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Pheromone-Triggered 'Flipflopping' Neural Signals Correlate with Activities of Neck Motor Neurons of a Male Moth, *Bombyx mori*

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ABSTRACT—Male silkworm moths, *Bombyx mori*, exhibited sidewise movements of the head when they showed zigzagging walking in response to pheromonal stimulation. When the moth changed the direction of walking, the head angles also changed to those associated with the walking direction. The sidewise movements of the head were thought to be regulated by neck motor neurons which innervated the first cervical ventral muscles and the ventral muscles through a second cervical nerve. It has been reported that the state transition, resembling the 'flipflop' operation of an electrical circuit, in the spike activity of descending interneurons running in the ventral nerve cord to a thoracic motor system appears to be important in the pheromone-modulated turning of male *B. mori*. We recorded the activity of neck motor neurons in the second cervical nerve and the flipflopping activity in the ventral nerve cord simultaneously with multiple suction electrodes, in order to clarify the physiological functions of such flipflopping signals involved in the behavior. We demonstrated that the activity pattern of some neck motor neurons was correlated with the state transition of a flipflopping activity pattern triggered by the pheromones. The result suggests that the flipflopping activity pattern is correlated with the instruction of the zigzag turning during the pheromone-mediated walking of *B. mori* males.

INTRODUCTION

In adult male silkmoths, *Bombyx mori*, pheromonal stimulation applied to the antennae elicits characteristic activities in brain interneurons whose axon descends via the cervical connectives to the thoracic ganglia (Olberg, 1983; Kanzaki *et al.*, 1994). These descending interneurons (DNs) have two distinct firing frequencies, high and low. Switching back and forth between the two states (i.e., high and low firing frequency states) occurs in response to sequential pulsed pheromonal stimulation (Olberg, 1983; Kanzaki *et al.*, 1994). The activity pattern was called a 'flipflop' one after the basic element of 'memory' in electronic circuit operation.

The flipflopping activity pattern has been classified into two types (i.e., 'FF' and 'ff') by Kanzaki *et al.* (1994) (see *Classification of two types of flipflopping activity patterns* ('*FF*' *and 'ff'*) in RESULTS). Thus, such a state transition, as that occurring in digital processing, takes place in the neural system of the insect brain. Although the flipflopping neural signals are thought to be important in the pheromone-modulated turning of *B. mori* males by the correlations in activities between the zigzag walking and the flipflopping DNs (Olberg, 1983; Kanzaki *et al*, 1994; Kanzaki and Shibuya, 1992), physiological correlates of the flipflopping activity in the motor system involved in the turning behavior are not yet known.

Like many other insect species, such as fly (Land, 1974; Liske, 1977; Sandeman and Markl, 1980; Hengstenberg *et al.*, 1986; Milde *et al.*, 1987; Strausfeld *et al.*, 1987) and locust (Shepherd, 1974; Kien, 1980) species, *B. mori* males exhibit a variety of sophisticated head movements when they turn during zigzag walking in response to pheromonal stimulation. In the study, the head movements of *B. mori* males were recorded by a high-speed video system during the zigzagging walking elicited by the pheromone. Since the behavioral study demonstrated that head movements were elicited in association with changing the directions of the zigzag walking of the moth, activity correlations between the flipflopping DNs in each connective and neck motor neurons, which regulates the head movements, were electrophysiologically studied.

In this report, we demonstrate that the activity pattern of some neck motor neurons in the second cervical nerve is synchronized to the state transition of the flipflopping activity pattern triggered by sequential pheromonal stimulation. The result suggests that the flipflop-like state transition in the descending spike activity is related to the instruction of the zigzag turning during pheromone-mediated walking.

MATERIALS AND METHODS

Insect

Adult silkworm moth, *Bombyx mori* (Lepidoptera: Bombycidae) males were used within 2 to 4 days after eclosion. All the males used in the experiments showed zigzagging walking with fluttering in response to pheromonal stimulation applied to the antennae (Kanzaki *et al.*, 1992).

Behavior

The head movements accompanying the walking of a male on a Styrofoam ball were recorded by a high-speed video camera system (Kodak, Ektapro EM) at 125 frames/sec. The video camera was positioned above the male moth. The moth was tethered to a steel bar on the dorsal part of the prothorax, which did not disturb the wing fluttering (Fig. 1Aa). The ball was not attached to the moth, but the

