

Summer 2016

Escape strategy of the cockroach (*Gromphadorhina portentosa*) to heat and looming stimuli

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Escape strategy of the cockroach (*Gromphadorhina portentosa*)
to heat and looming stimuli

An Honors Program Project Presented to
the Faculty of the Undergraduate
College of Science and Mathematics
James Madison University

by Jiangda Ou

May 2016

Accepted by the faculty of the Department of Biology, James Madison University, in partial fulfillment of the requirements for the Honors Program.

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PUBLIC PRESENTATION

This work is accepted for presentation, in part or in
full, at James Madison University, VA on 04/15/2016
and at Presbyterian College, SC on 04/02/2016.

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Acknowledgement

I would like to thank my advisor Dr. Corey L. Cleland for spending countless time, providing helpful advices to construct the thesis and the support of materials and equipment for conducting the experiments. I would also thank Dr. Kyle Seifert and Dr. Patrick Ludwig for giving helpful feedback to this study. In addition, this research cannot be finished without the funding from the Jeffress Foundation and the support from the Department of Biology at James Madison University.

Abstract

Escape responses to aversive stimuli have been observed in insects, including species of cricket, fly, locust, and cockroach. The goal of this study was to investigate the escape strategy of the Madagascar cockroach, *Gromphadorhina portentosa*. In regard to this species, Erickson and colleagues (2015) showed that electrical stimulation of both cerci and antennae together could generate an escape response. However, in other reports (Olsen and Triplehorn, 2014), it was observed that wind could not elicit the escape response. In this study, *G. portentosa* was stimulated by looming and heat stimuli. A 2.5" black ball approaching at 1 m/s was used to mimic a predator and a laser was used to apply heat stimuli to the cockroach's tarsi. The results showed that heat stimuli evoked robust turning and translation responses while the looming stimuli evoked small but significant translation but not turning. In conclusion, and in contrast to the literature, Madagascar cockroaches displayed robust escape responses to looming and especially heat stimuli.

Introduction

Insects are the most common group of animals on the earth (The IUCN Red List of Threatened Species, 2015), appearing everywhere in our daily life. Since insects can affect our life both positively and negatively (e.g. control the ecological balance, crop pest in agriculture), it is important to better understand their behaviors, such as their escape strategies, in order to prevent their negative effects.

Insects have diverse strategies to escape from their predators, including freezing, fighting and escaping (Domenici *et al.*, 2008, 2009; Card and Dickinson, 2008). For example, some cockroaches can fly away from dangers, spiders can produce silks to help them escape, and water striders can walk on water in order to escape from their terrestrial predators. However, one major strategy for escape is to move in the opposite direction from a stimulus. The basic components of escape behavior included turning, walking, jumping (Tauber and Camhi, 1995; Dupuy *et al.*, 2011) and flying (Fraser, 1977; Card and Dickinson, 2008). In real-world situations, insects employ multiple escape behaviors, which vary among insects such as crickets, a locust, flies, and cockroaches.

Escape Strategy – Cricket

Crickets escape from wind (Kanou *et al.*, 1999, 2006, 2014; Gras and Horner, 1992; Horner, 1992; Tauber and Camhi, 1995), touch (Dupuy *et al.*, 2011; Hiraguchi and

Yamaguchi, 2000) and looming objects (Dupuy *et al.*, 2011; Hiraguchi and Yamaguchi, 2000) by turning followed by either walking or jumping. Walking can be continuous (Kanou *et al.* 1999) or progress in bouts and pauses (Gras and Horner, 1992).

The escape rate of crickets to experimental stimuli varies greatly (5% - 95%), and was related to factors such as species (Kanou, 2006), age (Dupuy *et al.*, 2011), speed of stimulus (Kanou *et al.*, 1999, 2006), direction of stimulus (Dupuy *et al.*, 2011) and type of stimulus (Dupuy *et al.*, 2011; Hiraguchi and Yamaguchi, 2000).

The response rate varies across cricket species. *Grylloides sigillaus* has a higher response rate (94%), then *Gryllus bimaculatus* (58%; Kanou *et al.*, 1999, 2006). This difference suggests that species plays an important factor in predicting escape strategies. Age was also a factor. Younger crickets had a higher probability of successfully escaping from threats. Juvenile crickets, *Nemobius sylvestris* (1st- 3rd instars), had a high response to a looming object (40% - 100%), which was significantly higher than the older crickets (8% - 65% ; 7th – 9th instars and adults; Dupuy *et al.*, 2011). The reason younger crickets escape at higher rates may be because they are at greater risk of predation (Dangles *et al.*, 2006a).

The response rate also differed with stimulus direction, stimulus speed and the type of stimulus. The response rate for stimuli approaching from the front, side and back were 18% – 40%, 55% – 100% and 58 – 100%, respectively, showing a significantly higher response rate when the stimulation came from the side rather than from the front (Dupuy *et al.*, 2011).

The response rate was typically higher with higher stimuli speed. When the velocity of air-puff was 3.0 – 3.9 m/sec, the normal adult cricket response rate was 51% – 56%. However, if the velocity of air-puff dropped to 1.5 m/sec, the response rate dramatically decreased to only 6% (Kanou *et al.*, 1999). In *G. bimaculatus*, the response rate in response to touching was 52%, but increased to about 95% when crickets were stimulated by bending or pinching (Hiraguchi and Yamaguchi, 2000). In another species, *Nemobius sylvestris*, the response rate to a looming object was only 5 - 52% (Dupuy *et al.*, 2011).

Escape direction varied among cricket species, but all typically escaped in the opposite direction from the stimulus (100° - 170°, e.g. 0°: straight forwards to the stimulus, 180°: directly away from the stimulus, Figure 1). *G. bimaculatus*, escaped at 162° opposite to air-puff (Kanou, 1999), while *G. sigillatus*, a smaller species, escaped at approximate 100°. *N. sylvestris* escaped in the direction almost opposite to the approach of a

looming stimulus from back, front and side (180° ; Dupuy *et al.*, 2011). As mentioned above, *G. sigillaus* had a higher response rate, but *G. bimaculatus* had a higher accurate escape direction (closer to $\pm 180^\circ$; Kanou *et al.*, 2006). *N. sylvestris* escaped at 169° relative to the direction of the stimulus when an object stimulated from behind, and escaped at 156° when an object stimulated from the side (Figure 1; Dupuy *et al.*, 2011).

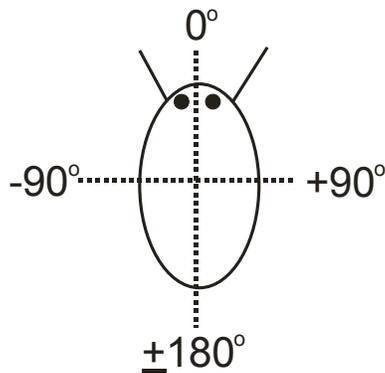


Figure 1. Typical angular coordinate system. The right turn was reported as a positive angle; the left turn was reported as a negative angle.

