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Original Research

Indicators of Postoperative Pain in Syrian Hamsters (*Mesocricetus auratus*)

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Despite the use of Syrian hamsters (*Mesocricetus auratus*) in research, little is known about the evaluation of pain in this species. This study investigated whether the frequency of certain behaviors, a grimace scale, the treat-take-test proxy indicator, body weight, water consumption, and coat appearance could be monitored as signs of postoperative pain in hamsters in a research setting. Animals underwent no manipulation, anesthesia only or laparotomy under anesthesia. An ethogram was constructed and used to determine the frequencies of pain, active and passive behaviors by in-person and remote videorecording observation methods. The Syrian Hamster Grimace Scale (SHGS) was developed for evaluation of facial expressions before and after the surgery. The treat-take-test assessed whether surgery would affect the animals' motivation to take a high-value food item from a handler. The hypothesis was that behavior frequency, grimace scale, treat-take-test score, body weight, water consumption, and coat appearance would change from baseline in the surgery group but not in the no-intervention and anesthesia-only groups. At several time points, pain and passive behaviors were higher than during baseline in the surgery group but not the anesthesia-only and no-intervention groups. The SHGS score increased from baseline scores in 3 of the 9 animals studied after surgery. The frequency of pain behaviors and SHGS scores were highly specific but poorly sensitive tools to identify animals with pain. Behaviors in the pain category were exhibited by chiefly, but not solely, animals that underwent the laparotomy. Also, many animals that underwent laparotomy did not show behaviors in the pain category. Treat-take-test scores, body weight, water consumption, and coat appearance did not change from baseline in any of the 3 groups. Overall, the methods we tested for identifying Syrian hamsters experiencing postoperative pain were not effective. More research is needed regarding clinically relevant strategies to assess pain in Syrian hamsters.

Abbreviations: AU, action unit; ICC, intraclass correlation coefficient; IP-HC, in-person behavior scoring in the home cage; SHGS, Syrian Hamster Grimace Scale; TTT, treat-take-test; V-BC, videorecording in the behavior cage; V-HC, videorecording in the home cage

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Pain experienced by laboratory animals can affect both animal welfare and research results. Little is known about the evaluation of pain in Syrian hamsters (*Mesocricetus auratus*) in the laboratory setting. However, various research models using Syrian hamsters involve surgery and are presumed to cause pain.[16,47,49] In 2018 alone, the USDA reported that 35,695 hamsters were used for research studies involving painful procedures.[48] Previously published behaviors exhibited by hamsters in response to pain include hunched posture with head down, reluctance to move, increased depression or aggression, extended sleep periods, and weight loss.[7,8,10,16,21] How these behaviors are affected by factors such as the type of painful stimulus, anesthetic protocol, handling procedures, and environmental conditions is unclear. The practicality of observing these signs in the research environment is uncertain and likely complicated by the nocturnal nature of Syrian hamsters and an assumed propensity of this species to mask pain, much like other prey species.[8,14,16]

A significant need exists for published data investigating whether behavioral observations or other clinical indicators can help recognize, quantify, or monitor pain in hamsters in a research setting. Detailed behavioral observations and well-controlled studies are needed to develop a system to assess postoperative pain in laboratory animals.[8,33] Moreover, little information is available on the efficacy of analgesic agents in hamsters.[1] The few studies of analgesics in hamsters rely on the mitigation of evoked pain responses (such as using a hot plate), which has limited relevance to clinical situations such as postoperative pain.[8,32,36,51] To date, no published literature has evaluated the efficacy or safety of analgesics to treat postoperative pain in hamsters. Validated real-time and practical methods for evaluating pain in Syrian hamsters would support the evaluation of analgesic efficacy in this species.

Various assessments have been developed to identify signs of pain in other species. Behavioral ethograms have been used to evaluate pain and analgesic efficacy in mice, rats, rabbits, and guinea pigs in the research environment.[5,6,20,23,25,34,35,39-41,53] Another tool used to evaluate pain in animals is the grimace scale,

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which has been developed for mice, rats, rabbits, ferrets, cats, sheep, pigs, horses, and even harbor seals.[3,4,9,11,13,15,19,22,26,30,37,45,50] The use of a proxy indicator, such as burrowing and time-to-integrate-to-nest in mice and time-to-consume in guinea pigs, can be used as an additional tool for the evaluation of pain.[5,17,18,35,38]

Because none of the previously mentioned assessment techniques were specific to hamsters, we here explored using these approaches to detect pain in Syrian hamsters that underwent laparotomy in a laboratory setting. We developed a species-specific ethogram and the Syrian Hamster Grimace Scale (SHGS). We also devised a novel proxy indicator of pain for use in Syrian hamsters, the treat-take-test (TTT), which is based on hamsters' natural behavior to hoard food.[16,46,49,52] Although water intake, body weight, and coat appearance are non-specific indicators of pain, we also measured these parameters.[5,19,23,33] Furthermore, we analyzed the effects of the presence of an observer and time of day. We hypothesized that behavior frequency, grimace scale, treat-take-test score, body weight, water consumption, and coat appearance would change from baseline in the surgery group but not in the no-intervention and anesthesia-only groups.

