Spatial perception through active sensing by cricket antennal mechanosensory system

(コオロギ触角機械感覚系のアクティブセンシングによる空間知覚)

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Abstract of Doctoral Dissertation

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Animals perceive their surroundings by using various modalities of sensory inputs to guide their locomotion by acquiring and integrating information of surrounding objects such as shape and location using multiple sensory organs. Crickets obtain sensory cues of spatial context mainly via antennal and visual systems, but it remains unclear whether the crickets modulate behaviors mediated by other sensory organs based on the spatial information. Crickets exhibit escape behavior in response to a short air-puff, which is detected by the abdominal mechanosensory organs called cerci and is perceived as a "predator approach" signal. In this thesis, in order to clarify if the crickets have an ability of spatial perception enough for a general use, I examined the effects contextual spatial information on the wind-elicited escape behavior, by using an open-loop treadmill system. In chapter 1, I examined whether the spatial perception of the cricket antennal mechanosensory system affects the wind-elicited escape behavior. I placed objects of different shapes at different locations with which the cricket actively made contact using its antenna, and then examined the changes in various locomotion parameters of the wind-elicited escape behavior. The crickets changed their movement trajectory in response to nearby objects to avoid collision with these obstacles during the cercal-mediated behavior. For instance, when a wall was placed at the center in front of the crickets so that it was

detected by both antennae, the forward movement triggered by the airflow from behind was suppressed and the escape trajectory curved bilaterally. In contrast, the one-sided frontal wall to be accessed by only one antenna significantly biased the movement toward the side opposite the wall. It was concluded that crickets modulated their escape behavior depending on spatial information of surrounding objects by using antennal inputs.

In chapter 2, to clarify how multisensory inputs are integrated to modulate the orientation behavior, I investigated the effects of both antennal and visual inputs on the wind-elicited escape behavior. A wall was placed in front of the cricket on one side so that only ipsilateral antenna could contact the wall. Then, the responses to airflow from behind were examined in four conditions: both antennae were intact or removed and in complete darkness or light, respectively. Crickets relied on the mechanosensory input from the antennae to localize frontal objects and modulated their escape behavior based on the spatial information obtained by the antennal mechanosensory system. On the other hand, the visual input had little impact on modulation of the walking direction induced by antennal inputs but reduced the walking distance and tuning movement and delayed the reaction time. These results suggest that crickets integrate information mediated by multiple sensory organs of different modalities to effectively avoid obstacles along the trajectory routes.

In chapter 3, I set two research aims. The first was to explore how bilateral comparison of right and left antennal inputs was involved in the localization of the forward objects. For this, I placed the wall in front of the cricket either at the center or one sided and compared the escape responses to the air puff from behind between when both antennae

were intact and when one antenna was ablated, respectively. When the antenna contralateral to the wall placed on one side was ablated, the movement biased to the wall-free side was remained but smaller than that in intact crickets. This suggests that the mechanosensory inputs from one antenna would be sufficient to localize the forward obstacle, but the bilateral comparison was more effective.

The second was to clarify whether crickets can measure the object size relative to their own body. I placed the wall with opening gap of different width that both antennae could detect, and examined the modulation of their escape response to the air puff from behind. Depending on the escape gap width, the crickets changed their behavior to either the straight-forward escaping or the movements biased to either side. This result showed that the crickets were likely able to perceive the size of the expected route sufficient to accommodate the own body size to avoid collision during escape behavior.

In conclusion, this study demonstrated that crickets were able to use the spatial information by active sensing with their antennal system to modify their behavior mediated by other sensory organs.

Contents

General introduction

Escape behavior as defense mechanism to predation risk

Escape behavior is a distinctly oriented locomotion in which animals move in the opposite direction to threats, such as predators, in order to increase, as much as possible, its distance from the threat (Card and Dickinson, 2008; Domenici et al., 2011a,b). Escape had been thought to be a stereotypic behavior. For example, C-start escape in fishes is a quick bending of the body on the contralateral side to the threat stimulus and is followed by a rapid fast acceleration to swim toward the opposite direction to the stimulus (Eaton et al., 2001). However, rather than robust and simple stereotypic behavior, the escape behavior requires to be flexibly changed adapting to the trigger stimulus, environments, and context. Animal's behavioral response depends on external stimulus (De Franceschi et al., 2016; Evans et al., 2019) and their internal state (Dickinson and Balleine, 1994). Animals firstly categorize the sensory stimuli into threatening and non-threatening signal (Evans et al., 2019). Then, depending on characteristics of the threatening stimulus, animals choose various defensive behaviors. For example, rats show freezing response to objects like raptors cruising above them, while they exhibit flight behavior if that object is approaching (De Franceschi et al., 2016). Crickets exhibit escape response, either running or jumping, to short air-puff, based on trade-off between speed and flexibility (Sato et al., 2019). These mean that animals have multiple defensive strategies and choose a specific behavior from them appropriate to the stimulus, considering their cost and benefit. In the escape behavior, decision of the moving direction is also important to

survive. Predators predict prey's movement and calculate their trajectory to ensure capture of the prey (Moore and Biewener, 2015; Combes et al., 2013), whereas preys try to escape in various directions to defy the predator's expectation (Domenici et al., 2008). Thus, escape behavior is one of the excellent models for neuroethological studies on the context dependence of behaviors and decision-making mechanisms.

Modulation of escape behavior by spatial context

Escape trajectories are often modulated depending on the environmental context (Domenici, 2010; Evans et al., 2019). When goldfish visually perceive an obstacle, they change their escape trajectory to avoid collision with it (Eaton and Emberley, 1991). Escape directionality of lizards and mice also depends on presence and location of shelter, detected visually and auditorily (Hennig et al., 1976; Vale et al., 2017). Insects use their antennae for exploration of surroundings and acquisition of the relevant spatial information, which are mediated by the repeated movement of antennal flagellum for active sensing to improve the sensory perception (Bermejo et al., 2005). During locomotion of insects, their antennae are involved in near-range orientation, including detecting, localizing, probing, and negotiating obstacles (Mitchinson et al., 2011, Dürr, 1999). However, it remains unknown whether the spatial information of the surroundings actively obtained by the antennal system modulate the escape behavior or not. In my thesis, I addressed this issue by examining the modulation of crickets' wind-elicited escape behavior by antennal mechanosensory stimulation.

Cercal-mediated escape behavior of crickets

Tactile stimulation to cockroach's antennae itself sometimes induces the escape behavior (Comer et al., 1994; Comer and Baba, 2011). To separate the contextual stimuli from trigger stimulus, I adopt the wind-elicited escape behavior, which was mediated by the cerci, an abdominal mechanosensory organ. Crickets detect surrounding air currents by the displacement of the filiform sensilla on the cerci (Landolfa and Miller, 1995) and exhibit an oriented escape behavior in response to short air puff (Gras and Hörner, 1992; Tauber and Camhi, 1995; Oe and Ogawa, 2013). The crickets moved in the opposite direction to the airflow stimulus both in running and in jumping during this escape behavior (Sato et al., 2019). The receptor neurons of the filiform sensilla project their axon into the terminal abdominal ganglion (TAG) in orderly manner (Jacobs and Theunissen, 1996; Paydar et al., 1999). The airflow information such as direction, velocity, and frequency are encoded by firing activity of the ascending projection neurons identified as giant interneurons (GIs) (Miller et al., 1991), which project their axon into the high centers such as cephalic and thoracic ganglia (Hirota et al., 1993). Descending signals from the brain where the directional information is processed are required to control the escape direction. Therefore, if the surrounding objects actively sensed by the antennae affects the cercal-mediated escape, it will be direct evidence of spatial context-dependent modulation of the escape behavior.

Multisensory integration to improve sensory perception

A single stimulus in specific modality can trigger the escape behavior, in general. In contrast, to comprehensively understand the environmental context, animals also integrate sensory information from multiple sensory organs with different modalities. Also in the escape behavior, the prey likely senses the surrounding context via multisensory integration to evade the predator in the environment. The combination of mechanosensory and visual cues results in the escape of rockpool prawn over longer distances and with greater directionality when compared to those triggered by mechanosensory stimulus alone (Guerin and Neil, 2015). Also, in wind-elicited escape behavior, crickets alter their escape trajectory elicited by a short air-puff depending on acoustic context represented as different sound frequencies (Fukutomi et al., 2015; Fukutomi and Ogawa, 2017). In addition, a wind stimulus applied to cockroaches that make contact with the wall using their antenna elicits different turning behavior when compared to animals that do not make contact (Ritzmann et al., 1991). Thus, the wind-elicited escape behavior that is mediated solely by the cerci, a different mechanosensory organ from the antennae, would be an ideal model to test the ability of antennal system in spatial perception-based behavioral modulation.

Goal of this thesis

The final goal of my thesis is to clarify if the insect's antennal system has an ability of spatial perception enough for a general use to the modulation of oriented behavior such

as the wind-elicited escape behavior in crickets. In chapter 1, I examined the effects of the antennal information such as object shape, distance, and orientation on the escape behavior. In chapter 2, by combining visual and mechanosensory inputs, the cross-modal effects were tested in the behavioral modulation. Lastly, in chapter 3, I tested how precisely the crickets detect the object shape and location through their bilateral antennal mechanosensory inputs.

Chapter 1

Modulation of wind-elicited escape behavior by antennal mechanosensory inputs

1.1 Introduction

Animals perceive their surroundings using various sensory inputs to guide their locomotion appropriately. In situations where visual cues are not available, such as in darkness or in very tight spaces due to surrounding objects, mechanosensory inputs provide effective cues to guide their path. For example, rodents employ their facial whiskers as a tactile sensor array to guide their locomotion (Prescott et al., 2011; Grant et al., 2018). In insects, the mechanosensory cues provided by antennae play an important role in guiding their locomotion. For example, cockroaches walk along a wall by contacting the wall using their antenna (Camhi and Johnson, 1999; Mongeau et al., 2013). These facts suggest that insects use mechanosensory inputs mediated by their antennae for appropriate course control in various environments (Staudacher et al., 2005).

However, it remains unclear whether the insect can use the spatial information of the surroundings perceived with their antennal system to modulate a behavior mediated by other sensory organs. The movement modulation in an oriented behavior adapting to the surrounding space would require the integration of different sensory inputs, one of which induces the behavior itself and the other provides spatial information of the surroundings. To confirm the general use of the spatial perception in a mobile behavior,

it is necessary to examine whether the spatial context such as the arrangement of objects affects the oriented behavior mediated by other sensory organs, rather than the behavior directly induced by the detected objects. This is because, if the animal is reflexively oriented to the object itself that causes the action, it can change its behavior depending on the position of the object without spatial perception. For example, the impacts of antennal stimuli on phonotaxis in female crickets have been investigated. Active contact with an object by the antennae of the crickets performing phonotaxis reduces forward velocity toward the sound source and suppresses phonotactic steering depending on the side where object is located and the distance (Haberkern and Hedwig, 2016). This finding suggests that the spatial perception of the cricket antennal mechanosensory system may affect the oriented behavior elicited by the stimulus mediated by other sensory organs. To address this issue, I used their escape behavior in response to short airflow, which was detected by the cerci, an abdominal mechanosensory organ (Gras and Hörner, 1992; Tauber and Camhi, 1995; Oe and Ogawa, 2013). As mentioned in General introduction, this wind-elicited escape behavior is one of the typical orientation behaviors, in which crickets move in correct opposite direction to the airflow stimulus. And this behavior is completely mediated by abdominal mechanosensory organ, cerci. Thus, this is an ideal behavioral model to investigate the ability of spatial perception as general use of the antennal mechanosensory system.

1.2 Materials and methods

1.2.1 Animals

I used the wild-type strain of field crickets for this study (*Gryllus bimaculatus* De Geer, 1773, Hokudai WT; Watanabe et al., 2018). The laboratory-bred adult male crickets (0.50–1.00 g body weight) were used in all the experiments. They were reared under 12 h light:12 h dark conditions at a constant temperature of 27°C. The guidelines of the Institutional Animal Care and Use Committee of the National University Corporation, Hokkaido University, Japan, specify no particular requirements for the treatment of insects in experiments. Before commencing the experiments, all crickets were checked to ensure that the legs, cerci, and antennae were intact. All experiments were conducted in the early hours of the animals' subjective night at room temperature (26–28°C).

1.2.2 Treadmill system

I monitored a cricket's locomotion in response to an air-puff stimulus with the same spherical-treadmill system that was installed within a sound-proofed dark box, as used in previous studies of our laboratory (Oe and Ogawa, 2013; Fukutomi et al., 2015; Fukutomi and Ogawa, 2017). An animal was tethered on top of an air-lifted Styrofoam ball (ø = 60 mm) using a pair of L-shaped insect pins that were stuck to the cricket's tergite with paraffin wax. The cricket's walking activity was monitored as rotation of the ball at a sampling rate of 200 Hz, using two optical mice that were mounted orthogonally around the ball. TrackTaro software (Chinou Jouhou Shisutemu Inc., Kyoto, Japan) was used to

measure the movement trajectory and to calculate parameters such as translational and angular turn velocities based on the measured ball rotation (Fig. 1-1).

1.2.3 Air-puff and tactile stimulations

An airflow stimulus was provided to the cerci of the cricket that was stationary for more than one second by a short puff of nitrogen (N_2) gas from a plastic nozzle (15 mm diameter) connected to a PV820 pneumatic picopump (World Precision Instruments, Sarasota, FL, USA). By adjusting the delivery pressure of the picopump, the velocity of the air-puffs was controlled at 0.68 $m\,s^{-1}$, which was measured at the center of the treadmill with a 405-V1 thermal anemometer (Testo, Yokohama, Japan). The duration of the air-puff stimulus was set to 200 ms. Eight air-puff nozzles were arranged around the inside wall of the arena, and the height of the nozzles was aligned with the same horizontal plane as the animal. The nozzle ends were positioned at a distance of 130 mm from the center of the treadmill and were spaced 45° apart. In the experiments to test the effects of antennal tactile stimulation, the air-puff stimulus was applied from either the posterior (180°) or lateral side (90°) of the animal. In a preliminary experiment to test the effects of bilateral ablation of antennae on the escape behavior, the stimulus was provided in sequence from eight nozzles that were spaced 45° apart (Fig. 1-2).

A vertical cylindrical rod (ø = 7 mm) or square plate (50 mm × 50 mm) was placed in different orientations and distances from the antennae. The objects were placed either in the “far” position, which was 5 mm proximal from the tip of the antenna or in the “near”

position, which was at half the antennal length (Figs. 1-3A, 1-4A). For different orientations of the object, the plate was placed either on the left side or in front of the cricket in the near position. The lateral plate was placed either at the anterior or posterior position (Fig. 1-10A). The frontal plate was centered or placed on one side in front of the animal (Fig. 1-13A).

1.2.4 Video recording of antennal movement

To check the frequency and duration of contact of the antenna with the object, the antennal movement was monitored using a high-speed digital camera (CHU30-B, Shodensha, Osaka, Japan) under red LED illumination. The voluntary movement of the antenna was recorded for 60 s in each trial at a frame rate of 90 frames/s with a resolution of 640 × 480 pixels. I manually counted the frames in which the antenna was in contact with the plate and calculated the frequency and duration of contact. I compared the antennal contacts to the anterior and posterior plates positioned on the lateral side of the animal, and also the contacts of ipsi- and contra-lateral antennae to the plate positioned on one side in front of the animal. For each condition, five trials of the measurement for 1 min were performed with an interval of 1 min between trials. Ten individuals were recorded in total for each experiment.

1.2.5 Experimental procedure

The common procedure to record escape movement of crickets using the treadmill system

is as follows. At first, a cricket was positioned on top of a Styrofoam ball by using a micromanipulator that moved a pair of L-shaped insect pins attached with its tergite. Next, an object was moved to the specific location against the animal by using a manipulator, and then I started recording the movement before and after the air-puff stimulus including a waiting period to confirm that animal was standing still for 1 second. (The waiting period was 11.57 ± 0.99 s in conditions with object presentation in Figs. 1-3 and 1-5). Since the object remained presented at a specific location during experiments in each condition, the animals were able to sense the object by contacting it with their antennae voluntarily during all trials including inter-trial intervals. I adopted three experimental arrangements with different representation of the objects and stimulations.

(i) Bilateral antennal cut

To examine the contribution of the antennal inputs induced directly by airflow stimulus to the wind-elicited behavior, I recorded the movement of crickets with intact antennae and those in which the antennae were bilaterally ablated at the base (Fig. 1-2A). Nothing was placed around the crickets and thus tactile stimulation of the antennae was eliminated. In this experiment, a single air-puff was delivered from each nozzle positioned at 0°, 135°, –90°, 45°, 180°, –45°, 90°, and –135°, in this order, with an inter-trial interval greater than one minute. For each individual, 40 trials (five trials for each stimulus angle) were recorded before and after the ablation of the antenna, respectively. To ensure that the animal had recovered from the damage of the antennal ablation, the experiments using the antenna-ablated crickets were performed more than 40 min after

the ablation. Twenty-six individuals were tested in total.

(ii) Different shapes and distances of object

To test the effects of object shape and distance, I adopted the following five types of stimulation conditions (Figs. 1-3A, 1-5A). The rod was placed near or far from the antenna, referred as “near pole” and “far pole”. The plate was placed near or far from the antenna on the side of the cricket, referred as “near wall” and “far wall”. In the control, neither rod nor plate was placed (control). The air puff was applied from the rear of the cricket or from its lateral side opposite to the objects. For each tactile stimulation condition, 10 trials were performed with an inter-trial interval greater than one minute. The stimulation conditions were tested in the following order, control, far pole, near pole, far wall, and near wall. Finally, the control condition was tested again to confirm that the escape movement was not adapted through the series of the experiment. The trials in this condition were referred to as “control2”. In total, 60 trials were performed for each individual. Twenty-four individuals were tested.

To check for a potential turbulence effect of the air-puff stimulation when the plate was positioned on the lateral side of the cricket, I measured the escape behaviors of the cricket in which both antennae were ablated from the base (Fig. 1-7). After more than 40 min of antennal ablation, the escape responses to the air-puff from behind (180°) or lateral side opposite to the plate (90°) were recorded in two different conditions: without the wall (control) and with the wall placed at the near position. For each individual, 20 trials (10 trials for each stimulus angle) were recorded. Twelve individuals were tested in total.

(iii) Different locations of wall

To test the effects of the object location, I adopted two types of experiments (Figs. 1-8A, 1-11A). In the first type, the plate was placed at the lateral side of the cricket either anteriorly or posteriorly relative to its head, referred as “anterior” and “posterior”, respectively, and the air-puff was applied from the contralateral side of the plate (Fig. 1-10A). The side edge of the plate was aligned with the base of the antenna. Three stimulation conditions, control, anterior, and posterior were tested in random order for each individual. In the second type of the experiment, the plate was either centered in front of the cricket or placed toward one side, referred as “center” and “one-sided”, respectively, and the air puff was applied from its rear (Fig. 1-13A). In the center stimulation condition, the center of the plate was aligned to the midline of the animal so that the cricket could access the plate with both antennae. In the “one-sided” condition, the side edge of the plate was aligned to the midline of the animal so that the cricket accessed the plate only with the antenna ipsilateral to the plate. The three stimulation conditions, control, center, and one-sided, were tested in random order for each individual. In both types of experiments, five trials were performed for each stimulation protocol with an inter-trial interval greater than one minute. In total, 15 trials were performed for each individual for each type of experiment. Thirty-three and 30 individuals were used in the first and second types of experiments, respectively. For the one-sided stimulation condition in the second type of experiment, the plate was placed on the right side of the animals in 15 crickets, and in another 15 crickets on the left side to counterbalance the

stimulation to the antenna bilaterally.

1.2.6 Data analysis

Behavioral data provided by the TrackTaro software were processed and analyzed offline, using algorithms customized using Python 3.7.1 (Jupyter Notebook version 5.7.4). First, I classified all recorded data from all trials into “wind-elicited response” or “no response” based on the walking speed, as used in previous studies using the treadmill system (Oe and Ogawa, 2013; Fukutomi et al., 2015; Fukutomi and Ogawa, 2017). If the walking speed exceeded 10 mm/s during the period from the stimulus onset to 250 ms after the stimulus onset and the maximum translational velocity was greater than 50 mm/s, the cricket was considered to respond to the air current (Fig. 1-1 A). If the cricket did not begin to move within this response definition period of 250 ms after the stimulus onset, that trial was considered as “no response”. The response probability was defined as follows:

$$\text{Response probability } = \frac{N_r}{N_r + N_n}$$

Where, N_r and N_n are the number of trials categorized as a wind-elicited response and a no response obtained for each stimulation condition, respectively. I focused on the initial responses to the air-puff stimulation, of which the response start was defined as the first time when the translational velocity exceeded 10 mm/s after stimulus onset; the finish was defined as the time when the velocity was less than 10 mm/s after the velocity exceeded 50 mm/s (Fig. 1-1 A, Oe and Ogawa, 2013; Sato et al., 2017, 2019). I measured

four locomotion parameters for this initial response: walking direction, turn angle, reaction time, and walking distance. Definition and calculation of these parameters are the same as in our previous study (Oe and Ogawa, 2013; Fukutomi et al., 2015; Fukutomi and Ogawa, 2017). The walking direction was measured as the angle between the body axis at the start point (red line in Fig. 1-1B) and the line connecting the start and finish points of the initial response (blue arrow in Fig. 1-1B). The turn angle was measured as the angle made by the body axes at the start and finish points (green line in Fig. 1-1B). The walking direction and turn angle were arranged for the forward direction as 0°, clockwise as plus, and counterclockwise as minus. I arranged the walking direction so that it ranged from 0° to ±180° (Fig. 1-1B). To compare the magnitude of the turning movement, unlike the walking direction, the turn angle was not arranged. The trajectory length of the initial response is referred to as the walking distance. The forward distance was defined as the travel distance of the forward movement during the initial response. The reaction time was defined as the time delay from the opening of the delivery valve in the picopump to the start of the response. Thus, it included a constant travel time of the airflow between the nozzle and the cricket.

1.2.7 Statistical methods

R programming software (version 3.5.2, R Development Core Team) was used for the statistical analysis. To avoid pseudo-replication, I used the mean value of the data obtained in the trials categorized as wind-elicited response for each individual as the

representative value for the statistical tests. Because the walking direction is a circular parameter, I calculated the circular mean angle of the walking direction for each individual. The turn angle was treated as a non-circular parameter just like reaction time and walking distance because it was measured as angular magnitude of rotation movement. All statistical tests for the significant effects among three or more groups were corrected with Bonferroni correction.

Prior to statistical testing of the non-circular parameters including turn angle, walking distance, and reaction time, I checked the distribution of the datasets, using the Shapiro-Wilk test. As the data for these parameters in all the experiments were not normally distributed, I used the Wilcoxon rank-sum test or Wilcoxon signed-rank test to assess the significance of the stimulation conditions. If some of the tested individuals did not respond, the Wilcoxon rank-sum test to test unpaired data set was used. If all tested-individuals responded, the Wilcoxon signed-rank test to test paired data set was used. The Wilcoxon signed-rank test was also used to assess the significance of the stimulation condition for the response probability.

Fisher's nonparametric test for the common median direction (Fisher, 1993; Pewsey et al., 2013) was used to assess the significance of the stimulation condition for the walking direction. In order to assess the significance of the stimulation condition for the angular dispersion around the mean of the walking direction and turn angle, I used the Wallraff test, for which the package "circular" was used (Agostinelli and Lund, 2017). To assess the significance of the trial order for the walking direction, I used the Kruskal

Wallis test.

1.3 Results

1.3.1 Effects of antennal mechanosensory inputs on the cercal-mediated escape behavior

First, to test whether the cricket's antennae also could sense the airflow stimulus and contribute to the escape behavior, I compared the response to the air puff of intact and bilateral antennae-removed crickets. If the antennal mechanosensory inputs were involved in the wind-elicited escape behavior, ablation of the antenna would alter the response to the stimulus from the anterior because the antenna was more sensitive to the frontal stimulus. Here, the air-puff stimulus was applied from eight angles around the cricket, and the stimulus-angle-related directionality in the escape behavior was examined. The antenna-ablated crickets responded to the air-puff stimuli applied from any angle, and their trajectory did not differ from that of intact crickets (Fig. 1-2A). The antenna ablation had little effect on either the walking direction or the turn angle for any stimulus angle (Fig. 1-2B-D). There was also no significant difference in angular dispersion around the mean angles of the walking direction between the cut and intact conditions (Table 1-1, Wallraff test). The reaction time was also not affected by antennal ablation (Fig. 1-2D). The turn angle, walking distance, and response probability were not affected except for the specific stimulus angles of 180º, 135º, and 90º, respectively (Fig. 1-2D, Table 1-1). This suggests that antennal ablation had little impact on responsiveness and directional control. In conclusion, my results indicate that any interaction of airflow with antennae did not contribute to the wind-elicited escape behavior of crickets.

