

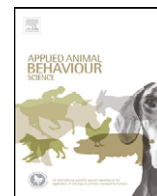


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Contents lists available at ScienceDirect

Applied Animal Behaviour Science

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



Some like it hot: Mouse temperature preferences in laboratory housing

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ARTICLE INFO

Article history:

Accepted 13 October 2008

Available online xxx

Keywords:

Animal welfare

Thermoregulation

Stress

Temperature preference

Mice

Home cage

ABSTRACT

In standard laboratory environments mice are housed at 20–24 °C. However, their thermoneutral zone ranges between 26 °C and 34 °C. This challenge to homeostasis is by definition stressful, and could therefore affect many aspects of physiology and behavior. We tested the hypothesis that mice under standard laboratory conditions are not housed at a preferred temperature, and predicted that this would be evident in thermotaxis and other behavioral responses to ambient cage temperature. We assessed the temperature preferences of C57BL/6J mice in standard laboratory housing from 4 to 11 weeks of age. Forty-eight mice (24 male and 24 female in groups of three) all born on the same day were randomly assigned to one of eight age treatments. One cage of males and one cage of females were tested each consecutive week. Mice were tested in a set of three connected cages with each cage's temperature set using a water bath. On days 1–3 each group of mice was acclimated to each of the three temperatures (20 °C, 25 °C, or 30 °C) in a random order. Then each group was given free access to all temperatures on days 4–6, and video taped continuously. The location of each mouse and the occurrence of three behavioral categories (Active, Inactive, and Maintenance) were recorded by instantaneous scan samples every 10 min over the 3 days, and time budgets calculated. While both sexes chose warmer temperatures overall ($P < 0.001$), they preferred warmer temperatures only for maintenance and inactive behavior ($P < 0.001$). This effect was most pronounced in females ($P = 0.017$). As temperature selection varied with time of day ($P < 0.001$), these behavioral differences cannot be due to ambient temperature dictating behavior. We conclude that C57BL/6J mice at 20–24 °C are not housed at their preferred temperature for all behaviors or genders, and that it may not be possible to select a single preferred temperature for all mice.

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1. Introduction

Mice under standard laboratory conditions are housed at 20–24 °C (Gordon, 1993, 2004; National Research Council, 1996) but their thermoneutral zone ranges between 26 °C and 34 °C (Gordon, 1993). This broad range is most likely due to differences between strains and methods of critical temperature measurement. However,

most researchers agree that the lower critical temperature of a single mouse is around 30 °C (Gordon, 2004) which is about 1 °C warmer than preferences of group housed mice (Gordon et al., 1998). This mismatch in standard laboratory housing and thermal neutrality temperatures is a challenge to homeostasis, and by definition, a stressor (Moberg, 2000), with the potential to alter many scientific measures taken from diverse areas of research. Housing animals at, or just below, standard housing temperatures is sufficiently stressful to have wide ranging effects. These mild cold stressors have been shown in mice to decrease growth (Yamauchi et al., 1983), organ weight (Yamauchi et al.,

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1983), immune function (Yamauchi et al., 1983), and increase basal metabolic rates (Gordon, 1985, 1990, 1993). In laboratory environments mice have little or no control over the cold temperatures to which they are exposed, and a lack of control typically exacerbates physiological stress responses (Moberg, 2000). We therefore set out to ask whether mice would attempt to use behavior to control which ambient temperature they spent their time in when given the chance.

Most information regarding thermoregulatory behavior of mice comes from thermoclines (which are a metal tube heated at one end and cooled at the other), or operant chambers with a lever and heat lamp (Weiss and Laties, 1961; Chen et al., 1998; Gordon et al., 1998). These environments tell us the basics about the thermal needs of mice but conditions inside them may differ from a typical mouse cage, possibly altering thermal preferences. Data from operant and thermocline studies have been extrapolated to all rodent housing conditions, whether the animals are single or group housed, with or without bedding, or in ventilated or static cages. Environmental differences between housing set ups, such as insulation of bedding material, air flow, and stocking density, affect the amount of heat lost which could be crucial when housing mice at mildly cold temperatures.