Materials and Methods

Animals and housing. Nine female and 8 male intact Syrian hamsters (LVG stock; age, 77 to 101 d; weight, 112 to 165 g on day 0 of the study) from Charles River Laboratories (Stone Ridge, NY) were used in this study. They were singly housed in open-top polycarbonate or high-temperature polycarbonate cages (R20 Rat Cage, Ancare, Bellmore, NY) with wire lids. Hamsters were provided bedding composed of aspen wood shavings (7093 Teklad shredded aspen, Envigo, Madison, WI), and the standard environmental enrichment consisted of cellulose bedding (Cellu-nest, Shepherd Specialty Papers, Watertown, TN), crinkle paper (Enviro-dri, FiberCore, Cleveland, OH), a small wood gnawing block (Bio-Serv, Flemington, NJ), and cardboard glove boxes (Halyard, Alpharetta, GA). The hamsters were acquired from a colony negative for *Bordetella bronchiseptica*, *Helicobacter bilis*, *H. hepaticus*, *Klebsiella oxytoca*, *K. pneumoniae*, *Lawsonia* spp., *Pasteurella multocida*, *Pseudomonas aeruginosa*, *Salmonella* spp., *Streptococcus pneumoniae*, β-hemolytic *Streptococcus* spp., *Clostridium piliforme*, endoparasites, and *Encephalitozoon cuniculi*. No health surveillance was done on arrival or while animals were on study. The hamsters were housed in their own room at an AAALAC-accredited institution (University of Minnesota, Minneapolis, MN), and the protocol was approved by the IACUC. Hamsters had ad libitum access to pelleted feed (2018 Teklad global 18% protein rodent diet, Envigo) and chlorinated municipal water delivered in glass bottles with rubber stoppers. The animal housing room was maintained on a 12:12-h light:dark cycle (lights on, 0600; lights off, 1800) with controlled temperature (72 ± 2 °F [22.2 ± 1.0 °C]) and humidity (30% to 70%). The cages were placed on the rack in positions determined by a random number generator.[12] Five of the female hamsters arrived with minor fight wounds that did not necessitate treatment, and only minimal scabbing was present 3 d later when the habituation period began. One hamster had a corneal ulcer on arrival and was treated (5 mg/kg carprofen SC daily for 3 d; Zoetis, Kalamazoo, MI); the ulcer completely healed with treatment. Hamsters were allowed to acclimate for 72 h after arrival (except for the one animal that was treated for the corneal ulcer), and then the 2-wk habituation period began. At the conclusion of the study, the animals in the surgery group were anesthetized with isoflurane and euthanized with intracardiac pentobarbital sodium and phytoin sodium (Euthasol-III Solution, Med-Pharmex, Pomona, CA); the other animals were transferred to a training protocol.

Study design. Animals were randomly allocated[12] to cohorts of 4 animals (2 females and 2 males per cohort, with one cohort having 3 females and 2 males), and each cohort moved through the study as a unit. Within each cohort, animals were randomly allocated[12] to undergo surgery ($n = 2$), anesthesia only ($n = 1$), or no intervention ($n = 1$). One cohort of the surgery group had an extra animal ($n = 3$) for reasons explained in the *Postsurgical complications* section.

During the 14-d habituation period, each animal was habituated to elements of the data collection procedure for 6 to 10 min between 0700 and 1900 daily. A video camera was used for observation. A study member (AE) or veterinary technical staff (BG, AS) accessed the cage and used a plastic cylindrical cup to move hamsters to a separate cage, where they underwent 5 min of further observation. The hamster was then weighed and offered a treat by hand after it returned to its home cage.

Each animal in a cohort underwent data collection procedures at 3 time points daily during days 0 through 6, with an intervention (surgery, anesthesia only, or no intervention) on day 3 (Figure 1). Day 0 was the first day after habituation. Hamsters were evaluated for 3 d after surgery in consideration of our institution's minimal required duration for postoperative analgesics. The hamsters were weighed once daily at 0700 on nonintervention days and at 1300 on day 3. The water bottles were weighed at the beginning of the 0700 time point and refilled as needed. The order of the animals for the data collection procedure was randomized each day.[12] To start each data collection procedure, the animal's cage was placed on the counter across from the rack for the 3 methods of observation, which occurred for 5 min each in the following order: in-person behavior scoring in the home cage (IP-HC), videorecording in the home cage (V-HC), and videorecording in the behavior cage (V-BC) (a separate barren container with a blue drape covering 3 walls; Gas Anesthetizing Box type AB 2, 10 × 8 × 8 in., Braintree Scientific, Braintree, MA). The hamster was then returned to its home cage for the TTT and coat appearance scoring. All procedures during the 1900 time point were performed by using a red-beam light from a headlamp and a red-beam clamp lamp. To minimize handling stress, hamsters were moved between cages by using a handheld cylindrical plastic cup. Between animals, the plastic cup and behavior cage were cleaned with hydrogen peroxide (dilution, 1:32; Rescue, Virox Animal Health, Oakville, Ontario, Canada). For the V-BC method, the observer was across the room; for the V-HC method, the observer was not in the room. IP-HC and V-HC scoring started when the hamster was roused from the nest.

Ethogram development. We compiled an original list of defined behaviors (Figure 2) for monitoring, including both normal behaviors for Syrian hamsters published from various sources and behaviors associated with pain in hamsters, mice, rats, guinea pigs, and rabbits.[2,6,23,41-43,52,53] To maximize the utility of the ethogram and minimize the effect of low-frequency behaviors, similar behaviors were grouped into a single category. Pain behaviors (incision grooming, back arch, fall/stagger, twitch, writhe, hind kick, dart, hunched posture with head down, vocalize, shift) were placed in the pain category. Similar to groups that previously assessed pain in guinea pigs and rabbits,[23,35] we designated behaviors that we expected to decrease or increase in response to pain as the active or passive, respectively. Walk, open rear, supported rear, manipulate bedding, hoard, scrabble, dig, gnaw/lick, bite wire bars, eat, drink, groom, shake, yawn and stretch, scent mark, wire bar hang/climb, and coprophagy were categorized as active behaviors. Pause, in nest, lay down, lay flat, and sleep/rest were categorized as passive behaviors.

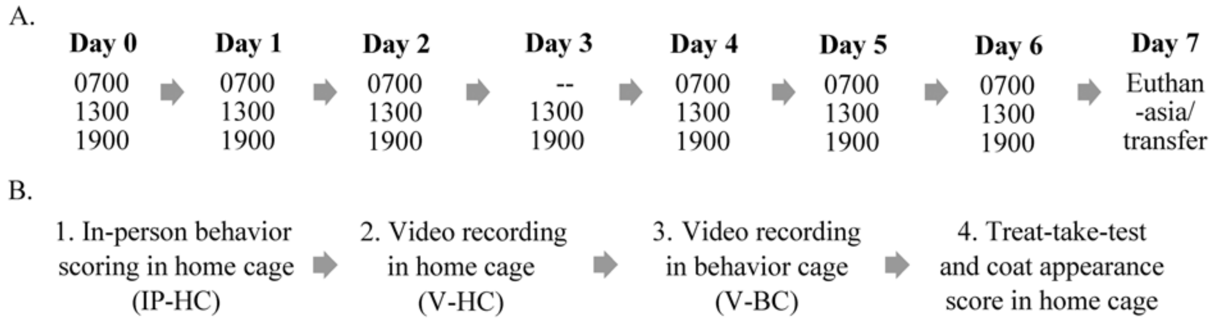

Figure 1. (A) The study timeline, with 3 time points daily. (B) The data collection procedure (steps 1 through 4) occurred sequentially at each time point. The data collection procedure did not occur at 0700 on day 3, because interventions occurred at that time point.

