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Effect of capsaicin on behaviour of cockroaches under dissimilar long-term temperature adaptation conditions

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Abstract

Optimal physiological and functional plasticity of an organism is altered outside the optimal temperature range. Acclimation to low or high ambient temperatures affects membrane lipid composition, and, therefore, protein function. In this study, we investigated the long-term temperature adaptation impacts of capsaicin, a ligand for transient receptor potential (TRP) channels, on cockroach behaviour. The effect of thermal acclimation to 14 °C (cold-acclimated) or 30 °C (warm-acclimated) on the insect's response to capsaicin was compared to the response to capsaicin in insects acclimated to 25 °C (optimal thermal conditions). We measured thermal preferences and escape latency from a high ambient temperature (50 °C) after the capsaicin treatment. Temperature acclimation to both 14 °C and 30 °C weakened the effect of the TRP ligand. In 25 °C-acclimated insects, strong desensitization to high ambient temperature was observed after multiple capsaicin treatment and acclimation to cold significantly lessened the desensitization level. In contrast, warm-acclimated cockroaches did not respond to subsequent doses of capsaicin with increasing desensitization. Moreover, acclimation to 30 °C reduced their sensitivity to capsaicin, so a higher dose was needed to match the thermal preference effect observed in 25 °C-acclimated individuals. Changes in thermoreception that occur after thermal acclimation could affect insects' resistance to a changing environment and modify the effectiveness of TRP ligands used as repellents and insecticides on pest species.

Key words: American cockroach, capsaicin, TRP receptors, thermal acclimation, thermal preferences.

Introduction

Temperature is an important factor affecting the physiological and behavioural functions of ectotherms. Ectotherms evolved several biochemical mechanisms to cope with the wide range of body temperature they experience, such as alterations in protein structures that lessen the effect of an increase in temperature (Tattersall *et al.*, 2012).

Temperature changes induce structural and functional modifications in insect cellular membranes. During cold exposure, membrane fatty acids become more unsaturated as they incorporate polyunsaturated and monounsaturated fatty acids into the phospholipids to maintain membrane fluidity (Michaud and Denlinger, 2006; Dooremalen *et al.*, 2011).

The function of membrane receptors is affected by their lipid environment. For example, a nicotinic acetylcholine receptor requires the presence of negatively charged phospholipids and cholesterol for undisturbed function. The degree of saturation of membrane phospholipids also determines the sensitivity of this receptor (McCarthy and Moore, 1992). Unsaturated fatty acids (which increase the bilayer fluidity) promote inactivation of voltage-gated sodium channels and N-type calcium channels but activation of calcium-activated potassium channels. Their high membrane level also induces desensitization of the nicotinic acetylcholine receptors and increases muscimol (agonist) binding to $GABA_A$. On the other hand, an increase in saturated fatty acid content (which decreases the bilayer fluidity) promotes a resting (non-desensitized) state of the nicotinic acetylcholine receptors and reduces muscimol binding to $GABA_A$ (Søgaard *et al.*, 2006). The function of thermoreceptors in TRP channels is also modulated by the local lipid environment. For example, cholesterol depletion in the membrane reduces the activity of TRPV1 and dTRPL (Startek *et al.*, 2019). After thermal acclimation, *Drosophila* show physiological changes that influence their resistance to extreme temperatures. Apart from changes in heat shock proteins levels, membrane composition, sugar or polyol concentration and metabolic rate change can affect temperature resistance (Hoffman *et al.*, 2003).

Capsaicin is used as an insect pest repellent and insecticide. In *Drosophila*, the alkaloid induced strong ovipositional aversion and compromised lifespan by causing intestinal dysplasia and oxidative immunity in midgut; these effects were mediated by activation of TRP Painless (TRPA subfamily) receptor (Li *et al.*, 2020). Capsaicin is reported to have broad-spectrum insecticidal activity against *Sitotroga cerealella* (Olivier), *Tribolum castaneum* (Herbst), *Sitophilus zeamais* Motschulsky, *Myzus persicae* Sulzer, *Bemisia tabaci* (Gennadius), *Hypera postica* (Gyllenhal) (Li *et al.*, 2019) or mosquitoes (Madhumathy *et al.*, 2007).

Capsaicin also affects thermoregulatory processes by acting on thermoreceptors in the TRP family. This natural alkaloid is responsible for the spicy taste of peppers and activates a mammalian heat receptor (TRPV1) (Caterina, 2007). Capsaicin was also shown to induce changes in thermoregulatory processes in the American cockroach, *Periplaneta americana* (L.). Responses similar to capsaicin-induced thermoregulatory responses (cold preference and a decrease in head temperature) were observed after allyl-isothiocyanate treatment, which is a TRPA receptor Painless activator (Maliszewska *et al.*, 2018a; 2019). However, so far only five TRP channels in *P. americana* have been found: TRPγ (TRPC subfamily) (Wicher *et al.*, 2006), TRP and

TRPL (French *et al*., 2015) and Nanchung and Inactive (TRPV subfamily) (Hennenfent *et al*., 2020).