Escape Strategy – Locust

In regard to the escape response of the locust, *Schistocerca gregaria*, the locust mostly jumped away from a looming stimulus, but in some trials walked away from the stimulus or only cocked its hind leg (Santer *et al.*, 2005).

When locusts were stimulated by a looming object, the response rate was higher than 95% (including jumping, cocking and walking). In terms of the escape direction, its jump was largely forward ($\pm 50^\circ$; Santer *et al.*, 2005). When the locust jumped away from the stimulus, it used its foreleg to pivot toward the final jump trajectory and then extended its foreleg while its hind leg was releasing to perform a jump behavior. There was no significant difference in regard to left and right escape trajectory. The foreleg motor program was independent of hind leg motor program, since the locust could decide the escape trajectory after its hind leg flexion (Santer *et al.*, 2005).

Escape Strategy – Fly

Regarding the escape response of the fruit fly, *Drosophila melanogaster*, the fly mostly jumped and flew away from a looming stimulus, but sometimes flew without first jumping (Card, 2012; Card and Dickinson, 2008).

The escape direction in flies was approximate 180° from the looming stimulus (Card, 2012), including a change of its trajectory by approximate 90° within 100ms during the initial response (Tammero and Dickinson, 2002). *Drosophila* jumped to the opposite side when a looming object came from the back or from the front side, but when a looming object approached from the side, it jumped at the angle between 36° - 72° and

72° - 108° (Figure 1). This forward bias response might be elicited by attractive odors or internal cues, which were always in the forward direction (Card, 2012).

Escape Strategy – Cockroach (*Periplaneta americana*)

In regard to the escape response of the cockroach, the escape response rate of *P. americana* was 100% when its femur or metathoracic leg was stimulated by touch (Comer *et al.*, 1994), 94% - 96% for air-puffs (Stierle *et al.*, 1993) and ~90° for air-puffs (Camhi and Tom, 1978). The initial movement of escape in the cockroach, *P. americana*, was first a pivot turn away from wind or live frogs, with the pivot point located in the posterior region of its body, followed by a walk. The escape response proved a successful strategy, allowing the cockroach to avoid predation in 18/19 instances with live frogs (Camhi and Tom, 1978).

Although cockroaches usually turned away from the stimulus, they did occasionally turn toward to the stimulus (3% - 19% based on Camhi and Tom, 1978, Comer and Dowd, 1987, Domenici *et al.* 2009, Stierle *et al.*, 1993). Furthermore, cockroaches sometimes turned away from the stimulus again after they turned towards to the stimulus, which was called “overshooting” by Domenici (2009). The distribution of turn angles were distributed differently for away and towards responses. Based again on (Camhi and Tom, 1978, Comer and Dowd, 1987, Domenici *et al.* 2009, Stierle *et al.*, 1993), the

range of away responses was much greater (35° - 72°) than the range of toward (15° - 37°) responses.

Sensory Behavior of Escape

The escape response may be mediated by different sensory stimuli (Ye *et al.*, 2003), such as cerci, vision, and antennae; or a combination of sensory modalities.

Cerci: Cerci, which are a pair of appendages at the backside of many insects, contain wind-sensitive filiform hairs that are highly and directionally sensitive to wind. The cerci have been studied mostly in Orthopteras and Dictyoptera, but their morphology, filiform hairs, and central targets varies broadly across insects (McGorry *et al.*, 2014).

Afferent activity is evoked by wind-mediated cercal displacement in all directions (Goldstein and Camhi, 1988). Subsequently, cercal afferents excite giant interneurons in the ganglia of the cockroach. These wind-sensitive interneurons (WSIs), located in the ventral intermediate tract (VIT) and dorsal intermediate tract (DIT), have been implicated in the wind-evoked response (Camhi and Nolen, 1981). Four different species of cockroaches, *P. americana*, *Blattella germanica*, *Blaberus craniifer* and *G. portentosa*, all possess ascending WSIs in the abdominal connectives, but the

magnitude of their response to wind varies (weakest in *G. portentosa*, strongest in *B. craniifer*; McGorry *et al.*, 2014), paralleling the magnitude of their escape response.

There is evidence that cercal receptors are necessary for the wind-evoked escape response. In the cockroach, *P. americana*, there was no detectable movement evoked by wind stimulation from any angle after the ventral cercal surfaces were covered with adhesive (Camhi and Tom, 1978), effectively ablating the cercal receptors. Further, cerci ablation decreased (from 350 mm s⁻¹ to 238 mm s⁻¹) escape velocity and escape distance (from 1017 mm to 771 mm; Ye *et al.*, 2003).

Similarly, in the cricket, *G. bimaculatus*, unilateral or bilateral cercal ablation (cerci were cut off at the base) reduced their response to wind dramatically. The response rate dropped from 46.0% to 0% after bilateral cercal ablation, and from 48.1% to 5.5% after unilateral cercal ablation (Kanou *et al.*, 1999). In *G. sigillatus*, the response rate also dropped from 97.2% to 2.7% following cercal ablation (Kanou, 2006). These results show that mechanosensory filiform hairs on the cerci are necessary for the escape response in crickets (Kanou *et al.*, 1999, 2006). However, there is evidence that the cercal receptor might not be absolutely necessary. The escape rate decreased, but the direction of the escape behavior of *G. sigillatus* did not change after the unilateral cercal ablation, suggesting that cercal receptors can change the response rate but not

determine the escape direction. However, the situation was different in *G. bimaculatus*, where both rate and direction were decreased (Kanou *et al.*, 2006)

Vision: The visual system varies among insects. In some insects, visual cues may be an important part of their escape strategy, because they (e.g. locust and fly) have been shown to require visual information for escape (Card, 2012; Santer *et al.*, 2005). However, in the cockroach, *P. americana*, there was no change in escape direction when vision was blocked (Ye *et al.*, 2003). Further, wind-evoked response rate in crickets were similar for both light and dark background (Kanou *et al.*, 2014).

In contrast, vision is important to the escape response to looming stimuli in the fly and locust (Card 2012). In *Drosophila*, visual escape is due to a pair of large descending interneurons, the giant fiber (GFs), and also involves additional central neurons (Card, 2012). In the visual system of the locust, the lobula giant movement detector (LGMD, O'Shea and Williams, 1974) and the descending contralateral movement detector (DCMD; Rind, 1984) respond to an approaching object (Judge and Rind, 1997). DCMD responses differed from different azimuthal (angle) directions (Guest and Gray, 2006), and there were at least three descending pathways (including DCMD, DIMD, and LDMCD) that could trigger the locust escape jump (Card, 2012).

Specific Aim

Although escape responses were readily evoked in the cockroach, *P. americana*, it is unclear if it can be evoked in the Madagascar cockroach (*Gromphadorhina portentosa*). Erickson and colleagues (2015) showed that electrical stimulation, of both cerci and antennae, could generate an escape response. However, in other reports (Clark and Triplehorn, 2014; Olsen and Triplehorn, 2014), it was indicated that wind could not elicit the escape response.