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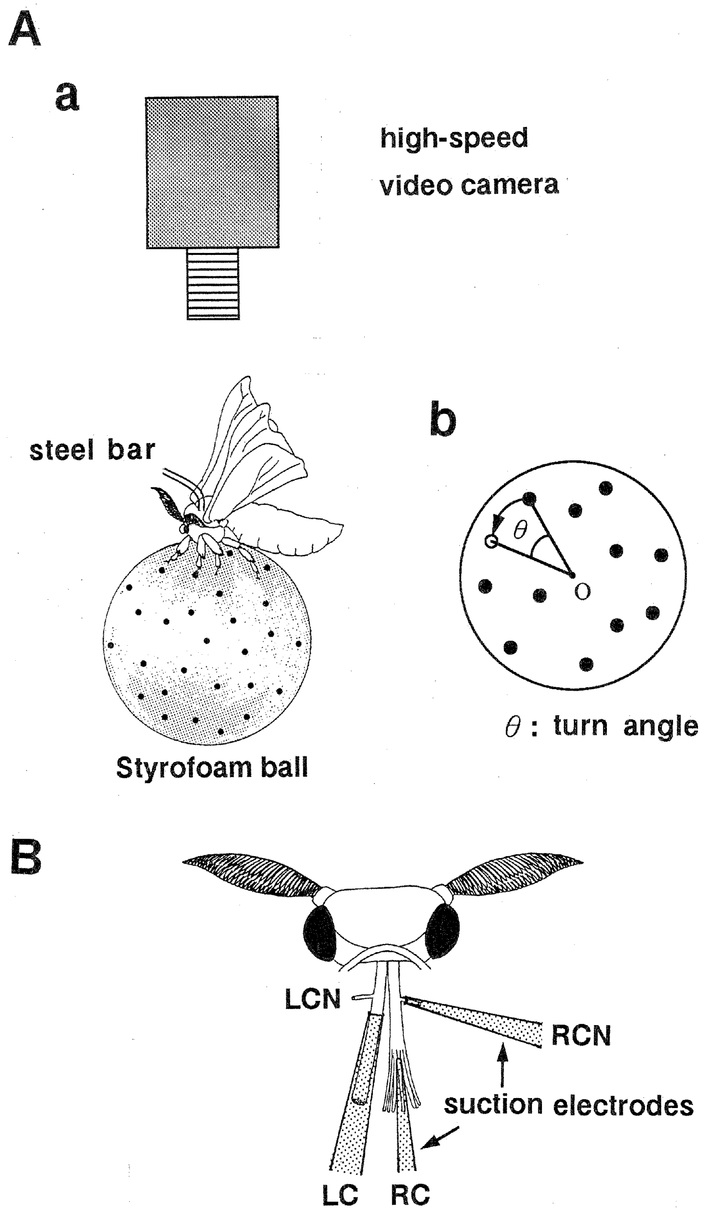

Fig. 1. Schematic diagram of the experimental preparations. (A) Head movements that accompanied the zigzagging walking were recorded by a high-speed video camera (125 frames/sec) positioned above the male. The male *Bombyx mori* tethered to a steel bar was walking on a Styrofoam ball (a). Walking directions of the moth were represented as the changes of the turn angles (θ) of the ball (b). (B) Electrical activity in response to pheromonal stimulation was recorded simultaneously from a whole left connective (LC), a small bundle (diameter: $<10\ \mu$m) of the dorsal part of the right connective (RC), and a whole right second cervical nerve (RCN) containing neck motor neurons, or vice versa, using three glass suction electrodes. LCN: left second cervical nerve.

moth kept the ball suspended by leg contact. The diameter of the ball was 3 cm and the weight (0.45 g) was almost the same as that of the moths. The ball turned in response to a reaction of the male's walking. The moth could actually lift and rotate a Styrofoam ball twice its body weight in a similar way, indicating that the moth had enough strength to lift and turn the ball used smoothly. This indicates that the inertia force of the ball was less than that of the moth.

Analysis of the behavior

The direction of the walking was represented as changes of the turn angles of the ball. The turn angles (θ) were calculated by a computer (NEC, PC-9801VX) frame by frame (every 8 msec) as changes of the x-y coordinates of the dots marked on the ball (Fig. 1Ab). Although absolute movements could not be measured with the methods used, the timing to change the walking direction could be measured precisely. The turn angles were plotted as a histogram by running a sum of each angle every 8 msec. That is, during the right turn the angle increases, whereas it decreases gradually during the left turn.

Since in many cases, even when the head was moving, moths seldom moved the antennae during the walking on the ball, distinctive positions on the antennae, such as a basal segment or a distal part of the antenna, were marked as measuring points for later analysis of the head angles. The head angles were represented as the angle between the body axis and the perpendicular line to the line connecting the measuring points on the antennae (Fig. 3A). The head angles were plotted as a histogram by running a sum of each angle every 8 msec.

Video analysis methods employed in this study were essentially similar to those described by Kanzaki *et al.* (1992). Video (RGB) signals of a personal computer (NEC, PC-9801 VX) and video recordings of the behavior were superimposed on a video monitor through a video-converter board. Consecutive locations of several measuring points on the antennae and on a Styrofoam ball were digitized and acquired by the computer. The positions of the points were digitized as x-y coordinates on the screen (640 pixels × 400 pixels), using the computer mouse accessory and saved on a hard disk.

Physiology

Physiological and morphological methods employed in this study were essentially identical to those described by Kanzaki *et al.* (1994).

After cooling (4°C, approximately 30 min) to achieve anesthesia, the abdomen and all legs of the moth were dissected and the dorsal part of the thorax including dorsal longitudinal muscles (wing depressor) were removed with the wings. The male was mounted ventral-side-up on a wax chamber. The head was immobilized by wax to prevent head movements. Mechanosensory and visual feedback information caused by moving the head was eliminated. Some small bundles of a connective of the ventral nerve cord (VNC) were prepared by surgically splitting the cut end of the connective with a sharpened Elgiloy orthodontic wire (tip diameter: 1–5 μm). As illustrated in Fig. 1B, three glass suction electrodes were simultaneously applied: one electrode was positioned against the descending, severed end of the small bundle (diameter: <10 μm), the second electrode was placed against the other whole connective (diameter: approximately 80 μm), and the third electrode was applied to a cut end of the whole second cervical nerve (CN) which contains neck motor neurons (NMNs). The electrodes were filled with saline solution (in mM: 140 NaCl, 5 KCl, 7 $CaCl_2$, 1 $MgCl_2$, 4 $NaHCO_3$, 5 Trehalose and 5 N-*tris* [hydroxymethyl] methyl-2-aminoethanesulfonic acid, pH 6.8).

