Journal of Thermal Biology 79 (2019) 149–154

Contents lists available at ScienceDirect

Journal of Thermal Biology

journal homepage: www.elsevier.com/locate/jtherbio

The heat is on: A device that reduces cold stress-induced tachycardia in laboratory mice

Cordelia E. Chan[a,1], Maia T. Hare[a,1], Gabrielle W. Martin[a], Christopher J. Gordon[b], Steven J. Swoap[a,*]

[a] Department of Biology, Williams College, Williamstown, MA 01267, USA
[b] Toxicity Assessment Division, National Health Effects and Environmental Research Laboratory, US Environmental Protection Agency, USA

ABSTRACT

Mouse vivaria are typically maintained at an ambient temperature (T_a) of 20–26 °C which is comfortable for human researchers. However, as this T_a is well below the mouse thermoneutral zone (TNZ) of 30–32 °C, typical vivarium temperatures result in cold stress for mice. Recently, a cage has been developed that provides variable cage floor heating, allowing mice to behaviorally regulate body temperature through thermotaxis. A hand warmer provides supplemental heat, elevating cage floor surface temperature for 13 + hours up to 30 °C. This provides a heated surface for the entirety of the light phase. Here, we test the ability of these local heat sources to remove physiological signs of cold stress in mice housed at room temperature by analyzing heart rate (HR), activity, and body temperature in three experimental conditions: 23 °C, 23 °C + heated surface, or 30 °C. The location of C57Bl/6 J mice within the cage was recorded using an infrared camera. In the presence of supplemental heat at a T_a of 23 °C, mice resided atop of the area of the heated surface 85 ± 3% of the 12-h light phase, as compared to 7 ± 2% in the absence of supplemental heat. Further, addition of supplemental heat lowered light phase HR and activity to that seen at a T_a of 30 °C. These results indicate that provision of a local heat source is successful in reducing cold-induced tachycardia in mice housed at typical vivarium temperatures without increasing the ambient temperature of the entire laboratory and subjecting researchers to heat stress.

1. Introduction

Mouse models have become a staple of biomedical and physiological research in recent years, and therefore understanding mouse physiology is crucial in improving applicability of these experimental models to human targets (Swoap et al., 2004, 2008; Gordon, 2012; Karp, 2012; Maloney et al., 2014). Discrepancies in rodent housing protocols are recognized sources of variability and create potential reproducibility challenges (David et al., 2013a,2013b). Ambient temperature (T_a) in animal facilities is a particularly notable source of variability (Gordon et al., 1998; Gaskill et al., 2009; Karp, 2012; Gordon, 2017). The T_a of animal facilities is typically set for human comfort at 20–26 °C, which is well below the 30–32 °C thermoneutral zone (TNZ) of mice (Gordon et al., 1998; Lodhi and Semenkovich, 2009; Gordon, 2017). Therefore, mice are in a state of constant cold stress during which they must engage physiological mechanisms for both elevated heat production and heat loss prevention (Talan et al., 1996; Swoap et al., 2004; Gordon, 2012). As such, the stress induced by sub-TNZ housing may lead to the potential of poor application to humans, who are not typically cold stressed (Maloney et al., 2014). Many experiments require unique housing considerations, and it is impractical to standardize rodent housing across laboratories. However, special care should be taken to reduce this cold-stress in order to reduce variability and increase reproducibility in mouse models (David et al., 2013a, 2013b). There are numerous examples of the significance of T_a in mouse models spanning a wide range of disciplines. For example, mice deficient in uncoupling protein 1 become obese when housed within their TNZ but do not when housed at typical laboratory T_a (Enerback et al., 1997; Feldmann et al., 2009). Sub-TNZ-housed mice spend less time asleep than TNZ-housed mice, demonstrating the importance of considering T_a in sleep research (Lo Martire et al., 2012). When using mice to model disease, it is crucial to take T_a into account, as it affects the animal's ability to combat infection (Karp, 2012; Speakman and Keijer, 2013; Hylander and Repasky, 2016). These are just a handful of examples, but the importance of T_a on mouse physiology spans many research fields.