Next, I tested whether the crickets could alter the escape behavior when they had the opportunity to place their antennae in contact with potential obstacles. I also examined how the crickets modulated the escape trajectories depending on the object shape and distance. Fig. 1-3 shows the responses to the air puff from behind when a cylindrical pole or square plate was positioned at different distances (Fig. 1-3A). The walking trajectories showed that the crickets tended to walk toward the free side, opposite to the objects, and this movement was most pronounced in the near wall condition (Fig. 1-3B). The data on the walking direction revealed that it was significantly biased toward the contralateral side of the plate in the near wall condition (Fig. 1-3C,D, Table 1-2). In contrast, there was no significant effect of either the plate placed at far position or the poles placed at near or far position. The turn angle, reaction time, walking distance, and response probability were not affected by the presence of objects regardless of their location (Fig. 1-4A,B, Table 1-2).

I further tested the effects of the pole or plate placed at different distances on the response to airflow applied from the side of the cricket (Fig. 1-5A). In the control condition with no object, the cricket moved in the direction opposite to the air-puff stimulus, as shown by gray traces in Fig. 1-5B, and the walking trajectories were distributed around -90° (Fig. 1-5C). However, when the objects were placed on the contralateral side of the stimulus, the cricket moved more backward, and the distributions of the walking direction were shifted (Fig. 1-5C). The backward bias in the walking direction was significant when the plate was positioned at the near position (Fig. 1-5D,

Table 1-3). The plate located at the far position also tended to bias the walking direction backwards, but this effect was not significant. The pole did not affect the walking direction, regardless of the location. These results suggest that crickets alter their walking direction to avoid hitting the obstacle depending on the obstacle shape and distance to the obstacles. The modulation in the walking direction would solely result from antennal contacts and not from air turbulence by the objects because the effect of the plate that was located at the near position was abolished by bilateral ablation of the antennae so that the crickets could not detect it (Fig. 1-7, Table 1-4). In contrast, neither the pole nor plate had any effect on the turn angle, walking distance, and response probability regardless of their location (Fig. 1-6, Table 1-3). There was a significant difference in the reaction time between control conditions 1 and 2, which were performed without objects before and after the experiments using other conditions with objects (Fig. 1-6). This might be due to after-effects of many previous encounters with obstacles. In summary, the results shown in Fig. 1-3 and Fig. 1-5 indicate that the crickets were able to sense the object shape and the distance to it and to modulate escape trajectory to avoid collision with obstacles by using their antennal mechanosensory system.

Next, I examined whether the crickets adapted or learned the open-looped experimental system, where all objects never moved even if the crickets walked on the treadmill. I checked if the effect on the walking direction by the near wall depended on the trial order, for the data shown in figure 1-3. There was no difference in the walking direction among the trials (Kruskal Wallis test, Fig. 1-8), suggesting that crickets did not

learn or adapt to the experimental condition. Also, I checked the spontaneous walking movements before the airflow stimulation when the wall was placed at near position. There were no differences in either the trajectory or direction of the spontaneous walking bouts. This result revealed that the spontaneous walking was not affected by the presence of the wall (Fisher's nonparametric test, Fig. 1-9).

1.3.2 Crickets could sense the location of obstacles with their antenna

Next, to test the ability of the antennal system to sense the location of the obstacles, I examined the effects of the location of the plate on the escape behavior. First, I placed the plate on the side of the cricket at two different positions, anterior or posterior (Fig. 1-10A). I predicted that crickets would change their movement direction depending on the plate location: they might move backwards when the plate is placed at the anterior position and forward when it is placed at the posterior position. The stimulus was applied from the contralateral side to the plate.

The walking direction was significantly biased backwards by the anterior plate. In contrast to my prediction, the posterior plate had no significant effect and did not enhance forward movement (Fig. 1-10B,C,D, Table 1-5). But the posterior plate also resulted in bimodal distribution of all trial data in the walking direction and separated the within-individual means into two clusters (Fig. 1-10C,D), suggesting the possibility that some individuals changed their trajectory by sensing the posterior plate. The bias in the walking direction caused by the anterior plate was coupled with a longer walking distance (Fig.

1-10D). It is likely that crickets might have to move longer distances in order to avoid the anterior wall. The turn angle, reaction time, and response probability were not affected by the presence of the plate in either position (Fig. 1-11A,B, Table 1-5). The lack of an effect of the plate positioned posteriorly might be due to the cricket's failure to detect it. To confirm this possibility, I observed the voluntary movement of the antenna against the plate placed at anterior or posterior position by using a high-speed camera. Although the crickets made less contacts with posterior plate than with anterior plate, they still did so on average about 200 times during recording period of 5 minutes (Fig. 1-12). There were no significant differences in the duration of each contact between the anterior and posterior positions of the plate (Fig. 1-12, Table 1-6). This indicated that the cricket could sense the plate placed at the anterior position more precisely, but it would have been possible to perceive the posterior plate, too. The changes in their escape behavior depending on the wall position suggest that crickets can sense the location of the objects with one antenna.

1.3.3 Single antennal modulation of escape in crickets.

The results thus far revealed that crickets could change their escape behavior by locating objects even with only one antenna. This was because it was difficult for the crickets to touch the laterally placed plate and pole with their contralateral antenna. If both antennae were used to detect the object, the crickets could perhaps perceive the object location more precisely. To examine this possibility, I studied the trajectory of the escape response

to the airflow from the rear when the plate was placed in front of the cricket at different positions namely, at the center or to one side (Fig. 1-13A). In this experiment, the crickets were able to touch the plate at the center in front of them with both antennae, while the plate located to one side could be touched by only the ipsilateral antenna. I predicted that the crickets in response to the airflow from the rear would move forward, biased to the free side and contralateral to the plate, when the plate was placed at one side. As expected, the plate placed on one side significantly biased the walking direction and turn angle to the free side opposite to the wall (Fig. 1-13B,C,D,E, Table 1-7).

On the other hand, when the wall was placed in front of the animal and aligned to the center, the trajectory was greatly altered. Both of the walking direction and the turn angle became widely and bimodally distributed and the number of movements decreased around 0° of the walking direction and turn angle although there was no significant difference in the median of these directional parameters compared to the control condition (Fig. 1-13C,D). As a result, the center wall increased the angular dispersion of the walking direction and turn angle significantly compared to the control and one-sided wall conditions (Fig. 1-13E, Table 1-7). Furthermore, there was no significant difference in the walking distance between the conditions), but the forward movement was significantly reduced in the center wall condition (Fig. 1-13E, Table 1-7). These results indicated that the center wall might enhance the lateral movement rather than the forward movement. The cricket could alter its escape movement depending on the position of the obstacle to effectively avoid collision with it. In addition, the reaction time significantly

increased when the plate was placed centrally in front of the animal (Fig. 1-13E). It might take a longer time for the cricket to make a decision on the escape direction. The plate placed in the frontal region of the cricket had no effect on the response probability regardless of its position (Fig. 1-13E, Table 1-7), suggesting that the obstacles on the escape route might not suppress the escape decision.

To observe how the intact crickets contacted the one-sided plate with both antennae, I monitored the antennae movements using a high-speed camera. I found that crickets rarely touched the plate with the contralateral antenna (Fig. 1-14, Table 1-6). Instead, the antenna ipsilateral to the plate actively contacted with not only surface but also its edge. Probably, the crickets, of which one antenna was ablated, detected medial edge of the one-sided front wall with the other antenna and turned around to avoid colliding it. These results imply that it is not always necessary for crickets to compare mechanosensory inputs from left and right antennae for the object localization.

1.4 Discussion

1.4.1 Object localization by antennal mechanosensing

In this study, the crickets were tethered on the treadmill so I could manipulate the shape, distance, and position of the stimulating objects. The crickets modulated their trajectory depending on the distance to the object, the object shape such as plate or rod, and the relationship between the object orientation and stimulus direction. The plate at the near position altered the walking direction, but that at the far position did not. In contrast, the pole had no effect regardless of the distance from the animal (Figs. 1-3, 1-5). In addition, the effects of the laterally placed plate differed depending on its anterior-posterior location (Fig. 1-10), and the plate placed in front of the cricket had different effects on the escape response from that placed laterally (Fig. 1-13). These results indicated that the crickets were able to perceive not only the objects that they came in contact with, but also their spatial information, including distance, location, and orientation.

Cockroaches use the antennal system to guide their locomotion in an environment with obstacles such as barriers and walls (Camhi and Johnson, 1999; Harley et al., 2009; Baba et al., 2010). When cockroaches actively touch an object placed close to their antenna, they exhibit different turning behaviors depending on the horizontal position of the object (Okada and Toh, 2000, 2006). These facts suggest that the cockroaches are able to identify the location and orientation of objects by using their antennal mechanosensory system. However, if the antennal inputs directly cause a movement in a specific relationship to the detected object position, the cockroach can avoid or localize an

obstacle. Thus, it is difficult to distinguish whether this orienting behavior is caused by the perception of the entire surrounding space or simply by a reflexive response to the tactile stimulation. In contrast, my present study directly demonstrates the spatial perception ability of the crickets' antennal system by examining the escape behavior elicited by airflow stimulus, which was not mediated by the antennae (Fig. 1-2). Crickets altered their escape behavior even for identical airflow stimulus applied in the same direction, depending on the location and orientation of the object. This suggests that the crickets may be able to perceive the entire arrangement of objects in the surrounding space.

1.4.2 Context-dependent modulation of escape behavior

The presence of an object detectable by the antennae altered the direction in which the cricket moved in response to the airflow. This wind-elicited movement is considered to be one of the escape behaviors in which the cricket perceives the airflow as a cue for the approach of a predator (Tauber and Camhi, 1995; Casas and Dangles, 2010; Dupuy et al., 2011). The direction of the wind-elicited escape in crickets is precisely controlled depending on the stimulus angle, similar to the rapid escape in flies and cockroaches (Domenici et al., 2008; Card, 2012). In the absence of an object, as in the control condition, the crickets fled in the opposite direction from which the air-puff stimulus came (Fig. 1-2; Oe and Ogawa, 2013). Consistent with previous studies, the directions of movement in the initial response to the airflow from either the rear or lateral side that was used in this study were also distributed around the front or contralateral direction to the stimulus

(Fukutomi et al., 2015; Sato et al., 2019). However, when a plate was placed on the antero-lateral side of the cricket, the direction that the cricket moved in was shifted to the opposite side of the plate for stimuli that were applied from the rear (Fig. 1-3), and the movement was shifted backwards for lateral stimuli, that were applied from the opposite side of the plate (Fig. 1-5). These shifts in the walking direction are thought to indicate avoidance of collisions with objects perceived as walls.

Cockroaches walking near a wall maintain a constant distance while keeping their antenna in contact with the wall (Camhi and Johnson, 1999), and surgically shortening the cockroach's antenna increases the collision rate to the wall (Baba et al., 2010). The results from my present study show that the crickets flexibly alter their escape trajectory to avoid collisions, depending on the angle of airflow stimulus, even if the same obstacles are placed in an identical position. For example, when the plate was placed at the near position on the lateral side of the crickets, their forward movement triggered by the stimulus applied from behind was biased toward the opposite side of the plate, while the lateral movement induced by the stimulus from the opposite side of the plate was altered toward the back (Figs. 1-10, 1-13). This suggests that crickets could modulate their behavior depending on the spatial relationship between the stimulus directly triggering the behavior and the environment, that is, the spatial context. The changes in escape trajectory depended on the location and orientation of the objects. The crickets moved toward a free space in the absence of obstacles (Figs. 1-10, 1-13). This fact suggests the ability of crickets to perceive the arrangement of objects in the surrounding space.

Interestingly, the escape response to the airflow from the rear was delayed and the forward movement was suppressed when the plate was positioned at the center in front of the cricket (Fig. 1-13E). Since running forward possibly would cause collision with the wall in the front, the crickets might have delayed their decision to start their escape movement. In contrast, the plate positioned to one side in front of the animal biased the escape toward the wall-free side but did not affect the reaction time. This implies that the response delay and the reduction in the walking distance are not simply due to the reactive inhibitory effects of the mechanosensory inputs from the antennae. It has been reported that high-frequency sounds that hint at the presence of predators reduce the response probability of wind-elicited escape (Fukutomi and Ogawa, 2017). The direction of the spontaneous walking before the air-puff stimulation when the wall was placed at near position (Fig. 1-9) shows that crickets spontaneously walked in various directions even with the wall in place, and there was no difference in the walking direction between with and without wall. This meant that the crickets neither simply kept the constant distance from the wall, nor preferred to move towards the wall (Camhi and Johnson, 1999, Okada and Toh, 2000). My results imply that crickets can flexibly change their escape behavior depending not only on acoustics but also on the spatial contexts sensed by the antennal system in the surrounding space. Thus, the crickets likely integrate the sensory inputs of multiple modalities to perceive the surrounding context and make decisions for an appropriate and successful escape.

1.5 Figures

(Figure 1-1)

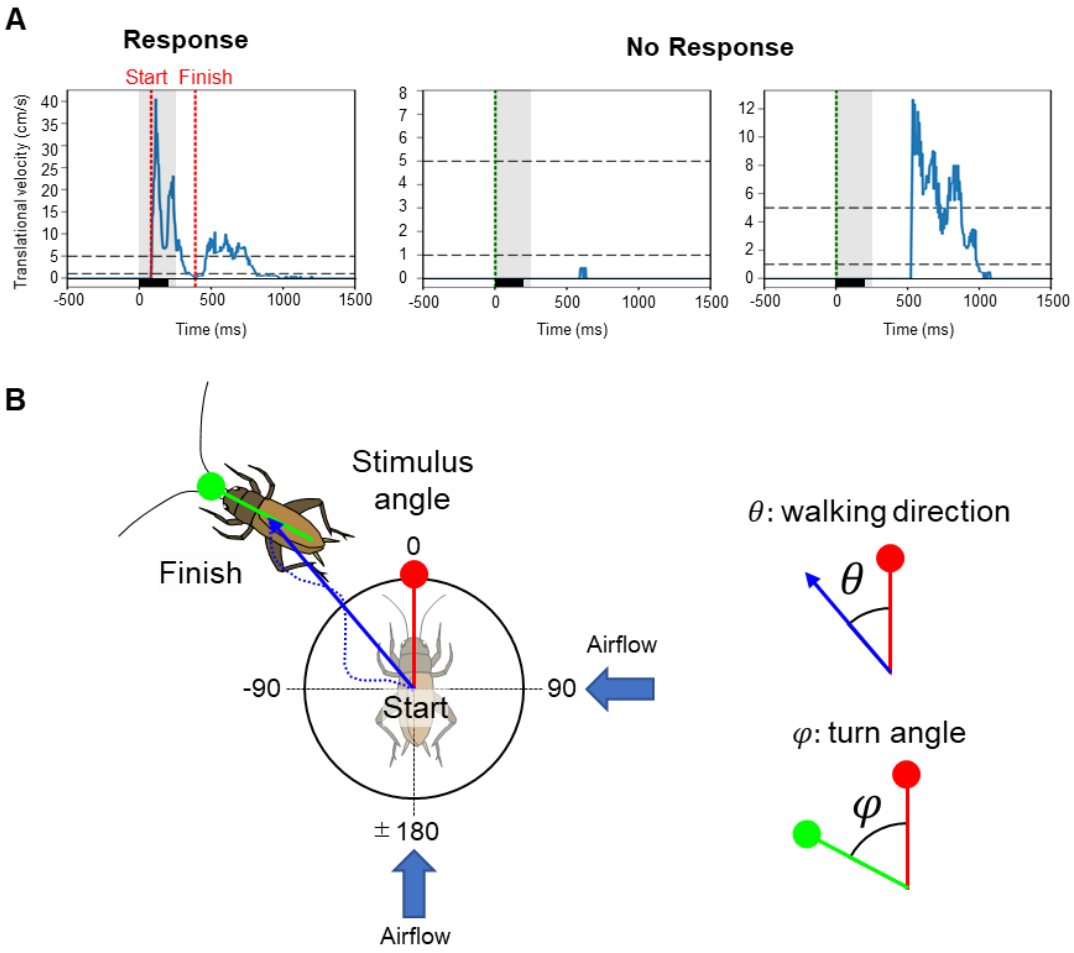

Fig. 1-1. Definition of behavioral response to air-puff.

(A) Typical time courses of walking velocity (blue traces) in response to air-puff for 200 ms (lower black traces). Based on their time course and magnitude, a trial was determined as 'response' or 'no response'. As shown in left panel, if a cricket started to walk and its maximum walking speed was > 50 mm/s (upper dashed lines) within 250 ms after the stimulus onset (indicated by the gray-shaded area), that trial was classified as a 'response'. The start of the initial response was defined as the first time when the translational velocity exceeded 10 mm/s (lower dashed lines) after stimulus onset; the finish of that was defined as the time when the velocity was less than 10 mm/s after the velocity exceeded 50 mm/s (red dotted lines). If a cricket did not move (center panel) or began to walk 250 ms or longer after the stimulus onset (left panel), those trials were classified as a 'no response'. **(B)** Definition of stimulus angle, walking direction (θ) and turn angle (φ) in the initial response to an air-puff stimulus. The left diagram shows the crickets at the start and finish points of the initial response and the walking trajectory on the virtual plane. The walking direction was measured as the angle between the body axis at the start point (red line) and the line connecting the start and finish points of the initial response (blue arrow). The turn angle was measured as the angle made by the body axes at the start and finish points (green line). Both walking direction and turn angles were arranged for forward as 0º, clockwise as plus, and counterclockwise as minus.

(Figure 1-2)

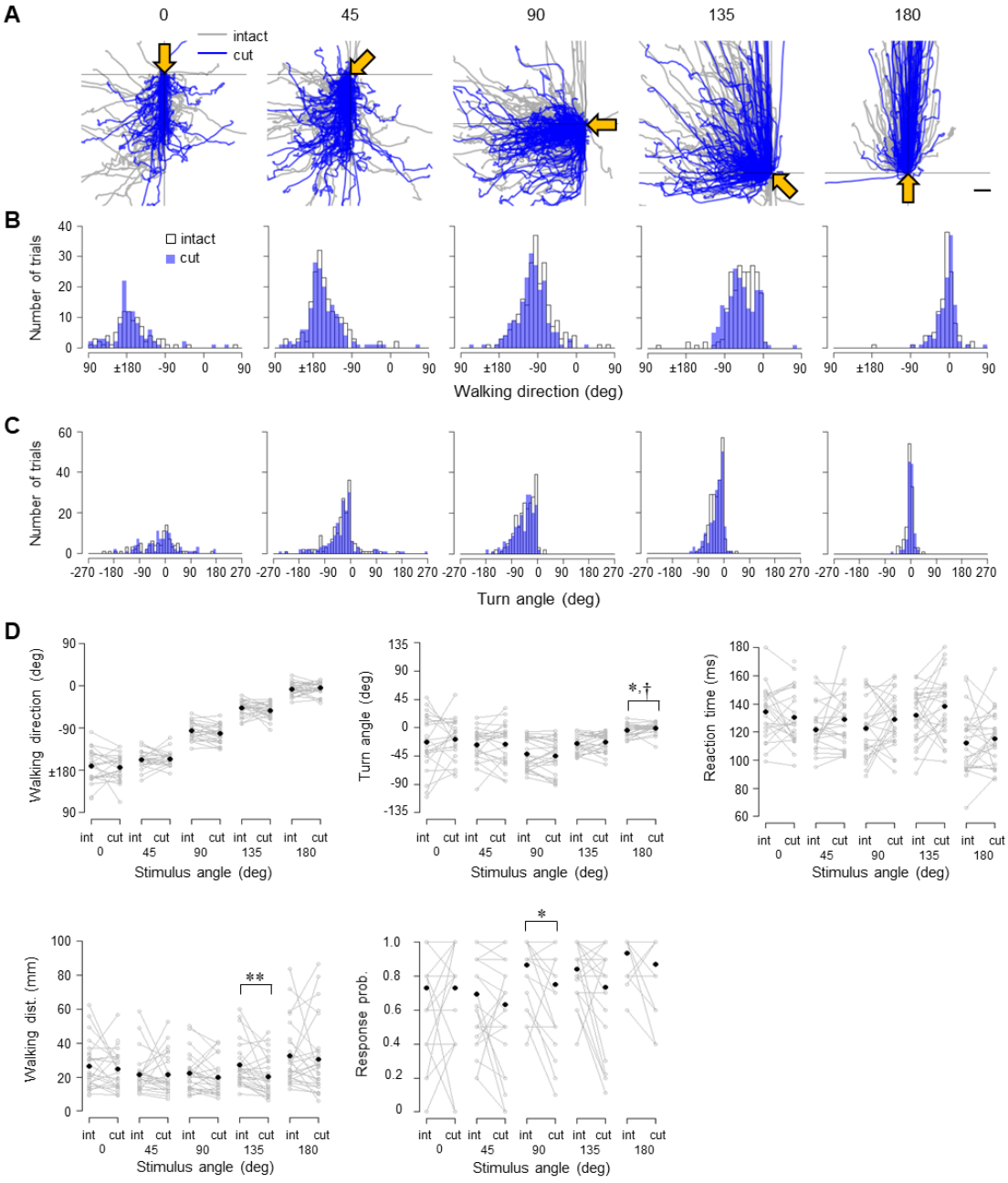
A
0
45
90
135
180
intact
cut
B
Number of trials
intact
cut
Walking direction (deg)
C
Number of trials
Turn angle (deg)
D
Walking direction (deg)
Turn angle (deg)
Reaction time (ms)
Walking dist. (mm)
Response prob.
Stimulus angle (deg)
int
cut

Fig 1-2. Effects of bilateral antennae ablation on wind-elicited escape behavior.

(A) Walking trajectories in the initial response to a puff of air from different angles. The data of the responses to the stimuli from the right and left sides were combined (45° and –45°, 90° and –90°, 135° and –135°). Gray traces show the trajectory of the intact crickets, and the blue traces show the trajectory of the crickets whose antennae were resected bilaterally. Scale bar shown in the right (180°) panel indicates 10 mm. **(B, C)** Distributions of walking direction (B) and turn angle (C) in the initial responses to stimuli from different angles. Open and blue bars indicate data from crickets with intact antennae and from antennae-ablated crickets, respectively. **(D)** Differences between the different conditions in the five locomotion parameters including, walking direction, turn angle, reaction time, walking distance, and response probability. N = 26 individuals. Five trials were performed for each stimulus angle for each individual. Gray open circles connected by lines indicate mean values for all trials in each individual. Black dots denote average across the population for each condition. There was no significant difference in the walking direction between the various stimulus angles. The other parameters were also not significantly different for stimulus angles that were close to the antenna. * $P < 0.05$, ** $P < 0.01$. (Wilcoxon signed-rank test). † $P < 0.05$ (Wallraff test).

(Figure 1-3)

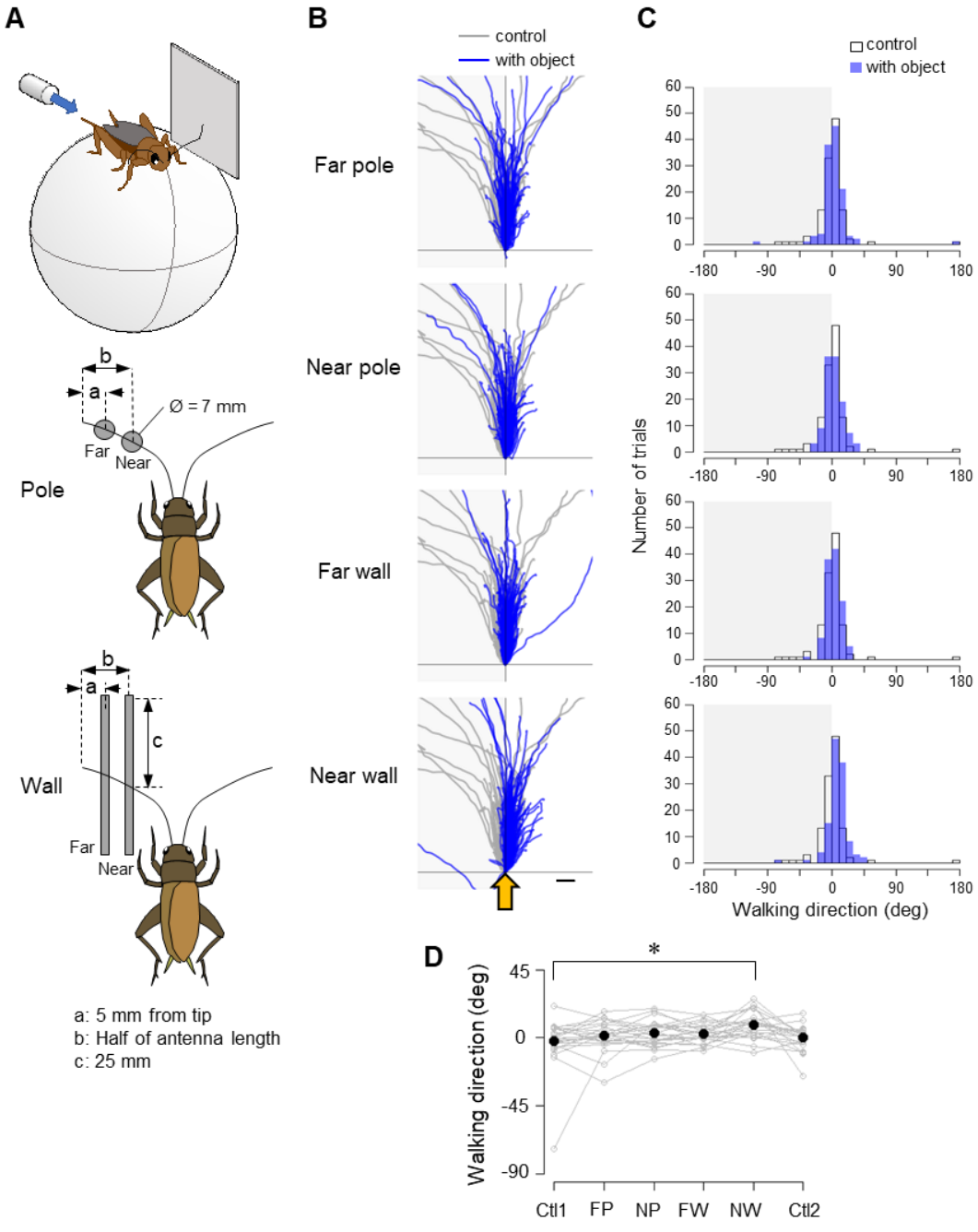
A
B
C
control
with object
Far pole
Near pole
Far wall
Near wall
Pole
Wall
Ø = 7 mm
Far
Near
a
b
c
a: 5 mm from tip
b: Half of antenna length
c: 25 mm
Number of trials
Walking direction (deg)
D
Walking direction (deg)
Ctl1
FP
NP
FW
NW
Ctl2
*

Fig. 1-3. Effects of antennal mechanosensory inputs on the escape response to airflow from behind.

