Behavioral responses of juvenile Steller sea lions to hot-iron branding

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ABSTRACT

Wildlife research frequently requires individual marking, such as hot-iron branding, however procedures used may alter an animal’s behaviors and cause pain. To date, studies have assessed the immediate responses to hot-iron branding, however no study has assessed how hot-iron branding affects the behavior of animals in the days following branding. Here we present the first data showing the post-branding behavioral responses in a marine mammal. Eleven captive juvenile Steller sea lions (*Eumetopias jubatus*) were observed for 3 days before and 3 days after branding. Four of six monitored behaviors changed after branding. The proportion of time sea lions spent in locomotion decreased from 0.07 (+0.02) before branding to 0.03 (+0.01) during the first 24 h following branding (back-transformed mean + SE) and returned to baseline during the second 24 h period. Wound-directed behavior (scratching, biting and head rubbing the branded area) increased from 0.0 (+0.0) before branding to 0.01-0.02 (+0.01) during the first 48 h after branding and returned to baseline thereafter. Time in the pool declined from 0.17 (+0.06) before branding to 0.05 (+0.04) during the first 24 h after branding and returned to baseline by the second 24 h period. The time spent with pressure on the branded side showed little change from the 0.08 (+0.03) before branding to 0.10 (+0.03) during the first 24 h after branding; however, this behavior decreased to 0.02 (+0.02) and 0.01 (+0.01) on following two days. These results show that hot-iron branding affects Steller sea lion behavior up to 72 h. Changes in these behaviours may be useful in assessing alternative effective analgesic protocols for this procedure.

Keywords: Behavior, *Eumetopias jubatus*, Hot-iron branding, Pain, Steller sea lions
INTRODUCTION

Animal conservation research often requires that individuals are marked and can thus be followed over an extended period. One marking technique for marine mammals is hot-iron branding. Brands provide researchers with a permanent and unique mark that can be used to identify individual animals throughout their life [19]. Hot-iron branding has been used to identify cattle and horses for centuries [14], and has been adapted for use in pinnipeds and non-domesticated ungulates [19, 23].

Changes in physical appearance and behavior can be used as reliable indicators of pain [22]. The immediate pain responses to hot-iron and freeze branding have been studied in cattle, with hot-iron branding causing increased pain-related behaviors including tail flicking, kicking, and vocalizations [26, 27] and greater escape-avoidance responses at the time of branding [11]. However, no published research has assessed an animal’s behavioral responses in the days following hot-iron branding.

Hot-iron branding of marine mammals has been criticized, resulting in a one-year suspension of hot-iron branding of Steller sea lions in the USA [3] and an indefinite suspension for elephant seals at Macquarie Island and Hooker’s sea lions in New Zealand [2, 7, 16]. Despite this concern, only four studies have been published that have examined the effects of branding in pinnipeds, and all four studies focused only on physiological and survival effects. Steller sea lions (Eumetopias jubatus) typically experience a general inflammatory response for up to two weeks after branding [18]. In harbor seal (Phoca vitulina) pups, 76% of hot-iron brands were found to have not healed at 9-10 weeks post-branding [4]. In Southern elephant seal (Mirounga leonina) pups most brands healed within one year, but brands with more peripheral skin damage
had longer healing times [30]. Longer-term monitoring has found no difference in survival between hot-iron branded and flipper-tagged Southern elephant seals [15].

The aim of our study was to describe the post-operative behavioral pain responses of juvenile Steller sea lions following hot-iron branding. This research was conducted on sea lions that were captured, brought into temporary captivity and underwent hot-iron branding, all as a part of different research project [17]. Permitting constraints did not allow for experimental treatment groups (e.g. with and without analgesics). We tested the hypotheses that: 1) the behavioral responses measured before branding would differ from the 3 days following branding, and 2) these differences would be most pronounced in the first 24 h period after branding.

Specifically, we hypothesized that animals would increase wound-directed behaviors, such as rubbing and scratching the brand, and spend less time lying on the branded side.

METHODS
Study design and animals
Research was conducted in collaboration with the Alaska SeaLife Center (ASLC) in Seward, AK, USA. All behavioral research conducted in this study was coordinated with the ASLC’s Transient Juvenile Steller Sea Lion Project [17] where an active hot-iron branding program is already in place.

