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Evaluation of Cage Mate–induced Postsurgical Trauma in Mice

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Although mice are social animals, individual housing is sometimes requested after surgery. We questioned whether pair-housing mice after surgery resulted in greater trauma to the surgical site as compared with single housing. We further evaluated the effect of individual housing after surgery on the wellbeing of mice that had previously been pair-housed. Female C57Bl/6 mice (age, 6 to 8 wk) were housed as follows: group A, individually housed before and after surgery (*n* = 10; all 10 mice underwent surgery); group B, pair-housed before surgery but individually housed after surgery (*n* = 10; all 10 mice received surgery); group C, pair-housed before and after surgery (*n* = 20; 10 mice underwent surgery but their cage mates did not); and group D, pair-housed before and after surgery (*n* = 10; all 10 mice underwent surgery). Dependent variables were body weight, body condition, grimace based on real-time scoring, nest building, time to incorporate into nest test (TINT) score, wound trauma score, and missing wound clips. Weight was significantly different between groups A and C both before and after surgery. Mean nest building scores were significantly higher for pair-housed (groups C and D) than for individually housed mice (groups A and B) after surgery while TINT scores were significantly higher for these same groups both before and after surgery. Mean values for body condition, grimace score, wound score, and number of wound clips missing did not differ significantly between any groups either before or after surgery. Taken together, these results suggest that pair housing mice after surgery benefited their wellbeing but did not increase trauma to the surgical incision site or disturb wound clips as compared with individually housed mice. Furthermore, separating previously pair-housed mice (group B) did not affect these measures as compared with individually housed mice (Group A) either before or after surgery.

Abbreviation: TINT, time to incorporate into nest test

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Introduction

Mice have been used as models for a variety of applications in biomedical research. In some cases, these models involve surgical manipulations. For example, surgical manipulation has been used extensively to study cardiovascular disease and orthotopic tumor implantation.[9,16,22,27] Surgical models require the use of aseptic technique and the provision of anesthesia, analgesia, and postoperative care that facilitates recovery of the animal and healing of the surgical wound.

Although mice are social animals, investigators sometimes request individual housing after surgery for mice that had been pair- or group-housed before surgery. One reason for this request is concern that cage mates may traumatize the incision site after surgery. However, no published data are available to support this belief, and these assertions are largely anecdotal or based on conjecture. Furthermore, little consideration is given to the dual effects of surgery and individual housing during postsurgical recovery on mice that had been pair or group-housed before surgery.

One study[25] reported that mice housed with an unoperated cage mate recovered better ("were less affected") in terms of behavioral and physiologic parameters after intraabdominal implantation of a telemeter as compared with individually housed mice. In that study,[25] skin incisions were closed with absorbable suture. The authors did not specifically comment on trauma to the incision site, other than to note an increase in self-grooming by individually housed mice. Results by others using a sham surgical embryo transfer procedure and wound closure with suture found little difference between socially housed and pair-housed mice in terms of behaviors associated with wellbeing, nor was any manipulation of the wound by cage mates noted.[10] In that study,[10] mice were housed either individually or in pairs both before and after surgery, thus preventing assessment of social separation initiated after surgery. Because investigators often initiate individual housing at the time of surgical recovery, we performed the current study to evaluate the effects of the combined initiation of postsurgical recovery and social separation.

Materials and Methods

Experimental preparation. All procedures were conducted in an AAALAC-accredited facility in accordance with standards described in the Guide *for the Care and Use of Laboratory Animals*[8] after review and approval by the University of Kentucky IACUC.

Female mice (C57Bl/6; age, 6 to 8 wk) were purchased from a commercial vendor (Envigo, Indianapolis, IN) and housed individually or in pairs for 1 wk prior to surgery. Mice were housed in individually ventilated 1145T microisolation caging (Techniplast, West Chester, PA), with Sani-Chip bedding Teklad 7115 (Envigo, Indianapolis, IN), were fed 2918 Teklad

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Irradiated Global 18% Protein Extruded Rodent Diet (Envigo, Madison WI), and received reverse-osmosis–purified water via automatic watering system. Rooms were maintained at 67 to 77 °F (19 to 25 °C) with relative humidity of 30% to 70%, and a 14:10-h light:dark cycle (on at 07:11 hours and off at 21:11 hours). Mice were free of common pathogens (viral, bacterial, and parasitic) based on vendor (Envigo) reports. Pair-housed mice were identified individually by marking the tails using a nontoxic marker. Each cage contained approximately 8 to 10 g of crinkled-paper nesting material (Enviro-Dri, Shepherd Specialty Papers, Kalamazoo, MI) and cotton nesting squares (Nestlets, Ancare, Bellmore, NY).

Experimental surgery. Mice were evaluated before surgery to ensure normal overall health, activity, and body condition. Each mouse assigned to surgery received a dose of Buprenorphine SR-Lab (1.0 mg/kg SC; Zoopharm, Fort Collins, CO) immediately before induction of anesthesia. Isoflurane (1% to 4%) was used for induction and maintenance of surgical anesthesia via a rodent anesthesia machine (Multi-Station Lab Animal Anesthesia Machine, Surgivet, Saint Paul, MN) and vaporizer (Ohmeda Isotec, Madison, WI). Immobility and lack of response to firm toe pinch were used to ascertain that a surgical plane of anesthesia had been reached. After aseptic preparation, a full-thickness, 3-cm skin incision was made on the dorsal midline to expose the underlying adipose and muscle for approximately 30 s. The incision was then closed with three 9-mm stainless steel wound clips (Braintree Scientific, Braintree, MA). During recovery from anesthesia, mice were placed in a cage with clean bedding that was in turn placed on a circulating water heating pad. Once ambulatory, mice were returned to the home cage appropriate to the treatment group (that is, single- or pair-housed).

