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Rearing Conditions Required for Behavioral Compensation after Unilateral Cercal Ablation in the Cricket *Gryllus bimaculatus*

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ABSTRACT—The rearing condition necessary for behavioral compensation after sensory deprivation was investigated in the cricket *Gryllus bimaculatus*. The right-cercus-ablated cricket was reared in a glass vial with a slightly larger diameter than the body length of the cricket. After two weeks of rearing in the vial, the air-puff-evoked escape behavior of the cricket was investigated. The response rate (relative occurrence of the escape behavior after a standard air puff) obtained was identical with that of crickets reared in a large cage. On the other hand, unlike crickets reared in a large cage, the distorted escape directional property of the cricket reared in the vial was not compensated at all. Control experiments proved that the restraint in the vial did not affect the motor system, and the air motion from environments was not essential for the compensational recovery of the escape direction. Therefore, the ablated crickets required spontaneous walking in order to compensate the directionality of their escape. A self-generated wind caused by spontaneous walking appears necessary for the crickets to realize the defect of their sensory system and to compensate the related escape behavior. A hypothesis for the compensation mechanism based on the efference copy signal is proposed.

Key words: cricket, escape behavior, behavioral compensation, plasticity

INTRODUCTION

The cricket *Gryllus bimaculatus* shows an escape behavior from an incoming air puff (Gras and Hörner, 1992; Kanou *et al.*, 1999, 2001). Since this behavior is also observed in cockroaches (Camhi and Tom, 1978; Camhi *et al.*, 1978), it must be a good adaptive behavior for evasion from attack by their predators. The motion of the air around the body is detected by a large number of mechanosensitive filiform hairs on the cerci, a pair of appendages at the end of abdomen (Bentley, 1975; Shimozawa and Kanou, 1984a, b; Kanou *et al.*, 1999, 2001). However, a cricket makes a misoriented escape after undergoing unilateral cercal ablation (Kanou *et al.*, 1999). Thus, the sensory inputs detected by filiform hairs on both cerci are necessary for the cricket to recognize accurately the direction of the air puff stimulus. A certain period after the ablation, however, the unilaterally cercal ablated cricket becomes capable of escaping toward an appropriate direction, i.e., behavioral compensation occurs (Kanou *et al.*, 1999). Such a behavioral compensation must arise from neuronal plasticity. Some giant interneurons

(GIs), for example, show functional changes after unilateral cercal ablation (Matsuura and Kanou, 1998b).

We have previously reported that a unilaterally cercal ablated cricket shows behavioral compensation in terms of both the response rate and the escape direction within two weeks of rearing in a large cage together with other ablated crickets (5–6 crickets) (Kanou *et al.*, 1999). It was found that crickets did not modify their behaviors in a “trial and error” fashion because there was no predator that attacked the crickets in the cage. The question is how the unilaterally cercal ablated crickets can accurately recognize the direction of an air motion and complete the compensational changes in their neural systems.

It has been reported that a unilaterally cercal ablated cockroach also shows compensational recovery in both the escape direction (Vardi and Camhi, 1982a) and the function of GIs (Vardi and Camhi, 1982b). However, the degree of compensation was less when the ablated cockroach was housed in a small plastic cup during the recovery period (Vardi and Camhi, 1982a). Although the compensation mechanism was not clarified, the rearing method used limited significantly the self-generated wind during free walking and ambient wind from environments. These results suggest that the stimulation of filiform hairs on an intact cercus is

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essential for the compensation. Actually, sensory deprivation by immobilizing the filiform hairs on an intact cercus prevented a compensational recovery of the escape direction of a cockroach (Volman and Camhi, 1988). Based on these facts, we speculate that the mechanism for behavioral compensation in cercal ablated crickets is similar to that in cockroaches because as far as we consider, detection of a self-generated wind is a unique way by which the insect can recognize the inconsistency between the actual direction of the wind and the direction sensed by its sensory system. In order to explore this, in the present study, we reared a unilaterally cercal ablated cricket in a small glass vial in order to limit the spontaneous walking of the insect and, after a certain period, investigated the degree of behavioral compensation for an air puff stimulus. Furthermore, control experiments were carried out in order to exclude other factors that might possibly prevent the compensational recovery of the escape direction. From the results obtained in the present experiments, we propose a hypothesis that explains how a unilaterally cercal ablated cricket compensates the direction of a wind-evoked escape behavior.

