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ORIGINAL PAPER

Troy Zars

Two thermosensors in *Drosophila* have different behavioral functions

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Abstract Insects inhabit extreme temperature environments and have evolved mechanisms to survive there. Small insects are especially susceptible to rapid changes in body temperature. Therefore, the rapid detection of environment and body temperature is important for their survival. Little, however, is known about the thermosensors that detect those temperatures. Using rapid thermosensitivity assays with temperature step gradients and a spatial learning paradigm (the heat-box) in which elevated temperature serves as the negative reinforcer, two thermosensors were identified and their behavioral functions assessed. A low-temperature thermosensor is located on the antenna, detects relatively low temperatures, and can detect spatial temperature gradients directly. Thus, the antennae can be used by *Drosophila* to quickly orient with respect to temperature cues. A high-temperature thermosensor of unknown location appears to have a roughly similar sensitivity to temperature differences as the low-temperature thermosensor (≤ 3°C) and is both necessary and sufficient for memory formation in the heat-box spatial learning paradigm. Therefore, the high-temperature thermosensor is important for remembering spatial positions in which dangerously high temperatures were encountered.

Keywords *Drosophila* · Spatial learning · Thermosensor · Antenna

Introduction

Insects inhabit a wide temperature distribution, being present from polar glaciers to high-temperature deserts. Necessarily, they have thermo-protective mechanisms to both survive and flourish in these extremes. Protection from death by frigid temperatures largely depends on the ability to supercool or on a tolerance for freezing while survival at high temperatures depends on physiological responses and cellular stress mechanisms (Feder and Hofmann 1999; Heinrich 1993; Sinclair 1999; Somme 1999). Most insects, however, are found in less hostile temperature environments. In these cases, some of the larger insects have physiological mechanisms for regulating at least part of their bodies' temperature (Harrison and Roberts 2000; Heinrich 1993). This phenomenon is largely applied to the maintenance of optimal flight muscle temperatures for flight at variable ambient temperatures. Small insects (less than about 50 mg) have the difficulty of having a large surface area to volume ratio. Thus, convection dissipates any heat generated from flight muscle contraction leaving the small insects unable to physiologically upregulate body temperatures in cold environments (Heinrich 1974; Heinrich 1993; Unwin and Corbet 1984). The opposite problem also exists for small insects, that is rapid rises in body temperature with heat source exposure (e.g. infrared radiation). It has been calculated that the temperature of a 10-mg fly could rise by 10°C with a 10-s exposure to sunlight (Heinrich 1993). The dependence of the body temperature of small insects on ambient temperature and other heat sources make it likely that behavioral mechanisms for maintaining body temperatures in a permissive range are important. Microhabitat selection is one mechanism (Heinrich 1993; Huey 1991). In an extreme example, mosquitoes and other small dipterans have been found to bask in the 'bowls' of arctic flowers, thus raising the body temperature of these insects approximately 6–15°C above ambient temperature (Hocking and Sharplin 1965; Kevan 1975).

The control of insect body temperature by physiological or behavioral means implies the presence of thermosensors. Indeed, thermosensors have been identified in several anatomical locations of different insect

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T. Zars
Theodor Boveri Institut für Biowissenschaften,
Lehrstuhl für Genetik und Neurobiologie,
Am Hubland (Biozentrum),
97074, Würzburg, Germany
E-mail: zars@biozentrum.uni-wuerzburg.de
Tel.: +49-931-8884455; Fax: +49-931-8884452

species. In *Cecropia* moths, a thermosensor in the thorax has been postulated because heating the thoracic ganglion (but not the flight muscles) induced flying behavior and increased heart rate (Hanegan 1973; Hanegan and Heath 1970). Using electrophysiological measurements and preferences on a temperature gradient, cockroaches were shown to have peripheral (antennal and tarsal) and central thermosensors (prothoracic ganglion) (Murphy and Heath 1983). In addition, a thermosensor has been postulated in the head of honeybees and yellowjackets as heating of the head (but not the thorax) induced honeycrop regurgitation, a behavior used for evaporative heat loss in these species (Coelho and Ross 1996; Cooper et al. 1985; Heinrich 1980a, 1980b). The presence of thermosensors in small insects has been proposed because of preferences on a temperature gradient and orientation toward heat sources (Flores and Lazzari 1996; Lazzari and Núñez 1989; Sayeed and Benzer 1996). In *Drosophila*, an antennal thermosensor and a second high-temperature sensor have been postulated based on flies' temperature preferences around 24°C (Sayeed and Benzer 1996). This preference was largely abolished with antennal removal. However 'antennaeless flies continued to avoid temperatures above 31°C' implying the presence of a second high-temperature thermosensor.