The number of impulses above a base line voltage level was measured and processed by a computer and shown as impulse-frequency histograms (0.5 sec bins).

The programs for processing neural signals and for behavioral analysis were written and compiled using Microsoft Quick Basic ver. 4.5 and Zortech C++ ver. 2.0 and run under MS-DOS 5.0.

Olfactory stimulation

An air-puff system similar to that described by Kanzaki *et al.* (1994) was used to deliver odors to the antenna. As olfactory stimuli synthetic (*E,Z*)-10,12-hexadecadien-1-ol (bombykol), the principal pheromone component of *B. mori* (Butenandt *et al.*, 1959), was used in these experiments. The olfactory stimulant was applied to a piece of filter paper (0.5 cm × 1 cm) as 50 μl of solution. Concentrations of odorants are expressed as the concentration on the filter paper in the stimulant cartridge. Two stimulant cartridges were positioned about 1 cm from each antenna. Each stimulation from the cartridge could be aimed selectively at one antenna or the other. From the other end of the cartridge a deodorized air puff (100 msec) was introduced into one of the stimulant cartridges by switching the continuous air stream (1 liter/min or approximately 1 m/sec) with a solenoid-driven valve to deliver the odorant to the antenna. After passing over the preparation, odorants were removed by gentle suction into an exhaust tube positioned nearby.

RESULTS

Classification of two types of flipflopping activity patterns ('FF' and 'ff')

We have reported the flipflopping activity patterns and the classification of these patterns in a previous paper (Kanzaki *et al.*, 1994). These are summarized briefly below.

The flipflopping activity has been defined by Olberg's criteria, that is, a state transition is at least a doubling or a halving of the prestimulus firing frequency which persists for at least 5 sec after the end of the stimulus (Olberg, 1983). Pure air stimulation hardly elicited state transition.

Even when the whole right or left connective was picked up in a suction electrode, some of the large unit activities showed a typical flipflopping activity pattern in response to brief sequential pheromonal stimulation (Fig. 2Aa, Ba). The flipflopping activity patterns that consistently accompanied some of the large unit activities recorded from the whole right connective (RC) and the whole left connective (LC) were antiphasic. In order to clarify the flipflop activity patterns, one suction electrode was applied to the whole right or left connective, and another electrode was applied to a small bundle split from the other connective (e.g., Fig. 1B).

Flipflop activity patterns recorded from a small bundle split from a connective (Fig. 2Ab) showed an antiphasic relationship to some of the large unit activities recorded from the other whole connective (Fig. 2Aa). On the other hand, flipflop activity patterns recorded from a small bundle split from one connective (Fig. 2Bb) exhibit a synchronized relationship to some of the large unit activities recorded from the other whole connective (Fig. 2Ba). We classified the flipflop activity patterns which showed an antiphasic relationship as 'FF' type (Fig. 2Ab) and those which showed a synchronized relationship as 'ff' type (Fig. 2Bb). Therefore, each connective contains both 'FF' and 'ff' DNs (e.g., Fig. 5B). The activity pattern classified as 'FF' recorded simultaneously from a right and a left connective is antiphasic. In some cases pheromonal stimulation failed to elicit the state transition or the state transition occurred spontaneously without pheromonal stimulation (e.g., Fig. 4).

Head movements of a male moth

Bombyx mori males performed a variety of head movements when they showed zigzagging turning in response to the pheromonal stimulation applied to the antennae. The head movements that accompanied the turnings were recorded by video at 125 frames/sec. Males were tethered to a steel bar on the dorsal part of the prothorax while walking on a Styrofoam ball. The most typical head movement that accompanied the zigzagging walking was sidewise. When the male turned to the right, its head showed sidewise movement to its right, and conversely for a left turn. When the moth showed a straight-line walking (a so-called forward 'surge'(Kanzaki *et al.*, 1992)), the head angle was fixed to the body axis (0°, Fig. 3A). Similar results were obtained even when both compound eyes of a male were painted black to prevent visual information (data not shown).

