Core Body Temperature Elevation in Adult Mice After Common Handling and Laboratory Procedures

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Summary

Common laboratory procedures are known to influence the core body temperature (CBT) of animals including mice. This includes procedures used to monitor CBT, like rectal thermometry, which presents a problem in studies where the CBT of animals is an important outcome. This experiment utilised an implantable telemetric capsule to examine the CBT response of male and female mice to common laboratory procedures. CBT was monitored for 60 min following four procedures: gentle handling, transport within the facility, exposure to a novel environment, and a rectal temperature measurement. Compared to baseline CBT, all procedures elicited a statistically significant rise in CBT, reaching a peak between 5-25 min after the procedure took place (gentle handling peak ΔT °C: female = 1.76; male = 2.82; transport peak ΔT °C: female = 0.99; male = 2.48; novel environment peak ΔT °C: female = 1.49; male = 2.30; rectal measurement peak ΔT °C: female = 1.66; male = 3.02). CBT was not statistically differentiable from baseline at 60 min post-procedure for any handling/laboratory procedure except for the rectal temperature measurement. Therefore, a method of CBT measurement that does not involve the handling of mice or disturbances to their environment is preferable to ensure CBT is not confounded by a thermal response. Furthermore, the inclusion of an undisturbed habituation period of at least 60 min prior to testing is necessary to reduce the confounding handling-induced thermal response, particularly where a valid measure of experimentally-induced CBT change is desired.

Key words

Radiotelemetry • Mouse • Body temperature • Animal handling

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Introduction

Rodents, in particular mice, are the most common subjects of biomedical studies [1]. The conditions and the manner in which mice are cared for, for example housing density, ambient temperature, handling practices, lighting and common laboratory procedures, are known to influence mouse behaviour and physiology [2-5], which in turn may influence a study's results. One aspect of mouse physiology that can be greatly influenced by some of these factors is core body temperature (CBT). Handling and laboratory procedures commonly used during routine husbandry practices or experimentation in mice can be perceived as stressful to mice [6], causing a rapid increase in CBT (known as stress-induced hyperthermia) that is accompanied by a rise in heart rate, plasma corticosterone and plasma glucose [7].

CBT is an important physiological outcome that is measured in many biomedical studies, therefore the influence that common laboratory practices and procedures have on the CBT of mice needs to be considered. However, as most temperature measurement practices require the physical manipulation of the mouse and/or its environment, the act of measuring CBT can itself greatly influence mouse CBT. For example, rectal thermometry is a commonly used method of CBT despite

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the procedure itself inducing an immediate elevation in CBT that takes approximately one hour to return to baseline levels [8-10]. CBT obtained from repeated rectal measurements within this time-period would be influenced by this response. Rectal thermometry is therefore most useful when obtaining only a single measure of CBT [11], and only on the assumptions that the measurement is completed before the onset of the CBT response to the procedure and that the procedure is performed identically between animals by experienced personnel. The first assumption can be difficult to satisfy given the rapidity of response (e.g. CBT starts to rise only one minute after the procedure, as shown in [9]). Also, a single measurement of CBT does not yield a comprehensive insight regarding the dynamic nature of CBT change observed in mice over time. The use of infrared thermography or a subcutaneously implanted microchip can lessen disturbances to mice but, as they measure superficial temperatures, they are not accurate measures of CBT itself [12]. Surgically implanted telemetric capsules or data loggers, on the other hand, are surgically implanted into the abdomen and can continuously record CBT deep within the body core without disturbance to the mice, thus providing a more accurate measure of CBT without any effects due to handling or rectal probe placement. These features make implantable telemetric capsules or data loggers the most appropriate method of assessing CBT in mice [11,12].

In addition to rectal temperature measurement, an experimental intervention may involve any of the following events: handling and/or restraint of mice for labelling or identification, in-house transport of mice within their home cage to a procedure room and the exposure of mice to an unfamiliar environment or cage. These procedures are potentially stressful to mice and are likely to cause an elevated CBT [6,13]. Consequently, the measurement of an intervention-mediated change in mouse CBT may be confounded if taken immediately after such procedures. To ensure the validity of a CBT measure taken in the context of an experimental intervention, it is important to minimise the handling/procedure-mediated rise in temperature (by choosing procedures that are less stressful), or preferentially, to let it subside (by including an undisturbed habituation period prior to an experimental intervention). To implement these strategies however, the CBT response of mice to the handling and procedures associated with an experimental intervention must be well characterised, for example by determining the maximum change of CBT relative to baseline, the time taken to reach the maximum CBT change and the duration of time needed to return to baseline CBT. The brief handling of male mice (picking up by the tail for 5 s) has been shown to cause a large increase in CBT that takes around one hour to return to baseline [14]. Picking up a mouse from the cage and restraining it in the hand (as if for an injection, for 15 s) also caused a large increase in the CBT of male mice, but this increase did not return to baseline after 60 min [15]. Whether the same CBT response is observed in female mice, or after mice are picked up using a less aversive method like tunnel handling [16] or resting on an open hand is not clear. Blenkus *et al.* [17] investigated the CBT response of male and female mice after handling (2-3 s) using either the tail or *via* a tunnel, and reported no difference between the two methods; however, the authors suggested that the stress of moving the cage and removal of bedding prior to handling may have hidden any potential effect. Gerdin *et al.* [18] investigated the CBT response of in-house transport (e.g. transport between rooms within a facility) in male and female mice, reporting that rectal temperature 60 min after transport was not different to rectal temperature immediately after transport. Given the issues with rectal thermometry described earlier, the lack of an appropriate baseline measure and absence of measurements throughout the transport procedure, these results do not provide sufficient information to effectively characterise the CBT response of mice to transport. Cage changing is well-known to cause a stress response [6,19], however the conditions of cage change (placement of mice into an identical-sized cage with fresh bedding, nesting materials and access to food and water) is likely to differ to the typical conditions of a novel cage used for an experimental intervention (e.g. different-sized cage without bedding, minimal nesting materials and often no access to food and water), so it is difficult to predict the CBT response caused by placement into a novel cage from the response to cage changing. Furthermore, the degree of CBT response to a procedure may be influenced by the stressfulness of a procedure and the strain of mice being investigated [14]. Therefore, the effects of handling and laboratory procedures on CBT should be examined within the context of the mice and procedures to be used in a specific experiment.

