Journal of the American Association for Laboratory Animal Science
Copyright 2016
by the American Association for Laboratory Animal Science

Vol 55, No 1
January 2016
Pages 29–34

Validation of a Behavioral Ethogram for Assessing Postoperative Pain in Guinea Pigs (*Cavia porcellus*)

Misha L Dunbar, Emily M David, Marian R Aline, and Jennifer L Lofgren*

Although guinea pigs (*Cavia porcellus*) have been used in research for more than a century and remain the most prevalent USDA-covered species, little has been elucidated regarding the recognition of clinical pain or analgesic efficacy in this species. We sought to assess pain in guinea pigs by using newer, clinically relevant methods that have been validated in other rodent species: the behavioral ethogram and cageside proxy indicator. In this study, 10 male guinea pigs underwent electronic von Frey testing of nociception, remote videorecording of behavior, and cageside assessment by using time-to-consumption (TTC) of a preferred treat test. These assessments were performed across 2 conditions (anesthesia only and castration surgery under anesthesia) at 3 time points (2, 8, and 24 h after the event). The anesthesia only condition served to control for the nonpainful but potentially distressing components of the surgical experience. Compared with those after anesthesia only conditions, subtle body movements were increased and nociceptive thresholds were decreased at 2 and 8 h after surgery. At 24 h, neither subtle body movement behaviors nor nociceptive thresholds differed between the 2 conditions. In contrast, TTC scores did not differ between the anesthesia only and surgery conditions at any time point, underscoring the challenge of identifying pain in this species through cageside evaluation. By comparing ethogram scores with measures of nociception, we validated select behaviors as pain-specific. Therefore, our novel ethogram allowed us to assess postoperative pain and may further serve as a platform for future analgesia efficacy studies in guinea pigs.

Abbreviation: TTC, time to consumption

Guinea pigs are one of the most frequently used USDA-covered species in research, with much of their popularity owed to their docile temperament, commercial availability, and easy maintenance.[2,5] According to the most recently published USDA annual report, nearly 200,000 guinea pigs were used in the research and education setting, and of these, approximately one third (31%) were in D or E pain and distress categories.[26] The Animal Welfare Act and *The Guide for the Care and Use of Laboratory Animals* require that postoperative pain be minimized unless scientifically justified.[1,6] Therefore, the need for validated methods to recognize pain and evaluate the efficacy of analgesics is imperative. However, little is known or standardized about how to recognize pain in this species. Guinea pigs, like most rodents, exhibit a 'conservation withdrawal' response and tend to hide overt signs of illness in the presence of an observer. This prey-species behavior makes discernment of painful behaviors more difficult due to guinea pigs' natural tonic immobility (freezing response).[2,11] Thus clinical signs of pain may go unnoticed until changes secondary to loss of normal behavior develop, such as dehydration, loss of body weight or condition due to decreased consumptive behaviors, or unkempt hair coat due to decreased grooming behaviors. These metrics are nonspecific, may fail to indicate pain intensity, and are retrospective in nature,[9,11] meaning that the animal may have experienced unalleviated pain for 12 h or more before the resulting clinical signs are apparent.

Received: 06 Apr 2015. Revision requested: 11 May 2015. Accepted: 16 Jun 2015.
Refinement and Enrichment Advancements Laboratory (REAL), Unit for Laboratory Animal Medicine (ULAM), University of Michigan, Ann Arbor, Michigan.
**Corresponding author. Email: jlofgren@umich.edu*

Published methods of pain evaluation in guinea pigs are predominantly based on nociceptive assays that measure evoked response to noxious stimuli. Commonly used tests such as the Hargreaves apparatus (or plantar test) measure thermal hypersensitivity states, whereas the modified Randall and Selitto and von Frey assays measure mechanical hypersensitivity.[15,27] All of these measures are reflexive in nature and are impractical for the clinical evaluation of spontaneous pain.[18] Measures that may serve as better correlates to clinical postoperative pain and that have been validated in mice, rats, and rabbits include the use of facial grimace scoring and behavioral ethograms.[9,10,12,13,18,20-23,25] Pain behaviors, as described in species-specific ethograms, and grimace scores exhibit strong correlation to each other and have the potential for use in guinea pigs.[10,13,28,] Furthermore, newer applications to assess animal health status have been explored in rodent species and include the use of 'proxy indicators' that can serve as quick and simple tools to facilitate cageside assessment. A proxy indicator is an indirect measure that represents the animal's normal spontaneous behavior when an observer is not present, and the absence of these behaviors may indicate an alteration in the animal's wellbeing, such as the presence of pain or illness. Nest building, for example, is a species-specific behavior that mice are highly motivated to perform for several fitness and survival functions.[8,19] Nest complexity, time to nest incorporation, and burrowing behaviors in mice have all been used successfully to assess postsurgical pain.[7,8,19] Guinea pigs are highly food-motivated, and they eat continuously due to their high metabolism.[2] Therefore this intrinsically motivated behavior might be developed as a proxy indicator of pain in this species. Accordingly, the latency to consumption of a highly palatable treat might vary depending on the magnitude of pain

that a guinea pig experiences postoperatively and thus may signal that additional examination is warranted.