The purpose of this study was to test the hypothesis that *Drosophila* has two thermosensors and that these are sensitive to different temperature ranges. To do this, surgically and genetically altered flies were tested for preferences in a thermosensitivity assay that has rapidly changing temperatures. This assay (compared to linear thermal gradients) may better reflect the rapidly changing temperatures insects encounter in the environment. In addition, the approximate sensitivities of these putative thermosensors and their behavioral significance were explored using a variation of the thermosensitivity assay and learning experiments.

Materials and methods

Flies were raised at 25°C and 60% humidity on standard corn meal/molasses medium (Guo et al. 1996) with an artificial 14-h light/10-h dark cycle. Flies between 2 days and 7 days of age were kept overnight at 25°C in new food vials with a dry filter paper before behavioral testing. They were not anesthetized. Canton S (CS) was used as the wild-type *Drosophila melanogaster* strain. To remove antennae, CS flies were immobilized in a shortened 200-μl pipette tip so that the antennae were just protruding from the tip end. The third antennal segments, including the aristae, were removed with a fine tipped forceps and are the CS n.a. flies used in all the experiments. Pilot experiments removing the complete antennae showed no increased thermosensitivity deficit compared to flies lacking only the third antennal segment (data not shown). The *bizarre* (*biz*) mutant was originally isolated as the non-phototactic mutant, SB8 (Benzer 1967). Homozygous *biz* mutant flies were recently published as defective in thermosensation (Sayeed and Benzer 1996). Both CS and *biz* flies have been maintained in Würzburg for more than 20 years. The *spineless*^{aristapedia} mutant (*ss*^{a}) transforms the third antennal segment into a leg-like structure (Stocker 1982; Struhl 1982) and was recently received from the Bloomington Indiana stock center.

Thermosensitivity assays

The thermosensitivity assays used a chamber (40 mm×5 mm×2 mm) with Peltier elements as floor and ceiling with the temperature of the front and back halves of the chamber independently controlled, as described in (Zars et al. 2000). The chamber is lined with an array of light gates through which the position of individual flies can be continuously monitored. In the first 'simple' thermosensitivity assay, a reference temperature of 24°C was always kept in one half of the chamber and the other half was stepped to 27, 30, 33, 37, 41, or 45°C. The side of the chamber set to the reference temperature changed every minute, thus forcing flies to make decisions about temperature preference. Depending on the temperature, all points in the chamber reached their final temperature within 2–6 s as measured by thermistors intimately contacting the Peltier elements. To measure thermosensitivity at different reference temperatures, a second 'interval' thermosensitivity assay was used. It kept 3°C or 6°C differences between chamber halves and raised the reference temperature by 3°C from a low of 15°C. Again, the low reference temperature was switched to opposite chamber halves every minute. The time spent on the high and low temperature sides was automatically measured and used to generate performance indices (below).

Learning experiment

The heat-box learning paradigm has been described (Zars et al. 2000) and is a modification of the original heat-box spatial learning paradigm (Wustmann and Heisenberg 1997; Wustmann et al. 1996). The same chambers were used in this and the thermosensitivity assays but both halves were kept at the same temperature and changed simultaneously. During a pretest phase of 30 s the chamber was maintained at the reference temperature of 24°C. However, if during the 4 min of training a fly ran across the midline the chamber heated to a predetermined temperature (in experiments described here, 30°C or 37°C). The chamber cooled to 24°C when the fly returned to the original side. Finally, the 3 min following training was the memory test and the punishing contingency was removed. The time spent in the punished and unpunished chamber-halves was automatically measured and used to calculate the performance index.

