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Role of thermosensitive transient receptor potential (TRP) channels in thermal preference of male and female mice

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Abstract

Transient Receptor Potential (TRP) ion channels are important for sensing environmental temperature. In rodents, TRPV4 senses warmth (25–34 °C), TRPV1 senses heat (>42 °C), TRPA1 putatively senses cold (<17 °C), and TRPM8 senses cool-cold (18–26 °C). We investigated if knockout (KO) mice lacking these TRP channels exhibited changes in thermal preference. Thermal preference was tested using a dual hot-cold plate with one thermoelectric surface set at 30 °C and the adjacent surface at a temperature of 15–45 °C in 5 °C increments. Blinded observers counted the number of times mice crossed through an opening between plates and the percentage of time spent on the 30 °C plate. In a separate experiment, observers blinded as to genotype also assessed the temperature at the location on a thermal gradient (1.83 m, 4–50 °C) occupied by the mouse at 5- or 10-min intervals over 2 h. Male and female wildtype mice preferred 30 °C and significantly avoided colder (15–20 °C) and hotter (40–45 °C) temperatures. Male TRPV1KOs and TRPA1KOs, and TRPV4KOs of both sexes, were similar, while female WTs, TRPV1KOs, TRPA1KOs and TRPM8KOs did not show significant thermal preferences across the temperature range. Male and female TRPM8KOs did not significantly avoid the coldest temperatures. Male mice (except for TRPM8KOs) exhibited significantly fewer plate crossings at hot and cold temperatures and more crossings at thermoneutral temperatures, while females exhibited a similar but non-significant trend. Occupancy temperatures along the thermal gradient exhibited a broad distribution that shrank somewhat over time. Mean occupancy temperatures (recorded at 90–120 min) were significantly higher for females (30–34 °C) compared to males (26–27 °C) of all genotypes, except for TRPA1KOs which exhibited no sex difference. The results indicate (1) sex differences with females (except TRPA1KOs) preferring warmer temperatures, (2) reduced thermosensitivity in female TRPV1KOs, and (3) reduced sensitivity to cold and innocuous warmth in male and female TRPM8KOs consistent with previous studies.

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CRediT authorship contribution statement

Mirela Iodi Carstens: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Methodology, Investigation, Formal analysis, Data curation. **Avina Mahroke:** Investigation. **Tudor Selescu:** Writing – review & editing, Validation, Methodology, Investigation. **E. Carstens:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors have none to declare.

Keywords

Thermal preference; Thermal gradient; Cold avoidance; Warm avoidance; TRP channel knockout1

1. Introduction

Homeothermic mammals maintain a constant internal body temperature by several mechanisms including behavior, seeking an environment at a preferred temperature. The temperature of the animal's environment is signaled by thermoreceptors sensitive to temperatures above and below thermoneutral, i.e. cold and warm receptors (Schepers and Ringkamp, 2010). Thermoreceptors express various thermosensitive transient receptor potential (TRP) ion channels that open within specific temperature ranges to activate their afferent axon. Transient receptor potential cation channel subfamily M (melastatin) member 8 (TRPM8) has been strongly implicated in signaling temperatures in the cool to cold range (Bautista et al., 2007; Colburn et al., 2007; Dhaka et al., 2007; Knowlton et al., 2013; Lewis and Griffith, 2022). Transient receptor potential cation channel subfamily A (ankyrin) member 1 (TRPA1) was originally reported to respond to intense cooling (Story et al., 2003) and more recently has been implicated in heat sensing as well (Hoffmann et al., 2013; Vandewauw et al., 2018), although roles for TRPA1 in both cold and heat sensing have not been replicated by others and thus remain controversial (for reviews, see Talavera et al., 2020; Zhang et al., 2022). Warmth sensing appears to involve multiple TRP channels including Transient receptor potential cation channel subfamily V (vanilloid) members 3, 4 and 1 (TRPV3, TRPV4, TRPV1) as well as TRPA1, among others (Pogorzala et al., 2013; Jeon and Caterina, 2018). It was recently reported that TRPV3 is negatively regulated by the transmembrane protein TMEM79 to influence warmth detection (Lei et al., 2023). TRPV1, TRPV2 and TRPM3 have also been implicated in detecting noxious and potentially damaging heat (Caterina et al., 1997, 1999, 2000; Vriens et al., 2011). The investigations of TRP channel roles in warm and cold sensing have frequently taken advantage of knockout (KO) mice in which one or more TRP channels have been genetically deleted. In the present study we wished to investigate the roles of TRPV1, TRPA1, TRPV4 and TRPM8 in temperature sensing using transgenic TRP channel KO mice in a two temperature preference test. We tested both male and female mice since most previous studies employing thermal preference tests of TRP channel KO mice did not test for potential sex differences, even though sex differences have been noted for thermoregulation and thermal preference and tolerance (Fernández-Peña et al., 2023). Moreover, mechanisms of regulating thermo-TRP channel function and expression by sex steroid hormones have been proposed (Asuthkar et al., 2015a, b; Pohóczky et al., 2016; Payrits et al., 2017; Ramírez-Barrantes et al., 2020; Gkika et al., 2020). Based on previous reports we hypothesized the following: (1) TRPM8KO mice of both sexes would exhibit reduced sensitivity to cold temperatures. (2) TRPV4KO mice of both sexes are likely to exhibit normal thermal preferences similar to wildtype (WT) mice, since TRPV3/V4 double KO mice did not exhibit any deficit in thermosensitivity (Huang et al., 2011). (3) TRPV1 and TRPA1 KO mice of both sexes are likely to exhibit subtle thermal preference differences compared to WTs.

We also tested mice on a continuous linear thermal gradient (4–50 °C) to more finely assess their thermal preference. It was previously reported that female C57Bl/6 mice preferred a warmer temperature compared to males in a thermal preference test of cages at different ambient temperatures (Kaikaew et al., 2017), and that female humans and rodents generally show a preference for warmer ambient temperatures (Fernandez-Peñna et al., 2023). We therefore tested the hypothesis that there is sexual dimorphism in thermal preference, with wildtype (WT) females exhibiting a preference for warmer temperatures than WT males on the thermal gradient. It is not known if there are sex differences in thermal preference among the TRP channel KO mice so this was additionally tested using the thermal gradient.

