Behavioral response to antennal tactile stimulation in the field cricket *Gryllus bimaculatus*

Jiro Okada and Seiryō Akamine

Graduate School of Fisheries Science and Environmental Studies

Nagasaki University

Nagasaki 852-8521, Japan

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Address for correspondence

Jiro Okada

Graduate School of Fisheries Science and Environmental Studies

Nagasaki University

Nagasaki 852-8521, Japan

Phone/Fax: +81-95-819-2762

E-mail: jokada@nagasaki-u.ac.jp

Abstract

We examined behavioral responses of the field cricket *Gryllus bimaculatus* to tactile stimuli to the antennae. Three stimulants of similar shape and size but different textures were used: a tibia from the hunting spider *Heteropoda venatoria* (potential predator), a tibia from the orb-web spider *Argiope bruennichii* (less likely predator), and a glass rod. Each stimulus session comprised a first gentle contact and a second strong contact. The evoked behavioral responses were classified into four categories: aversion, aggression, antennal search, and no response. Regardless of the stimulants, the crickets exhibited antennal search and aversion most frequently in response to the first and second stimuli, respectively. The frequency of aversion was significantly higher to the tibia of *H. venatoria* than to other stimulants. The most striking observation was that aggressive responses were exclusive to the *H. venatoria* tibia. To specify the hair type that induced aggression, we manipulated two types of common hairs (bristle and fine) on the tibia of the predatory spider. When bristle hairs were removed from the *H. venatoria* tibia, aggression was significantly reduced. These results suggest that antennae can discriminate the tactile texture of external objects and elicit adaptive behavioral responses.

Keywords: insect, cricket, antenna, tactile sense, behavioral response

Introduction

The antennae are the principal sensory organs of insects; they carry sensors for olfaction, gustation, mechanoreception, thermoreception, and hygroreception. Many studies have reported on the importance of insect antennae as chemical sensors that provide biologically relevant cues about food, mates, hosts, and habitats (Schneider 1964; Altner and Prillinger 1980; Chapman 1982; Steinbrecht 1984; Zacharuk 1985). Another function of insect antennae is as a tactile sensor for detecting not only the presence and location of external objects but also their physical characteristics such as shape, size, and texture (Staudacher et al. 2005). The tactile sense from antennae may be particularly important in nocturnal insects because it helps insects appropriately negotiate obstacles in the dark (Harley et al. 2009; Baba et al. 2010; Schütz and Dürr 2011). Insect antennae are characterized by their active movements at the basal joints, which may facilitate efficient collection of tactile information from the surroundings. Such an active tactile sense must involve signals from proprioceptors for antennal movements as well as signals from antennal exteroceptors. Although the central mechanisms are unclear, various antennal mechanoreceptors responsible for both exteroception and proprioception have been found in some insect species (Staudacher et al. 2005).

Behavioral correlates with antennal tactile/mechanosensory signals have been studied in relatively few insect species. For instance, in cockroaches, evasive responses (turning and/or fleeing from a stimulant) evoked by mechanical stimuli to antennae (Comer et al. 1994) were accompanied by activation of identified descending mechanosensory interneurons (Burdohan and Comer 1996; Ye and

Comer 1996). The wall-following behavior of cockroaches, in which they show high-frequency (>25 Hz) turns, depends on mechanical information from the flagella (Camhi and Johnson 1999). The object-guided tactile orientation behavior in searching cockroaches involves arrays of specific mechanosensitive hairs at the antennal basal segments (Okada and Toh 2000). In walking cockroaches, the negotiation of obstacles, i.e., choosing to climb over or tunnel under, is closely related to mechanical information received from the antennae (Harley et al. 2009). Similarly, in walking stick insects, antennal contact information plays a significant role in the adaptive control of leg movements when climbing objects (Schütz and Dürre 2011). Baba et al. (2010) showed that running cockroaches use antennal mechanosensory information about the presence and configuration of obstacles to avoid collision.