Within their TNZ, mice are able to maintain body temperature (T_b) via peripheral vasoconstriction, alterations in insulation through piloerection, thermotaxis, huddling, nest-building, and other behavioral mechanisms (Gordon, 2012). Group housed mice huddle together as a primary method of thermoregulation. Mice spend more time huddling when cold-stressed than when at their TNZ, demonstrating the

* Corresponding author.
E-mail address: sswoap@williams.edu (S.J. Swoap).
[1] Contributed equally to the manuscript and share first-author status.

https://doi.org/10.1016/j.jtherbio.2018.12.006
Received 31 August 2018; Received in revised form 21 November 2018; Accepted 9 December 2018
Available online 13 December 2018

significance of this behavior in maintaining T_b (Batchelder et al., 1983). Unfortunately, group housing is not always viable, as some experiments require individual housing. The behavioral mechanisms in which singly housed mice must participate are insufficient in maintaining T_b at typical room temperatures unless active heat production occurs (Talan et al., 1996; Swoap et al., 2004, 2008; Gordon, 2012). During the light period, mouse heart rate (HR) at room temperature (RT) is 550–600 beats per minute (bpm) and decreases approximately 25 bpm for every 1 °C increase in T_a to 350–400 bpm within the TNZ (Swoap et al., 2008), consistent with increased sympathetic drive to brown fat in mice in the cold (Kawate et al., 1993), suggesting an overall elevation of sympathetic tone at RT. Similarly, mean arterial pressure (MAP) of the mouse is elevated at RT, falling from 100 mmHg at 20 °C to 80 mmHg at 30 °C (Swoap et al., 2004, 2008). Because parasympathetic tone is dominant in healthy, resting humans at RT while sympathetic tone is dominant in mice at the same temperature, it was originally thought that the mouse cardiovascular system may provide a poor model for that of humans (Maloney et al., 2014). However, when mice are studied within their TNZ at 30 °C, they more closely resemble humans in that cardiac vagal tone sets resting HR (Swoap et al., 2008).

Gordon et al. have developed a cage that allows mice to behaviorally regulate body temperature through thermotaxis (Gordon et al., 2017). These cages utilize a chemically-activated hand warmer to heat a small portion of the cage floor to temperatures approaching 32 °C for 13 + hours and remain warm even as the hand warmer cools (Gordon et al., 2017). These cages allow mice to seek out warmer temperatures during the light phase and cooler temperatures during the dark phase when they are active, potentially removing cold stress within RT laboratory conditions. Mice in these cages were anecdotally reported to stay near the heated regions for many hours of the day (Gordon et al., 2017). Here, we 1) directly measure the position of the mouse in these cages using infrared thermography, 2) test the hypothesis that singly housing mice in cages with an activated hand warmer within standard RT laboratory conditions lowers their HR to that of mice housed within their TNZ, and 3) test the hypothesis that housing mice in cages with an activated hand warmer within standard RT laboratory conditions relatively reduces the activity typical of sub-TNZ, singly housed mice.

2. Methods

2.1. Animals and housing

C57BL/6J male mice were purchased from Jackson Labs (Bar Harbor, ME, USA). The age of the mice was 3–6 months old, and they weighed ~ 28 g before implantation of the telemeter. They were singly housed on a 12-h light: dark cycle, with lights on at 9 am, at 23 ± 1 °C and were given food and water ad libitum. The mice were housed using Envigo's Teklad, laboratory grade Sani-Chips for bedding at a depth of ~ 1 cm within conventional open-topped cages with wire lids and without microisolator lids. The ambient temperature of the room was maintained by the experimental room's temperature control within ± 1 °C and the relative humidity was controlled between 40% and 50%. All housing and protocols associated with this experiment were approved and overseen by the Williams College Institutional Animal Care and Use Committee.

2.1.1. Experiment #1

Mice (n = 5) were housed individually at 23 °C for 2–3 days in the specially-designed cages to allow time for the animal to grow accustomed to the cage before the addition of an activated hand warmer (See Fig. 1 (Gordon et al., 2017)). Commercially available, disposable hand warmers (HotHands, Philadelphia, PA, USA) are typically used to keep human hands warm in cold climates but ~~are~~ were used in these experiments as a means of a supplemental heat source so that the mouse could engage in thermotaxis (Gordon et al., 2017). The hand warmers have dimensions of 5.1 × 8.9 × 0.5 cm and generate heat from a nontoxic redox reaction when exposed to air. A single hand warmer was activated at the beginning of the light phase via exposure to room air, followed by insertion into the cage. An infrared camera (FLIR E60) was set up to take an image of the cage once per minute. We did not use the IR camera to measure the surface temperature of the mouse. Rather, the location of the mouse inside the cage was assessed using software that accompanied the camera (FLiR Tools+, version is 6.3.17227.1001) by examining the location of the warmest portion of the image, which was always the mouse. The experimental design consisted of 1) a 12-h light phase without a supplemental heat source, followed on the next day by 2) a 12-h light phase with a supplemental heat source. The design was intentionally not randomized to condition as we did not want the mouse to learn where a potential supplemental heat source might be located. A typical movie of mouse position is available in the Supplemental materials.