To further our understanding of such effects on CBT, the present experiment utilised an implantable telemetric capsule to test whether each of four handling

and laboratory procedures (gentle handling, a short transport between rooms within a facility, exposure to a novel environment and a rectal temperature measurement) caused a change in CBT, and where they did, how long it took for the change to occur, how long it took for CBT to return to baseline, and what the magnitude of this CBT change was (for male and female mice separately). The procedures investigated were chosen to match current research in our laboratory aimed at determining the effect of radiofrequency electromagnetic field (RF-EMF) exposure on mouse CBT [20], but they are also common to many biomedical studies investigating CBT change in mice and so this study's results may be applicable to other experimental interventions.

Animals, Materials and Methods

All experimental procedures were approved by the Animal Ethics Committee, University of Wollongong, Australia (AE21/11) and adhered to the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (National Health and Medical Research Council, 2021).

Animals and housing

Twelve male and twelve female C57BL/6 mice with a C3H background (wild type-like mice from a B6C3-Tg(*APPswe*, *PSEN1dE9*)85Dbo/JAusb breeding colony) were obtained from Animal BioResources, NSW, Australia. On arrival at the animal housing facility, mice were held in same-sex groups in Tecniplast GM500 individually ventilated cage housing units, lined with corn cob bedding, for at least two weeks to acclimatise to the new environment. Within the acclimatisation period, male mice were transferred to individual housing, while female mice were divided into groups of three mice per cage according to the animal ethics approval. The housing environment was maintained at a room temperature of 22±0.2 °C and operated on a 12-hour dark and 12-hour light cycle (07:00-19:00 h light, 19:00-07:00 h dark). The ambient temperature was the same throughout the animal facility including the holding room and all corridors, i.e. there was no change in ambient temperature when transporting mice between different rooms of the facility. Mice were allowed *ad libitum* access to water and standard laboratory chow. Environmental enrichment, including a house, chew block, tunnel and nesting materials, were present in all cages. Mice were handled regularly during the acclimatisation period and throughout the experiment, with tunnel transfer being the primary handling method (tail handling was not used at any point during the study). At the date of the first experimental procedure, the average (standard deviation) age of the mice was 38.9 (3.4) weeks.

AniPill implantation

The AniPill (BodyCap, Caen, France) is a proprietary temperature sensing telemetric capsule (8.9 mm × 17.7 mm, 1.7 g), with a stated accuracy of ± 0.2 °C (range 25-45 °C). At least 48 h after mice were separated into individual (male mice, to prevent fighting after surgery) or group housing (female mice), the AniPill was implanted into the intraperitoneal cavity *via* the flank instead of the traditional midline approach, similar to that described by Chappel and colleagues [21]. As described previously [20], mice were first inducted with isoflurane (5 %) and injected subcutaneously with saline and buprenorphine. The intraperitoneal cavity was accessed *via* a 1 cm incision in the right flank region of the lateral abdominal muscle and a bupivicaine line block was injected into the incision site. The AniPill was then placed into the intraperitoneal space, the muscle and skin were sutured and the skin was closed with flexible skin glue. Mice were maintained on 2.5 % isoflurane throughout the procedure. Finally, mice received a subcutaneous injection of meloxicam before recovering in oxygen and warmed in a clean home cage.

During recovery, the incision site was monitored regularly. One female mouse exhibited signs of abdominal pain at 24 h post-surgery and was given an additional subcutaneous injection of meloxicam (5 mg/kg). Additionally, two male mice required re-closing of the skin (within 24 h post-surgery). All mice had at least 7 days of recovery to allow adequate time for the healing of the surgical site and the return of normal activity and circadian body temperature cycles [21-23]. One AniPill in a female mouse ceased communication shortly after implantation, therefore it was excluded from further analysis.

Handling and laboratory procedures

Mice underwent four separate handling/laboratory procedures to represent the most common interactions between laboratory mice and a researcher, using a repeated measures design in the following order: (1) Gentle Handling, involving picking

up the mouse from its home cage using the tunnel, gently handling the mouse for two minutes and then returning it to the home cage; (2) Transport, where home cages were placed on a trolley and walked to a procedure room (occurred within a two-minute period) where they were left undisturbed for one hour; (3) Novel Environment, which followed the same steps as the Transport procedure, differing only in that after one hour, mice were placed into a 'new' cage (14 × 31 × 17 cm) containing one tissue only (male mice were placed individually into a new cage, whereas female mice were placed into a new cage with their cage mates), after which the mice were left undisturbed in the procedure room for one hour; and (4) Rectal Temperature Measurement, where mice were removed from their home cage using a tunnel, gently restrained by the base of the tail and a mouse rectal probe (World Precision instruments, Sarasota, USA) lubricated with Vaseline was inserted 2 cm into the rectum. The probe was left in place for approximately 5 s before being removed and the mouse was then returned to its home cage. For group-housed mice (females), the time between the Rectal Temperature Measurement of the first and last mouse in the cage was at most 4 min. Each procedure was performed on a different day early in the light phase (09:00-13:00) and no procedures took place on cage changing days.

CBT during the Transport and the Novel Environment interventions was measured once every 5 min (as opposed to the measurement once every 2 min for the Gentle Handling and Rectal Temperature Measurement intervention) in order to prolong the battery life of the AniPill device given the longer duration of these conditions.

