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Temperature-sensitive gating in a descending visual interneuron, DCMD

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Abstract Activity in neural circuits can be modified through experience-dependent mechanisms. The effects of high temperature on a locust visual interneuron (the descending contralateral movement detector, DCMD) have previously been shown to be mitigated by prior exposure to sub-lethal, elevated temperatures (heat shock, HS). Activity in the DCMD is reduced at high temperature in naïve animals (control), whereas HS animals show a maintained spike count at all temperatures. We examined whether this finding was due to direct effects of temperature on visual processing, or whether other indirect feedback mechanisms were responsible for the observed effect in the DCMD. Activity in the DCMD was elicited using a computer-generated looming image, and the response was recorded extracellularly. The temperature of visual processing circuits contributes directly to HS-induced plasticity in the DCMD, as maintaining the brain at 25°C during a thoracic temperature ramp eliminated the high frequency activity associated with HS. Removing ascending input by severing the thoracic nerve cord reduced DCMD thermosensitivity, indicating that indirect feedback mechanisms are also involved in controlling the DCMD response to increased thoracic temperature. Understanding how thermosensitive feedback within the locust affects DCMD function provides insight into critical regulatory mechanisms underlying visually-guided behaviors.

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Abbreviations

AP	Action potential
DCMD	Descending contralateral movement detector
HS	Heat shock

Introduction

Signaling between neurons can be modified by experience with adaptive behavioral consequences. The thermal operating range of neural processes can be extended by exposure to a short-term, high but sub-lethal temperature stress (heat shock, HS; Marcuccilli and Miller 1994; Robertson et al. 1996). There is considerable literature on the changes in cellular and molecular physiology following heat shock that are thought to contribute to thermal adaptation (for review see Hochachka and Somero 2002). Only recently, however, has acquired thermotolerance begun to be understood in terms of its effects on the function of neural circuits underlying animal survival (Robertson 2004a,b). Indeed, failure of neural circuitry at extremes of temperature occurs long before necrotic events in cells and tissues (Prosser and Nelson 1981; Moseley 1994), but with equally lethal consequences for the animal. Owing to the relative dependence of their internal temperature on external temperature, ectothermic animals are particularly at risk in high ambient temperature environments. Despite this, these animals are able to thrive under extreme as well as variable

temperatures, suggesting that adaptive mechanisms exist in these animals to protect function in harsh temperature environments. Consequently, studying ectothermic animals provides an excellent opportunity to further our understanding of the neural mechanisms of acquired thermotolerance following a heat stress.

In the locust in particular, heat shock has been shown to extend the maximum operating temperature of motor circuits (Dawson-Scully and Robertson 1998; Gray and Robertson 1998; Wu et al. 2001) leading to behavioral thermotolerance of flight rhythms (Robertson et al. 1996), escape jumping (Barclay and Robertson 2000), and ventilatory motor pattern generation (Newman et al. 2003). Underlying this thermotolerance are modifications to multiple signaling components including: central synapses (Dawson-Scully and Robertson 1998), neuromuscular junctions (Barclay and Robertson 2000), and conduction of action potentials (Wu et al. 2001; Money et al. 2005).

The locust visual system is a useful model for studying how sensory responses to a stimulus can be modified following heat stress to promote thermotolerance. Features of an approaching object are computed in the brain by the lobula giant movement detector (LGMD; Gabbiani et al. 1999), and the information is passed to the descending contralateral movement detector (DCMD) via a 1:1 chemical synapse (Rind 1984). The DCMD acts as a relay from the LGMD in the brain to motor centers in the thoracic ganglia, where it is thought to contribute to triggering escape behaviors (Gray et al. 2001; Gabbiani et al. 2004; Santer et al. 2005, 2006). In temperature naïve animals, activity in the DCMD is decreased at high temperature. Following HS however, this DCMD activity is potentiated (Money et al. 2005). In the present study, we examined the direct effects of temperature on visual processing. We have further explored whether other indirect feedback mechanisms contribute to the observed effect of temperature on the DCMD.

Materials and methods

Animal conditions

Adult male locusts (*Locusta migratoria*) in the gregarious phase were used for all experiments. Animals 3–5 weeks after final molt were taken from crowded cages maintained in the Department of Biology at Queen's University. Animals were fed wheat seedlings and a dry mixture of oats, bran, skim milk powder, and torula yeast; supplemented by sliced carrots. The photoperiod of the colony was set to a 12:12 light:dark cycle. Cage temperature was 25 ± 1°C, with a constant humidity of 23 ± 1%. Animals were assigned at random to either a control or a heat-shocked (HS) treatment group. HS locusts were placed in a 2 l ventilated plastic container in a humid incubator at 45°C for 3 h. After treatment, the animals were allowed to recover at room temperature (22°C ± 1°C) for 1–5 h. Control animals were placed in similar containers at room temperature for 4–8 h before an experiment.

Animal preparation

A semi-intact preparation was used to expose the locust's thoracic nervous system, and is described in detail in Robertson and Pearson (1982). All six appendages and two sets of wings were removed before dissection to limit movement. An incision was made along the dorsal midline and the animal was pinned open and dissected to expose the thoracic ganglia. The meso- and metathoracic ganglia were mounted onto a metal plate. A Peri-Star peristaltic pump (World Precision Instruments Inc., Sarasota, FL) was used to perfuse the preparation with standard locust saline containing (mM): 147 NaCl, 10 KCl, 4 $CaCl_2$, 3 NaOH, and 10 HEPES buffer (pH 7.2). The saline dripped into the left anterior portion of the thoracic cavity at a constant rate of 2.5 ml/min, flowed towards the posterior end of the thoracic nervous system, and exited through a cut in the posterior portion of the abdomen. Temperature of the preparation was controlled by passing a current through a coil of Nichrome wire wrapped around the glass pipette of the saline inflow. Temperature was monitored using a BAT-12 thermometer and probe (Physitemp Instruments, Inc., Clifton, NJ).

