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Effects of Laboratory Housing Conditions on Core Temperature and Locomotor Activity in Mice

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Drug developers worldwide assess compound safety and efficacy using measures that include mouse core temperature and locomotor activity. Subtle differences in animal housing conditions between institutions can alter these values, impacting scientific rigor and reproducibility. In these studies, adult male NIH Swiss mice were surgically implanted with radiotelemetry probes that simultaneously monitored core temperature and locomotor activity across various housing conditions. In the first study, ambient temperature was varied between 20 °C and 28 °C in groups of singly housed mice. Additional studies held the mice at a constant ambient temperature and examined the effects of cage density (housing animals singly or in groups of 3 or 6), bedding change and provision of nesting material, and the availability of a running wheel on core temperature and locomotor activity. Mice overwhelmingly maintained species-typical core temperatures across all ambient temperatures, across all housing conditions, when bedding was fresh or old, and with or without the provision of cotton squares as nesting material. However, engaging in wheel running and the combination of fresh bedding and cotton squares transiently increased core temperatures beyond the species-typical range. Similarly, the circadian distribution of locomotor activity was significantly disrupted by placing animals in cages with fresh bedding or nesting material, or by performing both of these manipulations concurrently during the light period. These findings suggest that standard husbandry practices and common housing conditions may transiently affect core temperature in adult mice. Furthermore, these practices may have profound and relatively long-lasting effects on motor activity and the regulation of circadian rhythms.

Abbreviations: U of M, University of Michigan; UAMS, University of Arkansas for Medical Sciences

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Arguments against animal use for biomedical research often state that results do not sufficiently translate from animal models to humans, and that safety and efficacy assays using animal models yield inconclusive results.[2] One strategy to combat these arguments emphasizes improving the "rigor and reproducibility"[23] of experiments. Rigorous experimental design and reporting of experimental methods should increase the likelihood that studies conducted by different lab groups will yield the same results, validating original findings and establishing consistency. Data that are not reproducible suggest that the animal model is unreliable.

A possible source of variability that may diminish rigor and reproducibility relates to differences in husbandry practices between institutions. Significant variability continues to exist with regard to housing conditions for laboratory animals, including temperatures, cage densities, bedding, nesting materials, and environmental enrichment. A recent example concerns the minimal ambient temperature for rodent housing, which was raised by several degrees in the eighth edition of the National Institutes of Health's Guide for the Care and Use of Laboratory Animals.[16] Some features of animal husbandry can alter commonly collected experimental data. Failure to consider management techniques may reduce reproducibility between labs and compromise the validity of some animal models used in biomedical research.

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Mouse body temperature and locomotor activity are common measures of interest in many in vivo studies in various biomedical disciplines. Historically, species-typical ranges for these 2 parameters have been difficult to determine in mice across the circadian cycle. Range estimates are often based on limited, and often unpublished, data sets, and are often coupled with subjective and anecdotal judgments regarding what is "normal" within a specific laboratory setting.[9] Furthermore, estimates of normal ranges for body temperature and locomotor activity in laboratory mice usually do not consider the effects of numerous housing factors and their interactions. Therefore, comparing across studies and extending previous work can be difficult due to these significant differences in environmental factors that affect the experimental variables of interest.

A common means of measuring mouse body temperature is by using a rectal thermometer.[12] The National Center for Infectious Diseases of the Centers for Disease Control and Prevention and the National Institutes of Health states that the average rectal temperature for a mouse is 36.5 ± 1.3 °C (see TABLE 1 "Thermoregulation Data on Common Research Animal Species").[24] The citation provided for this figure[13] states that "modification in activity is unquestionably a significant part of temperature adaptation," but does not measure or control for potential differences in locomotor behavior. Nevertheless, one report[13] found average rectal temperatures between approximately 36 °C (at a cold ambient temperature of approximately 15 °C) and approximately 38 °C (at a hot ambient temperature of approximately 35 °C) in mice, suggesting the capacity to thermoregulate across a wide range of ambient temperatures.

However, the process of inserting a rectal probe, as was done in the referenced study,[13] rapidly raises rectal temperature by about 0.5 to 1.5 °C,[26] and indeed this procedure is often used to model the feature of anxiety known as stress-induced hyperthermia.[1] Previous rodent studies have also shown that temperatures measured by a rectal probe may be lower (approximately 0.6 °C) and more variable than core temperatures recorded by chronically implanted devices.[4] In addition, procedures associated with measuring rectal temperature also affect locomotor activity.[4] Rodents do not seem to habituate to the rectal probe procedure, as neither hyperthermia nor locomotor effects decrease with repeated exposure.[4] All of these findings suggest that definitions of "normal" core temperature based upon samplings of rectal temperatures may be inaccurate.

To further study how common laboratory housing conditions affect core temperature and locomotor activity in mice, we conducted a retrospective analysis of data derived from approximately 10 y of studies conducted at the University of Michigan (U of M) and the University of Arkansas for Medical Sciences (UAMS) involving radiotelemetry in mice. Using data collected during studies of behavioral pharmacology and the neuropharmacology of drugs of abuse, we identified "non-injection control sessions" in which core temperature and locomotor activity were monitored across various housing conditions for at least 24 h. The resulting data set represented more than 20,000 samples of core temperature and motor activity across a broad range of conditions that are likely to be encountered in standard research settings, allowing a thorough analysis of the contributions of ambient temperature, cage density, bedding and nesting materials, and running wheel access on core temperature and locomotor activity in mice. All mice were of the same strain (NIH Swiss mice) and sex (male) and were of similar age.