MATERIALS AND METHODS

Animals

Adult female crickets, *Gryllus bimaculatus*, one week after the imaginal molt, were used. They were collected from a colony maintained in our laboratory. They were fed with "insect chow" (Oriental Koubo) and carrots. A shell vial filled with water and plugged with a cotton ball served as water supply and an egg-laying place. The temperature of the culture room was kept at $29 \pm 1^\circ\text{C}$ and the LD cycle was 12:12.

Behavioral experiments

The behavioral responses of crickets were investigated using an air puff stimulus. The air puff used was a jet of air produced by an air compressor (Hitachi, BEBICON 0.2OP-5S). We used an air puff with a peak velocity of 3.0 m/sec as a standard stimulus (Kanou *et al.*, 1999, 2001). The stimulus duration was 70 msec. Behavioral responses were recorded on a videotape and the

response angles relative to the stimulus angles (Fig. 1) were measured on a CRT screen. After plotting the data, the slope of the regression line and the correlation coefficient of the plots were calculated and employed as indices to assess the mean direction of the escape. When the averaged response direction is 180° opposite the stimulus source, the regression line will be $y=x$. The response rate, i.e., the relative occurrence of the escape behavior after a standard stimulus, was also investigated. The experimental setup and the stimulus profile were the same as those used in our previous studies (Kanou *et al.*, 1999, 2001).

It has been revealed that the compensation of the escape direction in unilaterally cercal ablated adult crickets was completed within 14 days of rearing in a large cage (Kanou *et al.*, 1999). Therefore, the behavioral investigation in the present study was carried out on the 14th, 15th and 16th days after the start of the conditioned rearing.

Cercal ablation

In the experiment of sensory deprivation, the right cercus of a cricket was cut at the base with a sharp razor blade. During the surgery, crickets were immobilized on a petri dish filled with ice for low-temperature anesthesia. Special attention was paid not to leave any wind-sensitive filiform hairs.

Conditions for insect rearing

In the experiments, we employed two different methods for insect rearing. In the first method, a cricket was reared in a glass vial. The inner diameter of the vial (38 mm) was slightly larger than the body length of the cricket. Another vial with a smaller diameter (25 mm), which was filled with water and plugged with a cotton ball for water supply, was hung inside the large vial (Fig. 2). The height of the space between the large and small vials was about 25 mm. Although the cricket kept in the space could drink water and eat insect chow scattered at the bottom of the large vial, it was restrained in the space and could not make any continuous walking. The cricket kept in the vial was almost completely isolated from any air motion coming from the outside of the vial.

In the second method, we used a tube made of wire mesh, which had diameter identical to that of the large glass vial (ref. Fig. 3). A large vial filled with water and plugged with a cotton ball was mounted on the wire-mesh tube. Although the motion of the cricket in the tube was also limited as in the first method, the cricket could experience air motion in the culture room caused by an air purifier (0.1–0.2 m/sec). The crickets sometimes experienced stronger air motion (0.5–1.5 m/sec), when the fan of the temperature controller