Despite the extensive use of guinea pigs in biomedical research and the availability of validated ethograms and proxy indicators for other rodent species, the published literature regarding pain evaluation in guinea pigs has, thus far, focused on nociceptive assays. Therefore, we determined that a clinically relevant measure of postoperative pain is urgently needed for this species. We hypothesized that after a surgical procedure, behaviors specific to pain in guinea pigs identified through the use of a behavioral ethogram would increase whereas the response threshold for mechanical pressure using the electronic von Frey assay would decrease. In addition, we anticipated that the desire for food and its consumption would decrease after surgery, leading to an increase in the time-to-consumption (TTC) latency. By comparing the 'gold standard' nociceptive assay, electronic von Frey measurement, with a novel behavioral ethogram and simple cage-side proxy indicator, TTC, we sought to establish not only a clinically relevant assessment tool for spontaneous pain but also to create a platform for future analgesic efficacy studies in guinea pigs.

Materials and Methods

Animals and housing conditions. Intact male pigmented guinea pigs SPF for pneumonia virus of mice, reovirus, lymphocytic choriomeningitis virus, and guinea pig adenovirus (n = 10; weight, 300 to 400 g; age, approximately 4 wk) were acquired from an inhouse breeding colony and maintained in an AAALAC-accredited animal facility (The University of Michigan, Ann Arbor, MI). The study was approved by the University Committee on Use and Care of Animals. Guinea pigs were singly housed in autoclaved polycarbonate guinea pig cages (Allentown Caging, Allentown, PA) in a temperature-controlled room (21 ± 2 °C) on a 12:12-h light-dark cycle. Single housing was necessary because the male guinea pigs were not all from the same litter; each animal was provided enrichment consisting of a hut and ball and had free access to food (5025 Guinea Pig Diet, LabDiet, St Louis, MO) and automated water in their home cage. At the conclusion of the study, all of the guinea pigs were adopted.

Acclimation. Animals were left undisturbed in their standard individually ventilated home cages for at least 48 h before handling. They then were handled by lab members (including those conducting the behavioral testing) for 10 to 20 min daily for 7 d to accustom the guinea pigs to human interaction, observation, and gentle handling. Hay and parsley stalks were determined to be the most widely and consistently consumed treats and were designated as 'preferred' treats for the duration of the experiment. For 2 wk, each guinea pig was acclimated daily to its assigned behavioral assessment cage, to all behavioral testing equipment, and to light touch on the abdomen to decrease reactiveness to touch during von Frey testing. For consistency, the same investigator performed all experimental manipulation once the study commenced.

Behavioral assessment cages. Custom acrylic cages were constructed on a modified housing rack. Clear acrylic facilitated high-definition videorecording by cameras affixed to one side of the rack. All other walls of the observation cages were opaque to prevent visualization of neighboring animals and room surroundings. Wire-mesh flooring (225 in^2 floor space) and a mirror placed at an angle on the shelf directly below the cage facilitated visualization of the guinea pig's abdomen and access for von Frey measurements.

Study design. To control for order effect, a random-number generator assigned the order of testing for each guinea pig within each time point. Transportation and anesthesia can alter normal behavior and activity but are required components that are performed on the day of a surgery.[13,18] Therefore, an anesthesia only condition was chosen to control for all of the distressing but nonpainful components of surgery, including transportation to surgical suite, anesthesia induction and recovery, eye lubrication, and surgical preparation. Each animal served as its own control and underwent both the anesthesia only and anesthesia plus surgery conditions at all time points. Comparison of these 2 conditions allowed for the isolation of pain-specific differences in behavior and nociception. Both conditions consisted of sampling time points at 2, 8, and 24 h after the manipulation, which were conducted at 1300, 1900, and 1100 the following day, respectively. This pattern placed one time point at 2 h into the dark cycle, when guinea pigs are more active. During sampling, spontaneous behavior was recorded, followed by von Frey and TTC testing. Guinea pigs were promptly returned to their home cages after recovery from experimental sessions and had free access to food and water. To allow for the study of pain-associated behaviors and to evaluate how long these behaviors persisted, analgesia was not provided until after the 24-h time point, when all animals received analgesics. A 14-d washout period occurred between the anesthesia only and anesthesia plus surgery conditions to permit full recovery after the anesthesia only condition.