The goal of this study was to investigate the escape strategy of the Madagascar cockroach, *G. portentosa*, to looming and heat stimuli. This species has been minimally studied, though currently popular as a pet, and moves slower and is more stationary compared with other cockroach species (e.g. *P. americana*). In this study, cockroaches were stimulated with a looming ball projected toward the cockroach and tarsi were individually stimulated with heat. The escape behaviors were recorded with high-speed video. The hypothesis of this study, based on previous studies of cockroaches and crickets, was the Madagascar cockroach would escape opposite to the looming object and the heat stimulus.

Methods

Insect

Adult Madagascar cockroaches (*Gromphadorhina portentosa*), 51 – 76 mm long, were used. Adults were wingless, slow moving and tended to remain stationary.

Cockroaches, purchased from a commercial vendor (New York Worms, Long Island, NY), were fed with carrots, dog food and water gel 3 times every week and kept in a transparent glass tank at 23 – 24 °C (room temperature) and 30 – 70% relative humidity with the ambient environmental light/dark cycle (Haynes, 2005). Experiments were conducted in the same environment during the daytime.

To prepare the cockroaches for the experiment, two small circular spots (the diameter approximately 1.0 mm; e.g. Figure 4) was marked with a white pen (AP ACM, Japan) on the center line of a cockroach without anesthesia, and then the cockroach was restrained by an 8.0 cm (internal diameter) acrylic tube and placed on a circular white primed canvas platform (25.5 cm in diameter; Figure 2). After about 3 minutes, the cover was removed gently. If the cockroach did not move, a trial was conducted.

Looming stimuli

The first series of experiments tested the response of the cockroach to looming stimuli. A ball (2.5" diameter black polystyrene) was the looming stimulus object, which was

projected at 1 m/s toward the cockroach at a 45° vertical angle. The ball never hit the cockroach (Figure 2). The ball stopped with its edge close to the edge of the cockroach, which still allowed the camera to record the entire cockroach from above vertically. When stimulating from the front (directions 0°, +45°, -45°; Figure 3A), care was taken to insure the ball would not hit cockroach's antennae.

Heat stimuli

Heat was delivered to the tarsi with a 980nm infrared laser (11 watts, B&W TEK, Wilmington, DE) focused by a condenser lens (Figure 2). In the event the cockroach did not respond, stimulus duration was limited to 1 second to prevent damage.

Video Recording

To record video, a high-speed camera (IDT, monochrome, 650 fps, 25mm lens, f 5.8 aperture) controlled by IDT software (Motion Studio) and a low heat LED ring were used (Figure 2). Each video was 3-second in duration and triggered at the onset of the laser or looming stimulus.

Experimental protocol

Each cockroach was stimulated from 8 directions for the looming experiments (Figure 3A) and 6 locations for the heat experiments (Figure 3B), which were then repeated 3

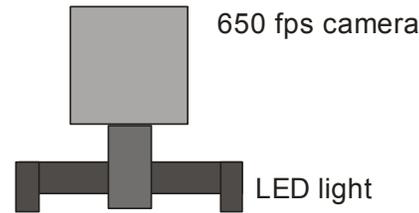
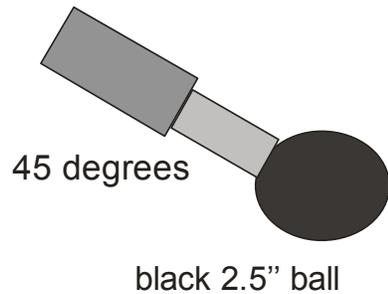
times for a total of 24 or 18 trials per cockroach. The sequence of 8 or 6 directional locations was randomized for each cockroach.

Movement Analysis

After the videos were recorded, ProAnalyst (Xcitex, Cambridge, MA) was used to track the movement of cockroaches based on the two marked spots on the back of the cockroach. The calculated mid-point of the cockroach was used to quantify *translation* (linear movement in the horizontal plane). The angle of the line between the two marked spots was used to determine the turn *angle*. Total angular change was specified as the maximum change in angle. Total translational direction and magnitude were specified as the change from beginning to the end of the movement..

Data were analyzed with custom routines written in Matlab (MathWorks, Inc., Natick, MA) by Corey Cleland. Statistics on movement translational direction and angle were computed with a Watson-Williams “circular ANOVA” test (Oriana, KCS, Wales, UK). Graphs were created in Sigmaplot (Systat Software Inc., San Jose, CA). Statistical alpha was set at 0.05. Boxplots represent median, 25-75% and 10-90%.

Looming Experiment



Heat Experiment

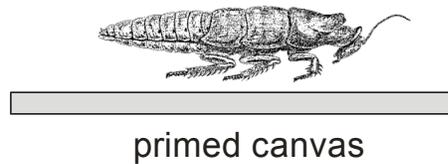
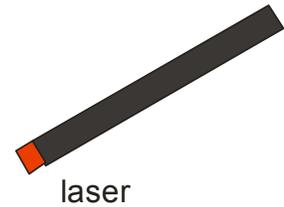


Figure 2. Experiential equipment and Set-up.

The primed canvas circular experiential platform (diameter was 25.5 cm). This platform was used as the experimental field, and a cockroach was demotivated to escape prior to the stimulus by an acrylic tube.

The ball looming system. The looming object, a 2.5" polystyrene ball, was propelled at the cockroach at 45 degrees with an air cylinder driven by 11 psi.

Camera, light and the laser. A high speed video camera (IDT, 650 fps, 25mm lens, $f/4$ aperture) recorded the movement. The laser (980nm infrared laser (11 watts) was to generate the heat to stimulate the cockroach's tarsi.