Eleven juvenile Steller sea lions, between 16 and 23 months of age, were captured in Prince William Sound, AK, USA, in August 2007 (three males), February 2008 (one male and one female) and August 2008 (four males and two females) under the Transient Juvenile Steller Sea Lion Project, as described by [17]. Upon initial handling, symbols were shaved in the animal’s fur on their dorsal side to facilitate identification prior to hot-iron branding. The animals were transported to the ASLC and held for up to 12 weeks prior to release.
The quarantine facility where the animals were held consisted of four adjoining pools (1 m x 4 m diameter and 3 m x 5 m diameter, 1.5 m deep), with unfiltered seawater from the directly adjacent Resurrection Bay. Each pool was enclosed by 122 m² of metal surface haul-out area. Animals could be housed individually or share access to multiple pools via sliding gates, however animals were housed together to the maximum extent possible.

All hot-iron branding was performed for the ASLC’s Transient Juvenile study, with no additional handling required for our behavioral observations. No animals were branded simply for the purposes of the current study. All research was conducted under Institutional Animal Care and Use Committee protocols AUP07-009 (ASLC), A07-0342 (UBC), 08-26 (UAF) and approved under NMFS permit #881-1890.

**Study procedures**

Sea lions were masked with 5% isoflurane in 100% oxygen for induction of anesthesia, after which all animals were intubated. Anesthesia was maintained with 1% to 3% isoflurane in 100% oxygen delivered via a semiclosed, partially rebreathing circuit.

Hot-iron brand marks consisted of a combination of four numerals. Brands were applied with specially designed stainless steel irons (each 10.2 cm high and 5.1 cm wide) that were heated in a propane-fueled forge until the irons were cherry red. Each branding iron then was applied to the left shoulder/flank area for 2 to 7 s each, as described by [18], with touch-ups to each numeral lasting for 1 to 4 s each. Anesthesia ended when the branding was complete. The average duration of anesthesia was 108.8 ± 16.4 min (mean ± SE).

**Behavioral observations**

All behaviors were monitored by live focal animal observations for a total of 6 days: 3 days before branding and 3 days immediately following branding. Days were calculated as 24 h
periods, with Day 0 starting immediately following extubation from anesthesia. With the exception of the day of branding, sampling occurred on all animals six times a day in 10 min periods, twice during each period of the day (morning, 0900-1100 hours; afternoon, 1300-1500 hours; and evening, 1700-1900 hours). Behaviors were recorded using point-in-time sampling (one scan every min for 10 min). On the day of branding, focal animals were observed after recovering from anesthesia, which was 1.5 h after extubation from anesthesia (based on [9]). During this initial observational period, sea lions were monitored continuously with behaviors recorded every min for 1 h. After the first hour of observation, 10 min observations resumed.

All observations were by a trained observer was hidden from the sea lions’ view behind a plastic blind or via one-way glass depending upon the location of the focal animals. Reliability of this observer’s scores was estimated by comparing scores with another trained observer for 196 scans on six animals. The two observers showed 97% agreement for time spent alert and complete agreement for all other behaviors scored.

We selected six behaviors related to post-operative pain using the following a priori rationale: (a) Wound-directed behaviors: based on work in other species that have assessed behavioural changes in the days following painful procedures (e.g. [20]), we predicted that sea lions would increase wound-directed grooming and decrease the time spent with pressure on the branded area during periods of lying and sitting; (b) Time spent in daily activities on land or water: animals have been known to increase or decrease activities after painful procedures depending on the use of the wounded area in performing that behavior. Based on work in other species (e.g. [6]), we anticipated a reduction in time spent in locomotion and time spent in the pool; (c) Time spent alert and attentive: animals exposed to noxious or painful stimuli increase attentiveness or time spent alert [25]. Therefore, we predicted that sea lions would increase time
alert after hot-iron branding; and (d) Time spent lying down: based on work in other species after painful procedures [10], we predicted that sea lions would decrease time lying after branding.

Mutually exclusive behaviors included locomotion, wound-directed grooming and lying time. Locomotion was scored when the sea lion moved on land (i.e. all four limbs are moved at least one body length either by walking on four flippers or by sliding its body across land with or without its full belly on the surface) or in water (i.e. swimming - actively propelling itself through the water at least one body length). Wound-directed grooming behaviors included scratching, biting and head rubbing the branded area. Sea lions scratched themselves using the front or rear flippers to scrape at their skin. Biting involved the use of teeth to grip or hold the left side branded area, typically in a fast repetitive motion. Head rubbing involved moving the head back and forth with pressure on skin surface. Sea lions were classified as lying down when their bodies were flat on the ground, including dorsal, ventral, and left and right side positioning; the head could be either lifted up or on the ground.