2.1.2. Experiment #2

2.1.2.1. Surgery. Mice (n = 4) underwent surgery for implantation of a telemetry device at three months of age. Mice were anesthetized with 5% isoflurane in an oxygen stream and maintained on 1–2% isoflurane throughout the surgery. Mice were kept on a 38 °C heating pad for the duration of the surgery. Three mice were implanted with blood pressure telemeters (HD-X10; Data Sciences International) in the left common carotid artery, as described previously (Butz and Davisson, 2001). This telemeter returned temperature, pressure, and activity. One mouse was implanted with an ECG telemeter (ETA-F20; Data Sciences International). This telemeter returned temperature, activity, and an ECG waveform from which heart rate was derived. For both telemeter types, the telemeter body was tunneled subcutaneously, placed in the peritoneal cavity, and sutured into the body wall. Meloxicam at 5 mg/kg BW (Henry Schein, USA) was given subcutaneously at the end of the procedure. Mice recovered in cages placed half on/half off a 38 °C heating pad for 48 h to ensure full recovery and then were housed individually for an additional eight days at 23 °C.

2.1.2.2. Physiological data collection. Ten-second waveforms from the implanted telemeters were collected once per minute, at a sampling rate of 1000 Hz, which allows the telemeter to get a good estimation of waveforms. Blood pressure waveforms showed a clear consistent HR, although the absolute arterial pressure was variable over time and between animals due to telemeter placement. Therefore, we report here only HR, T_b, and activity data. Activity was calculated on a minute-to-minute basis by the analysis software (Data Sciences International) as a change in signal strength from the telemeter.

2.1.2.3. Experimental setup. In experiment #2, mice were housed individually at 23 °C for 2–3 days in the specially-designed cages (see Fig. 1) to allow time for the animal to grow accustomed to the cage before the start of the experiment. Mice were then randomized into one of three environments: 1) Ambient temperature (T_a) of 23 °C, 2) T_a of 23 °C + heated surface, and 3) T_a of 30 °C (maintained by room controls to be between 29.5 and 30 °C). Each mouse spent 24 h in each of the three conditions, in a randomized cross-over fashion. The hand warmer was activated by shaking at the beginning of the light period and was removed after 24 h of data collection. iButtons (DS1921G, Thermochron) were taped to the underside of each aluminum platform for measurement of the region heated by the hand warmers. The iButtons sampled T_a once per minute with an accuracy of ± 1 °C.

2.1.2.4. Statistics. Data are presented as means ± standard error. A repeated-measures ANOVA was performed on each parameter (SPSS 21), followed by an LSD post-hoc test to probe for statistical significance.

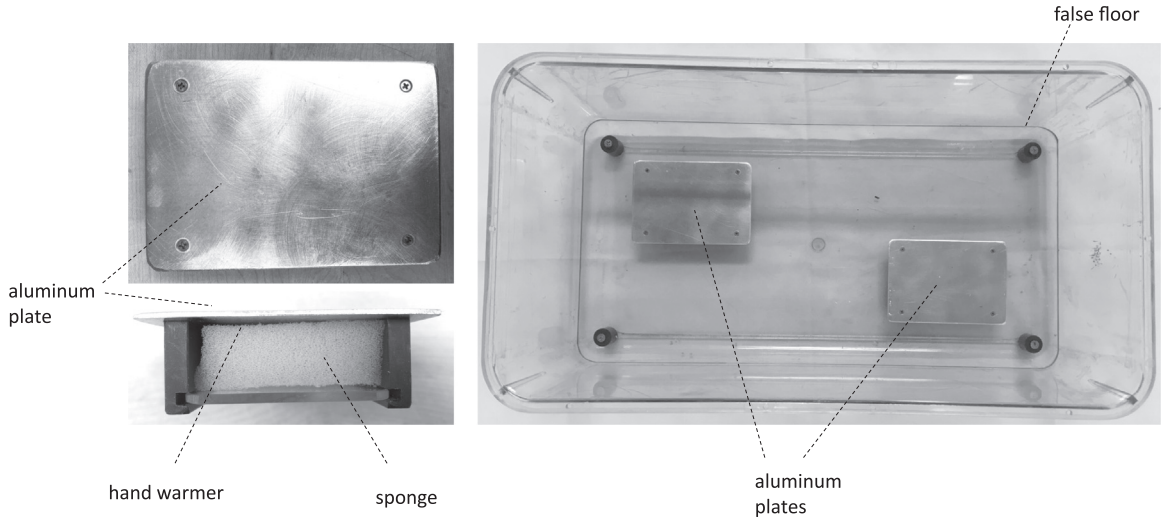

Fig. 1. Cages modified for the use of supplemental heat. An activated hand warmer was placed under only one of the aluminum plates to provide a supplemental heat source. For the experiments presented in these studies, the hand warmer was activated at the beginning of the light phase. (see (Gordon et al., 2017) for complete list of materials).

