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Changing Motor Patterns of the 3rd Axillary Muscle Activities Associated with Longitudinal Control in Freely Flying Hawkmoths

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ABSTRACT—The 3rd axillary muscles (3AXMs) in the mesothorax in hawkmoths are direct flight muscles and pull forewings back along to the body axis. The 3AXMs are regarded as steering muscles because of their changeable activities during turning flight under tethered conditions. We investigated activities of the upper unit of the 3AXMs during free flight with a micro-telemetry device and captured body and wing movements by high-speed cameras. The 3AXM was activated with 1 to 3 spikes per each wingbeat cycle but sometimes ceased to fire. The phase of the onset of the activities was, even though it was variable, close to the phase of the elevator muscle activities. Therefore the upper unit of the 3AXM activities would affect upstroke properties phasically including wing retractions. We focused on longitudinal flight control and identified a correlation between the phase of the 3AXM and body pitch angle, which is important kinematical parameter for longitudinal control in insect flight. The phasic changes of the 3AXM activities would support quick changes in longitudinal control.

Key words: flight muscle, muscle potential, telemetry, hawkmoth, free flight

INTRODUCTION

Flight maneuvers of hawkmoths are achieved by stroke patterns of two pairs of wings and accurate motor patterns of the various flight muscles. Understanding the relationships between these motor patterns and flight maneuvers will tell us the neural basis of insect flight control. Lepidoptera including hawkmoths have synchronous flight muscles whose motor commands correspond to muscle contractions and wing movements (Roeder, 1951). There are two types of flight muscles that move wings: direct muscles and indirect muscles. The direct muscles attach to base of the wings and move them directly, while the indirect muscles attach to the exoskeleton and move wings by distorting the structure of the thorax (Snodgrass, 1935). In Lepidoptera, the dorsal longitudinal muscles (DLMs, indirect wing depressors) and the dorso-ventral muscles (DVMs, indirect wing elevators) occupy large volume of the thorax (Eaton, 1971) for the basic motion of wing flapping while direct muscles are thin and act in various phases associated with visual stimuli and flight behaviors (Kammer, 1971; Kammer and Nachtigall, 1973; Wendler *et al*., 1993). Some direct muscles have identified roles for wing movements such as promotion, retraction, pronation and supination by their attachment to the wing sclerites (Snodgrass, 1935; Kammer, 1967; Rheuben and Kammer, 1987), therefore these direct muscles are regarded as being involved in flight control, especially directional control as steering muscles. The 3rd axillary muscles (3AXMs = pleurodorsal muscle PD2a, b, c; nomenclature after Eaton, 1971) are well-studied direct flight muscles in tobacco hawkmoths, *Manduca sexta* (Kammer, 1971; Rheuben and Kammer, 1987; Wendler *et al*., 1993). The 3AXMs in the mesothorax consist of three units (upper, middle and lower), are attached to the 3rd axillary sclerite and each unit is innervated by a single motor neuron respectively (Rheuben and Kammer, 1987). Since the 3rd axillary sclerite is situated posterior to the base of the wing, contraction of the 3AXMs pulls the wing back along to the body axis (wing retraction). Under the tethered flight of moths, changing phase and bursting of the 3AXM activities and associated asymmetrical wing retraction were observed in relation to the direction of turn that were spontaneous or induced by visual stimulation (Kammer, 1971; Kammer and Nachtigall, 1973; Rheuben and Kammer, 1987; Wendler *et al*., 1993). These changes of activities of the 3AXMs and associated wing retractions are

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also observed in the zigzag walking of non-flying silkmoths (Ariyoshi and Kanzaki, 1996; Kanzaki, 1998). Furthermore, Wendler *et al.* (1993) recorded the middle units of the 3AXMs on both sides in *M. sexta* at rest and reported that the middle units show not only asymmetrical activities to visual stimuli of yaw and roll but also symmetrical activities to thrust and lift stimuli even in non-flying animals. These results indicate that the 3AXMs would receive both symmetrical and asymmetrical motor command for longitudinal and lateral flight control; the activities of the 3AXMs would be a useful index of the motor output that represents directional control even in non-flying moths. Although these previous studies showed properties and roles of the 3AXMs under tethered conditions, actual activities and coordination with other flight muscles during free flight are still unknown. The observed dynamic change of phase and bursting might be strong effects on flight maneuvers, however, these changes might contain some exaggerate response to the stimuli because of the restriction of free movements. We investigated the 3AXM (the upper unit) activities and as references, indirect muscle activities on a single side during free flight of hawkmoths by dual-channel wireless transmission of muscle potentials. We report here that activities of the 3AXM are variable, however, its phase is close to phase of the indirect elevator muscle activities. Phase of the 3AXM activities is correlated with body pitch angle, which is an