Data analysis

For each testing procedure and mouse: Baseline CBT (CBT_{BL}) was defined as the average CBT across the 10 to 15-minute interval immediately prior to the start of the procedure (10 min for Gentle Handling and Rectal Temperature Measurement, 15 min for Transport and Novel Environment). The peak change in CBT ($CBT_{\Delta Peak}$) was defined as the difference between the peak CBT during the measurement period following the handling/laboratory procedure and CBT_{BL}; $CBT_{PeakLatency}$ was defined as the latency of the peak CBT. These definitions were used to allow for more-easily interpretable statistical results.

Statistical analyses

All statistical analyses were performed using SPSS (version 28, IBM, USA). Data (23 mice per handling/laboratory procedure; 12 male, 11 female) were assessed for normality both visually and using the Shapiro-Wilk test; all dependent variables were found to be sufficiently normal to use parametric statistics. Effect sizes are reported as partial eta-squared (η_p^2).

Hypothesis-testing analyses

Does the handling/laboratory procedure affect the subsequent 60 min of CBT? For each of the four handling/laboratory procedures separately, a mixed-design ANOVA with planned contrasts was used to determine whether handling/laboratory procedure affected CBT generally, where Time (32 levels: 0, 2, …, 62 min, for Gentle Handling and Rectal Temperature Measurement procedures; or 14 levels: 0, 5, …, 65 min, for Transport and Novel Environment procedures) was the within-subjects factor, Sex (2 levels: male; female) was the between-subjects factor, and CBT was the dependent variable. From this, a 1-df contrast compared CBT at baseline (0 min) to the average of the remaining interval (2 to 60 min) to determine whether handling/laboratory procedure affected CBT overall, and the interaction of this contrast with Sex was also assessed to determine whether sex moderated any effects of handling/laboratory procedures.

Do the handling/laboratory procedures differentially affect $CBT_{\Delta Peak}$? To determine whether the handling/laboratory procedures differentially affected $CBT_{\Delta Peak}$, a two-way mixed design ANOVA was conducted where the Procedure (Gentle Handling; Transport; Novel Environment; Rectal Temperature Measurement) was the within-subjects factor, Sex (male; female) was the between-subjects factor, and $CBT_{\Delta Peak}$ was the dependent variable. Only the main effect of Procedure and the interaction of this with Sex were assessed; for any significant results with >1 df, a *post hoc* test with Bonferroni correction was applied (adjusted p-values are reported).

Do the handling/laboratory procedures differentially affect peak $CBT_{PeakLatency}$? To determine whether the handling/laboratory procedure differentially affected $CBT_{PeakLatency}$, a two-way mixed design ANOVA was conducted where the Procedure (Gentle Handling; Transport; Novel Environment; Rectal Temperature Measurement) was the within-subjects factor, Sex (male; female) was the between-subjects factor, and

$CBT_{PeakLatency}$ was the dependent variable. Only the main effect of Procedure and the interaction of this with Sex was assessed; for any significant results with >1 df, a *post hoc* test with Bonferroni correction was applied (adjusted p-values are reported).

Exploratory analyses

To provide an indication of which (if any) specific time points were affected by handling/laboratory procedure, for each handling/laboratory procedure separately, each time point (2, 4, …, 62 min for Gentle Handling and Rectal Temperature Measurement procedures; or 5, 10, …, 65 min for Transport and Novel Environment procedures) was compared to baseline (0 min) using mixed model ANOVAs where Time (2 levels; within-subjects) and Sex (2 levels; between-subjects) were the independent variables and CBT was the dependent variable. Only the main effect of Time and the interaction of Time and Sex were considered. Note that as the study was not powered to conduct so many statistical tests, no Type 1 error adjustment was used, and the results must be treated as exploratory.

Results

It is important to note that the factor 'Sex' may be influenced by the different housing conditions (given that female mice were group housed while male mice were singly housed), and so care must be taken when interpreting any interactions with Sex.

Variability of baseline CBT

Both the within-subject standard deviation (s_w) and between-subject standard deviation (s_b) for CBT_{BL} was calculated (for each sex separately), to evaluate the variability of CBT_{BL} of each mouse before the handling/laboratory procedures and the variability of CBT_{BL} between individual mice, respectively. In our sample, the variability of CBT_{BL} was higher within each mouse before the handling/laboratory procedures (i.e. each day before the handling/laboratory procedure) (Male s_w=0.84; Female s_w=0.68) than it was between individual mice (Male s_b=0.53; Female s_b=0.46).

Hypothesis-testing analyses

Does the handling/laboratory procedure affect the subsequent 60 min of CBT? There was a significant increase in CBT from the baseline to the average of the 60-min interval following Gentle Handling ($F_{1,21}$=69.10, $p<0.001$; η_p^2=0.767). This did not interact with Sex ($F_{1,21}$=1.40, p=0.251; η_p^2=0.062). There was a significant increase in CBT from the baseline to the average of the 60-min interval following Transport ($F_{1,21}$=50.25, $p<0.001$; η_p^2=0.705). This interacted with Sex ($F_{1,21}$=12.28, p=0.002; η_p^2=0.369), such that the increase was larger in the males than females. There was a significant increase in CBT from the baseline to the average of the 60-min interval following the Novel Environment intervention ($F_{1,21}$=20.13, $p<0.001$; η_p^2=0.489). This did not interact with Sex ($F_{1,21}$=0.82, p=0.376; η_p^2=0.037). There was a significant increase in CBT from the baseline to the average of the 60-min interval following Rectal Temperature Measurement ($F_{1,21}$=89.10, $p<0.001$; η_p^2=0.809). This interacted with Sex ($F_{1,21}$=5.20, p=0.033; η_p^2=0.199), such that the increase was larger in the males than females.