Calculations

The performance index was calculated for the learning experiment and the thermosensitivity assays by subtracting the time spent on the 'punished' side or high temperature side from the time spent on the 'unpunished' side or low temperature side of the chamber all divided by the total time, respectively. In the learning experiment the performance index was calculated for the pretest, training, and memory test phases. The pretest performance index, indicating spontaneous side preference (typically less than 0.05), was subtracted from training and test phase performance indices and each was summarized as single bars.

Statistics

In the thermosensitivity and learning experiments repeated-measures ANOVAs were used. Duncan multiple post-hoc tests were used when warranted and differences were considered significant when P was less than 0.05.

Results

To determine if two thermosensors in *Drosophila* are responsible for detecting different temperature ranges, as suggested by Sayeed and Benzer (1996), flies with and without antennae were tested in a new thermosensitivity assay. Briefly, in this 'simple' thermosensitivity assay flies were placed in a small chamber that held one half of the chamber at a reference temperature of 24°C and the other half at a higher 'test' temperature. The flies were given 1 min to show a preference for one of the two temperatures and then were presented with another temperature pair with an even higher test temperature. The antennae were either removed surgically or by the genetic mutant *ss*^{a} (Stocker 1982; Struhl 1982). In addition, the *biz* mutant was tested as it was shown previously to have no thermopreference over a wide temperature range (Sayeed and Benzer 1996). When tested here, homozygous *biz* mutant flies were extremely sluggish (data not shown) and did not lend themselves to fast thermopreference tests. Therefore, heterozygous *biz* mutant flies (*biz*/+), with activity similar to wild-type and treated flies (data not shown), were tested. Wild-type CS flies showed the first significant response to a temperature of 27°C (Fig. 1), a difference in temperature from the reference of 3°C. All treated fly types failed to show a significant response to temperatures below 33°C but showed a clear preference for the reference temperature when the test temperature exceeded this level (Fig. 1). Thus, flies' thermosensitivity could be altered with surgical and genetic manipulation leading to a deficit either in sensing temperatures below 33°C or, alternatively, in detecting temperature differences smaller than 9°C.

To address this alternative, the sensitivity of altered flies was examined using the 'interval' thermosensitivity assay. This assay gave flies 1 min to choose between two chamber halves that were always 3°C or 6°C apart, starting at a low temperature of 15°C and increasing by 3°C to a maximum high temperature of 45°C (Fig. 2). The altered flies would show no response to 3°C or 6°C differences over the range of temperatures if they were defective in measuring temperature differences. If, in contrast, they were defective in measuring temperature per se (e.g. below 33°C) they would show reactions to temperature differences above some low threshold. Interestingly, wild-type flies showed the first significant response to 3°C differences at a high temperature of 27°C (Fig. 2A). The magnitude of the preference for the low temperature side gradually increased as the low temperature increased up to 39°C. For the 42°C/45°C choice the performance index decreased but the flies still showed a significant preference for the lower temperature. Antennae-less and heterozygous *biz* mutant flies (*biz*/+) showed the first significant response to 3°C differences at a temperature step from 30°C to 33°C (Fig. 2A). The response to 6°C temperature differences had similar thresholds, although the preference was higher. Wild-type flies responded to the same high temperature of 27°C (Fig. 2B). All three groups of treated flies had their first 6°C responses at the same high temperature of 33°C (Fig. 2B). Apparently, the genetically and surgically treated flies have a reduced range of thermosensitivity at the low end but are normal in their ability to detect temperature differences (3°C or 6°C) in the range of their thermosensitivity. Obviously, flies have a high-temperature thermosensor and this appears to be as sensitive to temperature differences as the antennal one. It, however, covers the upper temperature range.