Anesthesia. No more than 2 h prior to procedure time, guinea pigs were placed in fresh cages and transported to a procedure room. Anesthesia was induced by using 5% isoflurane in an induction box; after induction, guinea pigs were moved to a face mask where they were maintained on 2% to 3% isoflurane and monitored (pulse oximetry, heart rate, respiratory rate, and temperature). Animals were placed on an external heat source, eyes were lubricated, and caudal abdomens were shaved and aseptically prepared. Guinea pigs were then moved to a heated surgical table, draped, and monitored for the duration of the procedure (approximately 50 min of anesthesia total). Prior to recovery, permanent marker was used to mark the 2 areas designated for von Frey measurement: 1 cm above the scrotum and 1 cm below the xiphoid. Guinea pigs recovered under 100% oxygen and remained on a heating pad until they were normothermic and fully ambulatory.

Surgery. Anesthesia and aseptic surgical preparation was as described in the previous section. Separate autoclaved instruments and sterile drapes were used for each animal. The same veterinary surgeon performed all castrations, to ensure a consistent surgical stimulus. Gentle caudoventral pressure was applied to the abdomen to ensure the testicles were in the scrotum, and a small (1 to 1.5 cm) incision was made over each testicle, lateral to the penis. An open castration was performed by double ligation of the spermatic cord with sterile absorbable suture (Ethicon, Somerville, NJ), and the testicles were surgically excised. After monitoring of bleeding, each inguinal ring was closed with a single suture to reduce the risk of herniation. The scrotal incision was closed in 2 layers by using absorbable suture and skin glue (Vetbond, St Paul, MN). Areas designated for von Frey measurements were marked with permanent marker in the same manner as described previously. No intraoperative or perioperative complications occurred, and all animals recovered uneventfully.

Postoperative care. Carprofen (1mg/kg SC) was given to all guinea pigs at 24 h after surgery. To decrease the risk of postoperative infection, guinea pigs received sulfamethoxazole–

trimethoprim (30 mg/kg PO twice daily) for 7 to 10 d, beginning 24 h after surgery. Animals were weighed on a pediatric scale before the anesthesia only and anesthesia plus surgery conditions and daily for 7 d after surgery.

Videorecording. At each time point, guinea pigs were placed in the observation cages, and video cameras (HD Everio, JVC, Long Beach, CA) were used to record their behavior for 15 min in the absence of a human observer.

Electronic von Frey assay. Mechanical hyperalgesia was measured by using an electronic von Frey probe (IITC Life Science, Woodland Hills, CA) applied perpendicular to the ventral abdomen through the wire mesh flooring of the behavioral assessment cages. Incisional placement of the probe, that is within a 1-cm area surrounding the scrotal incisions, was chosen to reflect postoperative pain rather than placement on a footpad, which might be more representative of a secondary hyperalgesia. This approach has been used to evaluate analgesic efficacy in other veterinary species.[4,14] To control for the animal's response to nonincisional stimulation, the probe was applied to the cranioventral abdomen 1 cm below the xiphoid. The probe was applied with gradually increasing pressure (thus increasing the force in grams) until a response was elicited from the animal (jump, vocalize, or attempt to move away), giving a peak force measurement. For each animal, and in both locations, 3 peak force measurements were recorded, the mean of which was taken as the nociceptive threshold (in grams). To avoid sensitization, a 5-min rest period was provided between applications of the probe.

TTC score. After mechanical hyperalgesia testing, guinea pigs received preferred treats (hay and parsley). Because the study objective was to develop a cageside indicator that would be practical for clinical use in a postoperative setting, this task was performed in the home cage rather than the behavioral assessment cage. Guinea pigs were returned to their home cages, and time was recorded (in seconds), starting with the placement of the treat on the cage floor and ending at the initiation of consumption of the treat by the animal, to give the TTC score.

Analysis of videorecordings. A trained, blinded observer reviewed and scored 60 randomized videos according to the prescribed ethogram. Each video was divided into 9 clips of 10 s each. The total of 90 s of observation per time point was chosen because we sought to identify behaviors that were easily recognizable within a brief observation period and therefore practical for use by lab and vet staff. Clips were scored through fixed-interval time-span sampling (also referred to as one-zero sampling). In short, when a given behavior was present in a video clip, a score of 1 was assigned; if that behavior was absent, a score of 0 was assigned. The scores were summed for a total frequency of observation of the particular behavior for a given video. Analysis by this method was selected in light of published studies evaluating behavior in rodents and behavioral study design texts. Previous studies established manual scoring of video data as more sensitive and accurate than automated software for the detection of pain behaviors in rodents.[29] Furthermore, the one–zero time-sampling technique more accurately detects brief or intermittently performed behaviors, such as spontaneous abdominal contractions in the postsurgical condition, compared with instantaneous sampling. Lastly, one–zero sampling produces higher interobserver reliability than does continuous scoring; this feature is important when evaluating an ethogram for practical use in a clinically relevant setting.[3,16,24]

Behavior selection. An initial ethogram was drafted according to published ethograms in mice, rats, and rabbits.[9,,11-13,20,21,23] Analysis of a subset of recordings across each condition allowed for the identification of behaviors specifically performed by guinea pigs, including but not limited to coprophagy, grooming, head and neck flexion or extension, ear twitching, biting or licking of the wire floor, body turns, and ambulation. Because sufficient frequency and ease of recognition were considered paramount for developing a clinically relevant pain behavior assessment, we sought to identify those changes in behavior that were most frequent after surgery. Behaviors that were performed infrequently (fewer than 2 times across the scored video segments) were disregarded or combined with related behaviors to represent the most common types of behaviors observed, which included subtle body movement behaviors such as abdominal contraction (tensing of the abdomen resulting in the ventrum being momentarily, sometimes repeatedly, raised from the cage floor), back arching (rounding of the back resulting in a hunched appearance), twitching (spasmodic movements of the fur, skin, and muscles over the dorsum and flank), and weight shifting (transfer of body weight by shifting of limbs in the absence of locomotion; Figure 1).

