Journal of the American Association for Laboratory Animal Science
Vol 58, No 3
May 2019
Pages 1–10

Clinical, Physiologic, and Behavioral Evaluation of Permanently Catheterized NMRI Mice

Malene Kari Falkenberg,[1,†] Anne Charlotte Teilmann,[1,†] Trine Henriksen,[2] Jann Hau,[1] Henrik Enghusen Poulsen,[2] and Klas SP Abelson[1,*]

Vascular catheterization is becoming a popular technique in laboratory rodents, facilitating repetitive blood sampling and infusion in individual animals. In mice, catheterization is complicated by their small body size, which may increase the risk of postoperative complications that may both threaten catheter longevity and animal welfare. Less obvious complications to a permanent catheter may include subclinical infection, visceral tissue damage from disseminating microthrombi released from the catheter, and distress from being isolated from conspecifics and other experimental stressors. Such complications may go unnoticed and may affect animal welfare as well as confound research outcomes. This study investigated the implications of long-term arterial catheterization in NMRI mice by evaluating clinical, physiologic and behavioral parameters. Body weight and food and water consumptions were monitored during the study period. Fecal corticosterone metabolites were quantified as biomarkers of stress, and nucleic acid metabolites (8-oxo-7,8-dihydro-2′-deoxyguanisine and 8-oxo-7,8-dihydroguanosine) as biomarkers of oxidative damage. Behavioral dysfunction was studied by scoring animal welfare and nest building. Catheters were placed the right common carotid artery of mice; catheterized mice were compared with sham-operated and nonsurgical control mice. Except for an increase in the body weight of catheterized mice during the experimental period, clinical parameters (body weight and food and water consumptions) did not differ between groups. Physiologic parameters (oxidized nucleic acid metabolites and fecal corticosterone metabolites) were higher in control mice during the first week of experimentation compared with the end of study but did not differ between groups. Likewise, catheterization had no effect on behavioral parameters (nest building and animal welfare assessment). Long-term arterial catheterization of mice had no detectable implications on animal welfare in this study.

Abbreviations: 8-oxo-G, 8-oxo-7,8-dihydroguanosine; 8-oxo-dG, 8-oxo-7,8-dihydro-2′-deoxyguanisine; AWA, animal welfare assessment; FCM, fecal corticosterone metabolites

DOI: 10.30802/AALAS-JAALAS-18-000060

Blood sampling is one of the most frequently performed procedures in laboratory animals but is known to cause a stress response that can confound the study outcome.[13,34] The stress response is evoked by numerous stimuli related to the sampling, including handling, restraint, vascular puncture, blood loss, and possible anesthesia.[1,9,14,50,52] In studies where repeated blood sampling is required, a permanent vascular catheter potentially might overcome some of the stressors otherwise associated with blood sampling,[4] but catheterization itself might introduce other stressors, such as surgery and social isolation.[3,47]

Vascular catheterization of laboratory animals has been performed with success for many years in larger species such as rats, dogs, NHP, and pigs.[8,44,54] In mice, however, the technique is still fairly novel and is complicated by their small body size, which increases the demands on surgical skills and technical equipment. Although previous studies have shown that mice seem to recover well from surgery and quickly habituate to the catheter,[43,46,47] those studies mainly focused on stress parameters. A recent study suggests that catheterized mice have subclinical pathologic changes in well-vascularized organs, such as the kidneys, liver, and heart, and that cytokine levels increase after surgery and may stay elevated past a 3-d recovery period.[48]

Stress is a complex state in the animal, elicited when a stressor stimulates the HPA axis, causing a systemic increase in stress hormones that in turn causes a plethora of physiologic responses.[30,31,37,38] Corticosterone, the main effector hormone of the HPA axis in rodents, is released from the adrenal cortex and can be quantified noninvasively through metabolites excreted in feces.[17,51] Circulating corticosterone binds to glucocorticoid receptors, which are found on nearly all cells,[12] and increased blood concentrations of corticosterone have a wide range of physiologic and behavioral effects, which are necessary prerequisites to facilitate the fight-or-flight response to a stressor.[36] Although acute stress is a natural (and protective) adaptation to transient stressors, such as blood sampling in a laboratory setting, this stimulus may affect results based on the samples obtained. Chronic stress is more problematic in rodents and may inhibit physiologic processes, including reproduction, the immune system, and growth, as well as potentially induce a state of distress in animals.[7] Furthermore, prolonged periods of increased concentrations of stress hormones have been associated with pronounced oxidative damage, caused by increased levels of free radicals,[25] and overt modulation of animal behavior.[7] Therefore, chronic stress may compromise animal welfare and should be avoided in experiments, whenever possible. Moreover, as a dominant physiologic modulator, prolonged levels of stress hormones also confound experimental results,[13,34] leading

Received: 18 May 2018. Revision requested: 20 Jul 2018. Accepted: 16 Oct 2018.
[1]Department of Experimental Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark; and [2]Department of Clinical Pharmacology, Bispebjerg Frederiksberg Hospital, Copenhagen, Denmark.
[*]Corresponding author. Email: klasab@sund.ku.dk
[†]These authors contributed equally to the study

to increased variation within and between animals and poorly translatable animal research. Thus, permanently catheterized mice might experience multiple effects due to the procedure.

The aim of the present study was to use clinical, physiologic, and behavioral parameters to investigate the implications of long-term arterial catheterization on the welfare of laboratory mice. Clinical changes were monitored through measurements of body weight as well as food and water intake. Increased stress was detected by quantification of fecal corticosterone metabolites (FCM), and oxidative damage caused by catheterization was studied by assessing urinary levels of the DNA fragment 8-oxo-7,8-dihydro-2′-deoxyguanisine (8-oxo-dG) and its RNA equivalent 8-oxo-7,8-dihydroguanosine (8-oxo-G). Behavioral effects were studied by scoring animal welfare and nest building.

Our hypothesis was that mice with permanent catheters implanted in the right common carotid artery would experience decreased welfare compared with sham-operated mice, in which the carotid artery was ligated but not catheterized, and nonoperated control mice. We expected that this decrease in welfare was expected to be reflected by an increased level of the 8-oxo-dG and 8-oxo-G in urine, increased levels of FCM, loss of body weight, decreased food and water consumption, inferior animal welfare assessment (AWA) score, and decreased ability to make a high-quality nest.

Materials and Methods

This investigation was licensed by The Animal Experiments Inspectorate under the Danish Ministry of Environment and Food (license no. 2012-15-2934-00505) and approved by the local animal welfare committee. The study was conducted in accordance with the *Guide for the Care and Use of Laboratory Animals*[20] in an AAALAC-accredited facility. The facility followed the FELASA recommendations for health monitoring of rodent facilities,[29] where sentinel animals had tested positive for *Helicobacter* spp. but none of the other pathogens on the FELASA list.

Animals and housing. Male BomTac:NMRI mice were purchased at 6 wk of age (n = 30; Taconic, Ry, Denmark) and allowed 2 wk of acclimation before commencing the study. Mice were randomly allocated into 3 groups according to the experimental protocol: in one group, mice (n = 8) were catheterized in the right common carotid artery; another group (n = 10) was sham-operated, where the carotid artery was ligated but not catheterized; and the remaining mice (n = 10) comprised a nonoperated control group. Two mice were euthanized after catheterization after reaching preset humane end points, resulting in 8 mice in the catheterized group.

The mice were single-housed (Makrolon type II cages, Tecniplast, Buguggiate, Italy; dimensions, 268 × 215 × 141 mm) in an IVC system with aspen chips (Tapvei Oy, Kortteinen, Finland) as bedding material and wood wool (Tapvei) as nesting material. The mice were offered bite bricks (Tapvet, Kortteinen, Finland), cardboard houses (Brogaarden, Gentofte, Denmark), and cardboard tubes (Lilico, Horley, United Kingdom) for environmental enrichment. Feed (diet 1314, Altromin, Im Seelenkamp, Germany) and acidified tap water were provided without restriction, and a diurnal rhythm was maintained through a 12:12-h light:dark cycle (lights on, 0600) with a 30-min twilight before lights were turned off or on. Cage temperature was kept at 22 °C (± 2 °C), relative humidity was between 45% and 65%, and the air in each cage was exchanged approximately 75 times each hour.