(A) Experimental design. A cylindrical pole (ø = 7 mm) or square plate (50 × 50 mm) was positioned at the anterolateral position at different distances from the cricket. Far: 5 mm from the tip of the antenna. Near: Half of the antenna length. A puff of air was applied from behind the cricket. **(B)** Walking trajectories in the initial response to the air puff combined with the antennal stimulation. Gray traces show the trajectories under control condition with no objects. Blue traces show the trajectories under antennal stimulation conditions. Shaded region indicates the side on which the objects were placed. Yellow arrows indicate the direction of the air puff. Scale bar indicates 10 mm. **(C)** Distributions of walking direction under different conditions. Open and blue bars indicate the data from the control and antennal-stimulation conditions, respectively. **(D)** Walking direction under different conditions. N = 24 individuals. Five trials were implemented for each condition for each individual. Gray open circles connected by lines indicate mean values for all trials in each individual. Black dots denote average across the population for each condition. Data for the control condition were obtained for each individual twice, at the beginning (Ctl1) and at the end (Ctl2) of the experiment. FP, far pole; NP, near pole; FW, far wall; NW, near wall. * $P < 0.05$ (Fisher's nonparametric test with Bonferroni correction).

(Figure 1-4)

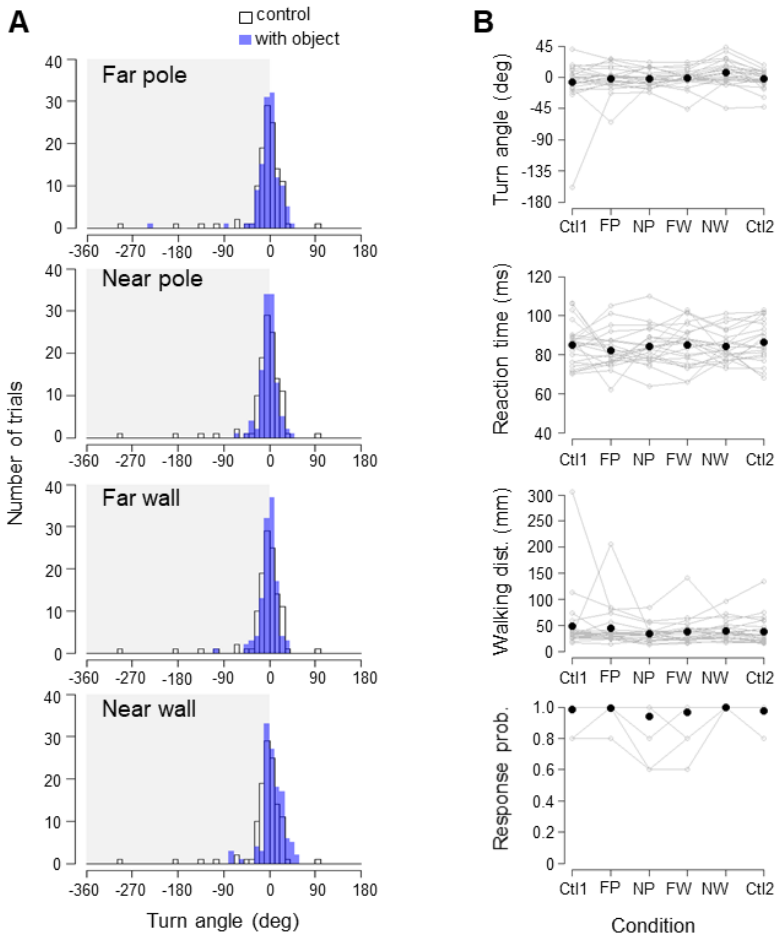

Fig 1-4. The locomotion parameters that were not significantly affected by antennal mechanosensory inputs in the escape response to airflow from behind.

(A) Distributions of turn angle under different conditions shown in fig. 1-3. Open and blue bars indicate the data from the control and antennal-stimulation conditions, respectively. **(B)** Locomotion parameters including turn angle, reaction time, walking distance, and response probability under different conditions shown in fig. 1-3.1. N = 24 individuals. Data for the control condition in A and B were obtained for each individual twice, at the beginning (Ctl1) and at the end (Ctl2) of the experiment. FP, far pole; NP, near pole; FW, far wall; NW, near wall.

(Figure 1-5)

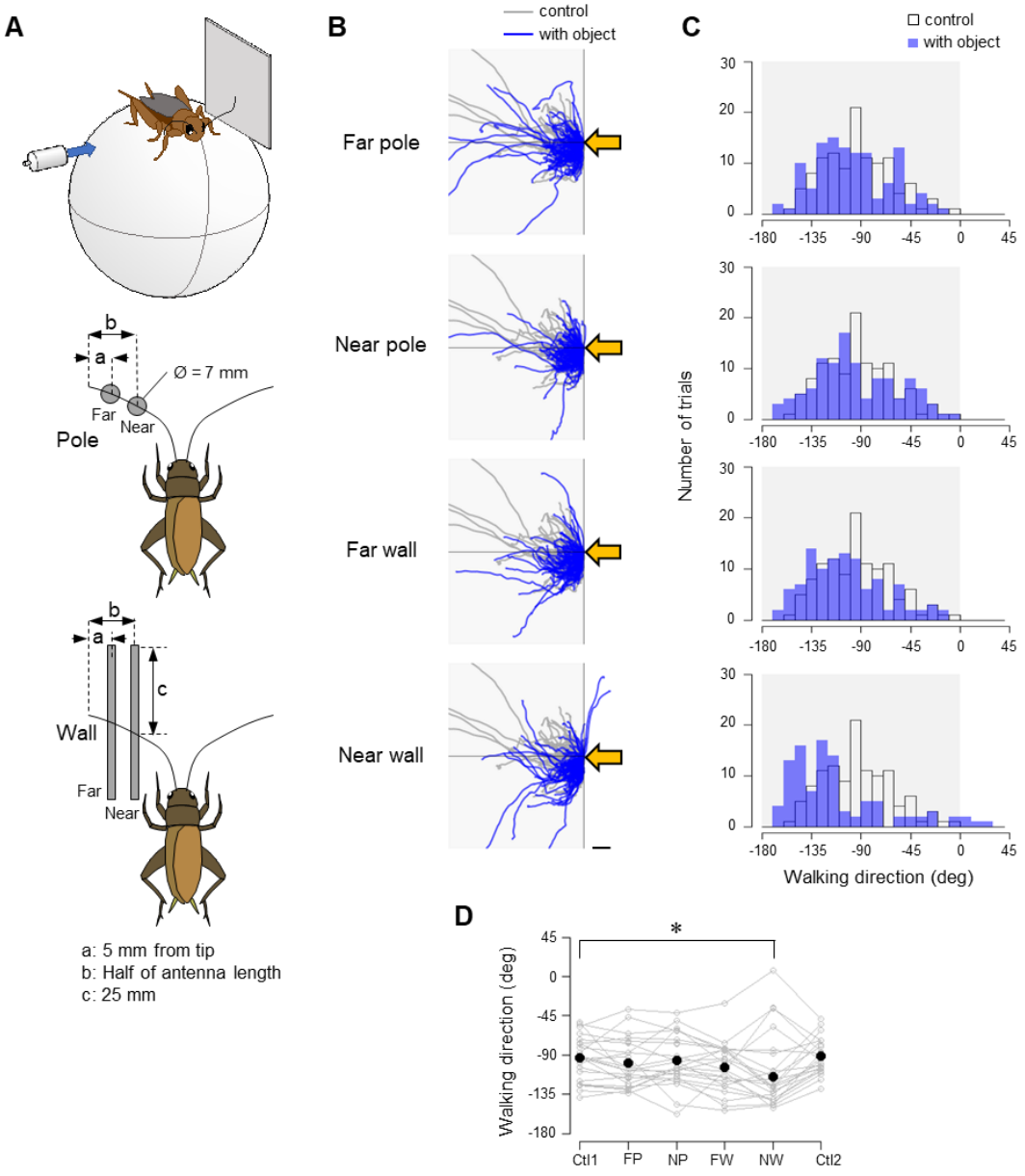
A
b
a
Ø = 7 mm
Far
Near
Pole
b
a
c
Wall
Far
Near
a: 5 mm from tip
b: Half of antenna length
c: 25 mm
B
control
with object
Far pole
Near pole
Far wall
Near wall
C
control
with object
Number of trials
30
20
10
0
-180
-135
-90
-45
0
45
Walking direction (deg)
D
*
Walking direction (deg)
45
0
-45
-90
-135
-180
Ctl1
FP
NP
FW
NW
Ctl2

Fig 1-5. Effects of antennal mechanosensory inputs on the escape response to airflow from the side contralateral to obstacles.

(A) Experimental design. As in figure. 1-3, a cylindrical pole (ø = 7 mm) or square plate (50 × 50 mm) was positioned at the anterolateral position at different distances from the cricket. Far: 5 mm from the tip of the antenna. Near: Half of the antenna length. Air puff was applied from the side contralateral to the objects. **(B)** Walking trajectories in the initial response to the air puff combined with the antennal stimulation. Gray and blue traces show the trajectories under control and antennal-stimulation conditions, respectively. Shaded region indicates the side on which the objects were placed. Yellow arrows indicate the direction of the air puff. Scale bar indicates 10 mm. **(C)** Distributions of walking direction under different conditions. Open and blue bars indicate the data from the control and antennal-stimulation conditions, respectively. **(D)** Walking direction under different conditions. N = 24 individuals. Five trials were implemented for each condition for each individual. Gray open circles connected by lines indicate mean values for all trials in each individual. Black dots denote average across the population for each condition. Data for the control condition were obtained for each individual twice, at the beginning (Ctl1) and at the end (Ctl2) of the experiment. FP, far pole; NP, near pole; FW, far wall; NW, near wall. * $P < 0.05$ (Fisher's nonparametric test with Bonferroni correction).

(Figure 1-6)

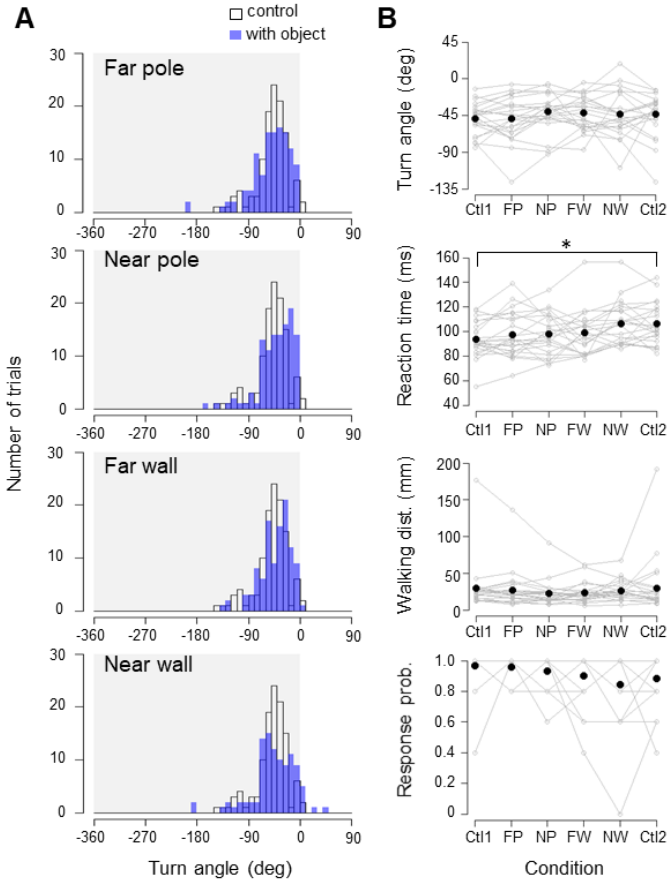

Fig 1-6. The locomotion parameters that were not significantly affected by antennal mechanosensory inputs in the escape response to lateral airflow.

(A) Distributions of turn angle under different conditions shown in figure 1-5. Open and blue bars indicate the data from the control and antennal-stimulation conditions, respectively. **(B)** Locomotion parameters including turn angle, reaction time, walking distance, and response probability under different conditions. N = 24 individuals. Data for the control condition in A and B were obtained for each individual twice, at the beginning (Ctl1) and at the end (Ctl2) of the experiment. FP, far pole; NP, near pole; FW, far wall; NW, near wall.

(Figure 1-7)

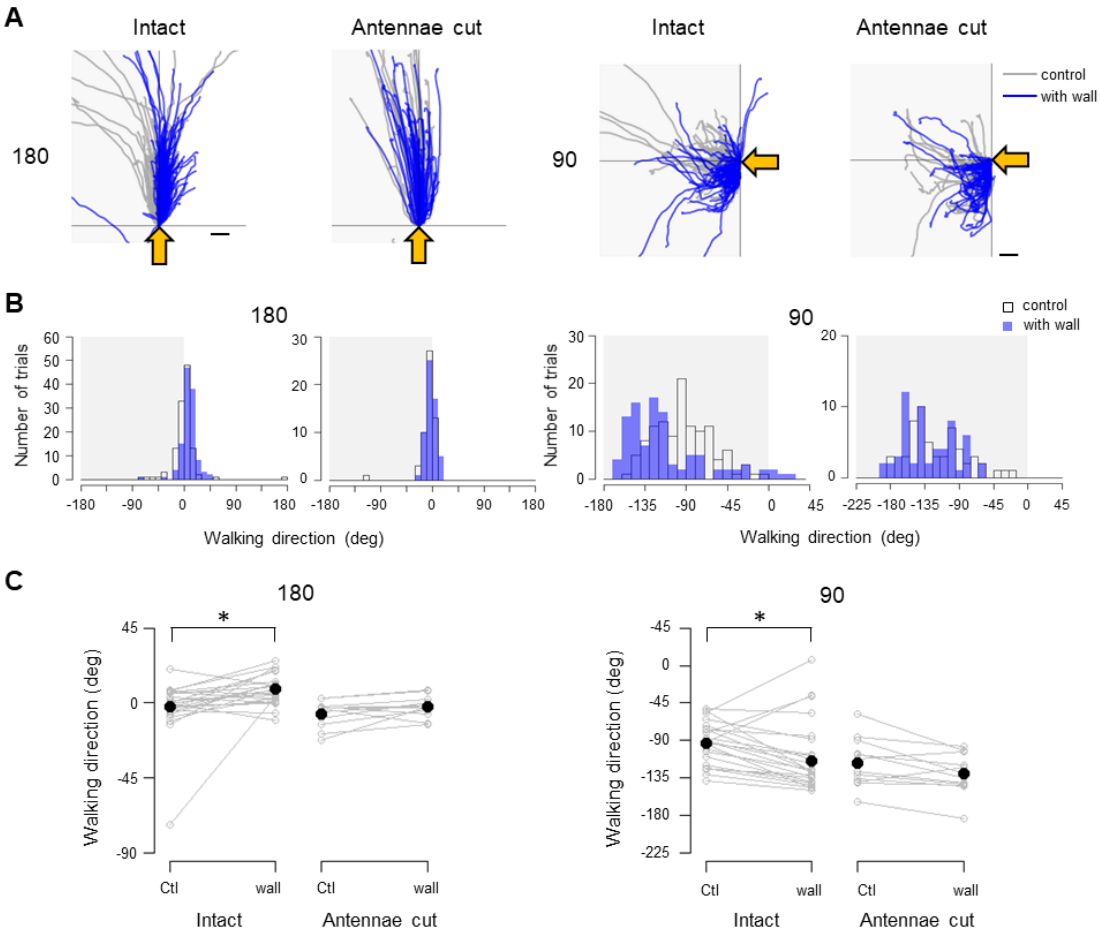
A
Intact
Antennae cut
Intact
Antennae cut
180
90
control
with wall
B
180
90
control
with wall
Number of trials
Walking direction (deg)
C
180
90
Walking direction (deg)
*
Ctl
wall
Intact
Antennae cut

Fig. 1-7. No influence of the plate on the escape behavior in response to air turbulence.

(A) Walking trajectories in the initial response to a puff of air from behind (180, left) or lateral side (90, right) of the intact crickets and those of which antennae were resected bilaterally. The data in intact crickets are the same as those shown in figures 1-3 and 1-5. Gray traces show the trajectories under the control condition without any objects. Blue traces show the trajectories when the plate was placed at the near position on the contralateral side to the airflow stimulus. Scale bars indicate 10 mm. **(B)** Distributions of the walking direction in the initial responses to stimuli from behind (180, left) or side (90, right) of the cricket. Open and blue bars indicate data from the control and when the wall was present, respectively. **(C)** Walking directions under different conditions in intact and bilaterally antennae-ablated crickets. N = 24 and 12 individuals for the intact and antennae-ablated crickets, respectively. Five trials were performed for each condition for each individual. Gray open circles connected by lines indicate mean values for all trials in each individual. Black dots denote average across the population for each condition. * $P < 0.05$ (Fisher's nonparametric test). When the antennae were ablated bilaterally, there was no significant difference in the walking direction between the presence and absence of the wall.

(Figure 1-8)

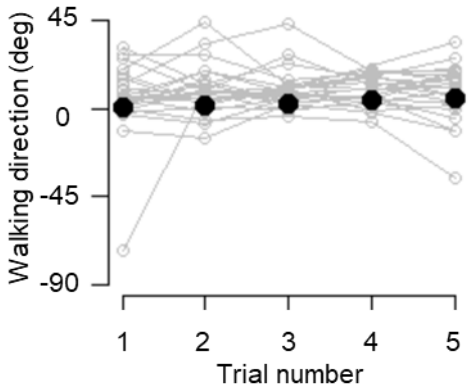

Fig. 1-8. The biased effects on the walking direction by the near wall did not depend on the trial order. Walking directions in the 1st to 5th trials under the near-wall condition shown in Fig. 1-3. There was no difference in the walking direction among the trials (P = 0.8379, Kruskal Wallis test). N = 24 individuals. Gray open circles connected by lines indicate the values for each order of trials in each individual. Black dots denote average across the population for each trial order.

(Figure 1-9)

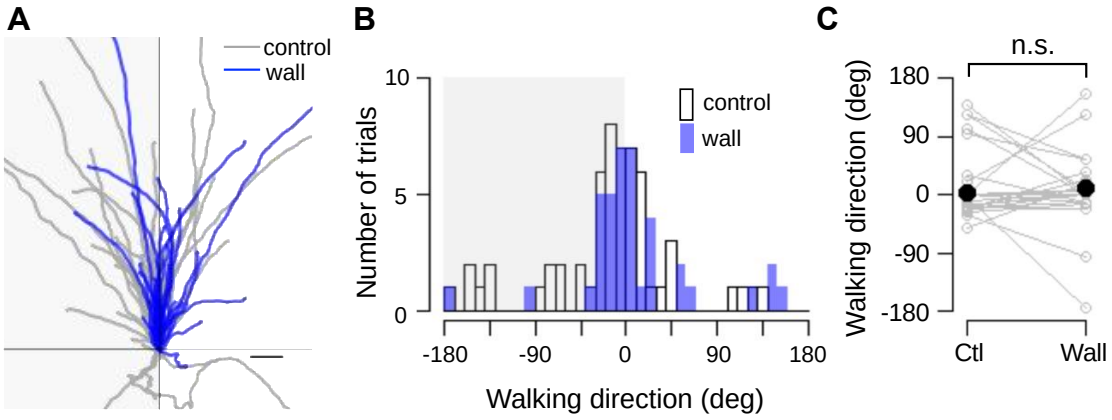

Fig. 1-9. No difference in the direction of the spontaneous walking bouts. **(A)** Trajectories of each bout during the spontaneous walking before the airflow stimulation under control (no wall) and near-wall conditions. All traces were aligned with the position and head direction at the bout start. The criteria of the start and end points for the walking bout was the same as that for the initial response to the airflow stimulus. **(B)** Distributions of walking direction for spontaneous walking bout under different conditions. Open and blue traces and bars indicate the data from the control and antennal-stimulation conditions, respectively. **(C)** Walking directions under different conditions. Gray open circles connected by lines indicate mean for each individual. Black dots denote average of means for all individuals. There was no difference in direction of the spontaneous-walking bouts ($P = 0.1607$, Fisher's nonparametric test). N = 24 individuals. The data were obtained from trials shown in Fig. 1-3.

(Figure 1-10)

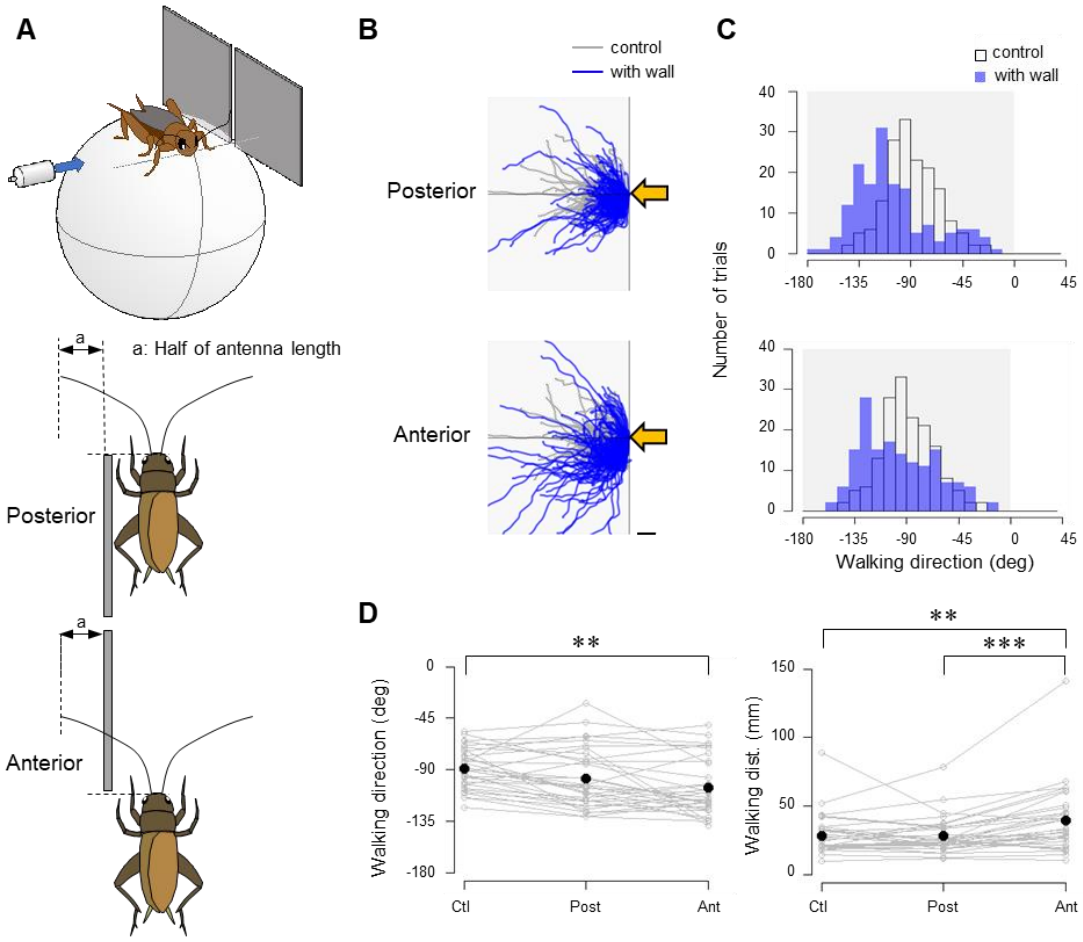
A
a
a: Half of antenna length
Posterior
a
Anterior
B
control
with wall
Posterior
Anterior
C
control
with wall
Number of trials
40
30
20
10
0
-180
-135
-90
-45
0
45
Walking direction (deg)
D
**
Walking direction (deg)
0
-45
-90
-135
-180
Ctl
Post
Ant
**
***
Walking dist. (mm)
150
100
50
0
Ctl
Post
Ant

Fig. 1-10. Positional effects of the side wall on the wind-elicited escape behavior.