Regarding tactile sensation, texture depends on the surface structure and mechanical properties of objects. Only a few studies have described the ability of insects to discriminate tactile texture. For example, by probing confronting objects actively with antennae, honeybees can recognize the difference between relatively large-scale texture patterns (Martin 1965; Erber et al. 1998). They also can discriminate flower petal microtexture with 10- μ m spatial resolution (Kevan and Lane 1985). In cockroaches, the escape response evoked by mechanical stimulation of the antennae is induced more frequently by a rough-surfaced stimulant than by a smooth-surfaced stimulant (Comer et al. 1994). A similar escape response is described in a pair of naturally occurring stimulants (wolf spider *Lycosa* sp. and a conspecific cockroach): cockroaches tend to escape from the hairy predatory spider but not from their conspecifics (Comer et al. 2003). Because washing the stimulants did not change the behaviors evoked, the key sensory cue appears to be tactile texture rather than cuticular chemicals (Comer et al. 2003). However, the tactile property of the stimulus responsible for the behavioral response is unknown.

The ultimate goal of our study is to elucidate the neural mechanisms underlying the texture discrimination by the active antennae of insects. As a first step towards this goal and being aware of the need of in-depth investigations of the underlying mechanisms, we extended the preceding behavioral study on cockroaches (Comer et al. 2003) to crickets. The field cricket *Gryllus bimaculatus* is an insect species whose antennal mechanosensory and motor systems are well documented (Honegger et al. 1990; Staudacher and Schildberger 1999; Gebhardt and Honegger 2001; Schoneich et al. 2011). Three stimulants of similar shape and size but different textures were used to evaluate differences in behavioral responses: a tibia from a hunting spider (potential predator), a tibia from an orb-web spider (less threatening predator), and a glass rod (unfamiliar artificial object). We also determined the type of tibial hair that elicits the behavioral response specific for the stimulation with the tibia of the predatory spider.

Materials and methods

Animals

Field crickets (*Gryllus bimaculatus* DeGeer) were obtained commercially and maintained in a laboratory colony at 24–27 °C on a 12-h light/dark cycle. Because preliminary tests showed no significant difference in behavioral responses, both sexes of adult crickets (25–31 mm in body length) were used for all experiments. Care was taken to use approximately the same number of males and females in each test group. To exclude visual cues completely in the stimulus presentation, the compound eyes and ocelli were cauterized to eliminate vision from the subject crickets at least 24 h before the experiments (Okada and Toh 2004). The test animals were maintained individually in plastic petri dishes (90 mm in diameter and 10 mm in height) and provided rodent pellets and water *ad libitum*.

The hunting spider *Heteropoda venatoria* (approximately 25 mm in body length) is commonly found in Japanese households. For this study, spiders were captured in the buildings of Nagasaki University during the night. They were fed a live cricket (*G. bimaculatus*) or cockroach (*Periplaneta americana*) once every few days. Orb-web spiders (*Argiope bruennichii*, approximately 20 mm in body length) were captured from the grasslands within the city of Nagasaki or from the suburbs. Only female adults were used for both spider species. Considering habitat and behavior, the free hunter *H. venatoria* is a natural enemy of the ground dweller *G. bimaculatus*, whereas *A. bruennichii* rarely preys on *G. bimaculatus* because it captures mainly flying insects such as homopterans and dipterans instead of ground dwellers (Miyashita and Shinkai 1995). Therefore, we consider *A. bruennichii* a nonpredator.

Scanning electron microscopy

To characterize hair types on spider legs, tibiae of *H. venatoria* and *A. bruennichii* were examined using scanning electron microscopy (SEM). In brief, freshly severed tibiae were fixed with 2% osmic acid solution for 1 h, dehydrated with a series of ethanol solutions, dried with a critical-point dryer (Samdri-PVT-3D; Tousimis, Rockville, MD), and coated with Pt/Pd using an ion sputter coater (E-1045; Hitachi Ltd., Tokyo, Japan). The specimens were viewed under a field emission scanning electron microscope (S-4800; Hitachi Ltd., Tokyo, Japan).