Non-mutually exclusive behaviors included time spent in the pool, time on the left branded side and the time spent alert. Time spent in the pool was calculated from activities that occur in the water (i.e. locomotion, floating and foraging). Time spent on the left branded side was scored when an animal was lying or sitting on land with the majority of their body weight and pressure on the left side of their body. Time spent alert was scored when an animal was attentive with both eyes open.

To determine sample size required, power calculations were computed using preliminary results from August 2007 data (Piface software Version 1.63). The analysis indicated that a sample size of between five and 10 individuals would be required to accurately identify the behavioral effects of hot-iron branding. Additionally, the sampling method used has been
Statistical analysis

The proportion of time sea lions spent displaying each behavior was averaged across the 3 days before branding (nominally Pre-brand) to generate one baseline measure per animal. For each of the 3 days following surgery (nominally Day 0, Day 1 and Day 2), one measure per animal was calculated to give the proportion of time each animal spent in each of the six behaviors. Proportional data were outside the range of 0.3 to 0.7; therefore, to condense the distribution and to allow for use in the parametric analyses, all data were arcsine square root transformed ($Y = \text{arcsine} \sqrt{p}$). Using a mixed model (SAS v9.1), with a compound symmetry covariance structure, an analysis was conducted to test the effects of hot-iron branding on the six behaviors. The model included the effect of day (Pre-brand, Day 0, Day 1 and Day 2). The residuals from the models were tested against the basic assumptions of normality and variance homogeneity, as well as plotted against the predicted values for the model. Three specified contrasts were run to compare Pre-brand with each of the 3 days following hot-iron branding (i.e. Pre-brand vs. Day 0, Pre-brand vs. Day 1 and Pre-brand vs. Day 2). In all cases, differences were considered to be significant at $p \leq 0.05$. For the simplicity of interpretation, data in Fig. 1 and Fig. 2 are presented back-transformed means with the positive SE.

RESULTS

Of the six behaviors measured, four changed after hot-iron branding; locomotion, wound-directed grooming behaviors, time in the pool and time spent with pressure on the left (branded) side.
The three mutually exclusive behaviors (i.e., locomotion, grooming the branded area and lying time) are illustrated in Fig. 1. Time in locomotion on land and in water decreased from 7% in the Pre-brand period to 4% in Day 0 \( (F_{1,30} = 4.40, p = 0.044) \), and returned to baseline by Day 1. Sea lions were rarely witnessed scratching, biting or rubbing their left side (area to be branded) in the Pre-brand period. However in the days following hot-iron branding wound-directed grooming increased, with sea lions occupying approximately 2% of their day on Day 0 and 1 after branding \( (F_{1,30} = 10.02, p = 0.004 \) and \( F_{1,30} = 5.71, p = 0.023 \), respectively) grooming their left (branded) side. Time spent grooming the branded area returned to Pre-brand levels by Day 2. For comparison, time spent grooming the right side showed no differences on Day 0, 1 or 2 when compared with the Pre-brand period \( (F_{1,30} = 0.47, p = 0.50, F_{1,30} = 0.18, p = 0.67 \) and \( F_{1,30} = 0.10, p = 0.76 \), respectively). Similarly, grooming other areas (e.g. head, rump) did not increase in Day 0, 1 or 2 when compared with the Pre-brand period \( (F_{1,30} = 0.48, p = 0.50, F_{1,30} = 0.57, p = 0.46 \) and \( F_{1,30} = 1.82, p = 0.19 \), respectively). No differences were found in lying time.

The three non-mutually exclusive behaviors (i.e. time in the pool, time spent with pressure on the left branded and time alert) are illustrated in Fig. 2. Prior to branding, sea lions spent 17% of their time in the pool. Time in the pool declined to 5% on Day 0 \( (F_{1,30} = 5.69, p = 0.024) \), but returned to Pre-brand levels on Days 1 and 2. The time sea lions spent with pressure on their left (branded) side showed little change from Pre-brand to Day 0 (N.S.), but decreased to near zero on Day 1 and 2 \( (F_{1,30} = 4.28, p = 0.047 \) and \( F_{1,30} = 7.01, p = 0.013 \), respectively). No differences were found in the time spent alert.