Laboratory mice are nocturnal/crepuscular, showing a peak of activity at the start of the dark phase, a series of less marked and more variable activity cycles during the rest of the night, and very little activity during the daylight hours (Mackintosh, 1981; Gordon et al., 1998; Latham and Mason, 2004). When active, mice create more heat, increasing their core temperature, and select cooler temperatures to increase heat loss (Gordon et al., 1998; Castillo et al., 2005). Conversely, mice select warmer temperatures when activity decreases (Ogilvie and Stinson, 1966; Gordon et al., 1998). For mice in normal laboratory environments, changes in temperature preference due to behavior and activity patterns are not known.

If these phenomena extrapolate from thermocline and operant work to standard housing, they could intensify the mild cold stress in the home cage, for three connected reasons. First, mice require spatial variation in ambient temperature to select a temperature. However, they do not receive the luxury of choice in standard laboratory conditions, where they are customarily housed at one temperature. Second, they may select different temperatures depending on the behaviors they are performing. In which case any set temperature would be inappropriate for some behaviors, some of the time. Third, preventing an animal's escape from, or control over, stressors in its environment magnifies their physiological impact (Moberg, 2000).

Therefore, our objective was to determine whether data from thermocline and operant experiments could be extrapolated to laboratory housing by examining the thermal preferences of mice, for different behaviors, under typical laboratory conditions, except that the mice could move between cages at different temperatures. We first hypothesized that mice would prefer environments warmer than the standard laboratory temperature, and

that those preferences would be closer to their thermo-neutral zone (Gordon, 1985, 2004; Gordon et al., 1998). Second, we hypothesized that the behavioral time budget would differ in the different temperatures. Exercise increases heat generation, therefore we predicted to see a weaker temperature preference for active behaviors. Third we hypothesized that there would be differences in temperature preferences between males and females. We predicted that females, being smaller, would have higher heat loss, and would prefer warmer temperatures than males. For similar reasons, we predicted that young mice would show a stronger preference for warmer temperatures than adult mice (Doi and Kuroshima, 1982).

2. Materials and methods

2.1. Animals and housing

A total of 48 C57BL/6J mice arrived at Purdue University, USA from Jackson Laboratories (Bar Harbor, ME, USA) at 26 days of age: 24 males and 24 females. Upon arrival the mice were randomly separated into same sex groups of three, assigned to one of eight ages as testing treatments (4–11 weeks of age) and ear notched for identification. The experimental unit was each group of three mice. The mice were housed in standard laboratory polycarbonate shoebox cages (Alternative Design, Siloam Springs, AR, USA; 18.41 cm *W* × 29.21 cm *D* × 12.7 cm *H*) with aspen shaving bedding (Harlan Teklad, Madison, WI, USA) and wire cage lids. The mice were kept on a 14:10 Light:Dark photoperiod (lights on at 06:00 h), at 60 ± 10% relative humidity and given food (Mouse diet 2019, Harlan Teklad, Madison, WI, USA) and water *ad libitum*. All housing and procedures associated with this experiment were approved by Purdue's Institutional Animal Care and Use Committee.

2.2. Thermal preference apparatus

We used three glass fish tanks (Fig. 1) as water baths to keep the mouse cages at a constant temperature. The water baths, heated by thermostatic electric fish tank heaters, were set to maintain constant ambient temperatures within the cages at 20 °C (a typical laboratory temperature), 25 °C, or 30 °C (corresponding to the lower and upper boundaries of a mouse's thermoneutral zone). Temperatures inside of each cage were confirmed prior to testing each day of the experiment by a thermometer just off the surface of the aspen bedding within the cage. Submerged cages were of the same make and size as cages in which the mice were housed prior to experimentation (Fig. 2). Approximately 0.65 cm of aspen bedding covered the floor of the cage, which was sufficient for mice to build shallow cup-like nests. *Ad libitum* food and water were located on top of all three cage lids within the experimental apparatus. The cages sat in a wire basket, immersed in the water baths up to 2.5 cm from the rim and secured with nylon straps that encompassed the water bath. Hard plastic hamster tubing (S.A.M., Penn Plax Inc., Hauppauge, NY, USA) was used to connect the three cages together through holes in the cage lids. Tube ends were approximately 7.5 cm from the cage floor. Two sets of apparatus were tested simultaneously (Figs. 1 and 2).

