks and head-rubs compared with DAC and ND. Head-rubbing, head-shaking and ear-flicking are useful behaviours for evaluating pain but quick transition from standing to lying is not a reliable behaviour to assess pain after hot-iron disbudding. In conclusion, hot-iron disbudding causes pain in calves for at least 3 hours and only the association of local anaesthesia with carprofen efficiently controls pain for 24 hours.

**Keywords**: calf disbudding; pain; plasma cortisol; behaviour; welfare

**Introduction**

Disbudding young female dairy calves is a routine procedure in most dairy farms. The objective is to reduce injury to other animals and humans caused by horned cows. The most common methods of destroying the growing horn tissue are: heat-cauterization, by applying on the horn base a device heated to above 600°C for ~30 seconds (thermal or hot-iron disbudding), and chemical-burning with sodium hydroxide paste (caustic disbudding). Thermal disbudding leads to the destruction of all the epidermal and dermal layers extending down to the subcutaneous tissue, but it may also cause tissue damage and oedema that extends beyond the burn-site increasing the sensitized area (Junger et al, 2002).

Pain-related distress can be assessed by measuring physiological and behavioural changes in animals submitted to the procedure compared with sham-disbudded ones (Morton et al, 1985; Molony and Kent, 1997; Mellor et al, 2005). Cortisol has been shown to be accurate to assess the occurrence of pain after hot-iron dehorning because the hypothalamic-pituitary-adrenal cortex axis is activated during the distress caused by the
procedure (Petrie et al., 1995; Graf and Senn, 1999; Doherty et al., 2007). Pain-related
behaviours recorded after disbudding have been: head-shaking, ear-flicking, rapid transition
from standing to lying and to standing again, and rubbing the head with the hind feet
(Morrise et al., 1995; Grøndahl-Nielsen et al., 1999; Doherty et al., 2007).

Local anaesthetic drugs act by inhibiting sodium-channels to impede nerve
depolarization and conduction. Lidocaine 2% is the most commonly used local anaesthetic
in cattle practice and its blocking effect persists for 60 to 90 minutes after injection (Muir et
al., 1995; Anderson and Muir, 2005).

Carprofen is a NSAID with a mode of action that is not entirely known, although it
is considered to be a relatively poor cyclo-oxygenase inhibitor. However, it has shown
analgesic properties similar to opioids after surgery (Nolan, 2005). Several studies on cats
(Al-Gizawiy et al., 2004), dogs (Lascelles et al., 1998), horses (Johnson et al., 1993) and
cattle Stilwell et al., 2008a) reveal that it is an excellent post-surgical analgesic. The half-
life of carprofen depends on the species, but has been established to be > 34 hours in 17-
week-old calves and 44 to 64 hours in adult cows (Delatour et al., 1996; Lees et al., 1996). A
long-lasting anti-inflammatory effect of carprofen has been found for cattle (Balmer et al.,
1997). The duration of the analgesic effect of carprofen in calves has not been established
although it has been shown to reduce cortisol and pain-related behaviours for 48 hours in
clamp-castrated calves (Stilwell et al., 2008a). Carprofen is a drug approved for cattle in
most of Europe and is currently used by many bovine practitioners.

Other non-steroidal-anti-inflammatory drugs (ketoprofen, meloxicam and flunixin-
meglumine) have been studied in calves after disbudding.

The objectives of this study were to assess pain-related distress in calves after hot-
iron disbudding by measuring physiological (plasma cortisol concentration) and
behavioural responses after different analgesic protocols with carprofen and lidocaine local nerve block.

**Material and Methods**

*Farm and animals*

All the experiments were done at the same 300 cow dairy farm. At this farm newborn calves are kept in individual hutches, bedded with straw, until weaning. Before weaning they are fed milk at 5% of body-weight in the morning and evening and have free access to grass hay, 18% protein calf starter and water. Weaning is done when the calf eats over half a kilogram of concentrate per day for three consecutive days. After weaning calves are moved to an open stable and have free access to concentrate, grass and alfalfa hay and water.