Catheterization. Mice in the catheterized group underwent surgery as previously described.[47,49] Anesthesia was induced in an induction chamber by using 5% isoflurane (Forene, Abbot Scandinavia, Stockholm, Sweden) delivered in 100% oxygen and maintained throughout surgery by using 2.5% to 3% isoflurane in oxygen provided through an anesthetic face mask (AgnThos, Lidingö, Sweden). An arterial catheter (MAC2B, SAI Infusion Technologies, Lake Villa, IL) was implanted in the right common carotid artery and advanced until the tip reached the proximal brachiocephalic trunk. The catheter was tunneled subcutaneously to the midscapular region in the neck and exteriorized through the skin, where it was secured with a single suture (6-0 polyglycolic acid suture, Ethicon, St Stevens Woluwe, Belgium). Catheter patency was confirmed through the aspiration of blood, which was returned to the animal by flushing with 50 µL of heparinized saline (25 IU/mL), before locking the catheter with 20 µL heparin:glycerol locking solution (Cath-LocHGS, SAI Infusion Technologies) and a steel plug (SAI Infusion Technologies).

Sham-operated mice were prepared for surgery in a similar manner as for catheterized mice. Briefly, the carotid artery were carefully dissected and ligated with a single ligature, without the implantation of a catheter.

Analgesia was given preemptively 1 h before surgery by means of buprenorphine (1 mg/ kg body) mixed in nut paste (Nutella, Ferrero, Pino Torinese, Italy), which was offered to the mice for voluntary ingestion.[2,22] To ensure adequate analgesia on the day of surgery, mice were injected subcutaneously with buprenorphine (0.1 mg/kg body weight) in the flank at the end of surgery. Adequate analgesia during recovery was ensured through the administration of buprenorphine in nut paste once daily for 2 d after surgery.

After surgery, the mice were allowed to recover in a cage heated to 28 °C in a quiet room. Recovery was monitored through daily inspections and measurements of body weight as well as food and water consumption for 2 d after surgery and then weekly throughout the study. Once each week and coordinated with the daily inspection, AWA was performed.[24] At all times, mice were handled by cupping to minimize stress.[19]

Urine and fecal sampling. Once a week, from 1 wk prior to surgery and throughout the study period, urine and feces were collected between 1600 and 1700, at the beginning of the active phase.

To obtain urine samples, the mice were transferred to cages without bedding or other material and allowed to urinate for 20 to 30 min. By using this method, typically ample urine was available for collection, which was transferred into microfuge tubes by using a Pasteur pipette. On a few occasions, less than 100 µL of urine was available after 30 min, and the mice were restrained by scruffing, and urine was collected directly into a microfuge tube. After urine collection, mice were transferred to clean cages containing new bedding and nesting material.

At 48 h prior to urine collection, all mice were moved to clean cages containing new bedding. Then, when urine was sampled, dirty bedding was collected from each cage, which thus contain a pooled, 48-h sample of excreted feces. Urine and fecal samples were stored at –20 °C until analysis.

Quantification of oxidized nucleic acid metabolites. Levels of 8-oxo-dG and 8-oxo-G were quantified by using a modified method involving ultraperformance liquid chromatography and tandem mass spectrometry,[18] where 8-oxo-dG as well as 8-oxo-G were analyzed through negative ionization instead of positive ionization.

Quantification of fecal corticosterone metabolites. All fecal boli from individual samples were collected and the total excreted levels of FCM were extracted and quantified by ELISA

(EIA4164, DRG Diagnostics, Marburg, Germany) according to the manufacturer's instructions. The total fecal sample was weighed and submerged in 96% ethanol (3 mL/g feces). All samples were incubated on a shaking table overnight for approximately 12 h to extract fecal corticosterone and FCM. The homogenate was centrifuged for 20 min at 3000 × *g* (Scanspeed 1236R, Labogen, Lynge, Denmark). The supernatant was decanted, the pellet discarded, and 1 mL of the supernatant was centrifuged for 15 min at 10,000 × *g* in a tabletop centrifuge (model 5415D, Eppendorf, Hamburg, Germany). A 300-µL aliquot of the supernatant was recovered by using a pipette and evaporated to dryness (model EZ2, Genevac, Ipswich, United Kingdom). The evaporate was resuspended in 300 µL assay buffer and analyzed by using a competitive corticosterone ELISA (EIA-4164, DRG Diagnostics) according to the manufacturer's instructions. The following cross-reactivities were reported for the assay: progesterone, 7.4%; deoxycorticosterone, 3.4%; 11-dehydrocorticosterone, 1.6%; cortisol, 0.3%; pregnenolone, 0.3%; and other steroids, <0.1%. Absorbencies at 450 nm (reference wavelength, 650 nm; Multiscan Ex, Thermo Fisher Scientific, Waltham, MA) were recorded.[28,43]

Body weight and food and water consumption. The body weight and food and water consumption of the mice in the catheterized and sham-operated groups were measured daily from 2 d before until 2 d after surgery to monitor postoperative recovery. Thereafter, all mice in all groups were weighed weekly and food and water intakes measured weekly throughout the entire study period until euthanasia. Food and water consumptions were calculated by subtracting the current weight of food and water from the weight at the previous measurement.

Assessment of nest building. Once each week, beginning 1 wk prior to surgery and continuing throughout the study period, cages were prepared between 1600 and 1700, at the beginning of the active phase, for nest-building tests. This test has been described comprehensively.[10] All nesting material was removed from the cage and replaced by clean 6.0 g of new nesting material, which were placed in a pile in the right front corner of the cage. The cardboard house was placed in the far left corner to stimulate nest building inside the house. Between 1000 and 1100 the following day, all nests were photographed, and all nesting material that was not incorporated into the nest was weighed, and the percentage of nesting material used in the nest was calculated according to the following formula:

$$\text{Nesting material used} = \frac{\text{amount of nesting material provided} - \text{amount material left outside nest}}{\text{amount of nesting material provided}} \times 100\%$$

The photographs were blinded, and nest quality was assessed by 2 independent observers, who were blinded to the study (Figure 1).

AWA. AWA (Figure 2) was performed between 1000 and 1100 once each week. Each mouse was inspected in the home cage from a distance for the scoring of body posture and movement. Then, the cage was placed on a nearby table, the lid removed, and the remaining parameters of appearance, natural behavior, fur quality, and degree of eye opening were scored. Each parameter was scored from 0 to 3, where 0 signified a normal appearance and 3 indicated a severe degree of impairment. Scores for all parameters were summed to obtain a total score for each animal.[21]

Statistics. Statistical analyses were performed by using SPSS version 22 (IBM, Armonk, NY) and Prism 5 for Windows version 5.01 (GraphPad Software, La Jolla, CA).

Parametric data were tested for normality according to the Shapiro–Wilk test. Data that belonged to a Gaussian distribution were analyzed by using multivariate ANOVA with Tukey posthoc testing, where group and week were defined as fixed factors. Data are presented as $F_{dfw, dfb}$, where df is the degree of freedom within and between groups, respectively. Nonparametric data were analyzed by using the Kruskal–Wallis test.

Correlation between the 2 sets of nest-quality scores from the 2 independent observers was calculated according to Spearman correlation coefficients. P values less than 0.05 were considered significant.

Retrospective power calculations were performed on FCM and 8-oxo-G data. At $\alpha = 0.05$, 1 SD of 6.27, and average FCM values of 23.39 and 30.91 for catheterized and control mice, respectively, the statistical power was calculated to be 97.7% for FCM data. Regarding 8-oxo-G data, $\alpha = 0.05$, 1 SD = 12.88, and average 8-oxo-G values of 39.68 and 44.90 for catheterized and control mice, respectively, resulted in a calculated statistical power of 33.4%.