2.3. Experimental design

One group of each sex was run simultaneously each week in two separate apparatuses. We took precautions to control for position bias and the potential effect of mice in adjacent cages by using visual barriers between cages, by rotating the temperature of the cages, and by alternating which set of apparatus each sex was tested in.

It took 6 days to test each group. The mice were confined to each of the three temperatures for 24 h in a random order the first 3 days. Tube caps allowed for exploration in the tube but restricted access to other cages. On days 4–6, the tube caps were removed and each mouse was placed in a separate temperature (i.e. cage) to begin the day, balanced across the 3 days, but was given free access to all cages. Social effects, such as seeking or avoiding other companions, could not be completely eliminated during these days due to this procedure.

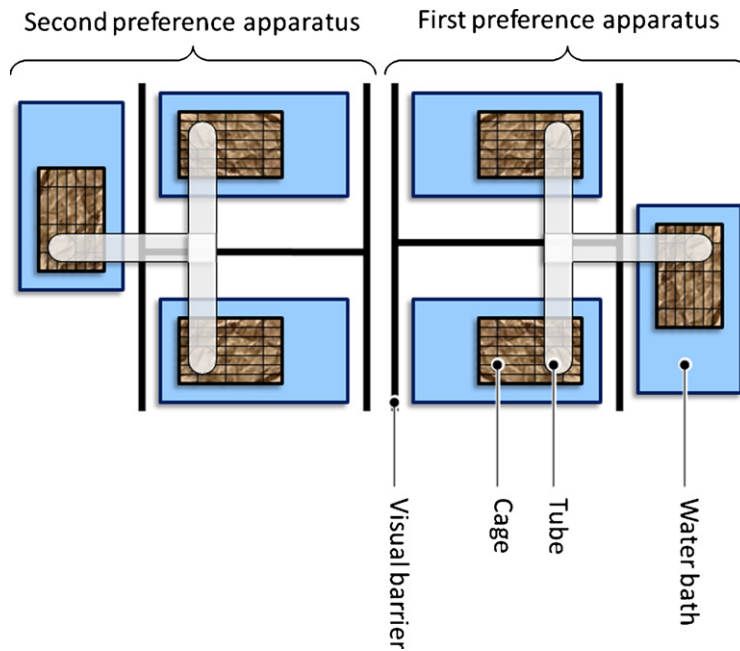


Fig. 1. Diagram showing the configuration of water baths and cages for testing cage temperature preferences for one group of male mice and one group of females simultaneously.

2.4. Behavioral observations and data processing

The mice were video taped continuously during days 4–6 for behavioral data collection using infrared cameras and illuminators, a multiplexer and timelapse video. The location and behavior of every mouse was recorded using instantaneous scan samples every 10 min. The ethogram was split into three broad categories of behavior (Inactive, Active, and Maintenance) (Table 1). These categories are ethologically relevant, in that mice typically transition between them in an activity cycle (i.e. Inactive → Maintenance → Active → Maintenance → Inactive) (Gaskill, B.N., Dufour, B., and Garner, J.P., unpublished data), and the behaviors also likely differ in their thermal balance.

Population time budgets were calculated for each group of mice by counting the total number of times each category of behavior was observed in each location (i.e. 20 °C, 25 °C, or 30 °C cages, and the tunnel) over the 3-day period, and dividing by the total of this count. Following this calculation, data from the tunnel were excluded from the analysis (as mice were rarely recorded in the tunnels).