Materials and Methods

Animals. All studies were carried out in accordance with the 7th edition of *The Guide for Care and Use of Laboratory Animals* (which was the most current version at the time these studies were conducted) as adopted and promulgated by the National Institutes of Health.[15] No new stipulations in the 8th edition of *The Guide*[16] would impact the methods used or the findings of these studies. Experimental protocols were approved by the IACUC at UAMS or at the U of M, both of which are accredited by AAALAC International. In addition, all experiments were conducted under IACUC-approved animal use protocols, including those involving anesthesia, pain, distress, and euthanasia. Male NIH Swiss mice (Envigo, Indianapolis, IN) 3 to 5 wk old and weighing 20 to 25g on delivery were housed 3 mice per cage (15.24 cm × 25.40 cm × 12.70 cm) before study. All studies used a sample size of 6 mice per group. Colony room conditions were maintained at 22 ± 2 °C at both institutions (but see below for variations in ambient temperatures of testing rooms) and 45% to 50% humidity, with lights set to a 12-h light/dark cycle. Mice were fed Lab Diet (Laboratory Rodent Diet no.5001, PMI Feeds, St Louis, MO) at U of M and Teklad Lab Diet (Envigo Teklad Diet, no. 8640, Indianapolis, In.) at UAMS. Both food and water were available ad libitum throughout testing. All mice were euthanized by CO_2 induced hypoxia, in accordance with American Veterinary Medical Association Guidelines on Euthanasia 2013, the most current version at the time of these studies.[3]

Implantation of telemeters for measurement of temperature and locomotor activity. After anesthesia with inhaled isoflurane (3% to 4%) at UAMS or a combination of ketamine (90mg/kg) and xylazine (10mg/kg) at U of M, the abdomen of each mouse was shaved and sanitized with both alcohol and iodine swabs. A 1.5 cm cranio-caudal incision was made on the ventral surface of the abdomen, providing access to the peritoneal cavity. A 15.5 mm × 6.5 mm cylindrical glass-encapsulated radio-telemetry probe (model ER-4000 E-Mitter, Mini Mitter, Bend, OR) weighing approximately 1 gm was then inserted. The incision was closed using absorbable 5-0 chromic gut suture material. Surgeries were performed at least 7 d before initiation of data collection, allowing time for incisions to heal and for mice to return to normal body weights. After surgery, all implanted mice received 1 mg/kg meloxicam SC for analgesia and were individually housed in 15.24 cm × 25.40 cm × 12.70 cm cages for at least 7 d.

Implanted transmitters produced activity- and temperature-modulated signals that were sent to a receiver (model ER-4000 Receiver, Mini Mitter) located beneath each cage. Every 5 min, the computer collected 2 data updates from the probes - core temperature (in °C) on one channel and locomotor activity (in counts) on the other. Each study represented one 24-hour period of telemetry data, recorded every 5 minutes, resulting in 1 daily data set per subject or 288 individual data sets of 5-min temperature readings per subject. At least 24 h of data were collected for all experimental conditions. Housing conditions during experimentation varied, depending on the particular variable being studied. Specific information on these conditions is provided below.

Ambient temperature. Historical data from control experiments performed at UAMS or U of M were analyzed to determine the effects of ambient temperature on core temperature and locomotor activity. For ambient temperature studies, both institutions singly housed mice implanted with radiotelemetry probes in 15.24 cm × 25.40 cm × 12.70 cm cages (standard static cages) prepared with approximately 75 g of wood chip bedding. Bedding was 3 to 4 d old during data collection and food and water were always available. All cages were placed on telemetry receivers 24 h before data acquisition began to allow for habituation to the new setting.

Radiotelemetry receivers at UAMS were placed inside light and sound-attenuating chambers (Model ENV-022M, Med Associates, St Albans, VT) to minimize environmental variability during tests. Each chamber at UAMS was equipped with a light (to maintain photoperiod), an exhaust fan, and a warm air heater to increase the ambient temperature (see Figure 1). The room was maintained at a "cool" ambient temperature of approximately 20 °C by an HVAC system. The "warm" ambient temperature of approximately 28 °C was maintained by warm air heaters attached to each chamber. For both the "cool" and "warm" conditions, ambient temperature was monitored every 5 min by data loggers placed within the chambers (Lascar EL-USB-1, MicroDAQ, Contoocook, NH) and also could be read in real time from a digital thermometer located in each chamber. The mean ambient temperature recorded by the data loggers during the targeted 20 °C "cool" condition was 20.2 °C (with a low of 18.9 °C and a high of 21.7 °C), while the mean ambient temperature recorded during the targeted 28 °C "warm" condition was 28.0 °C (with a low of 26.6 °C and a high of 29.5 °C) as previously demonstrated.[6] Radiotelemetry receivers at U of M were situated on an elevated shelf in a room maintained at a "normal" ambient temperature of 23 °C ± 2 °C. These receivers were partially isolated from each other by polycarbonate dividers.

Housing density. Historical data from control experiments performed at U of M were analyzed to determine the effects of housing density on core temperature and locomotor activity. All observations were made when the room was maintained

Figure 1. Experimental setting for thermoregulation and locomotor activity studies at UAMS showing a standard static home cage, 4W light to establish a photoperiod within the experimental space (upper right), exhaust fan (next to the light), and warm air heating vent to manipulate ambient temperature within the experimental space (upper left).

at a "normal" ambient temperature of 23 ± 2 °C, described in the section immediately above. For all experiments, a single mouse implanted with a radiotelemetry probe was placed into a new 15.24 cm × 25.40 cm × 12.70 cm cage prepared with approximately 75 g of wood chip bedding. Either 0, 2, or 5 cage mates, which were not implanted with telemetry devices, were also present in each cage. Thus, temperature and motor activity from only a single animal in each cage was monitored, regardless of the number of mice housed in the cage. Food and water were always available. After 3 to 4 d of habituation to their cage mates and the experimental space, data collection was initiated.