Experimental set-up

A cricket was placed in a cylindrical plastic arena (200 mm in diameter and 100 mm in height) and left alone for at least 3 min (Fig. 1). Initially, the cricket searched inside the arena but gradually stopped walking within a few minutes. After this accommodation period, the experimenter applied tactile stimuli to the cricket antennae using a solenoid stimulator. The stimulator consisted of a linear-sliding magnetic solenoid (44A-220-621-620; BLP Components Ltd., Suffolk, England), a manual switch, and a DC battery (Fig. 1). A tactile object (see below) was glued to the tip of the solenoid shaft. An experimenter held the stimulator, and the solenoid switch was controlled manually. When the experimenter pressed the switch, the stimulant was pushed forward. The stroke and velocity

of the stimulant were 2.4 mm and 330 mm/s, respectively. All experiments were recorded with a video recorder (Sony DCR-SR220) and replayed to confirm the classification of evoked behavioral responses.

Stimulants

A tibia of the 1st or 2nd walking leg and a distal part of the femur (10–20 mm in total length) were removed from a CO₂-anesthetized spider and glued to the shaft of the stimulator at the femur. Similarly, a glass rod (20 mm in length and 1.3 mm in diameter) was attached to the stimulator shaft. For the tibia of *H. venatoria*, two types of common hairs (bristle and fine) were manipulated. The bristle hairs were almost completely removed by pulling each hair with a forceps under a pair of binoculars. The fine hairs were removed by rubbing the tibia with an insect pin. Both the bristle and fine hairs were removed together by scrubbing the tibia with a small piece of adhesive tape. Thus, three kinds of tibiae with different textures were prepared and they were termed bristle-hair tibia, fine-hair tibia, and no-hair tibia, respectively (Fig. 5). In these hair-manipulated tibiae, the large spines were totally removed because our preliminary tests indicated that they had little effect on the tactile responses of crickets.

Stimulation, measurements, and analyses

The method for tactile stimulation followed the previous study performed in cockroaches (Comer et al. 2003). Each test comprised two successive contacts with one antenna. This stimulation procedure was also based on the observation that crickets encountering a live *H. venatoria* often probe it with their antennae until subsequent abrupt movement of the predator (Online Resources 5 and 6). The experimenter applied the 1st stimulus to the middle portion of the antenna manually so that the stimulant provided weak contact with the antenna and remained there for a few seconds. After about 5 s from the beginning of the 1st stimulus, the 2nd stimulus was applied as a single strong contact, using the solenoid stimulator, to the same region of the antenna. If the animal moved for >5 s after the 1st stimulus, the 2nd stimulus was applied 5 s after the motion ceased. Behavioral responses to the two successive stimuli were assessed and categorized in real time and reconfirmed by reviewing videotaped images. Each animal was tested 3–4 times a day with at least 3 h between tests during the subjective night period for crickets (0600 to 1800). A total of 94 to 113 tests were conducted using 22 to 26 crickets in each test group.

Differences in behavioral patterns between two test groups were determined using the chi-square test for independence prepared in an add-in statistical software (Excel Toukei 2008; Social Survey Research Information Co Ltd, Tokyo, Japan), and statistical significance was established at $P < 0.05$. For multiple comparisons between more than two test groups, the Bonferroni correction was used. In the chi-square test, if any expected frequency for a specific behavioral category was smaller

than 5, that category was discarded.

Classification of behavioral responses

Behavioral responses to tactile stimuli were classified into four types, including no response (NR) (Table 1, see also Online Resource 1 for more detailed classification). The aversion classification included four subtypes: run, walk, withdraw, and turn. The aggression classification included three subtypes: headstand, standing high (Loher and Rence 1978), and kick (Dumpert and Gnatzy 1977). Repetitive active antennal touch to the presented stimulant was classified as search. If the antennal movements were reflexive withdrawal from the presented stimulant and clearly distinguishable from the search response, such movements were regarded as NR. If more than two kinds of responses were observed successively with distinctive delays, the first behavioral response was recorded for the analyses.

Results

External morphology of tibial hairs in spiders

The body surfaces of the two spider species are covered with thousands of hairs. Differences in their external morphologies may reflect differences in their mechanical properties, which might explain species-specific tactile textures. In either spider species, the tibial hairs are classified into four types. Two of them were commonly observed and referred to as bristle and fine hairs. In each species, bristle hairs are relatively thicker in diameter and fewer in number than fine hairs (see below). Although the functional aspects of the two common hairs (bristle and fine) were unknown in the gross morphological observation in this study, most are speculated to be mechanosensitive or contact-chemosensitive (Foelix and Chu-Wang 1973; Harris and Mill 1977; Foelix 1985). The remaining two types were minorities in terms of number: large spines (Harris and Mill 1977) and trichobothria (Barth 2002), both of which are considered mechanosensitive.