2. Material and methods

The study was approved by the UC Davis Institutional Animal Care and Use Committee. Wildtype (WT) C57Bl/6 J, TRPV1KO (B6.129X1-Trpv1tm1Jul/J; strain #003770), and TRPM8KO (B6.129P2-TRPM8tm1Jul/J; strain #008198) mice of both sexes were obtained from Jackson Laboratories. TRPA1KO mice (Bautista et al., 2006) were a kind gift from Dr. David Julius, University of California, San Francisco. TRPV4KO mice (Suzuki et al., 2003) were originally obtained from Riken (B6.129X1-Trpv4tm1Msz) and kindly provided to us by Dr. Hongzhen Hu, Washington University, St. Louis. The TRPA1KO and TRPV4KO mice, as well as the other KO mice, were generated on a C57Bl6 background. All groups were age-matched and were tested over an age range of 8–20 weeks, i.e. young adults. The total numbers of animals were as follows: WT males 10, females 6; TRPV1KO males: 6, females 5; TRPA1KO males 6, females 8; TRPV4KO males 7, females 5; TRPM8KO males 7, females 7.

2.1. Thermal preference assay

This was determined using a two-temperature preference test. All tests were performed at room temperature (21.8 °C ± 0.98 SD). The apparatus consisted of two adjacent thermoelectric surfaces (each 13.3 in. × 6.37 in.; AHP-1200DCP, Teca Thermoelectric, Chicago, IL) that could be independently heated or cooled to a pre-set temperature (15 °C–45 °C) that was maintained within ±1.0 °C. A Plexiglas box enclosed both plates, separated by a center partition with a middle opening allowing the mouse to move freely between the two surfaces. Mice were habituated over 3 successive days (1 h/day) to the apparatus with both plates set at 30 °C. For preference tests, one plate was set at 30 °C and the other plate at the same, or a higher or lower temperature in 5 °C increments (i.e., 15, 20, 25, 30, 35, 40, or 45 °C), using a counterbalanced design. The mouse was randomly placed onto one of the plates and videotaped from above for 120 min, with all personnel leaving the room during videotaping. Videotapes were subsequently reviewed offline and the time the animal spent on each plate, as well as the number of times the mouse crossed between plates, was recorded by at least two observers blinded as to the temperature difference and the mouse's sex and genotype. Each mouse was only used in one preference test per day, with at least one day in between successive tests.

2.2. Thermal gradient assay

The gradient consisted of a metal bar 1.83 m (6 ft) in length, with Teca heating-cooling devices (AHP-11200DCP) at either end. This produced a near-linear temperature gradient from 4 to 50 °C. A Plexiglas enclosure was constructed around the gradient plate with a middle divider, forming two tracks along which mice could move freely. Mice were habituated to the gradient for 3 successive days (1 h per day) before testing. For testing, one mouse per track was placed in the middle of the gradient after which personnel left the room. At 5-min intervals (10-min intervals for TRPM8KOs) one investigator entered the room and measured the temperature at the rostral-most location where each mouse was positioned using an infrared laser thermometer (Cen-Tech), yielding a temperature for that location referred to as “occupancy temperature”. Data were separated into 4 time periods 0–30, 30–60, 60–90 and 90–120 min after the mouse was placed onto the gradient.

2.2.1. Statistical analysis—We conducted a post-hoc power analysis and determined that a n = 7 per group would have 80% power in showing a significant difference of 30% with a standard deviation of 20%. Thus our study is somewhat underpowered for many of the temperature differentials. This is because while we started with at least 5–6 or more animals per genotype/sex the number of animals decreased over time due to attrition.

To check for differences in the percent time spent on the 30 °C plate among the various temperature differentials for each sex and genotype, data were subjected to a nonparametric Kruskal-Wallis analysis of variance (ANOVA; Graphpad Prism, Boston MA). The numbers of plate crossings were analyzed in the same manner. Differences in percent time on 30 °C, or number of plate crossings, among the various temperature differentials were tested post-hoc using the Dunn’s test. To test for differences between WTs and each genotype across all temperature differentials, we performed Kruskal- Wallis ANOVA on the data percent time spent on the 30 °C plate, as well as number of plate crossings, with Dunn’s post-hoc test for WT-TRP KO differences. A $p < 0.05$ value was considered statistically significant. Data are represented as mean ± standard error of the mean (SEM).

Mean occupancy temperatures were calculated for each time period. For each genotype, occupancy temperatures for males and females were compared using an unpaired *t*-test. We also compared occupancy temperatures for males and females separately between each genotype and WT mice using an unpaired *t*-test. A value of $p < 0.05$ was considered to be significant.

3. Results

3.1 Thermal preference.

Fig. 1 plots the percent time animals spent on the 30 °C plate when the adjacent plate was set at the same or different temperatures. Note the degree of variance in individual animals (dots) as is typical for this type of preference test. WT mice of both sexes spent significantly less time on the coldest (15 °C) and hottest (45 °C) plates (Fig. 1A and B) and always showed the greatest preference for the 30 °C plate. This is manifested as a U-shaped curve for occupancy on the 30 °C plate as a function of temperature differential, with

significant differences between the coldest and hottest temperature differences compared to when both plates were set at 30 °C (Fig. 1A and B). This was also generally true for the TRPV1KO, TRPA1KO and TRPV4KO mice (Fig. 1C–H), with the exception that female TRPV1KOs and TRPM8KOs did not spend significantly less time on the hottest (40 and 45 °C) plates (Fig. 1D), suggesting that these genotypes are less sensitive to these temperatures. There were otherwise subtle but no striking differences in thermal preference comparing TRPV1KO males and TRPA1KOs and TRAPV4KOs of both sexes with WTs of both sexes. The TRPM8KOs showed the greatest deficits in thermal preference (Fig. 1I and J). Neither male nor female TRPM8KOs spent significantly less time on the coldest plates compared to WTs (Fig. 1D). Indeed, the TRPM8KOs did not spend significantly less time on plates below 35 °C (and TRPM8KO males exhibited a preference for 20 °C)(Fig. 1I and J). There was a significant sex difference for the percent time spent on the 30 °C plate for TRPV1KOs ($F = 14.2$, $p < 0.001$, multivariate ANOVA with post hoc Bonferroni test) and TRPM8KOs ($F = 6.4$, $p < 0.05$), but not for WTs, TRPA1KOs or TRPV4KOs.