Experimental design. Mice were housed as follows before and after surgery: group A, individually housed before and after surgery (n = 10 all having received surgery); group B, pair-housed before surgery and individually housed after surgery (n = 10 all having received surgery); group C, pair-housed before and after surgery (n = 20, 10 having received surgery and 10 not having received surgery); and group D, pair-housed before and after surgery (n = 10 all having received surgery).

Mice were evaluated daily by one of three trained individuals for the duration of the study to assess overall health, body condition and the integrity of the incision. Blinding was not possible due to lack of sufficient personnel. Seven dependent variables were measured. First, weight was measured in grams; each mouse was removed from its home cage and placed on digital scale (fitted with a plastic container) that had been tared to zero. Second, to score body condition: each mouse was observed during weighing to assign a body condition score using a scale from 1 (emaciated) to 5 (obese), with 3 representing a well-conditioned mouse.[24] Third, to assign facial grimace scores,[12] each mouse was observed in its home cage in real time and assigned a score of 0, 1, or 2 for each Facial Action Unit.[15] Fourth, nest building was evaluated cageside and scored from 0 for undisturbed nesting material to 5 for a fully domed nest.[5,7] Fifth, to determine the time to incorporate nest material (TINT),[4] a small (1 × 1 cm) piece of cotton square was added to each cage and a digital timer was started. After 10 min, each cage was evaluated to determine whether the nesting material had been incorporated into a resting–nesting area. A TINT score of 1 was assigned to mice that had incorporated the material into their nest within the established time limit, and a TINT score of 0 was assigned to mice that did not.[4] Sixth, wounds were evaluated based on edge apposition, redness, swelling, and discharge by using a modification of a previously described scale[2] and were scored from 0 (ideally healing wound with edges in apposition) to 4 (severe redness, swelling, discharge, or dehiscence) Table 1. Seventh, wound clips were evaluated in terms of missing or disrupted clips and were assigned one of the following scores: 0, all wound clips intact; 1, one wound clip missing or disrupted; 2, two wound clips missing or disrupted; 3, all three wond clips missing or disrupted. Mice were euthanized at the end of the study (2 wk after surgery) using CO2 as recommended in the *AVMA Guidelines for the Euthanasia of Animals*.[3]

Data analysis. Data were analyzed by using 2-way ANOVA with an α level of 0.05. The Tukey multiple comparisons test was performed to obtain detailed information on differences among the various study groups. When data values were missing, the analysis was performed by fitting a mixed model rather than repeated-measures ANOVA. All data were analyzed by using

Table 1. Postsurgery wound scoring

Wound score	Wound description
0	Perfectly healing wound, edges in apposition.
1	Mild redness or swelling around the wound.
2	Moderate redness or swelling or discharge or exposed subcutis.
3	Severe redness or swelling or discharge. Partial opening of the wound.
4	Severe redness or swelling or discharge. Complete opening of the wound.

Modified from https://www.pure.ed.ac.uk/ws/portalfiles/portal/55207565/s12917_018_1378_3.pdf

Table 2. Descriptive statistics for variables related to wellbeing

		Group			
		A (n = 10)	B (n = 10)	C (n = 20)	D (n = 10)
Weight (g)					
	Mean	22.4	22.0	21.0	21.9
	1 SD	1.4	1.8	1.2	1.5
Body condition score					
	Mean	3.0	3.0	3.0	2.9
	1 SD	0.2	0.2	0.00	0.2
Grimace score					
	Mean	0.0	0.1	0.0	0.0
	1 SD	0.18	0.22	0.07	0.15
Nest-building score					
	Mean	3.4	3.5	3.8	3.8
	1 SD	1.2	1.1	1.1	1.1
TINT score					
	Mean	0.4	0.5	0.9	0.9
	1 SD	0.5	0.5	0.3	0.3
Wound score[a]					
	Mean	0.2	0.1	0.0	0.0
	1 SD	0.4	0.3	0.2	0.0
Wound clip score[a]					
	Mean	0.1	0.1	0.2	0.0
	1 SD	0.3	0.3	0.7	0.1

Two-way ANOVA with an α level of 0.05. Tukey multiple comparisons test. When data values were missing, the analysis was performed by fitting a mixed model rather than repeated-measures ANOVA. All data are presented as mean ± 1 SD.
[a]Values for group C are based on n = 10

Prism (GraphPad Software, San Diego, CA), and results are presented as mean ± 1 SD (Table 2).

Results

Body weight. Intragroup weight data analysis revealed no statistically significant within-group differences in weights measured before and after surgery.

The mean body weight of all mice prior to surgery was 21.2 ± 1.1 g. The mean body weights for each of the 4 groups were: A, 21.6 ± 1.0 g; B, 21.5 ± 1.4 g; D, 21.3 ± 1.2 g. and C, 20.4 ± 0.8 g.

After surgery (experimental day 2), mice from all groups had a combined mean body weight loss of 0.96 g (5%). Losses for each group were as follows: D, 1.3 g (6%); A, 1.2 g (5%); B, 1.0 g (5%); and C, 0.3 g (2%). All mice returned to or surpassed their presurgical body weight between experimental day 3 (group C) and 4 (groups A, B and D). The mean recovery of the combined groups after reaching their lowest postsurgery weights was 2.9 g (17%) for the combined groups. Values for each group were: A, 17%; D, 15%; B, 14%; and C, 11%.

