Published in final edited form as:

Appl Anim Behav Sci. 2014 March 01; 152: 83–91. doi:10.1016/j.applanim.2013.12.011.

Pain-Suppressed Behaviors in the Red-tailed Hawk (*Buteo jamaicensis*)

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Abstract

Our ability to provide analgesia in wild and exotic patients is hampered by a lack of species-specific information on effective drugs and protocols. One contributing factor is the difficulty of applying data from traditional laboratory tests of nociception to clinical conditions frequently involving combinations of inflammatory, mechanical, and neuropathic pain. Pain-suppressed behaviors have become a valuable predictor of clinical utility in other species; in this study we extend this framework to red -tailed hawks in a wildlife hospital, in an attempt to develop a new, humane testing method for birds of prey. We scored six behaviors in hawks hospitalized either for orthopedic trauma or for non-painful conditions. These behaviors included: movement about the cage, grooming, head motions, foot shifts, beak clacks, and rouse. Movement, head motions, and beak clacks were all significantly reduced in hawks with recent orthopedic injury, but not in hawks with healed or minor injuries ($P < 0.05$ for all behaviors). However, it should be noted that due to stringent admission criteria, and the difficulties inherent in studying naturally-occurring injury in wild patients, this study only included -subjects in four experimental groups, and this limited our ability to fully investigate confounds within our data. A follow-up experiment was conducted to determine potential effects of buprenorphine, a μ opioid agonist, on the behaviors listed above. Buprenorphine in the absence of pain caused minor, non-significant decreases in most behaviors, and had no effect on head movement frequency. This suggests that head movements in particular may be sensitive to pain but not to sedative side-effects of buprenorphine. Overall, red -tailed hawks with recent orthopedic trauma show consistent and marked reductions in several normal maintenance behaviors. Head movements, reported for the first time in this study as a potential marker of pain in birds, in particular seem to be insensitive to sedative side effects of buprenorphine, while being a sensitive measure of affective state in hawks with painful injuries.

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These behaviors can be scored humanely and with minimal expense, and should be considered for further research on pain and analgesia in avian species.

Keywords

red-tailed hawk; pain-suppressed behaviors; pain; head movements; opioids; *buteo jamaicensis*

1. Introduction

Effective analgesia is a vital component of humane care, as well as a significant contributor to recovery for hospitalized patients. Inadequately treated pain increases circulating cortisol, slows healing, and decreases immune function (Grondahl-Nielsen et al., 1999; Hay et al., 2003; Stilwell et al., 2009). Despite its importance, clinical research on pain management in wildlife has been complicated by difficulties in access to study subjects, the high levels of variation in physiology and neuroendocrine responses, and the variability with which any given species may express or adapt to pain.

Behavioral assessment of pain has increased in popularity in both veterinary and pre-clinical research. Broadly, the types of behaviors assessed fall into two categories: pain-evoked (i.e., behaviors that are increased in response to pain), and pain-suppressed (i.e., behaviors that are decreased in response to pain) (Negus et al., 2006). Pain-evoked behaviors could include vocalizations, writhing, or postural changes (Paul-Murphy et al., 2009a, 2009b; Roughan and Flecknell 2001; Wright-Williams et al., 2007). Pain-suppressed behaviors could include decreases in locomotor activity (Stevenson et al., 2009) or appetite (Stevenson et al., 2006).

In general, pain-evoked behaviors have been more traditional components of pre-clinical research; these behaviors are often easier to recognize and may be more pain-specific than decreases in appetite or locomotion (Negus et al., 2006). In veterinary practice, most commonly recognized signs of pain tend to be of suppression rather than evocation. An owner might report a decrease in activity, appetite, or social interaction; or a laboratory mouse might display decreased nest-building. Spontaneous pain-evoked behaviors are more rarely described and tend to be fleeting; an animal might vocalize when putting weight on an injured limb, but response is unlikely to be sustained. This lack of obvious, consistent, pain-evoked behaviors has made it more difficult to recognize and treat pain even in familiar species; in wild birds, the challenges are greater.