Male WT mice exhibited more plate crossings at small (0–5 °C) temperature differentials (i.e., 25/30 °C, 30/30 °C and 35/30 °C), with significantly fewer crossings at larger temperature differentials in the hot and cold ranges (Fig. 2A and B). Female WTs exhibited a similar but non-significant trend (Fig. 2B). Mice would typically exhibit more crossings during the initial few minutes, followed by a gradual decline in plate crossings over the ensuing 60 min. This is best exemplified by WT mice at a 30/30 °C temperature differential as shown in Fig. 3. Initially there was a higher number of plate crossings/min followed by a gradual decline that was slightly more pronounced for males (Fig. 3A) than females (Fig. 3B) although there was no significant sex difference in regression lines. The pattern of more plate crossings at smaller vs. larger temperature differentials also generally applied to the male TRPV1KOs and TRPA1KOs, and to male and female TRPV4KOs (Fig. 2C, E, 2G, 2-H). Neither TRPV1KOs nor TRPA1KOs exhibited any significant difference in plate crossings across temperature differentials (Fig. 2D and F). The TRPM8KOs also deviated from the WT pattern, with more plate crossings at the largest temperature differentials in the cold range (significantly so for male TRPM8KOs at 15/30 °C). Both male and female TRPM8KO mice exhibited more plate crossings at the largest temperature differentials in the cold temperature range (15–25 °C) compared to WTs, and did not generally exhibit the trend to cross more frequently at small temperature differentials compared to WTs (Fig. 2I and J). There were significant sex differences for the number of plate crossings in all genotypes: WTs ($F = 7.8$, $p < 0.01$), TRPV1KOs ($F = 25.7$, $p < 0.001$), TRPA1KOs ($F = 22.9$, $p < 0.001$), TRPV4KOs ($F = 5.5$, $p < 0.05$) and TRPM8KOs ($F = 9.26$, $p < 0.01$), with females crossing more frequently compared to males (Fig. 2)

3.2 Thermal gradient.

We measured the temperature at the location of occupancy by the mouse on the thermal gradient (“occupancy temperature”). We reasoned that mice would eventually move to a location on the thermal gradient at which the surface temperature was acceptable. In general, all genotypes showed a broad distribution of occupancy temperatures which tended to shrink over time. The distribution of occupancy temperatures over the final 90–120 min period is shown in Fig. 4 for each sex and genotype. The mean occupancy temperatures

for each sex and genotype are shown in Fig. 5. There were significant sex differences, with the mean occupancy temperatures for females being significantly higher (30–34 °C) compared to males (26–27 °C) for all genotypes except TRPA1KOs for which no significant sex difference was observed. The mean occupancy temperature for TRPM8KO males was significantly lower compared to WT males ($p < 0.05$, unpaired *t*-test), consistent with the possibility that the TRPM8KO males were less sensitive to cooler temperatures and avoided them less.

4. Discussion

The main findings of our study are as follows. 1) TRPM8KO mice exhibited a reduced senstivity to cold as well as warm (35 °C) temperatures, consistent with previous findings and supporting our initial hypothesis. 2) TRPV1KO females exhibited no significant preferences across the temperature range, a novel finding that was not predicted. 3) TRPV1KO, TRPA1KO and TRPV4KO mice did not exhibit any major deficit in warmth sensing, supporting our initial hypothesis and partially consistent with prior studies. 4) In the thermal gradient assay there was a sex difference for all other genotypes except TRPA1KOs, with females preferring warmer temperatures. The sexual dimorphism for WTs is consistent with prior findings and is novel for TRP KO mice.

The thermal preference task is a valuable tool for testing the role of various thermoreceptors in establishing the animal's preferred temperature ranges with significance for behavioral thermoregulation. Thus, assaying temperature preference does not necessarily equate to temperature detection ability. The minimal temperature difference that an animal can detect (but not necessarily conduce to a preference) can be investigated using operant conditioning (Milenkovic et al., 2014; Yarmolinsky et al., 2016; Paricio-Montesinos et al., 2020) and was reported to be as little as 2.5 °C in trained mice (Isaacson and Hoon, 2021).

In the thermal preference task mice tended to prefer thermoneutral (25–35 °C) temperatures and avoided hotter and colder temperatures, with the exception of male and female TRPM8KOs that did not significantly avoid colder temperatures, as expected, and female TRPV1KOs that inexplicably did not show significant avoidance of any temperature (although showing the same general tendency as WT). We hypothesize that TRPV1-expressing afferents are more likely to be recruited for fine temperature discrimination in WT female mice. It was shown that in mouse DRG neurons, estradiol (E2) sensitizes TRPV1, decreases the neuronal thermal activation threshold in WT but not TRPV1KO mice, and enhances the channel's expression (Payrits et al., 2017). Therefore, we suggest an important role of TRPV1 in temperature transduction in wild type female mice and we infer that genetic ablation of TRPV1 induces a more pronounced deficit in female than in male mice.