To provide balanced replication across combinations of age and side of the apparatus, two consecutive age treatments (weeks of age at testing) were combined together to create the following Age-sets by weeks of age: 4 and 5; 6 and 7; 8 and 9; and 10 and 11.

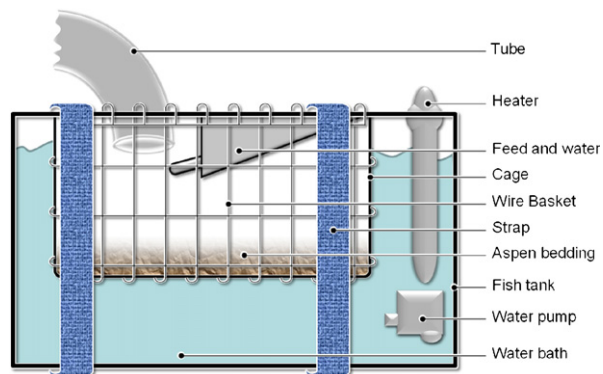


Fig. 2. Diagram depicting elements present in water bath and cage set up.

We also calculated a circadian budget, for each group of mice, by counting the total number of times mice were observed in each location during four blocks of time (00:00–05:59 h [dark]; 06:00–11:59 h [light]; 12:00–17:59 h [light]; and 18:00–23:59 h [2 h light, 4 h dark]) over the 3-day period, and dividing by the total of this count. Following this calculation, data from the tunnel were excluded from the analysis.

2.5. Analysis

All analyses were performed as split-plot ANOVA using GLM, in Minitab 14 for Windows. The assumptions of GLM (normality of error, homogeneity of variance, and linearity) were confirmed post hoc, and the data angular transformed to meet these assumptions (Grafen and Hails, 2002). Significant effects were then analysed using post hoc Tukey tests.

To avoid pseudoreplication, and accommodate repeated measures, the analysis was blocked by Group of mice, nested within Age-set and Sex. Group of mice cannot be treated as a random effect (there is not a meaningful wider population of groups of three mice representing unique and indivisible components of variance from which we selected our groups of three mice, and to which our results could pertain) (Newman et al., 1997), and was therefore treated as fixed (i.e. as a split-plot). Group, Sex, and Age-set (4 and 5; 6 and 7; 8 and 9; and 10 and 11 weeks of age) were crossed with Location (i.e. 20 °C, 25 °C, or 30 °C cages) and Behavior (Inactive, Maintenance or Active). Location tested the overall temperature preference of the mice (Hypothesis 1); the interaction Location-by-Behavior tested the dispersal of behaviors in the different temperatures (Hypothesis 2); the three-way interaction of Location-by-Behavior-by-Sex tested the preferences of both sexes (Hypothesis 3); and the interaction Age-set-by-Location tested the preference of temperatures as the mice aged (Hypothesis 4).

To control for the possibility that ambient temperature might be influencing behaviors within a cage, we examined the circadian budget. It could be that mice move from one area to another randomly, and ambient temperature triggers different behaviors, which last for longer than normal bout lengths. If this is true, the division of time between the locations would be the same regardless of time of day. On the other hand, if mice are selecting locations according to behaviors, we would expect location preference to change with circadian rhythm (Refinetti and Menaker, 1992; Gordon, 1993; Castillo et al., 2005). Our statistical model for testing the circadian rhythm was the same as above, except that Behavior was replaced by Hour-block.

Table 1
Ethogram of behaviors observed in the study.