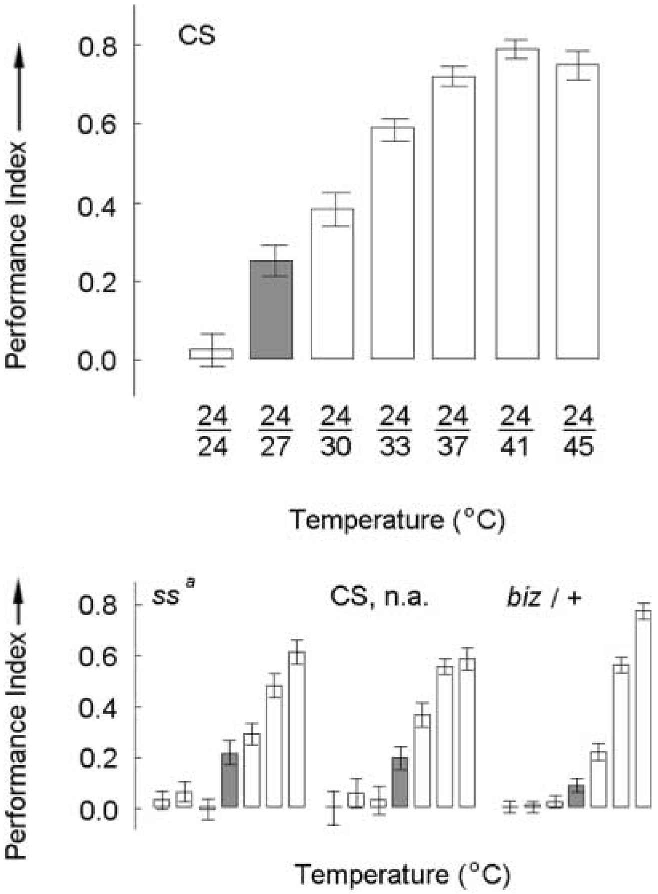

Fig. 1 Reduced thermosensitivity in surgically and genetically modified flies. The temperatures in the chamber halves are shown below the wild-type Canton S (CS) performance index values and were used for all experimental fly types; *error bars* are SEMs. A single temperature step was maintained for 1 min in this 'simple' thermosensitivity assay. All fly types show increased avoidance of the side of the chamber heated to higher and higher temperatures. The first temperature to which wild-type CS flies respond is 27°C (*gray bar*, $n=104$, $P<0.001$). Flies with genetically transformed antennae respond significantly to 33°C (gray bar, *ss*^{a}, $n=78$, $P<0.001$) as do CS flies without antennae (*gray bar*, CS n.a., $n=51$, $P<0.001$) and flies heterozygous for the *bizarre* mutation (*gray bar*, *biz*/+, $n=73$, $P<0.01$)

To explore the possibility that the two thermosensors might have different behavioral functions, the 'heat-box' learning paradigm was used under conditions in which the low, high, or both thermosensors could be used. In this assay, flies are free to run in a dark chamber and when they cross an invisible midline the whole chamber heats to a defined temperature (30°C or 37°C in this case). Normal flies quickly learn to avoid the side of the chamber associated with high-temperature punishment and continue to do so even when the punishment contingency is removed (Wustmann and Heisenberg 1997;