Recordings

Action potentials were recorded extracellularly from the DCMD. A suction electrode was placed on the dorsomedial surface of the connective anterior to the mesothoracic ganglion (0.5 mm). The preparation was grounded via a silver wire inserted through the abdomen in full contact with the thoracic saline. Identification of the DCMD was made by producing movement within the locust's visual field contralateral to the recording site. DCMD spikes were easily identified by their relative size compared to electrical noise or background activity. The signal was amplified using a Model P15 Preamplifier (Grass Instruments Inc., West Warwick, RI). Extracellular signals were digitized using a Digidata 1322A (Axon Instruments Inc., Union City,

CA) and recorded to a computer for offline analysis using Clampex 9.0.

Looming stimulus

The looming stimulus was a computer-generated video produced using Macromedia Flash MX 2004 software (Macromedia, Inc.) The video image was of an expanding solid black circle against a white background. The program was run from a dedicated computer with an ATI Radeon 256 MB video board. It was displayed by a digital projector (200 Hz refresh rate; Sharp XG-C55X, Sharp Electronics Inc.) onto a rear projection screen placed 7 cm from the eye of the animal. A quantum sensing light meter (Model LI-250A, LI-COR Inc.) placed directly on the surface of the screen gave a contrast ratio for the image of 0.92. The looming image had an apparent motion of 1 m/s on a collision trajectory towards the locust's right eye at 90° to the body axis. The image began at an angular size of < 1° and ended at a size of 30°. The angular size of the image on the retina at time (t) can be expressed as angle (t) = 2 tan–1 [$l/v \times (t)$], where (l) is the image's half-size, and (v) is its approach velocity. The video was presented at a rate of 100 frame/s, which was confirmed by use of a MotionScope 500 high-speed camera (Redlake Camera). Two signals from the output of the computer soundcard were used as inputs to the data acquisition to calculate time to predicted collision. These markers were embedded into the computer-generated image on the first and final frames of the stimulus trajectory toward the locust eye. The number of spikes elicited at room temperature by the computer-generated image was similar to the number of responses elicited by a mechanical target (Rind and Simmons 1992; Money et al. 2005) or by computer-generated dark squares used by other groups (Gabbiani et al. 1999; Matheson et al. 2004).

Treatments

Temperature was continually increased at a rate of 5°C every 90 s from 25 to 45°C. The response of the DCMD to a looming image was recorded once at each of the following sequentially increased temperatures: 25, 30, 35, 40, and 45°C, or until there was a failure of action potential generation. Responses in the DCMD were measured for three treatment groups: standard; brain temperature-controlled; and with a thoracically-severed nerve cord. In the standard treatment, dissection and saline perfusion were performed as described above. The brain temperature, as recorded on its dorsal surface, increased gradually during the thoracic temperature ramp, reaching a maximum of 32.8 ± 1.2 (mean ± SD). In the brain temperature-controlled treatment, a further dorsal dissection of the head was performed to expose the brain and optic lobes. A second saline perfusion was used to maintain the temperature of the brain region below 25°C, as monitored by a second thermocouple. Successful compartmentalization of the brain and thoracic regions was achieved, as temperature in the brain region did not significantly increase during the thoracic temperature ramp (one-way ANOVA; $F_{(4,46)} = 0.95$, $P = 0.45$). The compartmentalization was aided by using the gut as a physical barrier to saline flow. The third treatment group was identical to the standard treatment, except that it had the ventral nerve cord bilaterally severed posterior to the extracellular recording site, between the prothoracic and mesothoracic ganglion. This cut did not impair the ability to effectively distinguish the DCMD from background activity. A brief spike discharge (< 2 s) was associated with the nerve cord being severed, but no such further activity was observed. The act of severing the nerve cord did not appear to disrupt the general health of the animal throughout the course of the experiment. For all treatments, animals were separated into control and HS conditions as described.

Analysis of the stimulus triggered response

The number of action potentials elicited in the DCMD by the looming stimulus was determined in both control and HS animals at each temperature throughout a ramp. Animals that were poorly responsive at lower temperatures (< 30 spikes at 25°C) were eliminated from further analysis, as spike counts below this were found to be indicative of preparations with poor health. The sample size for all experiments diminished at high temperature, as DCMD failure made some animals non-responsive to the looming stimulus. The time of occurrence for each spike, relative to predicted collision, was determined at each temperature increment for all animals using ClampFit 9.0. Results were shown graphically using SigmaPlot 8.0. One- and two-way ANOVAs followed by multiple comparison analysis ($P < 0.05$) were used to determine statistical significance of spike count and instantaneous firing rate data. The response during a looming approach was examined by passing the spike time data through a Gaussian smoothing function (36 ms window) and was then plotted into 5 ms time bins. Mean firing rates represent the average value in each of 120 analyzed time bins (–560 ms → 40 ms). Repeated-Measures ANOVA (RM-ANOVA) statistics were used to assess differences between treatments.

Analysis of the peak firing rate and its timing relative to collision was accomplished by determining the bin with the highest firing rate for each animal and using this rate and timing data to produce a mean value across a given treatment. In reporting mean values, standard deviation (SD) is used for all data. All statistical analysis was performed using SigmaStat 3.0.

Results

DCMD activity is thermoprotected by HS

Spiking activity was reliably induced in the DCMD following the presentation of a computer-generated image with a collision trajectory set at 90° to the eye of the locust. A representative recording is shown in Fig. 1. The response of the DCMD to the looming stimulus was compared throughout a thoracic temperature ramp between control and HS locusts (Fig. 2). Control animals showed a significant thermosensitivity, with the number of spikes elicited decreasing with successive increases in temperature (one-way ANOVA; $F_{(4,53)} = 9.70$, $P < 0.001$). Following HS, the total number of spikes remained relatively constant throughout the temperature ramp (one-way ANOVA; $F_{(4,63)} = 0.74$, $P = 0.57$). There was a significant difference in the total spike count between control and HS animals (Fig. 2b; two-way ANOVA; $F_{(1,116)} = 16.52$, $P < 0.001$).