*Experimental Procedures and Design*

In this study the effects of the routine procedure of hot-iron disbudding was investigated for the first 24 hours.

The “Centro de Investigação Interdisciplinar em Sanidade Animal” (CIISA) Committee for post-doc studies, of the Lisbon Faculdade de Medicina Veterinaria, approved all animal use in this project. The disbudding protocol was the same as that usually carried out at the farm.

*Common procedures*

Disbudding was carried out between 10 and 11 a.m. during several days by the same operator, blind to the treatments. The iron was electrically-heated and applied over the horn
bud for ~30 seconds for each horn, producing a deep burn of the tissue at the base of the horn. A cold device was applied for the same time to the control calves (sham-disbudded).

Disbudding was done in groups of 4 to 6 animals a few days after weaning, corresponding to the age of 8 to 10 weeks.

Cornual nerve anaesthesia was achieved by the injection of 5 ml of 2% lidocaine (Anestesin®, Laboratorio Sorologico, Portugal), without adrenaline, just ventral to the lateral edge of the frontal bone, midway from the base of the horn to the lateral canthus of the eye (Noordsy, 1994; Greene, 2003). In control groups, a 0.9% saline solution was administered in the same way. Carprofen (2 ml, approx 1.4 mg/kg; Rimadyl®, Pfizer-Animal Health, Dundee, UK) was given i.v. 15 minutes before the procedure was carried out or, in controls, the same dose of a saline solution was given i.v. Animals’ approximate weight was estimated by body size.

Blood sampling (7ml) into a heparinised tube was by left jugular venipuncture. Blood was kept on ice then centrifuged and the plasma frozen (-20C). Cortisol was assayed in duplicate and measured by a validated solid radioimmunoassay, without extraction, using a commercial kit (Coat-A-Count; Diagnostic Product Corporation, Los Angeles, CA, USA) at the Faculdade de Medicina Veterinaria. The lowest detectable concentration of cortisol was 1.0 nmol/l. The inter-assay coefficients of variation was 6.5% for 1 ng/mL and 3.4% for 5 ng/mL and the intra-assay coefficients of variation was 5.6% (Multivalent Control Module, DPC, Los Angeles, CA, USA) (Rodbard, 1974).

Behaviour was assessed by an experienced veterinarian blind to the treatments. The frequencies of four pain-related behaviours (ear-flicking, head-shaking, head rubbing with hind foot and quick transitions from standing to lying and back to standing) were recorded by a veterinarian just before each blood sampling. The total behaviour incidence (sum of all
behaviours within each group divided by the number of animals in that group) was also calculated.

Animals

Twenty-eight female calves, mean age 88 ±17 days, were randomly assigned to four groups: DA: disbudded after lidocaine injection (n=7); DAC: disbudded after lidocaine and carprofen injection (n=7); D: disbudded after treatments with saline (n=7); ND: sham-disbudded after treatments with saline (n=8). Blood was collected 15 min before the procedure and then at 1, 3, 6 and 24 hours after disbudding. Behaviour was assessed for periods of 15 minutes at 15 min, 1, 3, 6 and 24 hours after disbudding.

One calf was eliminated from the DA group because of clinical disease signs shown during the experiment.

Statistical analysis

The between-day differences for plasma cortisol concentrations and behaviour incidence within groups were not significant so data were pooled.

The plasma cortisol analysis of variance was done with the PROC MIXED from SAS (SAS, 2004) using the following mixed linear model: $Y = X\beta + Z\gamma + \epsilon$, that includes the treatment and time and their respective interaction as fixed effects and the calves as the unknown random effect. In addition the Least Squares Means for each treatment*time
combination was calculated as well as the differences between the means and the respective t-test.

Distributions of the behaviour variables were shown by Levene and Shapiro-Wilkes tests to be non-normal, so non-parametric analyses were used. Behaviour means and standard errors were calculated using PROC GENMOD from SAS (SAS, 2004) with a model that included the effect of treatment and time and their respective interaction. The incidence of the four pain-related behaviours were then analyzed with the PROC NPAR1WAY from SAS (SAS, 2004), using Wilcoxon and Median tests to compare each pair of treatments (2x2) at different times.