In this study, we investigated whether acclimation to 14 °C (cold) or 30 °C (warm) changed the sensitivity of *P. americana* to capsaicin. We performed a set of experiments that evaluated the cockroaches' sensitivity to capsaicin according to changes in:

1/ Nociceptive response - in previous studies, we found out that multiple capsaicin treatments cause a "pharmacological blockade", decreasing heat sensitivity in cockroaches. Insects treated with multiple applications of capsaicin exhibited an increased escape latency from noxious heat (Maliszewska *et al*., 2018b). Therefore, here we decided to evaluate whether such state of 'heat desensitization' may be induced by multiple applications of capsaicin in insects acclimated to cold or warm before the capsaicin treatment.

2/ Thermal preference response - capsaicin was shown to induce a preference to colder environments (when the insects had an opportunity to choose ambient temperature) and a decrease in the head temperature of immobilized cockroaches (Maliszewska *et al*., 2018a). Here, we compared the response of cockroaches acclimated to 14, 25 and 30 °C to different concentrations of capsaicin. This allowed us to determine whether thermal acclimation changes the sensitivity of cockroaches to the TRP ligand. Our study shows the thermal response of acclimated cockroaches to different thermal regimes and to different concentrations of capsaicin.

Materials and methods

Insects

American cockroaches, *P. americana*, were obtained from our own breeding at Nicolaus Copernicus University in Toruń, Poland. They were reared in plastic containers at 25 ± 1 °C and under a 12:12 L:D photoperiod. All insects were fed the same diet, oat flakes and water, *ad libitum*. Only adult male cockroaches were selected for the experiments.

Tested substances

Capsaicin (M2028, ≥95%, from *Capsicum* sp., Sigma-Aldrich) was dissolved in ethanol and then diluted in cockroach physiological saline to concentrations of capsaicin ranging from 0.01 to 100 µM. The ranges were chosen based on a previous study (Maliszewska *et al*., 2018a). Control groups were treated with 1% ethanol. The tested substances (10 µl) were applied topically under the wings, on the mesothorax.

Experimental groups

We divided the cockroaches into six groups. Three groups were treated with capsaicin: Caps-Non-Accl (acclimated to 25 °C), Caps-Cold-Accl (acclimated to 14 °C) and Caps-Warm-Accl (acclimated to 30 °C).

Three other groups constituted the corresponding controls, and were all treated with 1% ethanol (Non-Accl, Cold-Accl, Warm-Accl) (figure 1).

Cockroaches acclimated to 25 °C were obtained from breeding and kept in plastic containers at 25 °C and 12:12

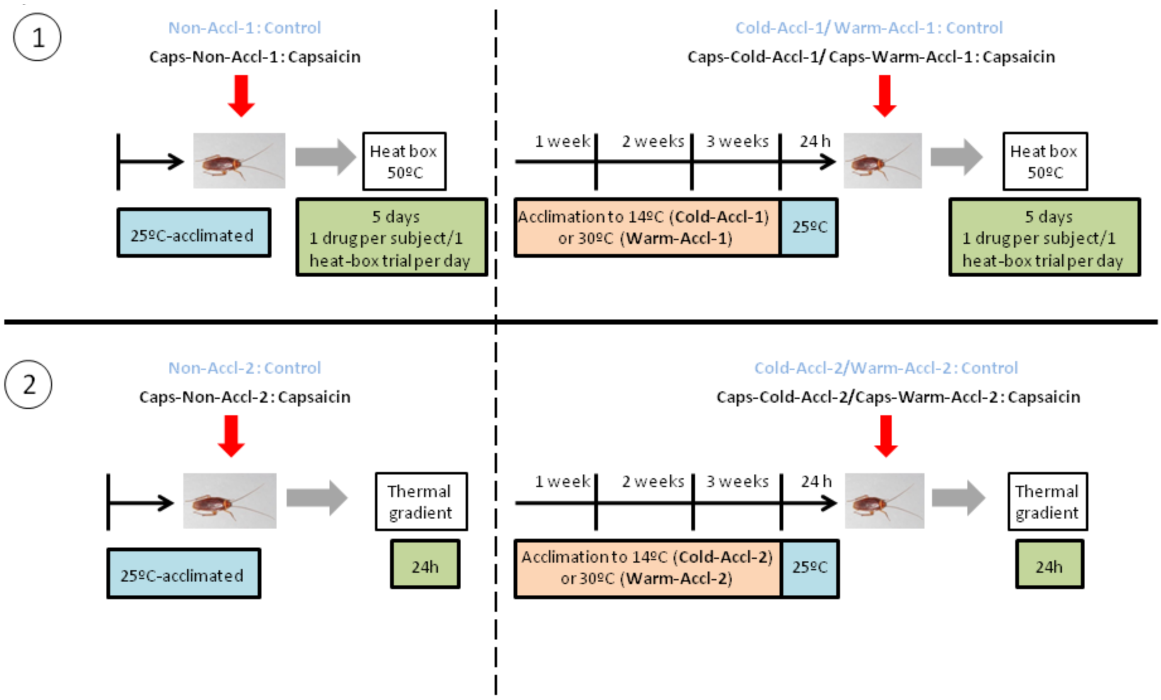

Figure 1. Sets of experiments. **1**) In the first set of experiments escape latency from heat was examined in cockroaches acclimated to 25 °C (Caps-Non-Accl-1), to 14 °C (Caps-Cold-Accl-1) or 30 °C (Caps-Warm-Accl-1) exposed to capsaicin (0.1 µM). **2**) In the second series of experiments acclimated cockroaches (Caps-Non-Accl-2 acclimated to 25 °C, Caps-Cold-Accl-1 acclimated to 14 °C and Caps-Warm-Accl-1 acclimated to 30 °C) were exposed to capsaicin of different concentrations (0.01-100 µM) and examined in the thermal gradient system.