Bedding and nesting material. Historical data from control experiments performed at UAMS were analyzed to determine the effects of cage bedding and nesting material on core temperature and locomotor activity. These observations all occurred with the room maintained at a targeted 20 °C "cool" ambient temperature, described in the section above (see Figure 1). For all studies, mice implanted with radiotelemetry probes were studied in 15.24 cm × 25.40 cm × 12.70 cm cages prepared with approximately 75 g of wood chip bedding that was either fresh or 3 to 4 d old. In addition, 5.1 cm × 5.1 cm pulped cotton fiber cotton squares, weighing approximately 3.0 grams, were provided to some mice immediately before initiation of data collection. Providing fresh bedding required removal of cages from the experimental space and the transfer of mice from the used home cage into a new home cage. As a control, mice housed in the "old" bedding condition were handled in the same manner immediately prior to data acquisition. Feed and water were available at all times.

Running wheel access. Historical data from control experiments performed at UAMS were analyzed to determine the effects of running wheel access on core temperature and locomotor activity. 24 h before data collection in these studies, cages were placed on radiotelemetry receivers in a room maintained at approximately 20 °C, as described in the section: Ambient Temperature (see Figure 1). For all studies, mice implanted with radiotelemetry probes were studied in 15.24 cm × 25.40 cm × 12.70 cm cages prepared with approximately 75 g of 3 to 4 d old wood chip bedding. Each cage contained a low-profile wireless running wheel hub (Model ENV-044, Med Associates, St Albans, VT), with or without the wheel attachment. The hub occupied approximately the same amount of physical space within the cage as did the full wheel assembly. Such placement controlled for decreased available cage space, but did not allow wheel-running behavior because the wheel itself was not present. Each wheel assembly was configured to transmit rotation data to a central computer every 5 min, and the clock on this computer was synchronized to the clock on the radiotelemetry computer. In this manner, wheel-running data and radiotelemetry data could be correlated for each subject. As in all other studies described, food and water were available ad libitum at all times.

Statistical analysis. Graphical presentation of all core temperature and locomotor activity data shows mean values in 5 min bins. Error bars are not shown to facilitate data presentation, but SEM (standard error of the mean) variability are presented in figure legends for highest, lowest, and mean core temperatures recorded over the 24 h observation period. Locomotor activity data were binned in 30-min means for time-activity figures, or averaged across total activity in light or dark periods. For Figure 2 (correlation between wheel-running activity and core temperature in individual mice), core temperature and wheel-running data were binned into 30-min averages (± SEM). Mean core temperatures were analyzed over the 24 h observation period by using a one-way analysis of variance. Posthoc testing of overall significant results was accomplished by planned comparisons between all experimental groups using the Tukey HSD test.

Results

Effects of ambient temperature. Mice housed at 20°, 23° or 28 °C maintained core temperatures within the species-typical range[24] throughout the observation period (Figure 3). Mean core temperatures for the 24 h observation period for mice housed at 20°, 23° or 28 °C did not significantly differ ($P = 0.566$). The highest core temperature recorded was observed in the mice housed at 20 °C, while the lowest core temperature recorded was observed in the mice housed at 23 °C. Mice housed at 28 °C exhibited the least variation (difference between highest and lowest mean temperatures). No statistical differences were obtained when the highest or lowest mean core temperatures were compared as a function of ambient temperature ($P = 0.602$ and 0.611, respectively).

Mice generally exhibited low levels of home cage activity across the circadian cycle, regardless of ambient temperature (Figure 4). Total activity counts for the 24 h observation period did not significantly differ as a function of ambient temperature ($P = 0.523$). The group housed at 20 °C exhibited the greatest nocturnal increase in locomotor activity, whereas both of the groups housed at warmer ambient temperatures did not clearly increase dark phase activity during the 24 h observation period.

Effects of cage density. Mice housed singly, 3 per cage or 6 per cage generally maintained core temperatures within the species-typical range throughout the observation period (Figure 5). Transient periods of apparent hyperthermia (temperatures higher than the species-typical maximum) occurred both in singly housed mice, and in mice housed 3 per cage. The magnitude of these higher temperatures was small (generally less than 1 °C), and the duration of these apparent hyperthermic responses was short (on the order of 20 min). In all cases, these deviations from the species-typical range of core temperature did not influence the overall mean temperature for any group.

Mice exhibited similar levels of home cage activity across the circadian cycle, regardless of housing density, and total activity counts across the 24 h observation period did not statistically differ between groups ($P = 0.660$) (Figure 6). An dark-phase increase in locomotor activity was not observed in any of the groups studied.