Category	Behavior	Description
Active	General locomotion	All locomotive behavior performed on the cage lid, climbing up the cage bars by the food hopper to reach the lid, and locomotion on the floor of the cage
	Rearing	Seen on the floor of the cage with all an animal's weight on its hind legs and front legs off the ground. Sniffing movements while on it's hind legs were commonly accompanied with this behavior
	Sniffing	Sniffing was also performed against the cage floor (ground), or in between the bars of the cage lid. Slight upward jerks of the head were seen
Maintenance	Settling and nest-building	Turning was characterized by the animal circling on the spot within the nest, commonly preceding sleep. Gathering was characterized by the animal reaching out of the nest and pulling sawdust to the edge of the nest. Gathering was distinct from locomotion in that the hind legs did not leave the nest, and each time the animal reached out of the nest it pulled its forelegs back in
	Grooming	All grooming behavior including licking the fur, grooming with the forepaws, and scratching with any limb. Grooming was usually performed in a sitting position with the animal's hind quarters in contact with the floor
	Feeding or drinking	The animal would rear up to gnaw at food pellets through the bars of the hopper. The forepaws would usually be used to hold the food pellet steady. The animal would rear up and lick the nipple drinker
Inactive	Sleeping	The animal was motionless, and either lying curled up on its side, or sitting curled up, with its face tucked into its body and out of sight of the camera. Occasionally interrupted by brief single twitches of the body
	Still and alert	The animal was sitting or curled up, but in contrast to sleep, the face was lifted. The animal either sat motionless, or would appear to be orientating its head to sounds outside of the cage

3. Results

We first hypothesized that mice would spend more time in temperatures warmer than 20 °C during days 4–6. As predicted the mice spent more time overall in 25 °C and 30 °C than 20 °C (Fig. 3) (GLM; $F_{2,64} = 69.59$; $P < 0.001$). Approximately 55% of the 3 days was spent in the 30 °C cages, 20% in 25 °C and 10% in 20 °C. Tukey tests showed that the percentage of time spent in each temperature was statistically different from one another (20 °C < 25 °C < 30 °C; $P < 0.05$).

We next tested our second hypothesis that mice would perform different behaviors in the three temperatures. Once again, as predicted, we found that the behaviors were not evenly dispersed across the temperatures (GLM;

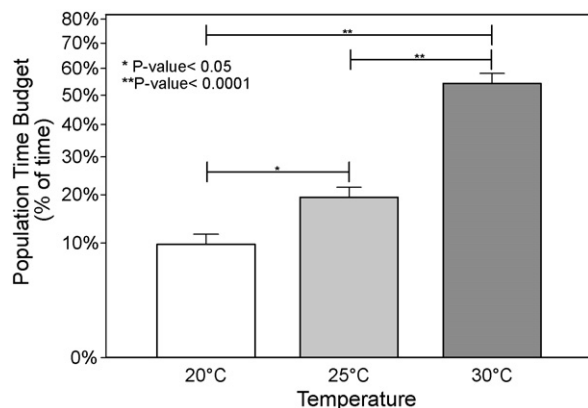


Fig. 3. Mean proportion of time spent by mice in cages at 20 °C, 25 °C, and 30 °C. LSM and SE are plotted and significant differences using Tukey comparisons are indicated by asterisks. The percentage of time over the 3 days is represented on the y axis as an angular transformed scale.

$F_{2,64} = 19.16$; $P < 0.001$; Fig. 4). Using Tukey tests, no statistical differences were found between the temperatures for active behaviors, but for inactive behaviors warmer temperatures were transitively preferred (20 °C < 25 °C < 30 °C; $P < 0.05$); and for maintenance behaviors there was a preference for 30 °C over the other two temperatures (20 °C = 25 °C < 30 °C; $P < 0.05$).

Our third hypothesis tested whether males and females have differences in temperature preference. As predicted we found a significant three-way interaction between Sex, Location, and Behavior (GLM; $F_{4,64} = 3.26$, $P = 0.017$; Fig. 5). The Tukey tests resulted in no statistical differences for active behaviors when comparing preferences within sex. For inactive behaviors females preferred 30 °C over the other temperature options (20 °C = 25 °C < 30 °C; $P < 0.0001$). The males showed similar results (20 °C < 25 °C = 30 °C; $P < 0.01$) but there was no difference between 25 °C and 30 °C. For maintenance behaviors, only females showed a significant temperature preference (20 °C = 25 °C < 30 °C; $P < 0.01$). When the two sexes were compared to one another only a difference in preference of approximately 10% was found at 25 °C for inactive behaviors ($P < 0.05$; Fig. 6).