There was generally an inverse relationship between the size of the thermal difference and number of plate crossings, with the highest number of plate crossings occurring when there was no temperature difference between plates (30/30 °C). The number of crossings decreased as the temperature difference increased in both hot and cold directions. This suggests that mice sampled both plate temperatures to determine which was more

comfortable, and then spent more time on the latter plate. In general, the number of plate crossings also decreased over time for each given temperature difference and genotype, implying reduced movement over time. Interestingly, while the temperature preference of TRPA1KO mice was almost the same as that of WT, both male and female TRPA1KO mice showed a significantly lower number of crossings between plates than all other genotypes. This suggests that TRPA1 modulates the level of locomotor activity through an as yet unknown mechanism. Interestingly, another TRPA1 mouse line (C57BL/6B6129P1/F2J) was reported to display an increased basal spontaneous activity (Bodkin et al., 2014).

4.1 Warmth.

TRPV3 and TRPV4 have been implicated in warmth sensing (Tominaga, 2007; Jeon and Caterina, 2018). TRPV4 expressed in keratinocytes was originally reported to respond to innocuous warming (Güler et al., 2002; Chung et al., 2003, 2004) and knockout mice lacking TRPV4 exhibited a shift toward preference of warmer temperatures compared to WTs (Lee et al., 2005). TRPV3 is a warmth-sensitive ion channel expressed in keratinocytes (Peier et al., 2002) and DRG cells (Xu et al., 2002). Knockout mice lacking TRPV3 were initially reported to exhibit a deficit in the detection of innocuous and noxious warming (Moqrich et al., 2005). Futhermore, warming of TRPV3 in keratinocytes was reported to activate sensory neurons via release of ATP (Mandadi et al., 2009). However, a more recent study reported that double-knockout mice lacking TRPV3 and TRPV4 did not exhibit any deficit in warmth detection (Huang et al., 2011), suggesting that the roles of TRPV3 and TRPV4 in warmth detection depend on the background strain. In the present study we did not investigate TRPV3KO mice, but we did not observe any deficits in thermal preference in the innocuous warm range for TRPV4 KOs, in support of the latter finding. A very recent study reported that mice lacking TMEM79, a negative regulator of TRPV3, exhibited a preference for warmer temperatures in a circular thermal gradient assay (Lei et al., 2023) in support of a role for TRPV3 and TMEM79 in warmth sensing. Synergism between TRPV3 and TRPV1 was suggested by the observation that TRPV3/TRPV1 double-knockout mice exhibited a greater deficit in the detection of noxious temperatures compared to single TRPV3 or TRPV1 knockout mice (Marics et al., 2014). This latter study only used male mice. Using a linear thermal gradient, TRPV1KO mice showed a tendency to spend more time on hotter areas of the gradient whereas TRPV3KO mice gravitated towards cooler temperatures. Similar results were obtained with TRPV1KO and TRPV3KO mice on a circular thermal gradient (Ujisawa et al., 2022; see below). Interestingly, however, double-knockout mice lacking both TRPV1 and TRPV3 were not different from WTs when tested on a linear temperature gradient for 30 min (Marics et al., 2014). In our study male TRPV1KOs did not show any deficit in thermal preferences compared to WTs, while female TRPV1KOs showed no significant differences in thermal preference for any temperature differential. A very recent study used an operant method to detect rapid changes in temperature (Isaacson and Hoon, 2021). Mice were trained to nose-poke one of two ports if they detected a change in floor temperature, and correct pokes were rewarded. Using this method mice were able to significantly detect temperature changes of as little as 2.5 °C. Following ablation of TRPV1-expressing neurons mice were unable to discriminate temperature differences above 35 °C (Isaacson and Hoon, 2021), suggesting a role for TRPV1-expressing neurons in warmth detection contrary to the present findings using the

two-plate thermal preference method. Finally, another recent study reported that KO mice lacking TRPV1 or TRPM2, and triple KO mice lacking TRPV1, TRPA1 and TRPM3, showed small reductions in sensitivity to warmth indicating that these ion channels are not absolutely required for the detection of innocuous temperatures (Paricio- Montesinos et al., 2020). Interestingly, in the same study loss or silencing of TRPM8 abolished warmth detection, suggesting that both warm- and cold-sensitive afferent input is necessary for warmth detection (Paricio-Montesinos et al., 2020). Our finding that neither male nor female TRPM8KOs avoided 35 °C (Fig. 2I and J) is consistent with a role for TRPM8 in warmth sensing. Overall, our present results with KO mice lacking individual TRPV1, TRPA1 and TRPV4 ion channels suggest that none of them plays an essential role in warmth detection. Since we presently used general KO mice, a caveat is that we cannot rule out the possibility that the absence of TRPV1, TRPA1, TRPV4 and/or TRPM8 in other tissues besides DRG and keratinocytes might have influenced the results.

4.2 Noxious heat.

Our results do not support a role for any individual TRP channel in mediating avoidance of the hottest temperatures within the noxious range (40, 45 °C). A recent study reported that triple KO mice lacking TRPV1, TRPA1 and TRPM3 exhibit a deficit in withdrawal from noxious skin heating (Vandewauw et al., 2018). Thus, a combination of TRP channels may ensure a useful redundancy for the detection of temperatures in the high noxious range.

4.3 Cold.

TRPM8KOs of both sexes showed the greatest differences in thermal preference. They exhibited no significant preference for temperatures in the 15–35 °C range compared to 30 °C (Fig. 1I and J), consistent with previous studies (Bautista et al., 2007; Colburn et al., 2007; Dhaka et al., 2007; Knowlton et al., 2013). TRPM8KOs males and females crossed between the reference plate at 30 °C and the plate at 15 °C more times (males significantly moreso) than WT mice (Fig. 2), similar to the results reported by Knowlton et al. (2013) in TRPM8KO mice and in mice whose TRPM8-expressing neurons were ablated. We presently observed that TRPM8KO mice tended to occupy lower temperatures on the thermal gradient compared to WTs (Figs. 4E and 5E), consistent with a previous study (Dhaka et al., 2007). This is also consistent with a recent study using a circular thermal gradient which obviated the problem that mice often gravitate to corners of a rectangular linear thermal gradient. Using the circular gradient, TRPM8KO mice spent significantly more time at colder temperatures (11.5–17.3 °C) and moved more slowly compared to all other phenotypes (WT, TRPV1KOs, TRPA1KOs, TRPV3KOs, TRPV4KOs and TRPM2KOs), consistent with a reduced avoidance of cold temperatures (Ujisawa et al., 2022). Another recent study investigating operant responses to rapid changes in temperature revealed that ablation of TRPM8-expressing neurons abolished the ability of mice to distinguish a 5 °C difference in temperatures below 25 °C (Isaacson and Hoon, 2021). Our present data are consistent with these latter studies and support an essential role for TRPM8 in cold detection. Our results support the findings of Bautista et al. (2007) regarding the lack of a significant role of TRPA1 in mouse temperature preference.