Comparatively little work has been done in most bird species, and what research exists has mostly used short-term, or phasic, nociceptive stimuli, and measured spinal withdrawal reflexes (Hoppes et al., 2003; Klaphake et al., 2006; Paul-Murphy et al., 1999; Sladky et al., 2006). The relevance of these types of tests to clinical pain is unclear (Le Bars et al., 2001); phasic stimuli are carried on different nerve fibers than sustained (tonic) pain, and the measured differences in withdrawal cannot be clearly attributed to effective analgesia as opposed to slowed motor function (Negus et al., 2006). A few recent studies have investigated butorphanol in captive psittacines using tonic inflammatory injury, and measuring pain-altered behaviors (Paul-Murphy et al., 2009a, 2009b). A similar

investigation compared anti-inflammatories and their effects on pain- altered behavior in chickens (Hocking et al., 2005). There is no comparable research in raptors.

Red-tailed hawks (*Buteo jamaicensis*) are ubiquitous throughout North America, are commonly kept in captivity for falconry and educational programs, and typically have a calmer demeanor than other raptors. Thus, data on better care for this species are likely to be of use in a variety of settings (zoos, wildlife hospitals, etc.), and they may be a good model species in which to begin investigation of pain and behavior in raptor s. Our project scored six behaviors: movement around the cage, grooming, head movements, foot shifts, beak clacks, and rouse (a brief episode of piloerection with a shake of feathers and resettling, generally seen in birds in relaxed settings and often associated with grooming). These behaviors were scored both shortly after admission, and several weeks later, in hawks with orthopedic injury and hawks admitted for non -traumatic conditions, in order to assess potential effects of hospitalization, injury, pain, and overall recovery. Our hypothesis was that all behaviors would be reduced in hawks with recent trauma, with the exception of grooming at injured areas, which we predicted to be increased.

Because this project operated within the ethical confines of clinical practice, all injured study subjects received buprenorphine (0.25 mg/ kg subcutaneously, twice daily) for pain relief. Opioids can have behavioral side-effects independent of their effect on pain. To determine whether buprenorphine could cause the behavioral depression seen in our trauma patients, a follow-up experiment (randomized and investigator blinded) was conducted using hawks with healed traumatic injury. Our hypothesis was that hawks administered buprenorphine and hawks administered saline (control) would not show different behavioral frequencies.

2. Materials and Methods

2.1 Experiment 1: Trauma-Suppressed Behaviors

2.1.1 Admission criteria and demographic information—Hawks were enrolled in the study if their presenting complaints included either fractures of one or more bones (Trauma groups) or were limited to non -painful conditions (Control groups). Birds were not enrolled in the study if there was concomitant head trauma, any likelihood of systemic infection (for example, West Nile Virus), anemia, rodenticide toxicity, or other clinical conditions judged likely to contribute to behavioral alterations for reasons other than pain. Some examples of presenting complaints for the control birds were: pine sap on wings; confiscated from inappropriate housing, but otherwise healthy; minor scrapes after entanglement in nets. Presenting complaints and initial health conditions were not uniform within groups, given the natural variation present in wildlife patients. However, every effort was made to standardize basic health and welfare. For each study subject, age was determined by plumage and eye color, and recorded as either hatch year, juvenile (1 to 2 y old), or adult (3 y or older).

2.1.2 Experimental Groups—In order to compare behaviors across both presence or absence of injury, and potential acclimation to hospital conditions, both Trauma and Control subjects were recorded at similar time points. These were the “A point” - collected 24–48 h

after admission; and “B point” - collected the day before birds were moved to outdoor cages to begin the reconditioning process. Thus this portion of the study includes four groups:

Trauma A (n = 5): birds with orthopedic trauma, whose trauma was judged by radiographic and physical examination to have occurred less than 4 d before recording. These recordings were taken 24–48 h after admission to Tufts Wildlife Clinic (TWC).