Age-set-by-Location was significant ($F_{6,64} = 2.88$; $P = 0.015$), but the Tukey tests did not reveal any changes in preference between Age-sets.

Our last analysis tested the possibility that ambient temperature may dictate the performance of behaviors. A two-way interaction between Location (i.e. 20 °C, 25 °C, or 30 °C cages) and Hour-block was found (GLM; $F_{6,154} = 7.26$, $P < 0.0001$). 30 °C was preferred at all times of day, but this preference was significantly stronger during the Hour-blocks 06:00–11:59 h and 12:00–17:59 h (light periods) than 00:00–05:59 h (dark period) and 18:00–23:59 h (2 h light, 4 h dark) (Contrast; $F_{1,154} = 37.6$; $P < 0.001$).

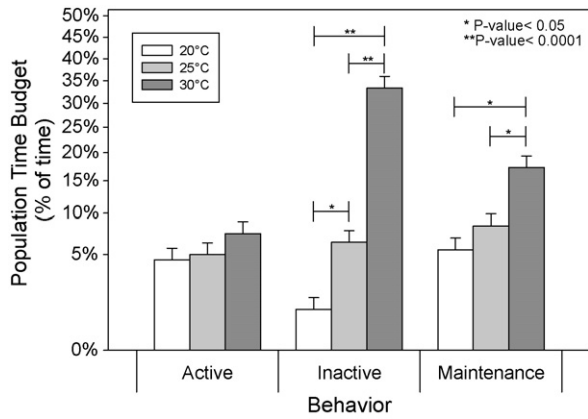


Fig. 4. Mean proportion of time spent by mice in behavioral categories at three temperatures. LSM and SE are plotted and significant differences using Tukey comparisons are indicated by asterisks. The percentage of time over the 3 days is represented on the y axis as an angular transformed scale.

4. Discussion

These data for the first time establish mouse temperature preferences in the home cage. Gratifyingly, these results match those from thermocline work, despite the potential limitation of thermoclines (such as no bedding material, odd cage configuration, and water and food at only one temperature). Importantly, this correspondence justifies extrapolation of other results from thermoclines to non-ventilated home cages. Overall, we found that mice preferred warmer temperatures than typical housing conditions. However, we found that mice in laboratory housing prefer warmer temperatures for maintenance and inactive behaviors but were indifferent when selecting a temperature for active behaviors under normal laboratory conditions. Our results agree with previous research in thermoclines, although preferences for maintenance behaviors have not been differentiated from general activity in this previous research (Yang and Gordon, 1996; Gordon

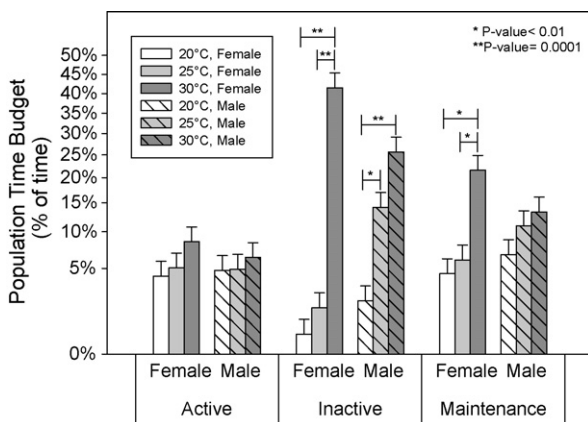


Fig. 5. Comparison within sexes of mice of mean proportion of time spent in behavioral categories at three temperatures. LSM and SE are plotted and significant differences from Tukey comparisons are indicated by asterisks. The percentage of time over the 3 days is represented on the y axis as an angular transformed scale.