Statistical analysis. All statistical analyses were conducted by using the software Prism (Prism 6 for Mac OS X, La Jolla, CA). Data were normally distributed, and two-way repeated-measures ANOVA with Tukey multiple comparisons for posthoc testing was used to compare anesthesia only and anesthesia plus surgery conditions across multiple time points. Differences were considered statistically significant when the P value was less than 0.05.

Results

Mechanical threshold according to electronic von Frey measurement. To control for an individual reactiveness to touch of each guinea pig, the difference in nociceptive threshold was calculated by subtracting the peak force applied at the scrotal incision (presumed to be more painful) from the peak force applied at the cranial abdomen (presumed to be nonpainful). Mechanical hyperalgesia was significantly increased at the 2-h ($P < 0.01$) and 8-h ($P < 0.05$) time points after surgery compared with anesthesia only but no difference between the 2 conditions at 24 h (Figure 2).

Comparison of behaviors. Review of all the videos for combined behaviors revealed no significant differences in movement, chewing, and licking behaviors between conditions or over time (Figure 3). However, subtle body-movement behaviors were increased after anesthesia plus surgery compared with the anesthesia only condition, specifically at 2 h ($P < 0.05$) and 8 h ($P < 0.01$), but not at 24 h (Figure 4).

Proxy indicator. TTC did not change after surgery at any time point. On average, the guinea pigs readily consumed their preferred food treats in a matter of seconds after both anesthesia only and anesthesia plus surgery conditions at all time points. Mean TTC (mean ± SEM) was nonsignificantly higher at 2-h time points in both conditions, but no significant differences were observed between conditions or across time points. (anesthesia: 2 h, 16.3 ± 4.1 s; 8 h, 9.9 ± 2.5 s; 24 h, 10 ± 2.7 s; surgery: 2 h, 13.7 ± 4.1 s; 8 h, 9.6 ± 3.1 s; 24 h, 7.4 ± 2.5 s).

Discussion

Here we developed and evaluated a novel guinea pig–specific behavioral ethogram and a cageside proxy indicator to assess postoperative pain behaviors in a castration model. The classic mechanical hyperalgesia assay, von Frey testing, and our novel ethogram both identified evidence of pain at 2 and 8 h but not

Category	Inclusive Behaviors
Movement	Forward or backward motion, turning the body or head, head or neck extensions, ambulation
Chew	May or may not be observed with coprophagy behavior
Bite or Lick Bars	Biting or licking of mesh floor in observational cage
Subtle Body Movements	Abdominal contraction, back arching, twitching, and weight shifting

Figure 1. Using published ethograms for mice, rats, and rabbits as a reference, we analyzed a subset of recordings across each condition to create a guinea pig behavioral ethogram. Behaviors that were performed infrequently (fewer than 2 times across the scored video segments) were disregarded (not shown), and a composite of the most common behaviors are shown. Related behaviors that tended to be performed in similar patterns were categorized together.

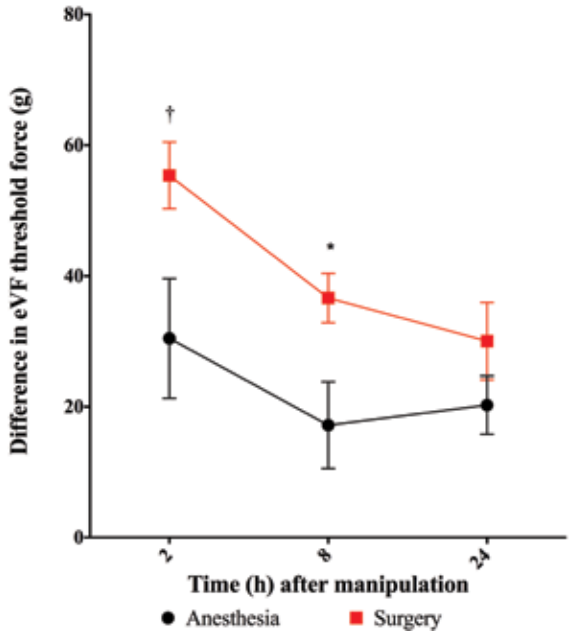

Figure 2. Guinea pigs (n = 10) underwent anesthesia only, followed by a 14-d washout period, and then were anesthetized for surgical castration. Mechanical hypersensitivity (mean ± SEM) was measured at 2, 8, and 24 h after either anesthesia only or surgery by using electronic von Frey assessment. To correct for an individual animal's natural reactiveness to touch, threshold difference was determined by subtracting the peak threshold at the scrotal incision from the peak threshold at the cranial abdomen, to isolate the response as pain-specific. Compared with that after anesthesia only, the nociceptive threshold difference after surgery was increased at 2 h (†, $P < 0.01$) and 8 h (*, $P < 0.05$) but not at 24 h after surgery.