In an example of *H. venatoria* (Fig. 2b), variations in the total length and shaft diameter of the bristle hairs were 0.2–2.0 mm and 10–20 μ m, respectively, and those of the fine hairs were 0.2–0.5 mm and 1–2 μ m, respectively. The bristle hairs protruded from the cuticular surface and were distally inclined. The fine hairs were more distally inclined and tended to cover the cuticular surface. A total of approximately 10 large spines arose from the anterior, posterior, and ventral surfaces of the tibia.

In an example of *A. bruennichii* (Fig. 2d), both the bristle and fine hairs on the tibia were similar in length (0.2–0.6 mm) but different in thickness (10–20 μ m and 5 μ m, respectively). Approximately 30 large spines were distributed along the tibia.

Behavioral responses to tactile stimulants

Crickets were exposed to three kinds of tactile objects, and the induced behavioral responses were classified into 4 types (Fig. 3, see Online Resource 1 for more detailed classification). For the 1st

stimulus, aversive responses were more frequent to the *H. venatoria* tibia (38/104 cases, see Online Resource 2 for video image) than to the *A. bruennichii* tibia (11/101) and the glass rod (4/102) (Fig. 3a). The most remarkable observation was that crickets showed aggressive responses exclusively to the *H. venatoria* tibia (6/104). Antennal search was the most frequent response to all three stimulants (Online Resource 3). We performed statistical tests for commonly observed response categories (aversion, search, NR) except aggression. The chi-square tests detected significant differences between the effects of stimulation with the *H. venatoria* tibia and the *A. bruennichii* tibia ($\chi^2 = 29.766$, $df = 2$, $P < 0.001$, with Bonferroni correction) and those with the *H. venatoria* tibia and the glass rod ($\chi^2 = 48.778$, $df = 2$, $P < 0.001$, with Bonferroni correction). On the other hand, no significant difference was found between the effects of stimulation with the *A. bruennichii* tibia and the glass rod ($\chi^2 = 4.236$, $df = 2$, $P = 0.1203$, with Bonferroni correction).

The behavioral response pattern to the 2nd stimulus using the *H. venatoria* tibia was also substantially different from those obtained with the nonpredator-derived stimulants (Fig. 3b). The aggression response was again observed to the *H. venatoria* tibia only (19/104, Online Resource 2). The most frequent response type to the three stimulants was aversion. In particular, the frequency of aversion to the *H. venatoria* tibia was high (81/104, Online Resource 3). Statistical tests for aversion, search and NR detected significant differences between the effects of stimulation with the *H. venatoria* tibia and the *A. bruennichii* tibia ($\chi^2 = 60.264$, $df = 2$, $P < 0.001$, with Bonferroni correction) and those with the *H. venatoria* tibia and the glass rod ($\chi^2 = 60.058$, $df = 2$, $P < 0.001$, with Bonferroni correction). The response patterns to the *A. bruennichii* tibia and the glass rod were similar. As in the 1st stimulation, no significant difference was detected between the effects of stimulation with the *A. bruennichii* tibia and the glass rod ($\chi^2 = 2.781$, $df = 2$, $P = 0.2489$, with Bonferroni correction).

We next examined whether the behavioral responses to the 2nd stimulus were influenced by the preceding 1st stimulus. Figure 4 shows responses to sudden strong contacts applied by the solenoid stimulator with the tibia of *H. venatoria*. Similar to the results of the 2nd stimulus in the normal procedure (see Fig. 3b, *H. venatoria*), crickets exhibited primarily aversive responses (83/104). However, the frequencies of the three other responses (aggression, search, NR) were considerably different from those to the 2nd stimulus in the normal procedure; the frequency of aggression response decreased, and those of search and NR responses increased. A statistical test for the three response categories (aversion, aggression, search) detected highly significant differences between the effects of the sudden strong stimulation and the normal 2nd stimulation ($\chi^2 = 12.907$, $df = 2$, $P = 0.0016$). It is therefore likely that the responses to the 2nd stimulus are affected by the preceding 1st stimulus.