(A) Experimental design. A square plate (50 × 50 mm) was placed laterally at the anterior or posterior position of the cricket. A puff of air was applied from the side contralateral to the plate. **(B)** Walking trajectories in the initial response to the air puff combined with the antennal stimulation. Gray and blue traces show the trajectories under the control and antennal-stimulation conditions, respectively. Shaded region indicates the side on which the plate was placed. Yellow arrows indicate the direction of the air puff. Scale bar indicates 10 mm. **(C)** Distributions of the walking direction under different conditions. Open and blue bars indicate data from the control and stimulation conditions, respectively. **(D)** Walking direction and distance under different conditions. N = 33 individuals. Five trials were implemented for each condition for each individual. Gray open circles connected by lines indicate mean values for all trials in each individual. Black dots denote average across the population for each condition. Ctl, control; Post, posterior; Ant, anterior. ** $P < 0.01$, *** $P < 0.001$ (Fisher's nonparametric test or Wilcoxon signed-rank test with Bonferroni correction).

(Figure 1-11)

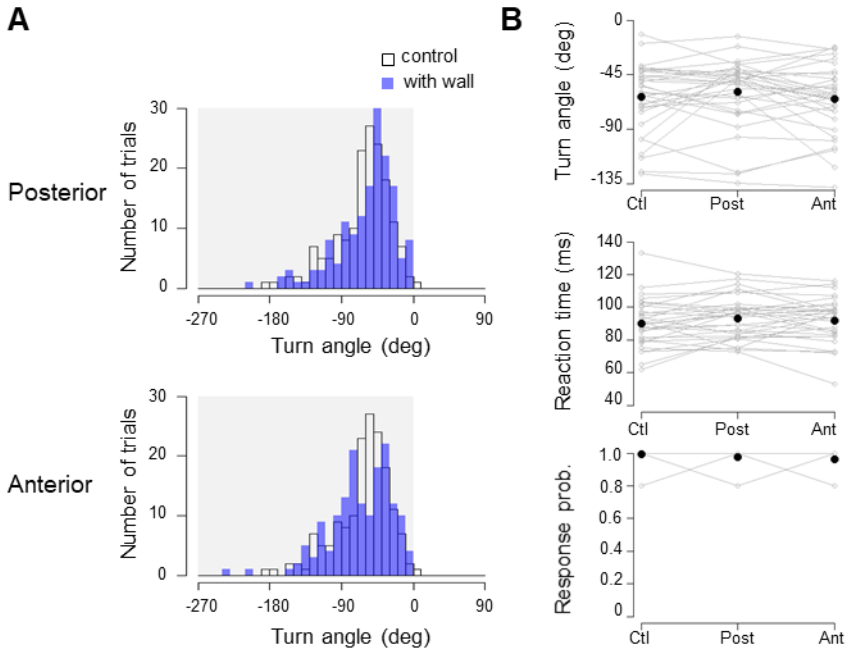

Fig. 1-11. The locomotion parameters that were not significantly affected by antennal mechanosensory inputs in the escape response to airflow from lateral side. **(A)** Distributions of turn angle under different conditions shown in figure 1-10. Open and blue bars indicate the data from the control and antennal-stimulation conditions, respectively. **(B)** Locomotion parameters including turn angle, reaction time, and response probability under different conditions shown in figure 1-10. N = 33 individuals. Ctl, control; Post, posterior; Ant, anterior. Five trials were implemented for each condition for each individual. Gray open circles connected by lines indicate mean values for all trials in each individual. Black dots denote average across the population for each condition.

(Figure 1-12)

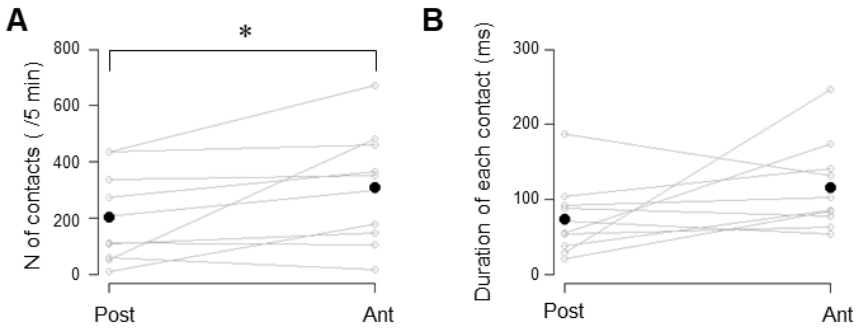

Fig. 1-12. Frequency and duration of contacts made by the antenna with the plate placed on the side of cricket.

The total number of contacts (**A**) and duration of a single contact (**B**) made by the antenna with the plate positioned anteriorly or posteriorly on the side of the cricket. N = 10 individuals. The total sampling duration for each condition was 5 min. Gray open circles connected by lines indicate mean values for all trials in each individual. Black dots denote average across the population for each condition. * $P < 0.05$ (Wilcoxon rank-sum test). Post, posterior; Ant, anterior.

(Figure 1-13)

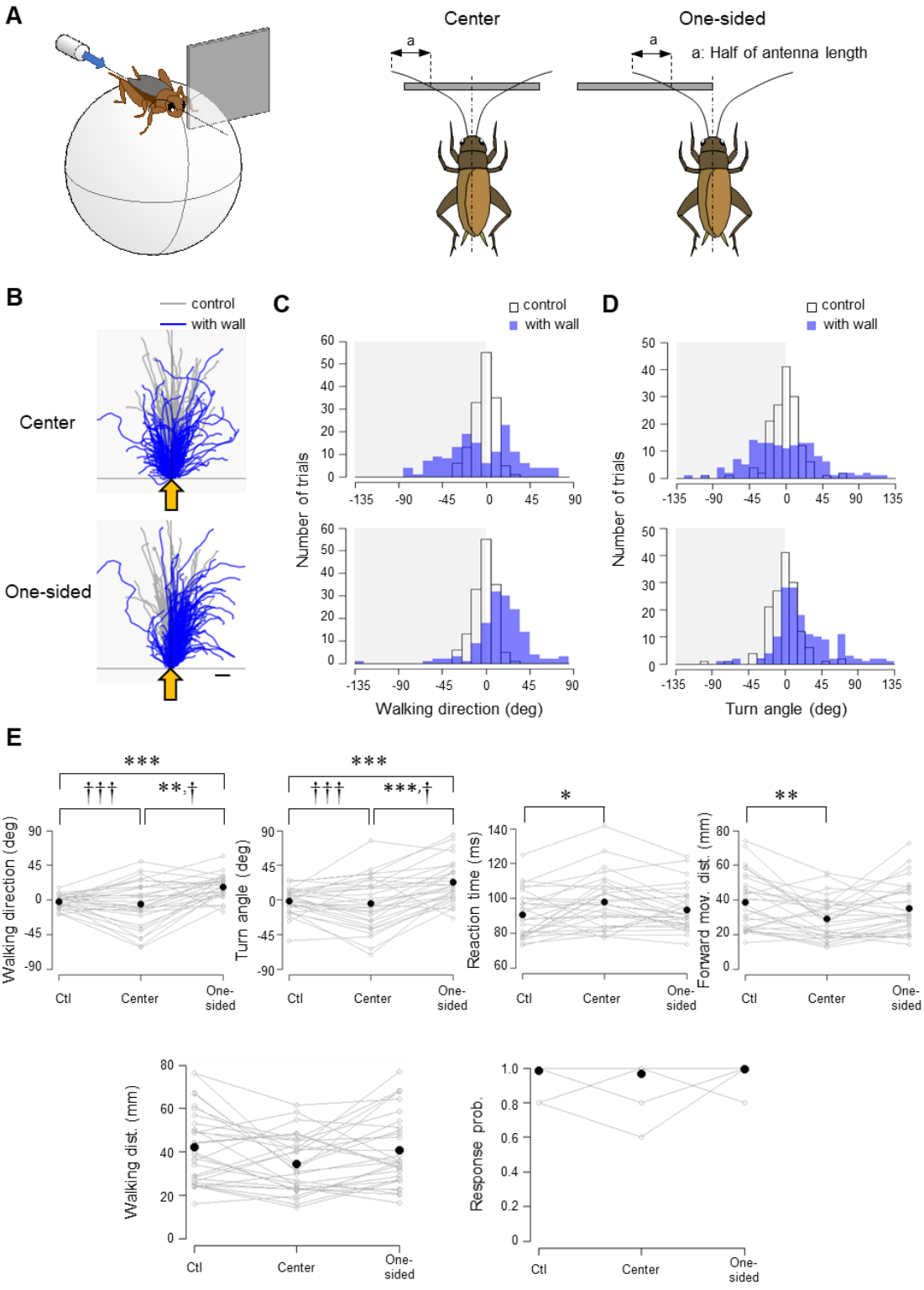
A
Center
One-sided
a
a
a: Half of antenna length
B
control
with wall
Center
One-sided
C
control
with wall
Number of trials
Walking direction (deg)
D
control
with wall
Number of trials
Turn angle (deg)
E
Walking direction (deg)
Turn angle (deg)
Reaction time (ms)
Forward mov. dist. (mm)
Walking dist. (mm)
Response prob.
Ctl
Center
One-sided

Fig. 1-13. Positional effects of the wall placed in front of the cricket on the wind-elicited escape behavior.

(A) Experimental design. A square plate (50 × 50 mm) was positioned in front of the cricket either at the center or toward one side. A puff of air was applied from behind the animal. **(B**) Walking trajectories in the initial response to the air puff combined with the antennal stimulation. Gray and blue traces show the trajectories under the control and antennal-stimulation conditions, respectively. Shaded region indicates the side on which the plate was placed. Yellow arrows indicate the direction of the air puff. Scale bar indicates 10 mm. **(C, D)** Distributions of walking direction (C) and turn angle (D) under different conditions. Open and blue bars indicate data from the control and stimulation conditions, respectively. **(E)** Walking direction, turn angle, reaction time, forward movement distance, walking distance, and response probability under different conditions. N = 30 individuals. Five trials were implemented for each condition for each individual. Gray open circles connected by lines indicate mean values for all trials in each individual. Black dots denote the average across the population for each condition. Ctl, control. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (Fisher's nonparametric test and Wilcoxon signed-rank test with Bonferroni correction). † $P < 0.05$, ††† $P < 0.001$ (Wallraff test with Bonferroni correction).

(Figure 1-14)

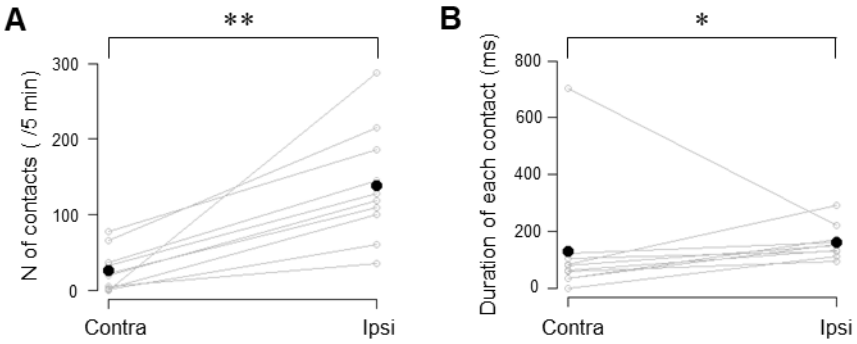

Fig. 1-14. Frequency and duration of contacts made by the antenna with the plate placed on the side or in front of cricket.

The total number of contacts **(A)** and duration of a single contact **(B)** made by the ipsilateral or contralateral antenna to the plate positioned to one side in front of the cricket. N = 10 individuals for each measurement. The total sampling duration for each condition was 5 min. Gray open circles connected by lines indicate mean values for all trials in each individual. Black dots denote average across the population for each condition. * $P < 0.05$, ** $P < 0.01$ (Wilcoxon rank-sum test). Contra, contralateral antenna to the wall; Ipsi, ipsilateral antenna to the wall.

1.6 Tables

Table 1-1. Statistical results to test effects of bilateral antennae ablation on locomotor parameters in the escape response to air-puff stimulus from 8 different angles.

Stimulus angle	Walking direction		Turn angle		Reaction time	Walking distance	Response probability
	Median	Angular dispersion	Median	Angular dispersion			
0º (front)	0.4767	0.4009	0.7287	0.1499	0.4936	0.789	0.752
45º	1	0.969	0.7362	0.0570	0.1787	0.5964	0.131
90º (side)	1	0.9126	0.2173	0.9126	0.1651	0.0890	0.0132
135º	0.5791	0.7143	0.4678	0.8981	0.1499	0.0032	0.0856
180º (behind)	0.2673	0.6212	0.0176	0.0422	0.3802	0.4227	0.1228

The numbers indicate *P* -values provided by Fisher's nonparametric test for the median of walking direction, Wallraff test for the angular dispersion of walking direction and turn angle, and Wilcoxon signed-rank test for other parameters. $N = 26$. The data were represented in Fig. 1-2.

Table 1-2. Statistical results to test effects of object shape and location on locomotor parameters in the escape response to air-puff stimulus from behind.

Tested conditions	Walking direction		Turn angle		Reaction time	Walking distance	Response probability
	Median	Angular dispersion	Median	Angular dispersion			
Ctl / Far pole	0.0833	0.7105	0.5457	0.5919	0.6231	0.8996	1
Ctl / Near pole	0.2482	0.5777	0.8553	0.4095	0.9441	0.0647	0.05447
Ctl / Far wall	0.0833	0.5637	0.4223	0.1671	0.8996	0.3449	0.1736
Ctl / Near wall	0.0195	0.7415	0.1243	0.665	0.7469	0.8334	1
Ctl 1 / Ctl 2	0.2482	0.7728	0.5088	0.5499	0.6033	0.1974	0.4237

The numbers indicate *P* -values provided by Fisher's nonparametric test for the median of walking direction, Wallraff test for the angular dispersion of walking direction and turn angle, and Wilcoxon signed-rank test for other parameters with Bonferroni correction. $N = 24$. The data were represented in Figs. 1-3 and 1-4.

Table 1-3. Statistical results to test effects of object shape and location on locomotor parameters in the escape response to air-puff stimulus from the side contralateral to the objects.

Tested conditions	Walking direction		Turn angle		Reaction time	Walking distance	Response probability
	Median	Angular dispersion	Median	Angular dispersion			
Ctl / Far pole	0.2482	0.6062	0.8143	0.2399	0.6022	0.4308	0.5877
Ctl / Near pole	0.0833	0.6501	0.0814	0.9343	0.2818	0.1253	0.2542
Ctl / Far wall	0.2482	0.7728	0.2727	0.2655	0.4803	0.2143	0.08898
Ctl / Near wall	0.0281	0.4187	0.6655	0.5513	0.0832	0.8085	0.1019
Ctl 1 / Ctl 2	0.5637	0.5919	0.1202	0.7887	0.0316	0.1153	0.2208

The numbers indicate *P* -values provided by Fisher's nonparametric test for the median of walking direction, Wallraff test for the angular dispersion of walking direction and turn angle, and Wilcoxon signed-rank test for other parameters with Bonferroni correction. *N* = 24. The data were represented in Figs. 1-5 and 1-6.

Table 1-4. Statistical results to test air-turbulence effects of walls placed at near position on walking direction in the escape responses.

Tested conditions	Stimulus angle	Antennae condition	Walking direction	Number of individuals
Ctl / Near wall	180º (behind)	intact	0.0195	24
		ablated	0.1025	12
	90º (side)	intact	0.0281	24
		ablated	0.4142	12

The numbers indicate *P* -values provided by Fisher's nonparametric test for the median of walking direction. The data were represented in Fig. 1-7.

Table 1-5. Statistical results to test positional effects of side walls on locomotor parameters in the escape response to air-puff stimulus from the side contralateral to the objects.

Tested conditions	Walking direction		Turn angle		Reaction time	Walking distance	Response probability
	Median	Angular dispersion	Median	Angular dispersion			
Ctl / Posterior	0.0802	0.1047	0.6088	0.6306	0.1391	0.9578	0.233
Ctl / Anterior	0.0041	0.0917	0.672	0.6768	0.1085	0.0017	0.0726
Post / Ant	0.2184	0.6862	0.2418	0.9949	0.396	< 0.0001	0.5653

The numbers indicate *P* -values provided by Fisher's nonparametric test for the median of walking direction, Wallraff test for the angular dispersion of walking direction and turn angle, and Wilcoxon signed-rank test for other parameters with Bonferroni correction. *N* = 24. The data were represented in Figs. 1-10 and 1-11.

Table 1-6. Statistical results to test differences in contacts made by the antenna with the walls placed at different positions.

Tested conditions (Wall positions)	Frequency	Duration
Posterior / Anterior	0.0273	0.1934
Ipsilateral / Contralateral	0.0002	0.0058

The numbers indicate *P* -values provided by Wilcoxon signed-rank test. $N = 10$. The data were represented in Figs. 1-12 and 1-14.

Table 1-7. Statistical results to test positional effects of walls placed in front of crickets on locomotor parameters in the escape response to air-puff stimulus from behind.

Tested conditions	Walking direction		Turn angle		Reaction time	Walking distance	Response probability	Forward distance
	Median	Angular dispersion	Median	Angular dispersion				
Ctl / Center	0.3017	0.0001	0.3707	0.0066	0.0279	0.0524	0.2031	0.004
Ctl / One-sided	< 0.0001	0.1379	< 0.0001	0.1379	0.7324	0.4771	0.7728	0.1642
Center / One-sided	0.0058	0.0266	0.0001	0.0266	0.9816	0.0832	0.2031	0.0524

The numbers indicate *P* -values provided by Fisher's nonparametric test for the median of walking direction, Wallraff test for the angular dispersion of walking direction and turn angle, and Wilcoxon signed-rank test for other parameters with Bonferroni correction. *N* = 30. The data were represented in Fig. 1-13.

Chapter 2

Cross modal effects of visual and antennal inputs on the cercal-mediated escape behavior

2.1 Introduction

Predators employ several strategies to catch a prey, including timing and direction of attack. Crickets as a prey animal adopt various defense behaviors ranging from freezing to flight (Baba and Shimozawa, 1997). Even for escaping, crickets choose appropriate behavior, suggesting that they have a complex strategy to escape (Sato et al., 2019). Environmental factors such as brightness, obstacles, and their habitat limits sensory modality to detect the predator's attack. To successfully evade the predator's attack, prey must detect the predator and strategically plan on "how", "to which direction" and "when" to escape. Visual cue such as 'looming stimuli' provides a crucible signal of the predator approaching in the escape planning. For example, speed and size of the looming visual stimulus account for flight and freezing in mice and zebrafish (De Franceschi et al., 2016; Yang et al., 2020; Temizer et al., 2015). Flies choose either long or short mode of escape taking off depending on the speed of the looming stimulus (von Reyn et al., 2014). The stimulus direction and presence of contextual stimulus are key sensory cues in planning the escape direction. Crickets basically move in opposite direction of the airflow stimulus (Oe and Ogawa, 2013), but their escape direction distinctively varies to confuse the predator's prediction (Domenici et al., 2008, 2011b). Multisensory stimulation by high-

frequency sound combined with airflow alters the movement direction and threshold for crickets' escape behavior (Fukutomi et al., 2015). This suggest that additional inputs of other sensory modalities possibly modulate the escape behaviors of crickets.

As shown in Chapter 1, cricket changed escape direction by detecting surrounding obstacles and delayed their escape start when they detected the wall in front by both antennae. Visual cues were also used in detecting not only approaching danger but also obstacles. For instance, if goldfishes visually detect the wall on the escaping path, they changed the direction of the escape response (Eaton and Emberley, 1991). Integration of multiple sensory cues alters behavioral performance in response to obstacle. Mice detect obstacles by their whiskers and rapidly steps over it to avoid collision, but if they detect it visually in addition to whisker input, the clearance speed decreases (Warren et al., 2021). Cricket has compound eye and ocelli that are adapted to visual perception of the environment at low light condition. Neural mechanisms underlying the reliability of compound eye in nocturnal navigation in insects has been reviewed by Warrant and Dacke (2016). However, it remains unknown if visual inputs indicating obstacles also alter the cricket movements in the escape behavior. I hypothesized that visual inputs also contribute to context-dependent modulation of escape. To test this hypothesis, I examined the effect of visual, antennal mechanosensory, and combined multisensory cues indicating obstacles on the escape behavior.

2.2 Materials and Methods

Animal, treadmill system, and airflow stimulator were the same as used in Chapter 1.

2.2.1 Stimulation

A white colored square plate of 50 × 50 mm was placed on one side in front of the cricket at distance of half the length of the antenna. The lateral edge of the plate was aligned to a midline of the cricket body. Two white LED array lights were placed on left and right sides above the treadmill to illuminate the plate. To enhance the contrast between the plate and background, a black paper sheets were put on the inside of the sound-proofed box used for all experiments. The turn on/off and the brightness of the LED lights were controlled out of the experimental box. Air-puff of nitrogen gas at velocity of 0.68 m/s was applied from behind of the cricket.

2.2.2 Behavioral Experiment

To examine the effects of different modalities of sensory cues on the wind-elicited escape behavior, I separated the crickets into four experimental groups based on antennal and visual stimulations. For each group, I tested the cricket's response in two conditions: without (control) and with the plate (with-wall). Thirty individuals were tested for each group, and 10 trials for each condition were performed in each individual. Inter-trial interval was longer than 1 minute. Crickets were tethered on the treadmill system for 5 minutes to adapt to the experimental environment before the airflow stimulation. During

this adaptation period, the crickets had a chance to perceive the plate. The test under control condition was followed by that under with-wall condition. The order of experimental group was shuffled so that the time of day to experiment for each group was not fixed. The four experimental groups are follows. Group A (negative control): In this group, cricket's antennae were ablated bilaterally at the base. The experiment was performed in darkness where the LED light was turned off. Crickets in this group were not able to sense the plate either tactilely or visually. Group B (visual-sensing group): In this group, the antennae were ablated bilaterally at the base, and the LED light was turned on. Cricket could sense the plate visually but not tactilely with antennae. Group C (Mechanosensory group): The crickets in this group have intact antennae, so they could sense the plate tactilely with their antennae. But they were not able to detect it visually because the LED light was turned off. Group D (multisensory group): The crickets in group have intact antennae and the LED light was turned on. So, they could sense the plate both visually and tactilely using their antennae.

2.2.3 Data analysis

Behavioral data were provided by TrackTaro software, processed, and analyzed by using the same procedure as in chapter 1. Criteria for the categorization of behavioral response, either "response" or "no response" was also the same as described in chapter 1. Also in this chapter, I focused on the initial response that was defined with the same criteria as in chapter 1 and measured four locomotion parameters for this initial response: walking

direction, turn angle, reaction time, and walking distance by using the same definition and calculation as in chapter 1. In addition, to compare the effects of the plate between the groups with different sets of individuals, I calculated the difference in the mean values of the locomotion parameters or the number of responded trials between 'control' and 'with-wall' conditions for each individual as follows:

$$d = p_{stm} - p_{ctl}$$

where d is the difference in the mean value of parameters indicating the magnitude of the effect by the plate, p_{stm} and p_{ctl} are the mean values in with-wall and control conditions, respectively.

2.2.4 Statistical methods

R programming software (version 4.1.1, R Development Core Team) was used for the statistical analysis. As used in chapter 1, to avoid pseudo-replication, I used the mean value of the data obtained in the trials categorized as wind-elicited response for each individual as the representative value for the statistical tests. The significance of the plate effects was assessed by using Fisher test for walking direction as angular parameter and Wilcoxon signed rank for other parameters. To compare the magnitude of the effects by the plate between the groups, the difference in mean value of each parameter was calculated for each individual, and for these differences the significance of the groups was assessed by using Wilcoxon rank-sum test including the walking direction. The statistical tests for the significant effects across the four groups were corrected using

Bonferroni correction.