Results

Oxidized nucleic acid metabolites. Multivariate ANOVA did not reveal significant difference in the levels of 8-oxo-dG between groups ($F_{10,34} = 0.671$, $P = 0.743$) or between weeks ($F_{12,87} = 0.711$, $P = 0.737$). Likewise, no overall significant difference in the levels of 8-oxo-G between groups ($F_{10,34} = 1.447$, $P = 0.203$) or between weeks ($F_{12,87} = 0.998$, $P = 0.457$) was found. However, when each group was considered separately, 8-oxo-G levels of control mice were significantly ($P = 0.007$) higher than the levels of sham-operated mice during week 1 (Figure 3).

FCM. Multivariate ANOVA did not find significant difference in FCM levels between groups at any time ($F_{8,44} = 1.014$, $P = 0.440$). However, control mice displayed a time-related change ($F_{9,65} = 2.836$, $P = 0.007$) in FCM, where levels were higher in week 1 compared with week 4 ($P = 0.001$) and higher in week 2 compared with weeks 3 ($P = 0.042$) and 4 ($P < 0.001$; Figure 4).

Body weight. Overall, multivariate ANOVA found that BW differed significantly among the 3 groups during weeks 1 through 3 ($F_{10,36} = 3.487$, $P = 0.003$). Control mice weighed significantly more than catheterized mice ($P = 0.011$) and sham-operated mice ($P = 0.003$) during week 1, and control mice weighed more than sham group in weeks 2 ($P = 0.002$) and 3 ($P = 0.021$). Body weight did not differ between groups during week 4 or at euthanasia. Overall, time had no significant effect on the body weight of mice within each group ($F_{12,82} = 1.49$, $P = 0.145$), except that catheterized mice weighed more ($P = 0.010$) during week 4 compared with week 1 (Figure 5).

Food and water consumptions. Multivariate ANOVA did not reveal significant difference in food consumption between groups ($F_{12,82} = 0.577$, $P = 0{,}791$) or between weeks ($F_{9,63} = 0.546$, $P = 0.835$). Similarly, water consumption did not differ significantly between groups ($F_{8,42} = 1.049$, $P = 0.416$) or between weeks ($F_{9,63} = 0.005$, $P = 0.184$; Figure 6).

Assessment of nest building. The Kruskal–Wallis test found that, in week 2, catheterized mice used significantly ($P = 0.029$) more nesting material than the other 2 groups. Otherwise, nest building did not differ between groups at any time point (week –1, $P = 0.457$; week 1, $P = 0.142$; week 3, $P = 0.065$; and week 4, $P = 0.669$; Figure 7).

We also separately analyzed the score sets from each of the 2 observers. Scores from observer 1 showed no significant difference

Score	Description
1	Nest material has not been manipulated; 90% to 100% of nest material is left intact
2	50% to 90% is nest material left intact
3	50% to 90% has been manipulated or moved but remains on the cage floor of cage, no clear nest
4	90% to 100% nest material is incorporated into an identifiable but flat nest
5	90% to 100% nest material is incorporated into a near-perfect, cave-like nest

Figure 1. Definition of nest scores. Using photographs, 2 independent, blinded observers scored nest quality.

Parameter	Description	Score
Appearance	Normal	0
	General lack of grooming	1
	Fresh ocular and/or nasal discharge	2
	Bloodstained or mucopurulent discharge from any orifice	3
Body posture and movement	Sitting, standing or move normally and upright	0
	Sleeping on side or curled up	0
	Less mobile but runs off, when touched	2
	Hunched up, unable to maintain an upright position, unwilling to move	3
Natural behavior	Awake, active, responding to surroundings	0
	Little responding to surroundings, less active, alert	2
	No responding to surroundings, very still, not alert	3
Fur quality	Smooth, shining fur	0
	Mild generalized piloerection	2
	Marked generalized piloerection	3
Degree of eye opening	Eye wide open	0
	Eyes slightly closed	1
	Eyes halfway closed	2
	Eyes completely closed	3
Total		0–15

Figure 2. Animal welfare assessment ethogram.

among the 3 groups at any time point (week –1, $P = 0.226$; week 1, $P = 0.140$; week 2, $P = 0.589$; week 3, $P = 0.978$; and week 4, $P = 0.702$). Scores from observer 2 showed significant differences prior to surgery, when catheterized mice had a significantly higher nest scores than sham-operated and control mice (week –1, $P = 0.027$; week 1, $P = 0.461$; week 2, $P = 0.089$; week 3, $P = 0.943$; and week 4, $P = 0.570$; Figure 8).

AWA. The Kruskal–Wallis test did not detect any significant differences in AWA score (mean ± 1 SD) among the 3 groups at any time point (week 1: 0.28 ± 0.53, $P = 0.070$; week 2: 0.21 ± 0.50, $P = 0.186$; week 3: 0.21 ± 0.50, $P = 0.186$; and week 4: 0.14 ± 0.36, $P = 0.168$; Figure 9).

Discussion

Carotid artery catheterization in mice is becoming a well-established procedure, especially for toxicologic and pharmacologic testing.[6,16,33,53] Despite its widespread use, this method is not without complications. Postoperative weight loss, stress, inflammation, and catheter occlusion are frequent complications of catheterization that may compromise animal welfare as well as the use of various mouse models.[24,43,47,48] Therefore, it is still crucial to evaluate the technique critically, because vascular catheterization most often is conducted for long-term purposes. We performed the present study to elucidate whether and to what extent long-term arterial catheterization affected parameters of animal welfare in laboratory mice.

Oxidative damage can be accelerated due to bacterial infection, trauma, tissue necrosis, and the presence of foreign bodies.[39] The 2 biomarkers of oxidative damage that we applied in the present study are widely used as explanatory variables for disease progression and development, chronic stress, and other physiologic stressors.[11,18,55] In the present study, the we quantified the DNA fragment 8-oxo-dG and its RNA equivalent 8-oxo-G as biomarkers of potential complications from surgery and catheterization. No effect of surgery or catheterization on these biomarkers of oxidative damage was found. However, the retrospective power calculation found very low power for 8-oxo-G values; in addition, calculated mean group difference was equally low (11%), and the present study failed to identify any difference between groups. Oxidative stress has been correlated with chronic stress, such that 8-oxo-G concentrations in subjects with the highest levels of urinary cortisol were approximately 60% higher than those of subjects with the lowest levels of cortisol.[25] The narrow difference between control mice and catheterized mice in the present study can thus be considered biologically irrelevant. Our study was not designed to detect such minor differences. Instead, we combined several parameters of physiologic stress to gain a complete and thorough picture of total loads on the mice. However, the higher levels of 8-oxo-G in control mice during the first week of experimentation were unexpected. Given that multiple hypotheses were tested in the present study, the chance of a rare event might increase due to type 1 error.