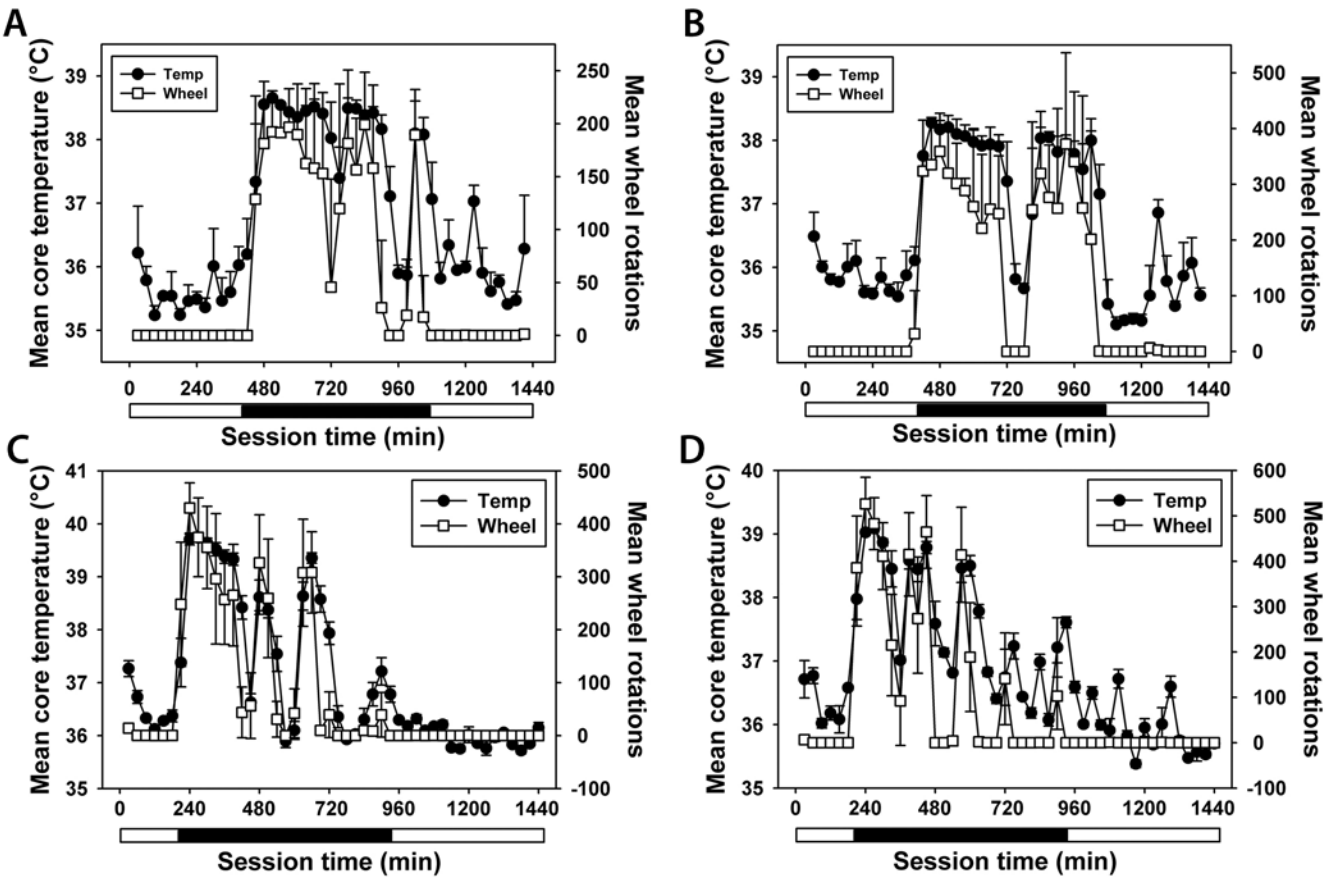

Figure 2. Effects of wheel-running behavior on core temperature in individual mice. Different durations and timing of wheel-running bouts preclude statistical averages across animals, so raw data from 4 representative mice are presented. *Abscissa:* elapsed experimental time (bar indicates light [white] and dark [black] phases). *Left ordinate:* core temperature, as recorded by implanted radiotelemetry probes, in °C. *Right ordinate:* wheel rotations. When not engaged in wheel-running activity, mouse core temperatures were generally approximately 36 °C, but rapidly rose to between 38 °C and 40 °C when mice engaged in wheel-running bouts. Core temperatures closely tracked wheel-running activity in all mice.

Effects of bedding and nesting material. Mean core temperatures for the 24 h observation period for mice housed in cages with 3 to 4 d old bedding or fresh bedding, with or without provision of a cotton square (all at an ambient temperature of approximately 20 °C), did not statistically differ ($P = 0.403$). However, the highest mean core temperature recorded was significantly different as a function of bedding condition ($P = 0.021$), and this overall difference was due to significantly higher maximal temperatures in mice housed with new bedding and given a cotton square as compared with mice housed in 3 to 4 d old bedding without a cotton square ($P = 0.015$). No statistical difference was detected in the lowest mean core temperatures recorded as a function of bedding condition ($P = 0.418$). Figure 7 shows that mice housed with 3 to 4 d old bedding, fresh bedding, or old bedding with a cotton square generally maintained core temperatures within the species-typical range throughout the observation period. However, simultaneously providing fresh bedding and a cotton square significantly increased core temperature above the species-typical range. This apparent hyperthermic effect was on the order of 1 °C, and lasted for approximately 2.5 h. Even when temperatures returned to the species-typical range, mice housed with new bedding and a cotton square exhibited higher temperatures than those in all other bedding conditions until approximately 4.5 h into the study.

Locomotor activity in this study was generally higher than in the previous experiments, because in these studies all mice were briefly removed from the experimental space and their home cage immediately before data acquisition to alter bedding conditions (or simply to control for these manipulations in the "old" bedding group). Nevertheless, a clear effect of bedding condition on motor activity was observed, as illustrated in Figure 8, with an overall statistical difference detected among groups ($P < 0.001$). This overall difference was due to the group housed with new bedding and a cotton square exhibiting significantly more locomotor activity than mice housed with old bedding ($P < 0.001$), new bedding ($P = 0.001$), or old bedding and a cotton square ($P = 0.012$).