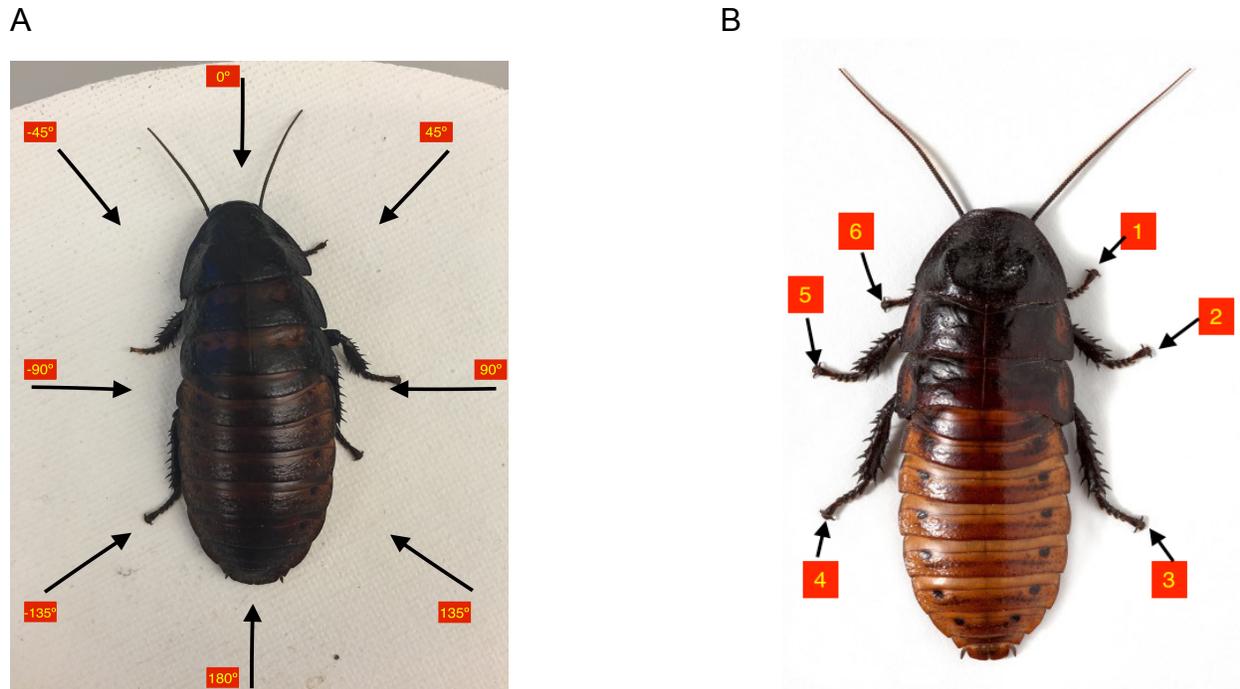


Figure 3. Stimuli direction.

(A) The looming stimuli. The cockroach was stimulated by a looming ball from 8 different directions.

(B) The heat stimuli. Six tarsi of each cockroach were simulated by heat generated by the laser.

Results

Aversive stimuli delivered to Madagascar cockroaches ($n=17$) evoked escape responses in 27.4% of 252 trials (11.4% heat, 50.5% looming). Figure 4 shows a typical escape response to a heat stimulus delivered to the back-left tarsus for video (A) and tracked locations (B). The cockroach both turned and translated in the direction

opposite to the stimulated tarsi (Figure 4). In the looming experiments, the cockroach also responded to the stimulus (Figure 5). However, the turn was typically small and often in the opposite direction. Further, the escape translation distance was shorter compared with the heat stimulation.

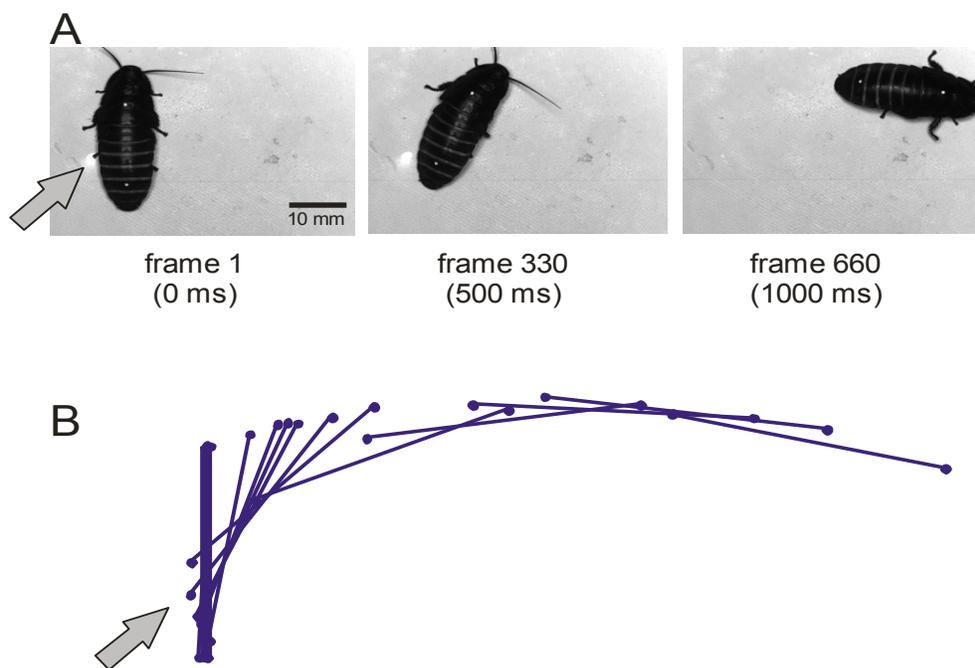


Figure 4. A typical escape trajectory of a Madagascar cockroach stimulated by heat. (A) 3 frames (1000 ms) of escape trajectory. (B) The stick diagram of the same escape trajectory as A. The filled circles correspond to the two tracked white marks on the cockroach. The arrows correspond to the stimulus location (back-left tarsus).

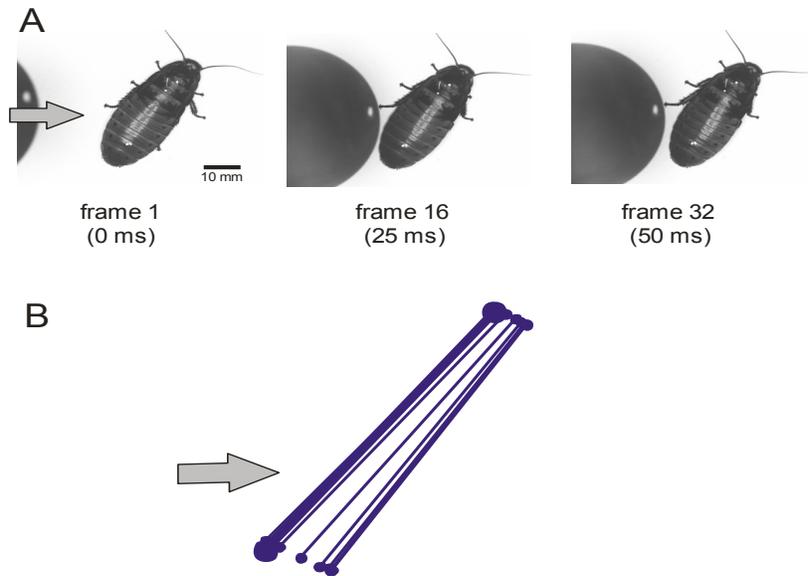


Figure 5. A typical escape trajectory of a Madagascar cockroach stimulated by a looming object. (A) 3 frames (50 ms) of escape trajectory. (B) The stick diagram of the same escape trajectory as A. The blue dots correspond to the two tracked white marks on the cockroach. The arrows correspond to the looming direction (back-left tarsus).

Overall, although escape translation of the cockroaches was directed away from both heat and looming stimuli, the total distance traveled (translation) was greater for heat than for looming stimuli (Figure 6A).