The mean body weight for all groups at the end of the study (experimental day 14) was 21.9 ± 1.5 g, with group values as follows: A, 22.4 ± 1.4 g; B, 22.0 ± 1.9 g; D, 22.0 ± 1.5 g; and C, 21.1 ± 1.2 g.

Overall mean body weight for all data combined across days was 21.8 ± 1.5 g. Group values were as follows: A, 22.3 ± 1.4 g; B, 22.0 ± 1.8 g; D, 21.9 ± 1.5 g; and C, 21.0 ± 1.2 g. A statistically significant difference in postsurgical mean body weight was detected between groups A and C ($P = 0.0154$), but significant differences were not identified between the other groups (Figure 1).

Body condition scores. As a measure of general health, body condition was scored daily by using an established system.[24] Mean body condition scores (Figure 2) did not exhibit a statis-

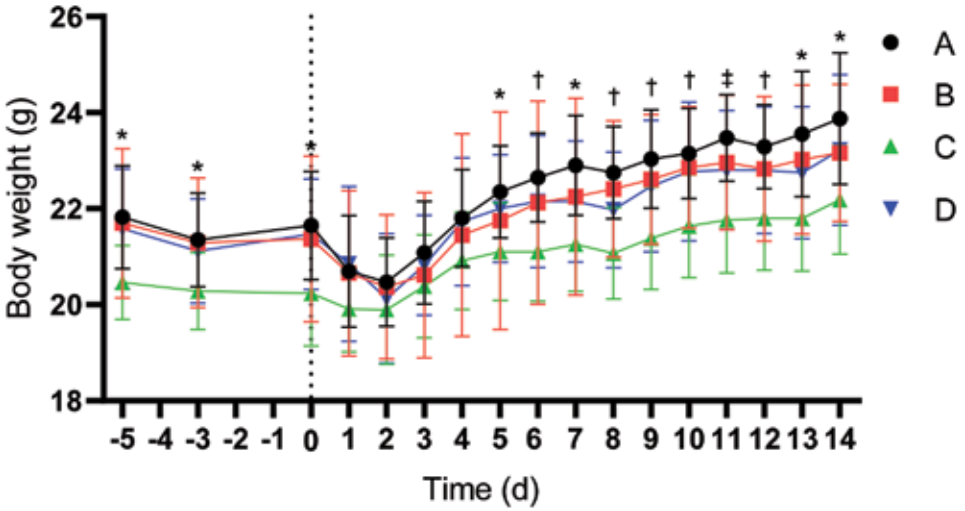

Figure 1. Body weight (mean ± 1 SD) values over time. Group A mice were single housed before and after surgery ($n = 10$ all having received surgery); group B mice were pair-housed before but single-housed after surgery ($n = 10$ all having received surgery); group C animals were pair-housed before and after surgery ($n = 20$, 10 having received surgery and 10 did not undergo surgery); and group D mice were pair-housed before and after surgery ($n = 10$ all having received surgery). Symbols indicate statistically significant differences (*$P \leq 0.05$; † $P \leq 0.01$; ‡ $P \leq 0.001$) between groups A and C at different time points. The vertical dotted line indicates the day of surgery (day 0).

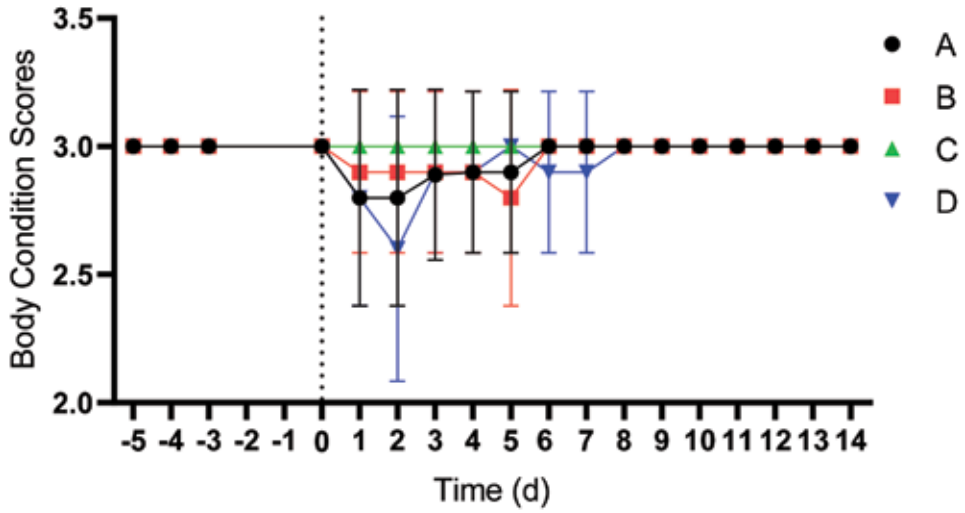

Figure 2. Body condition (mean ± 1 SD) values over time. Group A mice were single housed before and after surgery ($n = 10$ all having received surgery); group B mice were pair-housed before but single-housed after surgery ($n = 10$ all having received surgery); group C animals were pair-housed before and after surgery ($n = 20$, 10 having received surgery and 10 did not undergo surgery); and group D mice were pair-housed before and after surgery ($n = 10$ all having received surgery). No statistically significant difference was detected.

tically significant difference among groups ($P > 0.05$). Before surgery, the mean body condition score for all mice was 3.0. After surgery, mean body condition score for all mice was 3.0 ± 0.2, with group values as follows: C, 3.0; B, 3.0 ± 0.2; A, 2.9 ± 0.2; and D, 2.9 ± 0.2. Overall mean body condition score for all data combined was 3.0 ± 0.1, with group values as follows: C, 3.0 ± 0.0; B, 3.0 ± 0.2; A, 3.0 ± 0.2; and D, 2.9 ± 0.2.

