Journal of the American Association for Laboratory Animal Science
Copyright 2013
by the American Association for Laboratory Animal Science

Vol 52, No 1
January 2013
Pages 17–21

Nonsurgical Embryo Transfer Device Compared with Surgery for Embryo Transfer in Mice

Kendra H Steele,[1,*] James M Hester,[1] Barbara J Stone,[1] Kimberly M Carrico,[3] Brett T Spear,[2] and Angelika Fath-Goodin[1]

The use of a murine nonsurgical embryo transfer (NSET) device had been described previously for the transfer of blastocysts, morulae, DNA-microinjected embryos, and embryonic stem cell-containing embryos to create genetically modified mice. However, physiologic effects of the NSET device and traditional surgical methods had not been compared directly. Here we used electrocardiography and fecal corticosterone levels to monitor pseudopregnant mice that underwent anesthesia only, the NSET procedure with or without anesthesia, or surgery. These procedures were performed without the use of actual embryos, to focus on effects of the procedures themselves rather than on any physiologic effects due to the deposition of embryos. As compared with surgery and anesthesia, the NSET procedure was associated with less fluctuation in cardiac rhythm and lower levels of the stress biomarker fecal corticosterone. These results indicate that use of the NSET device avoids these physiological perturbations as well as other disadvantages of surgery (for example, postoperative pain and need for postoperative analgesia) and therefore provides a valuable refinement of existing mouse embryo transfer procedures.

Abbreviations: dpc, days post coitum; NSET, nonsurgical embryo transfer.

Genetically modified mice have been used extensively to identify gene functions, model genetic disorders, and study cell differentiation in mammalian cells.[5,15] Constructing transgenic mice is a complex process that involves the microinjection of DNA into a fertilized oocyte or the targeting of genes by homologous recombination in embryonic stem cells, followed by surgical transfer of embryos to the oviduct or uterine horn of a pseudopregnant mouse.[8,15] During surgery, incisions are made through the skin and muscle, part of the reproductive tract is externalized for transfer of the embryos, and the incision site is closed with wound closures.[29] This surgery requires significant technical expertise, time, space, money and necessitates anesthesia and analgesia to minimize pain and distress of mice. Considerable time is necessary to train surgical technicians, prepare the embryo recipient mice, perform the procedure, and monitor mice for postsurgical complications. In addition, multiple areas of the laboratory must be devoted to preparing mice, performing surgery, and for the recovery of mice from surgery. Furthermore, the surgical lab needs a plethora of specialized equipment, including fur clippers, a dissecting microscope, an assortment of surgical instruments, a glass-bead sterilizer, wound clips or sutures, analgesics, and all of the equipment required to anesthetize mice.[23] Clearly, refinements to these procedures that eliminate the need for surgery would simplify the process of embryo transfer.

The Animal Welfare Act[2] requires principal investigators to follow Russell and Burch's '3Rs' of animal research[30] to find alternative procedures to replace animals, reduce the number of animals used, and to refine methodologies to minimize pain or distress.[19] A major survival surgery can be stressful for any animal, and surgery puts animals at risk for hypothermia and infection.[6,12] Therefore, by replacing surgery, principal investigators are demonstrating to their funding source and to their IACUC that they are refining their techniques to reduce pain and distress in mice.

To this end, we have developed a nonsurgical embryo transfer (NSET) device to replace the surgery required for producing genetically modified mice.[4,16] The NSET device is a small, tapered catheter used to insert embryos directly through the cervix into the uterine horn of a mouse. The NSET procedure is much quicker than is surgical embryo transfer, does not cause obvious discomfort to the mouse, and eliminates the need for anesthesia and analgesia. An earlier study[16] showed that the NSET procedure is just as effective as surgery in producing a similar number of healthy pups. Therefore, the NSET device saves time and costs, requires less training and laboratory space, and, most importantly, replaces the traditional surgical procedure.