Effects of hair manipulations on behavioral responses

To clarify which type of hair on the *H. venatoria* tibia causes specific behavioral responses, three kinds of hair-manipulated tibiae were used (Fig. 5). When the bristle-hair tibia was presented to the crickets, they behaved similarly as when presented to the intact tibia (Fig. 5, Bristle hairs). The

most frequent responses to the 1st and 2nd stimulus were search (54/103) and aversion (75/103), respectively, and aggression was more frequently observed with the 2nd (26/103) than with the 1st stimulus (10/103). No significant difference was found between the effects of stimulation with the intact tibia (see Fig. 3, *H. venatoria*) and the bristle-hair tibia in regard to the 1st stimulus for aversion, aggression, and search ($\chi^2 = 1.234$, $df = 2$, $P = 0.5395$, with Bonferroni correction) as well as to the 2nd stimulus for aversion and aggression ($\chi^2 = 1.315$, $df = 1$, $P = 0.2515$, with Bonferroni correction).

The fine-hair tibia, which lacks bristle hairs, was surveyed in the next experiment (Fig. 5, Fine hairs). Compared with the intact tibia, the response frequencies were reduced for aversion and aggression but were increased for search and NR elicited by both the 1st and 2nd stimulus. Statistical tests detected significant differences between the effects of stimulation with the fine-hair tibia and the intact tibia in regard to the 1st stimulus for aversion, search, and NR ($\chi^2 = 15.758$, $df = 2$, $P < 0.001$, with Bonferroni correction) and to the 2nd stimulus for aversion, aggression, and search ($\chi^2 = 21.109$, $df = 2$, $P < 0.001$, with Bonferroni correction).

Finally, the crickets were exposed to the no-hair tibia (Fig. 5, No hairs). Aggression ceased following the complete removal of all hairs for both the 1st and 2nd stimulus. Significant differences were detected between the effects of stimulation with the no-hair tibia and the intact tibia in regard to the 1st stimulus for aversion, search, and NR ($\chi^2 = 11.092$, $df = 2$, $P = 0.0039$, with Bonferroni correction) and to the 2nd stimulus for aversion, aggression, and search ($\chi^2 = 21.304$, $df = 2$, $P < 0.001$, with Bonferroni correction).

The results of the hair manipulation experiments clearly suggest that the bristle hairs correlate with predator (*H. venatoria*)-specific behavior, namely, augmented aversion and aggression.

Discussion

Tactile sensing in cricket antennae

Comer et al. (2003) showed that sensory information originating from mechanoreceptors in the flagellum enables cockroaches (*Periplaneta americana*) to discriminate between conspecifics and predatory spiders. However, the previous study did not explain what kind of tactile feature contributes to the predator-specific behavioral response. Our study revealed that crickets may tactually discriminate between predatory (*Heteropoda venatoria*) and nonpredatory (*Argiope bruennichii*) spiders. In addition, we showed that commonly occurring bristle hairs on the leg of the predatory spider (*H. venatoria*) may provide crickets with information on impending danger. Our morphological characterization of the tibial hairs in the two spider species is still very preliminary and there are no mechanical data at all. The unknown mechanical properties of the bristle hairs on the tibia of *H. venatoria* may provide the cricket's antenna with particular tactile information. Such information might be important for survival in encounters between prey crickets and predatory spiders in the dark.

Although we used only *H. venatoria* in this study, there are many natural predators for *G. bimaculatus* such as other hunting spiders, insectivores, and rodents. They are not necessarily covered

with hairs similar to the bristles found in *H. venatoria*. Further behavioral, morphological, and biomechanical analyses using various hair textures are needed to provide hints towards an understanding of the causal relationships and the biological relevance of the tactile interaction between insects and their predators.