Does the handling/laboratory procedure differentially affect $CBT_{\Delta Peak}$? There was a significant main effect of Procedure ($F_{3,63}$=3.89, p=0.013; η_p^2=0.156), but this did not interact with Sex ($F_{3,63}$=1.07, p=0.367; η_p^2=0.049). *Post hoc* tests revealed a significant difference between the Transport and Rectal Temperature Measurement procedures (p=0.012; η_p^2=0.383), with Rectal Temperature Measurement causing a higher $CBT_{\Delta Peak}$ than the Transport procedure (Table 1). There were no other significant differences between handling/laboratory procedures ($p>0.114$).

Does the handling/laboratory procedure differentially affect $CBT_{PeakLatency}$? There was a significant main effect of Procedure ($F_{2.17,45.47}$=4.21, p=0.009; η_p^2=0.167), but this did not interact with Sex ($F_{2.17,45.47}$=2.07, p=0.135). *Post hoc* tests found $CBT_{PeakLatency}$ to differ significantly between the Gentle Handling and Transport procedures (p=0.03, η_p^2=0.321) and between the Transport and Novel Environment procedures (p=0.012, η_p^2=0.362), with the Transport procedure having the highest $CBT_{PeakLatency}$ (Fig. 1).

Exploratory analyses

What time points were affected by handling/laboratory procedures? CBT was larger than baseline from the first post-Gentle Handling time-point (+2 min) up until +56 min ($F_{1,21}\geq5.70$, $p\leq0.026$) (Fig. 2A). No differences were found from +58 min, indicating that there was no consistent deviation from baseline after this point ($F_{1,21}\leq3.17$, $p\geq0.09$). These results were affected by Sex between +8 to +18 min ($F_{1,21}\geq6.88$, $p\leq0.016$), such that the effect was larger in males than females, but not at other

times post-Gentle Handling ($F_{1,21}$≤4.19, p≥0.053). Note that, although CBTs were not significantly higher than baseline at the end of the recording period (+62 min), CBT from 8 of the 11 female mice and 5 of the 12 male mice did not return to baseline.

CBT was larger than baseline from the first post-Transport time-point (+5 min) up until +50 min ($F_{1,21}$≥9.33, p≤0.006) (Fig. 2B). No differences were found from +55 min, indicating that there was no consistent deviation from baseline after 55 min ($F_{1,21}$≤3.33, p≥0.082). These results were affected by Sex between +10 to +45 min ($F_{1,21}$≥5.00, p≤0.036), such that the effect was larger in males than females, but not at other times post-Transport ($F_{1,21}$≤3.64, p≥0.070). Although CBTs were not significantly higher than baseline at the end of the recording period (+65 min), CBT from 3 of the 11 female mice and 5 of the 12 male mice did not return to baseline.

CBT was larger than baseline from the first time-point after being placed in a Novel Environment (+5 min) up until +40 min ($F_{1,21}$≥9.8, p≤0.005) (Fig. 2D). No differences were found from +45 min, indicating that there was no consistent deviation from baseline after 45 mins ($F_{1,21}$≤3.81, p≥0.064). These results were affected by Sex between +5 to +10 min ($F_{1,21}$≥6.75, p≤0.017), such that the effect was larger in males than females, but not at other times post-procedure ($F_{1,21}$≤3.09, p≥0.093). Although CBTs were not significantly higher than baseline at the end of the recording period (+65 min), CBT from 2 of the 11 female mice and 6 of the 12 male mice did not return to baseline.

CBT was larger than baseline at all time-points post-Rectal Temperature Measurement ($F_{1,21}$≥6.69, p≤0.017) (Fig. 2C). These results were affected by Sex between +8 to +30 min ($F_{1,21}$≥4.73, p<0.041) and +38 to +40 min ($F_{1,21}$≥4.62, p≤0.043), such that the effect was larger in males than females, but not at other times post-procedure ($F_{1,21}$≤3.64, p≥0.070). Although CBTs remained significantly higher than baseline at the end of the recording period (+62 min), a small number of mice did return to baseline. Overall, 6 of the 11 female mice and 10 of the 12 male mice did not return to baseline.

Table 1. Peak core body temperature (CBT_{Peak}) and difference of the peak CBT from baseline ($CBT_{\Delta Peak}$) for male (n=12) and female (n=11) mice during four common handling/laboratory procedures. Data is presented as Mean ± standard deviation.

	CBT_{Peak} (°C)		$CBT_{\Delta Peak}$ (°C)	
	Male	**Female**	**Male**	**Female**
Gentle Handling	38.50 ± 0.99	37.71 ± 0.27	2.82 ± 0.84	1.76 ± 0.82
Transport	37.95 ± 1.12	37.26 ± 0.50	2.48 ± 0.82	0.99 ± 0.51
Novel Environment	38.46 ± 0.60	38.20 ± 0.39	2.30 ± 1.02	1.49 ± 0.34
Rectal Temperature Measurement	38.66 ± 1.33	37.70 ± 0.73	3.02 ± 1.00	1.66 ± 0.63