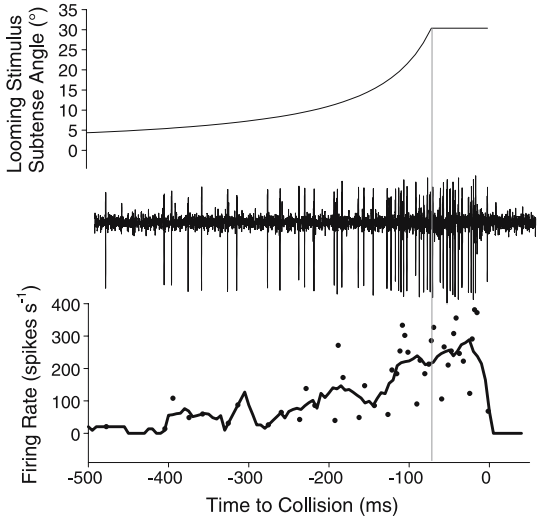

Fig. 1 Response of the DCMD to the looming approach of a computer-generated target. As the angular size of the target increases along its looming trajectory (*top*), the DCMD responds with spiking activity that is clearly distinguishable from background when recorded in the ventral nerve cord (*middle*). The DCMD spike time data (*bottom, dots*) was used to calculate a Gaussian-smoothed firing rate (*line*), which increases to a peak prior to predicted collision. The timing of target stop is indicated by the *solid line*

The DCMD firing pattern was also differentially affected by temperature in control and HS animals. Whereas no difference was found in the highest firing rate between control and HS animals at 25°C, the highest firing rate in HS animals increased significantly compared to control animals as temperature was increased (Fig. 2c; two way ANOVA; $F_{(1,114)} = 20.57$, $P < 0.001$). Both control and HS animals showed broad changes in spike timing at high temperature, with the most common interspike interval range shifting from 3–4 to 1–2 ms. Given that the overall spike counts were not increased in either treatment, this indicates that the spike train was clustered into bursts of higher frequency spikes. Further, the distribution of interspike intervals was significantly different between the control and HS treatments at 45°C, but not at 25°C (Fig. 2d, e; two-way ANOVA; $F_{(1,290)} = 14.61$, $P = 0.003$).

The firing pattern of APs in the DCMD, relative to time to collision, was similar in control and HS animals at 25°C (Fig. 3a; two-way ANOVA; $F_{(1,3360)} = 0.60$, $P < 0.44$). At 45°C, control animals had a significantly lower firing rate compared to the response of HS animals (Fig. 3b; two-way RM-ANOVA; $F_{(1,1186)} = 7.84$, $P = 0.019$). HS animals showed an increase in the firing rate throughout the response at 45°C, particularly at the peak level of activity. Whereas the timing of the peak activity was not different between control and HS animals at either 25 or 45°C (Fig. 3c), there was a significant increase in the peak firing rate of HS animals over control at 45°C (Fig. 3d; t test, $t = 2.88$, $P < 0.05$, $df = 10$). The increase in firing rate at 45°C toward the peak was punctuated with multiple smaller peaks. Most notable in the HS treatment were peaks at –260 and –175 ms. This response profile is in contrast to the more gradual rise found in both control and HS animals at 25°C.

Direct effect of temperature on DCMD activity

To examine how much of the HS effect was due to a direct conduction of heat to the DCMD in the brain, the temperature of the brain was held constant at 25°C during a thoracic temperature ramp. This was done using a separate saline perfusion (Fig. 4a; see methods for details). Under these conditions, the spike count of the DCMD response in both control and HS animals showed thermosensitivity (Fig. 4b; control; one-way ANOVA; $H_{(4)} = 12.76$, $P = 0.013$; HS; one-way ANOVA; $F_{(4,70)} = 5.88$, $P < 0.001$). HS animals, however, were still more thermotolerant than control