In the current study, we compared the responses of female CD1 mice that underwent embryo transfer surgery, general anesthesia without surgery, or the NSET procedure with or without anesthesia. Several markers of physiologic stress in mice include weight changes, heart-rate fluctuations, and corticosterone levels. The mammalian body responds to physiologic stress through the autonomic nervous system, which affects many body functions, including heart rate, lung function, and eye dilation. The use of anesthesia is known to affect the autonomic nervous system of mice,[18,31] and this characteristic was an important consideration in the current study, given that the NSET procedure does not require the use of anesthesia whereas surgery does. Electrocardiography is considered the 'gold standard' for measuring heart rate and heart rate variability, because it measures how fast, how strong, and how often the atria and ventricles contract, not just how often the heart, in general, contracts.[18] Heart rate variability has often been used to determine animals' responses to physiologic and environmental stress. Changes in volume status, arterial pressure, and autonomic tone all lead to beat-to-beat variation.[18] In the current study, we noninvasively recorded the electrocardiograms of the

Received: 21 May 2012. Revision requested: 06 Jun 2012. Accepted: 19 Jul 2012.
[1]ParaTechs Corporation, Lexington, Kentucky; [2]The Markey Cancer Center and Department of Microbiology, Immunology, and Molecular Genetics at University of Kentucky, Lexington, Kentucky; [3]Master of Public Health Program, University of Cincinnati, Ohio.
[*]Corresponding author. Email: kendrasteele@paratechs.com

mice in the various experimental groups. We also measured fecal corticosterone levels, a well-known biomarker of stress in mice.[11,14] Our data demonstrate that heart beat fluctuations and fecal corticosterone levels were higher in response to surgery compared with insertion of the NSET device. These data provide physiologic evidence that the NSET device causes less impact on these variables than does surgery for embryo transfer in mice.

Materials and Methods

Mice. All research was IACUC-approved. Vasectomized male (age, 11 wk) and female (age, 6 wk) CD1 mice were obtained from Charles River Laboratories (Wilmington, MA). On arrival at our facility, male mice were housed individually, and female mice were housed 3 to 5 per cage in animal rooms maintained at 20 to 22 °C with an average relative humidity of 35% under a 12:12-h light:dark cycle. Mice were housed in standardized ventilated microisolation caging having 81 in.^{2} floor space (Innovive, San Diego, CA). Mice had access to irradiated feed (Purina Picolab mouse diet, Cincinnati Lab Supply, Cincinnati, OH) and bottled sterile water (Innovive) ad libitum. Each cage contained irradiated corncob bedding (Innovive), a synthetic nesting material (Ancare, Bellmore, NY), and a sterilized paper roll. In addition, male mice each received an enrichment dome and wheel (Innovive).

Pseudopregnancy was achieved according to a previously described procedure.[25] Briefly, 2 female mice (age, 2 to 6 mo) were placed with a vasectomized male mouse in a single cage for 3 consecutive days for mating. Female mice were checked each morning for copulation plugs. Female mice with plugs were removed from the mating cage and housed together at 3 to 5 mice per cage.

For both experiments, pseudopregnant mice at 2.5 d postcoitum (dpc) were randomly assigned into 5 experimental groups: untreated control, anesthesia-only control, NSET + anesthesia, NSET only, and surgery + anesthesia. The control group had no procedures performed on them; anesthesia-only mice were anesthetized with 2.5% isoflurane for 10 min; mice in the NSET + anesthesia group were anesthetized with 2.5% isoflurane for 10 min, during which they underwent NSET; mice in the NSET group underwent NSET without being anesthetized; and the surgery group was anesthetized with 2.5% isoflurane and underwent a surgical procedure that took approximately 10 min. All anesthetized mice received meloxicam (5 mg/kg IP), an NSAID with analgesic and antipyretic properties.

Surgical and NSET procedures. Surgical embryo transfer was performed as previously described.[25] Briefly, each pseudopregnant mouse was anesthetized with 2.5% isoflurane. The incision site was shaved and disinfected, and an incision was made along the dorsal side of the mouse. The ovarian fat pad, ovary, oviduct, and upper uterine horn were exteriorized, and a small hole was made in the uterine horn by using a 26-gauge needle. No embryos were transferred during the surgery. The organs were placed back into the body cavity, and wound clips were used to close the incision site. Mice were allowed to recover in a clean cage on top of a warming pad for 1 h and until their behavior was normal.