A searching cricket often contacts an encountered object repeatedly with its antennae. This behavior could involve active tactile sensing. Whereas the behavioral aspects of active tactile sensing in insects are characterized using a limited number of species (in cockroaches, Okada and Toh 2006; Comer and Baba 2011; in stick insects, Schütz and Dürr 2011), the physiological mechanisms remain largely unknown. In the cricket, active tactile sensing with the antennae involves functional modules such as mechanoreceptors on the surface of the flagellum (Fudalewicz-Niemczyk and Rosciszewska 1973; Balakrishnan and Pollack 1997), proprioceptors that monitor antennal movements (Fudalewicz-Niemczyk and Rosciszewska 1973; Balakrishnan and Pollack 1997; Bartos and Honegger 1997), antennal motor neurons (Honegger et al. 1990), and interneurons that mediate antennal mechanosensory and motor information (Gebhardt and Honegger 2001, Schoneich et al. 2011). Presumably, these modules interact closely with each other to detect the tactile features of external objects efficiently via antennation. Among these modules, the flagellum may play a key role in texture discrimination because it makes direct contact with objects. To address the function of the flagellum in the tactile discrimination of cockroaches, Comer et al. (2003) performed a useful experiment in which both flagella were replaced with plastic fibers. We also used this method to study the cricket antenna, and behavioral responses to the predatory spider leg were compared between intact and “prosthetic” flagella. The replacement resulted in significant decreases in aggressive responses with both 1st and 2nd stimuli (1/103 cases for both) (see Fig. 3 for comparison). This is consistent with the hypothesis that flagellar mechanoreceptors are responsible for coding stimulus identity (Comer and Baba 2011).

What happens if the predator- or nonpredator-derived stimulant is presented to other parts of the body surface? In our preliminary study, the hind leg was stimulated with the tibia of *H. venatoria* and a glass rod, using the same procedure as that used for the antennae. Interestingly, the crickets behaved similarly to either stimulant (Online Resource 4). It is therefore likely that the hind leg has less ability, if any, than the antenna for discriminating the tactile properties of external objects.

Considerations regarding behavior

In this study, the evoked behavioral responses to the 1st or 2nd stimulus were consistent, regardless of the stimulant. Antennal search was the most frequent response to the 1st weak stimulus, and aversion was the most frequent response to the strong 2nd stimulus (Fig. 3). The former (antennal search) is considered as a probing process, while the latter (aversion) would be a reasonable response because crickets can rapidly avoid any abruptly moving object. In fact, when a searching cricket encountered a live *H. venatoria*, the cricket frequently contacted the spider’s body as long as the predator remained still (see Online Resource 5 for video image). This probing process may affect the

cricket's subsequent response to an abrupt movement of the confronting object. If the cricket detects dangerous tactile signals such as the bristle hairs of *H. venatoria*, the possibility of inducing the aversive response to the confronter's abrupt movement is presumably increased. Therefore, in this example, the crickets behaved in a context-dependent manner.

Aggressive responses were exclusively observed when the antennae were stimulated with the predatory spider leg (Fig. 3). In fact, crickets encountering a live *H. venatoria* during searching occasionally exhibited aggressive behaviors (see Online Resource 6). Previous studies have reported a correlation between aggression and sensory organs in crickets. In *G. bimaculatus*, the campaniform sensilla on the cerci, presumably activated by deformation of the cuticle, are involved in triggering a kick (Dumpeert and Gnatzy 1977). In the European house cricket *Acheta domestica*, headstand and stilt-stand responses to approaches of predatory wasps are triggered by unidentified mechanoreceptors on the cerci and antennae, respectively (Gnatzy and Heußlein 1986). Our study added a new example that the tactile texture derived from predatory spiders effectively triggers aggression if stimuli are applied to the antennae. However, the realistic effectiveness and suitability of the aggressive response is questionable considering the body size and strength of the predatory spider (*H. venatoria*) compared to those of the prey insect (*G. bimaculatus*). We therefore suspect that the aggressive response is a stereotyped behavior exhibited by the prey upon detecting danger signals from predators.

Involvement of chemical and visual cues in predator detection

The importance of antennal contact chemoreception for sex recognition is well-known in some cricket species (Rence and Loher 1977; Hardy and Shaw 1983; Balakrishnan and Pollack 1997; Tregenza and Wedell 1997; Iwasaki and Katagiri 2008). Olfaction or contact chemoreception is also a crucial sense for the avoidance of natural enemies in insects (reviewed by Dicke and Grostal 2001). One report found that field crickets (*Gryllus integer*) tend to avoid chemotactile cues derived from predatory spiders (*Hololena nedra*) that have preyed previously on conspecific crickets (Kortet and Hedrick 2004). Considering these findings, the role of chemical cues in the prey–predator relationship observed in this study should be examined carefully.