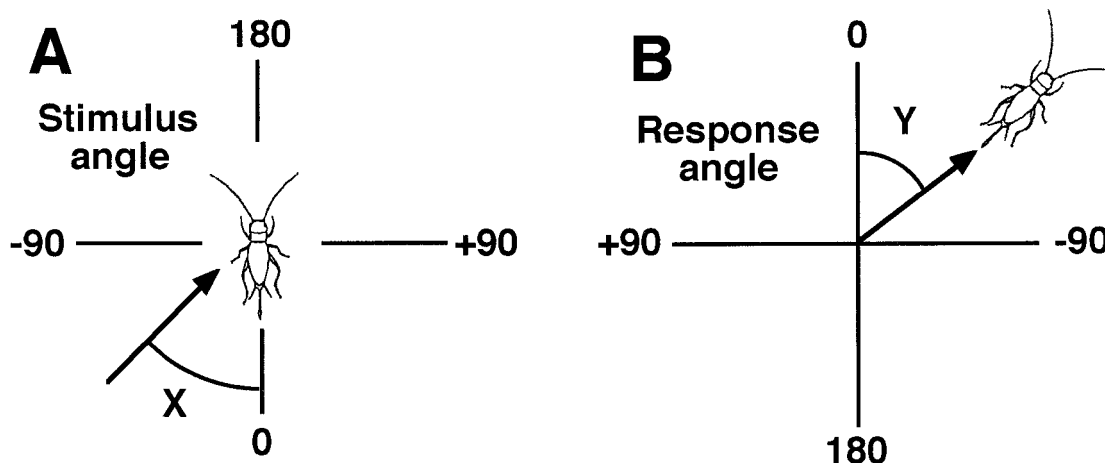


Fig. 1. Conventions for measuring stimulus (A) and response (B) angles.

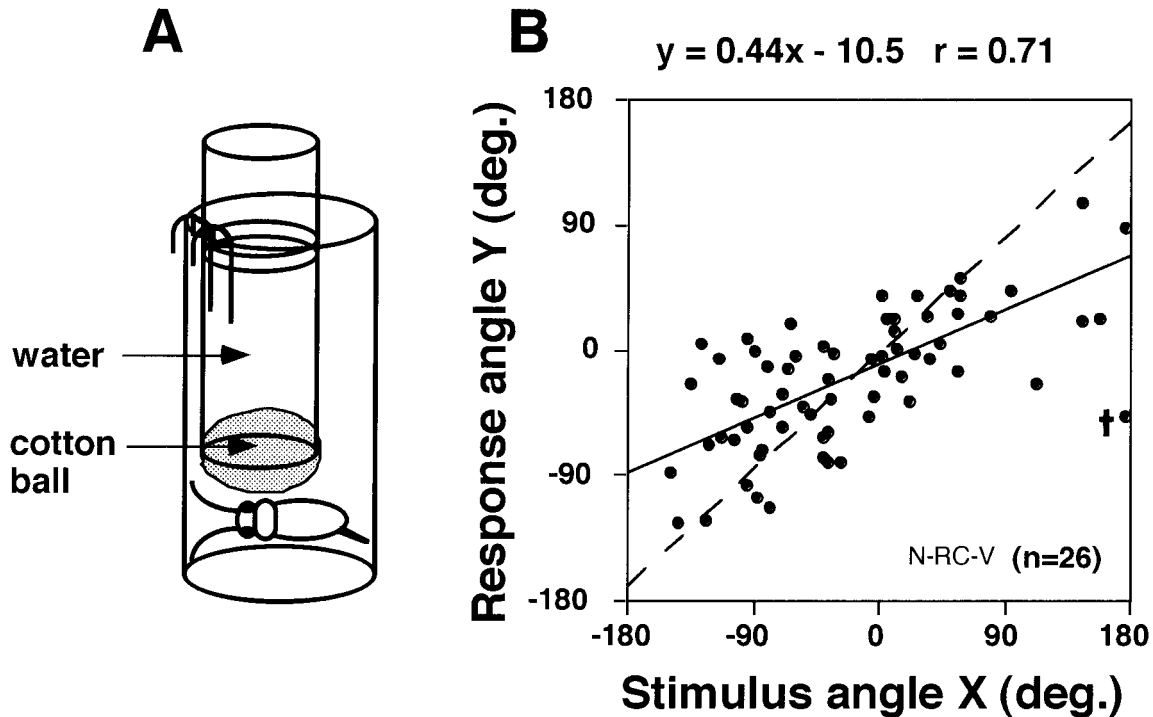


Fig. 2. A: A method of cricket rearing in a glass vial. See text for details. B: Stimulus and response angles of right-cercus-ablated crickets reared in a glass vial for two weeks. The slope of the regression line shows that no compensational change in the escape direction occurred in the crickets. A regression line (dashed line; $y=0.94x+0.4$) obtained from crickets reared in a large cage after a right-cercus ablation (Kanou *et al.*, 1999) is shown for comparison. Dagger: As the difference between ideal ($y=x$) and actual response angle exceeded 180°, the datum was excluded in the calculation of the regression line and the correlation coefficient.