at 24 h after surgery, thus validating the subtle body-movement behaviors as pain-specific. However, the use of a proxy indicator failed to differentiate postoperative animals from subjects that had undergone anesthesia only, highlighting the significant challenge of identifying clinical pain during cageside examination in this species.

Using the electronic von Frey assay, we identified significant increases in relative mechanical hyperalgesia at 2 and 8 h postoperatively when compared with anesthesia only. Due to strong individual personalities and responses to handling and touch, each guinea pig was used as its own control, to mitigate interanimal differences. To substantiate this measured hyperalgesia as pain-specific and associated with surgery, we evaluated the difference between the nociceptive thresholds taken at the surgical site and a distant nonpainful site, the cranial abdomen. This experiment was modeled after a study that used von Frey filaments to evaluate incisional pain in dogs after ovariohys-

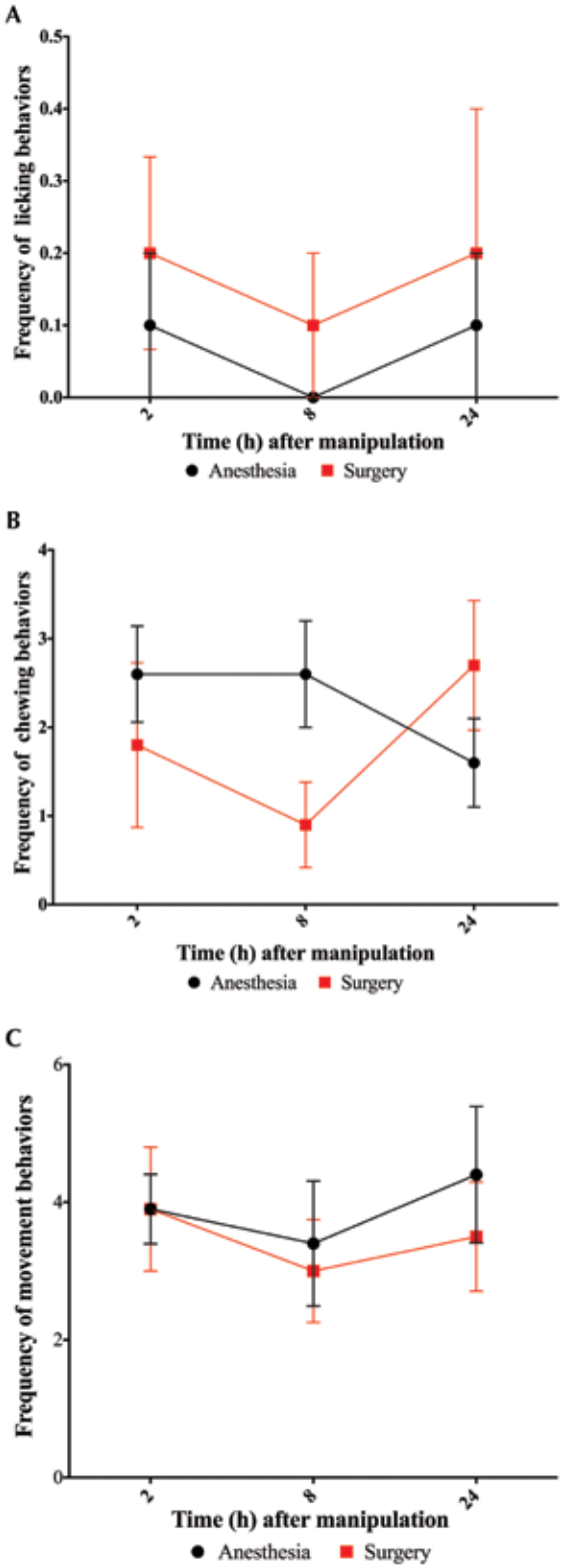

Figure 3. The frequency (mean ± SEM) of a behavior is the sum of the observations of that behavior across 9 clips (10 s each) taken at each time point. Compared with those during anesthesia only, (A) licking, (B) chewing, and (C) movement behaviors did not change significantly after surgery for guinea pigs (n = 10). Most behaviors showed a trend toward a decreased frequency at the 8-h time point, which coincided with the dark phase of the photoperiod.