**DISCUSSION**

In the three days following hot-iron branding sea lions increased wound-directed grooming and spent less time with pressure on their left branded side. Increased grooming
included head rubbing and scratching the branded area, similar to the wound-directed behaviors witnessed after tail docking and dehorning in calves [5, 29, 31], and in decapods after exposure to antenna-directed noxious stimuli [1].

Time spent with pressure on the branded side changed in the days following branding. The deck surface was metal, so the increased time on the branded side on Day 0 might have provided a cooling effect for the burn wound. The subsequent reduction in time on the branded side on Days 1 and 2 may be due to increased sensitivity to pain, or hyperalgesia associated with inflammation in the days after injury [13]. Burn injuries in animals that are not treated can lead to sensitivity to previously innocuous stimuli (i.e. allodynia; [24]).

The time sea lions spent in activities on land and water decreased following hot-iron branding. Specifically, sea lions decreased the time they spent in the pool and engaged in locomotion during the first 24 h after branding; both behaviors returned to Pre-brand levels by 48 h after branding. Pain is also known to affect locomotion activity [6, 21]; decreased time in the pool may also be associated with a sensitivity of the brand to salt water. Locomotion is often decreased in human burn victims if the burn location is in an area that is highly mobile or stretched during locomotion [8]. Sea lion recovering from abdominal surgery also showed reduced locomotion for 3 days following surgery [32].

Comparing changes in behaviors before and immediately after hot-iron branding is a first step in understanding pain responses. However, future studies should include branded and unbranded animals, as well as analgesia and anaesthetic control groups (with no hot-iron branding; [33]). Analgesia could involve pre-emptive administration of a local nerve block, the use of oral or intra-muscular administration of analgesics, and the use of local anaesthetic gels and sprays applied after branding. For example, administering a local anaesthetic to calves was
shown to reduce behavioral and physiological responses after dehorning [28]. Such studies should also include an assessment of the logistics of the procedure for field use (where branding typically occurs) where follow-up may be impossible.

Alternative methods of marking sea lions also warrant further investigation. It has been suggested that cold-iron branding may cause less pain. While hot-iron brands burn through the dermal layers and disrupt the hair follicles preventing new hair growth, cold branding damages the pigment-producing melanocytes but leaves the hair follicles intact allowing for regenerative growth of white hair [4, 23]. Studies on cattle show that cold-iron branding causes less of a pain response than hot-iron branding [11, 12, 26]. For some pinniped species, however, it has been shown that while cold-iron brands may heal faster, hot-iron brands are longer lasting and more legible [4, 15].

In summary, in the three days following hot-iron branding Steller sea lions spent more time grooming the branded area, less time with pressure on their left branded side, less time in the pool and less time in locomotion. These behavioral responses may be useful in monitoring pain following similar procedures in sea lions and other marine mammals and in developing alternative pain management strategies for this procedure.

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References


Figure 1. Mutually exclusive behaviors: back-transformed means (+ SE) for the proportion of time sea lions spent (a) in locomotion, (b) grooming the branded area and (c) lying down, before and after hot-iron branding. Time series data are shown as Pre-brand (mean of 3 d before branding), Day 0 (1st 24 h period after branding), Day 1 (2nd 24 h period after branding) and Day 2 (3rd 24 h period after branding). Significant differences for specified contrasts (Pre-brand vs. Day 0, Day 1 and Day 2) are denoted by: * \( (p \leq 0.05) \) and ** \( (p \leq 0.01) \).

Figure 2. Non-mutually exclusive behaviors: back-transformed means (+ SE) for the proportion of time sea lions spent (a) in the pool, (b) with pressure on their left (branded) side and (c) alert, before and after hot-iron branding. Time series data are shown as Pre-brand (mean of 3 d before branding), Day 0 (1st 24 h period after branding), Day 1 (2nd 24 h period after branding) and Day 2 (3rd 24 h period after branding). Significant differences for specified contrasts (Pre-brand vs. Day 0, Day 1 and Day 2) are denoted by: * \( (p \leq 0.05) \) and ** \( (p \leq 0.01) \).