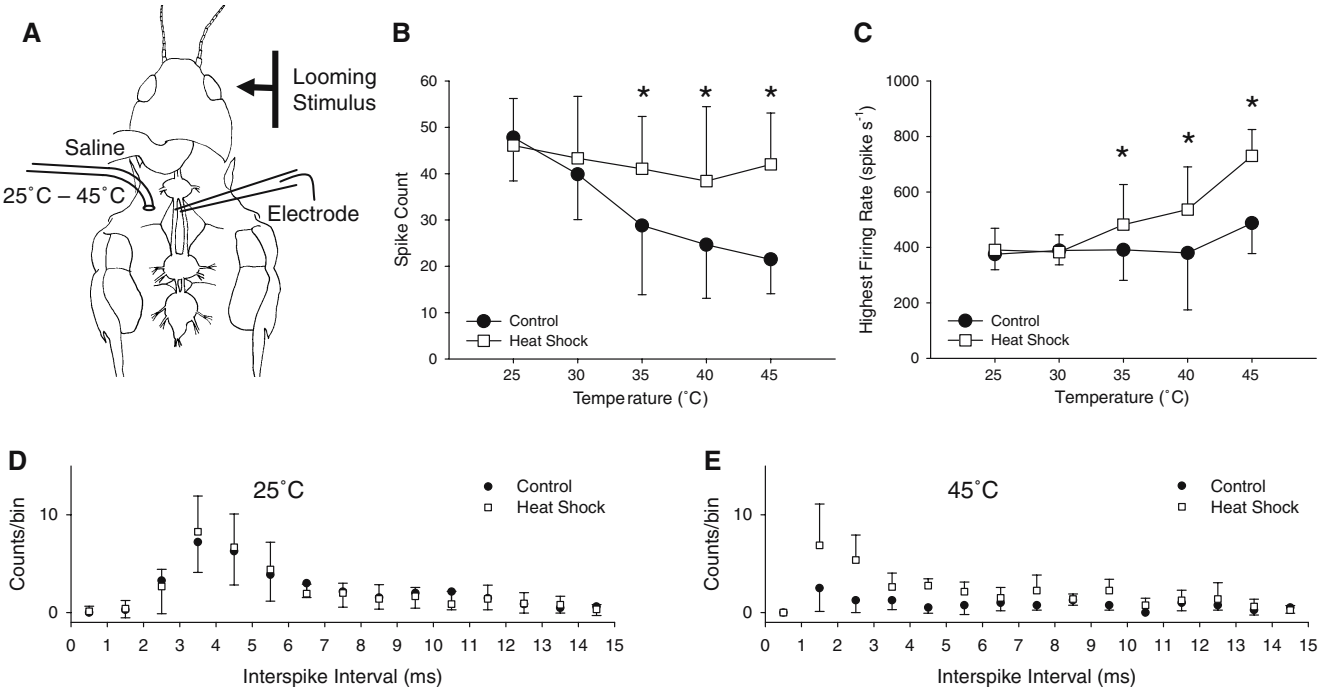

Fig. 2 The effect of thoracic temperature on the DCMD response to looming stimuli. *Asterisks* denote significance by multiple comparison ($P < 0.05$). **a** Experimental preparation. DCMD responses were recorded from the thoracic connective during a local increase in saline temperature delivered through a pipette. **b** The DCMD response was significantly more thermotolerant following HS. Spike count was lower in control animals with successive increases in temperature, but remained relatively constant following HS. **c** The highest firing rate in DCMD between any two spikes during the response was significantly higher in HS animals at high temperature. **d**, **e** Interspike interval (*ISI*) histogram in 1 ms bins for control and HS animals. There was no difference in the mode or mean ISI count between control and HS at 25°C. At 45°C, low ISIs increased in both control and HS, with significantly more ISIs in HS than in control animals. $N = 15$ control, 15 HS

when the brain temperature was held constant (Fig. 4b; two-way ANOVA; $F_{(1,111)} = 14.35$, $P < 0.001$).

Removing thermal influence to the brain region did, however, significantly impair other HS-induced phenomena. With maintained brain temperature, HS animals showed no increase in the maximum firing rate with increased thoracic temperature (Fig. 4c; two-way ANOVA; $F_{(1,131)} = 0.048$, $P = 0.83$). There was no shift in the mode of interspike interval with increasing temperature, which remained between 3–4 ms in control and HS animals at both 25°C and at 45°C (Fig. 4d, e). Similarly, interspike interval distribution showed no change between control and HS at a thoracic temperature of 45°C (Fig. 4e; two-way ANOVA; $F_{(1,493)} = 2.04$, $P = 0.17$).

The spike pattern in HS animals was not different from controls at either 25°C (Fig. 5a; two-way RM-ANOVA; $F_{(1,2880)} = 0.004$, $P = 0.95$) or 45°C (Fig. 5b; two-way RM-ANOVA; $F_{(1,2040)} = 1.84$, $P = 0.192$). DCMD spike rates throughout target approach in HS animals did not show high frequency spiking at 45°C, as was found when the brain temperature was not controlled. The timing of the peak was similar between control and HS and was not affected by thoracic temperatures (Fig. 5c). HS animals, however, showed a decreased peak firing rate at 25°C compared to control animals (Fig. 5d; Mann–Whitney rank sum test; $P < 0.05$). Also, the peak rate of HS animals increased from 25 to 45°C (paired t test; $t = 3.22$, $P < 0.01$, $df = 11$), although their peak firing rate did not differ from control values at this temperature. Together, these findings suggest that while direct effects of temperature on visual processing are required to produce HS-induced effects on high frequency activity, they can not entirely account for the continued thermosensitivity of DCMD spike count during constant brain temperature conditions.

Ascending input indirectly modifies DCMD activity

A change in DCMD activity occurred during a thoracic temperature ramp even when the brain temperature was maintained. This suggests that the DCMD may be modified by input from elements local to the site of the temperature change in the thorax. This was tested by severing the thoracic nerve cord posterior to the recording site (Fig. 6a). Following this treatment, spike count in response to a looming stimulus was similar

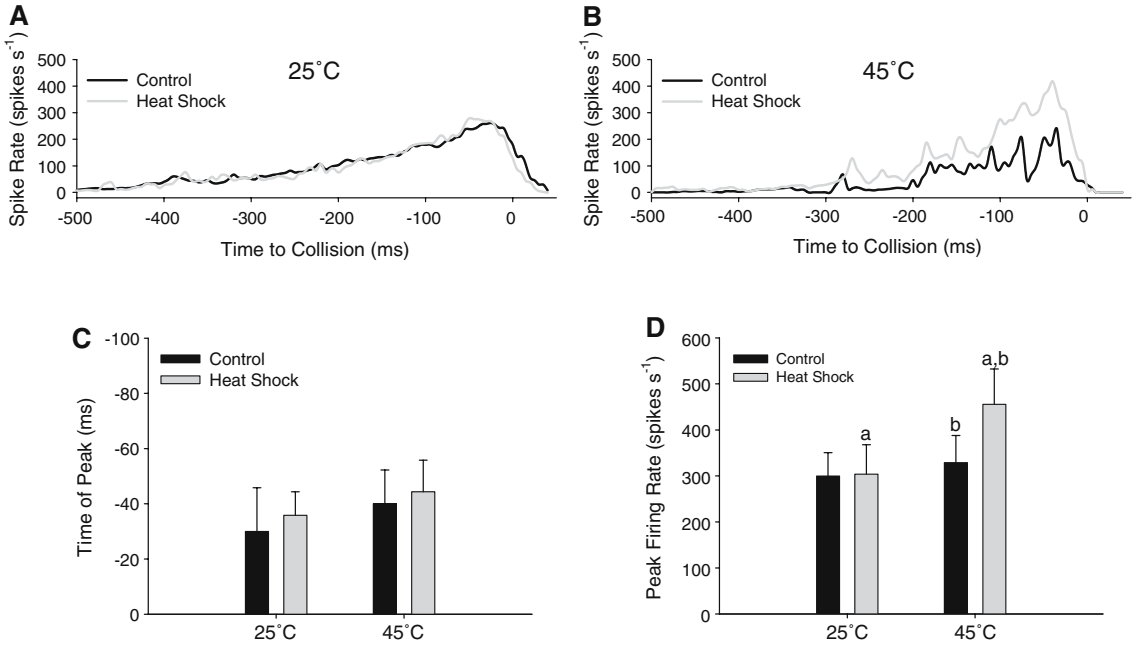

Fig. 3 Mean spike rate throughout looming approach in control and HS animals at 25 and 45°C. Significantly different groups are represented by the *same letter*. **a** At lower temperatures, both control and HS achieved similar firing rates. **b** At 45°C, multiple episodes of high frequency spiking were found in HS, with control responses significantly lower. **c** The DCMD peak time was not different between control and HS at either 25 or 45°C. **d** The DCMD peak firing rate of HS animals at 45°C was significantly higher than firing rates at 25°C as well as that of control animals at 45°C ($P < 0.05$)