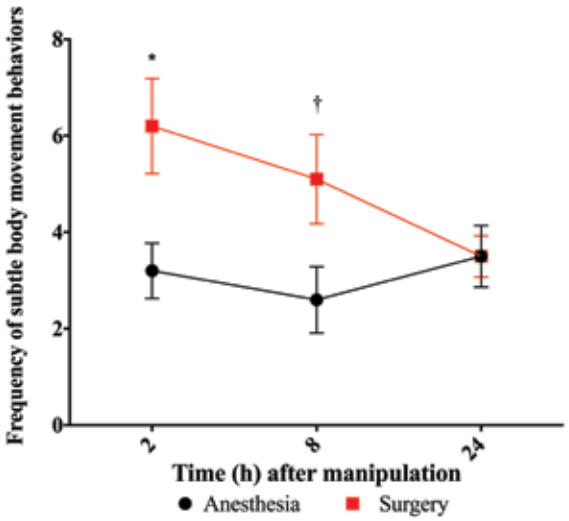

Figure 4. The frequency (mean ± SEM) of a behavior is the sum of the observations of that behavior across 9 clips (10 s each) taken at each time point. Comparison of the pain-specific behaviors observed in guinea pigs (n = 10) revealed that pain behaviors are significantly increased (*, $P < 0.05$; †, $P < 0.01$) at 2 and 8 h after surgery when compared with the frequency during anesthesia only.

terectomy.[4] In that study, in addition to probing the incisional area, the ventrolateral portion of each dog's thorax was tested to control for the animal's natural response to the filaments and to evaluate for the possibility of allodynia.[4] By taking the difference of the 2 thresholds in our study, we were similarly able to control for the guinea pig's individual responsiveness to touch.

Although the von Frey test is the gold-standard nociceptive assay that positively identified the presence of postoperative pain in our guinea pigs, it is impractical for use as a cageside assessment tool. Therefore, we sought to develop a cageside proxy-indicator test to rapidly identify guinea pigs that potentially are experiencing pain. In both guinea pigs and rabbits, overt responses to pain and distress are often masked in the presence of a human observer,[11] making it very difficult to effectively evaluate pain cageside. In fact, physical exam findings and in-person observations by trained laboratory animal veterinarians yielded minimal subjective indications of pain after surgery in the present study. We therefore developed the TTC score as a potential objective cageside method for evaluating postoperative pain in guinea pigs.

The premise of the TTC in guinea pigs was similar to use of burrowing or nesting behavior in mice: the adoption of a behavior the animal is naturally motivated to perform in the absence of a human observer but produces observable evidence that can readily be scored in a binary fashion.[7,8,19] In the current study, TTC latency did not differ between test conditions: all guinea pigs consumed the treat within seconds during both conditions and across all time points. In terms of practical use, this particular proxy indicator was not suitable for evaluating pain in this model. Because guinea pigs are highly food-motivated,[2] castration did not cause a severe enough pain stimulus to distract them from their intrinsic drive to search for food. The willingness of guinea pigs to readily consume their treats at time points when there was evidence of significant pain, as measured by using the von Frey assay and behavioral ethogram, further demonstrates the inherent difficulty in accurately assessing pain in this species through direct cageside observation. Before we completely rule out the use of this type of proxy indicator in guinea pigs, we are investigating strategies to improve the sensitivity of the TTC score. Possible improvements might include increasing the difficulty of retrieving the treat and evaluating the use of the TTC score in other guinea pig surgeries common in private practice and the research setting (that is, laparotomy). Even with modifications to the test, the potential remains that significant differences in TTC score may not be detectable when used with procedures causing less pain or once an animal is properly 'analgesed.' This situation suggests that a sensitive nonappetitive indicator might need to be explored.

Using remote videorecording and our novel ethogram, we identified and validated several behaviors as pain-specific for soft tissue surgery involving the lower abdomen in guinea pigs. In our ethogram (Figure 1), we refer to pain-associated behaviors as subtle body movements: a collection of small, transient body motions performed in a stationary position. Independent analysis of the behaviors in this category revealed significant increases in the performance of these behaviors after surgery compared with anesthesia alone. Because these behaviors trended together, they were grouped as related behaviors into the category 'subtle body- movements' to produce a more reliable composite measure when using the ethogram. In addition, because weight shifting increased significantly only during the postsurgical time points, we feel that this behavior is indeed reflective of postsurgical pain and not solely attributable to discomfort from the wire-mesh flooring of the observation cages. Similar pain behaviors to those we defined as back-arching, staggering, and writhing have previously been noted to increase postsurgically for procedures involving the abdomen in mice, rats, and rabbits.[11-13,20,22]