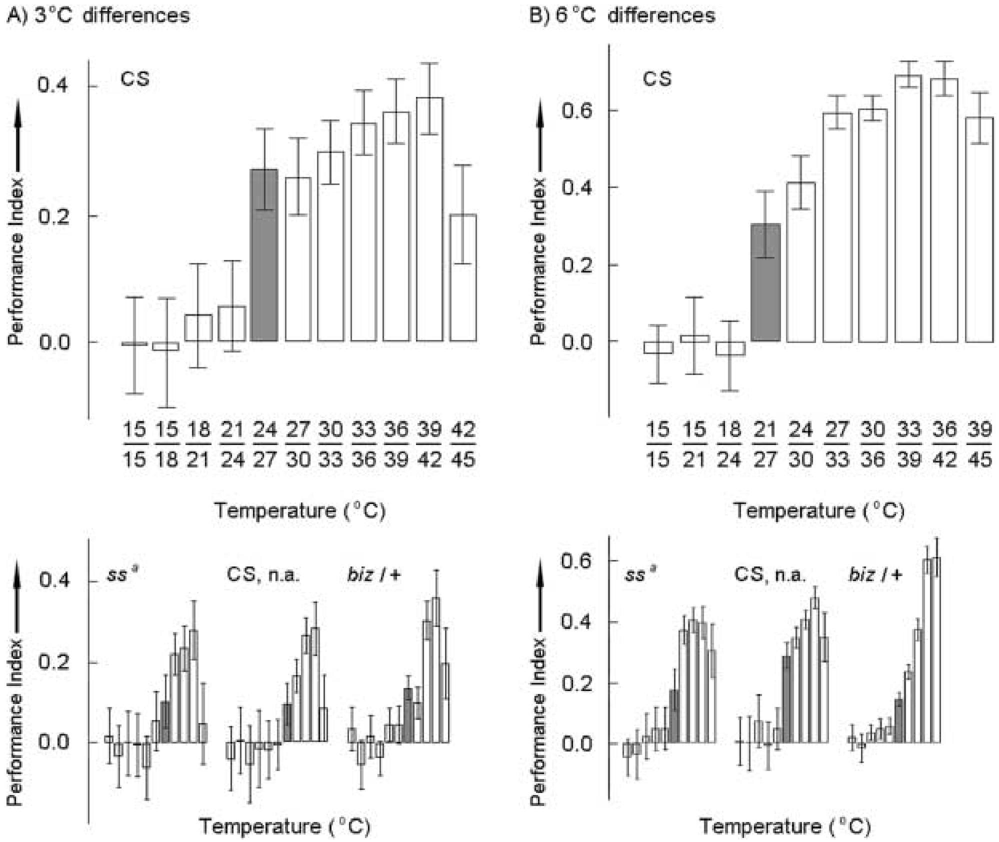

Fig. 2A, B Antennal removal and the *bizarre* (*biz*) mutation reveal a thermosensor responsible for measuring temperatures above 30°C. The 'interval' thermosensitivity assay tested flies' responses to 3°C (**A**) and 6°C (**B**) differences at reference temperatures starting from a minimum of 15°C and increasing by 3°C every minute. The temperature in the chamber halves are shown below the wild-type Canton S (CS) performance index scores and were used for all experimental fly types; *error bars* are SEMs. In wild-type CS flies, 27°C was the lowest temperature at which a 3°C difference was significant enough to warrant side preference (*gray bar*, $n=102$, $P<0.001$). This limit was shifted up to 33°C for flies lacking antennae (*gray bars*, ss^a $n=78$, $P<0.05$; CS n.a. $n=97$, $P<0.05$) and heterozygous for the *biz* mutation (*gray bar*, $n=78$, $P<0.01$). A 6°C difference was significant when the upper temperature was 27°C in wild-type CS flies (*gray bar*, $n=77$, $P<0.001$). This was, again, shifted to 33°C in flies lacking antennae (*gray bars*, ss^a $n=78$, $P<0.01$; CS n.a. $n=75$, $P<0.001$) and heterozygous for the *biz* mutation (*gray bar*, $n=77$, $P<0.01$). The response to 3°C differences at a 45°C upper temperature was reduced in wild-type and *biz* heterozygous flies but was still significantly different from zero (wild-type $P<0.001$; *biz*/+ $P<0.001$). Removal of antennae reduced 3°C responses to levels not different from zero at these temperatures. Responses to 6°C differences were also reduced at the 45°C upper temperature in all fly types except *biz*/+, although all were still significantly different from zero. Note scale differences in **A** and **B**