between these control and HS animals during an increase in temperature (Fig. 6b; two-way ANOVA; $F_{(1,108)} = 3.21$, $P = 0.076$). In fact, compared to cord-intact animals severing the nerve appeared to increase the maintenance of spike number in control animals at 45°C (*t* test, $t = 2.42$, $P < 0.05$, $df = 10$), whereas the spike count of HS animals was reduced at 45°C (*t* test, $t = 2.32$, $P < 0.05$, $df = 12$).

Both control and HS animals showed an increase in the DCMD's highest firing rate with increasing temperature. This difference was higher in control than HS (Fig. 6c; two-way ANOVA; $F_{(1,120)} = 12.00$, $P < 0.001$). Over the course of the entire response, however, the distribution of interspike intervals was not significantly different between control and HS animals at 45°C (Fig. 6d, e; two-way ANOVA; $F_{(1,319)} = 1.28$, $P = 0.28$).

The firing pattern of the DCMD to looming stimuli was not different between control and HS animals at either 25°C (Fig. 7a; two-way RM-ANOVA; $F_{(1,3000)} = 0.68$, $P = 0.42$) or at 45°C (Fig. 7b; two-way RM-ANOVA; $F_{(1,1440)} = 3.416$, $P = 0.089$). Compared to their non-severed counterparts, lesioned control animals had no significant difference in activity at 45°C (two-way RM-ANOVA; $F_{(1,1073)} = 2.743$, $P = 0.13$) while similarly severed HS animals had decreased activity throughout the response (two-way RM-ANOVA; $F_{(1,1553)} = 8.047$, $P = 0.014$). Following the ventral connective lesion, the timing of the peak activity varied widely in both control and HS (Fig. 7c). The peak appeared to be earlier at high temperature, although this difference was not significant. The peak firing rate increased in control animals from 25 to 45°C (Fig. 7d; repeated-measures *t* test; $t = 3.47$, $P < 0.05$, $df = 6$). Control and HS peak firing rates were not significantly different, however, firing rates for HS animals were highly variable. These results support the hypothesis that DCMD activity is affected indirectly by a thermosensitive input from the thorax or abdomen.

Discussion

Dual regulation of thermosensitivity in the DCMD

The locust descending contralateral movement detector (DCMD) is an interneuron responsible for relaying information about looming objects from the brain to motor centers within the thorax (Simmons 1980; Rind and Simmons 1992). In this study, the DCMD's response to a computer-generated looming stimulus

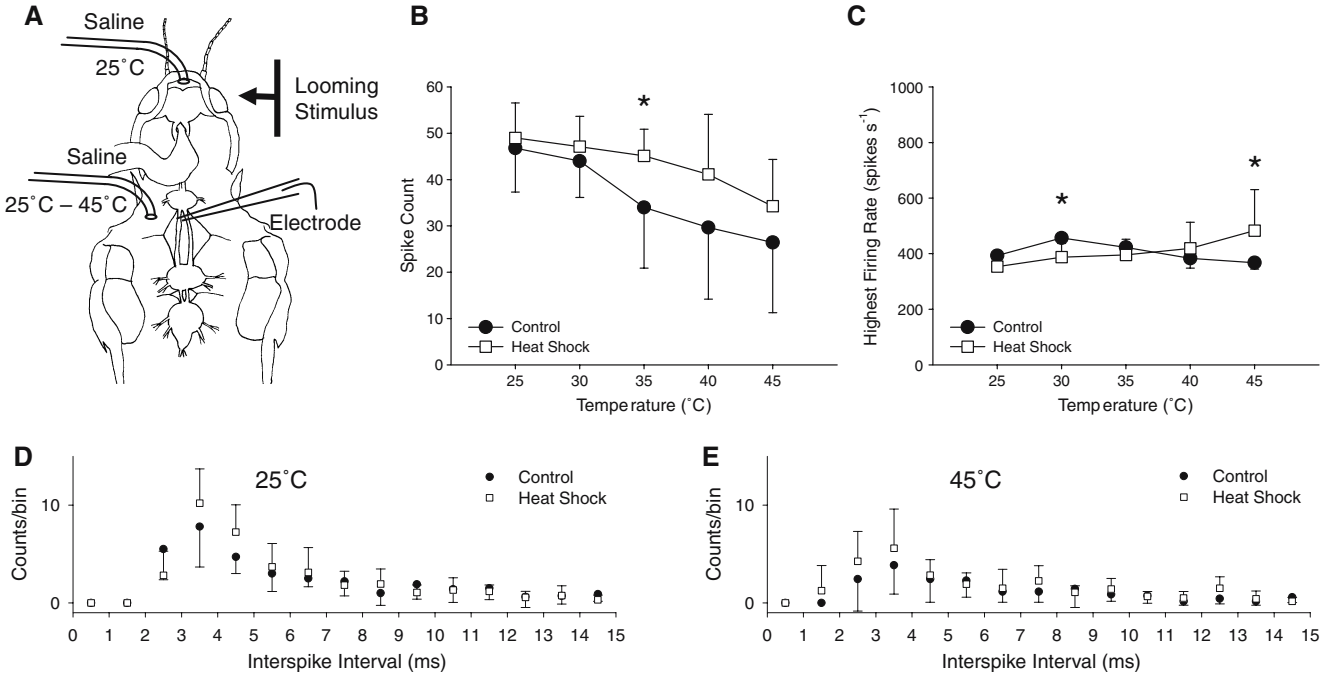

Fig. 4 Holding the brain temperature constant during a thoracic temperature ramp did not eliminate thermosensitivity. *Asterisks* denote significance at $P < 0.05$ (multiple comparison). **a** Brain temperature was maintained at 25°C through the use of a second saline perfusion while DCMD was recorded from the thoracic connective. **b** The DCMD response of both control and HS animals showed thermosensitivity. HS animals had significantly higher spike counts than control. **c** No clear increase in the maximum firing rate of HS animals was found when the brain temperature was maintained. **d**, **e** No difference was found in the mode or mean ISI between control and HS at 25 or 45°C. $N = 10$ control, 16 HS