3. Results and discussion

3.1. Experiment #1

A report that utilized these cages (Fig. 1) with activated hand warmers stated anecdotally that mice tended to stay atop of the region where the heated surface was located (Gordon et al., 2017). We measured the temperature of the bedding directly above the heated surface and bedding 10 cm away from the heat source. Bedding temperature directly above the heated surface reached a maximum of 30.0 ± 0.2 °C approximately 6 h after activation, and that maximum temperature lasted approximately 2 h (Fig. 2A).

To quantify the location of a mouse within the cage, we used infrared thermography, which took an image once per minute. We then analyzed the location of the mouse (n = 5) during the light phase with and without supplemental heat. Each image was analyzed for the position of the mouse, and placed into 20-min bins as either a) atop the heat source, or b) not atop the heat source (see supplemental data for a typical stream of images). The heated surface constituted 17% of the cage. When the heated plate was not warm the mice typically spent ~7% of the sampled time atop this region, choosing to sleep near the edges of the cage and not on the aluminum plate which was towards the center (Fig. 2). However, when the heated surface was warm, mice spend on average 85 ± 3% of the light phase on this small area (Fig. 2C). Indeed, mice stayed atop the heated surface virtually 100% of the time for two hours beginning about seven hours into the light phase, which is also seven hours after activation of the heat source (Fig. 2B).

3.2. Experiment #2

To assess the potential physiological effects of increasing local T_a, each mouse (n = 4) was randomly cycled through a 23 °C, 23 °C + heated surface, and 30 °C condition, spending 24 h in each. Three physiological variables (T_b, HR, and Activity) were measured. Fig. 3 depicts typical ECG tracings from a single mouse at the same time of day for each of the three conditions, and show qualitatively the higher HR at 23 °C vs. the other two groups. HR was calculated from ECG and blood pressure waveform tracings (not shown) during the two hours of maximum heated surface temperature, hours 6–8 (Fig. 4), and during the light and dark periods of each condition (Table 1).

3.3. Maximum heat production of heated surface (Hours 6–8)

The physiological parameters of the mice were averaged over the two hours of maximum temperature of the heated surface, and compared to the same two-hour period when the mice were housed at 30 °C or at 23 °C without supplemental heat. There was no main effect of housing conditions on the mouse T_b (Fig. 4A and B), $F(1, 3) = 1.3$, $p = 0.34$. There was a significant main effect of housing conditions on mouse HR (Fig. 4C and D), $F(1, 3) = 1.3$, $p = 0.017$. Within group statistics revealed that the HR in the 23 °C condition was higher than in both the 23 °C + heated surface and 30 °C condition, $p = 0.00$ and 0.017 respectively. There was no significant difference in HR between 23 °C + heated surface and 30 °C conditions, $p > 0.05$. The lowered HR in mice within the cages with an activated hand warmer is suggestive of autonomic activity that more closely resembles that of the mice housed within their TNZ, which have previously been shown to have HR set by vagal tone. There was a main effect of housing conditions on mouse activity (Fig. 4E and F), $F(1, 3) = 38.4$, $p = 0.04$. Within group statistics revealed that mouse activity in 23 °C was significantly higher than in both 23 °C + heated surface and 30 °C conditions, $p = 0.027$ and 0.04 respectively. There was no significant difference in activity level of the 23 °C + heated surface and 30 °C conditions, $p > 0.05$. These results support the hypothesis that mice are more active when housed below their TNZ and that mice are less active when housed in cages with activated hand warmers. These data are consistent with the findings shown in Fig. 2.

3.4. Twelve hour light/dark phase

Analyzing the light and dark periods of each condition determined whether the use of a supplemental heat source is suitable to remove cold stress in 23 °C-housed mice over a 24-h period (Table 1). During the light period, there was no main effect of housing conditions mouse T_b, $F(1, 3) = 2.2$, $p = 0.24$. As with the 2-h analysis of the warmest two hours of the floor surface, there was a main effect of housing conditions on mouse HR during the light entire period, $F(1, 3) = 22.8$, $p = 0.017$. HR over the entire light period during the 23 °C condition was significantly greater than both the 23 °C + heated surface and 30 °C conditions, $p = 0.002$ and 0.017 respectively (Table 1). The HR during the 23 °C + heated surface and 30 °C conditions were not statistically significant from each other ($p > 0.7$). During the light period, there was a no main effect of housing conditions on mouse activity $F(1, 3) = 0.022$, $p = 0.891$. This data indicates that increasing local T_a through hand