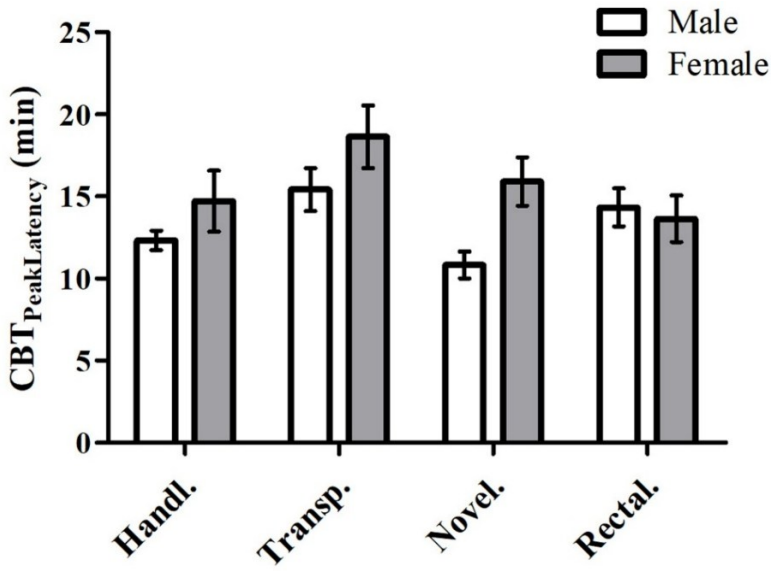


Fig. 1. Latency to reach peak CBT following gentle handling (Handl.), transport (Transp.), novel environment (Novel.) and rectal temperature measurement (Rectal.) in both male (n=12) and female (n=11) mice. Data are presented as mean ± standard error of the mean.

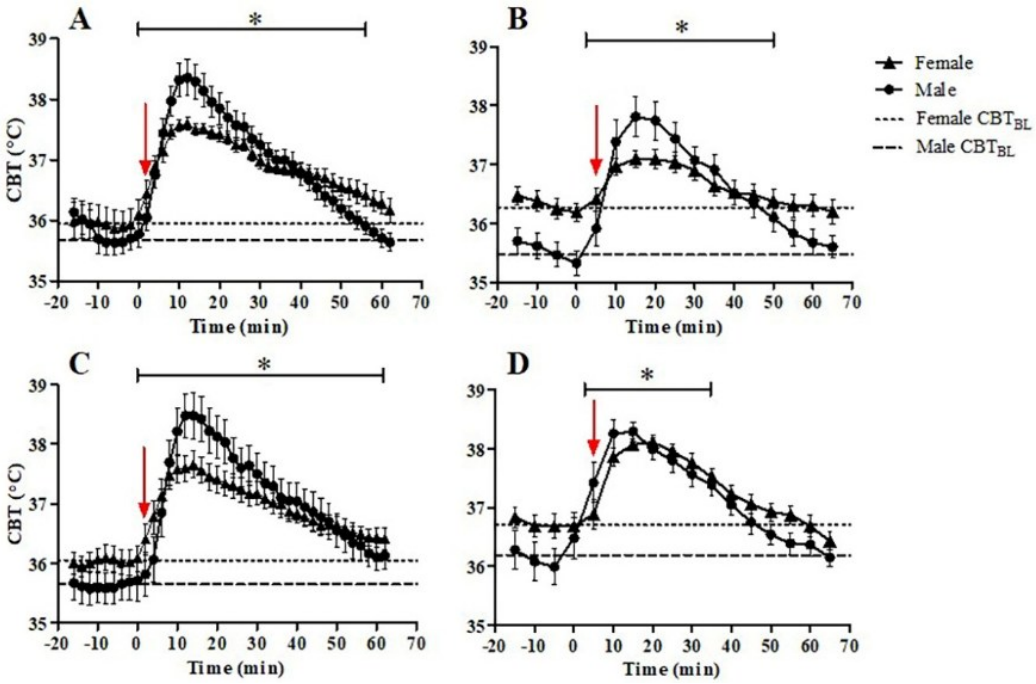


Fig. 2. Male (n=12) and female (n=11) mouse core body temperature (CBT) over a 60-minute period after (**A**) gentle handling, (**B**) home cage transport, (**C**) rectal temperature measurement and (**D**) placement into a novel environment. Time-point 0 represents the last CBT measure prior to each procedure. Data is presented as mean ± standard error of the mean. * $p<0.05$ (male and female data averaged) compared to baseline CBT (CBT_{BL}, the average CBT across the 10 (A,C) or 15-minute (B,D) interval immediately prior to the start of the procedure). Dotted lines indicate average CBT_{BL} for male and female cohorts. Red arrows indicate the first CBT measure after each procedure had occurred.

Discussion

This study investigated the CBT response of male and female mice after common handling/laboratory procedures: gentle handling, short transport within the facility, exposure to a novel environment, and the measurement of rectal temperature. All procedures resulted in a significantly elevated CBT immediately after the procedure in both male and female mice (with a peak change in CBT ranging between +1 and +3 °C), which lasted for at least 35 min. The procedure Rectal Temperature Measurement caused a higher peak CBT rise ($CBT_{\Delta Peak}$) than the Transport procedure and appears to take longer to return to baseline (CBT_{BL}), but there were no significant differences between other handling/laboratory procedures. There was some influence of the factor Sex on CBT following the handling/laboratory procedures, with the male cohort exhibiting a higher CBT at some points throughout the measurement period after all handling/laboratory procedures.

There were some gaps in the literature regarding the CBT response of mice to handling, transport and novel environment procedures that were addressed in the present study. Firstly, it was not clear whether a similar CBT response would be observed in mice that were handled using the tunnel compared to previous reports using tail handling. We found that the Gentle Handling procedure caused a peak CBT increase of 1.8 and 2.8 °C for female and male mice respectively and required an average of 58 min to no longer be statistically different from baseline CBT. In comparison, [14] reported a CBT increase of 1.7-2 °C in singly-housed male mice (of different strains) after being picked up and held by the tail for 5 s. Despite tunnel handling being considered a less aversive method of handling mice, CBT was increased to the same degree or higher in our mice though this is likely due to the length of time mice were handled by the experimenter (2 min on an open hand), rather than as a consequence of the method to retrieve mice from the cage. Secondly, the CBT response of mice to a short in-house transport had not been adequately investigated. We found the Transport procedure to cause an equivalent CBT response to the other procedures investigated (except for Rectal Temperature Measurement, which caused a greater CBT response), causing an average peak CBT increase of 1.0 and 2.5 °C for female and male mice respectively and requiring 50 min on average to no longer be statistically different from baseline CBT. Similarly, the CBT response in mice after placement into a Novel