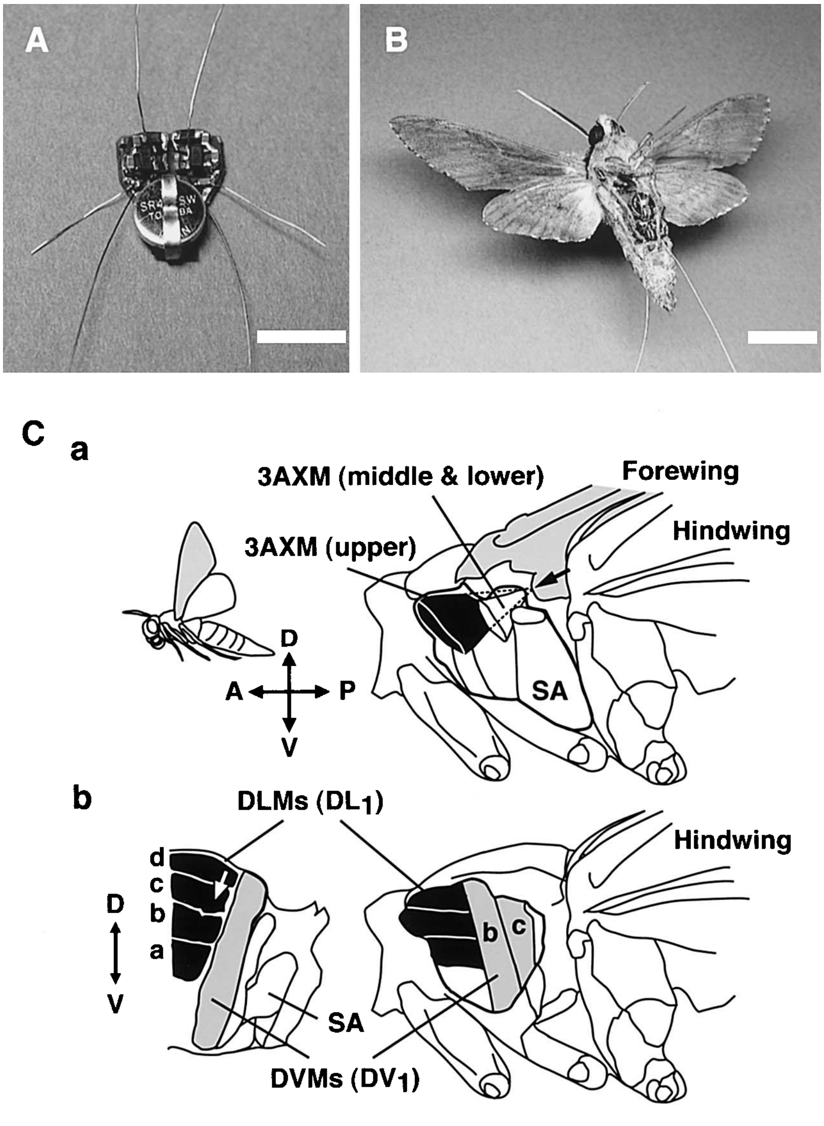

Fig. 1. A transmitter and anatomy of the flight muscles. (A) Appearance of the dual channel FM transmitter. Scale bar, 5 mm. (B) A hawkmoth with the transmitter. Two wires leading backward are 22 cm-long antennas for signal transmission. Scale bar, 10 mm. (C) Structure and recording sites of the flight muscles. (a) Lateral view of muscle structure in the mesothorax. Recordings were made from the upper unit (black) of the 3AXMs. All three units of the 3AXMs are attached to the 3rd axillary sclerite (arrow). SA: subalar muscle (a direct depressor muscle). (b) Frontal (left) and lateral view (right) of muscle structure. The lateral view shows more medial structures than (a). The DLMs (DL$_1$, black) consist of 5 bundles (a–e, bundle-e is not shown) while the DVMs (DV$_1$, gray) consist of three bundles (a–c, bundle-a is not shown). A recording electrode was inserted into the DLMs (DL$_{1c\text{–}d}$), adjacent to the area of the DVMs (DL$_{1b\text{–}c}$), for simultaneous recording of both muscles. The recording site is shown by a white arrow.