Figure 3 shows an example of correlations between the zigzagging walking and the head movements. In the preparation, the moth changed the walking direction from right to left at 1.5 sec and left to right at 5.6 sec after the onset of recording. When the moth changed the direction of walking,

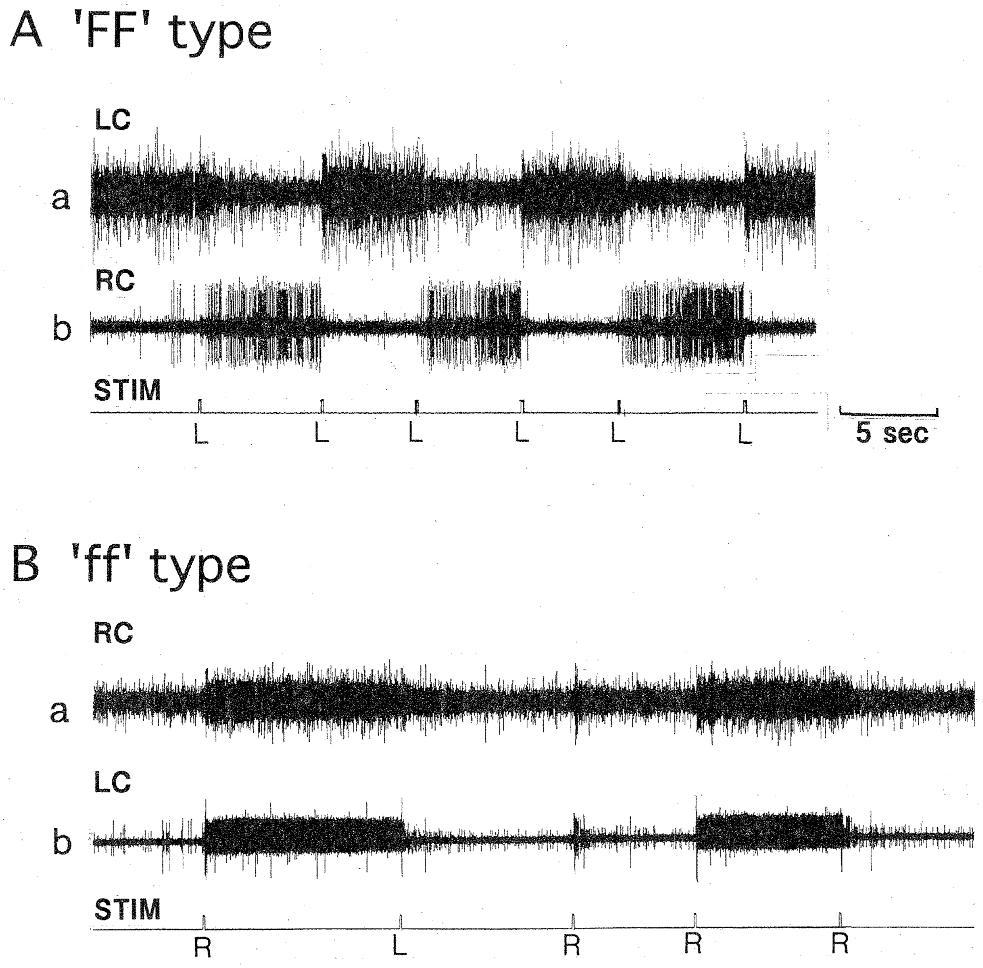

Fig. 2. Classification of two types of flipflopping state-transition patterns ('FF' and 'ff'). (A) The flipflopping activity pattern recorded from a small bundle split from a right connective (RC) had an antiphasic relationship to some of the large unit activities recorded from the whole left connective (LC). The flipflopping activity pattern in (b) is classified as 'FF' type. (B) The flipflopping activity pattern recorded from a small bundle split from a left connective (LC) had a synchronized relationship to some of the largest impulses recorded from the whole right connective (RC). The flipflopping activity pattern in (b) is classified as 'ff' type. STIM indicates the sequential pheromonal stimulation applied to the right (R) or left (L) antenna individually. Concentrations of the pheromones were 10 ng in (A) and 1 ng in (B).

the head angles also changed to those associated with the walking directions. This indicates that the timing to change the turn angle and the head angle is closely related at the behavioral level.

In a few cases, changes of the head movements began maximally about 0.1 sec earlier than those of the walking direction. This is because there is a time lag for the moth to begin to turn the ball by extending the foreleg to the ball.

Responses of neck motor neurons to pheromonal stimulation

This appropriate head movement is regulated by neck muscles and their neck motor neurons (NMNs). The second cervical nerve (CN, Fig. 1B) contains axons of some NMNs (Mishima and Kanzaki, 1995). The CN is described as ON6 in the case of the tobacco hornworm moth, *Manduca sexta* (Eaton, 1971, 1974). The CN is separated from each connective between the suboesophageal ganglion and the prothoracic ganglion (Fig. 1B). The CN innervated neck muscles include the first cervical ventral muscles (cv) and the ventral muscles (v). The cv arose on the dorsal edge of the furca and tapers to its insertion on the anterior arm of the cervicale. The v arose on the medial edge of the furca and inserts on the ventrolateral end of the postocciput. Muscle contraction of the v elicited by electrical stimulation caused a sidewise movement of the head toward the same side of the stimulation, which was similar to that observed during zigzagging walking (data not shown). The cv is also thought to play a role in the sidewise movement because of its basic arrangements. This suggests that excitation of the NMNs contained in the CN elicits the sidewise movement of the head toward the same side of the CN.