Wustmann et al. 1996; Zars et al. 2000). In Fig. 3, 4 min of training and 3 min of memory measured directly after training are summarized in single bars. Wild-type flies, having both high- and low-temperature thermosensors, performed the avoidance task during training and formed a memory as expected using punishing temperatures high enough to excite the high-temperature thermosensor (Fig. 3A). Interestingly, flies with only the high-temperature thermosensor (antennae-less) showed avoidance behavior during training and memory scores not different from wild-type flies using 37°C as punishment (Fig. 3A), although the ss^a mutant flies' scores were somewhat lower. Wild-type CS flies lacking antennae are the best for comparison with non-treated wild-type flies since genetic background differences are negligible. The reduced ss^a mutant flies' scores likely reflect differences in genetic background or pleiotropic mutant effects. Flies heterozygous for the *biz* mutation (*biz*/+), having a functional high-temperature thermosensor, showed no significant avoidance behavior or memory. When flies were allowed to use only the low-temperature antennal thermosensor in this learning task (using 30°C as punishment) they could perform the simple avoidance task but no memory was formed (Fig. 3B). Antennae-less flies showed neither avoidance behavior during training nor memory with this protocol, consistent with their defect in sensing temperatures below 33°C. Flies heterozygous for *biz* were not tested with the 30°C punishment as they already showed nearly no avoidance behavior using the 37°C punishment. Taken together, the low-temperature antennal thermosensor is sufficient but not necessary for orientation in the chamber during training and the high-temperature thermosensor is both necessary and sufficient for memory formation.

A comparison of performance in the thermosensitivity assays and the learning experiments in wild-type and altered flies shows that antennae provide improved temperature avoidance if a spatial gradient is present. In

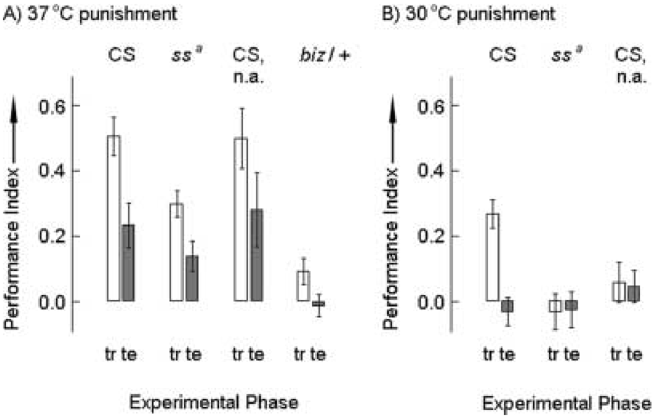

Fig. 3A, B The high-temperature thermosensor is necessary and sufficient for learning in the heat-box. Flies were trained for 4 min (*white bars*, *tr*) and tested for continued side preference in the 3 min directly following training (*gray bars*, *te*); *error bars* are SEMs. The punishing temperature was either 37°C (**A**) or 30°C (**B**). Surgical removal of antennae in wild-type flies (CS n.a.) and the homeotic antennal transformation mutation, ss^a, did not affect training and test phase performance using 37°C punishment ($P > 0.05$, $n = 65$; $P > 0.05$, $n = 102$, respectively). There was no significant avoidance behavior or memory score in flies lacking antennae when the punishing temperature was 30°C ($P > 0.05$, $n = 89$ for ss^a and $n = 57$ for CS n.a.). Wild-type Canton S (CS) flies showed significantly higher training and test phase performance than heterozygous *bizarre* (*biz*/+) mutant flies using 37°C punishment ($P < 0.001$, $n = 121$ for CS, $n = 102$ for *biz*/+). Wild-type CS flies showed significantly lower training avoidance and memory scores when the punishment was reduced to 30°C ($P < 0.01$, $n = 64$)

the simple thermosensitivity assay, wild-type CS flies detect a spatial temperature gradient and show a performance for the 24°C/37°C temperature difference at a level of 0.72 ± 0.026. Performance was significantly lower in the learning experiment in which a representation of a temperature gradient must be derived (0.49 ± 0.048; $p < 0.001$) (Wustmann et al. 1996; Wustmann and Heisenberg 1997). Also, when the high or punishing temperature was 30°C, the performance of wild-type CS flies in the simple thermosensitivity assay (0.38 ± 0.044) and the interval thermosensitivity assay (0.41 ± 0.068) was significantly higher than in the learning experiment (0.27 ± 0.04; $P < 0.001$ and 0.05, respectively). Antennae-less flies showed no significant performance differences in the simple thermosensitivity assay and learning experiment using the 24°C/37°C temperatures ($P > 0.05$). Heterozygous *biz* mutant flies showed a higher performance in the simple thermosensitivity assay compared to the learning assay (0.22 ± 0.055 versus 0.088 ± 0.034), although this was not significantly different ($P = 0.07$).