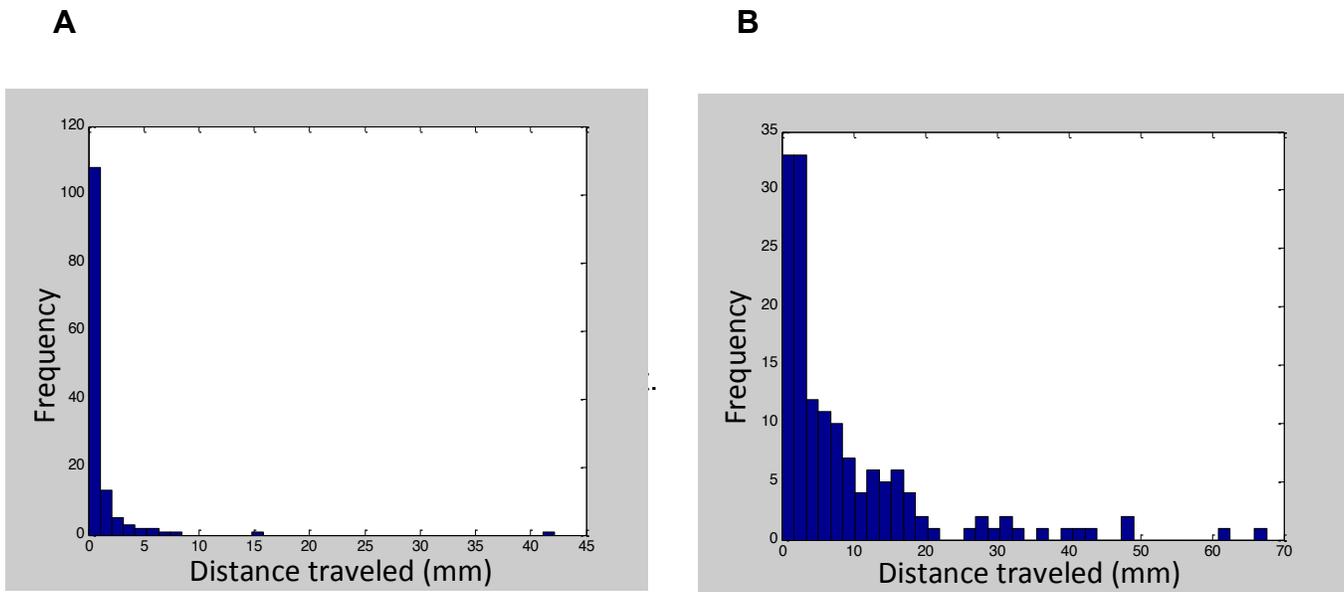


Figure 6. Frequency histograms of total movement. Frequency histograms of total translation for looming (left) and heat (right) evoked responses.

In the looming experiments (Figure 7), cockroaches turned (51/103, 49.5%) and translated (72/103, 69.9%) away from the stimulus. In contrast, for heat stimuli (Figure 8), cockroaches more reliably turned (132/149, 88.6%) and translated (145/149, 97.3%) away from the stimulus.

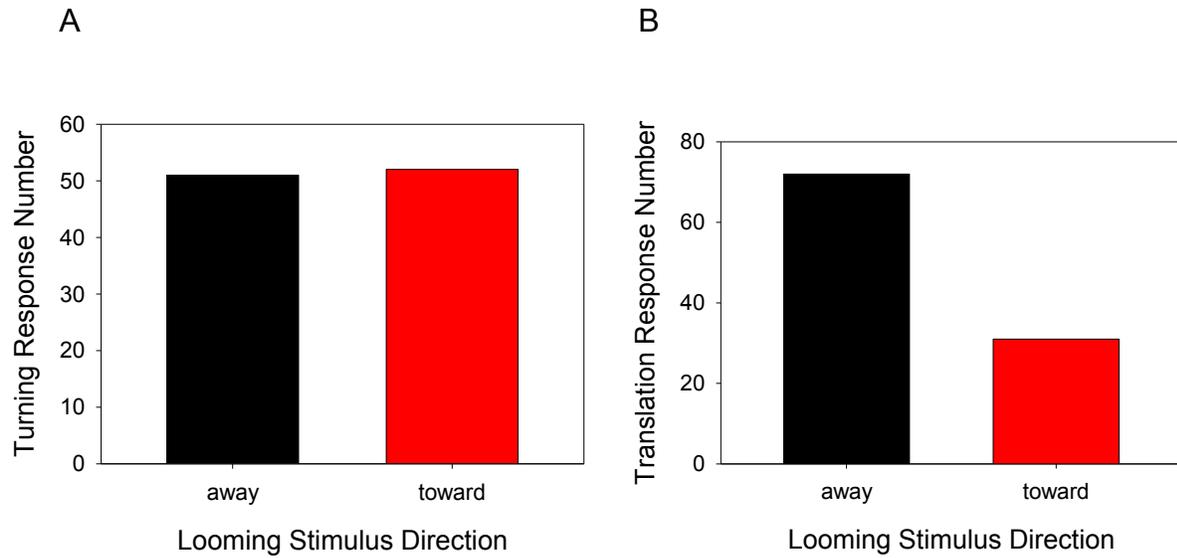
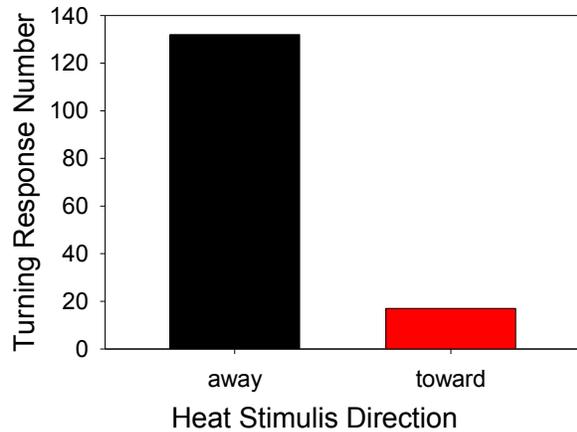


Figure 7 The escape trajectory stimulated by a looming object. (A) The escape direction of Madagascar cockroaches stimulated by a looming object (51 away, 52 towards, N = 103). (B) The escape translation of Madagascar cockroaches stimulated by a looming object (72 away, 31 towards, N = 103).

A



B

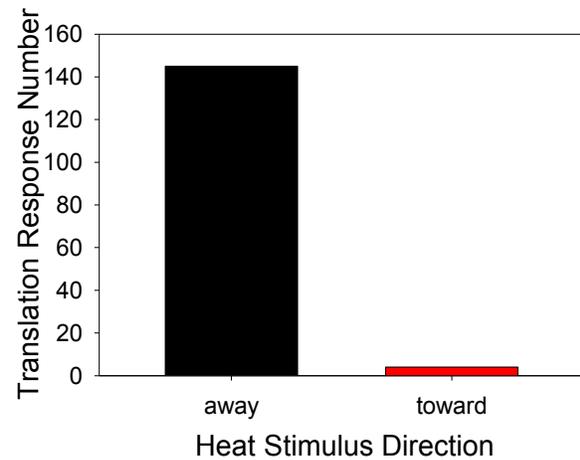


Figure 8. The escape trajectory stimulated by heat. (A) The escape direction of Madagascar cockroaches stimulated by heat (132 away, 17 towards, N = 149). (B) The escape translation of Madagascar cockroaches stimulated by heat (145 away, 4 towards, N = 149).