In the current study, we observed a significant increase in pain behaviors that mirrored the decrease in the nociceptive threshold at the surgical incision at 2 and 8 h after surgery when compared with anesthesia alone. These parallel observations validate that the identified behaviors were a direct response to surgery and subsequently were pain-specific. However, neither the nociceptive test nor our ethogram identified pain at the 24-h time point, indicating pain had abated by this time in our study. Although additional time points between 8 and 24 h might have revealed more precisely the return to a nonpainful state, and their lack could be viewed as a limitation to this study, we chose our experimental time points to reflect the actual times at which lab personnel would most likely perform postoperative monitoring. These times also corresponded to the typical dosing frequency of several commonly used analgesics (for example, opioids, or NSAIDs) in this species. In addition, our 8-h time point was 2 h into the dark cycle, because observations made only during the light phase can lead to poor pain assessment.[28] At this evening time point, we found evidence that pain persisted into the night. Prior studies in rodents demonstrated that pain experienced by an animal might be intensified during the dark cycle because these species are most active during this period.[17,18] Given our ethogram and the nociceptive evidence of the persistence of pain into the dark cycle and the significant challenge of accurately assessing pain during a cageside exam, we recommend that guinea pigs should receive analgesia for 24 h after castrations or surgeries of similar invasiveness. The decision to prolong analgesic treatment should also include consideration of the guinea pig strain, surgical procedure, and technical expertise of the surgeon.

Guinea pigs, like many other prey species, often suppress spontaneous pain behavior in the presence of a human observer,[11,12] thus making it difficult to observe and effectively score pain cageside. As we anticipated this would be a challenge to accurately assess clinical pain in this species, we used remote videorecordings. These recordings facilitated the detection of a range of subtle behavioral changes. As videos were scored and pain specific behavior was identified after the completion of the animal work, we did not have the opportunity to apply the ethogram cageside so we cannot definitively state that these behaviors

would or would not be performed in the presence of a human observer. While remote video assessment may not be practical in a clinical setting, it is important to have these kind of studies to understand how long pain persists and provide evidence-based recommendations regarding the duration of analgesia needed.

In summary, guinea pigs are vital animal models in research, and in the United States alone, nearly 60,000 guinea pigs are predicted to experience more than momentary pain or distress in research or educational settings. However, evidenced-based recommendations to guide management of their postprocedural pain are severely underdeveloped and further hindered by this species' natural freeze response to stress. Here, we have validated pain-specific behaviors and highlighted the challenges of identifying obvious signs of pain during cageside exam. These findings not only serve to support a recommendation for providing analgesia to guinea pigs for at least 24 h after a moderately invasive surgery, but they also may serve as a platform for future analgesia efficacy studies in this species.

Acknowledgment

We thank the Unit for Laboratory Animal Medicine at the University of Michigan for its funding contributions and Dr. Jean Nemzek for her editorial expertise. We also express our gratitude to the University of Michigan's Refinement and Enrichment Advancements Laboratory (REAL) for its continued support in projects such as this.