4.4 Thermal gradient occupancy temperatures: sex differences.

Using the more fine grained temperature variation ensured by the thermal gradient, we found that females of all genotypes except TRPA1KOs exhibited significantly higher occupancy temperatures on the thermal gradient compared to males of the same genotype (Fig. 5). Only the TRPA1KOs exhibited no sex difference, with TRPA1KO males exhibiting a higher mean occupancy temperature comparable to that of females and higher than WT males. Our thermal gradient data suggests that the sexual dimorphism in temperature preference is dependent on TRPA1, as the sex differences present in all other mouse lines were not recorded in TRPA1KO mice. The sex differences observed with the other genotypes are consistent with a previous study reporting that female C57Bl/6 mice preferred an approximately 1 °C higher ambient temperature compared to males in a thermal preference test of cages at different ambient temperatures (Kaikaew et al., 2017), a difference that was unaffected following gonadectomy. Our results are also consistent with the study of Gaskill et al. (2009) showing that mice prefer warmer cage temperatures for maintenance and inactive behavior, with females exhibiting a more pronounced preference. In another study utilizing an orofacial operant assay, female C57Bl/6 mice were less sensitive to a cool (18 °C) stimulus compared to males (Caudle et al., 2017). This was not due to any sex difference in biophysical properties of TRPM8 but appeared to involve sex differences in currents through voltage-sensitive K+ channels (IK) and hyperpolarization-activated cyclic nucleotide-gated channels (Ih) of TRPM8-expressing trigeminal ganglion neurons (Caudle et al., 2017). To understand the sex differences in temperature preference, the effects of steroid hormones on thermo-TRP channels should be considered, especially as testosterone was shown to be a potent agonist of rat, mouse and human TRPM8 (Asuthkar et al. 2015a, b, Alarcón-Alarcón et al., 2022). A more complex picture is revealed by another recent study reporting that orchidectomy of male mice and rats resulted in increased avoidance of a cold (18–21 °C) surface compared to shams, an effect that was rescued by infusion of exogenous testosterone (Gkika et al., 2020). This effect was lost in TRPM8KO mice, and the authors showed that testosterone reduced TRPM8-mediated currents via the cell surface androgen receptors. Thus, it might be argued that males having higher testosterone levels exhibit greater inhibition of TRPM8 compared to females, thus accounting for greater female sensitivity to cold. This idea receives support from a human study showing a correlation between lower testosterone levels and feelings of coldness in perimenopausal women (Gotmar et al., 2008). However, this does not explain the present finding that female TRPM8KOs exhibited a higher mean occupancy temperature on the thermal gradient than male TRPM8KOs. Based on our results, TRPA1 seems to be a determinant of the temperature preference difference between male and female mice. This sex difference might be partly explained by additional factors as follows. In humans, females generally have less muscle mass and 6–11% more body fat than males (Karastergiou et al., 2012), a ~23% lower resting metabolic rate (Arciero et al., 1993; 1993), and tend to be smaller than males, with a higher body surface to volume ratio such that they lose heat more quickly through the skin. These and other factors (Fernandez-Peña et al., 2023) might contribute to females losing more body heat and feeling colder than males at ambient temperatures. Such an argument may also apply to mice; 4-month old females also have significantly lower body mass than males while having comparable body fat and metabolic rates (Fischer et al., 2016).

As an aside, most laboratory rodents are housed at room temperature (20–24 °C) which is cooler than their normal thermoneutral zone of 26–34 °C (Gaskill et al., 2009). This potentially constitutes a chronic stressor and argues that vivarium temperatures should be warmer consistent with rodents' normal thermoneutral zone or otherwise ample nesting materials should be provided to the animals.

Many but not all studies report that humans also exhibit a significant sex difference in the acceptability of ambient environmental temperature, with females often preferring warmer temperatures (reviewed in Karjalainen, 2007, 2012; Schweiker et al., 2018; Greenfield et al., 2023; Fernandez-Peña et al., 2023). A better understanding of the factors that underlie sex differences in thermal preference has implications for establishing home and workplace environments acceptable to both sexes.

In conclusion, all mouse genotypes significantly avoided colder and hotter temperatures except for female TRPV1KOs that did not show significant thermal preferences across the temperature range. There was a reduced thermosensitivity in female TRPV1KOs, and reduced sensitivity to cold and innocuous warmth in male and female TRPM8KOs consistent with previous studies. The thermal gradient test revealed significant sex differences, with females of all genotypes, except TRPA1KOs, preferring warmer temperatures.