Discussion

Thermosensors

The thermosensitivity assays, together with antennal removal and the *biz* mutant, revealed two morphologically independent thermosensors. A thermosensor on the antenna is responsible for sensing relatively low temperatures and a second thermosensor senses the upper temperature range. Antennae-less and *biz* heterozygous (*biz*/+) flies were clearly defective in sensing temperatures below 33°C but not in sensing 3°C or 6°C differences. A similar loss of thermosensitivity was found in the cockroach and *Drosophila* when the peripheral thermosensors were removed (Murphy and Heath 1983; Sayeed and Benzer 1996). This was evident in the distribution of altered animals to higher temperatures on a thermal gradient compared to normal animals. Thermosensors have also been shown in the ventral ganglia of moths and the heads of honeybees, yellowjackets, and triatomine bugs (Coelho and Ross 1996; Cooper et al. 1985; Flores and Lazzari 1996; Hanegan 1973; Hanegan and Heath 1970; Heinrich 1980a, 1980b; Lazzari and Núñez 1989). Some of these might correspond to the thermosensors described here for *Drosophila*. The anatomical nature of the high-temperature thermosensor in *Drosophila* is currently unknown but may (at least in part) correspond to the ventral ganglion thermosensor found in moths and cockroaches and may also be in the antennae (see below).

The antennae can detect temperature gradients. There is an obvious difference in the magnitude of performance scores comparing flies with and without antennae in the thermosensitivity assay. Remarkably, this difference is gone in the training phase avoidance scores of the learning assay using the same temperatures. The effect of antennal removal is the lack of increased performance in the thermosensitivity assays compared to the learning assay. The difference between the assays is that in the first there is a spatial temperature gradient and in the second flies must generate a representation of a temperature gradient. This representation arises from the operantly derived temporal association of heat punishment with chamber position. That is, by a fly entering one chamber-half and being heated and when leaving that side having the chamber cool. The antennae-less flies' lack of increased performance score in the thermosensitivity assay likely indicates that these flies, again, determine the temperature gradient operantly. The advantage of the antennae is the determination of temperature gradients directly. This is presumably faster and leads to the dramatically higher thermosensitivity assay performance scores. Heterozygous *biz* mutant flies (*biz*/+) also showed twice the performance score in the thermosensitivity assay as in the learning paradigm. Since their antennae are intact and they still have the high-temperature thermosensor, it may be that they use an antennal high-temperature thermosensor to orient in the spatial temperature gradient. Interestingly, in triatomine bugs removal of a single antenna causes a feeding animal to misorient toward a warm 'food' source (Flores and Lazzari 1996; Lazzari and Núñez 1989) and the *Drosophila* antennae can be used to detect inter-antennal odor concentration differences (Borst and Heisenberg 1982). Thus, the antennae may be used by insects to orient with respect to relevant stimulus intensity differences.

The high-temperature thermosensor is necessary and sufficient for learning the contingency of spatial position and high-temperature punishment. This second thermosensor, responding only to elevated temperatures, might be an energy savings mechanism as memory formation would only be required when this sensor was active. The low-temperature thermosensor would then be used for orientation tasks with the presumably more common low temperature exposures.