References

1. **Animal Welfare Regulations** 2013. 9 CFR §§2.31.
2. **Bays TB.** 2006. Guinea pig behavior, p 207–238. In: Bays TB, Lightfoot T, Mayer J, editors. Exotic pet behavior: birds, reptiles, and small animals. St Louis (MO): Saunders Elsevier.
3. **Crockett CM, Ha RR.** 2010. Data collection in the zoo setting, emphasizing behavior, p 386-406. In: Kleiman DG, Thompson KV, Baer CK, editors. Wild mammals in captivity: principles and techniques for zoo management, 2nd ed. Chicago (IL): University of Chicago Press.
4. **Fitzpatrick CL, Weir HL, Monnet E.** 2010. Effects of infiltration of the incision site with bupivacaine on postoperative pain and incisional healing in dogs undergoing ovariohysterectomy. J Am Vet Med Assoc **237:**395–401.
5. **Harkness JE, Murray KA, Wagner JE.** 2002. Biology and diseases of guinea pigs, p 203–204. In: Fox JG, Anderson LC, Loew FM, Quimby FW. editors. Laboratory animal medicine, 2nd ed. San Diego (CA): Academic Press.
6. **Institute for Laboratory Animal Research.** 2011. Guide for the care and use of laboratory animals, 8th ed. Washington (DC): National Academies Press.
7. **Jirkof P, Cesarovic N, Rettich A, Nicholls F, Seifert B, Arras M.** 2010. Burrowing behavior as an indicator of postlaparotomy pain in mice. Front Behav Neurosci **4:**165.
8. **Jirkof P, Fleischmann T, Cesarovic N, Rettich A, Vogel J, Arras M.** 2013. Assessment of postsurgical distress and pain in laboratory mice by nest complexity scoring. Lab Anim **47:**153–161.
9. **Keating SC, Thomas AA, Flecknell PA, Leach MC.** 2012. Evaluation of EMLA cream for preventing pain during tattooing of rabbits: changes in physiological, behavioural, and facial expression responses. PLoS One **7:**e44437.
10. **Langford DJ, Bailey AL, Chanda ML, Clarke SE, Drummond TE, Echols S, Glick S, Ingrao J, Klassen-Ross T, Lacroix-Fralish ML, Matsumiya L, Sorge RE, Sotocinal SG, Tabaka JM, Wong D, van den Maagdenberg AM, Ferrari MD, Craig KD, Mogil JS.** 2010. Coding of facial expressions of pain in the laboratory mouse. Nat Methods **7:**447–449.
11. **Leach MC, Allweiler S, Richardson C, Roughan JV, Narbe R, Flecknell PA.** 2009. Behavioural effects of ovariohysterectomy and oral administration of meloxicam in laboratory-housed rabbits. Res Vet Sci **87:**336–347.
12. **Leach MC, Coulter CA, Richardson CA, Flecknell PA.** 2011. Are we looking in the wrong place? Implications for behavioural-based pain assessment in rabbits (*Oryctolagus cuniculi*) and beyond. PLoS One **6:**e13347.
13. **Leach MC, Klaus K, Miller AL, Scotto di Perrotolo M, Sotocinal SG, Flecknell PA.** 2012. The assessment of postvasectomy pain in mice using behaviour and the Mouse Grimace Scale. PLoS One **7:**e35656.
14. **Lomax S, Dickson H, Sheil M, Windsor PA.** 2010. Topical anaesthesia alleviates short-term pain of castration and tail docking in lambs. Aust Vet J **88:**67–74.
15. **Maes SS, Pype S, Hoffmann VL, Biermans M, Meert TF.** 2012. Antihyperalgesic activity of nucleoside transport inhibitors in models of inflammatory pain in guinea pigs. J Pain Res **5:**391–400.
16. **Martin P, Bateson P.** 2007. Measuring behaviour: an introductory guide, 3rd ed. Cambridge (UK): Cambridge University Press.
17. **Martínez-Gómez M, Cruz Y, Salas M, Hudson R, Pacheco P.** 1994. Assessing pain threshold in the rat: changes with estrus and time of day. Physiol Behav **55:**651–657.
18. **Matsumiya LC, Sorge RE, Sotocinal SG, Tabaka JM, Wieskopf JS, Zaloum A, King OD, Mogil JS.** 2012. Using the Mouse Grimace Scale to reevaluate the efficacy of postoperative analgesics in laboratory mice. J Am Assoc Lab Anim Sci **51:**42–49.
19. **Rock ML, Karas AZ, Rodriguez KB, Gallo MS, Pritchett-Corning K, Karas RH, Aronovitz M, Gaskill BN.** 2014. The time-to-integrate-to-nest test as an indicator of wellbeing in laboratory mice. J Am Assoc Lab Anim Sci **53:**24–28.
20. **Roughan JV, Flecknell PA.** 2001. Behavioural effects of laparotomy and analgesic effects of ketoprofen and carprofen in rats. Pain **90:**65–74.
21. **Roughan JV, Flecknell PA.** 2012. Evaluation of a short-duration behaviour-based postoperative pain scoring system in rats. Eur J Pain **7:**397–406.
22. **Roughan JV, Flecknell PA.** 2004. Behaviour-based assessment of the duration of laparotomy-induced abdominal pain and the analgesic effects of carprofen and buprenorphine in rats. Behav Pharmacol **15:**461–472.
23. **Roughan JV, Wright-Williams SL, Flecknell PA.** 2009. Automated analysis of postoperative behaviour: assessment of HomeCageScan as a novel method to rapidly identify pain and analgesic effects in mice. Lab Anim **43:**17–26.
24. **Saibaba P, Sales GD, Stodulski G, Hau J.** 1996. Behaviour of rats in their home cages: daytime variations and effects of routine husbandry procedures analysed by time-sampling techniques. Lab Anim **30:**13–21.
25. **Sotocinal SG, Sorge RE, Zaloum A, Tuttle AH, Martin LJ, Wieskopf JS, Mapplebeck JC, Wei P, Zhan S, Zhang S, McDougall JJ, King OD, Mogil JS.** 2011. The Rat Grimace Scale: a partially automated method for quantifying pain in the laboratory rat via facial expressions. Mol Pain **7:**55.
26. **United States Department of Agriculture.** [Internet]. 2014. Annual report animal usage by fiscal year; 2013. [Cited 1 January 2015]. Available at: http://www.aphis.usda.gov/animal_welfare/downloads/7023/Animals%20Used%20In%20Research%202013.pdf
27. **Vermeirsch H, Biermans R, Salmon PL, Meert TF.** 2007. Evaluation of pain behavior and bone destruction in 2 arthritic models in guinea pig and rat. Pharmacol Biochem Behav **87:**349–359.
28. **Whittaker AL, Howarth GS.** 2013. Use of spontaneous behavior measures to assess pain in laboratory rats and mice: how are we progressing? Appl Anim Behav Sci **151:**1–12.
29. **Wright-Williams S, Flecknell PA, Roughan JV.** 2013. Comparative effects of vasectomy surgery and buprenorphine treatment on faecal corticosterone concentrations and behaviour assessed by manual and automated analysis methods in C57 and C3H mice. PLoS One **8:**e75948.