Scoring frequency of behaviors. The occurrence of behaviors was recorded at each timepoint for the 3 observation methods using the one–zero sampling method.[27] As in a study validating a postoperative ethogram in guinea pigs,[5] we used this sampling method because it allows us to accurately capture intermittently occurring behaviors and helps to maximize interobserver reliability. Behaviors were recorded during the first 30 s of each minute of the 5-min session. Behaviors that were observed during those 30-s intervals were scored as 1, and behaviors that were not observed were scored as 0. The number of occurrences of each behavior were then summed for a total frequency for each time point (that is, when a behavior was seen during the first 30 s of each minute for all 5 min, the total frequency for that time point was 5). One female scorer (AE) who was blind to the interventions performed all cageside ethogram scoring. A different female scorer (AM) who was blind to the cohort, intervention, day, and time performed all videorecorded ethogram scoring. However, the scorers could see the shaved abdomens and incisions of the operated animals. The scorers used the same ethogram, and videos of select behaviors were generated for reference to help maximize agreement in scoring.

Videorecording. Behavior was videorecorded by using a camcorder (file type, AVCHD; image size, 1920 × 1080 pixels; infrared mode for dark-cycle recording; model FDRAX53/B, 4K HD Video Recording Camcorder, Sony, Tokyo, Japan). For the V-HC method, the camera was placed on a tripod, above the home cage and pointed down at an angle to maximize the amount of visible cage space. For the V-BC method, the camera was on the counter at cage level.

TTT score. A high-value food item was presented by hand to a hamster in its home cage such that the animal had to rear on its hindlegs to obtain the item. Banana chips and parsley were used, based on the animal's preference, as they were found during the habituation period to be the most high-value items. The treat was held in front and above the hamster's head for 2 min. When the hamster took the treat within 2 min after presentation, the TTT score was recorded as 1 and deemed a positive test result. When the animal did not take the treat within 2 min, the TTT score was recorded as 0 and deemed a negative test result.

Coat appearance score. The coat appearance was scored on a scale of 0 to 3, with 0 indicating a normal coat, 1 denoting piloerection, 2 as piloerection and unkempt, and 3 as severely unkempt with porphyrin accumulation. This system is similar to that in a published study evaluating the efficacy of 2 analgesics for postoperative use in rats.[34]

Interventions. ***Laparotomy.*** Anesthesia was induced by using 3% to 5% isoflurane USP (Piramal Critical Care, Bethlehem, PA) delivered in pure oxygen into an induction chamber (Gas Anesthetizing Box type AB 2, 10 × 8 × 8 in., Braintree Scientific). Anesthesia was maintained by delivering 1.5% to 3% isoflurane via nose-cone to hamsters in dorsal recumbency on a clean absorbent blue pad placed over an external heat source. The respiratory rate, mucus membrane color, and depth of anesthesia (toe pinch) were recorded at least every 15 min. Standard aseptic preparation of the animals, surgeon, and instruments was performed, and a sterile drape was used to cover the surgical area. Negative toe pinch was confirmed before the surgeon created a 1-cm linear longitudinal incision through the skin and linea alba by using a no.15 scalpel blade and tenotomy scissors, with the center at the umbilicus. The incisions were closed by using monofilament polydiaxonone suture (PDSII, Ethicon, Cincinnati, OH) in a simple interrupted pattern for the body wall and an intradermal pattern for the skin, with the addition of tissue glue (Vetbond, 3M Animal Care Products, Saint Paul, MN) as needed for the skin. The entire procedure (from induction to recovery) lasted 30 to 45 min.

Anesthesia only. Animals were anesthetized, aseptically prepared, maintained, recovered, and monitored as described for the laparotomy group, with the entire procedure from induction to recovery lasting 30 to 45 min.

No intervention. The animals in the no-intervention group were brought to the room where surgeries were performed and returned to their housing room with the rest of the cohort.

Syrian Hamster Grimace Scale picture generation and data collection. This procedure was adapted from *Rat Grimace Scale (RGS): The Manual*.[45] Images of hamster facial expressions were analyzed from 9 animals that underwent laparotomy, with each serving as its own control. By using the PlayMemories Home software (Sony) Save Frame feature, the images were generated from the 5-min V-BC videos taken at 1300 the day before surgery (day 2) and at 1300 on the day of surgery (day 3; approximately 6 h after the procedure). An observer (AE) who was blind to which day the videos were taken, watched blinded videos and captured still images at 1-min intervals when at least one side of the face was visible and resolution was optimal. Ninety images were generated, comprising 5 'baseline' images from day 2 and 5 'postsurgery' images from day 3 for each animal. The images were cropped to exclude the body, were brightened, and were presented to scorers in a digital slide presentation in a blind and random fashion by the observer.