The dependence of turning angle on stimulus location or direction was different for stimulation by looming or heat stimulation (Figure 9). For looming, the turning angle was always close to 0° and did not depend on stimulus angle (Figure 9A; $p = 0.1$, Williams-Watson). For heat, the translation direction varied between $\pm 50^\circ$ and depended significantly on stimulus location (Figure 9B; $p = 0.0001$, Williams-Watson).

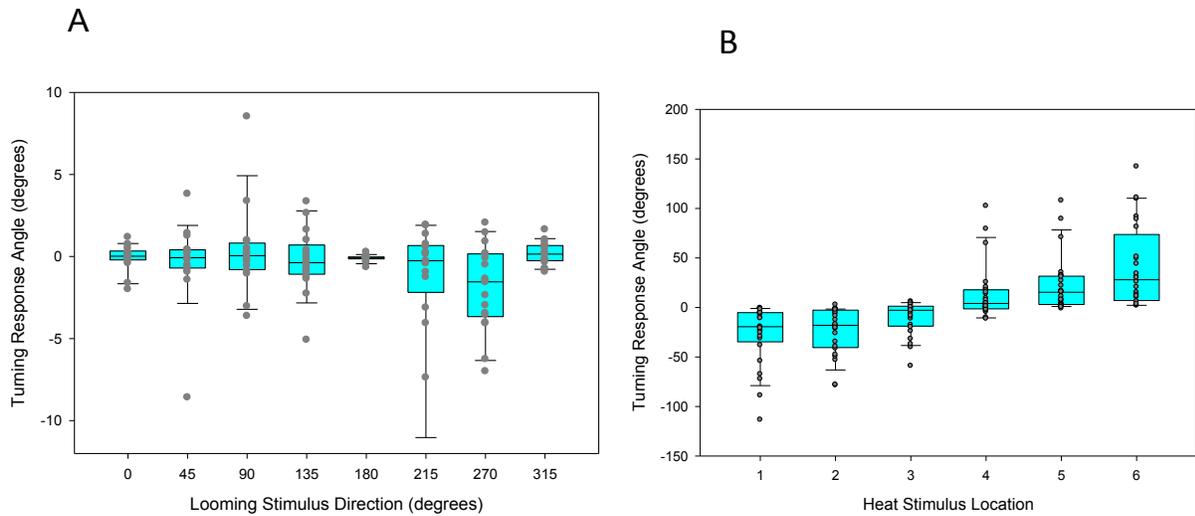


Figure 9. The turning angle of a Madagascar cockroach. (A) The turning angle for looming stimuli direction. The angle of turn did not depend on looming direction ($p = 0.1$; $n = 21/\text{angle}$, Williams-Watson). (B) The turning angle for heat stimuli location. The turn angle depended significantly ($p = 0.0001$, $n = 30/\text{angle}$, $r^2 = 0.416$, Williams-Watson) on heat location. The numbers correspond to tarsi (Figure 3B).

Since Madagascar cockroaches are symmetric, it was considered as the same stimuli in the looming experiment when a Madagascar cockroach was simulated by a looming object from the direction 45° , 90° , 135° and the direction -45° , -90° , -135° , respectively (Figure 3A); it was also considered as the same stimuli in the heat experiment when its tarsus was simulated from the direction 1, 2, 3 and the direction 6, 5, 4, respectively (Figure 3B). Consequently, stimuli and response from right-sided stimuli were reflected to the left-sided to combine results.

In the reflected analysis (Figure 10), similar results were obtained. There was no significant dependence of turn angle when cockroaches were stimulated by a looming object ($p = 0.07$, Williams-Watson). In contrast, for heat stimuli, the angle of turn depended significantly on stimulus location ($p < 0.0003$, Williams-Watson). Thus, heat-evoked turns depended not only on which side the stimulus was given, but also on the degree of laterality of the stimulus.

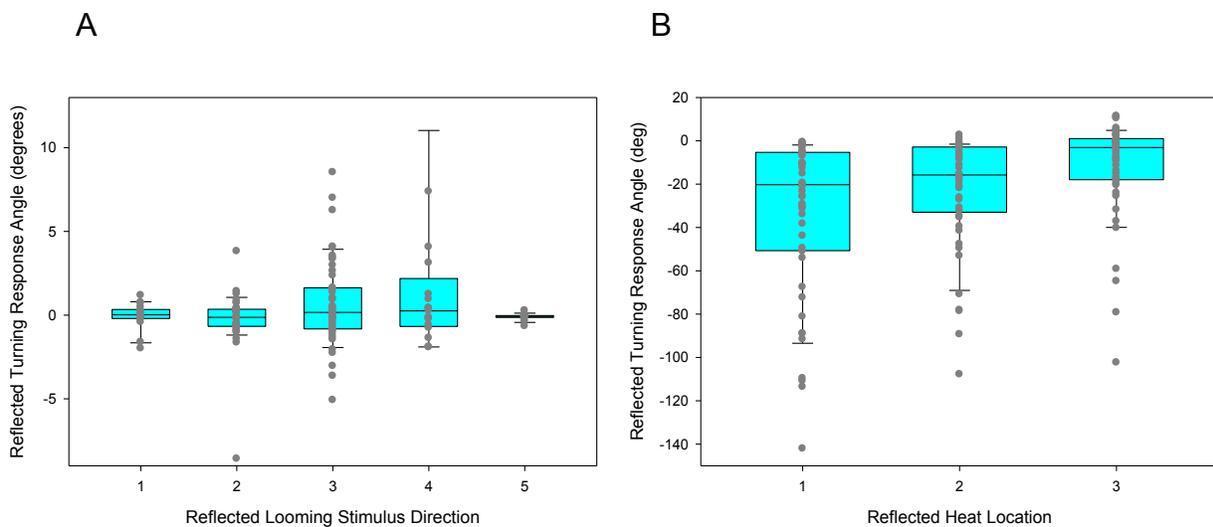


Figure 10. The reflected turning angle of a Madagascar cockroach. (A) The turning angle of a Madagascar cockroach did not depend on the reflected looming stimuli direction ($p = 0.07$, $N = 103$). (B) The turning angle of a Madagascar cockroach depended on the reflected heat stimuli location ($p < 0.003$, $N = 149$, $r^2 = 0.09$).

The dependence of translation on stimulus location or direction was also different for stimulation by looming or heat stimulation (Figure 11). For looming, the translation direction ranged from $\sim -180^\circ$ to $+180^\circ$ and depended on stimulus angle (Figure 11A; $p < 0.0001$). For heat, the translation direction varied similarly and also depended on stimulus location (Figure 11B; $p < 0.0001$, Williams-Watson). Since the slopes were $\sim 35^\circ$ and 54° per stimulus “step” (8 for looming = 280° , 6 steps for heat = 324°), the resulting direction of translation was close to directly away from the stimulus.