L:D with food and water, *ad libitum*. Some Non-Accl cockroaches (Caps-Non-Accl-1) were treated with capsaicin (0.1 μM) and used in heat nociception experiments (the first set of experiments). Another group of Non-Accl cockroaches (Caps-Non-Accl-2) was exposed to different concentrations of capsaicin (0.01 - 100 μM) and then evaluated for changes in their thermal preferences (the second set of experiments). Different cockroaches were used to test the effect of specific capsaicin concentration.

For cold and warm acclimation, cockroaches were placed in plastic containers, in the incubator (ILW 115 STD, POL-EKO APARATURA, Poland) with food and water, *ad libitum*. Cockroaches were acclimated to 14 °C or 30 °C for 21 days (12:12 L:D). Following the acclimation, insects were maintained at 25 °C for 24 hours before the start of the experiment. This eliminated any effects of acclimation temperature on behaviour because cockroaches taken out directly from 14 °C were dormant, which would seriously affect their thermal behaviour. Some of the acclimated insects were exposed to capsaicin (0.1 μM) and used in heat nociception experiments (groups Caps-Cold-Accl-1 and Caps-Warm-Accl-1). The rest of Cold-Accl and Warm-Accl insects were exposed to between 0.01 and 100 μM capsaicin and used to study thermal preferences (groups Caps-Cold-Accl-2 and Caps-Warm-Accl-2).

Procedures

The first set of experiments evaluated the responsiveness to capsaicin in cockroaches acclimated to optimal temperature (Caps-Non-Accl-1) and acclimated to cold or warm (Caps-Cold-Accl-1 and Caps-Warm-Accl-1) in a heat nociception assay. Because thermo-TRP channels function as nociceptors (Painless, Pyrexia) we measured the escape response of insects to nociceptive temperature (50 °C) after capsaicin treatment. Previous studies showed that capsaicin affects heat nociception in cockroaches (Maliszewska *et al*., 2018b). Cockroaches were acclimated to 25 °C (groups Caps-Non-Accl-1 and Non-Accl-1) or acclimated for 3 weeks to 14 °C (groups Caps-Cold-Accl-1 and Cold-Accl-1) or 30 °C (groups Caps-Warm-Accl-1 and Warm-Accl-1), and then transferred to 25 °C for 24 hours. After that, insects were exposed to 0.1 μM capsaicin (Caps-Non-Accl-1, Caps-Cold-Accl-1 and Caps-Warm-Accl-1 groups) or 1% ethanol (Non-Accl-1, Cold-Accl-1 and Warm-Accl-1 groups) and examined in the heat box. This procedure was repeated for five consecutive days (figure 1.1).

In the second series of experiments, we compared the thermal preferences of 25 °C-acclimated cockroaches (Non-Accl-2 and Caps-Non-Accl-2) and cockroaches acclimated to cold (Cold-Accl-2 and Caps-Cold-Accl-2) or warm (Warm-Accl-2 and Caps-Warm-Accl-2) after exposure to different concentrations of capsaicin.

In previous studies we showed that *P. americana* responds to TRP ligands by changing its thermal preferences (Maliszewska *et al*., 2018a). Thus, here we investigate whether ambient temperature changes the sensitivity of TRP channels to capsaicin according to changes in thermal preferences in the temperature gradient system. This second series of experiments was performed on Caps-Non-Accl-2, Caps-Cold-Accl-2 and Caps-Warm-Accl-2 cockroaches exposed to capsaicin of different concentrations to compare the behavioural response of each group. Cockroaches acclimated to 25 °C (Caps-Non-Accl-2), to 14 °C (Caps-Cold-Accl-2) or 30 °C (Caps-Warm-Accl-2) were exposed to different concentrations of capsaicin, from 0.01 to 100 μM, with different cockroaches used in each concentration experiment. Controls were exposed to 1% ethanol (Non-Accl-2, Cold-Accl-2 and Warm-Accl-2) and then examined in the thermal gradient system for 24 hours (figure 1.2).

Heat response in acclimated cockroaches

The latency to escape from 50 °C was evaluated using a previously described ‘heat box’ (Maliszewska *et al.*, 2018b). Briefly, the heat box is a dark chamber with an escape orifice on one side. Temperature inside the heat box was 50 °C. A single cockroach was placed inside the heat box immediately after application of capsaicin or 1% ethanol, and escape latency was measured. Escape latency was defined as the interval between releasing the animal into the box and its exit. The escape time is referred to as the escape latency in the text.

Each cockroach was tested five times - once a day for five days. Each trial included topical application of capsaicin or 1% ethanol ($n = 45 \pm 5$ for each treatment).

Thermal preferences

The cockroaches’ thermal preferences were determined in a thermal gradient system described in Maliszewska *et al.* (2018a). In brief, it consists of a long and narrow (5 × 60 cm) trough with temperature ranging from 10 °C to 40 °C. The cold end was cooled by water pumped out of a cryostat (Polyscience 9106, Niles, IL, USA), while the warm end was heated with hot water pumped out of thermostat (Polyscience 8006, Niles, IL, USA). The established temperature gradient along the trough was approximately 0.5 °C/1 cm. The trough was divided into 20 compartments of equal length in which ambient temperature was measured with a thermocouple before each experiment.