The activity of flipflopping state-transition was recorded in 70 preparations in response to sequential pulsed pheromonal stimulation applied to one antenna or the other. In 20 of these preparations pheromonal responses were simultaneously recorded from the whole CN and flipflopping

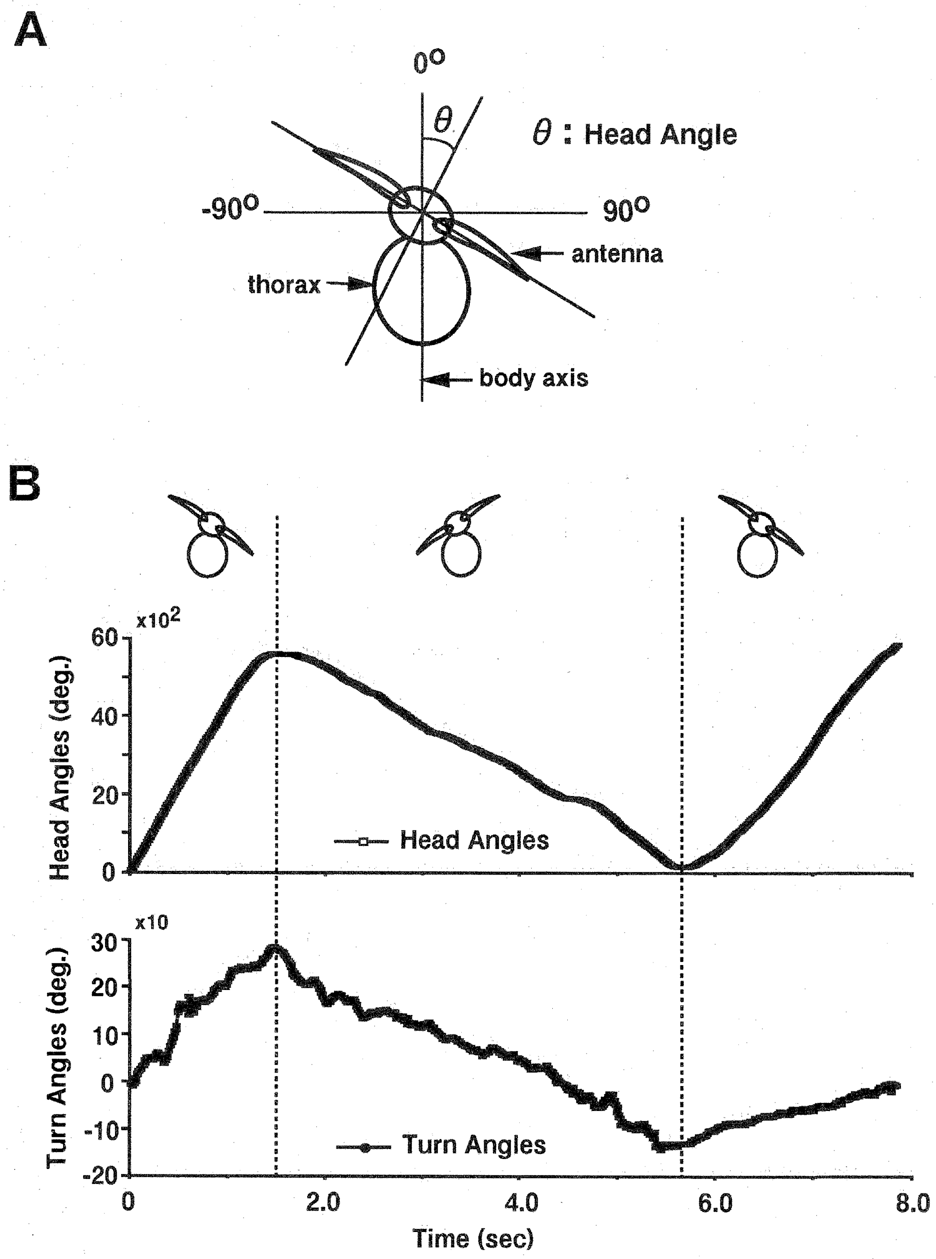

Fig. 3. Correlations between the sidewise movements of the head and the zigzagging walking. (A) Head angles (θ, degrees) were represented as in (A). (B) The plots indicate the correlations between the head angles and the turn angles of a moth. Insets illustrate the schematic head positions of the male moth. The head angles and the turn angles were both plotted as a histogram by running a sum of each angle every 8 msec. During the right turn the turn angle increases, whereas it decreases gradually during the left turn.

DNs in each connective.

Figure 4 shows an example of simultaneous recording from flipflopping DNs in both connectives and NMNs in the right second cervical nerve (RCN). Typical flipflopping responses were recorded from a small bundle of axons split from the dorsal part of the connective when sequential pulsed pheromonal stimulation (100 msec) was applied to the right (R) or left (L) antenna separately (Fig. 4Ac). The diameter of the recording bundle split from the dorsal part of the right connective (RC) was less than 10 μm. In many cases, even when the whole right (RC) or left connective (LC) was picked up in the suction electrode, some of the large unit activities consistently showed a flipflopping activity pattern. In that preparation, the flipflopping activity pattern recorded from the RC had an antiphasic relationship to that of some large unit activities recorded from the LC (Fig. 4Ac, Aa). According to our criteria described above (Fig. 2), the flipflopping activity pattern in Fig. 4Ac is classified as ‘FF’.

Figure 4Ab shows the recording of NMNs in the RCN, which was obtained simultaneously with the flipflopping activities of Fig. 4Aa and Ac. The activity pattern of the NMNs had a synchronized relationship to that of ‘FF’ DNs recorded from the RC (Fig. 4Ab, Ac).