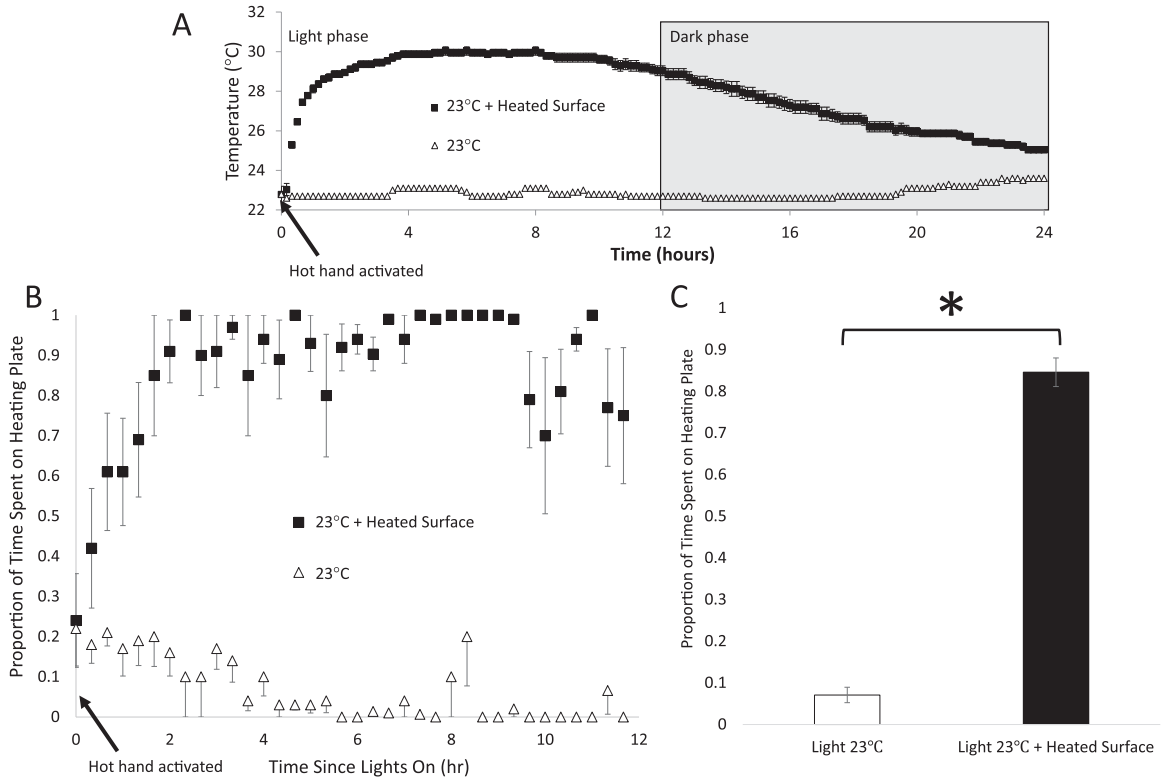

Fig. 2. Mice spend most of the light phase atop the heated surface at a T_a of 23 °C. A) The temperature of the bedding atop the heated surface was measured in four separate cages, as well as 10 cm away from the heated surface. The temperature of the bedding above the heated surface reached its maximum of 30.0 ± 0.2 °C approximately six hours after the activation of the supplemental heat. B) The location of C57Bl/6 J mice (n = 5) within the cage was determined once per minute and binned into 20-min bins. The location was quantified in the light phase in the presence of the heated surface (black squares) or in the light phase without the heated surface (clear triangles). C) The location of the mice over the entire 12-h period is quantified. Mice spent significantly more time on the bedding above the aluminum plate during the light phase when the plate was heated as compared to the previous light phase without a heated surface. Data shown as mean ± standard error. * $p < 0.05$.

warmers is comparable to housing mice in 30 °C vivaria. Therefore, increasing local T_a successfully reduces inferred sympathetic activity and cold stress in mice.

The heated surface remained warm for several hours into the dark period (see Fig. 2). During the dark period, there was no significant difference in activity or T_b among any of the three conditions, $F(1, 3) = 2.3$, $p = 0.23$ and $F(1, 3) = 0.75$, $p = 0.45$, respectively. There was, however, a significant effect on housing condition on mouse HR, $F(1, 3) = 53.2$, $p = 0.005$. Pairwise comparisons revealed that the HR during the 23 °C + heated surface condition was intermediate between