For all tests differences for which p<0.05 were considered significant.

**Results**

There were no differences in age between groups. DA (83 ±15); DAC (96 ±20); D (98 ±15); ND (76 ±11).

No differences were found between groups base-line plasma cortisol concentrations levels (Table 1). At 1 hour calves disbudded with no treatment showed higher cortisol than sham-disbudded and calves treated with regional anaesthesia and carprofen (P<0.01). At 3 hours calves treated only with lidocaine (DA) showed higher cortisol than sham-disbudded calves (P<0.05). Only D group showed differences when compared with baseline and only at 1 hour after the procedure.

There were no differences in transitions behaviour between groups at any time (Figure 1).

The other three behaviours’ incidences are shown in Table 2-4. Immediately after the procedure, D and DA showed significantly more head shakes than DAC and ND but D
showed more ear flicks and head rubs than all the other groups. One hour after disbudding D calves showed more head shakes and ear flicks than all other groups and more head rubs than DAC and ND. Calves treated with local anaesthesia showed more ear flicks (1, 3 and 6 h) and head rubs (3 and 6 h) than sham-dehorned animals. At no time did DAC showed any difference when compared with ND animals.

When assessing the total behaviour incidence (Table 5), D group shows more behaviours than D, DAC and ND (15 min); than DAC and ND (1 h); and than ND (3 h). Animals disbudded only with local anaesthesia show more signs at 15 min, 1, 3 and 6 hours when compared with DC and ND. Along time the disbudded-only animals showed a higher incidence of behaviours at 10 min than at all other times (P<0.05) and more behaviours at 1 hour than at 3, 6 or 24 (P<0.05). The group disbudded with lidocaine alone showed more behaviours at 10 min, 1 h and 3 hours when compared with the incidence at 24 hours (P<0.05).

DISCUSSION

Several studies have confirmed that hot-iron disbudding causes pain in calves for at least 2 hours (Petrie et al, 1995; Morisse et al, 1995; Doherty et al, 2007). The cortisol results of our Experiment 1 show that distress is present at 1 hour but no difference is evident at 3 hours when compared with sham-disbudded animals. However behaviour analysis shows a high incidence of altered behaviours at 3 hours, suggesting that, although not causing a noticeable rise in plasma cortisol, discomfort is present for longer than previously assumed. Although some studies with rats show that mechanical hyperalgesia is still present 2 weeks after full-thickness thermal burns (Summer et al, 2007), we did not find any evidence of pain-related distress in disbudded calves at 6 or 24 hours. This could
be due to species differences, a relatively smaller burned area or because we did not look at measures that effectively assess hyperalgesia and chronic pain.

Some studies have been contradictory as to the efficacy of regional anaesthesia. Petrie et al (1995), using 2% lidocaine, and Doherty et al (2007), using 2% and 5% lidocaine, concluded that regional anaesthesia is not very efficacious. In contrast, Grøndahl-Nielsen et al (1999) and Graf and Senn (1999) showed that cornual nerve block markedly attenuates behavioural and physiological response for the first two hours after the procedure. However all studies that looked at the struggling during the procedure agree that cornual nerve blocking is efficient in reducing signs of pain. Our results also show a positive effect by reducing the degree of struggling compared with animals disbudded with no anaesthesia. The Experiment 1 cortisol results indicate that regional anaesthesia is efficient in controlling pain for 24 hours, but analysing the pain-related behaviour incidence we show that pain is present as early as 1 hour and for 3 hours after disbudding. Cortisol levels in Experiment 2 also show that animals treated with regional anaesthesia suffer some distress immediately after the procedure when compared with sham-disbudded, although to a smaller degree than control disbudded ones. This could be due to the handling during disbudding but the fact that the sham-disbudded calves (submitted to the same handling) did not show an increase suggests that some pain is felt even when regional anaesthesia is given.