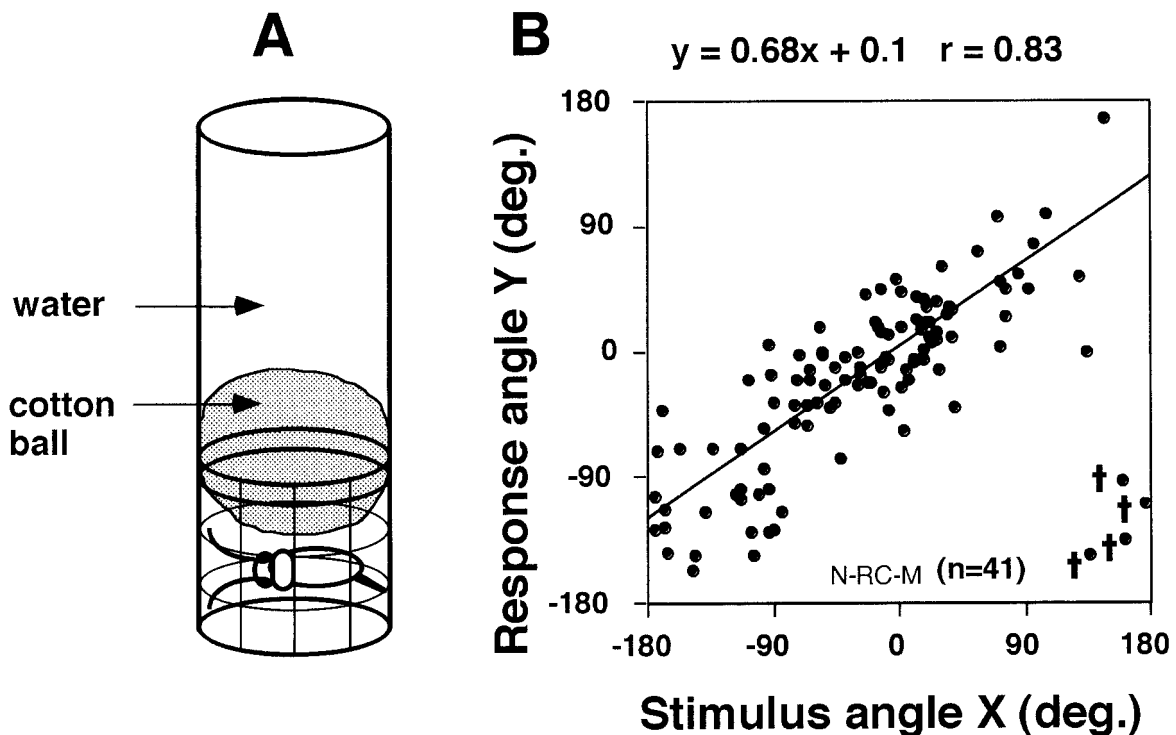


Fig. 3. A: A method of cricket rearing in a wire-mesh tube. See text for details. B: Stimulus and response angles of right-cercus-ablated crickets reared in a wire-mesh tube for two weeks. Similar to crickets reared in a glass vial, no compensational change in the escape direction occurred in these crickets. Dagger: Refer the legend of Fig. 2.

was functioning.

Statistics

Statistical differences were analysed by a z-test for response rate, a t-test for slope of regression line and a z-test for correlation coefficient after z-transformation.