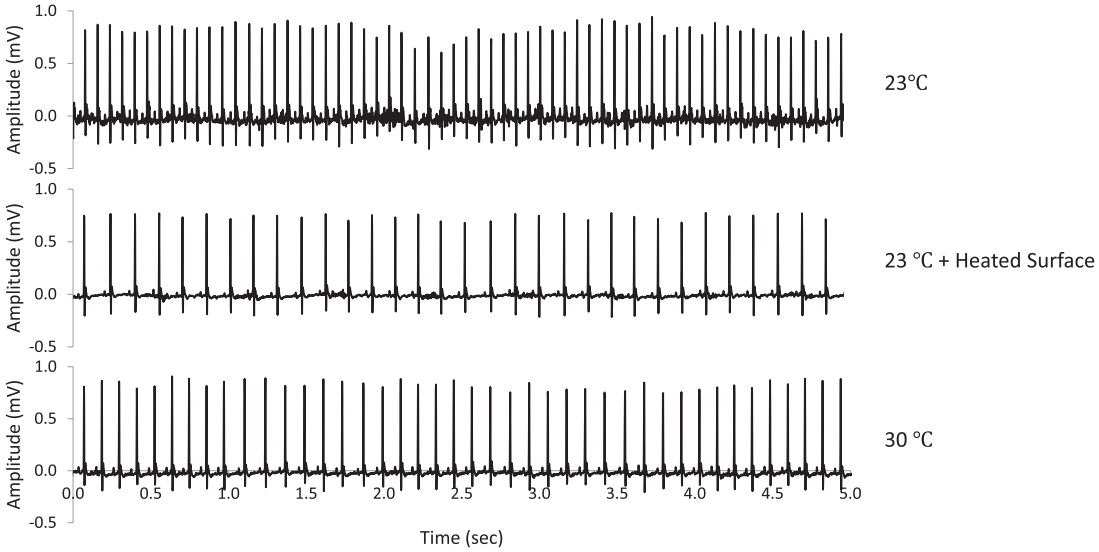

Fig. 3. ECG Waveform Comparison in an Individual Mouse. These waveforms were collected over a five-second period from a mouse was implanted with an ETA-F10 telemeter during the warmest two hours of the heated surface and the corresponding times during the 23 °C and 30 °C conditions. Note the greatly elevated HR at 23 °C from this mouse as compared to the slower HRs at 30 °C and 23 °C + heated surface.