Embryo transfer with the NSET device (ParaTechs, Lexington, KY) was performed as previously described.[16] Briefly, each pseudopregnant mouse was placed on a wire-top cage and allowed to grip the bars. The small and large specula (ParaTechs) were placed sequentially into the vagina to open and expose the cervix. The NSET catheter then was inserted through the large speculum, past the cervical opening, and into the uterine horn. No embryos were actually transferred into the uterus. The device and specula were removed, and the mouse was returned to its home cage.

Electrocardiography. Pseudopregnant mice (at 2.5 dpc) were randomly assigned to the 5 experimental groups (11 mice per group). As described in previous studies, a noninvasive electrocardiogram-monitoring device (The ECGenie, Mouse Specifics, Quincy, MA) was used to record electrocardiographic data from unrestrained mice without surgical implants.[17,24] Electrocardiograms were recorded the day before the experimental procedure (–1 d), 1 h before the procedure (–1 h), directly after the procedure or during recovery from anesthesia (0 h), and at 1, 3, 6, and 24 h after the various experimental procedures. Readings were obtained by placing each mouse on a platform that was instrumented with 3 monitoring electrodes. The electrocardiogram was measured passively through the paws as the mice explored the platform. The time necessary to record an acceptable cardiac signal depended on the mobility of the mouse and varied between 5 and 8 min. The raw data were analyzed by Mouse Specifics using their by EzCG analysis software. Calculated metrics included heart rate and heart rate variability.

Fecal sample collections and quantifying corticosterone levels. Pseudopregnant mice (at 2.5 dpc) were randomly assigned to the 5 experimental groups (15 mice per group). Fecal samples were collected the day before the procedure (–1 d), immediately before the procedure (0 h), and at 3, 6, 10, 24, 48, 72, 96, and 120 h after the procedure was completed. Samples were collected by transferring each mouse to a clean, empty cage, in which the subject remained for 30 min or until 3 fecal pellets were collected. Fecal pellets contaminated with urine were excluded from the study, because urine also contains metabolic corticosterone. Each sample was stored at −80 °C until processed. The time of collection and mouse weight were recorded.

A commercially available ELISA (Cayman Chemical Company, Ann Arbor, MI) was used to measure corticosterone levels in each fecal sample as described[32] and according to the manufacturer's directions. Briefly, each sample was resuspended in 95% ethanol (20 µL per 1 mg of fecal sample). After overnight vortexing of samples, the supernatants were collected by centrifugation (1083 × *g* for 15 min) and moved to clean 1.5-mL microcentrifuge tubes. The ELISA was performed by using the manufacturer's assay reagents and 1:100 dilutions of each fecal suspension. Dilutions of the manufacturer's corticosterone standard were used to construct a standard curve, and the level of corticosterone in the fecal suspensions was determined by comparison to the standard curve and by using the Cayman data analysis tool (www.myassays.com/corticosterone.assay).

Statistical analyses. Data are reported as mean ± 1 SD. The effect of treatment and time was evaluated by 2-way repeated-measures ANOVA (GraphPad Prism software, La Jolla, CA). Post hoc Bonferroni comparison was performed to identify statistical differences between groups at each time point whenever 2-way ANOVA analysis indicated a significant interaction between time and treatment group. A P value of 0.05 or less was used to indicate statistical significance.

Results

Decreased variance in heart rate after use of the NSET device. We noninvasively recorded electrocardiograms to measure heart rate and heart rate variability in our mice. Mean heart rates in the 3 groups of mice that were anesthetized (anesthesia-only control, NSET + anesthesia, surgery) decreased from 800 bpm to 400 to 500 bpm during the procedure and recovery, whereas those of untreated control mice and of mice that underwent