The overall distribution of motor activity across the circadian cycle was profoundly disrupted by bedding manipulations ($P < 0.001$ for the overall ANOVA). Figure 9 shows that mice housed in 3 to 4 d old bedding exhibited the characteristic pattern of significantly more locomotor activity during the dark phase than during the light phase ($P = 0.006$), but all other groups failed to show this species-typical motor response. Instead, both groups with new bedding displayed a reversal of this pattern, such that significantly more activity was observed in the light phase, both with ($P = 0.032$) or without ($P < 0.001$) the addition of a cotton square. A qualitatively distinct disruption of the allocation of motor activity across the circadian cycle was seen when a cotton square was added to cages containing 3 to 4 d old bedding, such that the amount of activity observed during the dark phase was not significantly different from that observed during the light phase for this group ($P > 0.05$).

Effects of running wheel access. Significant effects of wheel-running behavior on core temperature were previously obtained in a similar study.[32] In the present experiments (Figure 10),

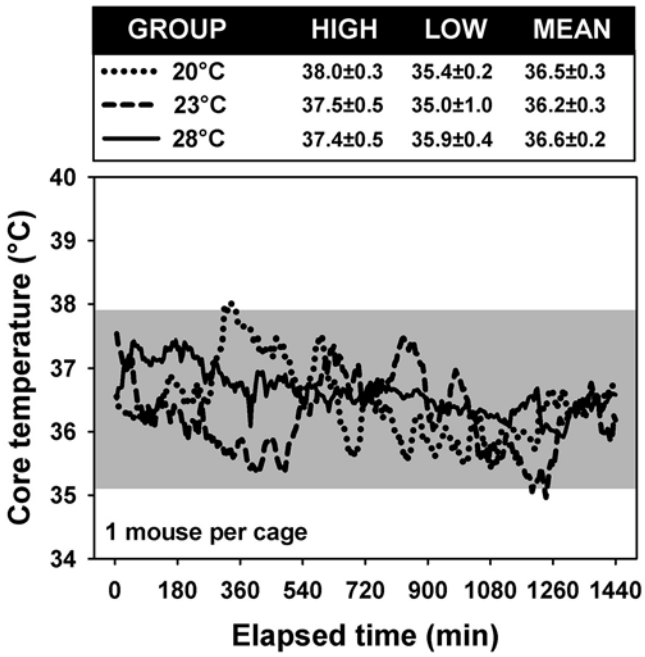

Figure 3. Effects of ambient temperature on core temperature. Gray region defines the species-typical range of core temperatures for mice. *Abscissa:* elapsed experimental time. *Ordinate:* core temperature, as recorded by implanted radiotelemetry probes, in °C. Average highest and lowest core temperatures for each condition are reported in the legend (in °C), as is the overall mean core temperature for each group. At no point were core temperatures significantly outside the species-typical range recorded, regardless of ambient temperature.

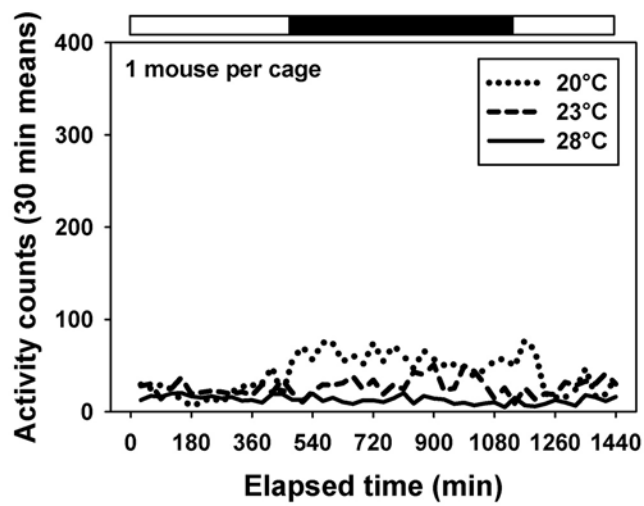

Figure 4. Effects of ambient temperature on locomotor activity. *Abscissa:* elapsed experimental time. *Ordinate:* activity counts, as recorded by implanted radiotelemetry probes, presented in 30 min averages. White and black bars above the figure illustrate light (white) and dark (black) phases. Home cage locomotor activity is generally low across the circadian cycle, but does increase during the dark phase (see Figure 9). No systematic differences in locomotor activity were observed as a function of ambient temperature.

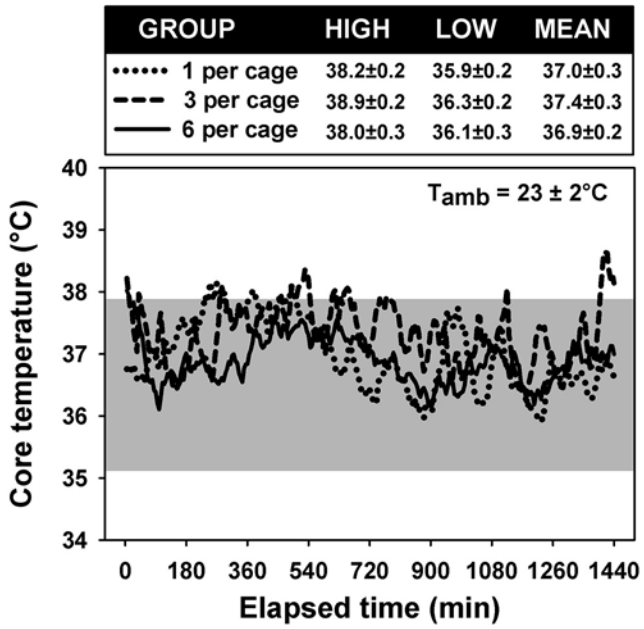

Figure 5. Effects of housing density on core temperature. All manipulations occurred at an ambient temperature of 23 °C. All other graph attributes as described in Figure 3. Core temperatures transiently exceeded maximal values defined in the species-typical range (typically by less than 1 °C, and for less than 20 min), but these slightly increased temperatures were not related to cage density.