Each tested cockroach was placed in the centre of the thermal system (at 23.8 ± 0.1 °C) immediately after the capsaicin or ethanol application, and its thermal preferences were recorded for 24 hours with a camera (Sony FDR AX33, Sony Electronics Inc., San Diego, CA, USA). Experiments started between 9:00 and 10:00 AM. The preferred temperature of each individual was established based on its position in the thermal gradient every 10 minutes during the first hour and then every 2 hours until the end of experiment. Twelve individuals were examined in each group. All experiments were performed in the 12:12 hours cycle of light and dark.

Data analysis

Using a Kolmogorov-Smirnow test, we determined the data were not normally distributed. To establish the effect of acclimation on the response to heat for cockroaches treated with capsaicin (groups Caps-Non-Accl-1, Caps-Cold-Accl-1, Caps-Warm-Accl-1) versus the control (Non-Accl-1, Cold-Accl-1 and Warm-Accl-1) we used a Kruskal-Wallis test. Individual groups differences were tested with a Mann-Whitney U test.

The Kruskal-Wallis test was also used to compare thermal preferences between groups Non-Accl-2, Cold-Accl-2, Warm-Accl-2 as control and Caps-Non-Accl-2, Caps-Cold-Accl-2 and Caps-Warm-Accl-2 treated with different concentrations of capsaicin. Difference among acclimated groups (14 °C, 30 °C or 25 °C) and among groups treated with different capsaicin concentration was determined with the Mann-Whitney U test.

Data are presented as medians with interquartile ranges. All analyses were performed in SPSS software ver. 28 (IBM, Armonk, NY, USA).

Results

Heat response in capsaicin-treated cockroaches is affected by acclimation

We evaluated heat response to nociceptive temperature in cockroaches from groups Non-Accl-1 and Caps-Non-Accl-1 (acclimated to 25 °C), Cold-Accl-1 and Caps-Cold-Accl-1 (cold-acclimated) and WarmAccl-1 and Caps-Warm-Accl-1 (warm-acclimated). Both the temperature acclimation and capsaicin treatment affected the escape latency (table 1).

Effect of cold acclimation on heat response

Capsaicin affected heat responses (figure 2). In the insects acclimated to 25 °C (Caps-Non-Accl-1), capsaicin significantly increased heat escape latency after the third ($p < 0.001$), fourth ($p = 0.001$) and fifth doses ($p < 0.001$) compared to control (Non-Accl-1). The latency after the fifth dose was significantly longer than after the first ($p < 0.01$) and second dose ($p < 0.05$). In cold-acclimated insects (Caps-Cold-Accl-1) the escape latency after the fifth dose of capsaicin was significantly longer compared to control (Cold-Accl-1; $p < 0.05$) and after the second dose ($p < 0.001$).

Acclimation temperature influenced the effect of capsaicin on the response to heat (table 2). In the cockroaches acclimated to 25 °C, a capsaicin-induced desensitization to heat was observed. Acclimation to cold diminished this reaction. Significant differences in the escape latencies were found between the 25 °C-acclimated (Caps-Non-Accl-1) and cold-acclimated (Caps-Cold-Accl-1) insects after the first, third and fifth doses (table 2).

Table 1. Results of statistical analysis with Kruskall-Wallis test of the heat response of 25 °C acclimated (Caps-Non-Accl-1), 14 °C-acclimated (Caps-Cold-Accl-1) and 30 °C-acclimated (Caps-Warm-Accl-1) American cockroaches treated with 0.1 µM capsaicin.

Main effects	df	H	p
Acclimation temperature	2	15.16	<0.001
Capsaicin treatment	1	25.47	<0.001
Capsaicin dose number	4	5.33	0.26

Effect of warm acclimation on heat response

The opposite effect of capsaicin was observed in the warm-acclimated insects (Caps-Warm-Accl-1; figure 2, table 2). The longest escape latency was found after the first dose of capsaicin ($p < 0.001$ compared to the Warm-Accl-1 group), with the effect decreasing with each subsequent dose. The latency after the first dose was significantly longer than after the second ($p < 0.001$), fourth ($p < 0.001$) or fifth doses ($p < 0.001$).

Acclimation to warm reversed the reaction to capsaicin, reducing its effect with each consecutive application in the same insect. Significant differences in escape latency between 25 °C-acclimated (Caps-Non-Accl-1) and warm acclimated (Caps-Warm-Accl-1) cockroaches were observed after the first and fifth doses (table 2).

A significant difference in escape latency was also found between cold- (Caps-Cold-Accl-1) and warm- (Caps-Warm-Accl-1) acclimated cockroaches treated with a single dose of capsaicin ($p < 0.001$) (table 2).

Acclimation weakens the effect of the capsaicin-induced preference for colder environment

We found a statistically significant effect of both the concentration of capsaicin and the acclimation temperature (table 3). In the capsaicin-treated cockroaches acclimated to 25 °C (Caps-Non-Accl-2), a statistically significant preference for a colder environment (compared to Non-Accl-2) was observed at all but the highest concentration of capsaicin (figure 3).