RESULTS

Escape behavior of right-cercus-ablated crickets reared in a glass vial

Each cricket was reared in a glass vial after right-cercus ablation (Fig. 2A). The escape behavior of the cricket was investigated on the 14th, 15th and 16th days after the ablation with a standard air puff (3.0 m/sec). The response rate obtained from 26 ablated crickets was 21.4% (67 responses / 313 trials: Table 1). This response rate (21.4%) was significantly higher ($P < 0.05$) than that measured one day after the ablation (5.5%; Kanou *et al.*, 1999). Therefore, compensational recovery of the response rate occurred even when the crickets were reared in the glass vial. The response rate of the unilaterally cercal ablated crickets reared in a large cage showed a fluctuation after the recovery to some extent (Kanou *et al.*, 1999). As the response rate obtained in the present study was within the fluctuating range, it was concluded that rearing in the vial did not affect the compensational recovery of the response rate.

The directional property of the air-puff-evoked escape behavior was also investigated in the crickets reared in a glass vial (Fig. 2A). The regression line and the correlation coefficient, which indicate behavioral directionality, were $y = 0.44x - 10.5$ and 0.71, respectively (Fig. 2B and Table 1). The directional properties obtained in the present study were again compared to those previously measured in the right-cercus-ablated crickets reared in a large cage (Kanou *et al.*, 1999; Table 1). In such crickets, the regression line and the correlation coefficient were $y = 0.94x + 0.4$ and 0.93, respectively (the regression line is shown in Fig. 2). Both the slope of the regression line and the correlation coefficient obtained in the present study were significantly smaller ($P < 0.05$) than those obtained in the previous study. The slope of regression line and the correlation coefficient of the unilaterally cercal ablated crickets reared in a large cage showed a fluctuation during a recovery period (Kanou *et al.*, 1999). As the slope of regression line obtained in the

present study was almost identical with the smallest one during the recovery period, it was confirmed that no compensational recovery of the escape direction occurred in the crickets reared in the glass vial.

In another group of crickets (48 crickets), the same experiment was carried out for further reconfirmation. The regression line of the group was $y = 0.42x - 4.8$ and the correlation coefficient was 0.70. The result again suggests that compensation of the escape direction did not occur in the crickets reared in the glass vial. Therefore, it appears that the unilaterally cercal ablated crickets require spontaneous walking for the compensation of the escape direction. We carried out the following experiments in order to exclude other possible reasons to explain the result and to confirm the above conclusion.

Escape behavior of right-cercus-ablated crickets reared in a wire-mesh tube

Rearing in the glass vial limits not only the spontaneous walking but also any air motion from the environment. As the sensation of such air motion may have some effects on the compensation of the escape direction, we carried out the same experiment in the right-cercus-ablated crickets reared in a wire-mesh tube instead of the glass vial (Fig. 3A). The escape behavior of these crickets was also investigated on the 14th, 15th and 16th days after the ablation.

The response rate measured in 41 crickets reared in a wire-mesh tube was 23.0% (116 responses / 504 trials; Table 1). It was not significantly different ($P > 0.05$) from the response rate measured in the crickets reared in a glass vial (21.4%). Therefore, the recovery of the response rate is not affected by air motion from the environment.

The regression line and the correlation coefficient of the ablated crickets reared in a wire-mesh tube were $y = 0.68x + 0.1$ and 0.83, respectively (Fig. 3B and Table 1). The slope of the regression line and the correlation coefficient were significantly smaller than those measured in the ablated crickets reared in a large cage ($P < 0.05$; $y = 0.94x + 0.4$, $r = 0.93$) and those were within the fluctuation during the recovery period. Therefore, no compensational recovery of the escape direction occurred in the crickets reared in the wire-mesh tube as in those reared in the glass vial, and it was confirmed that air motion from the environment is not essential for the crickets to compensate the

Table 1. Summary of the escape behavior of crickets reared under different conditions