The results from blood collected after the nerve block supposedly had subsided (Experiment 3) show a rise in cortisol in all treated calves although different calves responded at different times (data not shown). This is probably why no difference between groups is apparent at 90 and 120 min in Experiment 3 or at 3 hours in Experiment 1, when comparing disbudded with sham-disbudded animals, but for overall cortisol response
between the two groups in Experiment 3, the difference was evident. Graf and Senn (1999) and Grøndahl-Nielsen et al (1999) also showed a delayed increase in cortisol of lidocaine-treated animals at 180 and 210 min post-disbudding. Doherty et al (2007) did find a similar increase at 4 hours after blocking with 5% lidocaine but not when using 2% lidocaine. In contrast, behaviour differences are evident at 90 and 120 minutes (Experiment 3) and at 3 hours (Experiment 1) after disbudding suggesting that pain, probably due to extensive inflammation that follows deep thermal burns (Junger et al, 2002) is felt by calves when regional anaesthesia subsides. These results also show that the duration of nerve block varies between individuals, perhaps due to anatomical or physiological differences.

The use of both regional anaesthesia and a non-steroidal-anti-inflammatory drug (NSAID) is shown here to efficiently control pain-related distress after hot-iron disbudding. All previous studies using NSAID have used ketoprofen as the analgesic (McMeekan et al, 1998; Faulkner and Weary, 2000; Milligan et al, 2004). With this study we demonstrated that regional anaesthesia together with carprofen is equally efficient in reducing or eliminating the rise in plasma cortisol and pain-related behaviours from immediately after disbudding to 24 hours after the procedure.

Only two studies have looked at the effect of a NSAID given alone and preemptively: McMeekan et al (1998) showed that ketoprofen alone had no effect in controlling pain after scoop-dehorning and Stilwell et al (2008b) found the same result when using flunixin-meglumine after paste disbudding. In the present study we showed that carprofen, without regional analgesia, only reduces the intensity of the cortisol and behaviour response at 30 minutes after disbudding when compared with disbudded control animals. Carprofen alone also showed a trend towards the reduction of struggling during the procedure compared with non-treated animals. These results suggest that carprofen
alone does have an analgesic effect but not sufficient to eliminate pain caused by hot-iron disbudding.

We conclude that hot-iron disbudding of young calves is a procedure that causes severe pain during the procedure and for, at least, 3 hours. Regional anaesthesia is efficient in reducing struggling and pain signs for the first hour but does not prevent pain-distress when nerve blocking subsides. Carprofen given alone and pre-emptively does not reduce pain significantly although it does reduce the severity of the responses during the first hour. Only the combination of regional anaesthesia, 5 ml 2% lidocaine given s/c midway between the horn base and the lateral eye canthus, with i.v. carprofen resulted in reduced struggling, plasma cortisol and pain-related behaviours during the 24 hours after hot-iron disbudding, and so can ensure good welfare in the calves.

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Prof. Luísa Mateus for the cortisol assessment.

**References**


McMeekan CM, Stafford KJ, Mellor DJ, Bruce RA, Ward RN and Gregory NG 1998. Effects of regional analgesia and/or a non-steroidal-anti-inflammatory analgesic on the acute cortisol response to dehorning in calves. Research Veterinary Science. 64, 147-150


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Fig. 1 - Degree of struggling (mean ±SD) during hot-iron disbudding (scale from 0 = no struggling to 5 = severe struggling). DA₂ (n=7) – disbudded with cornual nerve blocking with lidocaine; DAC₂ (n=7) – disbudded with cornual nerve blocking with lidocaine and carprofen; DC₂ (n=8) – disbudded with carprofen alone; D₂ (n=7) – disbudded with no treatment. ND₂ (n=8) – sham-disbudded.