Trauma B (n = 6): birds with orthopedic trauma that had healed sufficiently to be placed in larger cages (as judged by radiographic and physical examination). These recordings were taken 14.3 ± 2.6 d after admission.

Control A (n = 5): birds admitted for non-traumatic causes, recorded 24–48 h after admission.

Control B (n = 3): birds admitted for non-traumatic causes, recorded 13.7 ± 5.7 d after admission.

A delay of 24–48 h after admission, before collection of “A point” data, was deemed advisable for two reasons. First, it reduced stress on study subjects, since they are extensively handled for physical examination and treatment on the day of admission. Second, it allowed for correction of non-pain-related metabolic issues such as dehydration or partial starvation that might contribute to behavioral alterations.

Four hawks presented to TWC with fractures already partially healed, and were included in Trauma B but not in Trauma A. Similarly, three hawks in Trauma A could not be recorded a second time due to clinical or logistical conflicts, and were not included in Trauma B. Control groups were subject to similar logistical issues. All statistics were performed without assumption of paired subjects (see Section 2.1.5, Statistical Methods).

2.1.3 Recording conditions—Seven recording sessions from the earliest part of the study were performed in clinic cages. These included three sessions in “black box” cages (measuring approximately 0.5 m × 0.5 m), with very low levels of ambient light (one Control A and two Trauma A sessions). It also included four sessions in indoor pens measuring 0.6 m × 1.4 m (one Control B and three Trauma B sessions).

All other sessions were recorded in a standardized testing crate, similar in size and ambient light level to the indoor pens. This consisted of a large dog kennel (0.7 m × 0.6 m × 1 m), adapted by drilling out holes to increase ambient light and provide placement for cameras. Birds were placed in the test crate, allowed to acclimate for 20–30 min, then recorded for 2 to 3 h.

All sessions were recorded using a set of four cameras placed at different angles and locations in each enclosure. One camera was a full-light fisheye lens, allowing the greatest visibility; the other three were low/ no-light capable lenses with infrared light sources. This ensured visibility in a variety of light conditions and in the entire cage. All sessions analyzed in this study were recorded between 10:00 h and 18:00 h (hours of full daylight).

2.1.4 Scoring procedure—Scoring was performed using JWatcher 1.0, an ethological analysis program (keystroke logger) developed by the University of California – Los Angeles and Macquarie University. Three behaviors were scored as “states” (having a measurable duration which allowed for calculation of time spent in each state, and considered mutually exclusive). These behaviors were: perching, grooming, and movement around the cage.

In addition, four behaviors were scored as “events”. These behaviors occurred regularly and in discrete bouts, but for very short periods of time, making it more accurate to count the number of bouts than to measure the time involved in each bout. These behaviors were scored only while the animal was perching, to reduce double-counting and overlap between behavioral categories. Behaviors scored as events included: head movements, foot shifts, beak clacks, and rouse. Head movements were defined as discrete, sharp motions of the head without movement of the body, and ranged from approximately 30°–180° in each movement. Foot shifts were changes of stance, either involving lifting a foot and replacing it, or leaning from one side to the other, without a change in location. Beak clacks were defined as a short bout of rapidly opening and closing the beak. Rouse was a behavior involving full-body piloerection with a short shake and resettlement of feathers.

To determine adequate scoring frequency, the first 10 sessions were scored in continuous 10 min segments for their entire length, with the results of each segment averaged to create a final score for each behavior for the entire session (referred to as long-form). Scores averaged from every other segment, and every third segment, were also calculated (referred to as short-form). Short-form results were almost perfectly correlated with the long-form results (r of 0.98 and 0.96, respectively). This indicated that the reduced sampling frequency would not significantly reduce the accuracy of the scores for each behavior. Given this, only short-form scoring was performed on the later recordings, scoring every other 10 min segment, with a minimum of six segments analyzed per session.