Environment (in this case being a smaller, otherwise empty cage except for half a tissue that has no access to food or water) was not clear. We found that the Novel Environment procedure also appears to induce an equivalent CBT response similar to the other handling/laboratory procedures, causing a peak CBT increase of 1.5 and 2.3 °C for female and male mice respectively and requiring an average of 40 min to no longer be statistically different from baseline CBT. This response is considerably lower than the CBT response reported for mice after cage change [14], indicating that differences in the conditions (e.g. cage size, presence of bedding and access to food and water) of relatively similar procedures may influence the degree of CBT response. Overall, the pattern of CBT response was similar between all procedures investigated, however the peak change in CBT and the latency to reach this peak change differed between some procedures and between the male and female cohort. These findings provide additional information to better characterise the CBT response of mice to these procedures.

In the case where CBT is a primary outcome or where changes in CBT will influence physiological parameters of interest, it is critical for the validity of a study to address the influence of handling procedures on CBT. However, this confound is often overlooked and not appropriately controlled (or not reported that it was controlled). In the present study, the smallest rise of CBT in response to a handling/laboratory procedure was approximately +1 °C, which, although transient in nature, is still a substantial rise in CBT. Such a rise in CBT is very likely to obscure the effect of an experimental intervention that causes CBT to increase to a lesser extent. The handling/procedure-induced increase to CBT in mice must be allowed to dissipate or return to the pre-handling/procedure 'baseline' for experimentally relevant changes to the CBT of mice to be observed. For example, exposure to RF-EMF has been reported to affect physiological functions [24]. Recently, our laboratory has found that the exposure of mice to a RF-EMF at 5 W/kg (the highest power of RF-EMF exposure studied), caused a CBT increase of +0.4 °C within the first 16 min of exposure [20]. Without controlling for the effect of handling/laboratory procedures, it is unlikely that this RF-EMF induced change to CBT would have been detected, or we may have misattributed changes in CBT that were caused by handling/laboratory procedures as being caused by RF-EMF. In such a case, the inclusion of an undisturbed habituation period prior to any experimental intervention (if mice have recently undergone handling or laboratory procedures) is appropriate and necessary so that any changes to CBT are not confounded by the effect of handling/laboratory procedures and can be attributed, where they occur, to the intervention.

It is important to consider what would be required to produce a sufficient baseline. Our study indicates that the rise in CBT following handling/ laboratory procedures is immediate (within 2-5 min) and can take up to 20 min to reach the peak CBT rise ($CBT_{\Delta Peak}$), with the latency of this peak ($CBT_{PeakLatency}$) dependant on the type of handling/laboratory procedure. Therefore, a habituation period of ≤30 min would not be sufficient to allow CBT to return to baseline. In fact, given the rapid rise in CBT, a habituation period of ≤30 min would mean that CBT measurements taken during this period would likely reflect the peak CBT rise caused by the handling/laboratory procedure. Most handling/laboratory procedures in this study required approximately one hour on average to return to baseline, therefore an appropriate habituation period in this cohort of mice would be ≥60 min, depending on the handling/laboratory procedures used. Further, some mice from each handling/laboratory procedure did not return to baseline after 60 min, which would suggest that 60 min is the minimum habituation length, but that this may not be sufficient for all mice. As different strains of mice may exhibit different degrees of response to stressors [14], it is important to test the effect of the handling/laboratory procedures and protocols that will be implemented in a study to determine whether the magnitude of this effect will be an important confound and, if so, the appropriate habituation period required to address this confound. As temperature and thermoregulation are influential in many biological processes [11], failure to account for the effects of general laboratory procedures and handling on mouse CBT may prevent valid interpretations of the results.

Our results support the need for careful consideration before rectal thermometry is used to obtain CBT measurements in mice. In this study, the Rectal Temperature Measurement procedure resulted in the lowest number of mice returning to their baseline CBT after the one-hour measurement period, with our exploratory analyses indicating that CBT may not return to baseline after one hour in this condition in male and female mice. This is longer than what has been reported by some other researchers [7,10,25]. As we concluded CBT measurements one hour after the Rectal

Temperature Measurement procedure, it is not clear how much longer would have been required for CBT to return to baseline in our cohort of mice. Since different strains of mice exhibit different degrees of response to stressors [14] and habituation to procedures may lessen the stress response [9, 26, 27], the strain of mice used in this study, combined with the absence of habituation to the procedure, may have extended the time needed post-procedure to reach baseline. Given that rectal temperature measurement is one of the most popular methods of measuring CBT in laboratory mice, the considerable time taken to return to baseline after a measurement is a notable disadvantage. Prior to an experimental intervention, a habituation period longer than one hour may be required for CBT to return to baseline after a rectal measurement. Another consideration for the use of rectal thermometry is that the anticipation of the procedure or even the act of disturbing the mice (e.g. entering a holding room and opening a mouse cage to begin rectal measurements) can increase CBT, such that the last mouse measured in a group-housed (or group-exposed) cage exhibits significantly higher CBT than the first mouse measured [28,29]. It would therefore be difficult to get accurate CBT measures when measuring a large cohort of mice (or a single mouse that has recently been disturbed) using rectal thermometry. A method of CBT measurement that does not involve the handling of mice or disturbances to their environment is preferable to ensure CBT is not confounded.