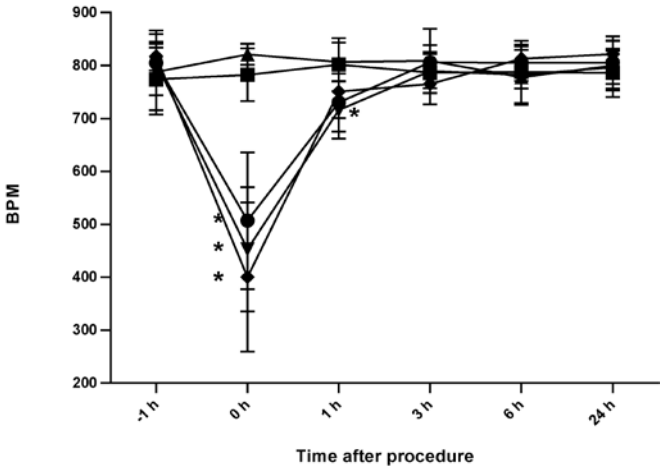

Figure 1. Heart rate (bpm; mean ± 1 SD; n = 11) at various times (h) after procedure in 2.5 dpc pseudopregnant CD1 mice in the following groups: untreated control (■), NSET (▲), NSET + anesthesia (▼), anesthesia-only control (●), and surgery (◆). *, Value is significantly ($P \leq 0.05$, Bonferroni post hoc test) different from that of the untreated control group.

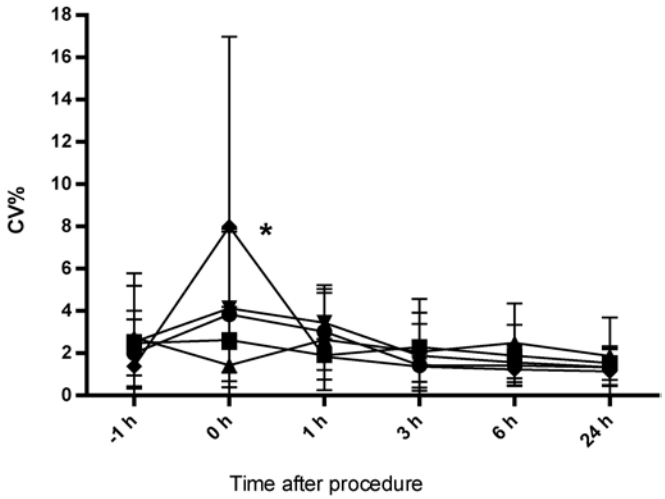

Figure 2. Coefficient of variance (CV%; mean ± 1 SD; n = 11) at various times (h) after procedure for 2.5 dpc pseudopregnant CD1 mice in the following groups: untreated control (■), NSET (▲), NSET + anesthesia (▼), anesthesia-only control (●), and surgery (◆). *, Value significantly ($P \leq 0.05$; 2-way repeated-measures ANOVA followed by Bonferroni post hoc analysis) different from that of the untreated control group.

NSET only remained unchanged (Figure 1). Comparison by 2-way repeated-measures ANOVA showed that treatment ($P < 0.0001$), time ($P < 0.0001$) and the interaction between these 2 factors ($P < 0.0001$) had significant effects on heart rate. In addition, matching between subjects was extremely effective ($P < 0.0001$). The heart rate of the NSET group differed significantly from those of the surgery, anesthesia-only, and NSET + anesthesia groups at 0 h ($P < 0.01$) and from that of the NSET + anesthesia group at 1 h ($P < 0.05$).

Variability in the beat-to-beat rhythm of the heart was measured as the coefficient of variance. A high-percentage coefficient of variance can indicate changes in cardiac function and the mouse's ability to respond to physiologic stress.[18] The coefficients of variance of mice that underwent surgery increased from 1.5% at baseline to 8% during the procedure and recovery period (Figure 2) and was significantly different from that of mice in the untreated control ($P < 0.0001$), NSET ($P < 0.0001$), and anesthesia-only ($P < 0.01$) groups; this parameter remained unchanged in the remaining groups, including those that received anesthesia. Among all groups, mice that underwent NSET had the least heart variability during recovery. In addition, 2-way repeated-measures ANOVA showed that time ($P < 0.0001$) but not treatment ($P = 0.9858$) was a significant source of variation overall; the interaction of time and treatment was found to be extremely significant ($P < 0.0001$). Matching between subjects was significant ($P = 0.0011$). These findings support a reduction in physiologic stress after use of the NSET device compared with surgery and anesthesia, likely because NSET eliminates the detrimental cardiac effects caused by anesthesia and surgery.