“State” behavior (grooming, movement) is reported in s per 10 min segment. Since the time spent perching varied between observation periods, “event” behaviors (head movements, foot shifts, beak clacks, rouse) are reported as events per min spent perching.

2.1.5 Statistical Methods—For an initial analysis, we used single-factor ANOVAs to compare scores for each behavior across the four experimental groups. The assumption of normal distribution was tested using the Shapiro-Wilk test with alpha set at $P < 0.05$. The scores did not show homogeneity of variance when tested using Levene’s statistic with alpha set at $P < 0.05$, and were square root transformed to correct this, yielding $P > 0.10$ for all behaviors. If an ANOVA revealed significant ($P < 0.05$) differences between groups, post-hoc analysis was conducted using the Tukey HSD test. This analysis was carried out in SPSS.

A second analysis was carried out to investigate possible effect of cage type independent of experimental group. For this, we used general estimating equations (GEE) models incorporating the behavioral frequencies within each 10 min segment (rather than a final averaged score). This increased the power of the test even within the relatively small sample sizes, while adjusting for correlation due to repeated measures on individual hawks. In

addition, this adjusted for the clustering of measurements within cage type as well as within hawk. GEE models are robust to non-normal data. All analyses were performed with SAS Version 10.

2.2. Buprenorphine trial

Five hawks with healed fractures received, in separate sessions, either buprenorphine at 0.25 mg/ kg, or an equivalent volume of 0.9% saline. The order of injections was randomized, and the investigator blinded as to the type of injection given. Given the small group size the order could not be fully balanced; three subjects received saline first and two received buprenorphine first. To minimize disruption of clinic operations, injections were given 24 h apart. This was judged to be an adequate washout period given the rapid elimination of systemic buprenorphine in birds (Paul-Murphy et al., 2004).

Behavioral scoring was conducted using the same procedure as described in Section 2.1, with two alterations. First, each recording session lasted 4 h rather than 2 h, to maximize the likelihood of detecting behavioral changes due to buprenorphine (Paul-Murphy et al., 2004). Second, scoring only occurred via short-form sampling, and eight 10 min periods were scored in each session. Since all scoring was performed in test crates, analysis via GEE was not required. Values for each behavior in each 10 min segment were averaged. The resulting values were normally distributed as indicated by the Shapiro-Wilk test. Averaged scores for each behavior were compared using paired t - tests.

2.3 Ethical approval

This project was approved by the Tufts Cummings School of Veterinary Medicine (TCSVM) IACUC (protocol #G2010-95). Care and handling were performed as per standard hospital procedures; any experimental procedure deemed by clinic staff to be potentially detrimental to subjects' recovery was discontinued immediately. All work was conducted in accordance with AABS standards of ethical animal use.

3. Results

3.1. Overview

Fifteen red-tailed hawks were enrolled in this study. As described in section 2.1.2, there was partial overlap of study subjects between groups. In total, two hawks were included in both Trauma A and Trauma B; and two hawks in both Control A and Control B. Thus a total of 19 recording sessions were included in the final analysis. For Trauma A and B subjects, fractures were of the humerus (n=3), radius and ulna (n=2), femur (n=2), or coracoid (n=2). The total number of recording sessions for each group, as well as estimated sex and age of study subjects, is presented in Table 1.

One Control A recording was excluded from analysis for head movements and beak clacks because camera placement made it difficult to count these accurately, but other behaviors were still clearly visible. The femur fracture patient in Trauma A was not included in analysis of movement or foot shifts.

3.2 Grooming and movement

Total amount of time spent grooming was highly variable in both trauma and control groups. Mean groom time for Trauma A was 43.7 ± 44.3 s/ 600 s; for Trauma B, 64.3 ± 45.5 ; for Control A, 50.7 ± 59.1 ; and for Control B, 66.2 ± 46.1 . The ANOVA on square-root transformed data [$F(3, 14) = 0.391$; $P = 0.761$] indicated no significant difference between groups.