Stress can be measured through quantification of the biomarkers corticosterone and corticosterone metabolites in blood, feces, urine, and saliva.[31] No effect of surgery or catheterization on FCM occurred in the present study. Quantification of FCM is considered minimally invasive compared with corticosterone quantification from blood samples,[17,40,52] but may be insensitive as a biomarker of mild stress.[23,40] For that reason, we quantified several biomarkers in the present study, because we believed

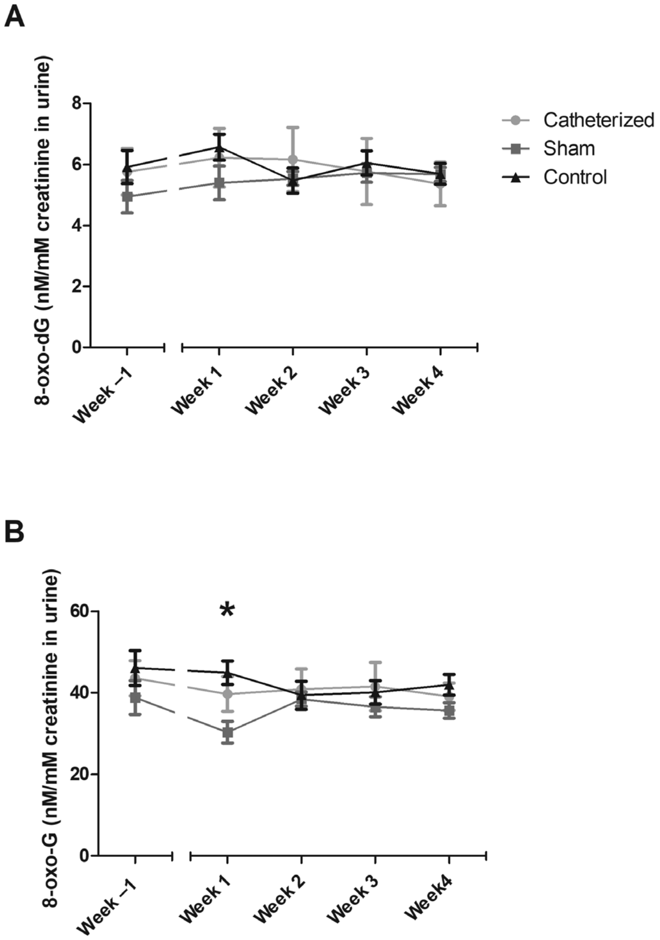

Figure 3. Urinary levels of 8-oxo-dG and 8-oxo-G. Urine samples were obtained during the week before surgery (week –1) and then once weekly throughout the 4-wk experimental period. Samples were analyzed for 8-oxo-7,8-dihydro-2′-deoxy guanosine (8-oxo-dG) and 8-oxo-7,8-dihydroguanosine (8-oxo-G). Levels of 8-oxo-dG levels did not differ between groups or weeks. Control mice had significantly (*, $P < 0.05$) higher 8-oxo-G levels than sham-treated mice in week 1. Otherwise, 8-oxo-dG levels did not differ between groups or weeks. Data are presented as mean ± SEM.

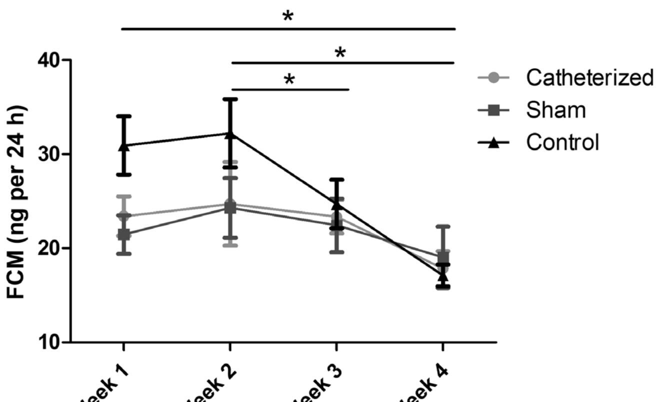

Figure 4. Fecal corticosterone metabolites. Fecal samples were obtained once weekly during the 4-wk experimental period and analyzed for fecal corticosterone metabolites (FCM), which did not differ significantly between groups. In control mice, higher FCM levels in weeks 1 and 2 were significantly (*, $P < 0.05$) higher than in weeks 3 and 4. Data are presented as mean ± SEM.

this strategy would increase the amount of information obtained and provide a more complete picture of the true state of the animals.

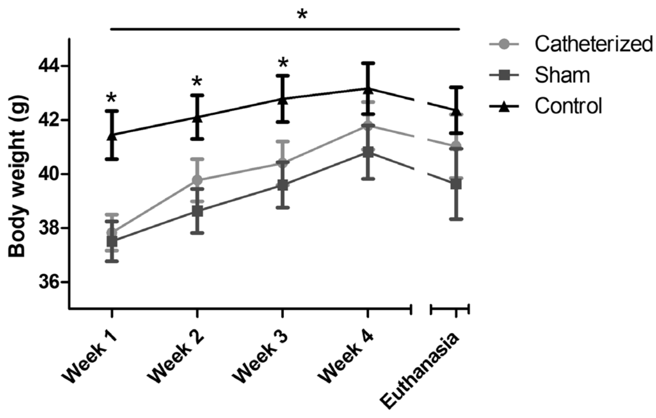

Figure 5. Body weight. Mice were weighed once weekly during the 4-wk experimental periodand immediately before euthanasia. Control mice weighed significantly (*, $P < 0.05$) more than both catheterized and sham-operated mice in week 1 and more than sham-operated mice in weeks 2 and 3. Catheterized mice weighed significantly more in week 4 than in week 1. Data are presented as mean ± SEM.

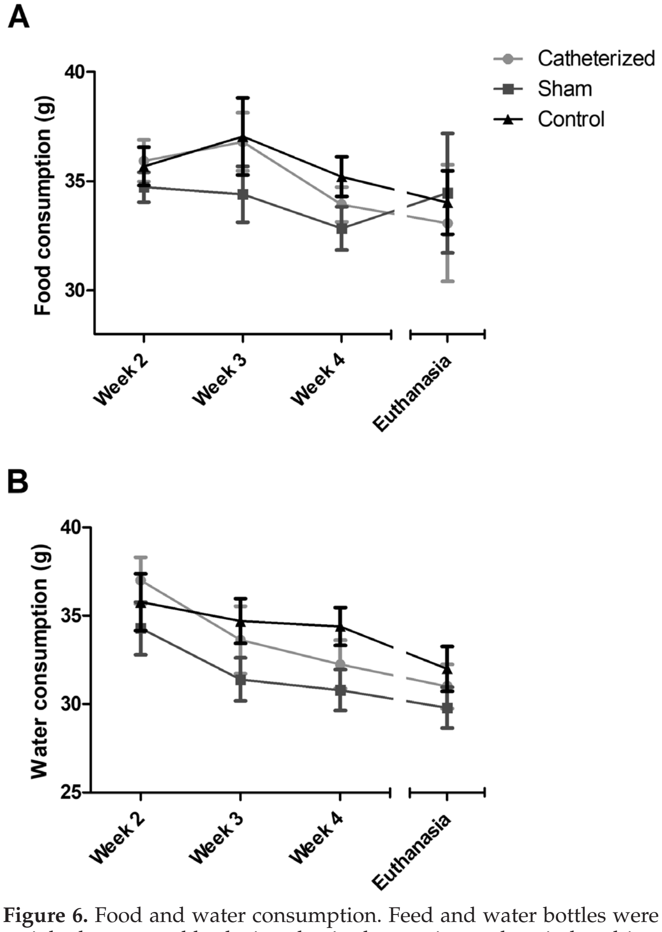

Figure 6. Food and water consumption. Feed and water bottles were weighed once weekly during the 4-wk experimental period and immediately before euthanasia. Food and water consumption were calculated by subtracting the obtained weight of food and water remaining from the weight at the previous measurement; thus, data collection began during week 2, because feed and water bottles were weighed for the first time in week 1. The amount of feed or water consumed did not differ between groups. Data are presented as mean ± SEM.