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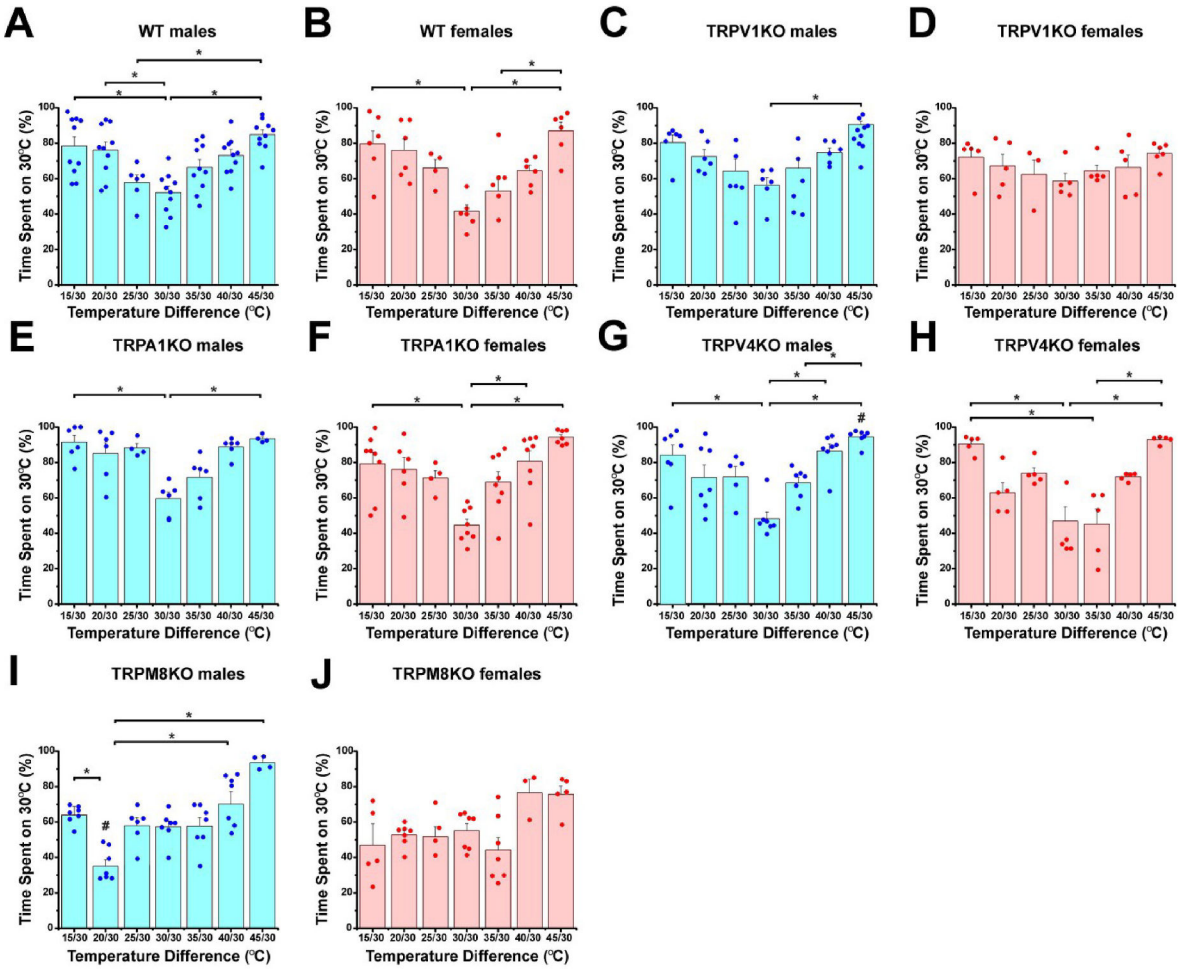

Fig. 1.

Thermal preference comparisons by genotype and temperature differential. Each graph plots the percent time spent on the 30 °C plate for each temperature difference. A: wildtype (WT) males. B: WT females. C: TRPV1KO males. D: TRPV1KO females. E: TRPA1KO males. F: TRPV1KO females. G: TRPV4KO males. H: TRPV4KO females. I: TRPM8KO males. J: TRPM8KO females. *: temperature difference statistically significant ($p < 0.05$, Kruskal-Wallis ANOVA with Dunns post-hoc test). #: significantly different from WT of same sex at same temperature difference ($p < 0.05$, Kruskal-Wallis ANOVA with Dunns post-hoc test for differences vs. WT at the given temperature differential). Numbers of mice: WT males 10, females 6; TRPV1KO males: 6, females 5; TRPA1KO males 6, females 8; TRPV4KO males 7, females 5; TRPM8KO males 7, females 7. Some of the temperature differentials have fewer data points due to attrition (range: 3–10). Bars plot means ± SEM.