Movement about the cage was significantly different between groups: $F(3, 14) = 8.461$, $P = 0.002$. Trauma A birds spent significantly less time moving around the cage than subjects in Trauma B, Control A or Control B groups ($P = 0.04$, 0.002 and 0.006 , respectively). Control groups were not significantly different from each other or from Trauma B. The transformed data are shown in Fig. 1. GEE results indicated that, after controlling for injury, hawks in test crates were not significantly different than hawks in black box cages ($P = 0.512$), but that hawks in pens were significantly *less* active than those in black box cages ($P = 0.004$). For reference, the non-transformed scores are reported in Table 2.

3.3 Head movements, beak clacks, foot shifts, and rouse

Frequencies of head movements and beak clacks were significantly different between groups: ($F(3, 14) = 13.9$, $P < 0.0005$; and $F(3, 14) = 7.20$, $P = 0.004$, respectively). Post-hoc analysis on head movement frequency indicated Trauma A was significantly lower than all other groups ($P < 0.001$ in all cases). Beak clacks occurred less frequently overall, but showed the same pattern, with Trauma A significantly less than all other groups ($P < 0.02$ in all cases). Trauma B and Control groups were not different for either behavior. GEE analysis indicated that, after controlling for injury, hawks in test crates and hawks in black box cages were not significantly different for head movements ($P = 0.186$) or beak clacks ($P = 0.100$); hawks in pens had lower scores for head movements than in black box cages ($P = 0.043$) but were not different for beak clacks ($P = 0.249$).

Foot shifts were also significantly different between groups [$F(3, 14) = 3.735$, $P = 0.037$]. Foot shifts occurred significantly less frequently in Trauma A than in Control B ($P = 0.03$). However, other groups were not significantly different. GEE analysis indicated that, after controlling for injury, hawks in test crates, pens, and black boxes were not significantly different ($P = 0.478$ and $P = 0.056$, respectively).

Rouse behavior occurred at a very low frequency, and differences between groups were not statistically significant ($F(3, 14) = 0.67$, $P = 0.584$). Results for rouse behavior, as well as head movements, foot shifts, and beak clacks are all shown in Fig. 2. Non-transformed scores for head movements, beak clacks, foot shifts, and rouse are all reported in Table 2.

3.4 Buprenorphine Trial

No significant changes were detected in any behavior following buprenorphine administration. The mean scores for each behavior, as well as P values ($n = 5$, $df = 4$ for all tests), are reported in Table 3. For comparison, corresponding mean scores for each behavior from Trauma A (which received buprenorphine for analgesia) and Control A (the most comparable non-buprenorphine treated group) are also reported.

4. Discussion

This study is, to the authors' knowledge, the first attempt to elucidate behavioral changes in captive birds with recent trauma, or in hawks given buprenorphine after recovery from injury. Overall, it appears that orthopedic trauma and its clinical sequelae are correlated with a marked decrease in normal maintenance behaviors. Buprenorphine administration may have contributed, in part, to this depression, but is unlikely to have caused the level of decrease we observed, and appears to have had no effect on the frequency of head motions in either injured or healed birds. Taken together, these findings suggest that measurement of pain-suppressed behaviors may be useful for assessing pain and analgesic response in avian species.

Though we initially hypothesized that grooming would be increased in injured birds, the design of this study was not ideal for assessing this behavior. Grooming in general was highly variable between individuals. Grooming at particular areas might be a more fruitful subject for investigation, but it was unexpectedly difficult to determine this accurately. Feathers frequently obscured visibility, and our subjects had a variety of injuries, making direct comparisons difficult.