Fecal corticosterone levels after insertion of the NSET device. At 3 and 6 h after the procedures, fecal corticosterone concentrations were significantly ($P < 0.05$) higher in CD1 mice that underwent embryo transfer surgery compared with untreated control mice (Figure 3). Fecal corticosterone levels in anesthetized mice (that is, anesthesia-only and NSET + anesthesia groups) did not differ from those of control mice. The corticosterone levels of the NSET and anesthesia-only groups at 3 h after the procedure were significantly ($P < 0.05$) lower than those of the surgery group. The fold increases in corticosterone (relative to baseline measurements) resulting from experimental treatment and time were analyzed via 2-way repeated-measures ANOVA. Time was a significant ($P < 0.0001$) source of variation, as was the interaction between time and treatment ($P < 0.0001$), whereas treatment alone was not a significant source of variation ($P = 0.3509$). In addition, matching between subjects was extremely effective ($P < 0.0001$).

Mice in all 5 groups had a 5% weight gain from day 0 to day 5 (data not shown), indicating that anesthesia, surgery, and NSET procedures do not affect the mice to the extent that they alter their eating habits. This result was expected, given that most surgeries that include meloxicam postoperatively do not result in weight loss.[7,35]

Discussion

Genetically modified mice are valuable tools for in vivo mammalian studies that include identifying gene functions and metabolic processes and studying autoimmune disorders and tumor biogenesis.[5,15,28,40] Obtaining genetically modified mice is a multistep process that involves the modification of the genetic make-up of a mouse by transferring genetically modified embryos to pseudopregnant recipients so that the offspring will express the altered DNA. Traditionally, embryo delivery has been accomplished through surgical manipulation,[29] but this process is costly and time-consuming, requires dedicated surgical space and substantial technical expertise, and, as found in the current study, is stressful to mice.

Many stressors activate the autonomic nervous system and increase activity of the sympathetic or parasympathetic pathways.[26] Anesthesia activates the parasympathetic pathway and suppresses the sympathetic pathway.[3,26,31] Mice anesthetized with isoflurane in the current experiments showed decreases in heart rate. Values returned to normal once the anesthetic affect deteriorated; therefore, isoflurane caused a short-lived physiologic response. The coefficient of variance is a measure of heart rate variability and specifically measures inconsistency between heart beats.[38] Many variables might affect the coefficient of variance, including body temperature, heart rate, and posture, all of which change in response to anesthesia.[3,12] A change in the coefficient of variance indicates that physiologic

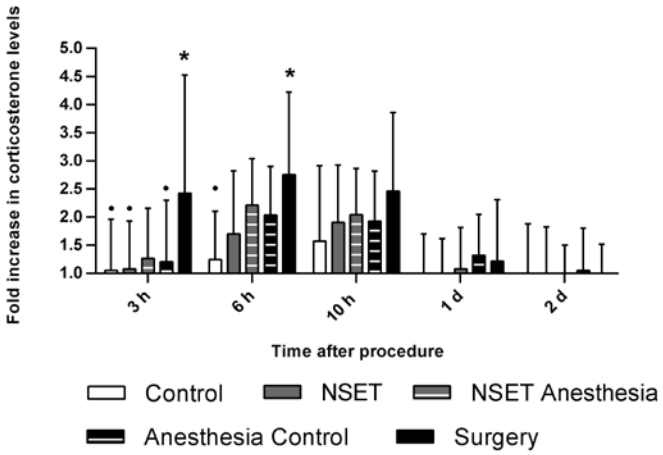

Figure 3. Fold increase (mean ± 1 SD; $n = 15$) in fecal corticosterone levels at various times after procedure for 2.5 dpc pseudopregnant CD1 mice. *, Value significantly ($P \leq 0.05$, Bonferroni post hoc test) different from that of the untreated control group. ●, Value significantly ($P \leq 0.05$, Bonferroni post hoc test) different from that of the surgery group.

stress has occurred, and this measure was much higher for mice in the surgery group than for any other group. This irregularity potentially was due to the decreases in body temperature and heart rate from anesthesia and the manipulation associated with the surgical procedure itself. In contrast, NSET mice exhibited no significant postprocedural heart rate variability, suggesting that using the NSET device is safer and less stressful than surgical embryo transfer in regard to cardiac function.