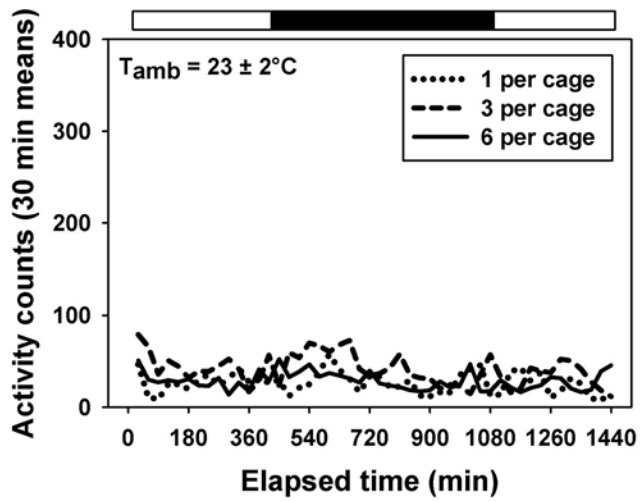

Figure 6. Effects of housing density on locomotor activity. All manipulations occurred at an ambient temperature of 23 °C. All other attributes as described in Figure 4. Home cage locomotor activity is generally low across the circadian cycle, but does increase during the dark phase (see Figure 9). No systematic differences in locomotor activity were observed as a function of cage density.

mean core temperatures for the 24 h observation period were significantly higher for mice housed in cages with a full running wheel assembly as compared with mice housed in cages with just the running wheel hub ($P = 0.011$). Similarly, the highest mean core temperature recorded was significantly greater in mice housed with the complete running wheel than in those housed with the hub only ($P = 0.032$). However, no statistical difference was detected in the lowest mean core temperatures recorded as a function of running wheel access ($P = 0.051$). Figure 10 shows that the core temperatures for these groups, though statistically different from one another, remained within the species-typical range.

Because wheel-running behavior was voluntary, mice ran at different experimental times and did so for different durations. For these reasons, we could not collapse wheel-running across subjects into a statistical mean. In addition, while mice were engaged in bouts of wheel-running, the radiotelemetry probes registered no locomotor activity because the position of the probe relative to the receiver was constant, making

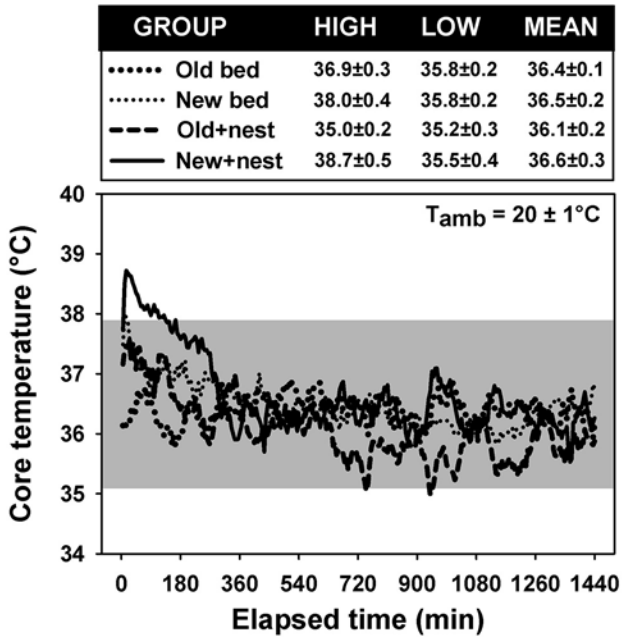

Figure 7. Effects of bedding condition on core temperature. All manipulations occurred at an ambient temperature of 20 °C. All other attributes as described in Figure 3. New bedding, or the addition of a cotton square increased core temperature as compared with values typically observed with old bedding, but the magnitude of this increase was small and within the species-typical range. However, the presence of new bedding and a cotton square significantly increased core temperatures outside the species-typical range for approximately 2.5 h.

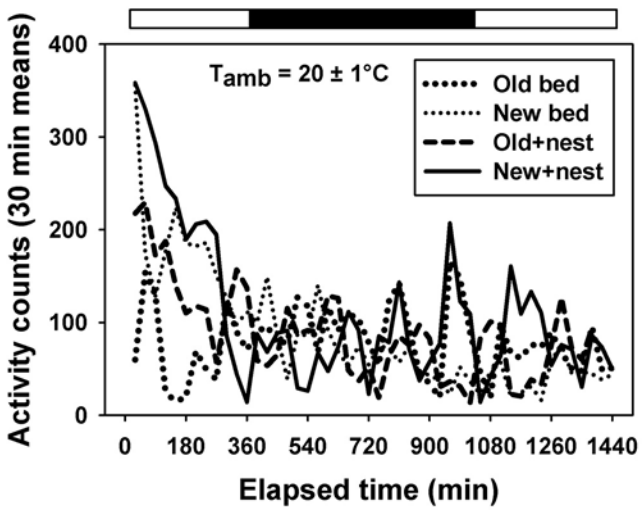

Figure 8. Effects of bedding condition on locomotor activity. All manipulations occurred at an ambient temperature of 20 °C. All other attributes as described in Figure 4. Home cage locomotor activity is generally low across the circadian cycle, but does increase during the dark phase (see Figure 9). New bedding profoundly increased locomotor activity for approximately 7 h, and the magnitude of this increase was somewhat more pronounced when a cotton square was also provided. Indeed, provision of a cotton square increased motor activity, even when bedding was not changed.