The drastic decrease in movement about the cage in the Trauma A birds is consistent with other studies on pain-suppressed behavior and supports our initial hypotheses (Roughan and Flecknell 2001; Stevenson et al., 2009; Weaver et al., 2010). Not only is movement of injured body parts painful in and of itself, but also, any movement has the potential to increase basal nociceptive sensitivity (Apkarian et al., 2005). Thus, a bird with a wing fracture may be reluctant to move its feet, even if it should be physically capable of doing so. This may also have contributed to the consistent decrease in other behaviors, especially head movements, which occurred even with injuries far away from the head or neck. Also, injured hawks may be reluctant to move and thereby display their injury to potential predators.

Head movement frequency was the single most reliable and useful measure to emerge from this project. Interestingly, the frequencies we found in Control A, Control B, and Trauma B subjects are nearly identical to those reported by O'Rourke et al. (2010), who scored head movements in RTHA in field and captive conditions. The similarity suggests that head movement frequency in healthy hawks alert to their surroundings may be generally consistent across a range of environments. Also, the results of the buprenorphine trial also demonstrate that head movements are insensitive to potential sedative side-effects of buprenorphine. To our knowledge, this is the first time that head movement frequency has been used as a measure of pain or affective state in birds. On a practical level, this behavior may also be the easiest to adapt to further projects as it occurs so much more frequently than any other behavior assessed here, and thus is more likely to be accurately measured in shorter time periods.

Beak clacks were likewise useful discriminators between experimental groups, unlike rouse behavior, which occurred very infrequently. That foot shifts followed a more complex pattern, occurring in Trauma B hawks at a frequency intermediate between Trauma A and

control values, may be an effect of the sample size of this study. It might also indicate that this behavior is slower or less likely to return to baseline values than head movements or beak clacks.

Overall, the limited sample sizes in each experimental group, as well as the different cage types used, do impose limitations on our ability to draw final conclusions from this study. GEE analysis does not show a clear pattern for an effect of cage type on behaviors, but it does not support the interpretation that the drastically decreased activity seen in Trauma A hawks was due to cage type alone, as it estimated that, after controlling for the effects of trauma, head movements and movement about the cage were actually increased in the black box cages. Our sample size did not permit analysis of the interaction of injury, time in hospital, or cage type; these need to be investigated in greater detail in further work.

Another factor that should be addressed in future work is the effect of stress on pain, or vice versa. In general, circulating and excreted corticosteroids (a common measure of stress) are increased in wild animals after introduction to captivity, regardless of injury status (Dickens et al., 2009; Gregory et al., 1996; Morgan and Tromborg, 2007). However, the magnitude and duration of this corticosteroid response varies by species, sex, season, and individual (Dufty and Belthoff, 1997). Though, in theory, stress from captivity would affect control and trauma subjects equally, in reality this may not be the case. Handling, temporary restraint, and minor procedures all consistently produce stress-induced analgesia in laboratory animals, and all are potential stressors for the subjects of this study in both control and trauma groups (Butler and Finn, 2009). However, long-term stress has also been shown to cause hyperalgesia (Clark et al., 2011; Rivat et al., 2007), so it is possible that the stress of captivity might actually increase behavioral differences due to pain. Overall, neither stress nor opioid administration alone is sufficient to explain the observed behavioral alterations in our study subjects. However, we cannot rule out an interaction of these factors (i.e., injured birds may react differently to opioid administration, or to hospitalization).

Another potential confounding factor is the effect of emaciation and dehydration, which are common in patients entering the wildlife hospital. This was controlled by eliminating any visibly debilitated hawks from the study, and by delaying initial behavioral assessment until treatment to correct these issues had begun. Preliminary data from a separate study indicates that non-trauma hawks present with moderate or severe emaciation at approximately the same frequency as hawks with orthopedic injury, so this factor is likely to have affected both Control A and Trauma A hawks (unpublished data). Overall, most behaviors occurred slightly more frequently in Control B hawks than in Control A, though this difference was not significant for any behavior. Thus there may be a slight effect of improvement in general health or acclimation to clinic environs, and this factor should be investigated in future work, ideally with larger sample sizes that can more readily distinguish multiple effects. However, the majority of the behavioral differences observed do appear to be related to injury and its clinical sequelae.