Acclimation to cold or warm weakened the effect of the capsaicin-induced preference for colder environments; so a higher concentration of capsaicin must be used to induce the same behavioural effect (preference for cold) as in observed in insects acclimated to 25 °C (figure 4).

Effect of cold acclimation on thermal preferences

Cold acclimation induced preference for 26.6 ± 1.7 °C (ColdAccl-2), which was significantly lower than recorded in the group acclimated to 25 °C (Non-Accl-2; 27.46 ± 1.0 °C; $p < 0.01$).

Capsaicin treatment exerted a minor effect on thermal preferences in cockroaches acclimated to 14 °C (Caps-Cold-Accl-2). Individuals exposed to the high concentration of capsaicin (10 µM) exhibited preference for the lowest temperature (25.7 ± 3.4 °C). In contrast, cockroaches

Table 2. Comparison of escape latency from nociceptive temperature (50 °C) between capsaicin-treated 25 °C-acclimated cockroaches (Caps-Non-Accl-1) and these acclimated to cold (Caps-Cold-Accl-1) and warm (Caps-Warm-Accl-1) (Mann-Whitney U test p-values for the first, third, and fifth dose of capsaicin; the p values for the second and fourth dose were not significant).

	First dose	Third dose	Fifth dose
Caps-Non-Accl-1 vs. Caps-Cold-Accl-1	0.005	0.011	0.04
Caps-NonAccl-1 vs. Caps-Warm-Accl-1	<0.001	ns	0.013
Caps-Cold-Accl-1 vs. Caps-Warm-Accl-1	<0.001	ns	ns