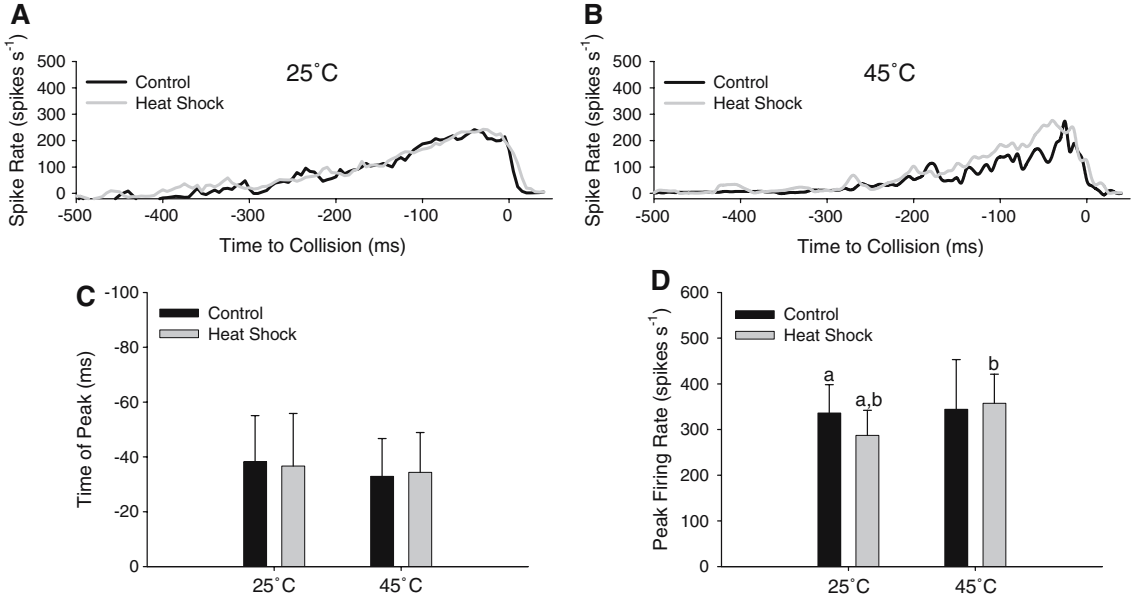

Fig. 5 Analysis of firing rate thermosensitivity during constant brain temperature conditions. Significantly different groups are represented by the *same letter*. HS treatment did not affect mean spike rate at 25°C (**a**) or at 45°C (**b**). **c** The time of the peak did not differ between control and HS at either 25 or 45°C. **d** The peak firing rate was decreased in HS animals at 25°C compared to control animals. However, the firing rates were equal at 45°C and were at similar activity levels as was seen in control animals at 25°C