Many methods are available to measure heart rate, but electrocardiograms are particularly advantageous because they reveal the rapidity, strength, and regularity of the heart beats and the intervals between heart beats.[18] Although arterial pressure can be monitored by using a tail-cuff or implanted telemetry system, these methods are invasive techniques that only calculate heart rate.[18,41] In contrast, the noninvasive system we used collects recordings from conscious mice as they explore a platform inside a 3-sided box. The free movement of the mice makes it challenging to collect readings at fixed intervals. Because the cardiac effects of stress may be short-lived, data may have been lost before the mouse achieved the appropriate position for recording. However, the noninvasive electrocardographic system can record copious information about cardiac function from numerous mice quickly. Future studies may include pulse oximetry, a well-accepted method for monitoring heart rate and blood oxygen saturation levels.[18,20] Although the mobility of the subject is limited, pulse oximetry can monitor heart rate without inducing a stress response.[18]

A second way to measure stress is to quantify corticosterone levels. Analysis of fecal corticosterone levels revealed significant increases at 3 and 6 h after surgery. In contrast, NSET did not raise corticosterone levels above control levels at any time point. Consistent with our cardiac data, this result indicates that use of the NSET device is less stressful to mice than is surgical embryo transfer.

Fecal corticosterone metabolites are an effective measure of rodent hypothalamic–pituitary–adrenal pathway activation over a prolonged period of time. However, fecal corticosterone is not a marker of acute stress, because the corticosterone must travel through the digestive tract before being collected. This lag may explain why we obtained the highest levels of fecal corticosterone at 6 h instead of immediately after surgery. Previous studies that measured radioactive fecal corticosterone or its precursor in the hypothalamic–pituitary–adrenal pathway, adrenocorticotropic hormone, found peak levels of corticosterone at about 9 h after injection,[21,33,34] but the time ranged from 4 to 12 h and depended on time of day of injection (that is, morning compared with evening) and mouse strain. These studies[21,33,34] also found that 47% to 60% of the injected corticosterone was in urine. In contrast, we measured biologically produced corticosterone only in the feces of CD1 mice (a different strain of mice than that in the cited studies), which may account for why we measured high levels of fecal corticosterone starting at 3 h, much earlier than in previous studies.[21,33,34] In addition, because we did not measure urinary corticosterone, we did not measure the total corticosterone produced. Alternatively, we might have analyzed serum corticosterone levels to measure acute stress. However, the collection of mouse serum is an invasive procedure that itself causes a stress response.[1,13,36] In contrast to collecting blood samples, collecting fecal samples for corticosterone analysis is noninvasive and has been validated in many studies.[21,32-34] Handling the mice during sample collection might be a source of stress, but because all mice experienced equivalent handling, any differences in fecal corticosterone levels likely result from differences in experimental procedure rather than from procedural handling.

A previous study[16] showed that the NSET device produced the same percentage of pups and chimeric pups as did surgery for embryo transfer in CD1 mice. The current study indicates that NSET has less effect on some measures of stress than does surgical embryo transfer. Future studies of NSET will address the efficiency of transfer for different stages of embryos and involve different strains of recipient mice.

Any invasive surgery can cause pain, and pain influences diverse physiologic functions that affect study outcomes.[10] Reduction of animal pain and stress should always be a goal of ethical scientific research. This expectation is reflected in the 3Rs[30] and mandates from oversight authorities.[19] Our data indicate that the NSET procedure is as effective as surgery for embryo transfer research purposes[16] but is associated with less fluctuation in cardiac rhythm and lower levels of the stress biomarker fecal corticosterone, indicating less physiologic impact on the mice than occurs with surgery. In addition, unlike the surgical method, the noninvasive NSET method would not be associated with surgical pain and therefore postoperative analgesia can be avoided. Together, these data indicate that the NSET device is an effective refinement for embryo transfer and, in keeping with the principles of the 3Rs, should be considered for research that requires the transfer of mouse embryos.

Acknowledgments

We thank Mouse Specifics for their generous support regarding the ECGenie instrument and for their blinded analysis of the data. Research reported in this publication was supported in part by the Office of the Director, NIH, under award numbers 2R44RR025737-02 and 8R44OD010958. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH. In addition, this technology was supported in part by an award from the Kentucky Cabinet for Economic Development, Office of Commercialization and Innovation, under the grant agreement KSTC-184-512-11-115 with the Kentucky Science and Technology Corporation.

References

1. **Abelson KS, 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. **Animal Welfare Act as Amended**. 2008. 7 USC 2131-2159.