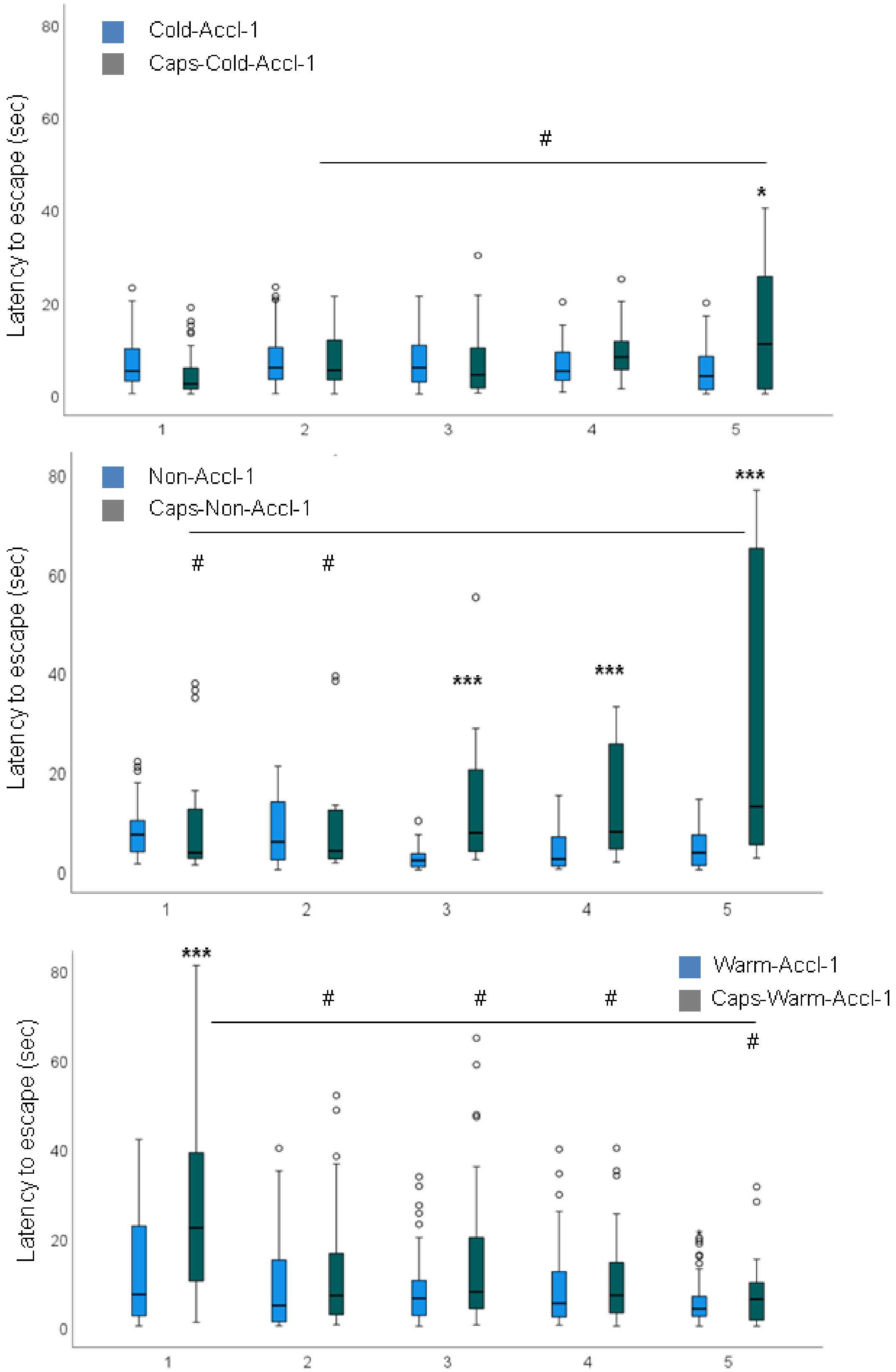

Figure 2. Latency to escape (seconds) from 50 °C of cockroaches acclimated to 25 °C, 14 °C or 30 °C and treated with 1% ethanol (Non-Accl-1; Cold-Accl-1; Warm-Accl-1 respectively) or capsaicin 0.1 μM (Caps-Non-Accl-1; Caps-Cold-Accl-1; Caps-Warm-Accl-1 respectively), of one to five doses (1-5). Each dose administration and escape reaction test were performed every 24 hours. * indicates significant difference between acclimated insects administered with different amount doses of tested substance comparing to non-treated group (* $p < 0.05$; *** $p < 0.001$); # indicates significant difference between insects treated with each subsequent dose of capsaicin at different acclimation temperatures. Mann-Whitney U test.

Table 3. Results of statistical analysis with Kruskall-Wallis test of the temperature preference of 25 °C-acclimated (Caps-Non-Accl-2), cold-acclimated (Caps-Cold-Accl-2) or warm-acclimated (Caps-Warm-Accl-2) American cockroaches treated with capsaicin from 0.01 to 100 µM concentration.

Temperature	df	H	p
Acclimation to 25 °C	2	17.2	0.004
Acclimation to 14 °C	2	12.5	0.03
Acclimation to 30 °C	2	23.73	0.0002
Capsaicin concentration			
0.01	5	7.65	0.02
0.1	5	16.23	<0.01
1	5	10.09	0.006
10	5	3.11	0.21
100	5	1.21	0.55
0 - control group (1% ethanol)	5	12.5	0.002

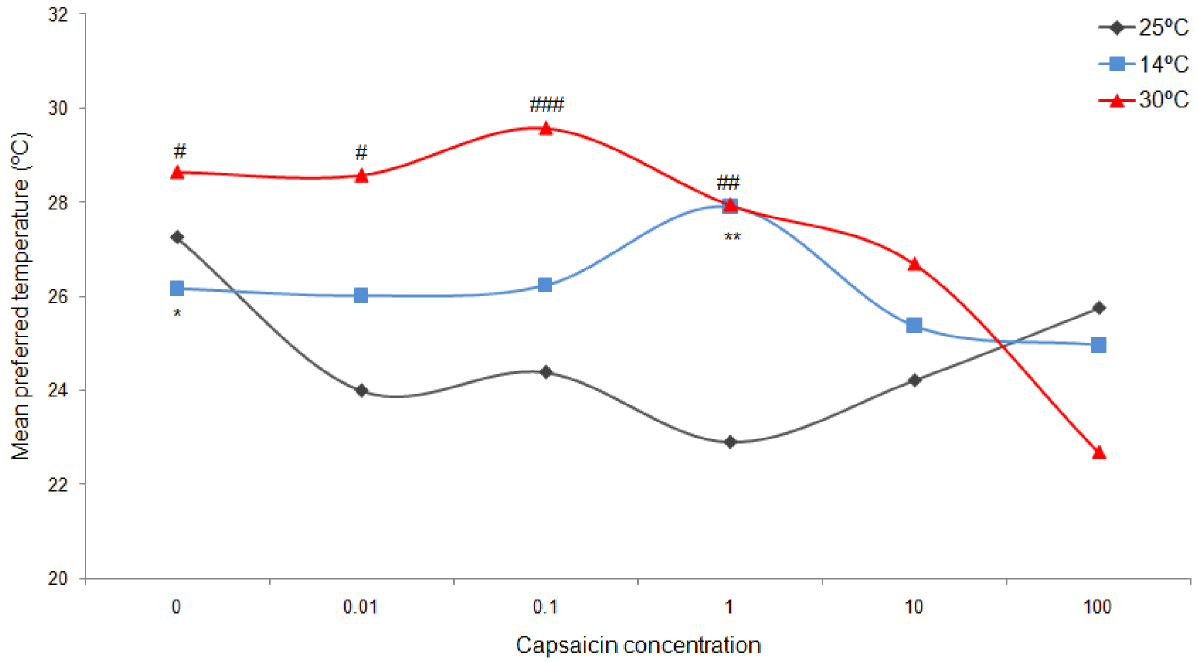

Figure 3. Mean preferred temperature (°C) in cockroaches acclimated at 25 °C (Caps-Non-Accl-2), at 14 °C (Caps-Cold-Accl-2) or 30 °C (Caps-Warm-Accl-2) exposed to different concentrations of capsaicin: from 0.01 to 100 µM, or 1% ethanol (Non-Accl-2, Cold-Accl-2 and Warm-Accl-2, respectively). * indicates significant difference in temperature preference between groups treated with different capsaicin concentrations in group acclimated to 14 °C and to 25 °C (* $p < 0.05$; ** $p < 0.01$); # indicates significant difference in temperature preference between cockroaches acclimated to 25 °C and warm administered with particular capsaicin concentration (# $p < 0.05$; ## $p < 0.01$; ### $p < 0.001$). Mann-Whitney U test.

exposed to 1 µM capsaicin preferred significantly higher ambient temperatures than insect acclimated to 25 °C ($p < 0.01$) (figure 3).