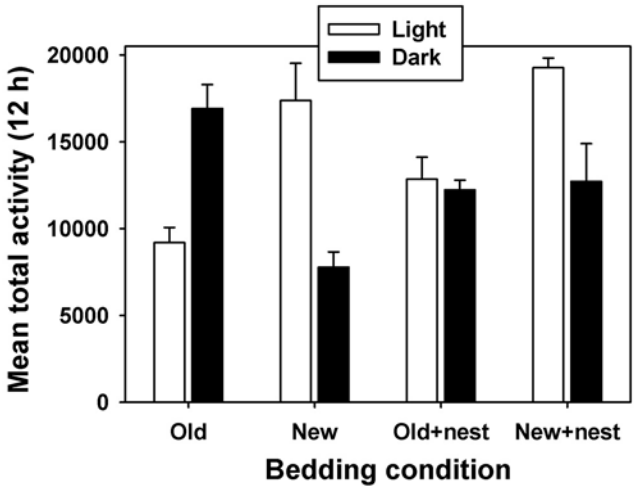

Figure 9. Effects of bedding condition on distribution of locomotor activity across the light / dark cycle. The normal circadian pattern whereby the majority of murine locomotor activity occurs during the dark phase is illustrated in the "old" bedding condition, but is disrupted in all other conditions. New bedding stimulates exploratory behavior, the majority of which occurs proximal to the introduction of new bedding during the light phase ("new" and "new+nest" conditions), inverting the species-typical circadian distribution of motor activity. Addition of a cotton square to old bedding also increases motor behavior in the light phase (when the cotton square was added), and decreases motor behavior in the dark phase, again disrupting the normal circadian pattern.

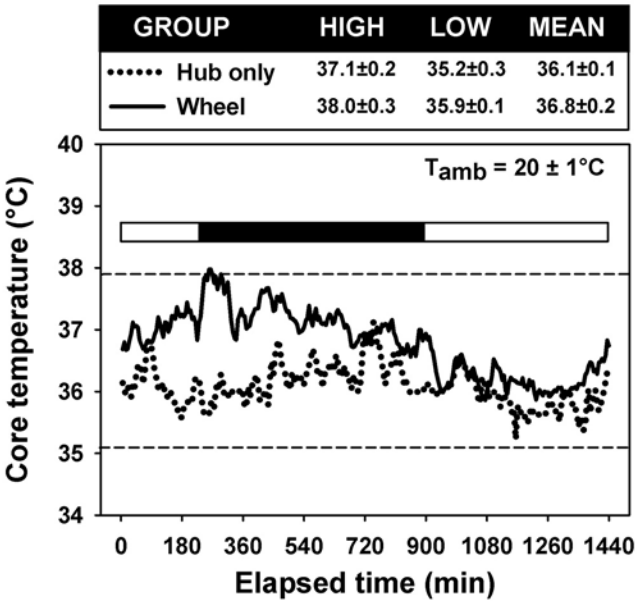

Figure 10. Effects of running wheel access on core temperature. All manipulations occurred at an ambient temperature of 20 °C. All other attributes as described in Figure 3. Provision of a running wheel hub did not alter core temperature, but the presence of the full wheel assembly did increase temperature compared with the hub control level. However, the magnitude of this temperature increase was small, and core temperatures remained within the species-typical range. Core temperature increases were driven by wheel-running activity (see Figure 2).

radiotelemetry of gross locomotor data highly variable and unreliable. Thus, Figure 2 presents data from 4 representative subjects housed with the full running wheel assembly, all of which illustrate a tight correlation between core temperature and wheel-running behavior. When mice were not engaged in wheel-running bouts, core temperatures were relatively stable within the species-typical range (approximately 36 °C for all mice). Running in the wheel resulted in a rapid and pronounced rise in core temperature, on the order of 2° to 4 °C, which transiently moved mice outside of the species-typical range. Temperatures quickly returned to normal levels when wheel-running ceased.

Discussion

Thermoregulation in the laboratory mouse is relatively insensitive to a variety of manipulations in housing conditions.[11] Despite transient instances of higher than expected core temperatures, mice mostly exhibited species-typical core temperatures when housed under the following conditions: 1) ambient temperatures between 20 °C and 28 °C, 2) housed singly or socially-housed in groups of 3 or 6, 3) with either fresh or old bedding, 4) with or without nesting material, or 5) with or without a running wheel available. The present findings involving manipulation of ambient temperature are consistent with a previous study using radiotelemetry to monitor core temperature, which reported only minor deviations from baseline (approximately 5% change) when mice were exposed to a more extreme cold stimulus (12 °C).[17] Behaviorally, mice typically respond to cold by huddling and/or nest building.[9,20] However, in the present experiments involving ambient temperature manipulations, mice were singly housed and no nesting material was present, eliminating the capacity of our subjects to engage in these behavioral thermoregulation strategies. Nevertheless, species-typical core temperatures were maintained when ambient temperature was reduced to 20 °C, implying that a shift in metabolic expenditure occurred to maintain normal temperature in a cold environment. Also consistent with this notion is the observation that the least variable core temperatures were recorded in the warmest (28 °C) ambient environment, suggesting that metabolic expenditure to maintain normothermia was minimal. This difference in metabolic expenditure should be kept in mind when rodents receive an infectious or inflammatory challenge that would create an additional metabolic demand.[18] Food and water were freely available, and we did not monitor consumption. Therefore, we were unable to correlate food intake with body weight and core temperature measures, and we cannot speculate on the impact of food intake on temperature regulation. However, laboratory conditions involving cool ambient temperatures and food restriction might present a significant challenge to thermoregulation in mice and should be carefully monitored.