Facial grimace scores. To assess the degree of distress experienced by mice, mean facial grimace scores were determined before surgery and during the postsurgical recovery period. Before surgery, mean grimace score for all groups combined was 0.1 ± 0.2; values for individual groups were as follows: D, 0.1 ± 0.3; B, 0.1 ± 0.2; A and C, both 0.0 ± 0.2. After surgery, the mean grimace score for all groups combined was 0.0 ± 0.1, with group scores as follows: B, 0.0 ± 0.2; A, 0.0 ± 0.2; D, 0.0 ± 0.2; and C, 0.0 ± 0.0.

Overall mean grimace scores (Figure 3) showed no statistically significant difference between groups ($P = 0.0628$). Overall mean grimace score for all data combined was 0.2 ± 0.1; individual group scores were as follows: B, 0.0 ± 0.2; A, 0.0 ± 0.2; D, 0.0 ± 0.1; and C, 0.0 ± 0.1). All mice had returned to the presurgery score of 0.1 by experimental day 2. Two mice in group B had positive grimace scores before surgery while they were still pair-housed and after postsurgical institution of individual housing (3 mice). Two mice in group C had positive grimace scores before surgery; one of these mice did not undergo surgery.

Nest-building scores. Nest building is a normal behavior practiced by mice both in the wild and in captivity. As such, the quality of the nest can be used as a measure of wellbeing, and specific scoring systems for nest-building quality have been developed.[5,7] In the current study, the mean nest-building score in the presurgery period for all groups combined was 3.2 ± 0.8; scores for individual groups were as follows: D, 3.5 ± 0.8; A, 3.2 ± 0.9; B, 3.1 ± 0.8; and C, 3.0 ± 0.9.

After surgery, the mean nest-building score for all groups combined was 3.7 ± 1.1, with values for individual groups as follows: C, 3.9 ± 1.0; D, 3.8 ± 1.2; B, 3.5 ± 1.2; and A, 3.4 ± 1.2. All mice returned to or surpassed their presurgical mean nest building score on experimental day 1 (group C) or 2 (groups A, B, and D).

The overall mean nest-building score for all data combined was 3.6 ± 1.1, with individual group scores as follows: D, 3.8 ± 1.1; C, 3.8 ± 1.1; B, 3.4 ± 1.1; and A, 3.4 ± 1.1. Mean nesting scores (Figure 4) exhibited statistically significant differences between groups A and C, A and D, B and C, B and D (all $P < 0.0001$), whereas no statistically significant difference was identified between groups A and B or groups C and D.

TINT scores. The TINT score allows evaluation of animal wellbeing by measuring how fast mice integrate additional nesting material into their existing nest. Before surgery, the mean TINT score for all groups combined was 0.9 ± 0.2, with individual group means as follows: C and D, 1.0 ± 0.0; B, 0.8 ± 0.4; and A, 0.6 ± 0.5.

After surgery, the mean TINT score for all groups combined was 0.7 ± 0.4, with individual group scores as follows: C, 0.9 ± 0.3); D, 0.9 ± 0.3; B, 0.5 ± 0.5; and A, 0.4 ± 0.5. Values returned to or surpassed their presurgical mean core on experimental day 3 (groups C and D) or 4 (group A). Group B never regained the mean presurgery TINT score.

The overall mean TINT score for all data combined was 0.7 ± 0.4; individual group scores were as follows: C, 0.9 ± 0.3; D, 0.9 ± 0.3; B, 0.5 ± 0.5; and A, 0.4 ± 0.5. Overall mean TINT score results (Figure 5) showed statistically significant differences between groups A and C, A and D, B and C, and B and D (all $P < 0.0001$) but not between groups A and B or C and D.

Wound scores. To evaluate the effect of social housing status on surgical wound healing, incision sites were scored daily throughout the duration of the study. Mean wound scores (Figure 6) were not significantly difference between groups ($P = 0.1558$). After surgery, the mean wound score for all groups combined was 0.1 ± 0.2; individual group scores were as follows: A, 0.1 ± 0.4; B, 0.1 ± 0.3; C, 0.0 ± 0.2; and D, 0.0 ± 0.0.

Surgical wound clip scores. The integrity of the wound clip closure was evaluated in order to assess trauma to the surgical site. After surgery, the mean wound clip score for all groups combined was 0.1 ± 0.3; values for individual groups were as follows: C, 0.2 ± 0.6; A, 0.1 ± 0.3; B, 0.1 ± 0.3; and D, 0.0 ± 0.1. No significan differences in mean wound clip scores (Figure 7) were detected between groups ($P = 0.7306$).