In this study, crickets were totally deprived of visual function by cauterizations of both compound eyes and ocelli. Such treatment would be useful for the complete obliteration of vision, but it may be rather unnatural given that normal crickets (*G. bimaculatus*) are reasonably active even in the light phase. In fact, visually guided antennal pointing is a well-known optomotor response exhibited by *G. bimaculatus* to immediate moving objects (Honegger 1981). Similarly, involvement of visual cues in antennal touch was recently described in American cockroaches (Comer and Baba 2011). It is therefore possible that visual information affects antennal tactile recognition and subsequent behavioral responses. This issue also needs to be investigated.

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Figure legends

Fig. 1 Experimental set-up. A stimulant was attached to the tip of a magnetic solenoid shaft. The switch of the stimulator was controlled manually by the experimenter. Each test comprised two successive contacts of the stimulant with one of the antennae. The 1st stimulus was a weak contact applied manually, and the 2nd stimulus was a single strong contact applied by the stimulator. All experiments were recorded with a video camera

Fig. 2 External morphology of two species of spiders. The tibia of the predatory spider *H. venatoria* is densely covered with long hairs (**a**). The tibial hairs of the less threatening predator *A. bruennichii* are relatively sparse and short (**c**). Scanning electron micrographs show the hair profiles precisely (**b**, **d**). In *H. venatoria*, the bristle hairs are thicker and more protrudent than the fine hairs (**b**). In *A. bruennichii*, the bristle and fine hairs are similar in length but the bristle hairs are thicker than the fine hairs (**d**). B, bristle hair; F, fine hair; S, spine; Me, metatarsus; Ti, tibia; Pa, patella

Fig. 3 Classified behavioral responses to three kinds of tactile stimulants. **a**. Responses to the 1st stimulus. Each numeral at the top of the column indicates the number of observations. Aggression response was observed to the stimulation with the tibia of *H. venatoria* only. **b**. Responses to the 2nd stimulus. Again, crickets showed aggressive responses to the *H. venatoria* tibia only. Behavioral categories for statistical tests: aversion, search, and NR for both **a** and **b**. *, significantly different; NS, not significantly different; NR, no response

Fig. 4 Behavioral responses to sudden strong stimuli with the *H. venatoria* tibia. A significant difference was detected for three behavioral categories (aversion, aggression, search) compared with the responses to the 2nd stimulus in the normal procedure (see Fig. 3b, *H. venatoria*)

Fig. 5 Effects of hair manipulations on behavioral responses. **a**. Responses to the 1st stimulus. The responses to the bristle-hair tibia were similar to those to the intact tibia (Fig. 3a, *H. venatoria*). Aggression was elicited significantly less with the fine-hair tibia and ceased completely with the no-hair tibia. Behavioral categories for statistical tests: aversion, aggression, and search for “bristle hairs”; aversion, search, and NR for “fine hairs” and “no hairs”. **b**. Responses to the 2nd stimulus. Again, aggression was elicited significantly less with the fine-hair tibia and ceased completely with the no-hair tibia. Behavioral categories for statistical tests: aversion and aggression for “bristle hairs”; aversion, aggression, and search for “fine hairs” and “no hairs”

Fig. 1

Fig. 2

Heteropoda venatoria

Argiope bruennichii

Fig. 3

Fig. 4

Fig. 5

Table 1. Classification of behavioral responses to tactile stimuli

Type	Subtype	Description
Aversion	Run	Rapid escape from the stimulant with turn and run
	Walk	Slow escape from the stimulant with turn and walk
	Withdraw	Withdrawal from the stimulant with backward walk
	Turn	Turning away from the stimulant
Aggression	Headstand	Raising the abdomen
	Kick	Turning, raising the abdomen, and kicking the stimulant with hind leg
	Standing high	Lifting up the body, frequently with rostrocaudal rocking
Search	—	Repetitive touch to the stimulant by antennae
No response	—	—