2.3 Results

2.3.1 Visual or antennal inputs are necessary to detect the obstacle in front

To test the contribution of antennal and visual inputs in the modulation of the wind-elicited behavior by the plate, I removed sensory inputs of each modality separately or both at once. First, to rule out the possibility that crickets detect the wall by any other sensory organ apart from the antennal and visual systems, group A was tested (Fig. 2-1A). This group was used as the negative control for other groups as well as to examine the air turbulence effect of the plate placed on one side in front of the cricket. In the group A, there was little difference in the escape trajectory induced by the air puff from behind, between the control and with-wall conditions (Fig. 2-1B). Even in the with-wall conditions in which the place was placed in front, the walking direction and turn angle were concentrated around 0º, meaning that crickets moved forward without turning regardless of the presence or absence of the wall (Fig. 2-2). There was no significant difference in any locomotion parameters between control and with-wall conditions (Fig. 2-3). These results suggest that crickets could not detect the wall in front when both their antennal mechanosensory and visual cues were absent.

2.3.2 Effects of visual cues on the wind-elicited escape.

To examine the effects of visual inputs indicating the obstacle on the wind-elicited escape, the group B was tested, where the LED light was turned on and both antennae were ablated (Fig. 2-4A). The escape trajectories indicated that the cricket moved forward but

decrease their movement distance in the with-wall condition (Fig. 2-4B). The walking direction and turn angle were not significantly changed by the wall in the distribution and mean angle (Figs. 2-5, 2-6A, B). In contrast, the reaction time was slightly but significantly increased, and the walking distance decreased (Fig. 2-6C, D). It was possible that the crickets that visually detected the obstacle delayed their decision to start the escape and reduced the forward movement to avoid collision with the wall. The decrease in the waiting time by the wall meant that the cricket less actively moved before the air-puff stimulation (Fig. 2-6E). The cricket visually sensing the obstacle in front might reduce the spontaneous walking activity. No effect on the walking direction by the wall placed on one side in front suggests that the visual cues alone did not provide enough information about the obstacle location, so crickets failed to modulate their escape direction. Response probability was not affected by the visual cue of the wall (Fig. 2-6F). In conclusion, the visual inputs indicating the wall in front decreased walking movement in the escape response and spontaneous activity.

2.3.3 Effects of mechanosensory cue provided by antennae

Next, the effects of antennal mechanosensory input were tested in group C, where the LED light was turned off and both antennae were intact so that they were able to contact the plate placed in front (Fig. 2-7A). The results indicated that crickets moved toward the contralateral side to the plate in response to the air-puff from behind (Fig. 2-7B). The distributions of the walking direction and turn angle were shifted to the wall free-side,

and the forward movements around 0º were decreased in the with-wall condition (Fig. 2-8). The escape behavior under the with-wall condition was significantly different in the mean values of the walking direction, turn angle, reaction time, walking distance, and waiting time to the air-puff stimulation from that under the control condition (Fig. 2-9A-E). The results were consistent with those in chapter 1. In conclusion, the large impacts in the directional and non-directional of parameters suggest that the antennal mechanosensory inputs provide crucial information about the surroundings, which could be used to modulate the oriented escape behavior.

2.3.4 Cross-modal effects of visual and antennal mechanosensory cues on the escape behavior

The results in the groups B and C revealed the individual effects by the visual and mechanosensory cues of the obstacle. Finally, I examined the cross-modal effects on the escape behavior when the visual and mechanosensory cues were presented simultaneously. The crickets in this group were able to sense the wall placed in front of them visually and tactilely with their antennae (Fig. 2-10A). The walking trajectories were biased to the contralateral side to the plate (Fig. 2-10B), and the distributions of the walking direction and turn angle were shifted toward the wall-free side (Fig. 2-11). Most of the locomotion parameters were greatly modulated by the multisensory inputs of the obstacle. The walking direction and turn angle were significantly moved to the wall-free side (Fig. 2-12A,B). The reaction time increased and the walking distance decreased,

significantly (Fig. 2-12C,D). The waiting time indicating the spontaneous walking activity decreased (Fig. 2-12E). These impacts on the behavioral parameters were similar to the results in the group C, in which the mechanosensory cue was available (Fig. 2-9). However, the changes in the reaction time, walking distance, and the waiting time were also observed in the group B with the visual cue (Fig. 2-6). Taken together, both visual and mechanosensory inputs contributed to modulation of the escape behavior adapting to the surrounding objects.

2.3.5 Different contributions to the behavioral modulation of visual and antennal mechanosensory inputs

The contributions to the modulation in the escape behavior of visual and mechanosensory inputs were examined by comparing the pooled data for the effects in all experimental groups. To compare the effects across the groups, I calculated the difference in the mean of the parameters between with and without wall (control) conditions for each individual in each group. The effects on the walking direction and turn angle were significantly larger for the multisensory and mechanosensory groups (groups C and D) than that for visual and senseless groups (groups A and B). However, there was no significant difference in these effects between multisensory and mechanosensory groups (Fig. 2-13A, B). This indicates that the mechanosensory cue would mainly resulted in the directional modulation in the multisensory group. The effects on the directional parameters in the visual group were not significantly different from that in the senseless group (Fig. 2-13A,

B). Thus, the visual cue would not have any contributions to the modulation of walking direction and turn angle. The reaction time, walking distance, and waiting time were affected by the wall sensed visually and tactilely, but there were little significant differences in these effects across the groups (Fig. 2-13D, E). This was possibly due to large variance in the effects by the wall among individuals.

2.4 Discussion

Crickets are nocturnal animals and hide within shelters such as a crave during the day in nature. Therefore, it is likely that they are often attacked by predators during the night when they are active, but sometimes they may be attacked and displaced from their hideout by predators during the day. In that situation, they need to escape seeking for a place of safety under bright daylight. For successful escape, animals need to perceive the predator approaching and surrounding objects to plan the appropriate escape route. Vision is very crucial for detection of the danger and surrounding objects in all species of animals including insects (Niebur and Koch, 1996; Garm and Nilsson, 2014; Buehlmann et al., 2020). Visual looming stimulus induces flight behavior to avoid collision or capture by predators (Dunn et al., 2016; Fotowat and Gabbiani, 2007). Nocturnal animals rely on mechanosensory systems such as antennae of insects or whiskers of rodents to guide their behavior. In chapter 1, I demonstrated that crickets changed their escape behavior by mechanosensory information mediated by their antennae. In this chapter, to clarify whether the visual cues improve the object perception in crickets, I examined the effects of visual, mechanosensory and multisensory inputs.

2.4.1 Visual inputs had different effects from antennal mechanosensory inputs on the escape behavior.

As well as diurnal animals, nocturnal insects use a visual cue to orient at nighttime such as celestial compass by moonlight and landmarks for path integration (Reviewed by

Warrant and Dacke, 2016). The cricket visual system has been studied in their polarization (Labhart 1988, 1999; Zufall et al., 1989; Henze et al., 2012; Labhart et al., 1984) and circadian rhythms (Sakura et al., 2003; Tomioka, 2000; Itoh et al., 1995; Abe et al., 1997). The physiological properties of photoreceptors of the cricket's compound eye were generally similar to those of other mainly nocturnal insect species such as cockroaches or stick insects (Frolov et al., 2014). Crickets are able to learn the spatial task combined with reward using visual cues, but their use of spatial memory to locate a food source is dependent on the individual personality trait (Doria et al., 2019). Polarization is one of the effective visual cues in spatial learning in insects such as cockroaches (Mizunami et al., 1998), crickets (Matsubara et al., 2021; Wessnitzer et al., 2008). The kind of spatial information visually acquired and used by insects depends on the behavioral context. The visual cue may play an important role in exploring shelters (Hale and Bailey, 2004), while my results indicated that the visual cue had little impact in the escape behavior. As shown by the results in this chapter, the visual cue of the wall in front had different impacts on directional and non-directional parameters.

When the crickets could detect the wall in front just visually, they did not change the escape direction. It is possible that the location of adjacent and large objects like a wall cannot be detected visually by the crickets. In contrast, the visual cues had a suppressive effect on the escape and spontaneous movements, which were revealed as the delay of the escape start and the decrease of the walking distance in the escape response and spontaneous walking activity. This result indicated that crickets visually perceived

the wall as an obstacle. Visual cues provide animals with pre-hand information about the obstacles ahead. Flies use this visual information for postural adjustment before escape (Card and Dickinson, 2008). It is possible that the crickets suppressed their forward movement to avoid collision with the visually detected obstacle ahead. In summary, visual cue may help cricket to prepare for the escape but not provide spatial information enough for directional modulation.

2.4.2 Crickets rely on the mechanosensory inputs to modulate the escape direction.

Even if the visual cue did not solely modulate the escape direction, it remained possible that the visual inputs in addition to the mechanosensory inputs enhanced the effects of the obstacle. In general, multisensory integration enhances neural and behavioral responses to multimodal stimuli compared with those of unimodal stimuli (Gu et al., 2008; McMeniman et al., 2014). Nocturnal birds such as owl have sensitive visual and auditory system that can identify the exact location of a prey in complete darkness (Roge, 1962; Ashida, 2015; Takahashi, 2010). In cricket's brain, several interneurons sensitive to both antennal mechanosensory and visual stimuli have been identified (Gebhardt and Honegger, 2001; Schöneich et al., 2011; Staudacher and Schildberger, 1999). The results in this chapter indicated that tactile stimulation to the antenna strongly biased the walking direction to the wall-free side for both as unimodal and in multimodal sensing conditions. However, the visual input did not enhance the modulation of the escape direction

comparing to the effects by the mechanosensory cue only. These results suggest that crickets would entirely rely on the antennal mechanosensory inputs to perceive surrounding objects around adjacent field. The tactile stimulation to antennae also evoked escape responses, which is not mediated by the cercal sensory system (Comer et al., 1994). It still unknown how the mechanosensory inputs provided by antennae and cerci were integrated. The modulation of the cercal-mediated escape behavior by the antennal mechanosensory inputs and the visual inputs imply the multisensory integration process of a threat signal and spatial information of surroundings.

2.5 Figures

(Figure 2-1)

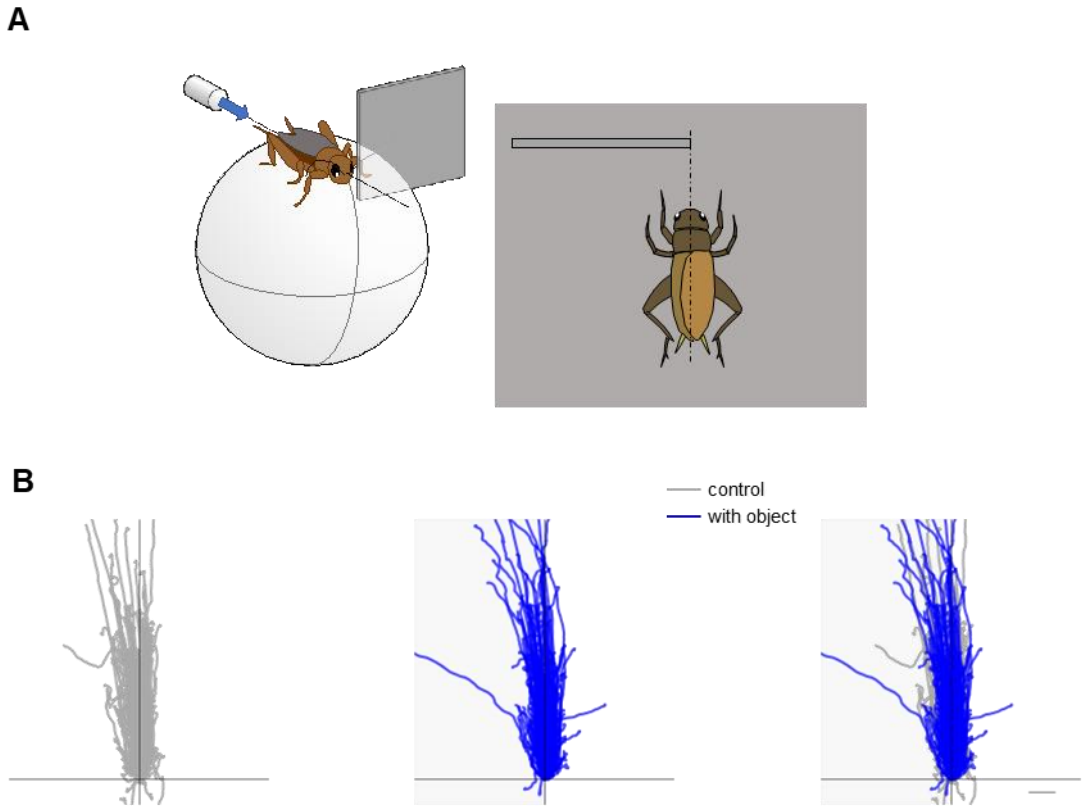

Fig. 2-1: Escape response under the conditions without sensory cue.

(A) Experimental arrangement for the condition without sensory cue. Cricket's antennae were bilaterally ablated at the base and light within the recording box was turned off. (**B**) Walking trajectory in the initial response to air-puff stimulus from behind in different conditions. Gray traces show the trajectories under control condition with no wall. Blue traces show the trajectories under wall condition. In right panel, the trajectories under these two conditions were overlaid.

(Figure 2-2)

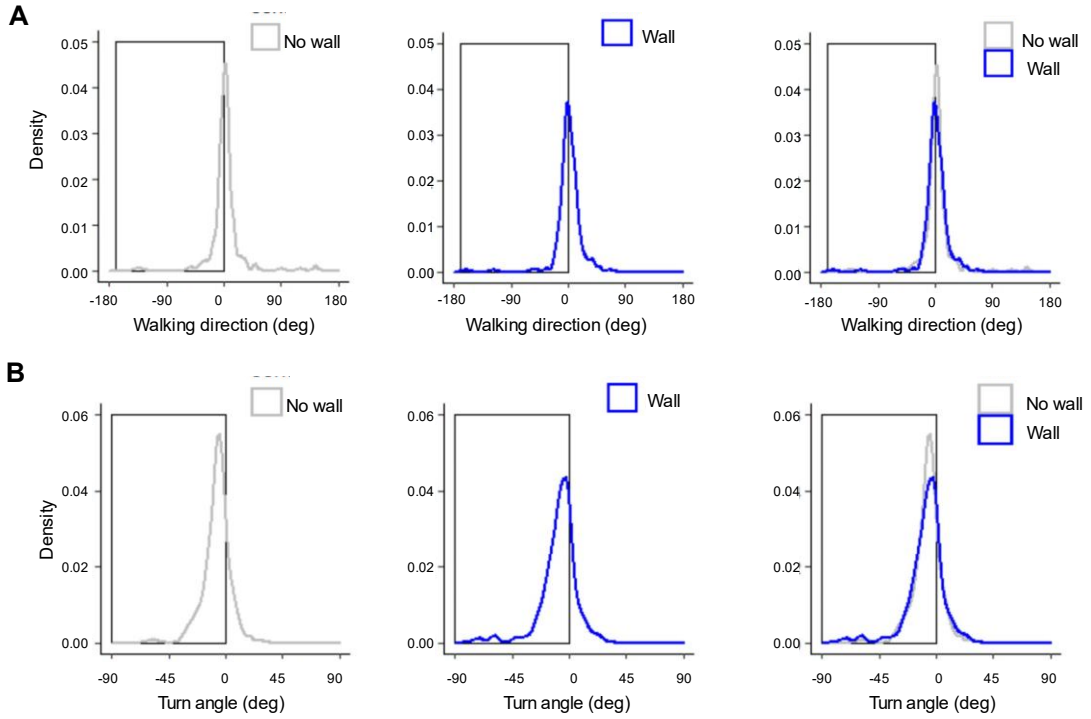

Fig. 2-2: Distributions of walking direction and turn angle under the conditions without sensory cue.

(A) Distributions of walking direction. The density plots show the proportion of walking direction in 300 trials for each condition. Gray and blue plots indicate the data from the control and wall conditions, respectively. In the right panel, the density plots for walking direction under these two conditions were overlaid. **(B)** Distributions of turn angle. The density plots show the proportion of turn angle in 300 trials for each condition. Gray and blue plots indicate the data from the control and wall conditions, respectively. In the right panel, the density plots for turn angle under these two conditions. The rectangles represent the wall side.

(Figure 2-3)

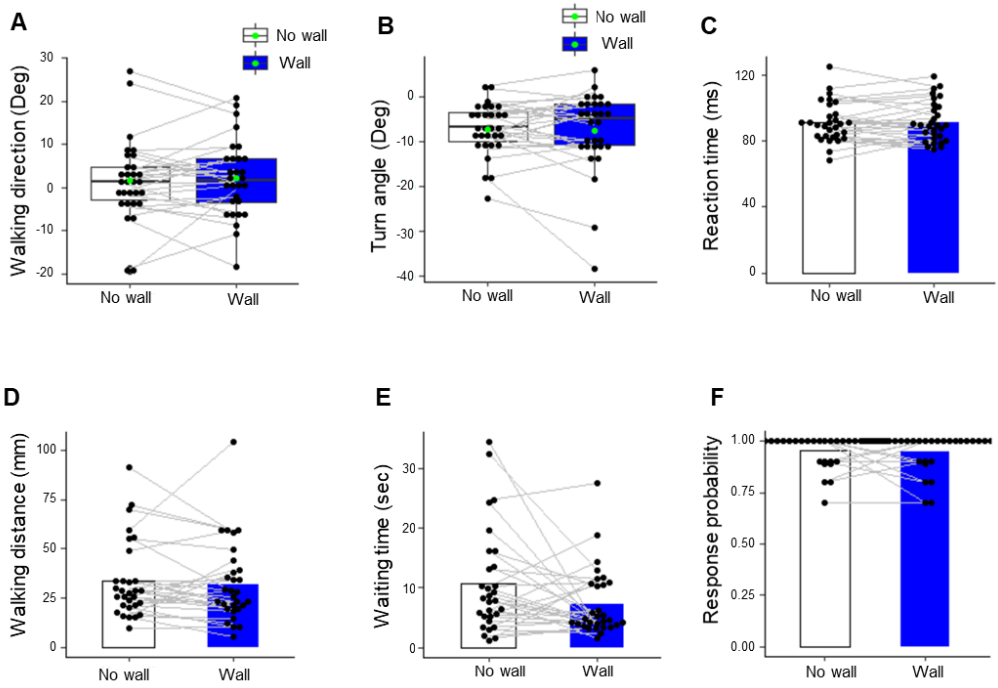

Fig. 2-3: No difference in the escape behavior under the condition without sensory cue.

Pooled data for the locomotion parameters, including walking direction (**A**), turn angle (**B**), reaction time (**C**), walking distance (**D**), waiting time (**E**), and response probability (**F**) under control and wall conditions. The box plot whiskers in **A** and **B** indicate the 1.5 x interquartile range of lower upper quartile; box limits indicate the lower, median, and upper quartile from the bottom to top across the population for each condition. The bar plots in **C-F** donate average across the population for each condition. Black dots connected by gray lines represent mean values for all trials in each individual, and green dots in **A** and **B** indicate average across the population for each condition. N = 30 individuals. Ten trials were implemented for each condition for each individual.

(Figure 2-4)

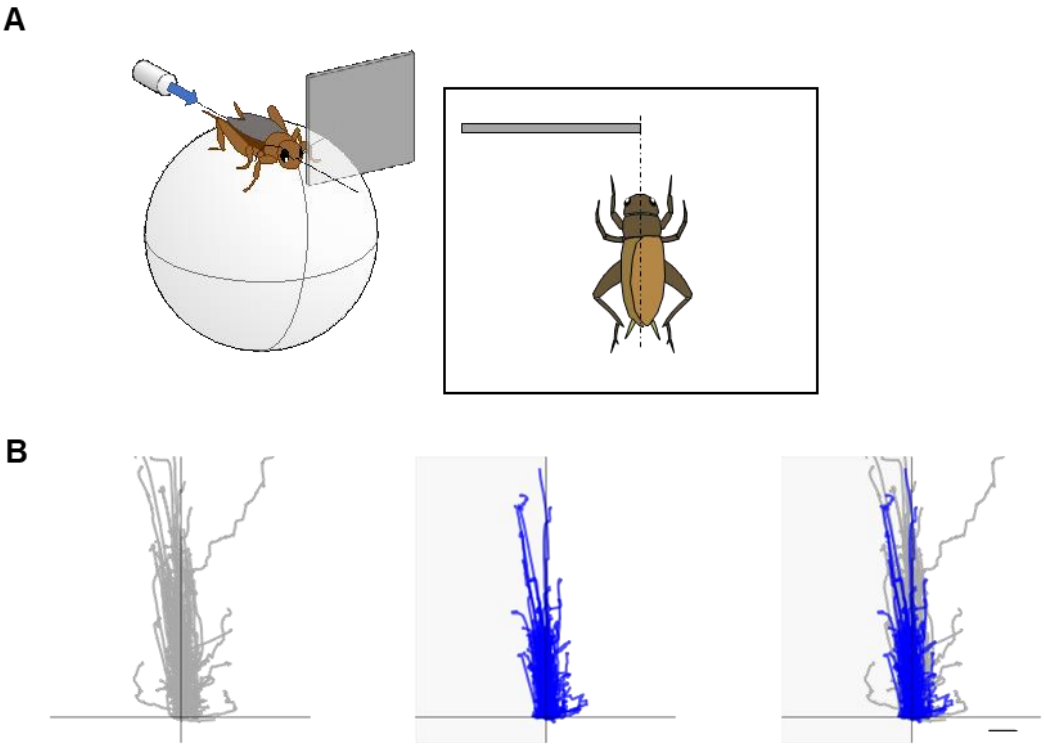

Fig. 2-4: Escape response under the conditions with visual cue.

(**A**) Experimental arrangement for the condition with only visual cue. Cricket's antennae were bilaterally ablated at the base and light within the recording box was turned on. (**B**) Walking trajectory in the initial response to air-puff stimulus from behind in different conditions. Gray traces show the trajectories under control condition with no wall. Blue traces show the trajectories under wall condition. In right panel, the trajectories under these two conditions were overlaid.

(Figure 2-5)

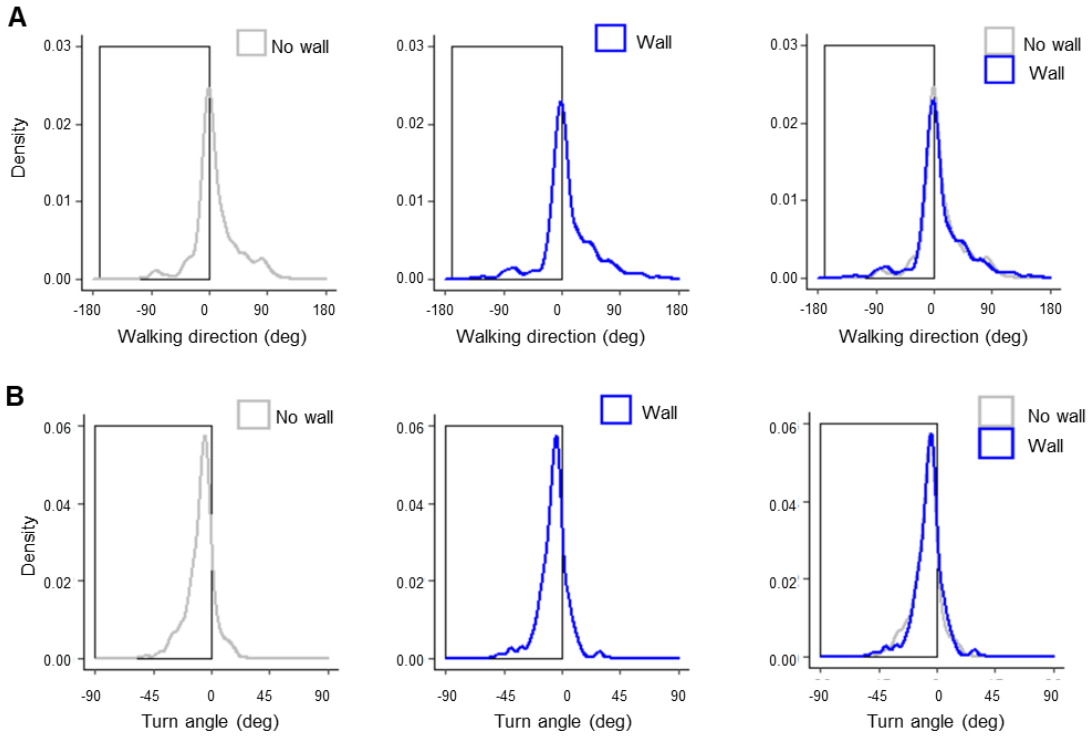

Fig. 2-5: Distributions of walking direction and turn angle under the conditions with visual cue.

(A) Distributions of walking direction. The density plots show the proportion of walking direction in 300 trials for each condition. Gray and blue plots indicate the data from the control and wall conditions, respectively. In the right panel, the density plots for walking direction under these two conditions were overlaid. **(B)** Distributions of turn angle. The density plots show the proportion of turn angle in 300 trials for each condition. Gray and blue plots indicate the data from the control and wall conditions, respectively. In the right panel, the density plots for turn angle under these two conditions were overlaid. The rectangles represent the wall side.