In the present study, the mice were allowed to recover for 2 d after surgery before further experimentation, perhaps explaining the lack of a FCM-quantifiable stress response, because any surgically induced stress may have normalized at this time. This

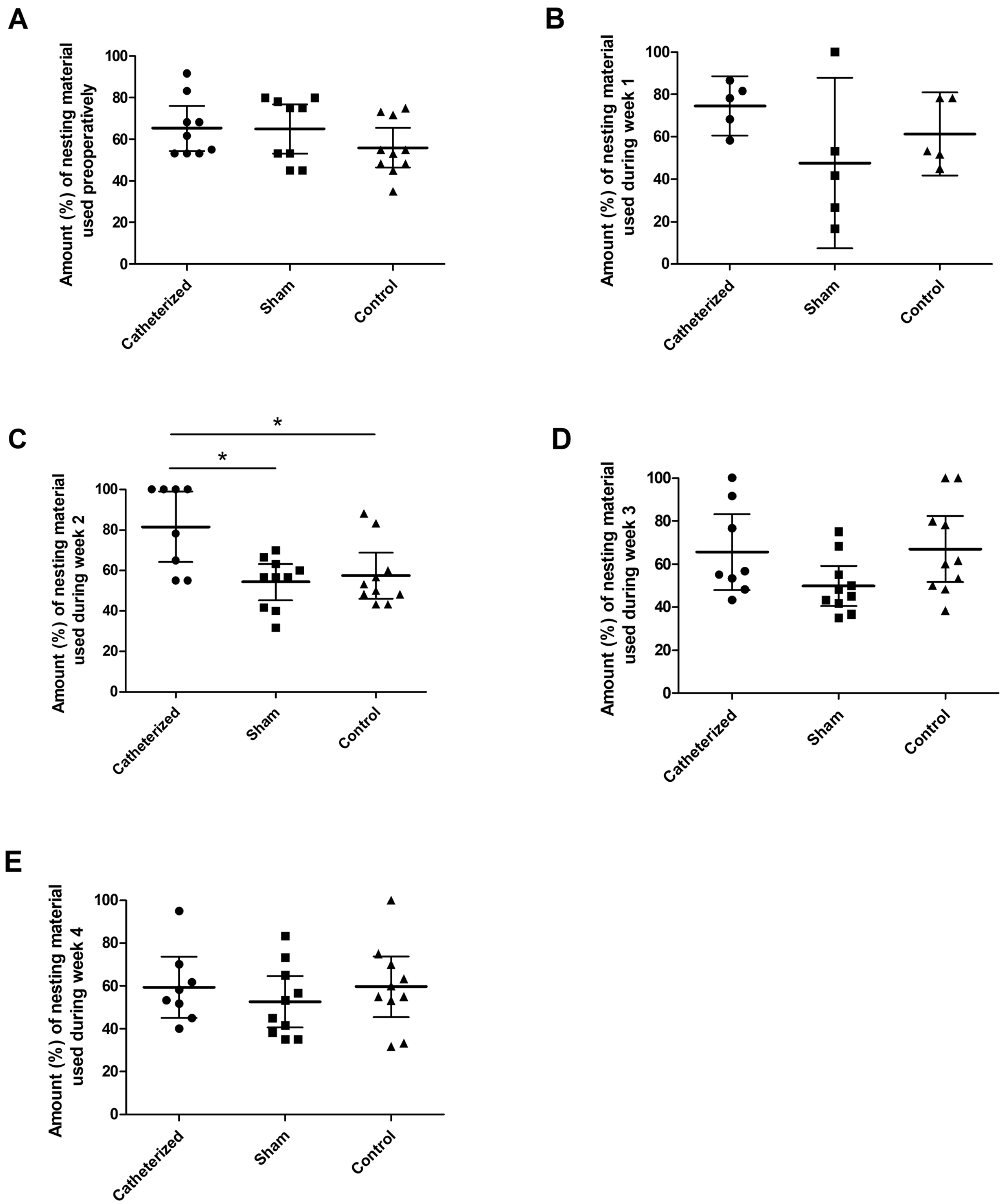

Figure 7. Nest quantity. The nest test was performed (A) from the week before surgery (Preoperative) and then (B through E) once weekly during the 4-wk experimental period; week 1 – week). The amount of nesting material used did not differ between groups. However, in week 2, control mice used more (*, $P < 0{,}05$) nesting material than sham-treated and catheterized mice. Data are presented as mean ± SEM.

normalization of the surgical stress response is in accordance with similar studies that used postcatheterization recovery periods of as long as 3 d.[35,43,46] The higher FCM levels in control mice the first week after surgery might be explained by decreased habituation to handling compared with the experimental mice, given that sham-operated and catheterized mice received pre- and postsurgical care in terms of analgesic administration as well as recording of body weight and food and water consumptions, whereas control mice did not. The elevated FCM levels in control mice gradually decreased during the study period, thus supporting this theory. All mice, including the control mice, were handled throughout the entire experimental period for weighing and sampling of feces and urine. The mice were handled by cupping to minimize stress.[4,19]

As expected, the body weights of sham-operated and catheterized mice decreased (albeit nonsignificantly) during the 2

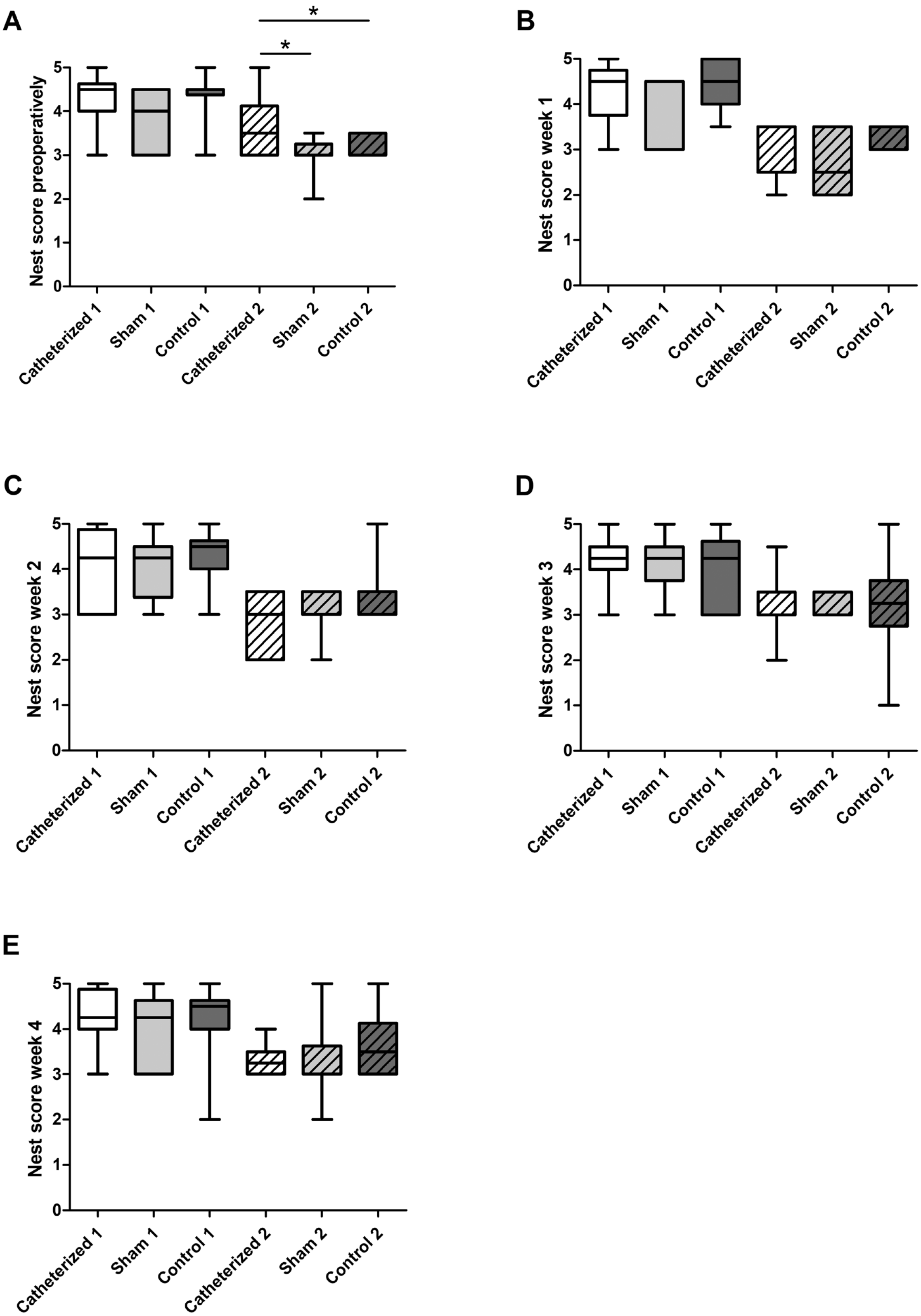

Figure 8. Nest quality. Nests were photographed and then scored according to the ethogram (Figure 1) during (A) the week before surgery (week –1) and then (B through E) once weekly during the 4-wk experimental period by 2 independent, blinded observers (1 and 2). No significant differences in the nest scores were found between groups. Data are presented as means with first and third quartiles, as well as minimal and maximal values.