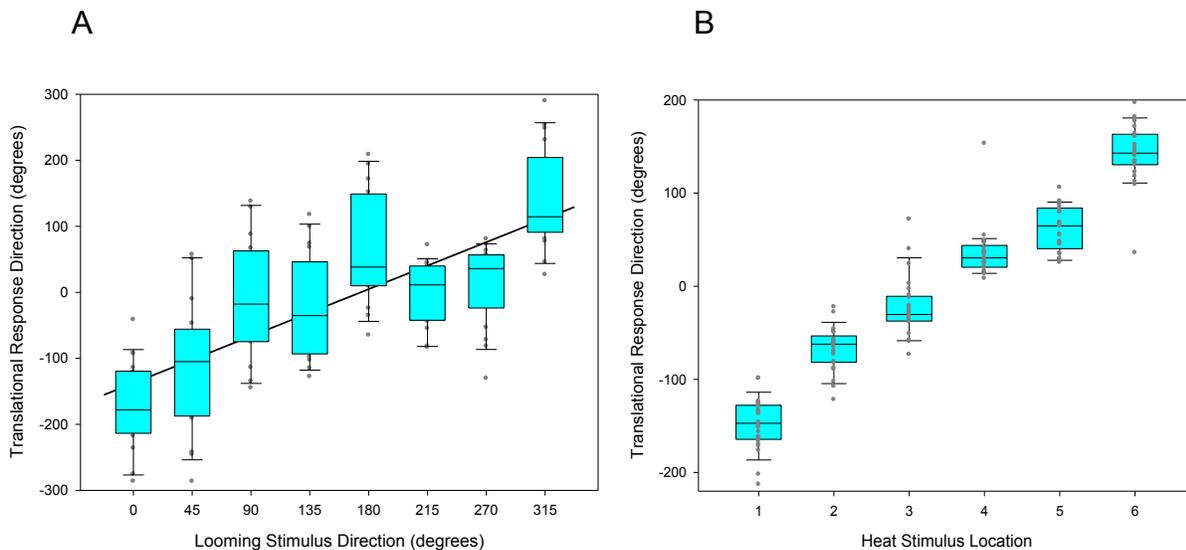


Figure 11. The escape translation direction of a Madagascar cockroach. (A) The escape translation direction for looming stimuli direction. The translation direction depended on looming direction ($p < 0.0001$, slope = $35.6^\circ/\text{angle}$, $N = 103$, $r^2 = 0.487$). (B) The escape translation direction for heat stimuli location. Responses direction depended significantly ($p < 0.0001$, slope = $54.2^\circ/\text{angle}$, $N = 149$, $r^2 = 0.906$) on heat stimulus location.

In the reflected analysis (Figure 12), translation direction for both looming ($p < 0.001$, Williams-Watson) and heat ($p < 0.001$, Williams-Watson) varied significantly with stimulus direction or location, demonstrating that the response depended on stimulus laterality (ipsilateral angle ranging from front to back).

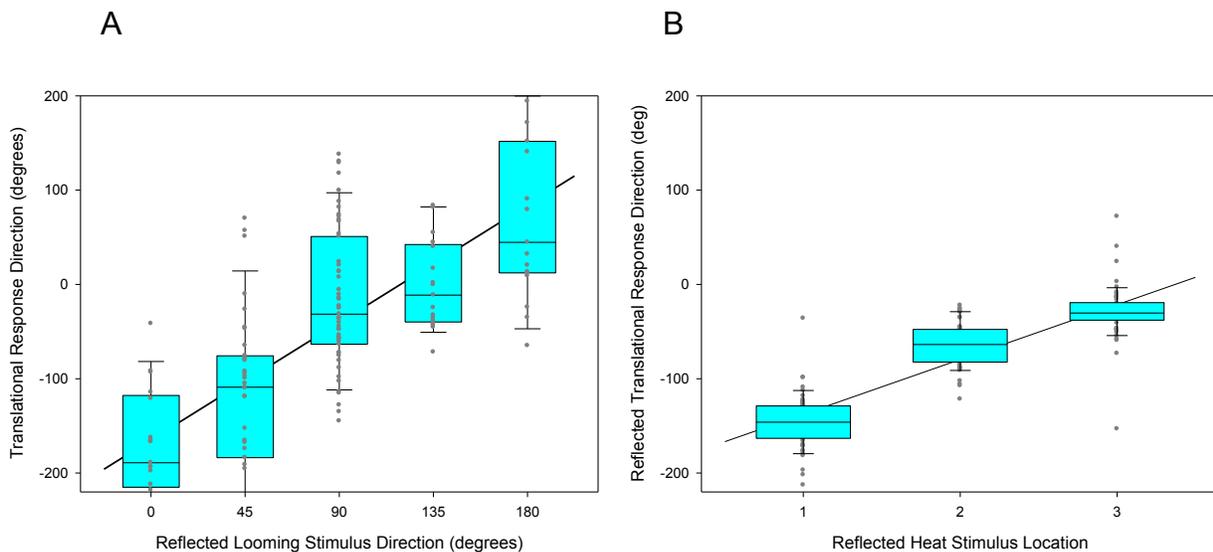


Figure 12. The reflected escape translation direction of a Madagascar cockroach. (A) The escape translation direction depended on the reflected looming stimuli direction ($p < 0.001$, $n = 62.2/\text{angle}$, $N = 103$, $r^2 = 0.46$). (B) The escape translation direction of a Madagascar cockroach depended on the reflected heat stimuli location ($p < 0.001$, $n = 58.1/\text{angle}$, $N = 149$, $r^2 = 0.72$).

Discussion

Summary

In contrast to a published report (Olsen and Triplehorn, 2014), the escape response of the Madagascar cockroach can be readily elicited by heat and looming stimuli, although the magnitude of the response to the looming stimulus used in these experiments was small compared to crickets (Camhi and Tauber, 1995) and cockroaches (Camhi and Tom, 1978). The escape response consisted of both a turn and translation with heat but only a translation with looming. Importantly, reflected analysis showed that the cockroaches response varied with both laterality (which side the animal stimulated) and degree of laterality (where on the side the animal was stimulated).

Comparison to Pervious Studies

Two reports suggested that there is neither a wind-evoked running escape response (Clark and Triplehorn, 2014) nor a terrestrial response or flight (Olsen and Triplehorn, 2014) in the Madagascar cockroach. Further, Erickson and colleagues were only able to evoke consistent escape responses with combined artificial (electrical) stimulation of both cerci and antennae. However, these results showed that this species responded to both heat and, to a lesser extent, looming. The explanation for the differences may be two-fold. First, responses, especially to looming, were small and may have been

missed. Second, heat, which produced the strongest responses, has not been previously used.

Sensory Mechanisms

Cercal activation of WSIs, located in the VIT, is to initiate the turning movement (Camhi and Nolen, 1981; Ye *et al.*, 2003). However, in *G. portentosa* the WSI provides weaker input to the premotor/motor neuron than in other cockroach species shown by lower spike counts (*P. americana*; McGorry *et al.*, 2014). Therefore, the cerci in *G. portentosa* may have less sensitivity or central strength when stimulated by wind.

However, there might also be possible that another sensory evoked the escape response in *G. portentosa* for the looming experiments, because *G. portentosa* might use the visual information to evoke the escape response when an object was approaching. Regarding the escape response to heat, while clearly there must be heat sensors in the tarsi, there are apparently no previous studies in the literature.