The facial features, termed 'action units' (AU), evaluated were: orbital tightening, whisker change, ear changes, and nose–cheek flattening. The scale was derived after initial observation of generated images and used elements as described in grimace scales developed for rats and mice.[22,45] Orbital tightening, whisker change, and ear changes were adapted from the Mouse Grimace Scale, and nose–cheek flattening was adapted from the Rat Grimace Scale. Normal hamsters had wide-open eyes, gently

Active behaviors	
Walk	Walk around cage, quadrupedal ambulation
Open rear	Stand on hindlegs with both forepaws off floor and walls
Supported rear	Stand on hindlegs with at least one forepaw touching wall or another surface
Manipulate bedding	Chew/manipulate/carry wood shavings or cardboard, place bedding in cheek pouch, could be nest modification
Hoard	Carry food or bedding to pile into nest or distinct hoard
Scrabble	Scraping with forepaws upward against wall while standing erect, usually includes one hindpaw
Dig	Scraping with forepaws directed at floor or wall
Gnaw/lick	Bite/lick at wall or wood block
Bite wire bars	Bite at wire bars
Eat	Chew/consume pellets, gnaw food, handle food with paws
Drink	Drink from water bottle
Groom	Rub forepaws over head, groom body, or scratch body with hindfoot
Shake	Sudden convulsive shake of whole body
Yawn and stretch	Lengthen body with mouth open
Scent mark	Press flank scent gland against a surface with an arched back, raised tail, distinct steps
Wire bar hang/climb	Hang/climb from wire bars, at least one hindlimb off floor
Coprophagy	Consume feces
Passive behaviors	
Pause	Pause immobile for >2 s, no other activity
In nest	Present in nest
Lay down	Sternal recumbency outside of nest
Lay flat	Lateral recumbency outside of nest
Sleep/rest	Motionless, lying on side or curled up, face tucked into body
Pain behaviors	
Groom incision	Groom abdominal incision
Back arch	Vertical stretch from crouched position with arched back (kyphosis)
Fall/stagger	Stagger or fall during ambulation, sudden crouch from rear or sudden loss of balance resulting in lateral recumbency, not associated with wire bar climbing
Twitch	Brief spasmodic contraction of back muscles
Writhe	Lateral contortion of flank abdominal muscles
Hind kick	Push back with both hindfeet at once
Dart	Moving suddenly and quickly
Hunched posture with head down	All 4 feet on floor, back arched with head lowered to floor, sustained (while not eating, grooming, sleeping, digging, interacting with bedding), may be while ambulating
Vocalize	Any audible vocalization
Shift	Shifting weight while standing still
Not categorized	
Urinate	Urination posture, stiffened with tail up, lordosis
Defecate	Defecation of feces

Figure 2. Behaviors that were recorded during observations are categorized. Because they are not specific to a category, urinate and defecate are not included in further analyses.

sloping whiskers, a distinct crease between their cheek and nose bulge, and slightly forward-facing ears. For orbital tightening, the palpebral fissure was narrowed, and hamsters appeared to be squinting. For whisker change, the whiskers stood out straight from the cheeks and appeared to clump together. For ear changes, the ears were folded and appeared narrower from the side and generally pointed caudally; as seen from the front, the ears were less visible or had tips that point laterally. For nose–cheek flattening, the separation (crease) between the nose and cheek area was less distinct, and the whole side of the face appeared to be flattened.

Four scorers were trained in SHGS and then evaluated hamster images. The scorers were 3 veterinary technicians (BG, AS, KF) with experience assessing animals for pain and 1 board-certified laboratory animal veterinarian (JH), all of whom are female. They were trained on the AU and score severity in a 30-min session using representative photos and received 3 photos for practice scoring. During the scoring sessions, scorers were given representative photos and written descriptions of the severity of each AU. Scorers rated the presence and intensity of each AU on a scale of 0 to 2 for practice images and the 90 experimental images. A score of 0 meant that the AU was not present, a score of 1 denoted that the AU was moderately visible, and a score of 2 was that the AU was pronounced. The total SHGS score for an image was the average of the intensity ratings for each AU. For each image, scorers assessed the animal as painful or not painful (pain–no pain). The reliabilities of the SHGS and the individual AU were determined via calculation of intraclass correlation coefficient (ICC), which quantifies the agreement among the scorers.[44]

Statistics. To assess the behavior frequency, we calculated the average frequency across behaviors for each animal on each day for each method separately. To assess changes in frequency after intervention, we computed the change from the average frequency before intervention for each after-intervention time point and compared the changes of hamsters that underwent surgery with those that did not by using Wilcoxon tests. To assess the utility of pain behaviors to identify animals in the surgery group, the sensitivity and specificity of using at least one pain behavior was computed, for each time point and for each method separately. To evaluate the SHGS and individual AU, ICC were computed and compared between scorers on a per-image basis and on a per-time point basis (averaging the 5 images per time point). The overall accuracies for the pain and control groups were computed. In addition, the change in SGHS from baseline to after surgery was computed, and the sensitivity and specificity of using SGHS to identify painful animals was computed overall and for each scorer. To assess the effect of an intervention on TTT, the average TTT score per group was computed before and after each intervention, both overall and by sex and time. A P value of less than 0.05 was used to define statistical significance.

Results

Frequency of behaviors. Initial assessment of the behavior frequency data did not reveal particular behaviors that were more promising candidates for indicating pain than others. We compared the frequency of pain, active and passive behaviors at each post-interventional time point with the baseline frequency (the average of days 0 to 2) for each group. The surgery group was significantly different (i.e., $P < 0.05$) from the other groups on changes from baseline in pain behaviors (Figure 3) and passive behaviors (Figure 4). These differences were not present at all time points or with all observation methods. No changes in active behaviors were detected for any of the groups. The V-HC method did not reveal statistically significant differences in any group.

The sensitivity and specificity of observing at least one pain behavior to identify hamsters that underwent surgery were evaluated (Figure 5). The specificity, which is the proportion of those in the non-surgery group with no pain behaviors, was relatively high (greater than 95%) for the V-BC and IP-HC methods at almost all time points. The sensitivity, which is the proportion of those in the surgery group with at least one of these behaviors, of the V-BC and IP-HC methods was considerably lower (50% or less) at almost all time points. Because we rarely observed pain behaviors by using V-HC, we did not analyze those data.