Effect of warm acclimation on thermal preferences

As with cold-acclimated insects, cockroaches acclimated to 30 °C (Caps-Warm-Accl-2) showed a stronger preference for cold after treatment with 100 µM of capsaicin compared to those treated with 0.01 µM ($p = 0.004$), 0.1 µM ($p < 0.01$) or 1 µM ($p < 0.01$) of the alkaloid and compared to control group (Warm-Accl-2; $p = 0.002$). Thus, to obtain the thermal preference observed in Caps-Non-Accl-2 cockroaches after treatment with 0.01 µM capsaicin (25.2 ± 5.2 °C), at least 10 µM or higher (100 µM) capsaicin concentration must be used in Caps-Warm-Accl-2 cockroaches (figure 3).

Discussion

Thermal acclimation affects insect thermal preferences. *P. americana* acclimated to 35 °C preferred lower temperatures than *P. americana* acclimated to 25 °C (Murthy, 1986). Kim *et al.* (2015) demonstrated that a 10-minute pre-exposure to 42 °C was sufficient to acclimate

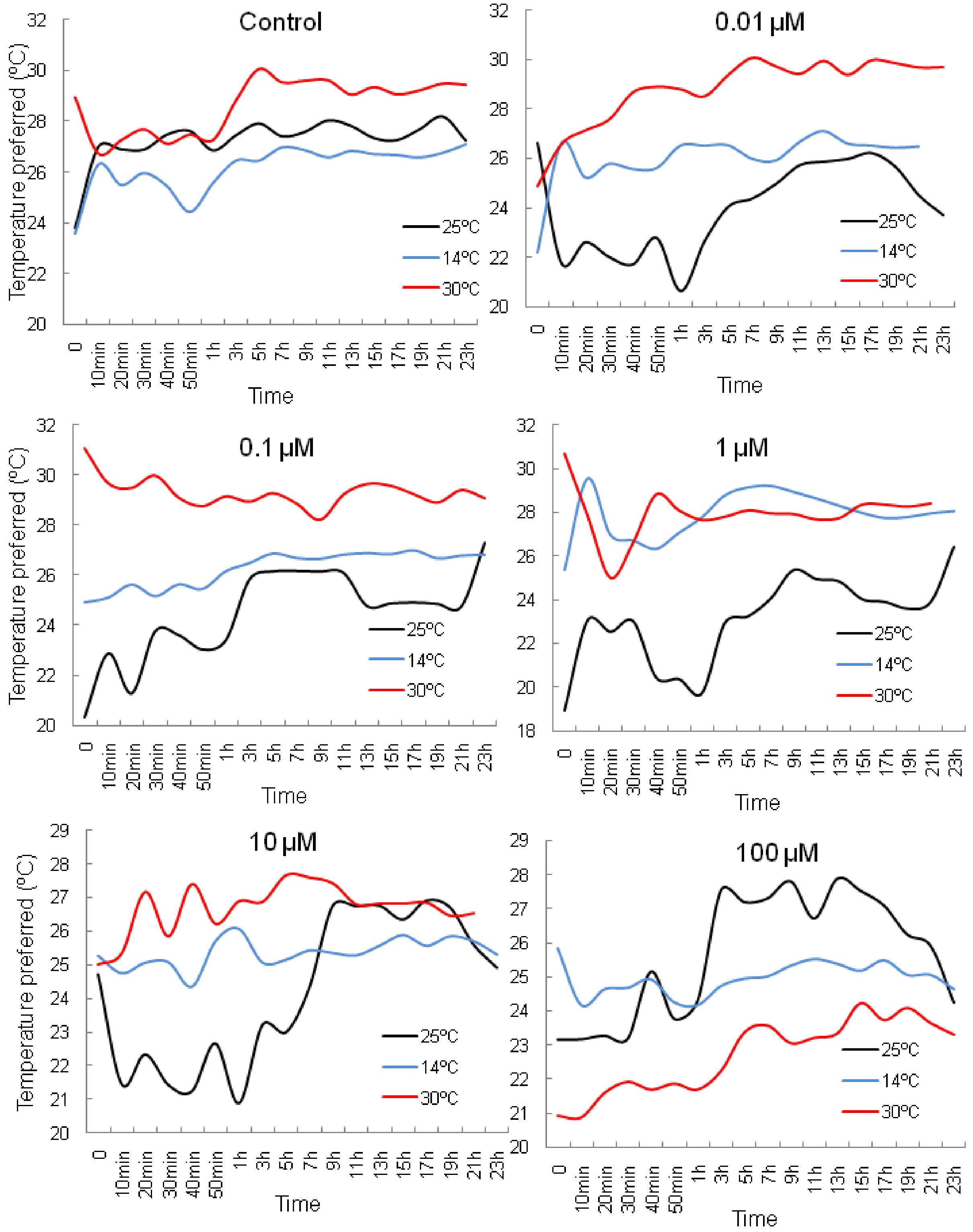

Figure 4. Distributions of cockroach positions during the 24 hours monitoring period in control group (1% ethanol) and after exposure to different capsaicin concentrations, from 0.01 µM to 100 µM. Black line represents cockroaches acclimated to 25 °C, blue line to 14 °C and red line to 30 °C.