(Figure 2-6)

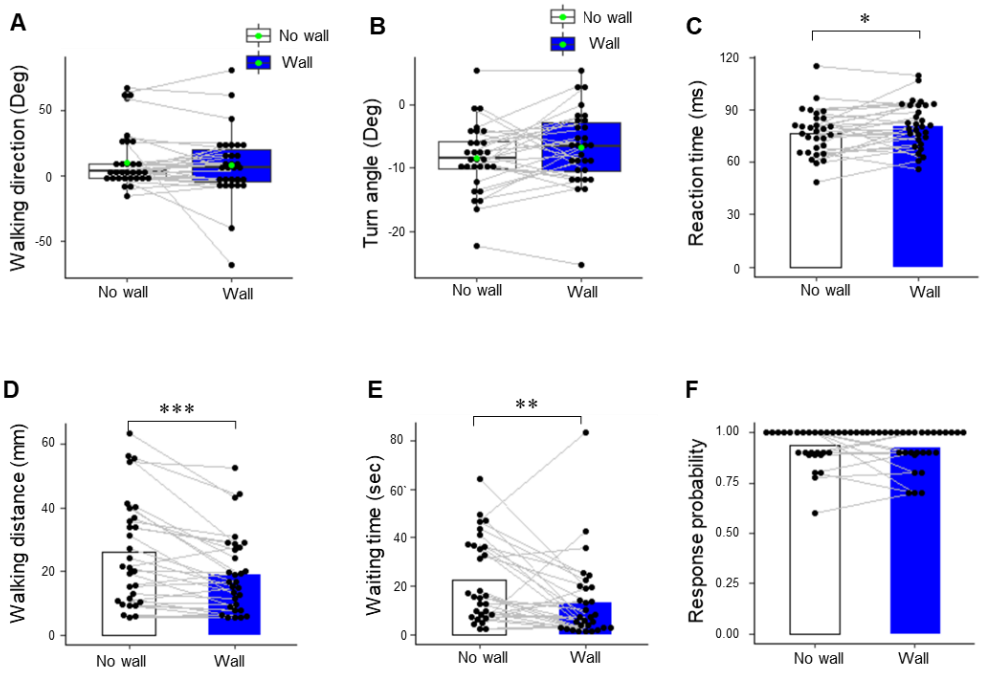

Fig. 2-6: Effects on the escape behavior by visual cue.

Pooled data for the locomotion parameters, including walking direction (**A**), turn angle (**B**), reaction time (**C**), walking distance (**D**), waiting time (**E**), and response probability (**F**) under control and wall conditions. The box plot whiskers in A and B indicate the 1.5 x interquartile range of lower upper quartile; box limits indicate the lower, median, and upper quartile from the bottom to top across the population for each condition. The bar plots in **C-F** donate average across the population for each condition. Black dots connected by gray lines represent mean values for all trials in each individual, and green dots in A and B indicate average across the population for each condition. N = 30 individuals. Ten trials were implemented for each condition for each individual. * $P <$ 0.05, ** $P < 0.01$, *** $P < 0.001$ (Fisher's nonparametric test for walking direction; Wilcoxon signed rank test for other parameters).

(Figure 2-7)

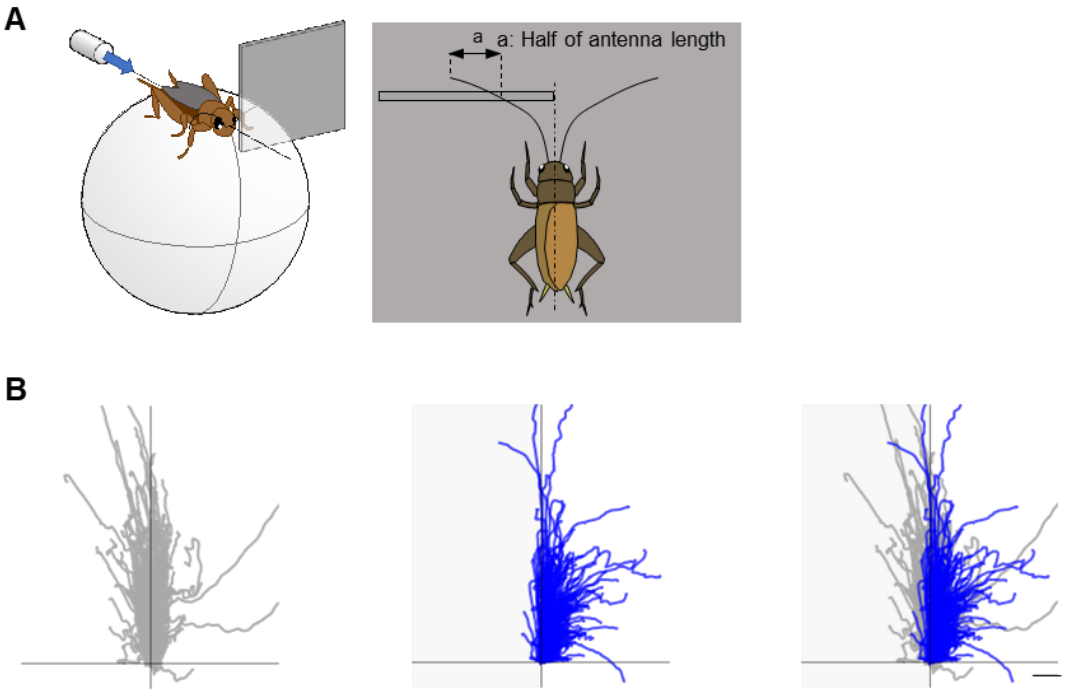

Fig. 2-7: Escape response under the conditions with antennal mechanosensory cue.

(A) Experimental arrangement for the condition with only antennal mechanosensory cue. Cricket's antennae were intact and light within the recording box was turned off. **(B)** Walking trajectory in the initial response to air-puff stimulus from behind in different conditions. Gray traces show the trajectories under control condition with no wall. Blue traces show the trajectories under wall condition. In right panel, the trajectories under these two conditions were overlaid.

(Figure 2-8)

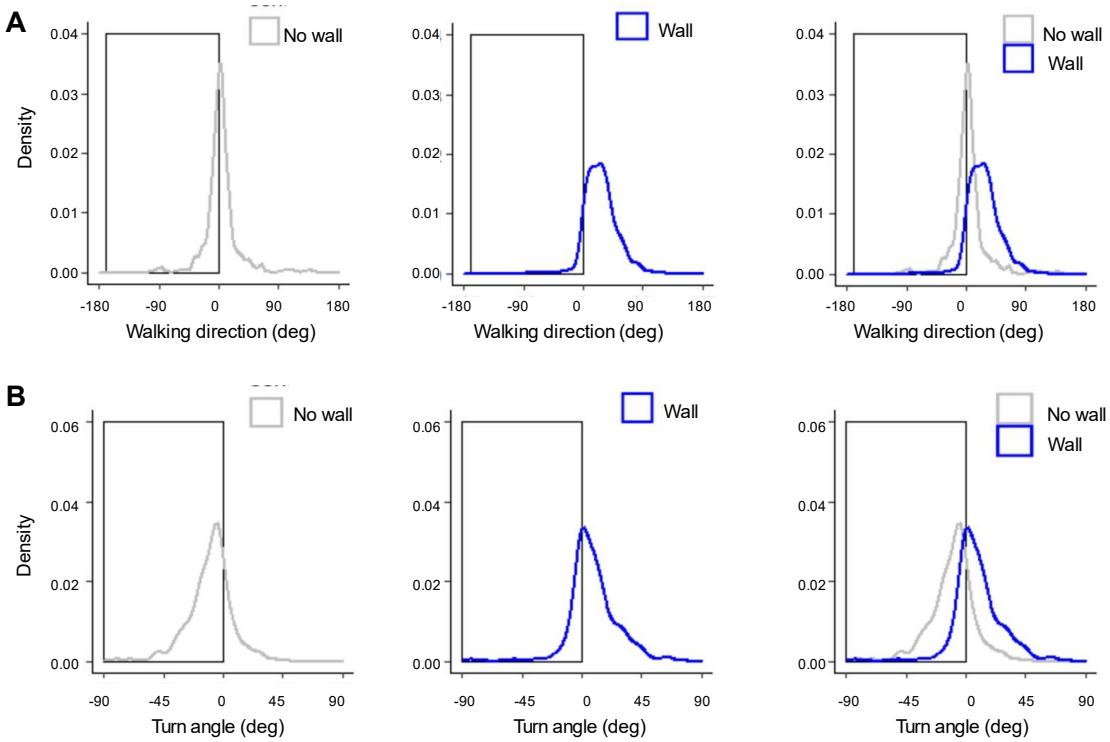

Fig. 2-8: Distributions of walking direction and turn angle under the conditions with antennal mechanosensory cue.

(A) Distributions of walking direction. The density plots show the proportion of walking direction in 300 trials for each condition. Gray and blue plots indicate the data from the control and wall conditions, respectively. In the right panel, the density plots for walking direction under these two conditions were overlaid. **(B)** Distributions of turn angle. The density plots show the proportion of turn angle in 300 trials for each condition. Gray and blue plots indicate the data from the control and wall conditions, respectively. In the right panel, the density plots for turn angle under these two conditions were overlaid. The rectangles represent the wall side.

(Figure 2-9)

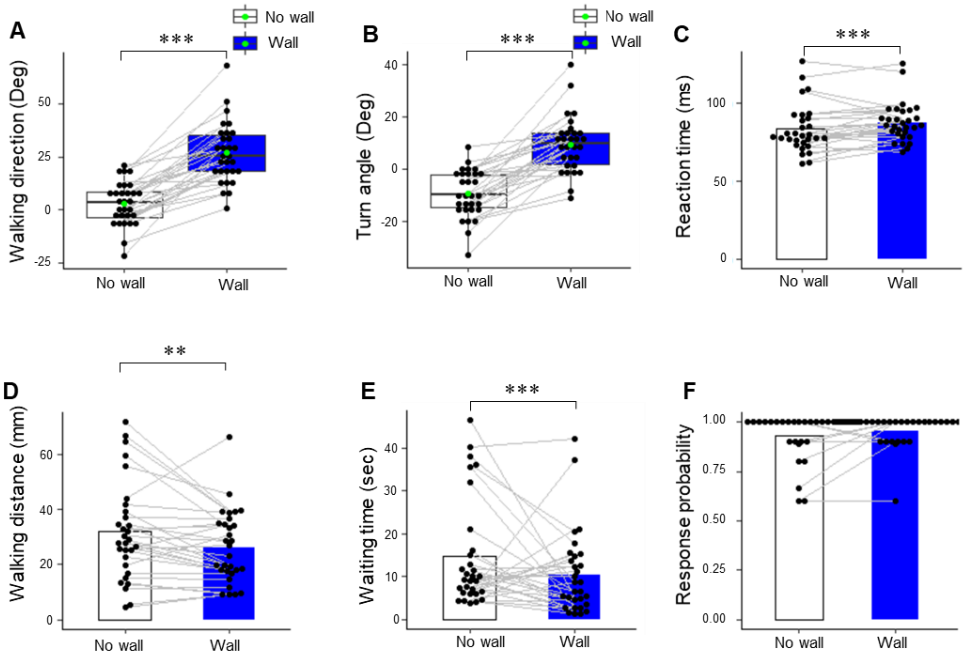

Fig. 2-9: Effects on the escape behavior by antennal mechanosensory cue.

Pooled data for the locomotion parameters, including walking direction (**A**), turn angle (**B**), reaction time (**C**), walking distance (**D**), waiting time (**E**), and response probability (**F**) under control and wall conditions. The box plot whiskers in **A** and **B** indicate the 1.5 x interquartile range of lower upper quartile; box limits indicate the lower, median, and upper quartile from the bottom to top across the population for each condition. The bar plots in **C-F** donate average across the population for each condition. Black dots connected by gray lines represent mean values for all trials in each individual, and green dots in A and B indicate average across the population for each condition. N = 30 individuals. Ten trials were implemented for each condition for each individual. ** $P <$ 0.01, *** $P < 0.001$ (Fisher's nonparametric test for walking direction; Wilcoxon signed rank test for other parameters).

(Figure 2-10)

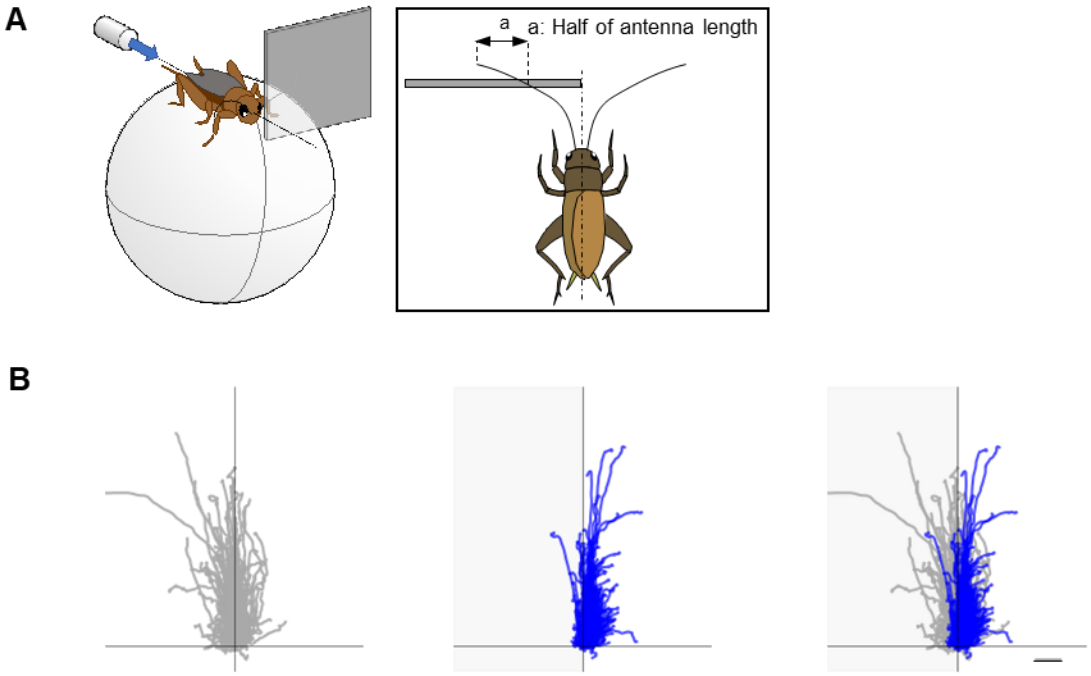

Fig. 2-10: Escape response under the conditions with multisensory cue.

(**A**) Experimental arrangement for the condition with both visual and antennal mechanosensory cues. Cricket's antennae were intact and light within the recording box was turned on. (**B**) Walking trajectory in the initial response to air-puff stimulus from behind in different conditions. Gray traces show the trajectories under control condition with no wall. Blue traces show the trajectories under wall condition. In right panel, the trajectories under these two conditions were overlaid.

(Figure 2-11)

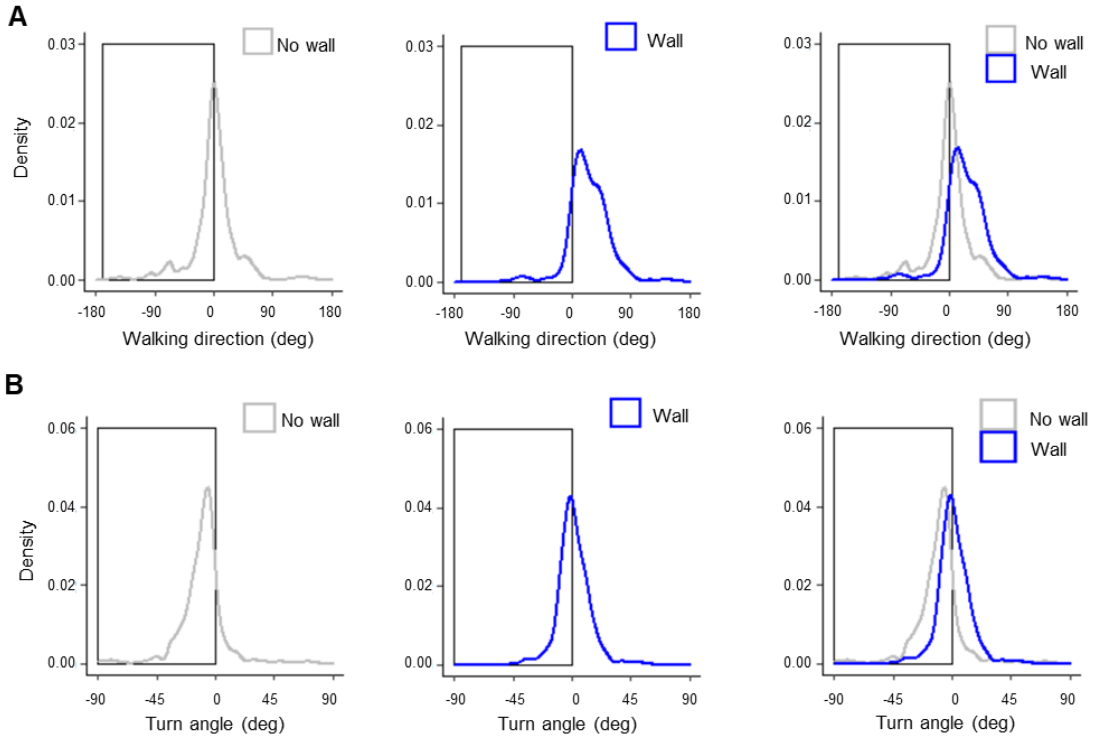

Fig. 2-11: Distributions of walking direction and turn angle under the conditions with multisensory cues.

(**A**) Distributions of walking direction. The density plots show the proportion of walking direction in 300 trials for each condition. Gray and blue plots indicate the data from the control and wall conditions, respectively. In the right panel, the density plots for walking direction under these two conditions were overlaid. (**B**) Distributions of turn angle. The density plots show the proportion of turn angle in 300 trials for each condition. Gray and blue plots indicate the data from the control and wall conditions, respectively. In the right panel, the density plots for turn angle under these two conditions were overlaid. The rectangles represent the wall side.

(Figure 2-12)

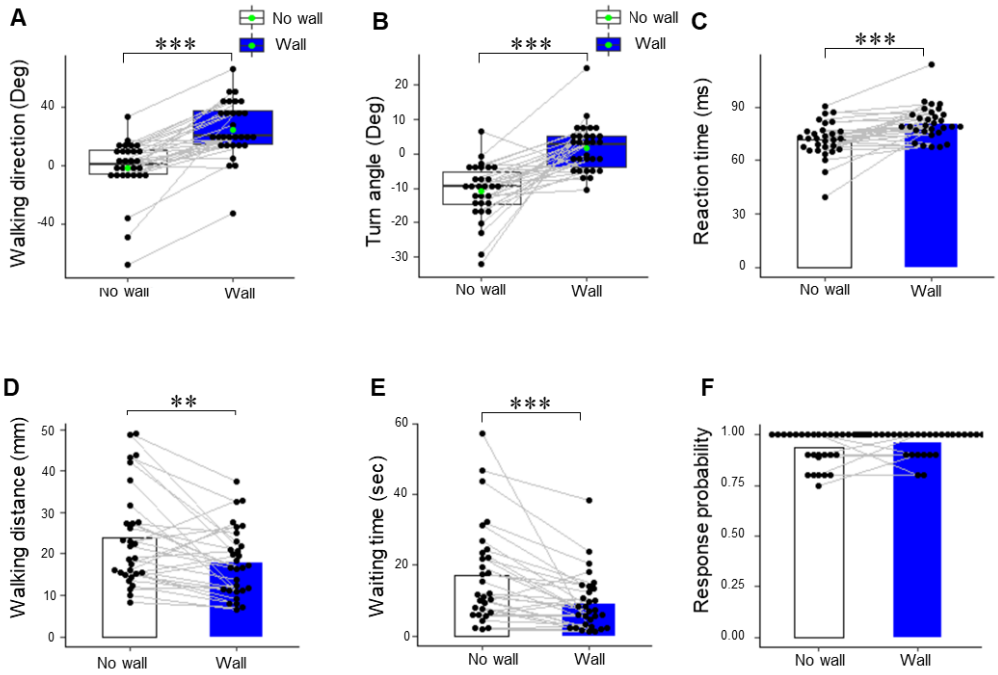

Fig. 2-12: Effects on the escape behavior by multisensory cue.

Pooled data for the locomotion parameters, including walking direction (**A**), turn angle (**B**), reaction time (**C**), walking distance (**D**), waiting time (**E**), and response probability (**F**) under control and wall conditions. The box plot whiskers in A and B indicate the 1.5 x interquartile range of lower upper quartile; box limits indicate the lower, median, and upper quartile from the bottom to top across the population for each condition. The bar plots in **C-F** donate average across the population for each condition. Black dots connected by gray lines represent mean values for all trials in each individual, and green dots in A and B indicate average across the population for each condition. N = 30 individuals. Ten trials were implemented for each condition for each individual. ** $P <$ 0.01, *** $P < 0.001$ (Fisher's nonparametric test for walking direction; Wilcoxon signed rank test for other parameters).

(Figure 2-13)

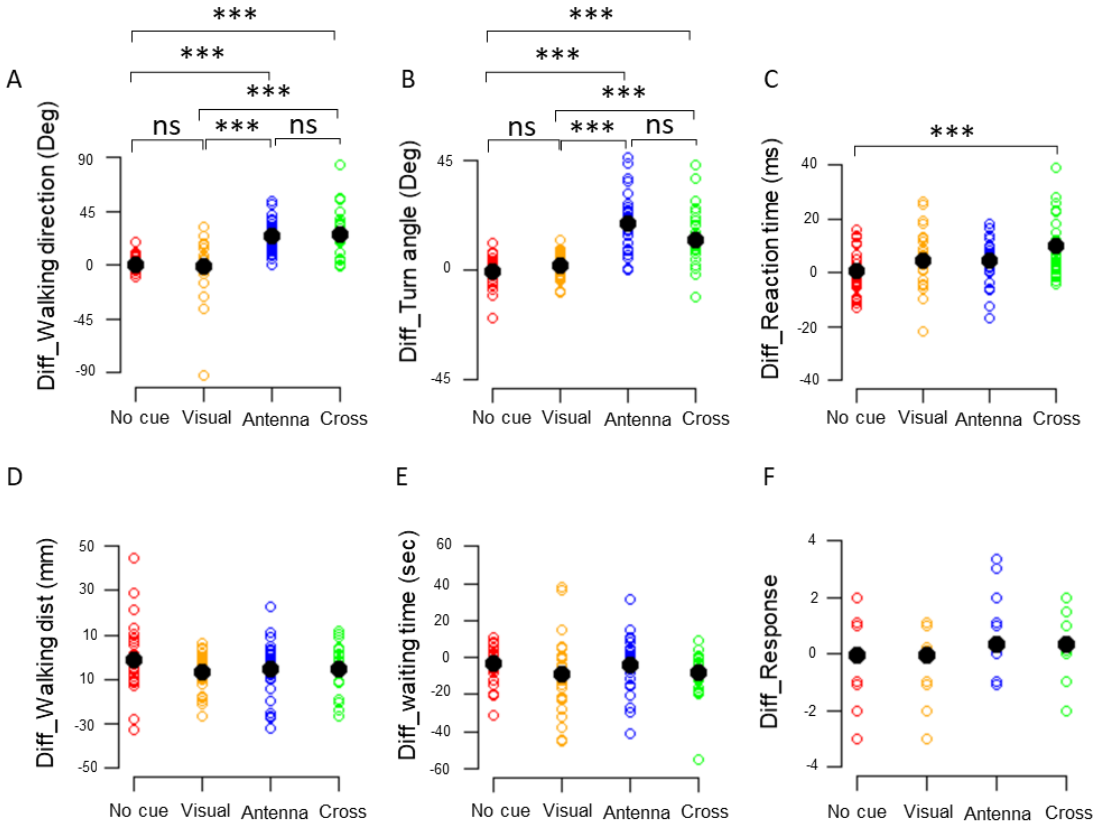

Fig. 2-13: Modulations of escape behavior by a wall in front under different sensory conditions.

Differences in locomotion parameters, including walking direction (**A**), turn angle (**B**), reaction time (**C**), walking distance (**D**), waiting time (**E**), and response probability (**F**), between the conditions with wall and no wall. Red, yellow, blue, and green dots represent the difference in the mean value for each individual, under the conditions with no-sensory cues, visual, mechanosensory, and multisensory (mechanosensory and visual). N = 30 individuals. *** $P < 0.001$ (Wilcoxon rank-sum test with Bonferroni correction).