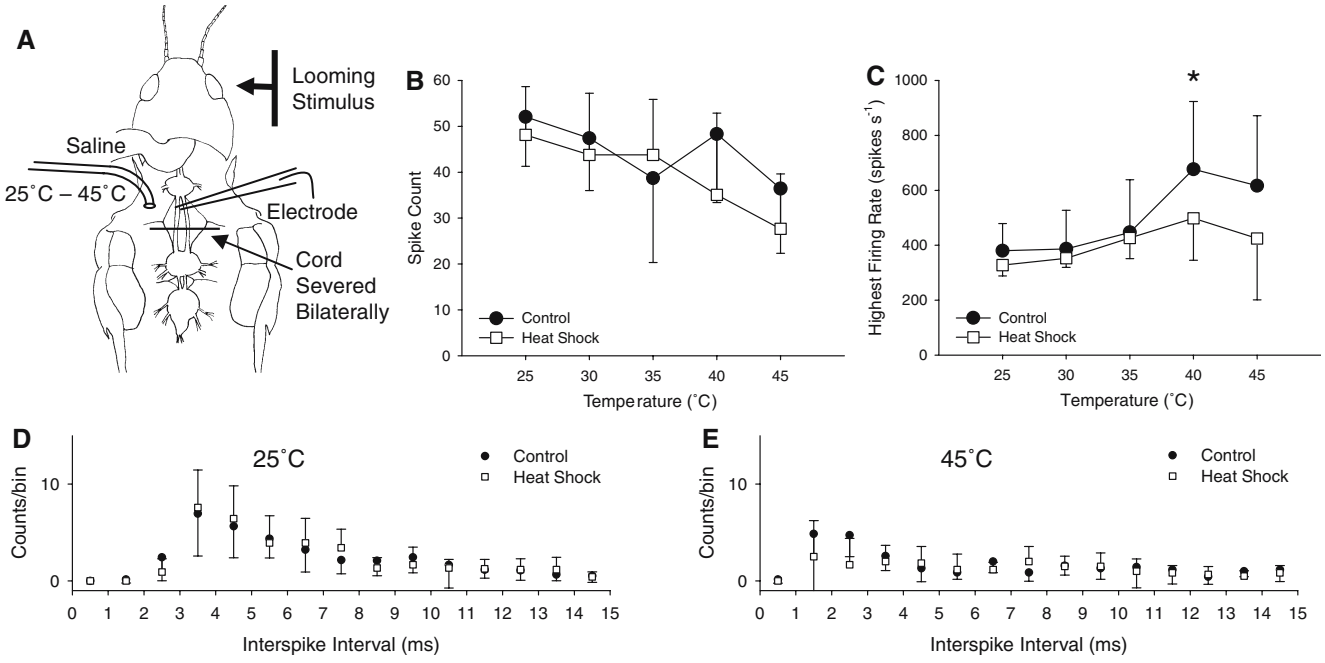

Fig. 6 Severing the thoracic nerve cord affects DCMD thermosensitivity. *Asterisks* denote significance at $P < 0.05$ (multiple comparison). **a** The cut was made bilaterally and posterior to the recording electrode. **b** The number of spikes produced in response to a looming image was similar between control and HS with increasing temperature. Control responses were improved compared to non-severed counterparts. **c** The maximum firing rate was increased in both control and HS at high temperature, with a greater increase in control animals. **d**, **e** The mode of the ISI histogram decreased as temperature increased in both control and HS. Control and HS animals had ISI distributions that were not significantly different. $N = 14$ control, 14 HS

was examined during a ramped increase in thoracic temperature. The response profile was altered in both control and HS animals at high temperature. At 45°C, control animals generated fewer DCMD action potentials. By contrast, HS animals had a maintained number of action potentials at 45°C with short bursts of

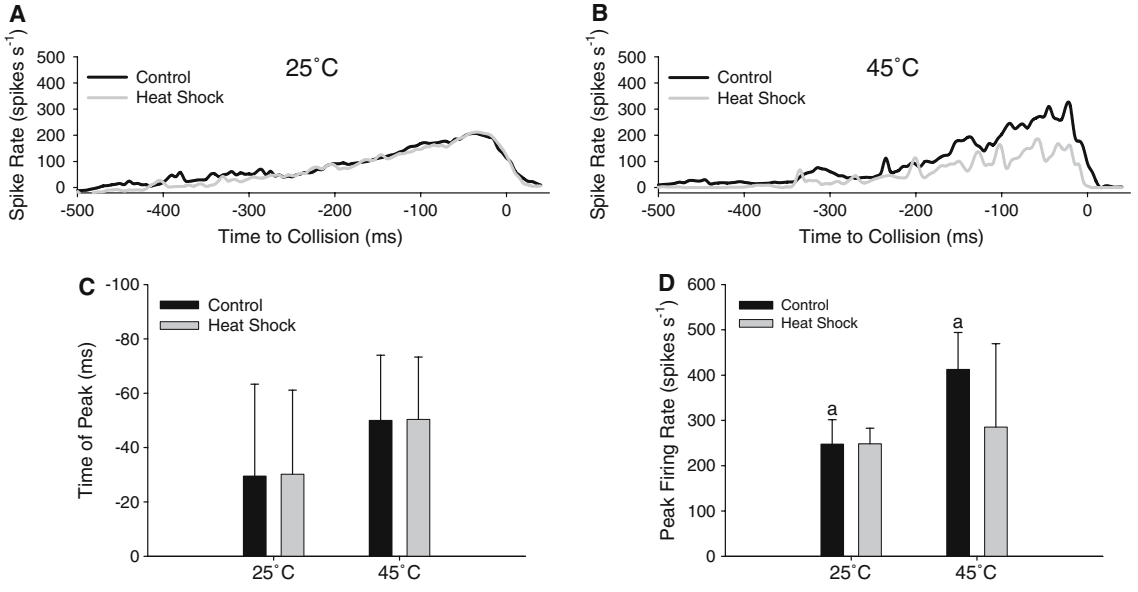

Fig. 7 Severing the nerve cord affects DCMD mean spike rate. Significantly different groups are represented by the *same letter*. **a** Control and HS animals were similarly responsive to looming stimuli at 25°C. **b** HS animals showed reduced activity throughout much of the response at 45°C. **c** Timing of the peak was not different between control and HS animals at either 25 or 45°C. **d** Peak activity was significantly increased in control animals, but not HS, at 45°C

spikes occurring at a higher rate than occurred at 25°C. Similar results have also been obtained previously using a mechanically-looming target (Money et al. 2005).

Despite the considerable plasticity shown by HS animals, the DCMD of both control and HS animals displayed some similarities in their response to high temperature, characterized here as a shift in the interspike interval histogram. Both treatments showed an increase in low interspike intervals at increased temperature. In control animals, these changes occurred simultaneously with a decrease in the overall spike count. In HS, however, these temperature-dependent phenomena occurred with a spike count that in many cases was maintained at its 25°C level. This resulted in a potentiation of the temperature-dependent aspects of the response profile, and may in large part account for the clear differences between control and HS response to approaching objects. It is important to note, however, that while HS dramatically alters the DCMD response profile, it does not fundamentally change the time of the peak relative to collision. The timing of the peak firing rate has been shown to be related to an angular threshold of the approaching target (Gabbiani et al. 1999), and has also been correlated with behavioral output (Hatsopoulos et al. 1995). As HS strengthens this part of the response, these effects can be viewed as enhancing the animal's capacity to respond to approaching objects at high temperature. Whether the HS effects shown here translate into an increase in escape behavior is unknown. However, evidence of DCMD's role in generating a gliding escape behavior has been recently described (Santer et al. 2006), and sustained high frequency spiking in DCMD (> 150 Hz) is required to produce sufficient summation to elicit the glide. HS-induced plasticity is further demonstrated in the increased number of high frequency bursts of activity prior to the point of peak activity, resulting in a series of secondary peaks. Other such aspects of the DCMD response have been implicated as important in behavior (Matheson et al. 2004). The short bursts of activity shown here could be sufficient to produce significant excitation in post-synaptic motor circuits, which would facilitate the initiation of relevant behaviors.