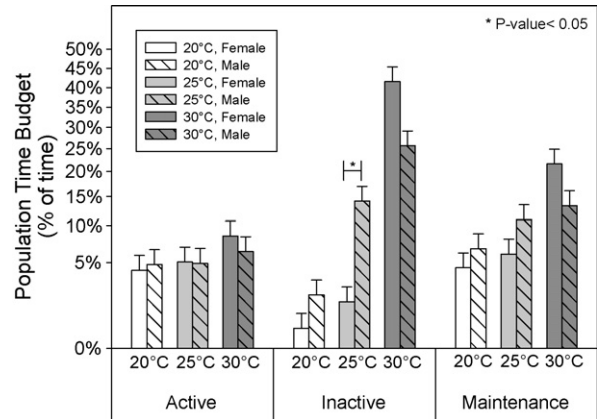


Fig. 6. Comparison between sexes of mice of mean proportion of time spent in behavioral categories at three temperatures. LSM and SE are plotted and significant differences from Tukey comparisons are indicated by asterisks. The percentage of time over the 3 days is represented on the y axis as an angular transformed scale.

et al., 1998; Castillo et al., 2005). Our findings were perhaps due to maintenance and inactive behaviors overall requiring less energy and thus producing less heat. Rearing and sniffing typically occur within the context of active exploration, while settling, nest-building, and grooming typically occur while the animal is stationary and (especially in the case of settling and nest-building) in the nest site. Similarly, eating and drinking typically occur outside of the context of intensive exploratory activity. Consequently, the mice may not have been cold stressed when active but may have been when performing less energy demanding behaviors, or may have even been heat stressed when active in warmer ambient temperatures. If so, we might expect that if we had used even warmer temperatures the mice might have shown a preference against them when active. Alternatively, the increase in activity might have been a thermoregulatory response as an increase in activity is sometimes seen in cooler environments (Weiss and Laties, 1961; Gordon, 2004). However, the weight of existing evidence argues against activity as a thermoregulatory response in rodents (Gordon, 1993).

Females demonstrated more of a thermal preference compared to males when engaged in maintenance and inactive behaviors. This too could be due to the differences in metabolic demands between behaviors and sexes (Jakobson, 1981; Gordon, 1993, 2004), and strengthens the interpretation that mice are selecting particular temperatures for particular behaviors. When the sexes were compared to one another we found a difference in preference for inactive behaviors: only 30 °C was preferred by females, but both 25 °C and 30 °C were preferred by males, suggesting that the minimum preferred temperature for males is lower than females. A possible explanation for this difference could be due to sexual dimorphism within this strain. Females are generally lighter than males and therefore females have a higher surface area to volume ratio which increases the amount of heat lost to the environment (Jakobson, 1981; Gordon, 1993). It is possible that this difference in heat loss could alter thermoregulation and preferences between sexes. In addition, the mice

used in this study, C57BL/6J, become obese later in life. This increased fat deposition alters the surface area to volume ratio further, increasing heat retention. Thus, older more heavily insulated mice may be less affected by cooler temperatures than younger mice. Regrettably, mouse weight was not recorded during this experiment and therefore any weight differences between the sexes could not be determined. Anecdotally, we did not see vast differences in body size between the 4- and 11- week-old mice nor did we see any evidence of obesity during the period of testing.

Sex hormones also effect thermoregulation. For instance progesterone is known to increase body temperature in the rat (Freeman et al., 1970). Prepubescent mice with smaller amounts of circulating sex hormones and less insulation may show different preferences than sexually mature, older mice. Unfortunately, we did not identify any preference differences between the three choice temperatures related to age at testing in this experiment. It is possible that the differences are present but the experiment did not have enough resolution to identify differences in preferences less than 5 °C and the range of temperatures provided may not have been wide enough. In addition, a longer study may be needed to identify differences in preferences due to age and stages of life.