Chapter 3

Requirement of bilateral comparison in antennal mechanosensory system and its spatial resolution

3.1 Introduction

Bilaterians that are animals with bilaterally symmetrical body generally have a pair of sensory organs, which are well positioned in their left and right sides. These animals can compare bilateral inputs from sensory organs on both sides to localize the stimulus source and induce an oriented behavior. For example, crayfishes use bilateral information provided by both antennae to improve the performance of their orientation behavior (Kraus-Epley and Moore, 2002). The bilateral comparison of mechanosensory inputs between right and left antennae is necessary for crayfish to navigate in T-maze (McMahon et al., 2005). Rats actively move their whisker depending on the nature of the contacted object, however, partial trimming of some whiskers on one side abolishes simultaneous movements of the whisker and altered the whisker-dependent behavior (Sellien et al., 2005). Not only the mechanosensory system but also other sensory systems employ the comparison of bilateral inputs. For instance, hawk moths detect temporally asymmetric inputs of odor stimuli between their antennae to orient the odor source, indicating that they compare olfactory inputs bilaterally to discriminate spatial and temporal differences (Parthasarathy and Willis, 2021). Lobster and crayfish use bilateral comparison also in their antennal olfactory system to orient toward chemical sources (Atema, 1996; Beglane

et al., 1997; Kraus-Epley and Moore, 2002). Biaural difference is fundamental mechanism of the sound localization. Comparing the auditory inputs between right and left ears, owls precisely localize the prey (Roger, 1962). These studies suggest that bilateral comparison could be a basic strategy for object localization.

The results in chapter 1 indicated that crickets were able to detect the shape, distance, and location of the surrounding objects by using their antennal mechanosensory system. They discriminated the location of the plate on the side that could be contacted only by a single antenna ipsilateral to the plate (Fig. 1-3). This suggests that cricket detects the edge of the plate by using one antenna and modulates their movement in the escape behavior. This means that edge detection of the contacted object plays an important role in object localization. For the plate in front, however, the cricket could bring their both antennae in contact with it, so the cricket might be able to determine the object location by comparing the antennal mechanosensory inputs on both sides. Thus, to explore how the bilateral comparison was involved in the localization of the forward objects, I unilaterally ablated the cricket's antenna and examined its effects on the modulation of the escape behavior by the obstacle in front.

In addition, I explored how accurately the crickets assessed the object scale relative to own body size. Angelfish choose one of two escape movements, either single or double bending, depending on their body size. Small fishes prefer double-bending response while larger ones use single bending response (Domenici and Blake, 1993b). This suggests that angelfishes choose the escape response with consideration of their own

body size. Perception of the size of the obstacle relative to the own body size would be also important for animals to determine the travel path or the escape to shelter. Cockroaches move along a wall keeping a certain distance to the wall by touching their antenna (Camhi and Johnson, 1999). Shortening of antennal flagellum increase the rate of collision to the obstacle (Baba et al., 2010; Okada and Toh, 2003). This suggests that cockroaches possibly use the antennae to measure the object size relative to their body. However, it remains unclear whether the insects are able to perceive the width of travel route such as escape path. To address this issue, I examined whether the cricket could modulate the escape behavior depending on the width of a gap between the forward walls, which might be measured by bilateral comparison and edge detection by both antennae.

In this chapter, focusing on whether crickets compared bilaterally their antennal mechanosensory inputs to localize the object in front and measured the width of gap as the escape path, I examined the modulation of the escape behavior by the walls in front of the cricket.

3.2 Materials and Methods

Animals, treadmill system, and airflow stimulator were the same as used in chapter 1. All experiments were performed in darkness within the sound-proof box at 1 to 6 hours after the start of the subjective nighttime under the raring condition.

3.2.1 Tactile stimulation

I conducted two types of experiment. In the first type of experiment, a square plate of 50 × 50 mm was placed at either center or one side in front of the cricket, as in chapter 1 (Fig. 3-1 A). In the second type of experiment, a square plate of 50 × 50 mm walls with a squared open of different width (0, 4, 8, 12, 16, and 20 mm) were placed at the center in front of the cricket (Fig. 3-3A). The dead end of the plate gap was too deep for crickets tethered on the treadmill to contact with it by their antenna. The crickets were also unable to contact with any of the lateral edges of the plate placed at the center in front by their antenna.

3.2.2 Behavioral experiment

In the first experiment, to test the utilization of the bilateral comparison to localize an object, I examined the behavior of the unilaterally-antenna-ablated crickets in response to an air-puff stimulus applied from the rear. The movement of the intact crickets was monitored in the “control” condition without the plate and in the 2 “wall” conditions where the plate was placed at the center or one side in front of the cricket. Then, the

antenna contralateral to the plate on one side was ablated at the base. After the ablation, the animal was left undisturbed for more than 40 minutes, after which its movements were recorded under the 3 conditions including control, center wall, and one-sided wall. The plate location and the antennal ablation were counterbalanced on both sides. That is, for 15 crickets, the plate was positioned on the left side and the right antenna was ablated. For other 15 crickets, the plate was positioned on the right side and the left antenna was ablated. For each of control and wall conditions, five trials were performed with an inter-trial interval greater than 1 minute. In total, 30 trials were performed for each individual. The order of conditions was reshuffled for different individuals. Thirty individuals were tested.

In the next experiment, to examine whether the crickets could measure the size of escape path relative to their body, I adopted six plates with opening of different gap-widths. For each individual, the movement of the crickets was monitored in the "control" condition without the plate and in the 6 "wall" conditions where the plate with a gap of different widths was placed at the center in front of the cricket. For each condition, five trials were performed with an inter-trial interval greater than one minute. In total, 35 trials were performed for each individual. The order of conditions was reshuffled for different individuals. Thirty individuals were tested.

3.2.3 Data analysis

Behavioral data were provided by TrackTaro software, processed, and analyzed by using

the same procedure as in chapter 1. Criteria for the categorization of behavioral response, either “response” or “no response” was also the same as described in chapter 1. Also in this chapter, I focused on the initial response. Also in this chapter, I focused on the initial response that was defined with the same criteria as in chapter 1 and measured four locomotion parameters for this initial response: walking direction, turn angle, reaction time, and walking distance by using the same definition and calculation as in chapter 1. In addition, to compare the effects of the antennal ablation on the walking direction, I calculated the difference in the mean angles of the walking direction between ‘control’ and ‘with-wall’ conditions for each individual as follows:

$$d = p_{stm} - p_{ctl}$$

where d is the difference in the mean angle of walking direction indicating the magnitude of the effect by the plate, p_{stm} and p_{ctl} are the mean values in with-wall and control conditions, respectively.

3.2.4 Statistical methods

R programming software (version 4.1.1, R Development Core Team) was used for the statistical analysis. As used in chapter 1, to avoid pseudo-replication, I used the mean value of the data obtained in the trials categorized as wind-elicited response for each individual as the representative value for the statistical tests. The significance of the plate effects was assessed by using Fisher non-parametric test for walking direction as the angular parameter and Wilcoxon signed rank for other parameters. Wilcoxon signed rank

test was also used to assess the significance of the antennal ablation for the difference in the walking direction. The statistical tests for the significant effects across the different conditions were corrected by using Bonferroni correction. To analyze the dependency of the locomotion parameters on the width of the opening gap on the plate, I used linear regression analysis as follows:

$$(locomotion\ parameter) = a(gap\ width) + b$$

And I tested significance for the regression slope (a).

3.3 Results:

3.3.1 Unilateral antenna-lesion affected the modulation of escape behavior by the obstacle in front.

In chapter 1, it was revealed that crickets could modulate their escape behavior depending on the location of the obstacles such as antero-posterior and medial-lateral positions. Did the crickets need to compare the left and right antennal inputs to localize the object? Or did even one antenna provide enough information about the object location by detecting edges of the plate to modulate the escape behavior? To answer this, I ablated one antenna and examined the modulation of the escape behavior triggered by the air-puff stimulus applied from behind when the plate was positioned in the front, either at the center or one side ipsilateral to the intact antenna (Fig. 3-1A). In intact crickets, as similar to the result presented in chapter 1, the plate placed at the center spread the escape movements to left and right, which was indicated by significance in the variance of the walking direction (P = 0.0013, Wallraff test, Fig. 3-1A,B, Fig. 3-2A,B). Whereas the one-sided plate significantly biased the walking direction and turn angle to the wall-free side (Fig. 3-1A,B, Fig. 3-2A,B). However, the cricket of which the antenna was unilaterally ablated exhibited different behaviors. When there was no plate in front of the cricket under control condition, they moved to the side of intact antenna in response to the air-puff. Also when the wall was placed at the center, the unilaterally antenna-lesioned crickets walked and turned towards the side of intact antenna (Fig. 3-1B,C,D). This indicated that the crickets of which one antenna was lost tended to move towards the side where the other side of

antenna provided sensory information. Interestingly, the walking direction seemed to be more biased to the intact antenna side rather than that in the intact crickets. There was significant difference in the effect of the center wall, which was indicated by the difference values in the walking direction between control and wall conditions (Fig. 3-2G). It was likely that unilateral tactile information provided by the remained antenna enhanced the biased walking toward the intact antenna side. These results suggest that crickets would require bilateral inputs to localize the wall correctly. In contrast, the wall placed on one side in front of the unilaterally antenna-lesioned cricket biased the movement to the wall-free side as well as intact crickets (Fig. 3-2A,B). The crickets might localize the one-sided wall by detecting its medial edge. However, this bias effect by the one-sided wall was significantly smaller in the ablated crickets than that in intact ones (Fig. 3-3H). Although the wall placed at the center elongated the reaction time in both intact and unilaterally antenna-lesioned crickets, the one-sided wall increase that only in the ablated crickets (Fig. 3-2C). This suggests that it takes longer time for the unilaterally antenna-lesioned crickets to decide the escape direction. The walking distance was affected by neither wall position nor antennal ablation (Fig. 3-2D,E). There was little impact on the response probability by the antennal ablation (Fig. 3-2F). Taken together, crickets could use both comparison of bilateral antennal inputs and edge detection to localize the forward obstacles.

3.3.2 Crickets modulated escape behavior depending on the gap width of travel path.

To clarify whether crickets can measure the object size relative to their own body, I examined the modulation of their escape response to the air puff by the frontal wall with opening gap of different width. If crickets measure the space for the escape path by comparing left and right antennal inputs, the locomotion parameters in the escape behavior would be modulated with the gap width. The escape trajectory and distributions of the walking direction and turn angle were altered depending on the width of gap (Figs 3-3B, 3-4A, 3-5B). For the plate with no gap (0 mm), the crickets moved and turned either left or right as the results for the center plate in chapter 1 and figure 3-1. The greater the gap width, however, the cricket tended to walk straight forward (Fig. 3-3B), and both directional parameters, walking direction and turn angle, became concentrated at 0º indicating the center forward (Fig. 3-4A, 3-5A). As a result, the angular variance of the walking direction decreased depending on the gap width (Fig. 3-4B, Table 3-1). These results indicated that crickets altered their escape direction depending on the space of the travel path to escape. Meanwhile, the dependency of the turn angle variance on the gap width was not significant (Fig. 3-5B, Table 3-1). This might be because the inter-individual variability was greater than intra-individual variability for the turn angle. Significance of the effect by the forward plate was observed in the walking direction variance for the gap width from 0 mm to 12 mm (Fig. 3-4B). Considering the width of cricket's body (6.95 ± 0.07, N = 30), it is possible that the crickets measure whether the

travel route was wide enough for their body size to pass through by using antennal system. The reaction time became shorter, and the walking distance increased as the gap width increased, and these parameters significantly depended on the gap width (Fig. 3-6A,B, Table 3-1). This indicated that cricket likely delayed the escape start and suppress the escape movement depending on the size of the expected escape route. In addition, the waiting time for the air-puff stimulation also increased with the gap width (Fig. 3-6C, Table 3-1). These results suggest that the size of travel path in the forward space also affected spontaneous walking activity in the crickets. Taken together, by using antennal system, the crickets were likely able to perceive the size of the expected route sufficient to accommodate the own body size to avoid collision during escape behavior.

3.4 Discussion:

Spatial perception of the surroundings such as distance and orientation to the objects is crucial for animals to survive. The distance to predator determines the survivability of preys because it affects success rates of both predator's attack and defense by preys (Wainwright et al., 2001; Tammero and Dickinson, 2002). Bilaterian animals acquire sensory information from a pair of sensory organs to guide them in keeping a distance from the threat. The integration of bilateral inputs from left and right sensory organs is also necessary not only to detect the location of threat but also to perceive the surrounding space. When animals determine a travel route in a confined space, they need to predict the size of the space to be traveled in relation to the own body size. In this chapter, I examined whether crickets compared bilateral antennal mechanosensory inputs to localize an object and modulated their escape behavior depending on the size of the perceived travel path.

3.4.1 Crickets compared bilateral antennal mechanosensory inputs to localize the objects.

Comparing the inputs from the left and right antennae is useful for obtaining a more accurate picture of the surrounding environment. For example, in navigation to localize an odor source, insects sample chemicals using a pair of antennae as chemical sensors and compared the sensory inputs to orient (Eiras and Jepson, 1994; Willis, 2008; Takasaki et al., 2012). The bilateral comparison of antennal inputs has also been reported for

mechanosensory cues. Crayfish have been reported to compare tactile inputs from both antennae to determine the turning direction (McMahon et al., 2005).

I predicted that crickets used two different methods to localize the object by their antennal mechanosensory system detection of the object edge and bilateral comparison of right and left inputs. Previous studies have indicated that lack of bilateral information from antenna affects crayfish's ability to orient to odor sources (Kraus-Epley and Moore, 2002). In my study, even if one antenna was ablated, the crickets walked to the opposite side of the plate placed on one side. Probably, the crickets, of which one antenna was ablated, detected medial edge of the one-sided front wall with the other antenna and turned around to avoid colliding it. These results imply that it is not always necessary for crickets to compare mechanosensory inputs from left and right antennae for the object localization.

However, when detecting the center plate that the cricket's antennae could not contact with the side of edge, the unilaterally antenna-lesioned crickets moved toward the side of intact antenna contacting with the wall. In other words, the crickets with only one antenna were not able to correctly localize the object without edge detection. This result suggests that bilateral information provided by right and left antennae was required to localize the object of which the edge could not be detected. In addition, the walking trajectory of the unilaterally antenna-ablated crickets was biased toward the side of intact antenna even under no wall condition. This may be because the ablated antenna did not provide any information about the presence or absence of an object, but the intact antenna actively

provided "absence" information of the obstacle. Thus, they were oriented toward the intact antenna side under no wall conditions. The perception of the “absence” of objects is one of the important functions of active sensing, for which animals repeatedly scan the environment by moving their sensory organ voluntarily (Bermejo et al., 2005; Nelson and MacIver, 2006). Furthermore, the bias effect on walking direction by the one-sided plate was larger in intact cricket than that in unilaterally ablated cricket. The behavioral modulation by the one-sided wall would be enhanced in intact crickets by the information of “absence of object” provided by the antenna on the wall-free side even if it did not contact with anything. This result also suggests bilateral comparison of left and right antennal inputs indicating ‘presence’ or ‘absence’ of the object would be also used for spatial perception by crickets. Insects likely perceive the surrounding space including object location based on the edge detection and bilateral comparison by active sensing with their antennal mechanosensory system.

3.4.2 Crickets have an ability to perceive the escape space relative to their own body size.

When both antennae detected the edge of two walls separated by a gap, the crickets gradually changed their escape behavior depending on the gap width. Interestingly, for the wall with an opening gap wider than 16 mm, which was twice of about their average body width, crickets moved forward as well as for no wall condition. This suggests that crickets measured the space of forward path relative to their own size. Animals change

their behavior depending on the size of the surrounding object such as a shelter, comparing with their own body size. Smaller crabs escape to small sized shelters while larger crabs prefer bigger ones (Bateman and Fleming, 2015). It is possible that animals consider the size of travel route or shelters in escape behavior. Insects may perceive the surrounding space available for future movements based on the edge detection and bilateral comparison by using active sensing with their antennal mechanosensory system.

Spatial information on the 'size' of the surroundings and animal's own body is important to modulate their orientation behavior (Webster et al., 2001). Frogs choose their escape mechanism depending on their body size (Bateman and Fleming, 2015), and cockroaches also use the antenna to measure the distance to surrounding barriers to prevent collision with them (Camhi and Johnson, 1999; Okada and Toh, 2003). In this study, I hypothesized that crickets could measure whether the width of travel path is sufficient for the escape route, by using their antennae. If crickets can measure the space relative to its body size, they will alter the direction of their escape behavior depending on the width. When perceiving that the forward space is too small for crickets to pass through, they will move to both sides to avoid collision during the escape behavior. For larger space where crickets can easily pass through, hence they will escape forward in response to the stimulus from rear.

I tested six different widths of gaps that were determined considering the average body size of crickets, about 7 mm. As a results, the wall with an opening gap narrower than 12 mm biased the movement to left or right side. For the wall with wider gaps, the

crickets walked straight forward as well as for control (no wall) condition. Other locomotion parameters in the escape response such as reaction time and walking distance also changed to suppress the forward movement, depending on the gap width of the wall. These results clearly suggest that crickets could measure the surrounding space to modulate their movements depending on the available space. The escape behavior is also modulated based on the size of obstacles weighted by another sensory modality. For example, fishes initiates escape when the visually detected obstacle takes a specific angle in the visual field (Garm et al., 2007a). Thus, the cricket's antennal mechanosensory system would have a resolution enough to modulate their behavior adapting to surrounding spatial information.

3.5 Figures

(Figure 3-1)

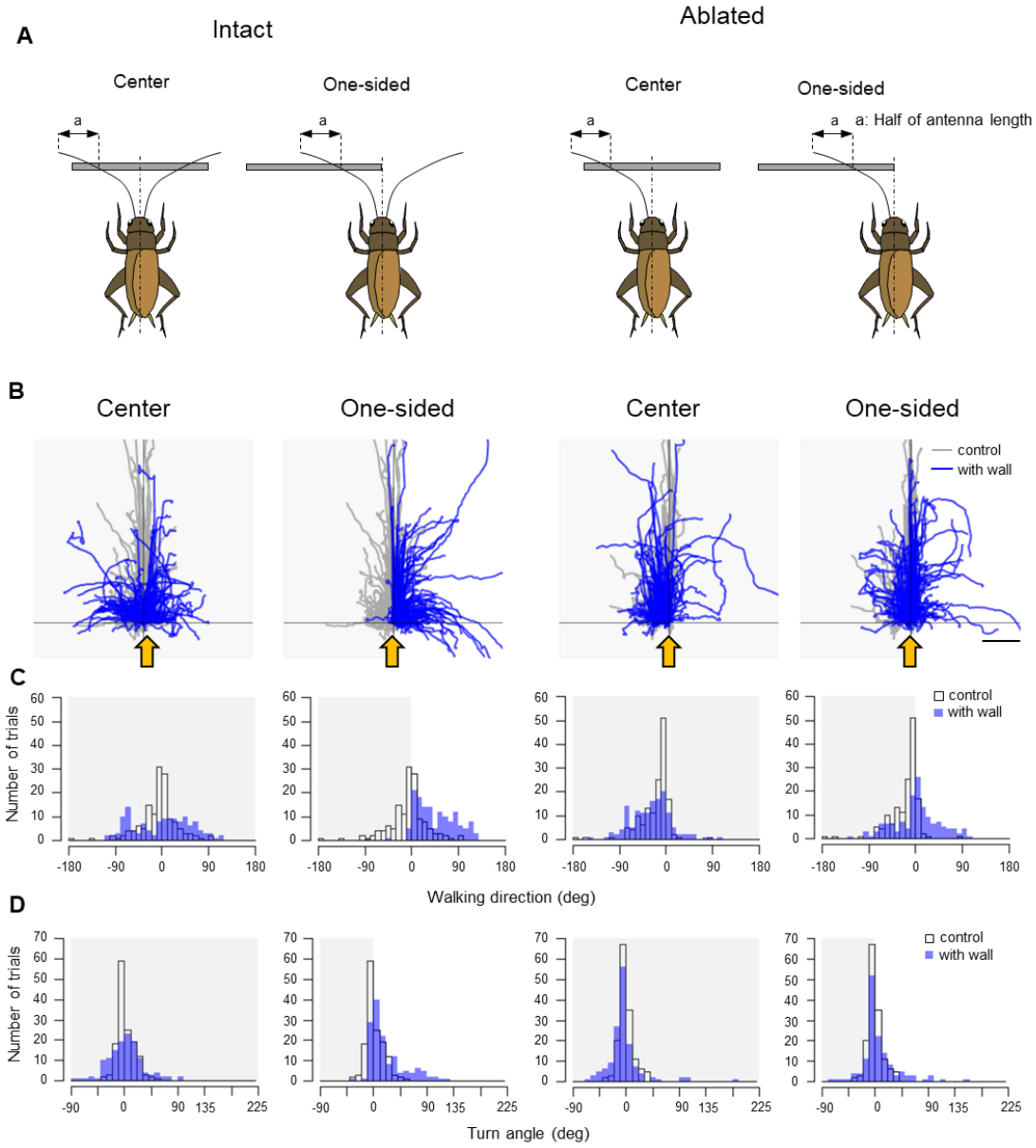

Fig. 3-1. Impacts by unilateral lesion of the antenna on the modulation of escape behavior.

(**A**) Experimental design. A square plate was positioned at the center or one side in front of the cricket. Air puff stimulus was applied from behind the animal. In unilateral-cut condition, the one antenna contralateral to the one-sided plate was resected. (**B**) Walking trajectory in the initial response to air-puff stimulus from behind in intact (2 left panels) and antenna-ablated crickets (2 right panels). Gray and blue traces show the trajectories under control (without wall) and antennal-stimulation (with wall) conditions, respectively. Shaded regions indicate the area where the plate was positioned. Yellow arrows indicate the direction of the air puff. Scale bar indicates 10 mm. (**C, D**) Distributions of walking direction (**C**) and turn angle (**D**) under different conditions in intact (2 left panels) and antenna-ablated crickets (2 right panels). Open and blue bars indicate data from the control and wall conditions, respectively. N of trials for each condition = 150.

(Figure 3-2)

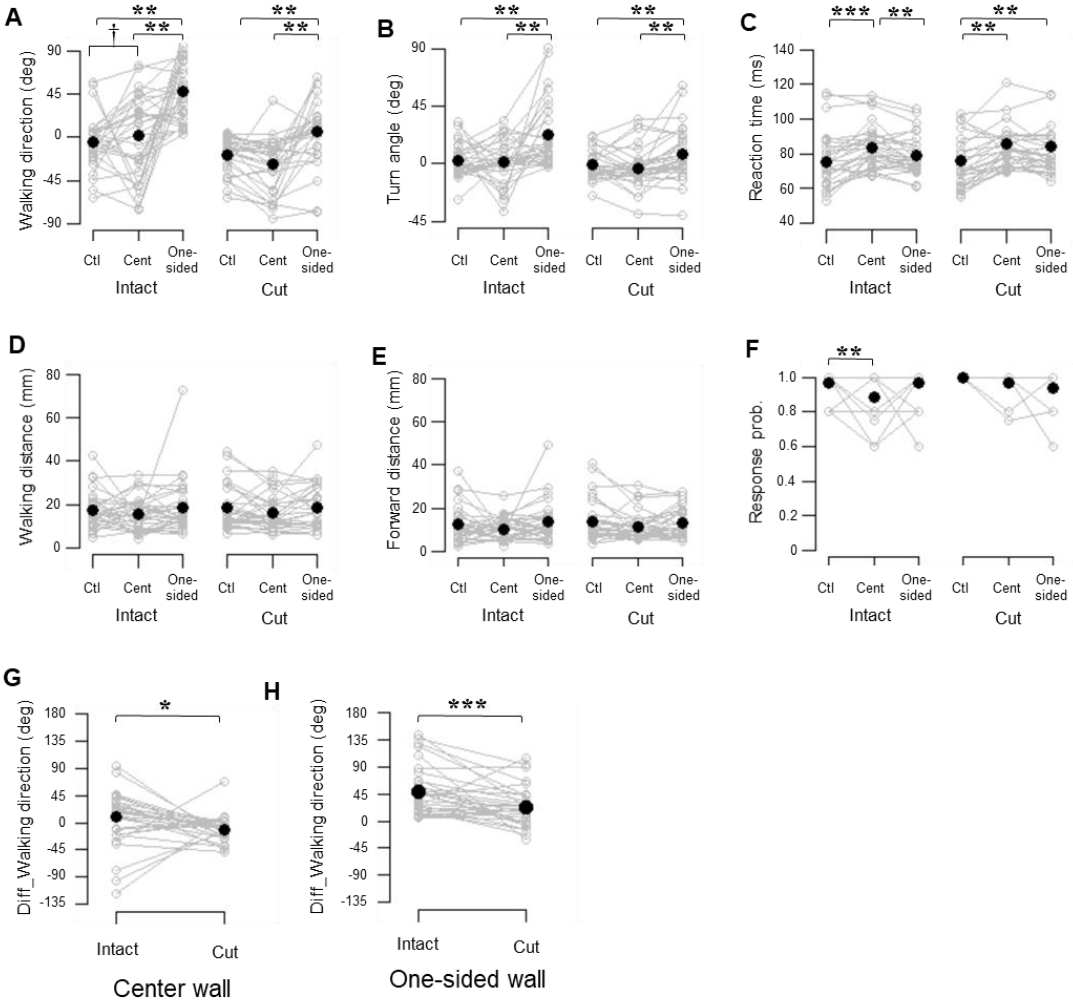
A
B
C
D
E
F
G
H
Walking direction (deg)
Turn angle (deg)
Reaction time (ms)
Walking distance (mm)
Forward distance (mm)
Response prob.
Diff_Walking direction (deg)
Ctl
Cent
One-sided
Intact
Cut
Center wall
One-sided wall

Fig. 3-2: Crickets possibly compared left and right antennal inputs to localize the forward object.