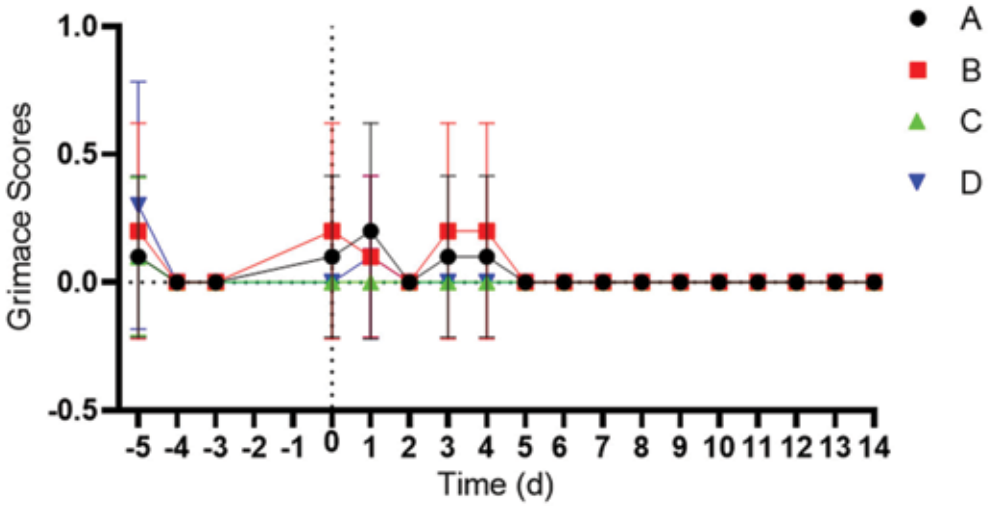

Figure 3. Grimace (mean ± 1 SD) values over time. Group A mice were single housed before and after surgery ($n = 10$ all having received surgery); group B mice were pair-housed before but single-housed after surgery ($n = 10$ all having received surgery); group C animals were pair-housed before and after surgery ($n = 20$, 10 having received surgery and 10 did not undergo surgery); and group D mice were pair-housed before and after surgery ($n = 10$ all having received surgery). No statistically significant difference was detected.

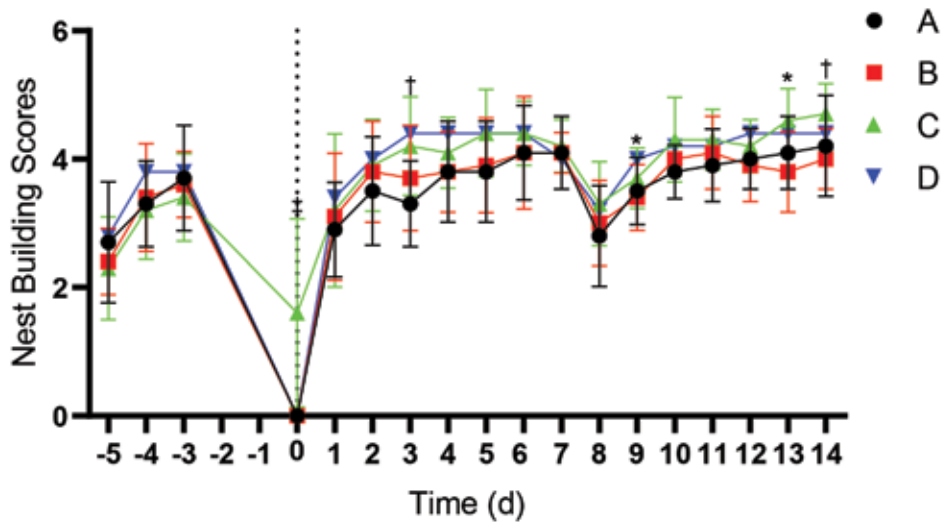

Figure 4. Nest-building (mean ± 1 SD) values over time. Group A mice were single-housed before and after surgery (n = 10 all having received surgery); group B mice were pair-housed before but single-housed after surgery (n = 10 all having received surgery); group C animals were pair-housed before and after surgery (n = 20, 10 having received surgery and 10 did not undergo surgery); and group D mice were pair-housed before and after surgery (n = 10 all having received surgery). Symbols denote statistically significant differences (*$P \leq 0.05$; † $P \leq 0.01$; ‡ $P \leq 0.001$) between groups A and C (days 6, and 9), A and D (day 9), B and C (days 6, 19, and 20), B and D (day 15), and C and D (days 6 and 15).