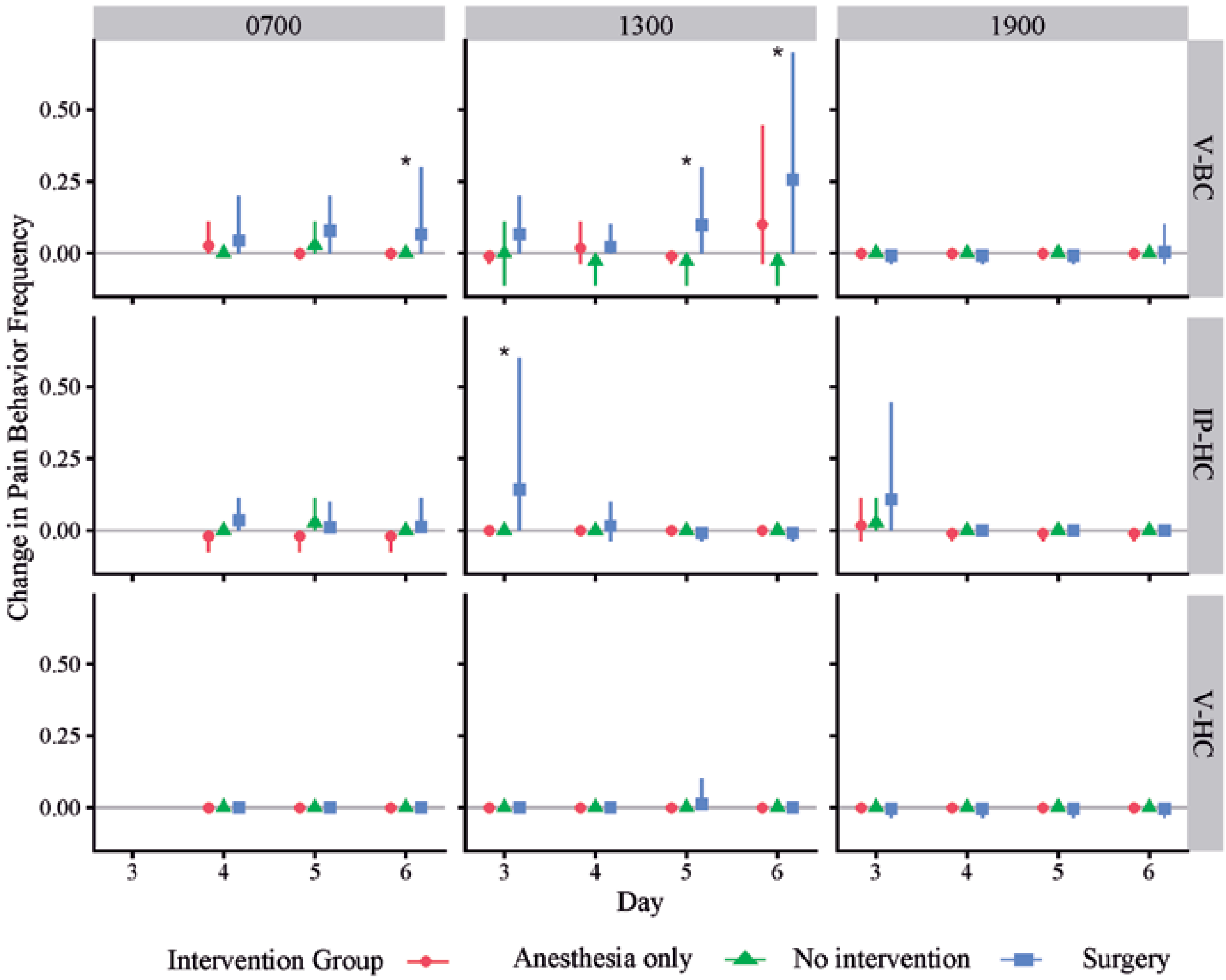

Figure 3. The change in the frequency (mean and range) of pain behaviors after intervention from the baseline frequency (average of days 0 through 2) for each animal at each timepoint is shown, by method of observation and time of day. *, Value differs ($P < 0.05$) between the surgery group and the anesthesia-only and no-intervention groups.

Some patterns in behavior frequency were seen depending on the observation method and time of day but were not examined for statistical significance. Animals generally exhibited more active and fewer passive behaviors with the V-BC method. More active behaviors were observed at 1900 than at either 0700 or 1300 across all methods. Animals in home cages generally had more passive behaviors at 1300 than at 0700 or 1900.

Postsurgical complications. The first hamster that underwent laparotomy sustained iatrogenic skin removal from a 1×0.5-cm region on the medial aspect of the right hindleg, due to attempted removal of skin glue. The animal frequently groomed the area through day 4. The dermis remained intact and a dry scab developed by 0700 on day 4. The animal appeared to favor this limb slightly and was touch-sensitive at the affected area on days 3 and 4. By day 6, the lesion was healed almost completely, with only a 4-mm linear scab present. Daily averages showed that this hamster had the highest frequency of pain behaviors on days 3 and 4 based on the IP-HC method. The animal did not have an increase in SHGS after surgery. The amount of skin glue was reduced for the remaining surgeries. An animal was added to the third cohort, for a total of 5 hamsters.

One animal in the surgery group had a bruised toe on the fifth digit of the left hind paw after escaping from its cage on the morning of day 6. On clinical examination by the area veterinarian, the animal did not appear to be in pain and did not receive analgesics. Based on daily averages, this animal had the highest frequency of pain behaviors on day 6 based on the V-BC method and was the only animal that showed pain behaviors on that day according to the IP-HC method.