Figure 4B and C illustrate the impulse-frequency histograms (0.5 sec bins) of the activity shown in Fig. 4A. A synchronized state-transition pattern was consistently observed between the RCN and RC (Fig. 4C). Even when the state transition occurred spontaneously, synchronized activity

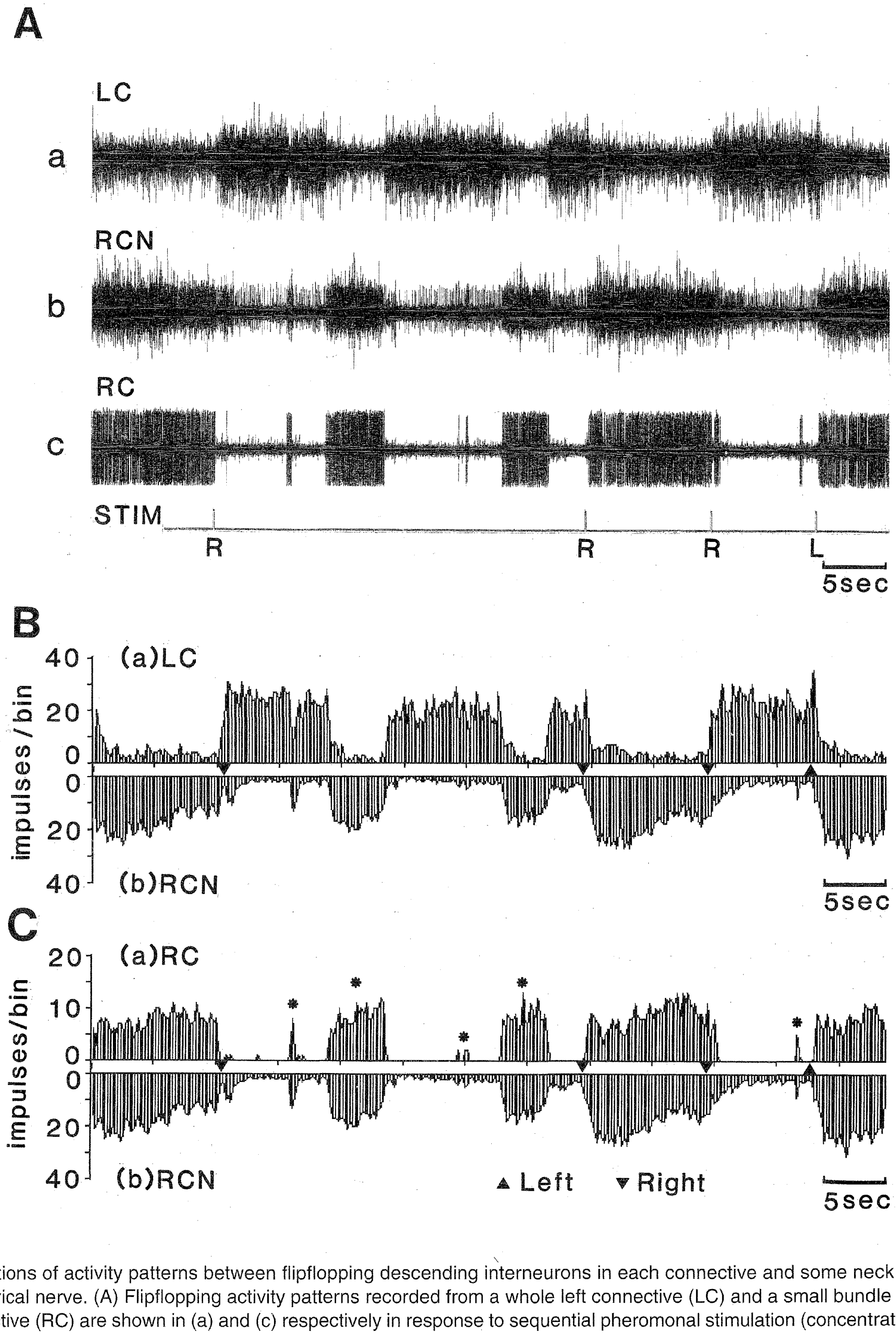

Fig. 4. Correlations of activity patterns between flipflopping descending interneurons in each connective and some neck motor neurons in a second cervical nerve. (A) Flipflopping activity patterns recorded from a whole left connective (LC) and a small bundle of the dorsal part of right connective (RC) are shown in (a) and (c) respectively in response to sequential pheromonal stimulation (concentration: 10 ng, duration: 100 msec). Stimulation (STIM) was individually applied to the right antenna (R) or left antenna (L). Pheromonal responses of neck motor neurons in the right second cervical nerve (RCN) were simultaneously recorded (b). (B, C) Impulse-frequency histograms (0.5 sec bins) of (A) are illustrated. The frequencies are indicated in the y-axis with opposite direction between LC and RCN (B), and between RC and RCN (C). Even when the state transition occurred spontaneously (∗), synchronized activity pattern between the RC and RCN is consistently maintained. Triangles (▲,▼) indicate the stimulation applied to the left (L) or right antenna (R), respectively.

pattern was consistently maintained between the RCN and the RC (∗ in Fig. 4C).