d of postoperative monitoring. A transient loss in body weigh after surgery is a known effect of the physiologic stress of surgery,[5] which why we consider this decrease in body weight as a component of postoperative recovery rather than an outcome of the experiment. Other authors have found that body weight as well as food and water consumptions were useful indicators for pain and discomfort related to surgery.[42] Furthermore, other studies reported that postoperative analgesia had no effect on

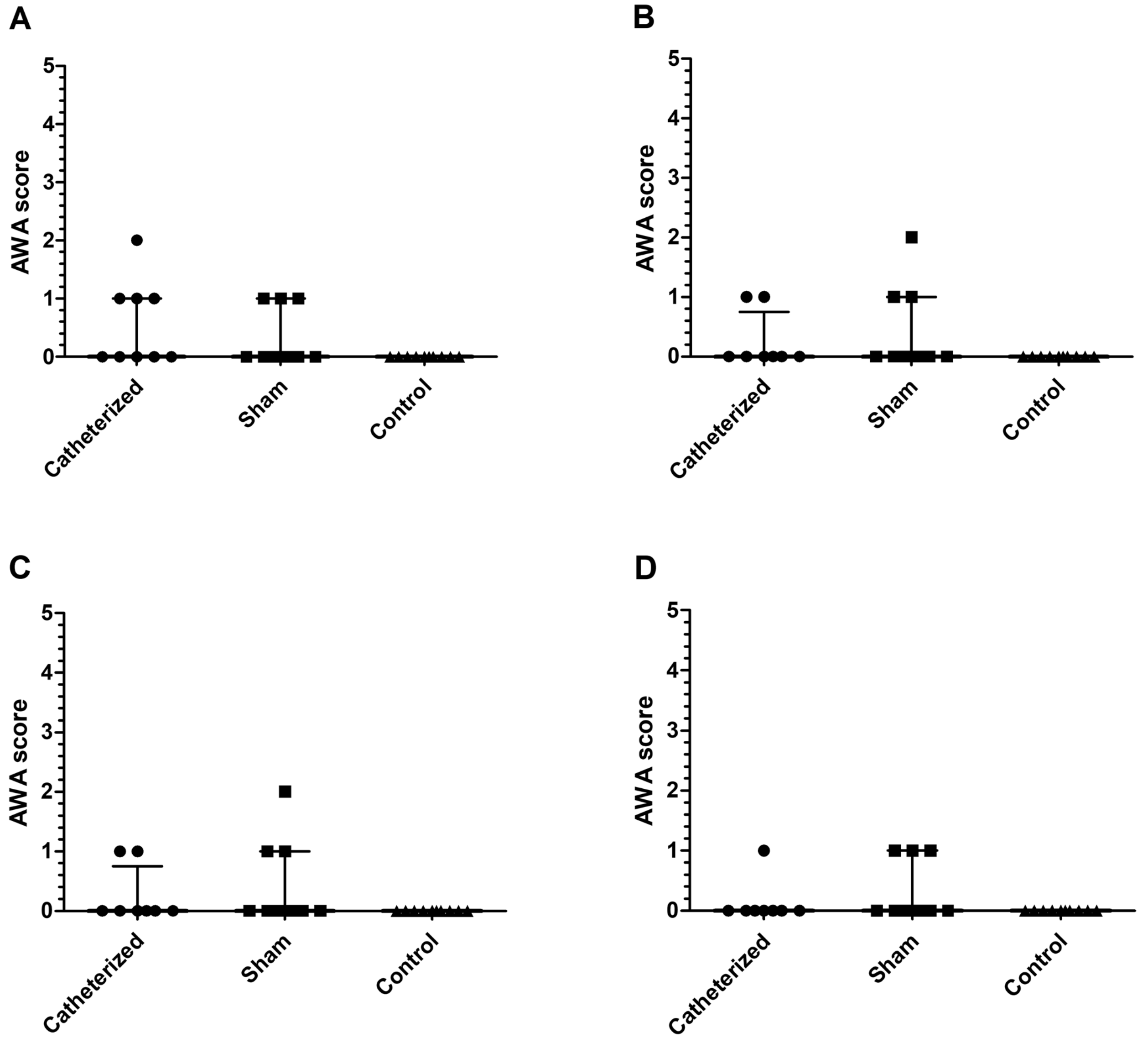

Figure 9. Animal welfare assessment. Animal welfare was assessed according to the ethogram (Figure 2) once weekly during (A) week 1, (B) week 2, (C) week 3, and (D) week 4 of the experimental period. Scores (median with interquartile range) did not differ between groups.

postoperative weight loss in rats, although those that received analgesia had less corticosterone excretion, suggesting greater wellbeing, than controls.[26,43] In the present study, the body weights of sham-operated and catheterized mice increased faster than that of control mice after the postoperative weight loss. This pattern may indicate improving wellbeing in the sham-operated and catheterized mice, paralleling the recovery of the loss in weight after surgery. After having reached presurgical levels on day 24 after surgery, sham-operated and catheterized mice did not differ from control mice in regard to body weight.

Nest building, a complex and species-typical behavior in many species including mice, has proven to be sufficiently sensitive to discriminate between states affecting animal welfare. Because nest building is highly motivated under normal circumstances, impaired nest-building behavior may be indicative of reduced animal welfare.[10,15,32] The nest test that we used in the present study was modified from a previous author's,[10] who describes the use of pressed cotton squares. Instead, we provided a pile of new, clean nesting material after removing all old material, because this modification worked well previously in our laboratory. We placed the nesting material outside the mouse house, to encourage the mice to move the material into their house, where we expected them to build their nest. During the first week after surgery, 4 of the 8 catheterized mice did not move the nesting material into the cardboard house but instead used the pile of material where it was placed. These catheterized mice likely were less motivated to move the nesting material inside their house than typically, given that they may still have been affected by the surgery, thus diluting natural behavior in this instance. Because we weighed the amount of nesting material incorporated into the nest to obtain objective scores of the nest, these 4 mice that did not move the nesting material inside the house were scored as having used all nesting material, erroneously giving the impression that catheterized mice used more nesting material during this week than other groups. This situation is a clear disadvantage compared with the original method,[12] which forces the mice to work in terms of shredding the cotton square to create nesting material. All other mice successfully moved the nesting material and built their nests inside the houses.

As described previously,[10] nest quality was assessed and scored blinded by 2 independent observers. The Spearman rank correlation coefficient showed a low correlation of 0.62 between the 2 observers, indicating the uncertainty associated with subjective parameters and why such parameters must never be used independently but should be interpreted along with objective measurements.

The AWA did not detect any effects due to catheterization or sham surgery. The use of an AWA protocol may be challenging, given that mice are prey animals. They therefore will try to hide any signs of weakness, including stress and suffering, especially in the presence of humans.[15] However, an AWA protocol—similar to that we used in the current study—was a significant predictor of tumor burden in a murine xenograft model.[21] Catheterization, as performed in the present study, may have had less of an effect on the wellbeing of mice compared with the murine xenograft model, thus affecting our AWA scores to a lesser degree.