The ultimate goal of this project was to determine whether behavioral alterations due to pain were likely to be a reliable measure for cage-side or experimental investigation of pain and analgesia in wild red-tailed hawks, especially given the variation in presenting complaint

and relatively uncontrolled nature of wildlife patients. It would appear that three of the measures reported here (movement about the cage, beak clacks, and especially head movements) are robust enough to account for variations in severity of injury, as long as some care is taken with patient selection. The major deficiency with this model is that all of the behaviors reported might be considered *pain-suppressed* behaviors; we did not discover any active behaviors that can be specifically attributed to pain. An ideal model for analgesic research would include both pain-elicited and pain-suppressed behaviors (Negus et al., 2006). Other studies have noted shaking, crying, trembling, or postural changes as pain-elicited behaviors in a variety of species (e.g., Firth and Haldane, 1999; Grondahl-Nielsen et al., 1999; Hay et al., 2003; Leach et al., 2009; Marchant-Forde et al., 2009; Morton et al., 2005). Interestingly, though common dogma is that prey species do not exhibit signs of pain, these behaviors have been described in both predator and prey species. Red-tailed hawks in the wild do not exhibit the complex vocalizations or social groupings of, for example, corvids or psittacines; they may therefore have a smaller behavioral repertoire available for the expression of pain, or have no reason to express these behaviors in the absence of a bonded conspecific. Or, conversely, it may be that active pain-related behaviors did occur in these subjects, but eluded the investigator. Postures that mitigate pain, for example, seem likely to occur, but the variability in trauma locations and severity amongst the injured subjects may have made it more difficult to detect specific pain-related behaviors.

The other critical component of a model for research on analgesia is that it be responsive to analgesic or anti-hyperalgesic drugs. It is impossible to fully determine whether the model described here will fulfill this requirement, because we included no study subjects who were painful but did not have their pain addressed in some form. There is no direct evidence that buprenorphine is an effective analgesic in red-tailed hawks at the doses used, and certainly its administration did not return the Trauma A hawks to normal behavior. This may indicate that the model is insensitive to behavioral effects of buprenorphine (or other opioids), or that buprenorphine is not an effective analgesic for this type of injury in red-tailed hawks (Paul-Murphy et al., 1999); though fentanyl, another mu opioid agonist, has been shown to have some analgesic effect in RTHA (Pavez et al., 2011). Or it may be that buprenorphine *is* an effective analgesic, and that the observed behavioral depression in Trauma A hawks would have been even greater had they not received it.

5. Conclusion

Overall, our results suggest that behavioral suppression as a result of trauma in hospitalized red-tailed hawks may be a useful indicator of pain status, and an effective behavioral endpoint for future investigations of analgesics in this species. Trauma is associated with drastic reductions in several measures of voluntary activity, as compared to non-painful hawks or as compared to hawks with mostly-healed injuries. These behavioral alterations are consistent with research in pre-clinical trials on laboratory animals, as well as other studies of pain-altered behavior in veterinary medicine. Buprenorphine, which hawks admitted for orthopedic trauma all received, is unlikely to have caused the depression seen in study subjects. We propose that future studies on pain management in red-tailed hawks may benefit from inclusion of measures of pain-suppressed behaviors, as a humane, non-invasive, and reliable tool for investigation.

Acknowledgments

This project was funded by NIH Training Grant T32 OD011165 (Project Director, Dr. Bob Bridges), and by the National Wildlife Rehabilitators Association. Neither source had input into study design or decision to publish results.

We would like to thank Dr. Glenn Stevenson of the University of New England for his contributions to analysis and content. We would also like to thank the staff of the Vermont Institute of Natural Sciences for their help in acquiring the data that led to this experimental design.

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Fig. 1.