In a few examples, 'FF' and 'ff' responses were simultaneously recorded by a single suction electrode as shown in Fig. 5B. In the preparation, suction electrodes were applied to a small bundle of axons split from the dorsal part of the RC, whole LC, and whole RCN (Fig. 5A). Only the responses recorded from the RC and from the RCN are shown. The activity pattern of the large impulses and that of the small ones exhibit an antiphasic relationship. Since the activity pattern of the large impulses had an antiphasic relationship to that recorded from the LC (data not shown), the pattern of the large impulses is classified as 'FF' and that of the small impulses is classified as 'ff' according to our criteria (Fig. 2). The spike activity of some NMNs was synchronized to that of the flipflopping activity pattern classified as 'FF', but not to that of 'ff'.

In all the preparations (n=20), the flipflopping activity pattern classified as 'FF' and the activity pattern of some NMNs were synchronized when these two activities were recorded from the connective and the CN on the same side.

DISCUSSION

Bombyx mori males perform a variety of sophisticated and synchronized head movements when they show zigzagging turning in response to the pheromonal stimulation. Even though both compound eyes of a male moth were painted black to prevent visual information, the moth showed a similar pattern of head movement in response to the pheromonal stimulation. Thus, in *B. mori* males the head movements are affected by pheromonal stimulation without any visual information. The head movements were recorded by a high-speed video system during the zigzagging walking of a tethered male moth (Fig. 3). As observed in flies (Milde *et al.*, 1987; Strausfeld *et al.*, 1987), when the male *B. mori* turned to the right, its head showed sidewise movement to its right, and vice versa. The timing to change the turning direction was consistently synchronized to that of the sidewise movements of the head (Fig. 3).

The morphological organization of the neck motor system (i.e., neck motor neurons and their target neck muscles) has been well studied in flies (Strausfeld *et al.*, 1987). It has been reported that the sidewise head movement of the flies is regulated by oblique horizontal muscles (OH1, 2) supplied by the CNM2 (neck motor neurons) in the cervical nerve and others (Strausfeld *et al.*, 1987). In *B. mori* males, the second cervical nerve (CN) innervated neck muscles including the cv and v. Since the artificial contraction of the v caused a similar sidewise movement to that observed during the zigzagging walking, some NMNs innervating the v in the CN play the role of regulating the sidewise movement that accompanies zigzagging walking. Basic arrangements of the cv and v of *B. mori* males also suggest a similar function of these neck muscles (Mishima and Kanzaki, 1995).

The flipflopping signal carried by DNs in the VNC, which link the brain and the thoracic motor system, are thought to be important for instructing the pheromone-modulated turning of *B. mori* males (Olberg, 1983; Kanzaki *et al.*, 1994). However, the physiological correlations between the flipflopping activity

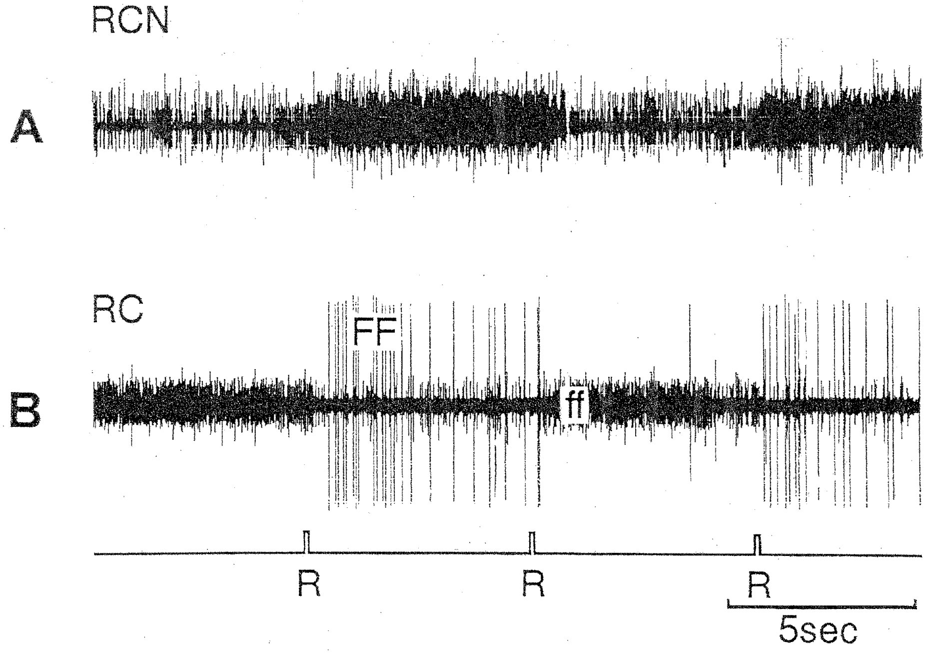

Fig. 5. Activity pattern of some neck motor neurons is synchronized to that of 'FF' type DNs. (A) Pheromonal responses of some neck motor neurons in the right second cervical nerve (RCN). (B) Flipflopping patterns recorded by a single suction electrode applied to a small bundle of the dorsal part of the right connective (RC). According to our criteria (Fig. 2), large impulses and small impulses are classified as 'FF' and 'ff' respectively (see text). Pheromonal concentration was 0.1 ng.

pattern and the motor activity pattern involved in the behavior are not yet known. In the present study, activity correlations between the flipflopping DNs in each connective and NMNs in the CN were electrophysiologically studied since our behavioral study demonstrated that head sidewise movements were elicited in association with changing the directions of the zigzag walking of the moth (Fig. 3).