SHGS. An increase in the SHGS score from baseline to postsurgery of at least 46% (range, 46% to 595%) was seen in 3 (animals C, F, and G) of the 9 hamsters (Figure 6). The scores for AU had a similar pattern (Figure 6). The average SHGS score for the surgery group was 0.48 (range of 0.20 to 1.26) for baseline and 0.70 (range of 0.19 to 1.84) after surgery. Using a SHGS score of 1.25 or higher to identify painful animals produced a specificity of 94% and a sensitivity of 28%. The threshold score of 1.25 was based on receiver operating characteristic curve analysis. The individual scorers' specificity ranged from 89% to 100%, and sensitivity ranged from 22% to 33%. The interrater reliability of the SHGS on a per-image basis was represented by an ICC of 0.79. The ICC of each AU on a per-image basis was 0.87 for orbital tightening, 0.55 for whisker change, 0.68 for ear changes,

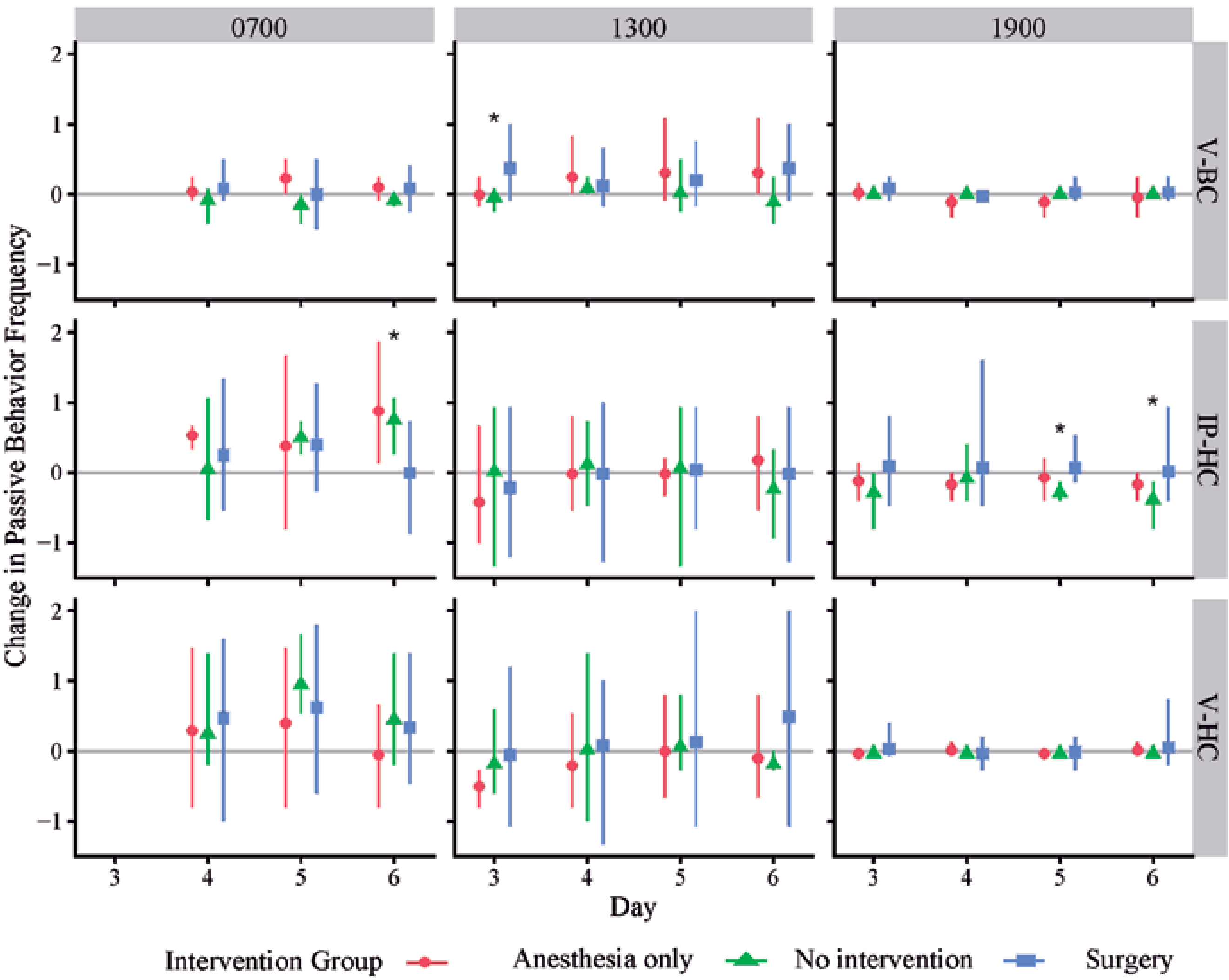

Figure 4. The change in the frequency (mean and range) of passive behaviors after intervention from the baseline frequency (average of days 0 through 2) for each animal at each time point is shown, according to method of observation and time of day. *, Value differs ($P < 0.05$) between the surgery group and the anesthesia-only and no-intervention groups.

and 0.58 for nose-cheek flattening. On a per-time point basis, the SHGS ICC was 0.90. The ICC of each AU on a per-time point basis was 0.94 for orbital tightening, 0.63 for whisker change, 0.82 for ear changes, and 0.78 for nose–cheek flattening. For the pain–no pain determination, the scorers had a 31% accuracy rate for postsurgery images and a 91% accuracy rate for baseline images.

The 3 hamsters with the largest increases in SHGS from baseline to postsurgery showed pain behaviors on days 3 and 4 with the IP-HC method and on day 6 with the V-BC method. However, these 3 hamsters also showed pain behaviors before surgery; one showed pain behaviors on day 2 via the IP-HC method, and the other 2 showed pain behaviors on day 0 via the V-BC method.

TTT score. The TTT scores were averaged across all animals in each intervention group. In the surgery group, 76% and 79% of hamsters had positive scores on days 0 through 2 and days 3 through 6, respectively. In the anesthesia-only group, 69% and 70% of hamsters had positive scores on days 0 to 2 and on days 3 to 6., respectively. In the no-intervention group, 78% and 70%, respectively, scored positively on days 0 to 2 and on days 3 to 6. Averaged over the whole testing period, 95% of hamsters had positive scores at 0700, 98% at 1300, and 35% at 1900. Averaged over all time points, 83% of female hamsters and 66% of males scored positively. For the 1900 time point, 56% of females scored positively, as compared with 12% of male hamsters.

Body weight, water consumption, and coat appearance score. The body weight and water consumption data were too variable to determine the effect of intervention on these values. Therefore, these data were not analyzed. The coat appearance score was always 0.