The circadian rhythm of temperature preference found was similar to previous data in thermoclines (Gordon et al., 1998). Furthermore, these circadian preferences are inconsistent with mice choosing behavior in response to ambient temperature. Behavior is under the control of circadian rhythm but ambient temperature is constant throughout the day. Therefore, if the mice's behavior was dictated by temperature there would be no circadian temperature preference. Instead these data imply that mice chose particular temperatures in order to perform particular behaviors.

Anecdotally it has been suggested that microenvironments inside a cage may compensate for low room temperatures. However, internal cage temperature is typically only about 1 °C warmer than room temperatures (Gordon, 2004), and in thermoclines huddling only affects preference by about 1 °C as well (Gordon et al., 1998). Therefore these results clearly show that mouse temperature preferences are not being met the majority of the day, especially when inactive.

One downfall of simple preference testing, however, is that we do not know whether the choice by mice of a warmer temperature is imperative to their well-being. Measuring how much a mouse is willing to work or pay for access to a warmer environment would allow us to evaluate how essential it is to them (Dawkins, 1990; Mason et al., 1998). In retrospect, it would have been very informative to have observed home cage time budgets during the 3 days of forced temperature exposure. Measuring the frequencies of behaviors in each temperature and comparing them to data taken during free access, would have provided further evidence to distinguish between mice responding passively to temperature or choosing a temperature for a particular behavior. Collecting animal weight and amount of food consumed throughout the experiment would also have provided

information on insulation and energy available for heat production. Similarly, measuring core temperature would have allowed us to assess effects on thermoregulation directly. However, the water-baths prevented us from using radio-telemetry to monitor core temperature.

Although a non-preferred option is not necessarily aversive or stressful, mice prefer temperatures in the thermoneutral zone, and being outside of the thermoneutral zone is a physiological stressor. Therefore, the small amount of time spent in cold temperatures for inactive and maintenance behaviors suggest the mice might perceive a 20 °C home cage as stressful, possibly indicating compromised welfare when performing these behaviors. While lack of preference does not absolutely indicate impaired welfare, it does point to a stressor that requires some behavioral or physiological coping by the animal. Although there was a general preference for warmer temperatures, we saw that mice's temperature preference was affected by sex and behavior. Therefore, it makes little sense to select one temperature as being the preferred or optimal temperature, and finding one temperature for all sexes and housing setups is not feasible. This may help explain why preferred temperatures in this experiment and in thermoclines and operant chambers differ from physiologically optimum temperatures. Yamauchi et al. (1983) found that temperature range at which a variety of physiological parameters were unaltered is between 20 °C and 26 °C (in these experiments mice could not control ambient temperature), but preference research has consistently shown that adult mice will spend more time in 26–34 °C (Ogilvie and Stinson, 1966; Gordon, 1984, 1993; Gordon et al., 1998).

A possible solution, to both the apparent inconsistency between these literatures, and the issue of there being no one preferred temperature, would be to provide nesting material to mice, allowing them to insulate themselves during behaviors with reduced metabolic heat generation. Indeed this may explain why nesting material has been found to be a beneficial enrichment for mice (Olsson and Dahlborn, 2002) and why nesting material decreases food intake and increases body weight (Dahlborn et al., 1996). Investigating how nesting material affects temperature preferences in typical housing would therefore be a fruitful direction for research into both mice's ambient cage temperature needs, and the design and efficacy of mouse enrichment. This is the focus of our ongoing research.

5. Conclusions

Based on previous research, mouse well-being and scientific results are affected by ambient temperatures. Laboratory mice are housed at temperatures below their thermoneutral zone. Here we have shown that, when given a choice, mice prefer to spend more time in temperatures closer to their thermoneutral zone. However, this preference is also affected by sex, current behavior, and time of day. Therefore while one temperature for all housing combinations would be convenient, it may not be conducive to mouse well-being.

Acknowledgements

We thank Megan Lee for assisting with video observations and animal care, Jason Fields for animal care, and Erica Davis for pilot work. Authors B.N.G., E.A.P., J.R.L., and J.P.G. conceived and designed the experiment and B.N.G. and S.A.R. executed the experiment.

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