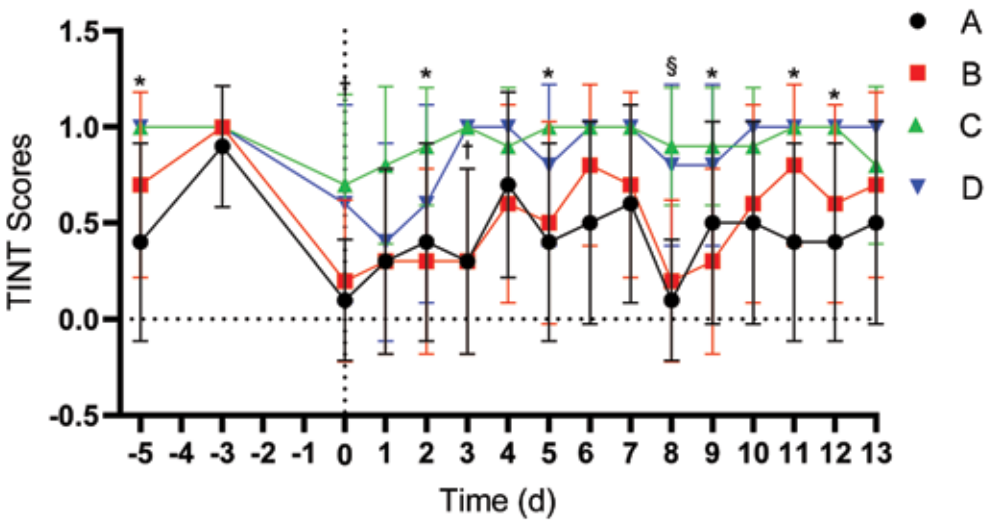

Figure 5. Time to Incorporate into Nest Test (mean ± 1 SD) values over time. Group A mice were single-housed before and after surgery (n = 10 all having received surgery); group B mice were pair-housed before but single-housed after surgery (n = 10 all having received surgery); group C animals were pair-housed before and after surgery (n = 20, 10 having received surgery and 10 did not undergo surgery); and group D mice were pair-housed before and after surgery (n = 10 all having received surgery). Symbols denote statistically significant differences (*, $P \leq 0.05$; †, $P \leq 0.01$; ‡, $P \leq 0.001$; §, $P \leq 0.0001$) between groups A and C (days 1, 6, 9, 11, 14, 17, and 18), A and D (days 1, 9, 14, 17, and 18), B and C (days 6, 8, 9, 14, and 15), and B and D (days 9 and 14).

Discussion

Results of the current study suggest that pair housing mice after surgery does not lead to increased trauma to the surgical site, wound clip disruption, or reduced overall wellbeing compared with individually-housed mice. The data also support a positive effect of pair-housing on mouse wellbeing after surgery. Our data also indicate that separating previously pair-housed mice appeared to have no additional impact on measured parameters as compared with mice that were individually housed before and after surgery.

Successful outcomes for research involving mouse surgical models depend on a variety of factors, including proper aseptic technique, effective pain relief, and surgical and clinical acumen of personnel. Although the technical aspects of surgery must be conducted with proficiency, the animal's wellbeing is also a priority. Therefore, the opportunity for normal social interaction is a consideration. Preference for housing mice individually after surgery is based on the belief that separation from cagemates will reduce the likelihood of trauma to the incision site and improve wellbeing by preventing cage mate dominance, particularly from mice that have not undergone surgery. We sought to explore the influence of social separation initiated at the time of surgery on animal wellbeing and assessed the following parameters both before and after surgery: body weight, body condition, facial grimace, nest construction quality, TINT, surgical wound healing, and integrity of the wound closure with wound clips.

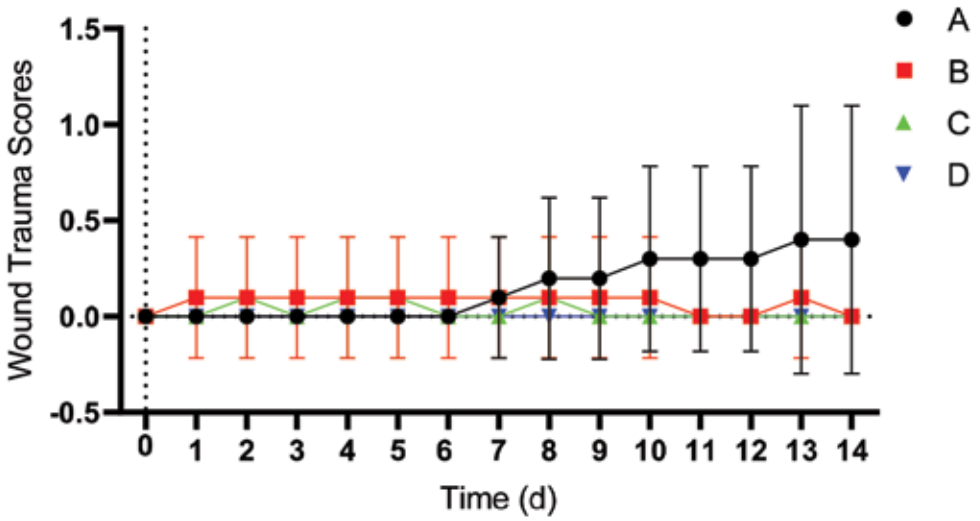

Figure 6. Wound score (mean ± 1 SD) values over time. Group A mice were single-housed before and after surgery (n = 10 all having received surgery); group B mice were pair-housed before but single-housed after surgery (n = 10 all having received surgery); group C animals were pair-housed before and after surgery (n = 20, 10 having received surgery and 10 did not undergo surgery); and group D mice were pair-housed before and after surgery (n = 10 all having received surgery). No statistically significant difference was detected.