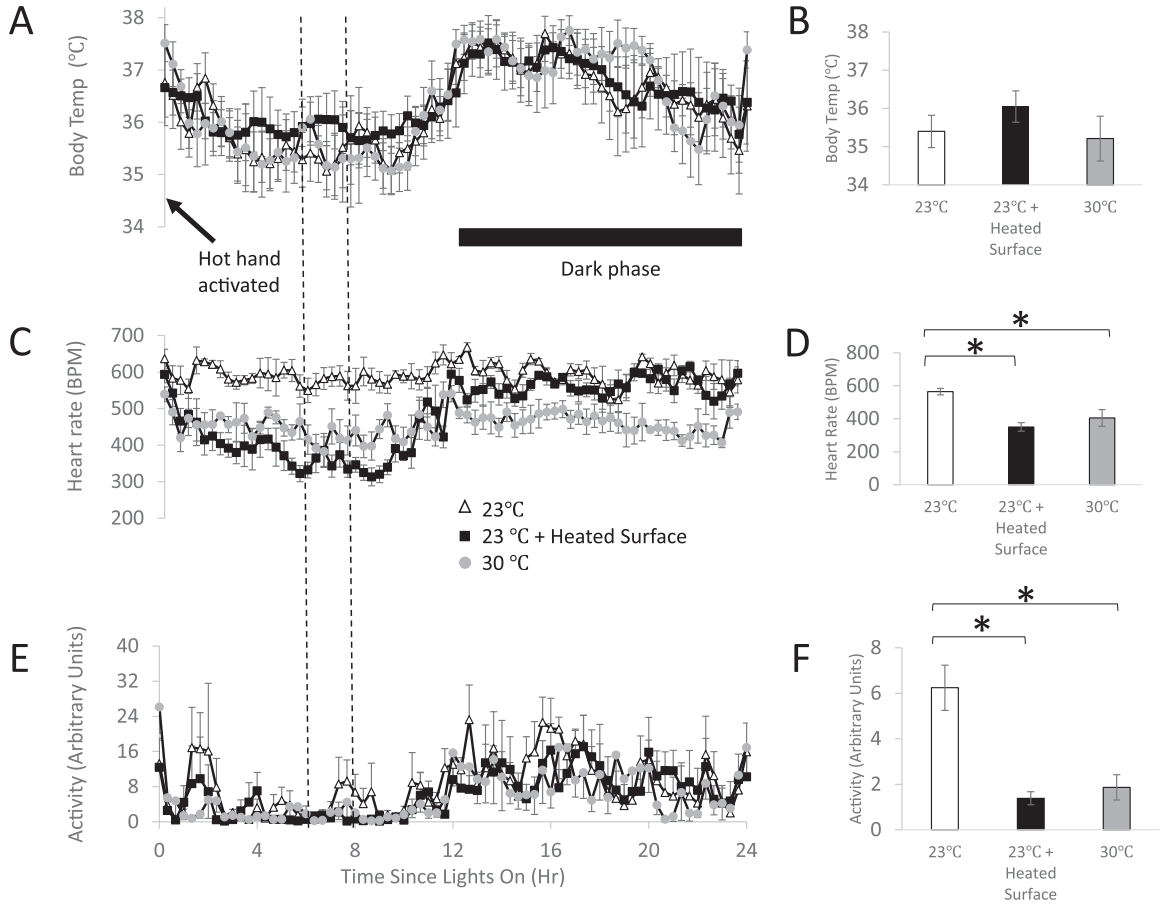

Fig. 4. The Effect of a Local Heat Source on Mouse T_b, HR, and Activity. Body temperature (A, B), heart rate (C, D), and activity (E, F) of C57BL/6 J mice (n = 4) are shown in three conditions: 1) T_a of 23 °C, 2) T_a of 23 °C with an activated hand warmer at time = 0, and 3) T_a of 30 °C conditions. Data was collected once per minute, and collapsed into 20-min bins for this graph. The mean body temperature, heart rate, and activity of mice was calculated during the warmest two hours of the heated surface (e.g. hours 6–8) as denoted by the dotted black lines, and shown in parts B, D, and F. * $p < 0.05$ for the comparison shown.

Table 1
Body temperature (T_b, °C), heart rate (HR, bpm), and activity (arbitrary units) of C57Bl/6J mice (n = 4) during 12-h light and dark periods.

	23 °C	23 °C + heated surface	30 °C
Light phase T_b (°C)	35.7 ± 0.5	36.0 ± 0.4	35.5 ± 0.5
Light phase HR (bpm)	562 ± 17	403 ± 23[a]	413 ± 42[a]
Light phase activity (AU)	5.8 ± 0.6	4.0 ± 0.7	6.3 ± 3.4
Dark phase T_b (°C)	36.9 ± 0.4	36.8 ± 0.5	37.0 ± 0.4
Dark phase HR (bpm)	597 ± 10	559 ± 12[a]	460 ± 27[a,b]
Dark phase activity (AU)	12.9 ± 1.7	12.0 ± 0.9	11.6 ± 1.7

AU = arbitrary units.
[a] $p < 0.05$ vs. 23 °C.
[b] $p < 0.05$ vs. 23 °C + heated surface.

the 23 °C and 30 °C condition (Table 1). The HR during the 23 °C + heated surface condition was significantly lower than the HR during the 23 °C condition, p = 0.004, but higher than the HR during the 30 °C condition, p = 0.017. These results are consistent with our observation that the heated surface remains warm through part of the dark period, and that supplemental heat continues to influence mouse HR. Furthermore, the lack of significant difference that we found in the activity of the three conditions during the dark period suggested that the mice are most likely seeking out cooler temperatures rather than remaining inactive on the heated surface. Therefore, these cages may be preferable to housing mice in 30 °C rooms not only by decreasing researcher discomfort but also by allowing mice to seek out a cooler T_a in the dark period, which may remove the potential of heat stress during the part of their circadian rhythm in which they prefer cooler temperatures (Gordon et al., 2017).

An important consideration is that this experiment only used male mice. It is likely that the findings found here for male mice will translate to female mice as the smaller female, with a larger surface area to volume ratio, may be more sensitive to cool temperatures than male mice. This remains to be determined experimentally. Further, we only examined C57BL/6 mice in the current study, so care must be taken not to generalize to all mice. We note that the physiology of rats, despite their much larger size relative to mice, is also sensitive to ambient temperature (Swoap et al., 2004). We therefore feel it is likely that other strains of mice will also be sensitive to changes in ambient temperature conditions. A third caveat that may be important is the caging type. We used conventional caging with wire lids. Because caging type can vary from facility to facility, and that variance could impact the physiology of the mice, it will be important to test whether supplemental heat will have an impact within multiple cage types (Rosenbaum et al., 2010).

To sum, we used a supplemental heat source at the beginning of the light period when mice are least active and prefer the warmest T_a. The lowered HR in mice housed in cages with activated hand warmers (over both the hottest two hours of the heated surface and the entire light period) indicated that these cages can significantly reduce cold stress in mice, and therefore may allow for a more predictive model system while maintaining comfortable laboratory environments for researchers (Karp, 2012; Gordon, 2017). There are, however, costs to implementing these cages into a laboratory setting. The use of disposable hand warmers for daily housing can be cumbersome, so perhaps the next step in

this research is designing a cage that can selectively heat a portion of the cage automatically. The current set of experiments serve as a proof of principle that allowing mice to regulate behaviorally their body temperature significantly reduces physiological signs of cold stress when housed at a typical vivarium temperature. When modelling human metabolism, obesity, cardiovascular disease, and other T_a dependent fields, we recommend considering usage of a supplemental heat source that allow for thermotaxis that can that reduce mouse cold stress on the mouse.

Acknowledgements

This work was support by a grant to SJS (NIH 1R15HL120072-01A1).

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jtherbio.2018.12.006.

References

Batchelder, P., Kinney, R.O., Demlow, L., Lynch, C.B., 1983. Effects of temperature and social interactions on huddling behavior in mus-musculus. Physiol. Behav. 31 (1), 97–102.