	Number of insects used	Number of trials	Number of responses	Response rate (%)	Regression line	Correlation coefficient	
intact / cage				53.7±5.0	$y = 0.90x - 1.3$	0.87	Kanou <i>et al.</i> , 1999
intact / mesh	22	267	161	60.3	$y = 0.89x + 4.3$	0.91	present study
ablated / cage	41	612	79	12.9	$y = 0.94x + 0.4$	0.93	Kanou <i>et al.</i> , 1999
ablated / vial	26	313	67	21.4	$y = 0.44x - 10.5$	0.71	present study
ablated / mesh	41	504	116	23.0	$y = 0.68x + 0.1$	0.83	present study

intact: intact crickets, ablated: right-cercus-ablated crickets, cage: reared in a large cage, vial: reared in a glass vial, mesh: reared in a wire-mesh tube

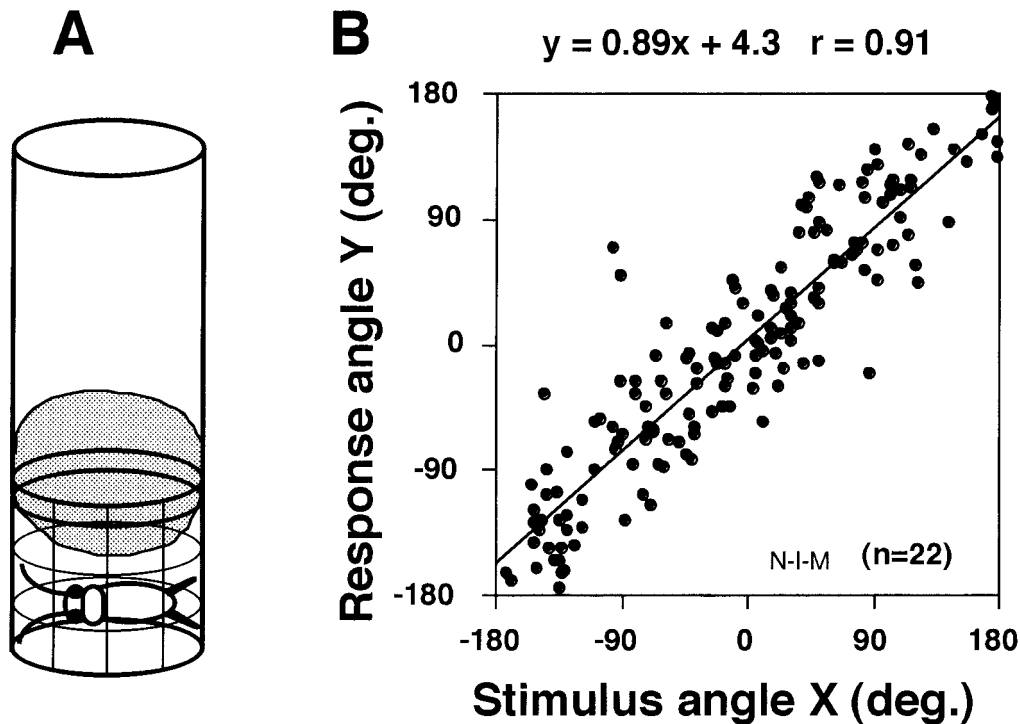


Fig. 4. A: Each intact cricket was reared in a wire-mesh tube. B: Stimulus and response angles of intact crickets reared in a wire-mesh tube for two weeks. The escape direction is as accurate as that in crickets reared in a large cage.

escape direction.

Escape behavior of intact crickets reared in a wire-mesh tube

Rearing under restraint may prevent compensational recovery of the escape direction by affecting the motor system, e.g., it may cause degeneration of leg muscles. In order to explore this, each intact cricket was reared in a wire-mesh tube (Fig. 4A). Similar to other experiments, the escape behavior of the intact cricket was investigated on the 14th, 15th and 16th days from the start of rearing.

The response rate measured in 22 crickets was 60.3% (161 responses / 267 trials; Table 1). It was not significantly different ($P > 0.05$) from the response rates measured in intact crickets reared in a large cage (Kanou *et al.*, 1999; Table 1). Therefore, it was confirmed that the restraint did not affect the response rate.