In flies (Land, 1974; Liske, 1977; Sandeman and Markl, 1980; Hengstenberg *et al.*, 1986; Milde *et al.*, 1987; Strausfeld *et al.*, 1987) and locusts (Shepherd, 1974; Kien, 1980), the head movements observed during standing, walking, or flying behavior, are elicited by visual and mechanosensory stimulation. In our preparation, the head of the *B. mori* male was immobilized to prevent head movements. Visual feedback and mechanosensory information caused by moving the head were eliminated under this condition. The activity of some NMNs in the CN was elicited by olfactory stimulation (Figs. 4, 5). It is obvious that the head movement is elicited by olfaction in *B. mori* males. The activity of the NMNs, moreover, had a correlation with the flipflopping state-transition signals produced in the brain (Figs. 4, 5). All of our physiological results in the present study demonstrate that the flipflopping activity pattern classified as 'FF' shown by DNs and the activity of some NMNs in the CN are consistently synchronized when recorded from the same side of the connective and the CN. This indicates that the state-transition signal descending the VNC to the thoracic motor system is related to the turning during pheromone-mediated walking. In other words, when the DNs which show 'FF' are on the high-firing state, *B. mori* males turn to the the same direction of the DNs. Conversely, when the DNs are on the low-firing state, the males turn to the opposite direction to the DNs. Thus, the flipflopping activity pattern carried by DNs can play a role as a command to instruct the directions of the zigzag turns.

In addition, when the state transition of NMNs was simultaneously recorded from the LCN and RCN, they had an antiphasic activity pattern in response to pheromonal stimulation (Mishima and Kanzaki, unpublished). This is consistent with that of the activity pattern of the 'FF' DNs recorded simultaneously from the LC and the RC, and is antiphasic.

Recent findings using several flying moth species suggest that, in addition to optomotor anemotaxis (i.e., flying straight upwind), male moths employ a self-generated zigzag program to locate the pheromone source (Kennedy, 1983; Willis and Arbas, 1991; Arbas *et al.*, 1993; Mafra-Neto and Cardé, 1994). We have suggested in previous papers that the *B. mori* walking toward the pheromone source is instructed by a self-generated zigzag turning program which is triggered by intermittent pheromonal stimulation to the antennae (Kanzaki *et al.*, 1992; Kanzaki, 1995). A single pulse stimulation (100 msec) with pheromone to either antenna elicits a forward surge, zigzag turns and following looping (turns of more than 360°) (Kanzaki *et al.*, 1992). Similar results are obtained even when both compound eyes of *B. mori* males were painted black to prevent visual information (Kanzaki *et al.*, 1992). *Bombyx mori* males walk straighter upwind to fast-pulsed (>2 Hz) than to slowly-pulsed (<1 Hz) plumes (Kramer, 1975, 1986; Kanzaki *et al.*, 1992). In flying moth species, Mafra-Neto and Cardé (1994) demonstrated that males of almond moths, *Cadra cautella*, fly straighter upwind to fast-pulsed plumes (4.7 Hz) than to slow-pulsed plumes (0.6 Hz). Characteristics of the zigzagging tracks of *B. mori* males are similar to those of the flying moths. This suggests that the pheromone-triggered zigzagging behavior in flying and walking moth species are both instructed by a similar self-generated program relying on the similar neural mechanisms.

In the present study, we demonstrated strong evidence that the pheromone-triggered flipflopping activity pattern carried by DNs in *B. mori* males can play a role as a command for instructing the directions of the zigzag turns. In many cases, spontaneous state-transition of flipflopping activity occurred repetitively just after a single pulsed stimulation of the pheromone. Even when the flipflopping DNs showed spontaneous state transitions, NMNs contained in the CN consistently exhibited a synchronized activity pattern (Figs. 2, 4C∗). It seems that the repetitive state transitions of flipflopping DNs may be the instructions for the self-generated zigzag turns of *B. mori* males.

We have predicted in previous papers, that the particular neuropil area in the protocerebrum of the brain, the lateral accessory lobe (LAL), is important for producing the flipflopping activity pattern (Kanzaki *et al.*, 1994; Kanzaki, 1995). We also morphologically characterized the flipflopping DNs which have dendritic arborizations in the LAL (i.e., group I and group II DNs) and some 'FF' DNs are contained in the group II DNs (Kanzaki *et al.*, 1994; Kanzaki, 1995). In the *M. sexta* males group-I like DNs show a similar flipflopping activity pattern (a so-called 'state-dependent activity pattern') in response to sequential stimulation of their pheromones (Kanzaki *et al.*, 1991, 1992). Group-I and group-II like DNs were morphologically characterized in a related sphingid, the sweet potato hornworm moth, *Agrius convolvuli* (Kanzaki, unpublished data). Similar functional links in the LAL system may exist in *B. mori*, *M. sexta* and *A. convolvuli*. There is a possibility that pheromone-triggered zigzag maneuvers of both walking and flying moth species may be instructed by similar 'flipflopping' activity patterns carried by DNs.

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