The overall mean temperatures for mice housed singly, as compared in groups of 3 or 6, did not differ, nor did the lowest recorded temperature differ by group. However, maximal core temperatures were higher in the mice housed 3 per cage than in mice housed 6 per cage, but neither of these groups differed significantly from singly housed mice. Social housing of laboratory mice elicits a wide range of effects on physiology and behavior, including reduced stress reactivity, improved immune function, and less aggressive behavior and toxicity.[33] Based on these findings, increasing housing density would be expected to have only minor effects on temperature in mice, although extreme crowding would likely have a more significant impact.

The studies on bedding and nesting material suggest that various housing manipulations might have interacting effects on core temperature, as mice placed into cages with both fresh bedding and cotton squares, as provided by many facilities simultaneously during routine cage change procedures, were the only group to display reliable and significant core temperatures that were outside of the species-typical range. These findings somewhat conflict with a previous study using radiotelemetry in mice, which reported no significant effects of the addition of 8 g Enviro-Dry nesting material on core temperature in male or female C57BL/6NCrl, BALB/cAnNCrl, or Crl:CD1(ICR) mice.[8] However, in addition to the different composition of the nesting material, these studies[8] also averaged temperatures across 28 d, which may have masked an initial effect of nesting material on core temperature, such as that reported in our study. "Novelty-induced hyperthermia" has been documented both in rats[31] and in mice.[29] This effect may account for the higher temperatures observed in this study when mice were housed with fresh bedding and cotton squares. However, increased exploratory or nest-building behavior, possibly due to increased wakefulness from cage change[7] or the amount of bedding provided and together with the ability of mice to burrow,[10] seems unlikely to account for these higher temperatures, because levels of locomotor activity as high as those presently reported have previously been observed in response to the administration of certain drugs yet had no apparent hyperthermic effects.[5] To the extent that "novelty-induced hyperthermia" is considered a marker of a stress response[14] or an indicator of anxiety in mice,[25] the present results may suggest that relatively routine husbandry procedures such as cage changes or rotation of environmental enrichment objects might induce some noxious effects in the mouse. A prudent strategy might be to temporally separate these necessary husbandry activities from experimental observations as much as possible to minimize potential research confounds.

The profound, but short-lived, effects of wheel-running behavior on core temperature suggest that this common environmental enrichment device may have a greater effect on murine physiology than is typically realized. Running wheel access provides numerous beneficial effects to laboratory rodents, including reductions in anxiety-like behaviors and decreased stress-reactivity,[28] but the current experiments and a previous study[32] demonstrate that wheel-running behavior also significantly alters temperature in mice. Thus, in studies that use core temperature is an experimental variable, running wheel access should be limited to periods where data collection is suspended. Similarly, research involving chronic drug administration may be confounded by wheel-running activity, as diverse pharmacological endpoints from opioid-induced analgesia[21] to rewarding effects of cocaine[22] have been shown to differ in mice as a function of wheel-running behavior. Indeed, selective breeding for high levels of wheel-running behavior results in widespread neurobiological alterations in mice.[27]

In contrast to core temperature, which was remarkably stable across most of the housing manipulations studied here, locomotor activity was extremely sensitive to changes in housing conditions. In contrast to dedicated environments for automated detection of locomotor activity, our telemetry system records movement in the home cage. When data collection begins, subjects are well-adapted to this setting, and baseline home cage activity is generally low. Habituation to novel experimental environments is typically required prior to assessment of drug effects on locomotor activity, but such habituation occurs naturally in our studies as subjects live in the experimental space during the period of recovery from telemetry probe implantation. Nevertheless, simply placing mice into new cages with fresh bedding strongly stimulated locomotor activity, and the magnitude and duration of this effect was comparable to or greater than that observed with administration of psychostimulant drugs (see, for example [8], where locomotor activity was quantified in mice using the same equipment as here described). If the new cage also contained nesting material, the locomotor response was even more pronounced and long-lasting. Performing these manipulations during the light phase, as was done in the present studies, reversed the species-typical allocation of motor behavior across the light / dark cycle, such that a greater percentage of total activity occurred during the light phase. Cage

change has been shown to increase wakefulness in mice[7] and because mice normally spend more time asleep during the light phase, increased activity during the light phase could occur with a reduction in sleep. Such disruption of circadian rhythmicity due to acute sleep deprivation could also affect behavioral and neural activities in laboratory mice[19] and confound experimental results obtained the day of or even the day after the cage change.

In summary, evidence is presented here that core temperature in the laboratory mouse is relatively resistant to environmental conditions and the standard husbandry practices likely to be encountered in a research setting. In contrast, locomotor activity was quite sensitive to the simultaneous provision of fresh bedding and nesting material in the form of cotton squares. These common husbandry practices induced significant and prolonged stimulation of motor behavior, hyperthermic effects, and a disruption of circadian rhythms. Some of these effects may be related to the well-described phenomena of novelty-induced exploratory behavior and hyperthermia, and if so, may decrease in intensity as mice habituate to these procedures with repeated exposure. Nevertheless, prudent practices dictate that interventions related to home cage bedding and or nesting material should not be initiated proximal to behavioral testing. As demonstrated, many environmental conditions can clearly elicit a variety of behavioral and physiologic effects in laboratory mice. These effects, though usually subtle, may become more pronounced under certain conditions including the availability of novel enrichment. A further understanding of these effects is necessary to decrease experimental error and variability in data collection. This understanding will only come with continued research in this area. Future studies should not only expand the species under observation, but should also expand to include female subjects. Furthermore, these findings seem especially noteworthy as environmental enrichment initiatives continue to become standard practice in AAALAC International accredited animal research facilities. Lastly, facility directors and husbandry staff, as well as investigators and their technical personnel, should recognize that certain enrichment manipulations may dramatically alter basal behavioral and physiologic parameters in mice and carefully consider the timing of these manipulations, as well as the choice of specific devices used to satisfy enrichment goals.

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