The regression line and the correlation coefficient obtained from such crickets were $y = 0.89x + 4.3$ and 0.91, respectively (Fig. 4B and Table 1). Both the slope of the regression line and the correlation coefficient were the same as those obtained in intact crickets reared in a large cage (Kanou *et al.*, 1999; Table 1). Therefore, the motor system of the cricket functioned normally even after long-term restraint.

DISCUSSION

We have shown that in unilaterally cercal ablated crick-

ets, compensational recovery of the escape direction requires the rearing condition under which the insect can move freely. That result is identical with that reported in the compensational recovery of the escape direction in unilaterally cercal ablated cockroaches, *Periplaneta americana* (Vardi and Camhi, 1982a). Therefore, neural compensation that underlies the behavioral compensation seems to be based on the same mechanism in both crickets and cockroaches. In crickets, it was proved that long-term restraint in the vial did not affect the motor system, and isolation from ambient air motion had no effect on the compensation of the escape direction. Furthermore, it is possible to assume that other factors such as CO₂ concentration and humidity in the glass vial, which have been suggested by Vardi and Camhi (1982a), have no relation to the compensation because the unilaterally cercal ablated crickets reared in a wire-mesh tube and in a glass vial show no difference in the compensational recovery of the escape behavior (Figs. 2 and 3). Therefore, the only requirement for the crickets to compensate the escape direction is spontaneous walking after the unilateral cercal ablation.

How does free walking affect the related neural system and compensate the escape direction? In order to reform the neural system, the cercal ablated insect has to realise that the wind-direction information processed in its sensory system is incorrect, and also has to recognize accurately the direction of the wind. As has been suggested in cockroaches (Volman and Camhi, 1988), a self-generated wind caused by spontaneous walking (i.e., self-stimulation of the

cercal sensory system) is the most likely candidate for the reformation because the CNS of the insect should know the direction of the body motion and the direction of air motion, which is opposite to the body motion. Therefore, comparison of the actual activity and the expected activity of some CNS neurons during spontaneous walking is one way for the cricket to recognize whether the sensory system mediates correct wind information or not. It has been revealed that GIs in the cricket CNS carry both directional and intensity information of an air-motion (Tobias and Murphey, 1979; Murphey *et al.*, 1977; Kanou, 1991, 1996; Matsuura and Kanou, 1998a) and show compensational functional changes after unilateral cercal ablation (Matsuura and Kanou, 1998b). Furthermore, the activities of the lateral and medial GIs (LGI and MGI) in the cricket, *Acheta domesticus*, are suppressed during walking, and it is assumed to be caused by an efferent control (Murphey and Palka, 1974). On the other hand, other small unidentified cricket GIs are excited during walking (Daley and Delcomyn, 1980a). In the cockroach, *Periplaneta americana*, the GIs are also under efferent control, i.e., the dorsal GIs are excited while the ventral GIs are inhibited during walking (Delcomyn and Daley, 1979; Daley and Delcomyn, 1980a, b). Therefore, GIs are the most likely candidates for such CNS neurons.

For further physiological investigation of the compensation mechanism in the cricket neural system, we made a tentative working hypothesis as follows (Fig. 5). Spontaneous walking is initiated by an Efference (motor output) signal generated from a motor center located somewhere in the

CNS (probably in thoracic ganglia). The motor center also generates a copy signal of the Efference (an Efference copy or a Corollary discharge (CD)). The signal affects each GI in different manners, i.e., some GIs are excited and others are inhibited (Murphey and Palka, 1974; Delcomyn and Daley, 1979; Daley and Delcomyn, 1980a, b). The Efference copy is also sent to a neural circuit that we tentatively call an Operator. In the Operator, the expected response of a particular GI is estimated. The estimation is based on both expected inputs from filiform hairs generated by self-stimulation and the excitatory or inhibitory effects of the Efference copy. Then, the expected response of the particular GI is compared with the actual response (Reafference) of the GI in a neural circuit tentatively called a Comparator. When sensory apparatuses are intact or the sensory information from filiform hairs is correct, the two responses must be the same and no signal for compensation is generated. However, when sensory apparatuses have some damage, for example, one of the cerci is missing, there will be a difference between the responses. In such a case, an Error signal based on the inconsistency between the responses is sent to the particular GI from the Comparator for functional compensation. Such a reformation process must be required to occur repeatedly because it takes almost two weeks for the complete compensation of the escape direction (Kanou *et al.*, 1999). With regard to this hypothesis, we are now analyzing the functional properties of some descending neurons in the connective just anterior to the TAG during spontaneous walking in crickets (Kanou *et al.*,