Mean time (square root-transformed) spent moving about the cage (s/ 10 min) by hawks in trauma-suppressed behavior study. Hawks in Trauma A, regardless of type of fracture, were almost completely immobile. Asterisks indicate significant differences between the indicated group and Trauma A (* indicates $P < 0.05$, ** indicates $P < 0.01$, and *** indicates $P < 0.005$). Trauma B and Control groups were not significantly different. Error bars indicate SEM.

Fig. 2. Mean frequencies of head shifts (A), beak clacks (B), foot shifts (C), and rouse (D) per minute spent perching (square root -transformed) by hawks in trauma-suppressed behavior study. Asterisks indicate significant differences between groups (* indicates $P < 0.05$, ** indicates $P < 0.01$, *** indicates $P < 0.005$). For head shifts (A) and beak clacks (B), Trauma A was different than all other groups, and those groups were not different from each other. For foot shifts, only Trauma A and Control B were significantly different. Error bars indicate SEM.

Table 1

Estimated demographics of hawks in trauma-suppressed behavior study.

Experimental Group	N	Sex	Age
Control A	5	2 F, 2 M, 1 undetermined	2 adults, 2 juveniles, 1 hatch year
Control B	3	1 F, 2 M	2 juveniles, 1 adult
Trauma A	5	4 F, 1 undetermined	3 adults, 2 juveniles
Trauma B	6	4 M, 1 F, 1 undetermined	3 adults, 3 juveniles

Table 2

Scores for movement about the cage, head movements, beak clacks, foot shifts, and rouse, for hawks in the trauma-suppressed behavior study.

Group	Movement s/10 min $\pm$ S.D.	Head Movements per min perching $\pm$ S.D.	Beak clacks per min perching $\pm$ S.D.	Foot shifts per min perching $\pm$ S.D.	Rouse per min perching $\pm$ S.D.
Control A	114.2 $\pm$ 53.8	30.3 $\pm$ 10.9	1.03 $\pm$ 0.38	2.17 $\pm$ 0.88	0.26 $\pm$ 0.21
Control B	130.8 $\pm$ 123.2	36.8 $\pm$ 18.7	1.24 $\pm$ 0.70	3.21 $\pm$ 1.84	0.26 $\pm$ 0.33
Trauma A	4.22 $\pm$ 5.43	7.50 $\pm$ 2.71	0.21 $\pm$ 0.13	0.52 $\pm$ 0.53	0.11 $\pm$ 0.14
Trauma B	54.5 $\pm$ 25.3	28.5 $\pm$ 5.57	1.06 $\pm$ 0.54	1.56 $\pm$ 0.97	0.16 $\pm$ 0.09

Values reported reflect actual time spent in each behavior, not transformed scores.

Table 3

Mean frequencies and relative change in behaviors in hawks with healed trauma, after saline (SAL) and buprenorphine (BPN) administration. Buprenorphine administration decreased foot shifts, beak clacks, and movement, but the decrease was not statistically significant. Buprenorphine administration had no apparent effect on head movement frequency.

Behavior	SAL		BPN		Percent decrease	P value _{BPN}	Cont. A	Trauma A	Decrease	Percent dec.	P value _{TRAUMA}
Head mvmt (events/min)	30.8	29.8	1	3.20%		0.877	30.3	7.5	22.8	75.20%	0.0006
Foot shifts (events/min)	2	1.4	0.6	30.90%		0.317	2.2	0.5	1.7	77.30%	0.08
Beak clacks (events/min)	0.6	0.4	0.1	22.80%		0.137	1	0.2	0.8	79.40%	0.018
Movement (s/10 min)	87.1	49.6	37.5	43.10%		0.159	114.2	4.23	109.7	96.30%	0.002

P value_{BPN} indicates the result of paired t-tests performed on each behavior comparing BPN and SAL treatments. For comparison, non-transformed scores for the same behaviors in hawks with no injury (Cont. A) and recent injury (Trauma A) are reported, along with corresponding p values from post-hoc t-tests comparing those groups. Recent trauma was associated with a marked decrease in all behaviors.