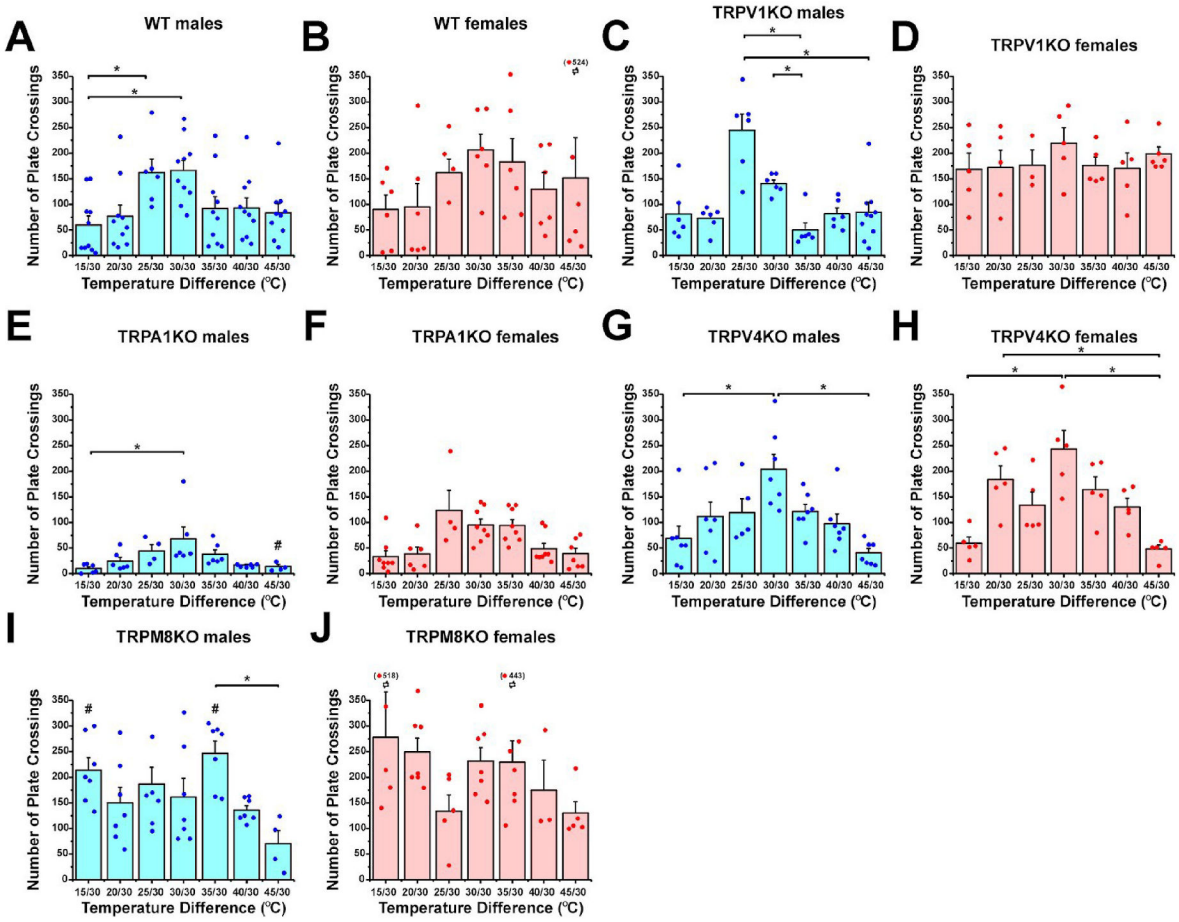

Fig. 2.

Plate crossings by genotype and temperature differential. Each graph plots the number of crossings between thermoelectric plates for each genotype and temperature differential. A: WT males. B: WT females. C: TRPV1KO males. D: TRPV1KO females. E: TRPA1KO males. F: TRPV1KO females. G: TRPV4KO males. H: TRPV4KO females. I: TRPM8KO males. J: TRPM8KO females. *: temperature difference statistically significant ($p < 0.05$, Kruskal-Wallis ANOVA with Dunns post-hoc test). #: significantly different from WT of same sex at same temperature difference ($p < 0.05$). Numbers of mice: WT males 10, females 6; TRPV1KO males: 6, females 5; TRPA1KO males 6, females 8; TRPV4KO males 7, females 5; TRPM8KO males 7, females 7. Some of the temperature differentials have fewer data points due to attrition (range: 3–10). Bars plot means ± SEM.

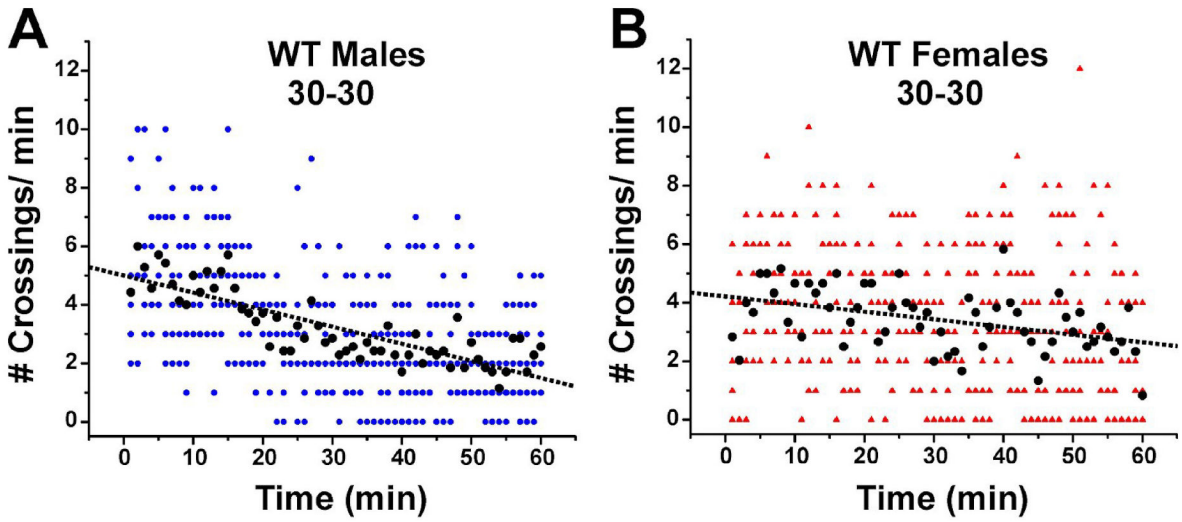

Fig. 3.

Graph plots number of crossings vs. time with both plates set at 30 °C for WT mice. A: males. ●: individual data; ●: means. Thick dashed line shows linear fit of means. B: females. ▲: individual data; ●: means. Thick dashed line shows linear fit of means. Error bars omitted for clarity.