(**A-F**) Population data for the locomotion parameters, including walking direction (**A**), turn angle (**B**), reaction time (**C**), walking distance (**D**), forward movement distance (E), and response probability (F) under control and two conditions with wall in intact (left) and antenna ablated crickets (right). (**G,H**) Angular differences in walking direction for center wall (**G**) and one-sided wall (**H**) between the control and wall conditions in intact and antenna-ablated crickets. Gray open circles connected by lines indicate mean values for all trials in each individual, and black dots donate average across the population for each condition. N = 30 individuals. Five trials were implemented for each condition for each individual. Ctl, control; Cent, center wall; One-sided, one-sided wall. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (Fisher's nonparametric test and Wilcoxon signed rank test with Bonferroni correction). † $P < 0.05$ (Wallraff test with Bonferroni correction).

(Figure 3-3)

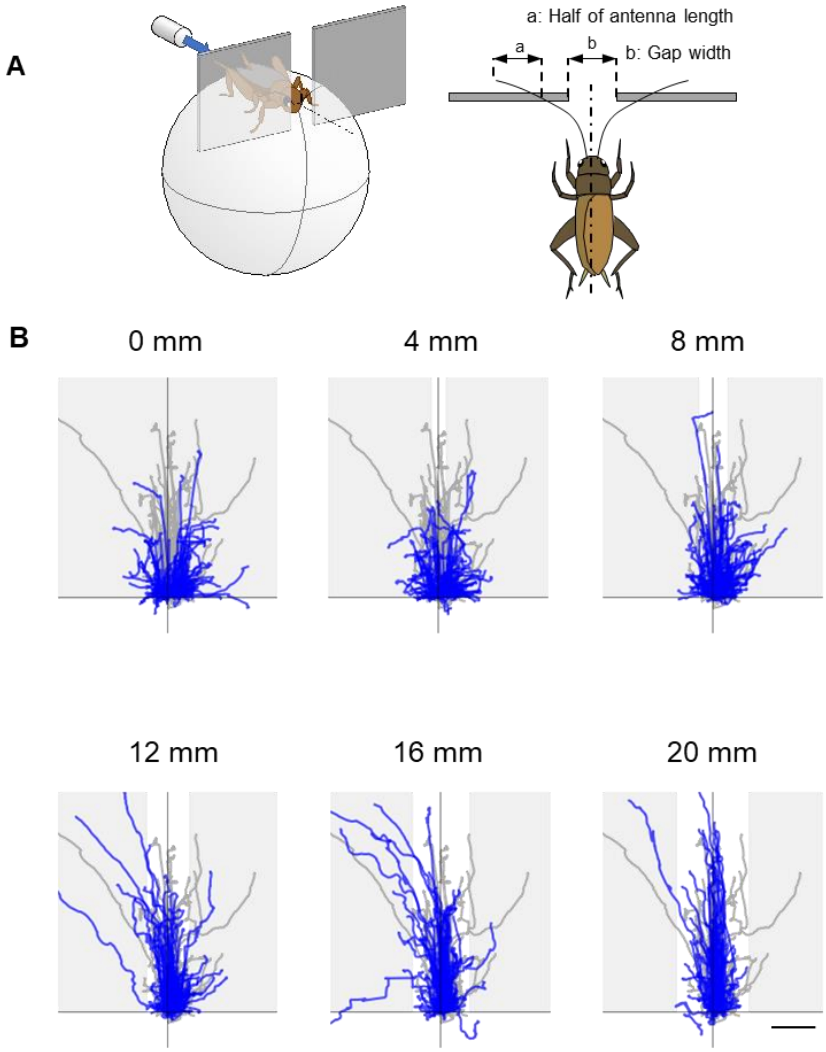

Fig. 3-3: Escape response when the plate with an opening gap was placed in front of the cricket

(A) Experimental design. A square plate with an opening gap of different width, 0, 4, 8, 12, 16, and 20 mm, was placed at the center in front of the cricket. **(B)** Walking trajectory in the initial response to air-puff stimulus from behind in control condition and in the antenna-stimulated condition with the front plate with different gaps. Gray and blue traces show the trajectories under control and antennal-stimulation conditions. Scale bar indicates 10 mm. Shaded regions indicate the area where the plate was positioned.

(Figure 3-4)

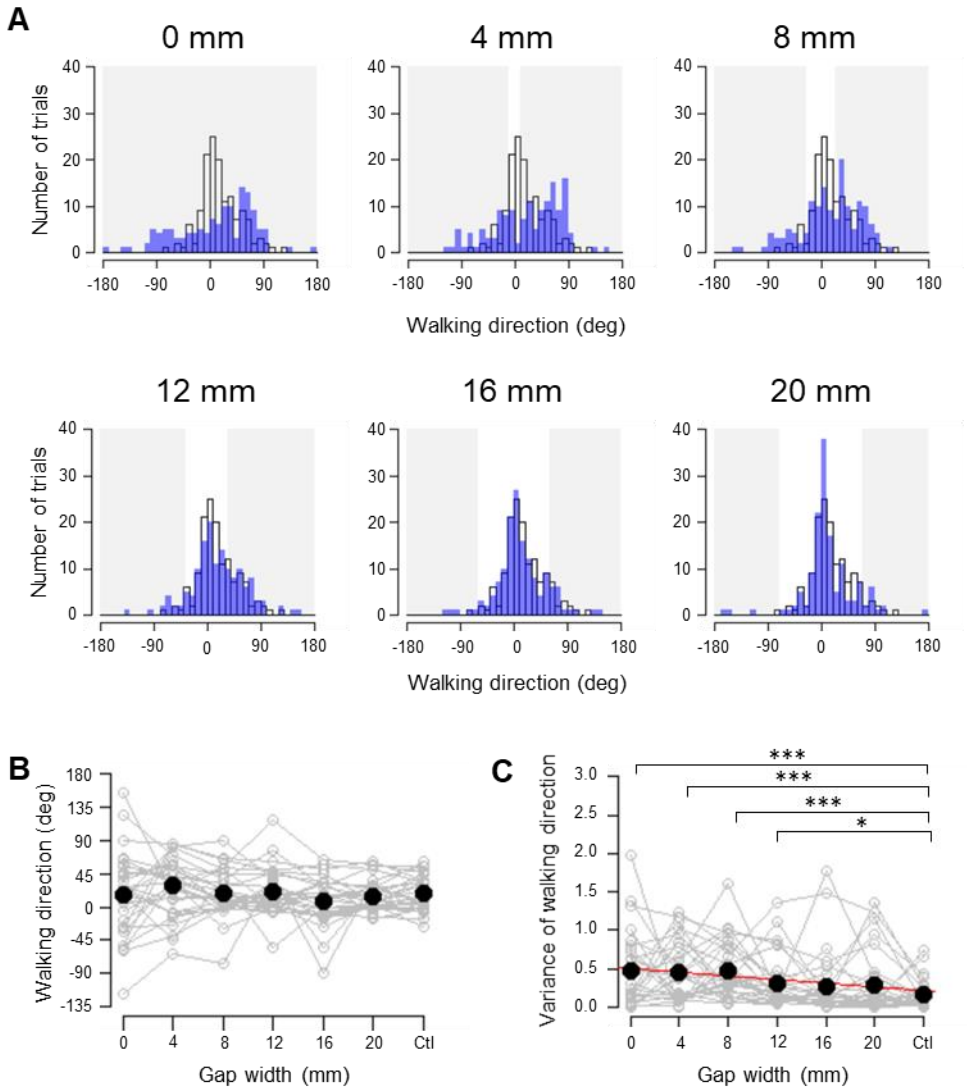
A
0 mm
4 mm
8 mm
12 mm
16 mm
20 mm
Number of trials
Walking direction (deg)
B
Walking direction (deg)
Gap width (mm)
C
Variance of walking direction
Gap width (mm)
***
***
***
*

Fig. 3-4: Effects on walking direction by the forward plate with an opening gap.

(A) Distributions of walking direction under control and the antenna-stimulated conditions with the front plate with different gap widths. Open and blue bars indicate the data from the control and wall conditions, respectively. N of trials for each condition = 150. Shaded regions represent the area where the plate was covered. **(B, C)** Population data for mean **(B)** and circular variance **(C)** of the walking direction. Gray open circles connected by lines indicate mean values or circular variance for all trials in each individual, and black dots donate average across the population for each condition. A red line in C indicate the regression lines with significant slope ($p < 0.05$ by test for regression slope, Table 3-1). N = 30 individuals. Five trials were implemented for each condition for each individual. * $P < 0.05$, *** $P < 0.001$ (Wilcoxon signed rank test followed by Bonferroni correction).

(Figure 3-5)

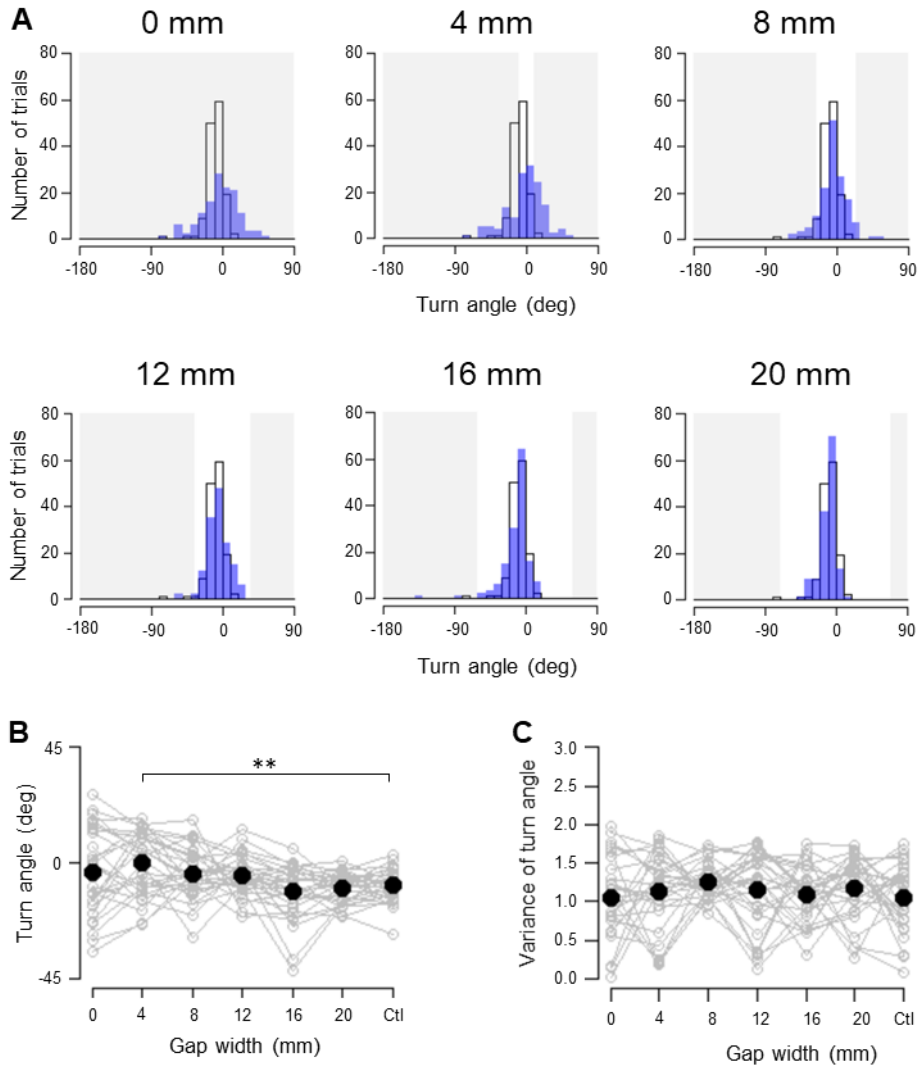
A
0 mm
4 mm
8 mm
12 mm
16 mm
20 mm
Number of trials
Turn angle (deg)
B
Turn angle (deg)
**
Gap width (mm)
C
Variance of turn angle
Gap width (mm)
Ctl

Fig. 3-5: Effects on turn angle by the forward plate with an opening gap.

(A) Distributions of turn angle under control and the antenna-stimulated conditions with the front plate with different gap widths. Open and blue bars indicate the data from the control and wall conditions, respectively. N of trials for each condition = 150. Shaded represent the area where the plate was covered. **(B, C)** Population data for mean **(B)** and circular variance **(C)** of the turn angle. Gray open circles connected by lines indicate mean values or circular variance for all trials in each individual, and black dots donate average across the population for each condition. There was no significant correlation between the variance of the turn angle and the gap width. N = 30 individuals. Five trials were implemented for each condition for each individual. *** $P < 0.001$ (Wilcoxon signed rank test followed by Bonferroni correction).

(Figure 3-6)

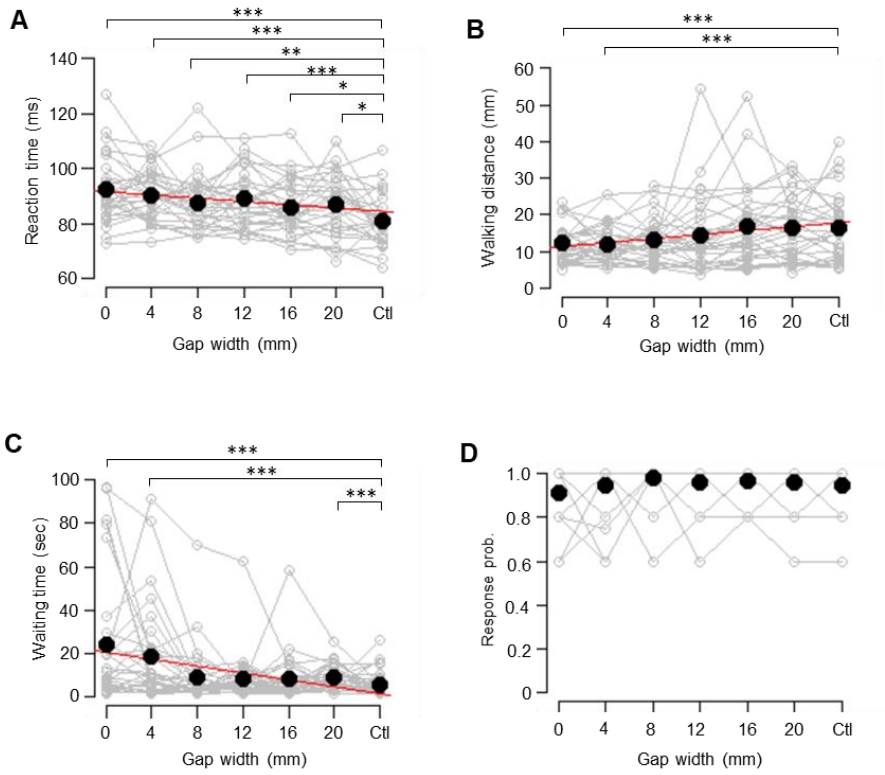

Fig. 3-6: Reaction time, walking distance, and spontaneous walking activity depended on the opening gap width.

Population data for the locomotion parameters, including reaction time **(A)**, walking distance **(B)**, waiting time **(D)**, and response probability **(D)** under different conditions. Gray open circles connected by lines indicate mean values for all trials in each individual, and black dots donate average across the population for each condition. A red line indicates the regression lines with significant slope ($P < 0.05$ by test for regression slope, Table 3-1). N = 30 individuals. Five trials were implemented for each condition for each individual. *** $P < 0.001$ (Wilcoxon signed rank test for other parameters).

3.6 Table

Table 3-1. Summary of regression analysis

Parameter	Slope	intercept	P-value	R^2
Variance of walking direction	-0.04648	0.493	0.008	0.039
Variance of turn angle	0.011	1.117	0.541	0.002
Walking distance	1.087	11.488	0.002	0.053
Reaction time	-1.188	91.545	0.010	0.037
Waiting time	-3.1833	20.819	0.0001	0.088

General discussion

Spatial perception by active sensing of cricket antennal mechanosensory system

The cricket, which was tethered on the treadmill, actively sensed the objects by moving its antennae freely and scanning the surrounding space. For spatial perception, “active sensing” is one of the most reliable ways to acquire contextual cues from the environment. In mechanosensory active sensing, animals voluntarily move their sensory organs across the surrounding objects to acquire information about their environment and to enhance the searching space and sampling frequency. Rodents use their facial whiskers not only as passive sensory organs, but also actively move them to perceive surrounding objects (Mitchinson et al., 2007; Deutsch et al., 2012; Voigts et al., 2015; Bush et al., 2016). Insects move their antennae to actively sample the surrounding space and are able to identify obstacles, recognize conspecifics and predators, actively track objects, and probe surface textures (Staudacher et al., 2005; Okada and Toh, 2006). Repeated probing an object surface with the antennae provides more accurate spatial information and periodic sensory update about the object condition. By using a high-speed video camera, I observed that cricket actively moved their antennae and repeatedly touched objects accompanied with bending of the antenna. The bending angle of the antenna likely plays a role in object localization. Cockroaches follow a wall with bending of the antenna to keep a specific distance to it (Johnson and Camhi, 1999). The video data of antennal contacts with the anterior and posterior walls on the side indicated that crickets touched the anterior wall more frequently than the posterior one. Behavioral results also indicated

that the anterior wall significantly affected the escape direction, but posterior wall had no effect. Taken together, it is possible that the more frequently the antenna contacts, the more greatly the escape behavior is affected. Thus, my results suggest that active sensing by a cricket's antennal system provides advanced spatial information to perceive the surrounding space, which allows the crickets to modulate their behavior mediated by different sensory organs.

Learning ability and spatial awareness in crickets

Since the experimental system I used was controlled in open-loop, and any object did not move as the cricket walked, it was still possible that mismatch between the feedback signals of self-motion and the sensory inputs may affect animal's behavior. However, this open-looped system was required for my experiments so that I can quantitatively manipulate the object position, and orientation relative to cricket's fixed position. To solve this problem, I focused on the initial response which so shortly lasted that the sensory feedback may not affected the behavior. In addition, as shown in Fig. 1-8, crickets did not learn the experimental conditions. Although the wall presence did not affect the spontaneous movement, the escape trajectory was clearly displaced in the direction of avoiding the object. These suggest that crickets would alter their behavior in anticipation of 'possible' collision with obstacles. In addition, when the wall was located in the direction of the escape movement, the reaction time increased, and the walking distance decreased. These results suggested that crickets did not reflexively change the escape

behavior by the antennal inputs. Rather, they likely alter the escape behavior considering the surrounding space and expected escape path.

The results mentioned above imply the possibility of 'spatial awareness' in crickets. They were able to modulate their behavior flexibly, based on the relationship between the airflow direction and the spatial arrangement of objects perceived by the antenna, as shown in chapter 1. Furthermore, ability to measure the gap width as the escape route seems to be a cognitive behavior. Previous studies have illustrated that insects modulated their walking based on the contacted object (Camhi and Johnson, 1999) and avoided collision using the antenna (Baba et al., 2000). These behavioral changes are possible even with reflexive circuits, and do not necessarily require the spatial awareness. In contrast to these previous findings, the behavioral modulations I found would not result from simple reflex circuits, rather may require 'internal representation' of spatial arrangement in surroundings. Taken together, from all my results, it is possible that crickets have spatial awareness. However, more well-designed behavioral tasks like for mammals will be necessary to prove directly spatial awareness of insects, in addition to clarify the neural representation of spatial arrangement by antennal mechanosensory inputs.

Neural mechanism for spatial-context-dependent modulation of escape behavior

It was revealed that the crickets modulated their escape behavior based on the spatial information of surrounding objects mediated by the antennal mechanosensory system.

These behavioral results suggest the presence of neural integration of the stimulus information such as airflow speed and direction detected by the cerci and the spatial information of surroundings sensed by the antennae. However, it remains unknown how cricket's nervous system integrates the cercal and antennal sensory information. Studies on cricket cercal and antennal mechanosensory neural process has been reviewed. The mechanosensory afferents of filiform hairs on the cerci are projected into the TAG, and the airflow information processed by local circuit is conveyed to the high centers such as brain and thoracic ganglia by ascending projection neurons including identified giant interneurons (Hirota et al., 1993; Jacobs et al., 2008). The antennal mechanosensory system, on the other hand, is used to sample near-head space (Schütz and Dürr, 2011; Horseman et al., 1997; Dürr, 1999). Various types of mechanoreceptive sensilla are distributed to the antenna in insects (Staudracher et al., 2005). These antennal mechanosensory neurons project their axons to dorsal lobe and the ventral areas of flagella afferent (VFA) region in the deutocerebrum of the brain (Yoritsune and Aonuma, 2012). In cockroach, the hair plate on the basal segment of antenna plays a major role in antennal object detection (Okada and Toh, 2000, 2001). These previous physiological studies suggest that neural circuit in the brain would be involved in the integration of mechanosensory inputs from both cercal and antennal mechanosensory organs. In addition, some previous studies of ablation experiments revealed that modulation of the cockroaches' escape required neural connection between the cephalic and thoracic ganglia (Rigdel and Ritzmann, 2005), and that intact connection between suboesophageal

and prothoracic ganglia disconnected with brain could lead to continues walking but failed to respond to objects on its path in cockroaches (Rigdel and Ritzmann, 2005). It has also been reported that descending signals from the cricket brain are necessary to regulate the escape direction (Oe and Ogawa, 2013). Some descending neurons sensitive to mechanical stimulation of antennae and cerci have been identified in the cricket brain (Staudacher and Schildberger, 1999; Gebhard and Honegger, 2001). The spatial contextual information presented by the insect's antennal system may be processed in the brain and used to control their movement for successful escape via descending neurons.

Cross-modal modulation of escape behavior

Animals receive multiple modalities of sensory cues during locomotion, which arise from visual, chemical, and mechanical stimuli in the environment. They obtain a relevant information from visual cues to modulate their behavior. Ants use visual cues for path integration and homing (Narendra, 2007a). Flies and locust detect a visual looming stimulus as threatening cues and initiate avoidance behavior (Tamnero and Dickinson, 2002b; Blender and Dickinson, 2006; Fotowat and Gabbiani, 2007). Also in cricket, shelter-like visual stimulus affects the escape behavior (Kanou et al., 2014). Tactile cues provide more reliable information of nearby objects. In nocturnal animals such as mice and insects, whiskers and antennae are used to acquire tactile information on a close range, and their movements are rapidly modulated based on it (Warren et al., 2021). Phonotactic steering and velocity in crickets were suppressed by tactile cue to the antenna (Haberkern

and Hedwig, 2016). In general, integration of multisensory cues enhances robustness and success rate of animals' behavior. Cockroaches integrate tactile and visual cues to control their escape behavior (Ye et al., 2003), and ants use multimodal integration to improve navigation performance (Buehlmann et al., 2020). Bats improves avoidance behavior in the presence of visual and auditory cues together, compared with any of these cues presented alone (Jones and Moss, 2021). Also in mice, when visual cue is combined with tactile cue by the whisker, the clearance speed and accuracy are greater than that in single modality of sensory cue (Warren et al., 2021). In my result, however, the multisensory cues did not enhance the modulation of escape behavior compared with only mechanosensory cue. And the visual cue solely had slight suppressive effects on the escape behavior. It is likely that crickets mainly rely on the tactile cues provided by antennal system to perceive nearby objects in surroundings.

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Research achievements

Publication

Ifere, N.O., Shidara, H., Sato, N. and Ogawa, H. (2022). Spatial perception mediated by insect antennal mechanosensory system. *J Exp Biol* jeb.243276.

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Presentations

International

1. **Ifere, N.O**., Shidara, H., Sato, N. and Ogawa, H. Mechanosensory antennal inputs modulate cercal-mediated escape behavior in crickets. The 50th annual meeting of Society for Neuroscience. Online. P578.07, Nov. 8-11, 2021. (Virtual Poster)

Domestic

1. **Ifere, N.O**., Shidara, H., Sato, N. and Ogawa, H. Antennal mechanosense of obstacles modulates the movement trajectory in wind-elicited escape behavior of crickets. The 61st annual meeting of Hokkaido branch in Zoological Society of Japan. Online. Program book P.7, March 20, 2021. (Oral).
2. **Ifere, N.O**., Shidara, H., Sato, N. and Ogawa, H. Spatial perception of obstacle by antennal mechanosensory system in the cricket. The 44th annual meeting of Japan Neuroscience Society. Online. 4P-134, July 28-30, 2021. (Virtual Poster).

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