Conclusions

These results demonstrate that Madagascar cockroaches, like other insects and in contrast to the existing literature, execute an escape behavior in response to aversive stimuli. Surprisingly, however, heating of their tarsi evoke far stronger responses than

looming stimuli that mimic predators. These results raise questions about the normal degree of threat faced by Madagascar cockroaches from looming predators and terrestrial sources of heat.

References

- Camhi, J.M., Nolen, T.G. (1981). Properties of the escape system of cockroaches during walking. *Journal of Comparative Physiology A*. 142:339-346
- Camhi, J.M., Tom, W. (1978). The Escape Behavior of the Cockroach *Periplaneta americana*. *Journal of Comparative Physiology*. 128:193-201.
- Camhi, J.M., Tom, W., Volman, S. (1978). The Escape Behavior of the Cockroach *Periplaneta americana*. *Journal of Comparative Physiology*. 128:203-212.
- Camhi, J.M., Tauber, E. (1995). The Wind-evoked escape behavior of the cricket *Gryllus bimaculatus*: Integration of behavioral elements. *The Journal of Experimental Biology*. 198: 1895-1907.
- Card, G.M., Dickinson, M.H. (2008). Visually mediated motor planning in the escape response of *Drosophila*. *Current Biology* 18, 1300 – 1307.
- Card, G.M. (2012): Escape behaviors in insects. *Current opinion in Neurobiology*. 22:180-186.
- Clark, A.J., Triplehorn, J.D. (2014). Mechanical properties of the cuticles of three cockroach species that differ in their wind-evoked escape behavior. *DOI 10.7717/peerj.501*
- Comer, C.M., Dowd, J.P. (1987). Escape turning behavior of the cockroach. Changes in directionality induced by unilateral lesions of the abdominal nervous system. *Journal of Comparative Physiology A*: 160: 571-583.

- Comer, C.M., Mara, E., Murphy, K.A., Getman, M., Mungy, M.C. (1994). Multisensory control of escape in the cockroach *Periplaneta americana* II. Patterns of touch-evoked behavior. *Journal of Comparative Physiology A*: 174:13-26.
- Dangles, O., Casas, J., Coolen I. (2006). Textbook cricket goes to the field: the ecological scene of the neuroethological play. *Journal of Experimental Biology* 2006 209:393-398.
- Domenici, P., Booth, D., Blagburn, J.M., Bacon, J.P. (2008). Cockroaches keep predators guessing by using preferred escape trajectories. *Current Biology*, 18(22): 1792-1796.
- Domenici, P., Booth, D., Blagburn, J.M., Bacon, J.P. (2009). Escaping away from and towards a threat - The cockroach's strategy for staying alive. *Communicative & Integrative Biology* 2:6, 497-500.
- Dupuy, F., Casas, J., Body, M., Lazzari, C.R. (2011). Danger detection and escape behaviour in wood crickets. *Journal of Insect Physiology*. 57:865-871.
- Erickson, J.C., Herrera, M., Bustamaante, Shingiro, A., Bowen, T. (2015). Effective stimulus parameters for directed locomotion in Madagascar hissing cockroach biobot. *Journal.pone.0134348*
- Fraser, P.J. (1977). Cercal ablation modifies tethered flight behavior of cockroach. *Nature*. Vol. 268: 523-524.

- Goldstein, R.S., Camhi, J.M. (1988). Modulation of activity in sensory neurons and wind-sensitive interneurons by cercal displacement in the cockroach. *Journal of Comparative Physiology A* 163:479-487
- Gras, H., Horner, M. (1992). Wind-evoked escape running of the cricket *Gryllus bimaculatus* I. Behavioural analysis. *Journal of Experimental Biology*: 171, 189-214.
- Guest, B., Gray, J. (2006). Responses of a looming-sensitive neuron to compound and paired object approaches. *Journal of Neurophysiology*. 95:1428.
- Haynes, M. (2005). Husbandry Date Sheet. *TITAG Husbandry Information*. (Terrestrial Invertebrate Taxon Advisory Group). Retrieved 12 Oct., 2015.
- Hiraguchi, T., Yamaguchi, T. (2000). Escape behavior in response to mechanical stimulation of hindwing in cricket, *Gryllus bimaculatus*. *Journal of Insect Physiology* 46 (2000) 1331-1340
- Judge, S., Rind, F. (1997). The locust DCMD, a movement-detecting neurone tightly tuned to collision trajectories. *The Journal of Experimental Biology*. 200:2209-2216.
- Kanou, M., Ohshima, M., Inoue, J. (1999). The Air-puff Evoked Escape Behavior of the Cricket *Gryllus bimaculatus* and its Compensational Recovery after Cercal Ablations. *Zoological Society of Japan*. 16:71-79.

- Kanou M., Konishi, A., Suenaga, R. (2006). Behavioral Analyses of Wind-evoked Escape of the Cricket, *Grylloides sigillatus*. *Zoological Society of Japan*. 23:259-364.
- Kanou M., Matsuyama, A., Takuwa, H. (2014). Effects of Visual Information on Wind-Evoked Escape Behavior of the Cricket, *Gryllus bimaculatus*. *Zoological Science*, 31(9):559-564.
- McGorry, C.A., Newman, C.N., Triplehorn, J.D. (2014). Neural responses from the wind-sensitive interneuron population in four cockroach species. *Journal of Insect Physiology* 66 (2014) 59-70.
- O'SHEA, M., Williams, J.L.D. (1974). The anatomy and output connections of a locust visual interneurone: the lobula giant movement detector (LGMD) neurone. *Journal of Comparative Physiology*. 91:257–266.
- Olsen, A.C.K., Triplehorn, J.D. (2014). Neural responses from the filiform receptor neuron afferents of the wind-sensitive cercal system in three cockroach species. *Journal of Insect Physiology*. 68: 76-86.
- Rind, F.C. (1984). A chemical synapse between two motion detecting neurones in the locust brain. *The Journal of Experimental Biology*. 110:143–167.
- Santer, R.D., Yamawaki, Y., Rind, F.C., Simmons, P.J. (2005). Motor activity and trajectory control during escape jumping in the locust *Locusta migratoria*. *Journal of Comparative Physiology A* 191: 965-975.

Tammero, L.F, Dickinson, M.H. (2002). The influence of visual landscape on the free flight behavior of the fruit fly *Drosophila melanogaster*. *Journal of Experimental Biology* 2002 205: 327-343

Tauber, E., Camhi, J.M. (1995). The Wind-Evoked Escape Behavior of the *Gryllus Bimaculatus*: Intergration of Behavioral elements. *The Journal of Experimental Biology*.198.1895-1907.

The IUCN Red List of Threatened Species (2015-4). Summary Statistics. 2016.4. Table 1 - Numbers of threatened species by major groups of organisms (1996–2015).

Ye, S., Leung, V., Khan, A., Baba, Y., Comer, C.M. (2003). The antennal system and cockroach evasive behavior. I. Roles for visual and mechanosensory cues in the response. *Journal of Comparative Physiology A*. Vol.189, Issue 2, pp89-96.