T. castaneum to the extremely high temperature of 52 °C. Kim *et al.* (2015) also shown that activation of TRP channel Painless is required for rapid acclimation to high temperatures, whereas TRP channel Pyrexia protects the beetles from acute heat stress without acclimation. This could be particularly important for managing invasive species, which are often able to adapt to various environmental temperatures. Lü *et al.* (2014) demonstrated with *B. tabaci* that TRP channel play an essential role in the organisms' heat tolerance and this potentially facilitated its invasiveness.

Changes in insect thermal preference after acclimation to different ambient temperatures may result from changes in membrane lipid composition that occurs in cold or warm environments. Baldus and Mutchmor (1988) reported changes in the fatty acid composition after temperature acclimation in *P. americana*. Low temperature acclimation induced an increase in unsaturated fatty acids, such as eicosadienoic and arachidonic, while acclimation to 30 °C resulted in an increase in saturated fatty acid content (myristic, pentadecanoic, palmitic) in the nerve cord. Such changes in membrane lipid composition may affect the activity of membrane proteins, including TRP channels. In *Drosophila melanogaster* Meigen, TRP and TRPL channels activation is mediated by mechanical forces in the membrane. When the proportion of polyunsaturated phospholipids is decreased 7-fold by the diet, *Drosophila* TRP and TRPL channel sensitivity is reduced 2 to 3-fold (Randall *et al*., 2015).

Our results indicate that the behavioural response to capsaicin in cockroaches is altered outside the optimal temperature range. In the insects placed within an optimal thermal range (25 °C), strong desensitization to high ambient temperature was observed after multiple levels of capsaicin treatment. This reaction was diminished in both cold- and warm-acclimated cockroaches, but most significantly in warm-acclimated cockroaches, as they did not respond to subsequent doses of capsaicin with increasing desensitization. Acclimation to cold and warm also reduced the effect of capsaicin on cockroach thermal preferences. We demonstrated that acclimation process reduced the sensitivity to capsaicin by 10,000 - fold: the dose must be as high as 100 μM to induce the same behavioural response in acclimated cockroaches as the dose 0.01 μM for those acclimated to optimal thermal range.

Temperature acclimation may reduce the effectiveness of capsaicin through changes in membrane lipid composition, which modify the membrane lipid-channel interactions. Although we did not measure changes in lipid saturation, other studies (Baldus and Mutchmor, 1988; Crocket, 1998; Dooremalen *et al*., 2011) suggest that the observed effects of temperature acclimation depend on changes in membrane lipid composition. Linoleic acid was shown to inhibit TRP channels indirectly, as a modifier of lipid-channel interactions (Parnas *et al*., 2009). Changes in membrane lipid saturation followed by thermal acclimation are well known (Overgaard *et al*., 2008). Here, we show that acclimation to warm temperatures reduces insects' sensitivity to capsaicin. This may stem from a decrease in unsaturated lipid content and an increase in the saturated lipid content. Moreover, cholesterol levels in the membrane may increase during warm acclimation (Crocket, 1998) and an increase in cholesterol in membranes decreases sensitivity of TRPV1 to capsaicin (Ciardo *et al*., 2017). Similarly, we show here that cockroaches acclimated to a cold temperature demonstrated decreased sensitivity to capsaicin. An increase in membrane polyunsaturated lipid content followed by cold-acclimation may affect TRP channel function. Some TRP channels are activated by PUFA (ligand for TRPV, TRPA, TRPC members), but others are inhibited by these acids (TRPM) (Elinder and Liin, 2017). Rats acclimated to different ambient temperatures demonstrated the same response to exogenous opioids treatment. The cold- and warm-acclimated rats showed hyperthermic response to PL-017 (mu-opioid receptor selective agonist) application (Handler *et al*., 2001).

Optimal physiological plasticity of L-type calcium channels function in the temperature range of normal development was demonstrated in *D. melanogaster* (Frolov and Singh, 2013). At 30 °C, they observed molecular bond destabilization that led to weaker interactions between channels subunits and therefore a decrease in conductance and kinetic changes.

In this study, the effect of temperature acclimation on the output of neurons expressing thermo-TRP was not a factor as the acclimated cockroaches were transferred to 25 °C for 24 hours before evaluating their heat response and thermal preference.

Proper thermosensation mechanisms are required for insects' survival. Changes in thermoreception that occur after thermal acclimation affect insects fitness and resistance to changing environment. This could be especially important in pest species, in which thermal acclimation changes may affect sensitivity to TRP ligands used as repellents or insecticides, including capsaicin (Li *et al*., 2019). Our results show the influence of temperature on sensitivity to capsaicin.

Ambient temperature is one of the most important factors determining the sensitivity to toxins. Moreover, the "climate-induced toxicant sensitivity" theory states that susceptibility of organisms to toxicants are altered by climate change factors. Pre-exposure of the mosquito, *Culex pipiens* L., to heat diminished the effectiveness of chlorpyrifos, reducing the lethal impact of this pesticide (Meng *et al*., 2020). Thus, determining the effect of ambient temperature on pest insects' sensitivity to chemicals is crucial to developing an effective strategy for their control.

Conclusions

Acclimation to cold and warm temperatures affects cockroaches' sensitivity to the TRP ligand - capsaicin. The insects' responsiveness to capsaicin diminished with higher doses of capsaicin needed to induce the same response observed in insects at optimal temperature range (a preference for colder environments). The results show that thermal acclimation induces changes in thermoreception, which, in turn, may modify the response of pest species to TRP ligands used as repellents and insecticides.

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