Different letter indicates differences between groups.
Table 1 – Mean ±SD plasma cortisol (nmol/L) of calves disbudded with hot-iron in Experiment 1. DA₁: calves disbudded after treatment with lidocaine; DAC₁: calves disbudded after treatment with lidocaine and carprofen; D₁: calves disbudded without treatment; ND₁: calves sham-disbudded.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>-5 min</th>
<th>+ 1h</th>
<th>+ 3h</th>
<th>+ 6h</th>
<th>+ 24h</th>
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<tbody>
<tr>
<td>DA₁</td>
<td>6</td>
<td>18.21Åa ± 5.53</td>
<td>16.95Åa ± 5.53</td>
<td>25.17Åa ± 5.53</td>
<td>28.19Åa ± 5.53</td>
<td>17.11Åa ± 5.53</td>
</tr>
<tr>
<td>DAC₁</td>
<td>6</td>
<td>24.00Åa ± 5.12</td>
<td>15.54Åa ± 5.12</td>
<td>21.11Åabb ± 5.12</td>
<td>14.87Åab ± 5.12</td>
<td>35.98Åb ± 5.12</td>
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<tr>
<td>D₁</td>
<td>7</td>
<td>15.64Åa ± 5.12</td>
<td>33.89Åb ± 5.12</td>
<td>20.95Åab ± 5.12</td>
<td>16.51Åab ± 5.12</td>
<td>25.13Åab ± 5.12</td>
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<tr>
<td>ND₁</td>
<td>8</td>
<td>10.64Åa ± 5.12</td>
<td>7.17Åa ± 5.12</td>
<td>10.09Åb ± 5.12</td>
<td>12.40Åb ± 5.12</td>
<td>15.66Åa ± 5.12</td>
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Different lower case superscript letters indicate difference between groups for which P<0.05
Table 2 – Incidence (mean ±SD) of four different behaviours (head shake, ear flick, head rub and transitions from standing to lying) for calves disbudded with hot-iron in Experiment 1. Observational period: 15 min. Treatment groups: DA₁ – calves disbudded after treatment with lidocaine; DAC₁ – calves disbudded after treatment with lidocaine and analgesia (carprofen); D₁ – calves disbudded without treatment; ND₁ – calves sham-disbudded.

<table>
<thead>
<tr>
<th>Group</th>
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<th>Time from disbudding</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>+15m</td>
</tr>
<tr>
<td>DA₁</td>
<td>6</td>
<td>1.50 ±0.33&lt;sup&gt;bA&lt;/sup&gt;</td>
</tr>
<tr>
<td>DAC₁</td>
<td>7</td>
<td>0.57 ±0.53&lt;sup&gt;bA&lt;/sup&gt;</td>
</tr>
<tr>
<td>D₁</td>
<td>7</td>
<td>6.14 ±1.35&lt;sup&gt;cA&lt;/sup&gt;</td>
</tr>
<tr>
<td>ND₁</td>
<td>8</td>
<td>0.57 ±0.53&lt;sup&gt;bA&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
Different lower case superscript letters indicate difference between groups for which $P<0.05$.

Different upper case superscript letters indicate difference across time for which $P<0.05$.