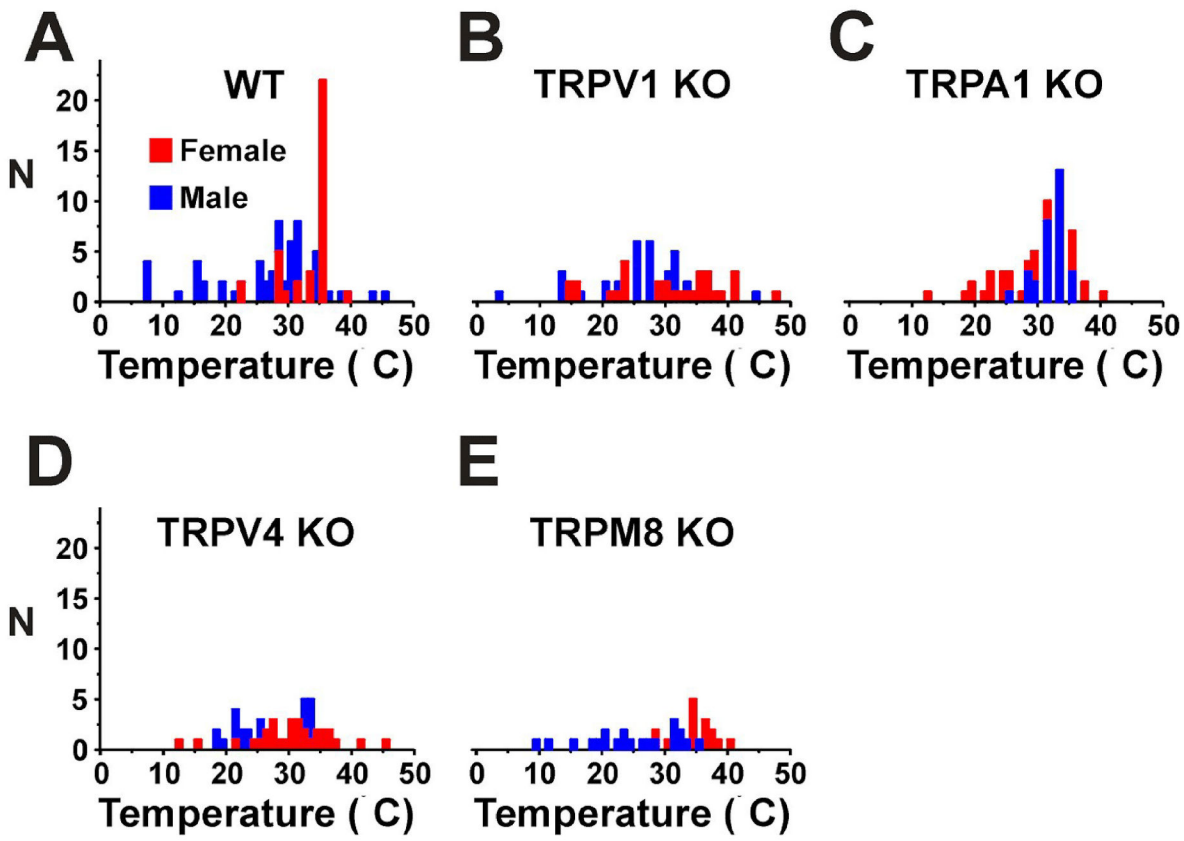

Fig. 4.

Distribution of occupancy temperatures. Mice were placed on a linear thermal gradient and the temperature at the site of occupancy of each mouse was measured every 5 min (or 10 min for TRPM8KOs). Each graph plots the number of occupancy temperatures sampled for each mouse every 5 or 10 min in the time period 90–120 min after placement on the gradient. Each group included 5–10 individuals. A: WTs. B: TRPV1KOs. C: TRPA1KOs. D: TRPV4KOs. E: TRPM8KOs. Numbers of mice: male WT: 10, female WT: 6, male TRPV1KO: 6, female TRPV1KO: 6, male TRPA1KO: 6, female TRPA1KO: 9, male TRPV4KO: 6, female TRPV4KO: 5, male TRPM8KO: 7, female TRPM8KO: 7.

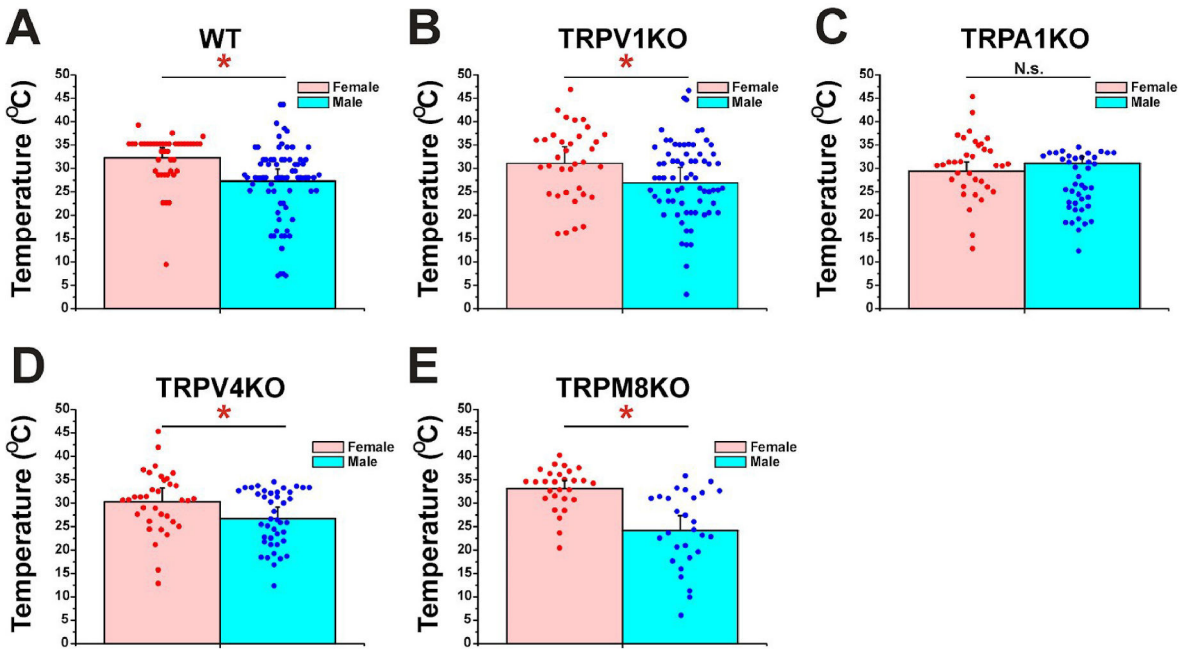

Fig. 5.

Mean occupancy temperatures on the thermal gradient for males and females of various genotypes, measured 90–120 min after being placed on the gradient. A: WTs. B: TRPV1KOs. C: TRPA1KOs. D: TRPV4KOs. E: TRPM8KOs. *: $p < 0.05$, unpaired *t*-test. Error bars: SEM. Numbers of mice: male WT: 10, female WT: 6, male TRPV1KO: 6, female TRPV1KO: 6, male TRPA1KO: 6, female TRPA1KO: 9, male TRPV4: 6, female TRPV4KO: 5, male TRPM8KO: 7, female TRPM8: 7.