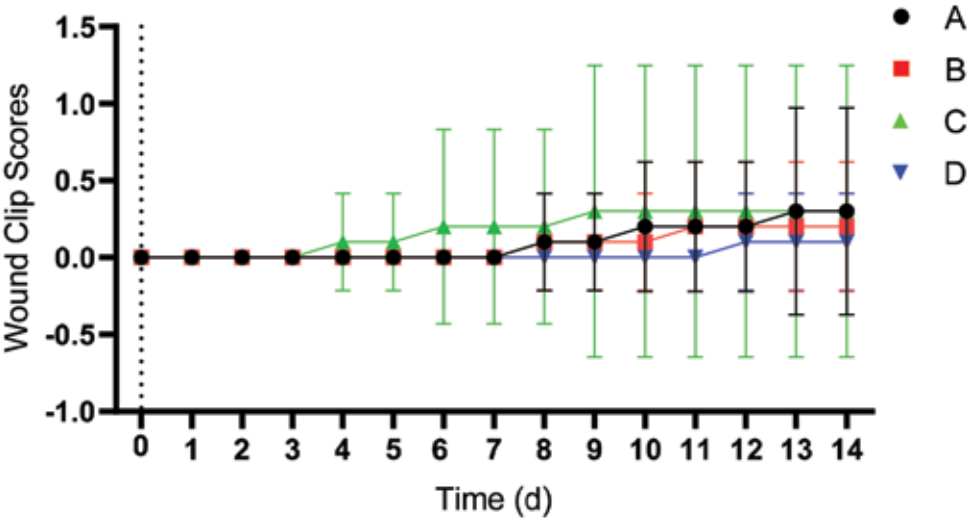

Figure 7. Surgical wound clip score (mean ± 1 SD) values over time. Group A mice were single-housed before and after surgery (n = 10 all having received surgery); group B mice were pair-housed before but single-housed after surgery (n = 10 all having received surgery); group C animals were pair-housed before and after surgery (n = 20, 10 having received surgery and 10 did not undergo surgery); and group D mice were pair-housed before and after surgery (n = 10 all having received surgery). No statistically significant difference was detected.

Our study found a statistically significant difference in mean body weight between groups A (individually housed before and after surgery) and C (pair-housed before and after surgery). Because group A mice were individually housed throughout the study, we attributed this difference to social isolation, decreased voluntary exploratory behavior and increased food intake.[25,26] In one review, the authors[19] concluded that stress can "trigger both orexigenic-like and anorexigenic-like responses reflecting a variety of intrinsic and external factors such as individual differences, (palatable) food availability and/or the type of stress." However, the effect of individual housing on body weight is not clear in mice. In one study, individual housing of male mice was associated with significant reductions in body weight,[17] whereas another study[20] found through meta-analysis that individual housing had no significant effect on body weight of mice. Therefore, the effect of individual compared with social housing on body weight is uncertain, as is the value of change in body weight as a metric for wellbeing. We cannot determine whether the changes in body weight in our study were a direct outcome of differences in housing condition, related to the surgical intervention, or due to some other unknown factors. Because all mice (except for the 10 in group C) received the same surgical intervention and analgesics, we consider that the body weight results are more likely to reflect differences in housing condition rather than surgery-related pain or distress.

A previous study[13] explored food intake in normal mice when exposed to soiled bedding from mice with pancreatic ductal adenocarcinoma and found that they consumed less food than mice exposed to soiled bedding from control mice. However, group C in our study lost the least weight (0.34 g) and recovered or surpassed their presurgical weight in 3 d as compared with 4 d in the other 3 groups. We speculate that this outcome could be due to a positive effect of the unoperated mice on the wellbeing of mice that had undergone surgery.

Body condition is an established means of evaluating the general health of animals, with loss of body condition indicating a decline in overall health.[24] In our study, body condition scores did not differ significantly between any of the treatment groups. The use of analgesics and other nonpharmacological methods to manage postsurgical pain and distress, including a clean, stable environment with bedding and nesting material, may have contributed to effective management of single housing, social isolation, and postsurgical pain and distress, ameliorating any negative effects in psychologic, biologic, and behavioral parameters. Given the lack of significant differences in body condition scores among groups in our study, the initiation of individual housing at the time of surgery does not seem to influence the overall health status of mice.

Facial grimace scoring has been used to evaluate pain and distress in mice.[12,15,23] Although the accuracy of cageside grimace assessment is uncertain, it still provides a simple and quick evaluation of the pain and distress experienced by an animal. The facial grimace scores in our study showed no statistically significant difference between groups and our data suggest that change in social housing status after surgery did not affect grimace scores. However, one unoperated mouse and some individually and pair-housed mice showed positive grimace scores both before and after surgery. These observations reinforce the imprecision of this method but may also indicate that the conditions triggering a facial expression (that is, a Facial Action Unit[12,15]) coded as positive were likely multifactorial. For example, all mice receiving surgery were anesthetized with isoflurane and treated with analgesics, both which would be expected to reduce pain and grimace after surgery. However, this interpretation contrasts with that of another study[14] reporting buprenorphine alone had no effect on grimace scores, but isoflurane anesthesia resulted in an increase in grimace scores in DBA/2 mice. Another study[21] found that pain and stress levels after subarachnoid hemorrhage were not improved after treatment with meloxicam or carprofen. The authors of that study also concluded that neither of the 3 analgesics used were effective in controlling pain after traumatic brain injury.[21] However, the preparation (Temgesic, Essex, Munich, Germany) and dosage rates used in that study (0.1 mg/kg) were different than those we used. Similarly, carprofen, buprenorphine, and a combination of the 2 failed to improve recovery after mammary fat pad removal surgery.[1] Taken together, these published papers bring into question our interpretation of the effect of analgesics on grimace scores. Another consideration is that mice, as a prey species, may hide signs and symptoms of pain when confronted with a potential predator.[23] This aspect of mouse behavior could also have influenced facial grimace scores.

The group that developed the Mouse Grimace Score[12] used CD1 mice, whereas we used C57Bl/6 mice in the current study. According to some investigators,[23] a common assumption is that a scale developed in one strain of mice will perform equally well under different circumstances. This view fails to account for potential differences in strain used, methods, etc. We have not identified literature in which the grimace score was used to measure conditions other than pain. Thus, grimace score data may not be appropriate for detecting effects other than pain.