This study is not without its limitations, which should be addressed in future research. Since CBT did not return to baseline within the 60-minute measurement period for approximately 50 % of the mice, it is unclear how long post-intervention would be required. Extending the post-procedure measurement period past 60 min would have provided clarification. Relating to this point, the Novel Environment intervention was performed approximately one hour after a transport to a procedure room. Our data indicated that 60 min after the Transport intervention, 8 mice (5 male; 3 female) did not return to baseline – therefore the CBT of some mice may have still been elevated when calculating the Novel Environment intervention baseline CBT. That is, given that $CBT_{\Delta Peak}$ was calculated using this (potentially elevated) baseline CBT, the actual $CBT_{\Delta Peak}$ in these mice may be higher than what was reported. The difference in housing conditions between the female and male mice may also represent a limitation in that socializing was not possible for the males, including huddling behaviour. This may have made it easier for males to thermoregulate than females (assuming the latter did engage in huddling behaviour), provided a more naturalistic (and potentially less stressful) environment for the females, but also may have increased stress in females given that auditory and olfactory stress communication was possible between female cage mates [30]. These possibilities were not assessed in the present study, and unfortunately it was not possible to group-house male mice in this study due to the risk of aggression and fighting after the surgical procedure. Accordingly, the above-described differences between the male and female groups may have been confounded by factors other than sex. Another possible limitation is that we did not examine the effects of habituation, i.e. the familiarisation of mice to a procedure, to the handling/laboratory procedures on the magnitude or duration of the CBT response. In this study, the mice were regularly handled and had experienced transport to another room on two occasions prior to the experiment. Conflicting evidence has shown there to be little to no effect [6,10,25] or some effect [26,27] of habituation to a procedure in lessening this rise in CBT, so this effect is not a large concern in the present study. Finally, it should also be noted that, although it would be ideal, it is not possible to obtain a true ‘stable’ baseline CBT in mice. The CBT in mice is capable of large fluctuations in short periods of time [31] and is closely associated with activity levels [15]. There was some variability in baseline CBT, both between individual mice and within each mouse across the different days of testing, indicating that mice may have exhibited different activity levels (some may have been asleep, resting or awake and active) during the 10 min prior to each intervention used to calculate the baseline CBT, therefore increasing the variability of $CBT_{\Delta Peak}$. Other factors outside our control also contributed to the variability of baseline CBT. Each procedure was performed on a different day (but within the same time period, 09:00-13:00), and we could not completely control the movement or noise within the facility by other researchers or facility staff. Efforts were taken to minimize the effects of any disturbances, such as only performing a procedure at least an hour after morning welfare checks by facility staff had occurred. There were no disturbances within the holding/experimental room in the one-hour measurement period after the handling/laboratory procedure, so it is assumed the main source of CBT variability during this period is from the activity levels of the mice. Although this difference in

activity level cannot be avoided, it remains critical to control for the effect of handling/laboratory procedures as much as is feasible so that experimental effects on CBT are minimally confounded. Rather than a 'stable' baseline, perhaps it is more realistic for studies to aim for an 'appropriate' baseline, wherein baseline CBT is calculated in mice that have not recently been disturbed or undergone handling/laboratory procedures (e.g. ≥1 h since disturbance).

Conclusions

Common laboratory procedures including gentle handling, transport, placement into a novel environment and performing rectal temperature measurements induced large increases in the CBT of male and female mice. The rise in CBT was transient, reaching a peak after 5-25 min before gradually reducing towards the pre-procedure baseline over time. However, at one-hour post-procedure not all mice had returned to baseline, with this lack of return most common after rectal temperature measurement. Accordingly, whether a one-hour habituation period prior to handling/procedures in mice is appropriate may be dependent on the particular procedures performed and so would usefully be optimised for each study. In addition, studies examining CBT in mice may need to reconsider the use of CBT measurement practices that involve direct interactions with the mice, like rectal thermometry. It is important for any study investigating CBT change induced by an experimental intervention to determine the effect of the handling procedures and protocols used in their experiments so that an appropriate habituation period prior to CBT measurements can be implemented.

Conflict of Interest

There is no conflict of interest.

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Abbreviations

CBT, core body temperature; CBT_{BL}, baseline CBT; $CBT_{\Delta Peak}$, peak change in CBT; $CBT_{PeakLatency,}$ latency of the peak CBT; RF-EMF, radiofrequency electromagnetic field.

References

1 Gordon CJ. Thermal physiology of laboratory mice: Defining thermoneutrality. J Therm Biol 2012;37:654-685. https://doi.org/10.1016/j.jtherbio.2012.08.004

2. Gozalo AS, Elkins WR. A Review of the Effects of Some Extrinsic Factors on Mice Used in Research. Comp Med 2023;73:413-431. https://doi.org/10.30802/AALAS-CM-23-000028

3. Saré RM, Lemons A, Smith CB. Behavior Testing in Rodents: Highlighting Potential Confounds Affecting Variability and Reproducibility. Brain Sci 2021;11:522. https://doi.org/10.3390/brainsci11040522

4. Škop V, Xiao C, Liu N, Gavrilova O, Reitman ML. The effects of housing density on mouse thermal physiology depend on sex and ambient temperature. Mol Metab 2021;53:101332. https://doi.org/10.1016/j.molmet.2021.101332

5. Toth LA, Trammell RA, Ilsley-Woods M. Interactions Between Housing Density and Ambient Temperature in the Cage Environment: Effects on Mouse Physiology and Behavior. J Am Assoc Lab Anim Sci 2015;54:708-717.