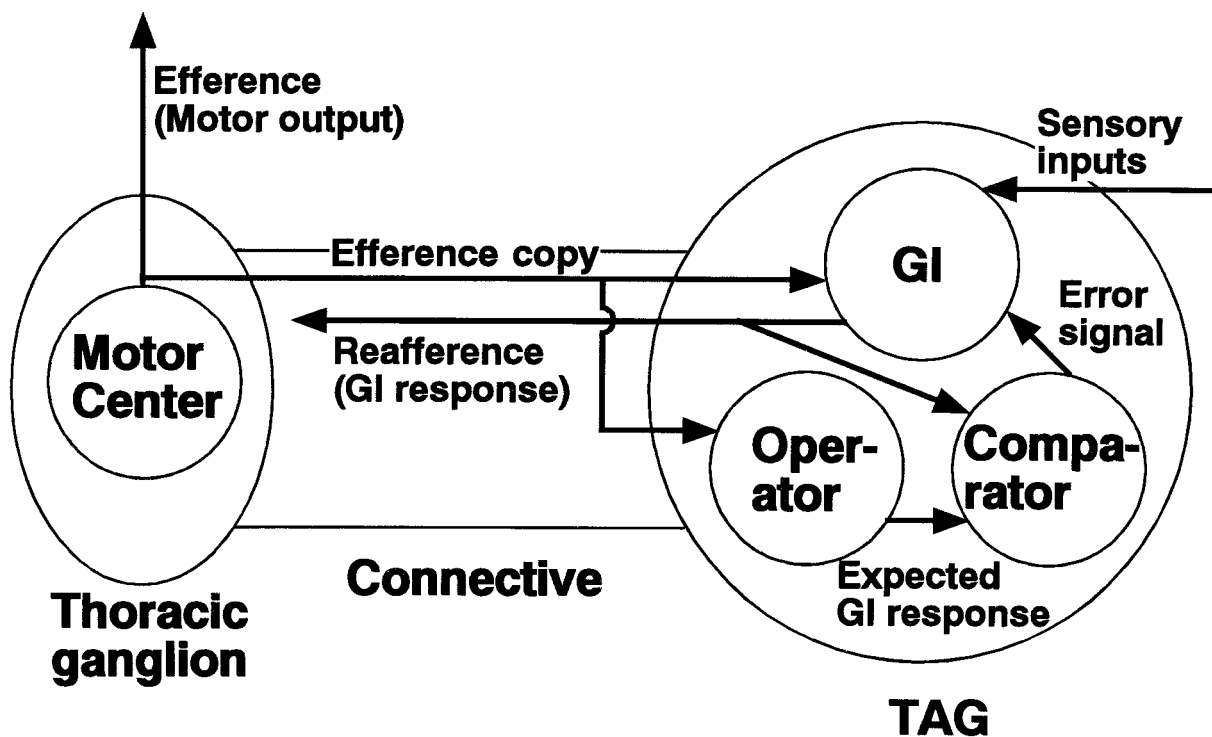


Fig. 5. Schematic drawing of a tentative hypothesis explaining the compensation mechanism of the cricket escape direction. Solid lines and arrows indicate information flow without showing individual neurons. See text for details.

unpublished). Although CD interneurons that are activated during leg motion are identified in thoracic ganglia in cockroaches (Delcomyn, 1983), the CD neuron affecting the GIs has not been identified yet. Functional and morphological identification of such descending neuron(s) will be a great step for further understanding of the compensation mechanism.

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