A final consideration, as discussed by others,[15] is that "baseline mouse grimace scores are not zero, as is often anticipated." If this statement holds true, our current results (especially those during the presurgery period) may reflect normal variation and not pain or distress related to either changes in housing condition or surgery.

The quality of nest construction by mice is widely regarded as an effective indication of animal wellbeing.[5,6] In our study, nest-building scores exhibited statistically significant differences between groups that were individually housed after surgery (A and B) and those that were pair-housed after surgery (C and D), suggesting that animals pair-housed after surgery experience increased wellbeing compared with those single-housed, and that a change in social housing status made immediately following surgery has no impact on nest building scores. This difference may reflect cooperation in nest building[6] in groups C and D, which were pair-housed both before and after surgery.

One group of authors[23] warned that "single housing of animals that could otherwise be socially housed is not desirable, as it can contribute to unnecessary stress in a recovering animal." Furthermore, other group of authors[18] reported that in a cecal manipulation study in which mice underwent laparotomy and their ceca were exteriorized and gently held in moist gauze for 3 min, individually housed mice drank more medicated (ibuprofen) water than group-housed mice, thereby demonstrating that the social environment significantly influenced postoperative recovery and self-administration of analgesics. This interpretation raises the question whether the lower nest-building scores in groups A and B were influenced by postsurgical pain, the single-housing condition, or the combined effects of both.

Another study[11] found that quality of nest construction is inversely correlated with the degree of invasiveness of the experimental procedures and that nest-building behavior provides a robust indicator of animal wellbeing. However, the surgical procedure used in our study was less invasive and arguably less painful than that used in the referenced study[11] (sham embryo transfer). Also, in our study, all mice that underwent surgery received analgesia, which could also contribute to less pain and better nest-building scores. However, establishing that nest-building scores reflect effective pain alleviation may require further study, as suggested previously.[11]

If unoperated mice provided a 'caregiver effect,' we speculate that it would have presented as a higher nest-building score. Given that groups C and D had similar nest building scores, we argue that the presence of a cage mate improves wellbeing, whether that cage mate has also undergone surgery or not.

The TINT provides a method of evaluating wellbeing in mice that supports and reinforces evaluation of nest-building behavior. Our TINT scores paralleled those of nest building, with groups that were pair-housed having significantly greater scores than did mice that were individually housed. As with nest building, our data support the idea that housing with a cage mate, whether it underwent surgery or not, may have compensated in some way for limitations due to surgery, given the lack of significant differences in TINT or nest building scores between groups that were pair-housed after surgery (C and D). Mice that were pair-housed both before and after surgery with a cage mate that did not undergo surgery (group C) took 5 d to return to presurgery TINT scores, whereas mice that were individually housed before and after surgery or pair-housed before and after surgery with a cage mate that underwent surgery (groups A and D) only required 4 d, suggesting a faster return to normal behavior.

Our data suggest that animals pair-housed after surgery experience increased wellbeing compared with those housed individually and that a change in social housing status made immediately after surgery has no effect on TINT scores.

A putative rationale for single housing of mice after surgery is to prevent trauma to the wound site and disruption to healing of the incision. Our scoring system indicated that 3 of the mice that were single housed both before and after surgery (group A) had traumatized incisions; of these, 2 received a score of 1 and one received a score of 2. Among mice that were pair-housed before surgery and then singly-housed after surgery (group B), 3 had traumatized surgical wounds, all of which scored as 1. Among mice housed with a cage mate that had not undergone surgery (group C), 2 mice had evident surgical wound trauma (both with a score of 1), whereas mice that were pair-housed with a cage mate that also underwent surgery (group D) had no wound trauma reported.

Our data suggest that change in social housing status after surgery had no effect on the healing of surgical wounds.

Missing wound clips were noted in at least one mouse in each group (2 in group A). Although we could not determine whether the surgical wound trauma experienced by mice in group C and the missing wound clips in one mouse in that group were self-induced or the result of cage mate aggression, the frequency and severity of surgical wound trauma in mice that were individually housed suggests that such trauma is not due to cage mate aggression but rather is self-induced.

These data suggest that change in social housing status after surgery had no effect on the surgical closure with wound clips.

Our data are undoubtedly influenced by the mouse strain and sex and the specific surgical model, and variations in these parameters may lead to different outcomes. Also, we missed several data collection time points during our study, thus potentially reducing the strength of our data. Grimace scores were collected in real time, and results may not be as valid as those collected via video recordings and posthoc analysis. In addition, our findings may be related to our use of a different mouse strain than that used to develop the MGS. Future studies to answer these questions by using additional mouse lines, both sexes, and group housing animals may be valuable.

It may be difficult to fully elucidate if the changes in body weight, nest building and TINT scores observed in the present study were a direct outcome of changes in housing condition, related to the surgical intervention and secondary pain or distress or merely reflect normal random variation. Our data demonstrate that damage to the surgical site was minimal in pair-housed mice. Interestingly, the data support a positive effect of pair housing on animal wellbeing following surgery, though mice moved from pair-housing to single housing at the time of surgery (Group B) had measures of wellbeing that were similar to mice that were single housed both before and after surgery, thus indicating that an abrupt change in social environment at the time of surgery did not have an added impact. Combined with assessments of wellbeing, it appears that there is little validity to the rationale that single-housing of mice following surgery should be practiced as a measure to enhance post-operative recovery and healing.

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