3. **Appel ML, Berger RD, Saul JP, Smith JM, Cohen RJ.** 1989. Beat to beat variability in cardiovascular variables: noise or music? J Am Coll Cardiol **14:**1139–1148.
4. **Barrette VF, Adams MA, Croy BA.** 2012. Endometrial decidualization does not trigger the blood pressure decline of normal early pregnancy in mice. Biol Reprod **86:**66.
5. **Bockamp E, Maringer M, Spangenberg C, Fees S, Fraser S, Eshkind L, Oesch F, Zabel B.** 2002. Of mice and models: improved animal models for biomedical research. Physiol Genomics **11:**115–132.
6. **Bradfield JF, Schachtman TR, McLaughlin RM, Steffen EK.** 1992. Behavioral and physiologic effects of inapparent wound infection in rats. Lab Anim Sci **42:**572–578.
7. **Brennan MP, Sinusas AJ, Horvath TL, Collins JG, Harding MJ.** 2009. Correlation between body weight changes and postoperative pain in rats treated with meloxicam or buprenorphine. Lab Anim (NY) **38:**87–93.
8. **Bronson SK, Smithies O.** 1994. Altering mice by homologous recombination using embryonic stem cells. J Biol Chem **269:**27155–27158.
9. **Burrows HL, Nakajima M, Lesh JS, Goosens KA, Samuelson LC, Inui A, Camper SA, Seasholtz AF.** 1998. Excess corticotrophin releasing hormone-binding protein in the hypothalamic–pituitary–adrenal axis in transgenic mice. J Clin Invest **101:**1439–1447.
10. **Dunwoody CJ, Krenzischek DA, Pasero C, Rathmell JP, Polomano RC.** 2008. Assessment, physiological monitoring, and consequences of inadequately treated acute pain. J Perianesth Nurs **23:**S15–S27.
11. **Engler H, Bailey MT, Engler A, Stiner-Jones LM, Quan N, Sheridan JF.** 2008. Interleukin 1 receptor type 1-deficient mice fail to develop social stress-associated glucocorticoid resistance in the spleen. Psychoneuroendocrinology **33:**108–117.
12. **Fox LK, Flegal MC, Kuhlman SM.** 2008. Principles of anesthesia monitoring—body temperature. J Invest Surg **21:**373–374.
13. **Gärtner K, Büttner D, Döhler K, Friedel R, Lindena J, Trautschold I.** 1980. Stress response of rats to handling and experimental procedures. Lab Anim **14:**267–274.
14. **Glasner A, Avraham R, Roseene E, Benish M, Zmora O, Shemer S, Meiboom H, Ben-Eliyahu S.** 2010. Improving survival rates in 2 models of spontaneous postoperative metastasis in mice by combined administration of a β-adrenergic antagonist and a cyclooxygenase 2 inhibitor. J Immunol **184:**2449–2457.
15. **Gordon JW, Scangos GA, Plotkin DJ, Barbosa JA, Ruddle FH.** 1980. Genetic transformation of mouse embryos by microinjection of purified DNA. Proc Natl Acad Sci USA **77:**7380–7384.
16. **Green M, Bass S, Spear BT.** 2009. A device for the simple and rapid transcervical transfer of mouse embryos eliminates the need for surgery and potential postoperative complications. Biotechniques **47:**919–924.
17. **Heier CR, Hampton TG, Wang D, Didonato CJ.** 2010. Development of electrocardiogram intervals during growth of FVB/N neonate mice. BMC Physiol **10:**16.
18. **Ho D, Zhao X, Gao S, Hong C, Vatner DE, Vatner SF.** 2011. Heart rate and electrocardiography monitoring in mice. Curr Protoc Mouse Biol **1:**123–139.
19. **Institute of Laboratory Animal Research.** 2011. Guide for the care and use of laboratory animals, 8th ed. Washington (DC): National Academies Press.
20. **Iyriboz Y, Powers S, Morrow J, Ayers D, Landry G.** 1991. Accuracy of pulse oximeters in estimating heart rate at rest and during exercise. Br J Sports Med **25:**162–164.
21. **Kalliokoski O, Hau J, Jacobsen KR, Schumacher-Petersen C, Abelson KS.** 2010. Distribution and time course of corticosterone excretion in faeces and urine of female mice with varying systemic concentrations. Gen Comp Endocrinol **168:**450–454.