The distinct DCMD responses of control and HS animals to temperature indicate that visual processing is capable of temperature-dependent plasticity. The findings of this study could be useful in identifying how HS is having its effect, the mechanism of which is presently unknown. The DCMD acts as the output for this motion-detecting visual circuit, with its firing rate mirroring that of its presynaptic partner LGMD (Rind 1984). Computation of looming objects is thought to be achieved by the LGMD, which receives retinotopically organized excitation and feedforward inhibition (Rowell et al. 1977; Rind 1996; Gabbiani et al. 2005). As such, HS could act on the DCMD or any presynaptic components of the visual circuit. A different balance between the excitatory and inhibitory inputs onto the LGMD dendrites in control and HS could account for the different response profiles at high temperature. However, changing the strength of the feedforward inhibition relative to the excitatory drive would be expected to change the timing of the peak (Gabbiani et al. 2005). As this was not the case in the present experiment, this is not likely how HS is acting in this system. Alternatively, the difference between control and HS animals may result from a modulation of spike transmission ratio across the LGMD:DCMD synapse. Short-term plasticity has been demonstrated synaptically in other visual circuits (Blitz and Regehr 2003). A facilitation following HS is supported by known effects of HS to stabilize function at locust central synapses (Dawson-Scully and Robertson 1998) and neuromuscular junctions alike (Barclay and Robertson 2000). HS also modifies the intrinsic excitability of the DCMD, and at high temperature induces post-inhibitory rebound activity and action potentials with large after-depolarizations (Money et al. 2005). These events can increase activity at spike-initiating zones (Jensen et al. 1996) and improve conduction reliability in axons (Bowe et al. 1987; McIntyre et al. 2002). At high temperature, a HS-induced increase in DCMD excitability could account for the differences found between control and HS responses to looming stimuli. As these mechanisms are not mutually exclusive, HS could potentially act on multiple targets.

When the brain was held at a constant temperature, both control and HS animals still showed significant thermosensitivity. The decrease in spike count occurred without significant change to other temperature-sensitive elements of the response, namely the pattern of interspike intervals. Elimination of these effects indicates that they are dependent upon visual processing circuits experiencing an increase in temperature. However, deleterious effects on spike count are still seen in the absence of temperature increase. This reduction in spike count is lessened following HS. The direct effect of temperature on visual processing, therefore, cannot explain all of the observed phenomena. This indicates that an indirect modulation occurred in response to an increase in thoracic temperature. To test the hypothesis that an ascending input from the thorax was responsible for the indirect thermosensitivity, the nerve cord was bilaterally

severed above the mesothoracic ganglion. This experimental manipulation eliminated much of the thermosensitivity found in control animals. Following the lesioning, control locusts showed significantly more DCMD activity than normal at high temperature. These findings demonstrate that ascending thoracic nerve inputs play a role in setting the level of DCMD activity.

Motor activity in the locust can suppress the DCMD response to visual cues, as shown during a visual saccade (Zaretsky and Rowell 1979; Zaretsky 1982), during antennal cleaning (Rowell 1971), or during a jump (Heitler 1983). Each of these responses is thought to represent a form of corollary discharge; the problems associated with reafference are mitigated through the inhibition of sensory areas that are likely to be affected (Poulet and Hedwig 2002; Webb 2004). Severing the connectives between the mesothoracic and metathoracic ganglia has been shown to prevent the DCMD suppression associated with motor activity during a locust defensive jump (Heitler 1983), indicating that ascending neural inputs from the thorax can modulate DCMD activity. Heitler (1983) concluded that the suppression resulted from increased central arousal related to a behavior, and not to proprioceptive feedback associated with the action. In addition, Rowell and O'Shea (1980) showed that spontaneous activity recorded in the thoracic connective results in IPSPs at DCMD integrating segments that reduce the probability of spike initiation. In our experiments, responses in control animals were suppressed not from the initiation of a specific motor task, but rather during an increase in temperature. However, the basal level of activity in thoracic and abdominal circuits would be expected to increase at high temperature (Xu and Robertson 1994), which in turn would explain the increased level of DCMD inhibition in temperature-naïve control animals.

The severing of the connective had contrary effects on the DCMD in control and HS animals at 45°C. Function was improved in control animals whereas HS animals displayed a marked decrease in activity, suggesting that ascending input affects visual circuits differentially between these treatments. Differences in the level of ascending activity between control and HS animals may account for the different responses shown by each at high temperature. Alternatively, the difference could be due to a HS-induced change in the effect the ascending input has on visual circuits. Membrane potential in the DCMD is more hyperpolarized at high temperature following HS (Money et al. 2005), which may affect the amplitude of synaptic events compared to control animals. The known differences in DCMD excitability between control and HS animals may result in diverse outcomes to the same input. This question needs to be resolved by making intracellular recordings from DCMD neuropil, in which synaptic inputs onto the DCMD can be quantified (Rowell and O'Shea 1980). These experiments should allow for a determination of the impact thoracic inputs have on setting DCMD activity levels, and how this is modified following HS.

Functional significance

In its natural environment, the locust is routinely exposed to high temperature. Gregarious locusts reach thoracic temperatures of 45°C within a few minutes while basking in the sun (Uvarov 1966), indicating that the time frame of heating in the present study would be realistically encountered in a natural setting. The results obtained in the present study illustrate a thermotolerance mechanism used by locusts daily to adjust and maintain function in the DCMD.

The DCMD is thought to be involved in the generation of escape responses (Rind and Simmons 1992; Gray et al. 2001; Gabbiani et al. 2004; Santer et al. 2005, 2006). Peak activity in DCMD has been shown to be associated with the femoro-tibial flexion needed for locust jumping (Hatsopoulos et al. 1995). Maintaining DCMD activity at high temperature should strengthen femoro-tibial flexion, and could enhance predator evasion following HS by improving the likelihood of success in triggering an escape behavior. The primary role of the ascending input is likely to gate DCMD activity during thoracic behaviors that produce self-motion in the visual field. A second role, however, appears to be to provide feedback to sensory visual circuits about the thoracic thermal environment, modifying DCMD activity in a temperature-dependent manner. In the behaving animal, temperature gradients are produced along the body axis during times of high activity (Church 1960; Weis-Fogh 1956). This creates different thermal environments between the site where visual cues are processed and the site of the consequent motor action. The ascending feedback may act to compensate for this variation through changes in the level of DCMD activity, allowing the DCMD to most effectively trigger a behavioral response despite the constraints placed on the circuitry by increased temperature.

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