Discussion

The hypothesis that the frequency of behaviors, grimace scale, TTT score, body weight, water consumption, and coat appearance would change from baseline in the surgery group but not in no-intervention and anesthesia-only groups was found to be true for the frequencies of pain and passive behaviors and for SHGS. Our analysis indicated that these measures have high specificity and low sensitivity for the identification of pain, thus suggesting that these methods are not useful as screening tests for pain and have limited utility for identifying postoperative pain in Syrian hamsters.

The surgery group was significantly different from the other groups in change from baseline for the pain or passive behaviors at several time points, but these changes were modest, sporadic,

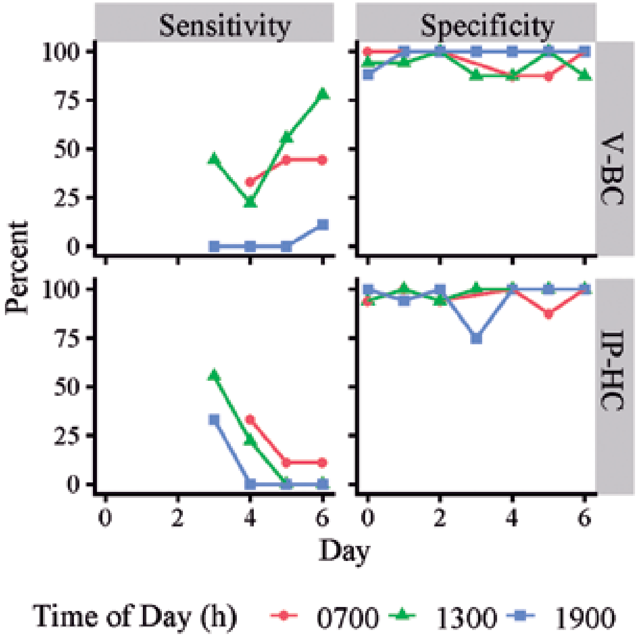

Figure 5. The sensitivity and specificity of observing at least 1 pain behavior identifying a hamster in the surgery group is shown for the V-BC and IP-HC methods over the course of the study. Sensitivity and specificity are not shown for 0700 on day 3 because interventions were performed at that time.

and inconsistent between animals, time points, and methods, and therefore were unlikely to be adequate for clinical use. We saw an increase in pain behaviors from baseline for hamsters in the surgery group (significantly different than the control groups) on day 3 with the IP-HC method, and on day 5 and 6 with the V-BC method. The change in frequency of passive behaviors from baseline for hamsters in the surgery group was significantly different from the control groups on day 3 with the V-BC method, and day 5 and 6 with the IP-HC method. In previous studies by others, rats showed behavioral evidence of pain for 2 d after laparotomy,[34] guinea pigs and rats showed behavioral evidence of pain chiefly within the first 24 h after surgery,[5,6,20] and rabbits showed behavioral evidence of pain for 3 d after surgery.[23] Although 2 studies reported higher grimace scores and lower tail-flick thresholds in rodents at night (when they were more active) than during daytime,[28,29] our study did not yield consistent evidence of increased pain at night.

Hamsters with postsurgical complications had the highest frequency of pain behaviors in the surgery group on the days that the complications occurred. Because the main purpose of this study was to identify methods to identify pain in Syrian hamsters and address the paucity of literature in this area, we decided to report our findings even though they may complicate our interpretation of laparotomy pain. Even with the inclusion of these animals in the data, the frequency changes identified by using our methods were of minimal clinical use. Further work investigating whether these or other nonevoked methods are effective in identifying and mitigating pain other than that experienced due to a simple laparotomy incision could be useful.

We chose our 3 methods of observation on the presumption that exploration of a barren environment (such as the behavior cage) and the presence of an observer would affect the behavior frequency. The behavior frequency differed depending on the method of observation, which we believe contributed to the differences in sensitivity between IP-HC and V-BC. The differences between these methods may have been due to interobserver variability, despite efforts to minimize this effect. The ability to pause and review videos may have allowed the V-BC observer to recognize more subtle behaviors than could be seen in real time. The presence of an observer at cage level might have reduced the sensitivity of the IP-HC method. This finding suggests that Syrian hamsters (as a prey species) hide their pain in the presence of an observer, which is comparable to responses in guinea pigs and rabbits.[5,23,35]

Similar to behavior frequency, the grimace scale was not effective for identification of postoperative pain in Syrian hamsters. SHGS had a low sensitivity for identifying postoperative hamsters: an increase in SHGS score occurred in only 3 of the 9 postsurgical animals. One possibility is that the laparotomy did not produce sufficient discomfort. The lack of abnormal coat appearance identified during this study may be consistent with this theory. Testing the utility of a grimace scale for identifying joint or visceral pain of moderate duration may reveal different results, given that previous work showed variable utility of the grimace scale in mice depending on the pain model tested.[24] Individual hamsters showed considerable variation in how each animal responded to surgery and between images taken within the same 5-min period, indicating that hamsters differed individually and temporally in their propensity to grimace. We expected to see an increase in SHGS at 5 to 6 h after laparotomy, although peak grimace scale scores of rats in a previous study peaked after 4 h.[45] In addition, the videos of our hamsters were shorter than those used in other studies to develop grimace scales.[22,45] Had we generated more videos or collected them earlier, our findings might have been different. Our method was intended to gather images to provide an evenly distributed representation of expressions during a 5-min period. The SHGS did have high interrater reliability, especially when considering multiple images taken at a particular time point. The SHGS ICC value on a per-image basis is only slightly lower than in other publications for various species.[19,22,45] Consistency between scorers was highest for orbital tightening and lowest for whisker change, but the scores for each individual AU were similar as compared to the SHGS. The correlations between animals that showed an increase in SHGS and those that exhibited pain behaviors strengthened the argument that the SHGS was highly specific. SHGS images were captured from V-BC videos, which were collected with an observer across the room. Concealing pain behaviors from an observer may have contributed to the low SHGS sensitivity and suggests that using the SHGS at cage level may not be sensitive enough to identify painful hamsters, although this remains to be tested. The literature shows mixed results in terms of assessing grimace scores by direct observation, with some reports of success in evaluating rats but less success in mice.[24,31] Despite the low sensitivity of SHGS, specificity was high, suggesting its potential for utility after further refinement.