Table 3 – Mean ±SD plasma cortisol (nmol/L) of calves disbudded with hot-iron in Experiment 2. DA$_2$: disbudded after treatment with lidocaine; DAC$_2$: disbudded after treatment with lidocaine and analgesic (carprofen); DC$_2$: disbudded after treatment with analgesic (carprofen); D$_2$: calves disbudded without treatment; ND$_2$: calves sham-disbudded.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>-5 min</th>
<th>+ 10 min</th>
<th>+ 30 min</th>
<th>+ 50 min</th>
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<tbody>
<tr>
<td>DA$_2$</td>
<td>7</td>
<td>12.94 ±6.47$^{bA}$</td>
<td>44.94 ±6.47$^{aC}$</td>
<td>15.16 ±6.47$^{aK}$</td>
<td>8.31 ±6.47$^{bL}$</td>
</tr>
<tr>
<td>DAC$_2$</td>
<td>7</td>
<td>19.06 ±6.47$^{bB}$</td>
<td>32.69 ±6.47$^{aA}$</td>
<td>12.76 ±6.47$^{bL}$</td>
<td>8.10 ±6.47$^{bL}$</td>
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<tr>
<td>DC$_2$</td>
<td>8</td>
<td>15.14 ±6.05$^{A}$</td>
<td>83.77 ±6.05$^{bB}$</td>
<td>91.69 ±6.05$^{bB}$</td>
<td>72.02 ±6.47$^{bB}$</td>
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Table 4 – Incidence (mean ±SD) of four different behaviours (head shake, ear flick, head rub and transitions from standing to lying) for calves disbudded with hot-iron in Experiment 2. Observational period: 10 min. Treatment groups: DA₂ – calves disbudded after treatment with lidocaine; DAC₂ – calves disbudded after treatment with lidocaine and analgesia (carprofen); DC₂: disbudded after treatment with analgesic (carprofen); D₂ – calves disbudded without treatment; ND₂ – calves sham-disbudded.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>0 - 10m</th>
<th>+20 - 30m</th>
<th>+40 - 50m</th>
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<tr>
<td>DA₂</td>
<td>7</td>
<td>1.71 ±1.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.71 ±0.76&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.14 ±1.35&lt;sup&gt;bc PQ&lt;/sup&gt;</td>
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Different lower case superscript letters indicate difference between groups for which P<0.05.
Different upper case superscript letters indicate difference across time for which P<0.05.
Table 5 – Mean ±SD plasma cortisol (nmol/L) of calves disbudded with hot-iron in Experiment 3. DA₃: disbudded after treatment with lidocaine; ND₃: calves sham-disbudded.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>-5 min</th>
<th>+ 90 min</th>
<th>+ 120 min</th>
<th>+ 150 min</th>
<th>Mean post-disbudding</th>
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<tbody>
<tr>
<td>DA₃</td>
<td>8</td>
<td>14.11 ±3.91ᵃ</td>
<td>25.56 ±3.91ᵇᶜ</td>
<td>18.50 ±3.91ᵇᶜ</td>
<td>31.37 ±3.91ᵇᶜ</td>
<td>25.15 ±3.91ᵇᶜ</td>
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<tr>
<td>ND₃</td>
<td>5</td>
<td>14.24 ±4.52ᵃ</td>
<td>15.35 ±4.52ᵃ</td>
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<td>16.97 ±4.52ᵃ</td>
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Different lower case superscript letters indicate difference between groups for which P<0.05.
Different upper case superscript letters indicate difference across time for which P<0.05.
Table 6 – Incidence (mean ±SD) of four different behaviours (head-shake, ear-flick, head-rub and transitions from standing to lying) for calves disbudded with hot-iron in Experiment 2. Observational period: 15 min. Treatment groups: DA3 – calves disbudded after treatment with lidocaine; ND3 – calves sham-disbudded.

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<th>Time from dehorning</th>
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<tr>
<td>DA3</td>
<td>8</td>
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<tr>
<td>ND3</td>
<td>5</td>
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Different lower case superscript letters indicate difference between groups for which P<0.05.

Fig. 1 – Incidence of transitions shown by hot-iron disbudded calves after local anaesthesia (DA), local anaesthesia plus carprofen (DAC), no treatment (D) or sham-disbudded (ND). For all comparisons p>0.05.

Fig. 2 – Incidence of head shakes shown by hot-iron disbudded calves after local anaesthesia (DA), local anaesthesia plus carprofen (DAC), no treatment (D) or sham-disbudded (ND). Different upper case letters indicate differences (p<0.05).
Fig. 3 – Incidence of ear flicks shown by calves’ hot-iron disbudded after local anaesthesia (DA), local anaesthesia plus carprofen (DAC), no treatment (D) or sham-disbudded (ND). Different upper case letters indicate differences $p<0.05$.

Fig. 4 – Incidence of head rubs shown by calves’ hot-iron disbudded after local anaesthesia (DA), local anaesthesia plus carprofen (DAC), no treatment (D) or sham-disbudded (ND). Different upper case letters indicate differences $p<0.05$. 
Fig. 5 – Total behaviours shown by hot-iron disbudded calves after local anaesthesia (DA), local anaesthesia plus carprofen (DAC), no treatment (D) or sham-disbudded (ND).

Different upper case letters indicate differences at each time and different lower case letters indicate differences across time (p<0.05).