22. **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.
23. **Longenecker G, Kulkarni AB.** 2009. Generation of gene knockout mice by ES cell microinjection. Curr Protoc Cell Biol Chapter **19:**1–36.
24. **Mabe AM, Hoover DB.** 2011. Remodeling of cardiac cholinergic innervations and control of heart rate in mice with streptozotocin-induced diabetes. Auton Neurosci **162:**24–31.
25. **Nagy A, Gertsenstein M, Vintersten K, Behringer R.** 2003. Manipulating the mouse embryo: a laboratory manual, 3rd ed. Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press.
26. **National Research Council.** 2008. Recognition and alleviation of distress in laboratory animals. Washington (DC): The National Academies Press.
27. **New World Encyclopedia Contributors.** [Internet]. 2008. Autonomic nervous system. New World Encyclopedia. [Cited 12 June 2012]. Available at: http://www.newworldencyclopedia.org/p/index.php?title=Autonomic_nervous_system&oldid=831950.
28. **Nilsson LM, Forshell TZ, Rimpi S, Kreutzer C, Pretsch W, Bornkamm GW, Nilsson JA.** 2012. Mouse genetics suggests cell-context dependency for Myc-regulated metabolic enzymes during tumorigenesis. PLoS Genet **8:**e1002573.
29. **Parker JM, Austin J, Wilkerson J, Carbone L.** 2011. Effects of multimodal analgesia on the success of mouse embryo transfer surgery. J Am Assoc Lab Anim Sci **50:**466–470.
30. **Russell WMS, Burch RL.** 1959. The principles of humane experimental technique. London (UK): Methuen.
31. **Tan TP, Gao XM, Krawczyszyn M, Feng X, Kiriazis H, Dart AM, Du XJ.** 2003. Assessment of cardiac function by echocardiography in conscious and anesthetized mice: importance of the autonomic nervous system and disease state. J Cardiovasc Pharmacol **42:**182–190.
32. **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.
33. **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.
34. **Touma C, Sachser N, Möstl E, Palme R.** 2003. Effects of sex and time of day on metabolism and excretion of corticosterone in urine and feces of mice. Gen Comp Endocrinol **130:**267–278.
35. **Tubbs JT, Kissling GE, Travlos GS, Goulding DR, Clark JA, King-Herbert AP, Blankenship-Paris TL.** 2011. Effects of buprenorphine, meloxicam, and flunixin meglumine as postoperative analgesia in mice. J Am Assoc Lab Anim Sci **50:**185–191.
36. **Vachon P, Moreau JP.** 2001. Serum corticosterone and blood glucose in rats after 2 jugular-vein blood sampling methods: comparison of the stress response. Contemp Top Lab Anim Sci **40:**22–24.
37. **Walker MK, Boberg JR, Walsh MT, Wolf V, Trujillo A, Duke MS, Palme R, Felton LA.** 2012. A less stressful alternative to oral gavage for pharmacological and toxicological studies in mice. Toxicol Appl Pharmacol **260:**65–69.
38. **Weiergräber M, Henry M, Südkamp M, de Vivie ER, Hescheler J, Schneider T.** 2005. Ablation of $Ca_v2.3$/E-type voltage-gated calcium channel results in cardiac arrhythmia and altered autonomic control within the murine cardiovascular system. Basic Res Cardiol **100:**1–13.
39. **Wright-Williams SL, Courade J-P, Richardson CA, Roughan JV, Flecknell PA.** 2007. Effects of vasectomy surgery and meloxicam treatment on faecal corticosterone levels and behavior in 2 strains of laboratory mouse. Pain **130:**108–118.
40. **Zhang L, Yu W, Schroedter I, Kong J, Vrontakis M.** 2012. Galanin transgenic mice with elevated circulating galanin levels alleviate demyelination in a cuprizone-induced MS mouse model. PLoS ONE **7:**e33901.
41. **Zhao X, Ho D, Gao S, Hong C, Vatner DE, Vatner SF.** 2011. Arterial pressure monitoring in mice. Curr Protoc Mouse Biol **1:**105–122.