Our proxy indicator, the TTT score, was not useful in identifying postoperative pain in Syrian hamsters. The surgery did not affect the hamsters' propensity to accept the offered treat, suggesting that the hamsters were highly motivated to perform this behavior. Similar results were found for guinea pigs offered a high-value treat after surgery.[5,35] TTT scores were influenced by the time of day and sex but not by intervention group. Therefore, we do not advocate using the TTT as a diagnostic test to evaluate pain in Syrian hamsters after laparotomy.

The study had several pitfalls. The first was that, due to the presence of many enrichment items in the home cage, the hamsters often were not visible for the V-HC method. The

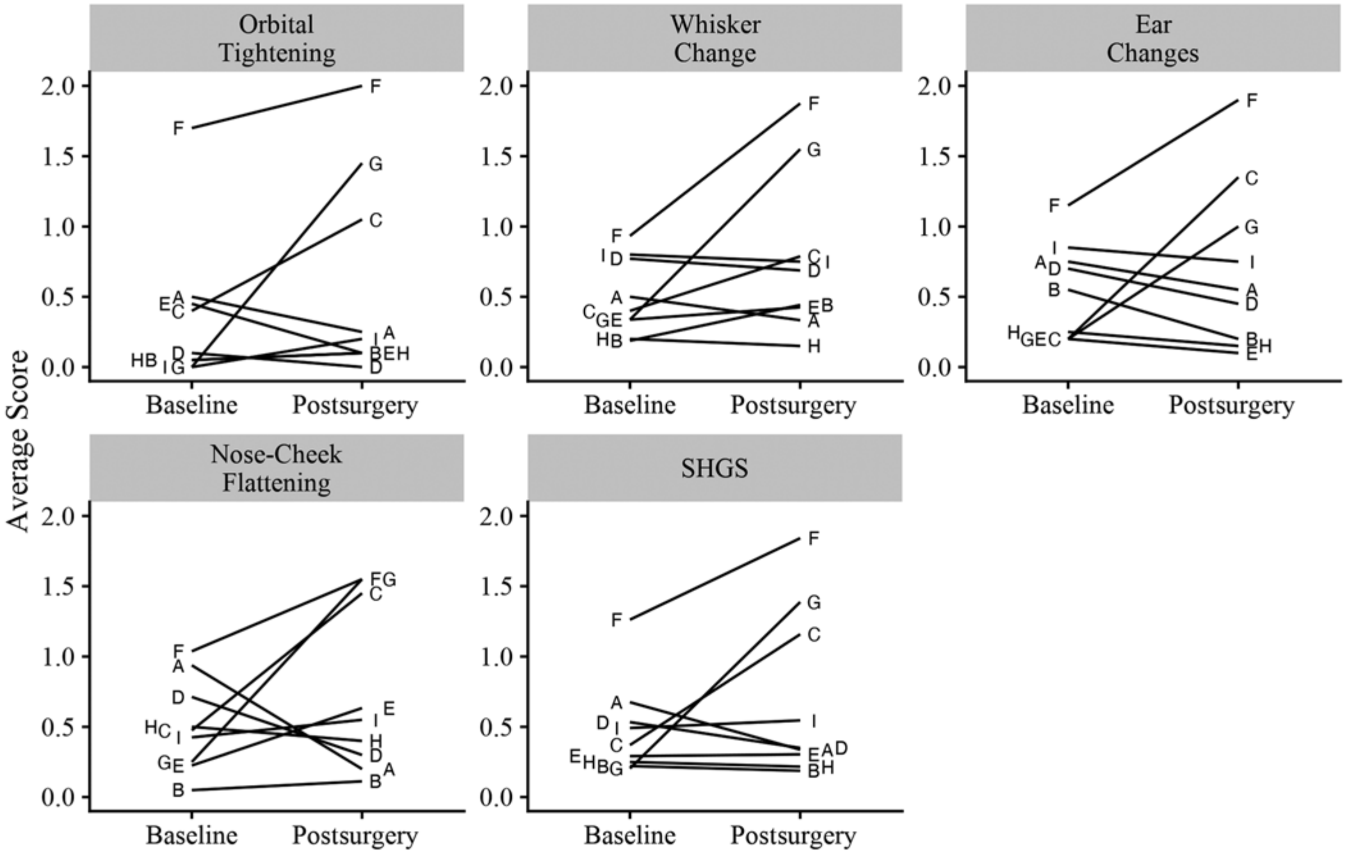

Figure 6. Individual action unit and SHGS scores of individual animals (A through I) are shown. Each point denoted by a letter is the average score of that animal's 5 images for each time point. Values for the day before surgery (baseline) and 6 h after surgery (postsurgery) are shown.

environmental enrichment may have improved animal welfare but often obstructed the video camera view. The poor visibility of behavior made it difficult to compare the V-HC method with IP-HC and to evaluate the effect of the observer on the behavior of the hamsters in their home cages. The presence of an observer in the room for the V-BC method might also have affected the SHGS findings, as mentioned earlier. Furthermore, due to the shaved abdomens, the ability to blind the observers for assessment of behavior frequency was limited. Another pitfall of the study was the variability of the body weight and water consumption data. Body weight variability was likely due to the presence of feed in the cheek pouches, which was not removed to avoid handling-associated stress. The water bottles had to be removed from the cages to access the animals; this practice caused water spillage from the bottles, and therefore artificially increased the apparent water consumption for a given day and likely contributed to the variability in these data.

In conclusion, our findings suggest that behavior frequency, SHGS, TTT, body weight, water consumption, and coat appearance were ineffective for evaluating postoperative pain in Syrian hamsters. Procedures that are presumed to be painful warrant the provision of analgesia, despite the lack of indicators of pain. Although pain-related behaviors were observed infrequently, they were seen intermittently for as long as 3 d after laparotomy. We therefore will continue our institution's practice of recommending the administration of analgesics for 3 d after an invasive surgery. Exploration of methods to evaluate pain and analgesic efficacy in Syrian hamsters in a research setting are still needed, especially assessments that remove the influence of an observer.

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