Because the catheters were kept closed for the entire experimental period, the risk and frequency of catheter occlusion were not taken into consideration in this study. Daily maintenance of catheters is a potential source of stress, and catheter occlusions are significant adverse events.[45] Therefore the exclusion of these factors from the current study provides an advantage to the catheterized mice, compared with real-life toxicologic and pharmacologic testing using arterial catheterization. We chose this scenario so to study the isolated effects of permanent catheterization without the effects of catheter maintenance and related potential stress.

To manage pain, buprenorphine was administered perioperatively to mice that underwent surgery. The mice were allowed a 2-d recovery after surgery, during which period buprenorphine was administered in the morning. No experimentation other than a daily AWA to monitor welfare and recovery was conducted during the recovery period. Given its half-life of 9 h after voluntary ingestion,[27] we considered that buprenorphine would be almost completely eliminated by 24 h after the last dose and thus likely did not influence the results.

The present study found minimal effects of carotid catheterization on animal welfare and on the associated mouse model. However, because various characteristics differ between strains and stocks,[41] the results of the current study reflect effects on male catheterized BomTac:NMRI mice. Less robust strains, such as inbred or gene-manipulated mice, may prove more sensitive to this technique, and additional research is necessary to completely examine the effects of carotid catheterization in mice.

In conclusion, we found no gross effects of sham surgery or catheterization on any of the parameters studied. Provided appropriate and adequate surgical skills as well as postoperative care, catheterization alone does not seem to affect animal welfare to a greater extent. Minor influences from catheterization may, however, introduce small confounding factors in the experimental outcome, as this study was not designed to detect very small effects. More research is needed to further elucidate how this method affects animal welfare and wellbeing.

Acknowledgments

We thank Michael Falkenberg for his tremendous effort regarding data processing and statistical assistance.

References

1. **Abelson KSP, Adem B, Royo F, Carlsson HE, Hau J.** 2005. High plasma corticosterone levels persist during frequent automatic blood sampling in rats. In Vivo **19:**815–819.
2. **Abelson KSP, Jacobsen KR, Sundbom R, Kalliokoski O, Hau J.** 2012. Voluntary ingestion of nut paste for administration of buprenorphine in rats and mice. Lab Anim **46:**349–351. https://doi.org/10.1258/la.2012.012028.
3. **Arakawa H.** 2018. Ethological approach to social isolation effects in behavioral studies of laboratory rodents. Behav Brain Res **341:**98–108. https://doi.org/10.1016/j.bbr.2017.12.022.
4. **Balcombe JP, Barnard ND, Sandusky C.** 2004. Laboratory routines cause animal stress. Contemp Top Lab Anim Sci **43:**42–51.
5. **Baumans V, Brain PF, Brugére H, Clausing P, Jeneskog T, Perretta G.** 1994. Pain and distress in laboratory rodents and lagomorphs. Report of the Federation of European Laboratory Animal Science Associations (FELASA) Working Group on Pain and Distress accepted by the FELASA Board of Management November 1992. Lab Anim **28:**97–112. https://doi.org/10.1258/002367794780745308.
6. **Butz GM, Davisson RL.** 2001. Long-term telemetric measurement of cardiovascular parameters in awake mice: a physiological genomics tool. Physiol Genomics **5:**89–97. https://doi.org/10.1152/physiolgenomics.2001.5.2.89.
7. **Carstens E, Moberg GP.** 2000. Recognizing pain and distress in laboratory animals. ILAR J **41:**62–71. https://doi.org/10.1093/ilar.41.2.62.
8. **Christison GI, Curtin TM.** 1969. A simple venous catheter for sequential blood sampling from unrestrained pigs. Lab Anim Care **19:**259–262.
9. **Council of the European Communities.** [Internet]. 2011. Report from the Commission to the Council and the European Parliament. Seventh report on the statistics on the number of animals used for experimental and other scientific purposes in the member states of the European Union. [Cited 11 March 2019]. Available at: http://www.understandinganimalresearch.org.uk/files/7914/1207/5212/eu-animal-use-statistics-2013.pdf
10. **Deacon RMJ.** 2006. Assessing nest building in mice. Nat Protoc **1:**1117–1119. https://doi.org/10.1038/nprot.2006.170.
11. **Deng XS, Tuo JS, Poulsen HE, Loft S.** 1998. Prevention of oxidative DNA damage in rats by Brussels sprouts. Free Radic Res **28:**323–333. https://doi.org/10.3109/10715769809069284.
12. **Desborough JP.** 2000. The stress response to trauma and surgery. Br J Anaesth **85:**109–117. https://doi.org/10.1093/bja/85.1.109.
13. **Diehl KH, Hull R, Morton D, Pfister R, Rabemampianina Y, Smith D, Vidal JM, van de Vorstenbosch C.** 2001. A good-practice guide to the administration of substances and removal of blood, including routes and volumes. J Appl Toxicol **21:**15–23. https://doi.org/10.1002/jat.727.
14. **Dyreforsøgstilsynet**. [Internet]. 2015 Annual report. Ministry of the Environment and Food—the Danish Veterinary and Food Administration. [Cited 11 March 2019]. Available at: https://www.foedevarestyrelsen.dk/Dyr/dyrevelfaerd/Dyreforsoegstilsynet/Sider/forside.aspx [In: Danish].pdf
15. **Gaskill BN, Karas AZ, Garner JP, Pritchett-Corning KR.** 2013. Nest building as an indicator of health and welfare in laboratory mice. J Vis Exp **82:**1–7.
16. **Grouzmann E, Cavadas C, Grand D, Moratel M, Aubert JF, Brunner HR, Mazzolai L.** 2003. Blood sampling methodology is crucial for precise measurement of plasma catecholamines concentrations in mice. Pflugers Arch **447:**254–258. https://doi.org/10.1007/s00424-003-1140-x.
17. **Harper JM, Austad SN.** 2000. Fecal glucocorticoids: a noninvasive method of measuring adrenal activity in wild and captive rodents. Physiol Biochem Zool **73:**12–22. https://doi.org/10.1086/316721.
18. **Henriksen T, Hillestrom PR, Poulsen HE, Weimann A.** 2009. Automated method for the direct analysis of 8-oxo-guanosine and 8-oxo-2′-deoxyguanosine in human urine using ultraperformance liquid chromatography and tandem mass spectrometry. Free Radic Biol Med **47:**629–635. https://doi.org/10.1016/j.freeradbiomed.2009.06.002.
19. **Hurst JL, West RS.** 2010. Taming anxiety in laboratory mice. Nat Methods **7:**825–826. https://doi.org/10.1038/nmeth.1500.
20. **Institute for Laboratory Animal Research.** 2011. Guide for the care and use of laboratory animals 8th ed. Washington (DC): National Academies Press.
21. **Jacobsen KR, Jorgensen P, Pipper CB, Steffensen AM, Hau J, Abelson KSP.** 2013. The utility of fecal corticosterone metabolites and animal welfare assessment protocols as predictive parameters