6. Balcombe JP, Barnard ND, Sandusky C. Laboratory routines cause animal stress. Contemp Top Lab Anim Sci 2004;43:42-51.

7. Groenink L, van der Gugten J, Zethof T, van der Heyden J, Olivier B. Stress-induced hyperthermia in mice: hormonal correlates. Physiol Behav 1994;56:747-749. https://doi.org/10.1016/0031-9384(94)90237-2

8. Poole S, Stephenson JD. Core temperature: Some shortcomings of rectal temperature measurements. Physiol Behav 1977;18:203-205. https://doi.org/10.1016/0031-9384(77)90122-6

9. Van Der Heyden JA, Zethof TJ, Olivier B. Stress-induced hyperthermia in singly housed mice. Physiol Behav 1997;62:463-470. https://doi.org/10.1016/S0031-9384(97)00157-1

10. Zethof TJ, Van der Heyden JA, Tolboom JT, Olivier B. Stress-induced hyperthermia in mice: A methodological study. Physiol Behav 1994;55:109-115. https://doi.org/10.1016/0031-9384(94)90017-5
11. Gordon CJ, Spencer PJ, Hotchkiss J, Miller DB, Hinderliter PM, Pauluhn J. Thermoregulation and its influence on toxicity assessment. Toxicology 2008;244:87-97. https://doi.org/10.1016/j.tox.2007.10.030
12. Meyer CW, Ootsuka Z, Romanovsky AA. Body Temperature Measurements for Metabolic Phenotyping in Mice. Front Physiol 2017;8:520. https://doi.org/10.3389/fphys.2017.00520
13. Meijer MK, Spruijt BM, van Zutphen LFM, Baumans V. Effect of restraint and injection methods on heart rate and body temperature in mice. Lab Anim 2006;40:382-391. https://doi.org/10.1258/002367706778476370
14. van Bogaert MJ, Groenink L, Oosting RS, Westphal KGC, van der Gugten J, Olivier B. Mouse strain differences in autonomic responses to stress. Genes Brain Behav 2006;5:139-149. https://doi.org/10.1111/j.1601-183X.2005.00143.x
15. Clement JG, Mills P, Brockway B. Use of telemetry to record body temperature and activity in mice. J Pharmacol Methods 1989;21:129-140. https://doi.org/10.1016/0160-5402(89)90031-4
16. Hurst JL, West RS. Taming anxiety in laboratory mice. Nat Methods 2010;7:825-826. https://doi.org/10.1038/nmeth.1500
17. Blenkuš U, Gerós AF, Carpinteiro C, de Castro Aguiar P, Olsson IAS, Franco NH. Non-Invasive Assessment of Mild Stress-Induced Hyperthermia by Infrared Thermography in Laboratory Mice. Animals 2022;12:177. https://doi.org/10.3390/ani12020177
18. Gerdin A-K, Igosheva N, Roberson L-A, Ismail O, Karp N, Sanderson M, et al. Experimental and husbandry procedures as potential modifiers of the results of phenotyping tests. Physiol Behav 2012;106:602-611. https://doi.org/10.1016/j.physbeh.2012.03.026
19. Rasmussen S, Miller MM, Filiupski SB, Tolwani RJ. Cage change influences serum corticosterone and anxiety-like behaviors in the mouse. J Am Assoc Lab Anim Sci 2011;50:479-483.
20. Sylvester E, Deng C, McIntosh R, Iskra S, Frankland J, McKenzie R, Croft RJ. Characterising core body temperature response of free-moving C57BL/6 mice to 1.95 GHz whole-body radiofrequency-electromagnetic fields. Bioelectromagnetics 2024;45:387-398. https://doi.org/10.1002/bem.22527
21. Chappell MG, Koeller CA, Hall SI. Differences in postsurgical recovery of CF1 mice after intraperitoneal implantation of radiotelemetry devices through a midline or flank surgical approach. J Am Assoc Lab Anim Sci 2011;50:227-237.
22. Helwig BG, Ward JA, Blaha Md, Leon LR. Effect of intraperitoneal radiotelemetry instrumentation on voluntary wheel running and surgical recovery in mice. J Am Assoc Lab Anim Sci 2012;51:600-608.
23. Leon LR, Walker LD, DuBose DA, Stephenson LA. Biotelemetry transmitter implantation in rodents: Impact on growth and circadian rhythms. Am J Physiol Regul Integr Comp Physiol 2004;286:R967-R974. https://doi.org/10.1152/ajpregu.00380.2003
24. Parizek D, Visnovcova N, Hamza Sladicekova K, Misek J, Jakus J, Jakusova J, et al. Electromagnetic fields - do they pose a cardiovascular risk? Physiol Res 2023;72:199-208. https://doi.org/10.33549/physiolres.934938
25. Bae DD, Brown PL, Kiyatkin EA. Procedure of rectal temperature measurement affects brain, muscle, skin, and body temperatures and modulates the effects of intravenous cocaine. Brain Res 2007;1154:61-70. https://doi.org/10.1016/j.brainres.2007.03.078
26. Cabanac A, Briese E. Handling elevates the colonic temperature of mice. Physiol Behav 1992;51:95-98. https://doi.org/10.1016/0031-9384(92)90208-J
27. Thompson CI, Brannon AJ, Heck AL. Emotional fever after habituation to the temperature-recording procedure. Physiol Behav 2003;80:103-108. https://doi.org/10.1016/S0031-9384(03)00224-5
28. Borsini F, Leci A, Volterra G, Meli A. A model to measure anticipatory anxiety in mice? Psychopharmacology (Berl) 1989;98:207-211. https://doi.org/10.1007/BF00444693
29. Vinkers CH, Groenink L, van Bogaert MJV, Westphal KGC, Kalkman CJ, van Oorschot R, et al. Stress-induced hyperthermia and infection-induced fever: Two of a kind? Physiol Behav 2009;98:37-43. https://doi.org/10.1016/j.physbeh.2009.04.004
30. Takahashi A. Toward Understanding the Sex Differences in the Biological Mechanism of Social Stress in Mouse Models. Front Psychiatry 2021;12:644161. https://doi.org/10.3389/fpsyt.2021.644161

31. Gordon CJ. The mouse thermoregulatory system: Its impact on translating biomedical data to humans. Physiol Behav 2017;179:55-66. https://doi.org/10.1016/j.physbeh.2017.05.026