The neuronal components of the *Drosophila* low- and high-temperature thermosensors are currently unknown. However, temperature receptor neurons (warm and cold cells) have been found in the antennae and ventral ganglia of several insect species. Cold cells have been identified in different insects, e.g., cave beetles, stick insects, saturniid moths, migratory locusts, mosquitoes, crickets, ticks, and cockroaches (Altner and Loftus 1985; Ameismeier and Loftus 1988; Davis and Sokolove 1975; Gödde and Haug 1990; Hess and Loftus 1984; Itoh et al. 1984; Loftus 1968; Loftus and Corbière-Tichane 1981; Nishikawa et al. 1985; Tichy and Loftus 1987). These generally increase their firing rate to decreases in temperature and decrease their firing rate to increases in temperature. Anatomical and physiological investigations indicate that (in the cockroach) cold cells project to the antennal lobe where they innervate interneurons and projection neurons. These in turn communicate warm and cold information to the mushroom body calyces and lateral protocerebrum (Nishikawa et al. 1995; Zeiner and Tichy 2000). Warm cells, responding to rises in temperature with increased neuronal activity, have been identified in spider and tick tarsi, mosquito and cave beetle antennae, and the cockroach prothoracic ganglion (Anton and Tichy 1994; Davis and Sokolove 1975; Ehn and Tichy 1994, 1996; Hess and Loftus 1984; Janiszewski 1986; Loftus and Corbière-Tichane 1981; Murphy and Heath 1983). As most non-blood feeding insects studied thus far have at least cold-receptors, it is likely that the *Drosophila* thermosensors also contain cold-receptors. These must be located in the antennae and somewhere else (perhaps in the ventral ganglion as in the cockroach). Determining the physiological and anatomical characteristics of the temperature sensing neurons in *Drosophila* awaits future experimentation.

The finding that the antennal low-temperature thermosensor is not needed for learning in the heat-box influences the conclusions of memory localization experiments. Using localized rescue experiments and the *rutabaga* type I adenylyl cyclase transgene, parts of the ventral nerve chord, the median bundle, and the antennal lobes were implicated as underlying the learning process in the heat-box (Zars et al. 2000). Since antennal removal does not dramatically affect learning in the heat-box and the antennal lobes primarily receive sensory information from these structures, the antennal lobes are not likely to be important for this learning task. Where the high-temperature thermosensor is located and how it is connected with the neuronal circuitry underlying spatial information remains to be seen.

Thermal behavior and physiology

Thermopreference is asymmetric. Wild-type flies did not respond to 3°C or 6°C differences when both temperatures were below 24°C but did show an obvious lower temperature preference when the higher temperature exceeded 27°C. Similar asymmetric preferences have been seen in the cockroach (Murphy and Heath 1983) and in several ectothermic species (DeWitt and Freidman 1979). This asymmetry may be related to a higher survivability at low temperatures since most species' body temperature are near their upper 'lethal body temperature' limit (McFarland 1993). Thus, small increases in temperatures toward that limit are likely to be more predictive of danger than small temperature decreases. The indifference that flies showed for low temperatures in the thermosensitivity assay is in contrast to the results of thermal gradient experiments. Sayeed and Benzer (1996) showed that flies avoid both high and low temperatures. This was evident in the flies' rather narrow distribution around 24°C. Based on the results of the thermosensitivity assays used here, the predicted distribution of flies on a thermal gradient would be random below 24°C but have a sharp cut-off above this temperature. The differences in the amount of time flies were allowed to display their preference (1 min versus 20 min) or differences in the test apparatus might account for this discrepancy. The implication is that the salience of a given temperature depends on its temporal and spatial context.

Temperatures above 30°C are physiologically important for *Drosophila*. Fly stocks can be maintained at temperatures from 12°C to 32°C, although rearing temperatures above 29°C frequently lead to male (but not female) sterility (Ashburner 1989). Indeed, death results from temperatures greater than 32°C (Lamb and McDonald 1973). The time for which flies can survive at an elevated temperature decreases as the temperature increases. For example, mean survival time of adult flies at 37°C is approximately 2 h but exposure to 40°C for 30 min results in fewer than 40% of the flies surviving (Lamb and McDonald 1973; Morrison and Milkman 1978). Thus, as a fly is exposed to rising temperatures it becomes increasingly important for it to remove itself from the situation. In addition, flies have a heat shock response to short high temperature exposure that 'hardens' flies to a second exposure and increases survival at elevated otherwise lethal temperatures (Brettencourt et al. 1999; Gilchrist and Huey 1999; Hoffmann et al. 1997). This investment in a physiological thermal protection is another indication of the importance of detecting and avoiding high temperatures. Perhaps it is not coincidental that the high-temperature thermosensor is also sensitive to temperatures above 30°C as it could provide the means for detecting and avoiding this potentially lethal situation.

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