Butz, G.M., Davisson, R.L., 2001. Long-term telemetric measurement of cardiovascular parameters in awake mice: a physiological genomics tool. Physiol. Genom. 5 (2), 89–97.

David, J.M.C., Arion, F., Wang, Hongkai, Stout, David B., 2013a. The hidden cost of housing practices: using noninvasive imaging to quantify the metabolic demands of chronic cold stress of laboratory mice. Comp. Med. 63 (5), 386–391.

David, J.M.K., Scott, Lamkin, Donald M., Stout, David B., 2013b. Individually ventilated cages impose cold stress on laboratory mice: a source of systemic experimental variability. J. Am. Assoc. Lab. Anim. Sci. 52 (6), 738–744.

Enerback, S., Jacobsson, A., Simpson, E.M., Guerra, C., Yamashita, H., Harper, M.-E., Kozak, L.P., 1997. Mice lacking mitochondrial uncoupling protein are cold-sensitive but not obese. Nature 387 (6628), 90–94.

Feldmann, H.M., Golozoubova, V., Cannon, B., Nedergaard, J., 2009. UCP1 ablation induces obesity and abolishes diet-induced thermogenesis in mice exempt from thermal stress by living at thermoneutrality. Cell Metab. 9 (2), 203–209.

Gaskill, B.N., Rohr, S.A., Pajor, E.A., Lucas, J.R., Garner, J.P., 2009. Some like it hot: mouse temperature preferences in laboratory housing. Appl. Anim. Behav. Sci. 116 (2), 279–285.

Gordon, C.J., 2012. Thermal physiology of laboratory mice: defining thermoneutrality. J. Therm. Biol. 37 (8), 654–685.

Gordon, C.J., 2017. The mouse thermoregulatory system: its impact on translating biomedical data to humans. Physiol. Behav. 179, 55–66.

Gordon, C.J., Becker, P., Ali, J.S., 1998. Behavioral thermoregulatory responses of single- and group-housed mice. Physiol. Behav. 65 (2), 255–262.

Gordon, C.J., Puckett, E.T., Repasky, E.S., Johnstone, A.F., 2017. A device that allows rodents to behaviorally thermoregulate when housed in vivariums. J. Am. Assoc. Lab Anim. Sci. 56 (2), 173–176.

Hylander, B.L., Repasky, E.A., 2016. Thermoneutrality, mice, and cancer: a heated opinion. Trends Cancer 2 (4), 166–175.

Karp, C.L., 2012. Unstressing intemperate models: how cold stress undermines mouse modeling. J. Exp. Med. 209 (6), 1069–1074.

Kawate, R., Talan, M.I., Engel, B.T., 1993. Aged C57BL/6J mice respond to cold with increased sympathetic nervous activity in interscapular brown adipose tissue. J. Gerontol. 48 (5), B180–B183.

Lo Martire, V., Silvani, A., Bastianini, S., Berteotti, C., Zoccoli, G., 2012. Effects of ambient temperature on sleep and cardiovascular regulation in mice: the role of hypocretin/orexin neurons. PLoS One 7 (10), e47032.

Lodhi, I.J., Semenkovich, C.F., 2009. Why we should put clothes on mice. Cell Metab. 9 (2), 111–112.

Maloney, S.K., Fuller, A., Mitchell, D., Gordon, C., Overton, J.M., 2014. Translating animal model research: does it matter that our rodents are cold? Physiology 29 (6), 413–420.

Rosenbaum, M.D., VandeWoude, S., Volckens, J., Johnson, T., 2010. Disparities in ammonia, temperature, humidity, and airborne particulate matter between the micro- and macroenvironments of mice in individually ventilated caging. J. Am. Assoc. Lab Anim. Sci. 49 (2), 177–183.

Speakman, J.R., Keijer, J., 2013. Not so hot: optimal housing temperatures for mice to mimic the thermal environment of humans. Mol. Metab. 2 (1), 5–9.

Swoap, S.J., Li, C., Wess, J., Parsons, A.D., Williams, T.D., Overton, J.M., 2008. Vagal tone dominates autonomic control of mouse heart rate at thermoneutrality. Am. J. Physiol. Heart Circ. Physiol. 294 (4), H1581–H1588.

Swoap, S.J., Overton, J.M., Garber, G., 2004. Effect of ambient temperature on cardiovascular parameters in rats and mice: a comparative approach. Am. J. Physiol. Regul. Integr. Comp. Physiol. 287 (2), R391–R396.

Talan, M.I., Kirov, S.A., Kosheleva, N.A., 1996. Nonshivering thermogenesis in adult and aged C57BL/6J mice housed at 22 degrees C and at 29 degrees C. Exp. Gerontol. 31 (6), 687–698.