of tumor development and animal welfare in a murine xenograft model. In Vivo **27:**189–196.
22. **Jacobsen KR, Kalliokoski O, Hau J, Abelson KSP.** 2011. Voluntary ingestion of buprenorphine in mice. Anim Welf **20:**591–596.
23. **Jacobsen KR, Kalliokoski O, Teilmann AC, Hau J, Abelson KS.** 2012. The effect of isoflurane anaesthesia and vasectomy on circulating corticosterone and ACTH in BALB/c mice. Gen Comp Endocrinol **179:**406–413. https://doi.org/10.1016/j.ygcen.2012.09.012.
24. **Jacobsen KR, Kalliokoski O, Teilmann AC, Hau J, Abelson KSP.** 2012. Postsurgical food and water consumption, fecal corticosterone metabolites, and behavior assessment as noninvasive measures of pain in vasectomized BALB/c mice. J Am Assoc Lab Anim Sci **51:**69–75.
25. **Joergensen A, Broedbaek K, Weimann A, Semba RD, Ferrucci L, Joergensen MB, Poulsen HE.** 2011. Association between urinary excretion of cortisol and markers of oxidatively damaged DNA and RNA in humans. PLoS One **6:**1–6. https://doi.org/10.1371/journal.pone.0020795.
26. **Kalliokoski O, Abelson KSP, Koch J, Boschian A, Thormose SF, Fauerby N, Rasmussen RS, Johansen FF, Hau J.** 2010. The effect of voluntarily ingested buprenorphine on rats subjected to surgically induced global cerebral ischaemia. In Vivo **24:**641–646.
27. **Kalliokoski O, Jacobsen KR, Hau J, Abelson KS**. 2011. Serum concentrations of buprenorphine after oral and parenteral administration in male mice. Vet J **187:**251–254. https://doi.org/10.1016/j.tvjl.2009.11.013.
28. **Kalliokoski O, Jacobsen KR, Teilmann AC, Hau J, Abelson KS.** 2012. Quantitative effects of diet on fecal corticosterone metabolites in 2 strains of laboratory mice. In Vivo **26:**213–221.
29. **Mähler Convenor M, Berard M, Feinstein R, Gallagher A, Illgen-Wilcke B, Pritchett-Corning K, Raspa M.** 2014. FELASA recommendations for the health monitoring of mouse, rat, hamster, guinea pig and rabbit colonies in breeding and experimental units. Lab Anim **48:**178–192. https://doi.org/10.1177/0023677213516312.
30. **McEwen BS.** 2017. Allostasis and the epigenetics of brain and body health over the life course. JAMA Psychiatry **74:**551–552. https://doi.org/10.1001/jamapsychiatry.2017.0270.
31. **Möstl E, Palme R.** 2002. Hormones as indicators of stress. Domest Anim Endocrinol **23:**67–74. https://doi.org/10.1016/S0739-7240(02)00146-7.
32. **National Research Council (US), Committee on Pain and Distress in Laboratory Animals.** 1992. Recognition and alleviation of pain and distress in laboratory animals. Washington (DC): National Academies Press.
33. **Pacher P, Nagayama T, Mukhopadhyay P, Bátkai S, Kass DA.** 2008. Measurement of cardiac function using pressure–volume conductance catheter technique in mice and rats. Nat Protoc **3:**1422–1434. https://doi.org/10.1038/nprot.2008.138.
34. **Parasuraman S, Raveendran R, Kesavan R.** 2010. Blood sample collection in small laboratory animals. J Pharmacol Pharmacother **1:**87–93. https://doi.org/10.4103/0976-500X.72350.
35. **Royo F, Björk N, Carlsson HE, Mayo S, Hau J.** 2004. Impact of chronic catheterization and automated blood sampling (Accusampler) on serum corticosterone and fecal immunoreactive corticosterone metabolites and immunoglobulin A in male rats. J Endocrinol **180:**145–153. https://doi.org/10.1677/joe.0.1800145.
36. **Sapolsky RM.** 2000. Stress hormones: good and bad. Neurobiol Dis **7:**540–542. https://doi.org/10.1006/nbdi.2000.0350.
37. **Sapolsky RM.** 2015. Stress and the brain: individual variability and the inverted U. Nat Neurosci **18:**1344–1346. https://doi.org/10.1038/nn.4109.
38. **Sapolsky RM, Romero LM, Munck AU.** 2000. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocr Rev **21:**55–89.
39. **Sies H.** 1997. Oxidative stress: oxidants and antioxidants. Exp Physiol **82:**291–295. https://doi.org/10.1113/expphysiol.1997.sp004024.
40. **Siswanto H, Hau J, Carlsson HE, Goldkuhl R, Abelson KSP.** 2008. Corticosterone concentrations in blood and excretion in faeces after ACTH administration in male Sprague–Dawley rats. In Vivo **22:**435–440.
41. **Sluyter F, Van Oortmerssen GA.** 2000. A mouse is not just a mouse. Anim Welf **9:**193–205.
42. **Stasiak KL, Maul D, French E, Hellyer PW, Vandewoude S.** 2003. Species-specific assessment of pain in laboratory animals. Contemp Top Lab Anim Sci **42:**13–20.
43. **Sundbom R, Jacobsen KR, Kalliokoski O, Hau J, Abelson KSP.** 2011. Postoperative corticosterone levels in plasma and feces of mice subjected to permanent catheterization and automated blood sampling. In Vivo **25:**335–342.
44. **Swindle MM, Nolan T, Jacobson A, Wolf P, Dalton MJ, Smith AC.** 2005. Vascular access port (VAP) usage in large animal species. Contemp Top Lab Anim Sci **44:**7–17.
45. **Teilmann AC, Falkenberg MK, Hau J, Abelson KSP.** 2014. Comparison of silicone and polyurethane catheters for the catheterization of small vessels in mice. Lab Anim (NY) **43:**397–403. https://doi.org/10.1038/laban.570.
46. **Teilmann AC, Jacobsen KR, Kalliokoski A, Hansen AK, Hau J, Abelson KSP.** 2012. The effect of automated blood sampling on corticosterone levels, body weight and daily food intake in permanently catheterized male BALB/c mice. In Vivo **26:**577–582.
47. **Teilmann AC, Kalliokoski O, Sorensen DB, Hau J, Abelson KS.** 2014. Manual versus automated blood sampling: impact of repeated blood sampling on stress parameters and behavior in male NMRI mice. Lab Anim **48:**278–291. https://doi.org/10.1177/0023677214541438.
48. **Teilmann AC, Rozell B, Kalliokoski O, Hau J, Abelson KS.** 2016. Carotid catheterization and automated blood sampling induce systemic IL6 secretion and local tissue damage and inflammation in the heart, kidneys, liver and salivary glands in NMRI mice. PLoS One **11:**1–14. https://doi.org/10.1371/journal.pone.0166353.
49. **Teilmann AC, Thomsen MB, Ihms EA, Pate N, Hau J, Abelson K.** 2017. Ultrasonographic and histological evaluation of the effects of long-term carotid catheterization on cardiac function in NMRI mice. Lab Anim **52:**17–28. https://doi.org/10.1177/0023677217706724
50. **Thanos PK, Cavigelli SA, Michaelides M, Olvet DM, Patel U, Diep MN, Volkow ND.** 2009. A noninvasive method for detecting the metabolic stress response in rodents: characterization and disruption of the circadian corticosterone rhythm. Physiol Res **58:**219–228.
51. **Touma C, Palme R.** 2005. Measuring fecal glucocorticoid metabolites in mammals and birds: the importance of validation. Ann N Y Acad Sci **1046:**54–74. https://doi.org/10.1196/annals.1343.006 PubMed
52. **Touma C, Palme R, Sachser N.** 2004. Analyzing corticosterone metabolites in fecal samples of mice: a noninvasive technique to monitor stress hormones. Horm Behav **45:**10–22. https://doi.org/10.1016/j.yhbeh.2003.07.002.
53. **Wong P, Pham R, Whitely C, Soto M, Salyers K, James C, Bruenner BA.** 2011. Application of automated serial blood sampling and dried blood spot technique with liquid chromatography–tandem mass spectrometry for pharmacokinetic studies in mice. J Pharm Biomed Anal **56:**604–608. https://doi.org/10.1016/j.jpba.2011.06.022.
54. **Yoburn BC, Morales R, Inturrisi CE.** 1984. Chronic vascular catheterization in the rat: comparison of 3 techniques. Physiol Behav **33:**89–94. https://doi.org/10.1016/0031-9384(84)90018-0.
55. **Yoshida R, Ogawa Y, Kasai H.** 2002. Urinary 8-oxo-7,8-dihydro-2'-deoxyguanosine values measured by an ELISA correlated well with measurements by high-performance liquid chromatography with electrochemical detection. Cancer Epidemiol Biomarkers Prev **11:**1076–1081.