important parameter for longitudinal control in insect flight. Although changing activities of the 3AXM during free flight are small compared to previous works in tethered flight of *M. sexta*, the correlation indicates that various flight maneuvers of hawkmoths would be achieved by slight but effective changes of flight muscle activities.

MATERIALS AND METHODS

Radio-telemetric recording of muscle potentials

2–5 day old adult male hawkmoths, *Agrius convolvuli* (Lepidoptera: Sphingidae) were obtained from our laboratory colony. Adult hawkmoths were kept under a 16 hr: 8hr light: dark cycle at 25°C and 60% relative humidity. The experimental procedure of the radio-telemetric recording was described previously (Ando *et al.*, 2002). The dual-channel FM transmitter was attached to the ventral side of the abdomen of a hawkmoth (Fig. 1A, B). The weight of the transmitter (0.25 g) including a silver-oxide battery (0.13 g; SR416, Toshiba, Japan) occupied approximately 25% of weight of the male moth (1.0 g). Recording electrodes (copper or silver wires of 80–100 μm in diameter, insulated except at the tip) were inserted into the upper unit of the 3AXMs (Fig. 1Ca; see nomenclature, Rheuben and Kammer, 1987) through a hole in the cuticle made by an insect pin (since we recorded only from the upper unit of the 3AXM, we described the unit as the 3AXM unless otherwise noted in the following text and fig.). Reference electrodes (silver wire of 100 μm in diameter) of each channel were inserted into the abdominal haemocoel. Recording of indirect muscle activities was made from the DLMs ($DL_{1c\text{–}d}$) and the DVMs (DV_1), nomenclature after Eaton (1971). Simultaneous recording of both the DLMs and the DVMs were made by a single electrode that was inserted into the DLMs, adjacent to the area of the DVMs (Fig. 1Cb, white arrow). Transmitted signals were received by an antenna (SK-11AS, DX Antenna, Japan) and demodulated by two FM tuners (TX-480, Yamaha, Japan). These signals and a trigger cue for camera recording described below were recorded onto a DAT recorder (RD-125T, Teac, Japan).

Flight conditions and high-speed video recording of free flight

The experimental room was darkened and the flight experiments were carried out 1–4 hr after the beginning of the dark period. Room conditions were 24–27°C and 40–50% relative humidity. Loaded moths flew in a glass cage measuring 45 cm (L) ×30 cm (W)×30 cm (H). There was neither wind nor any odor to attract the moth in the cage. Flight behaviors were captured by two Kodak Ektapro high-speed video cameras at 1000 frames s^{-1} and the cage was illuminated by four incandescent white lights (40W × 4) with infrared-transmissible (>800 nm) acrylic sheets. The intensity of illumination on the floor of the cage was 2.0 lx. The video cameras were aligned along their optical axis, facing each other. Images from the two cameras were stored onto VCR (SLV-R5, Sony, Japan) respectively. We used two cues for precise synchronization between the video images and the muscle potentials: one was a trigger signal that indicated the end of the high-speed video recording and it was recorded onto both a DAT and a videotape. Another cue was an infrared LED illuminated by amplified voltage of received muscle potentials and it was also filmed by the high-speed cameras with flight behaviors.

Parameters of muscle activities

Recorded muscle potentials were sampled by a computer by a data acquisition system (Powerlab/8SP, AD Instruments, CO, USA) at sampling rate of 10 kHz. We calculated cycle length, phase of the onset of the muscle activities and burst duration as parameters of muscle activities. Cycle length is the time interval between the first spikes in successive depressor bursts (Fig. 2). Phase of the onset of DVM and the 3AXM activities were calculated as dividing latency (shown as negative value) of the timing of these muscle activities by the cycle length of the DLMs and shown as degrees (Fig. 3Aa).

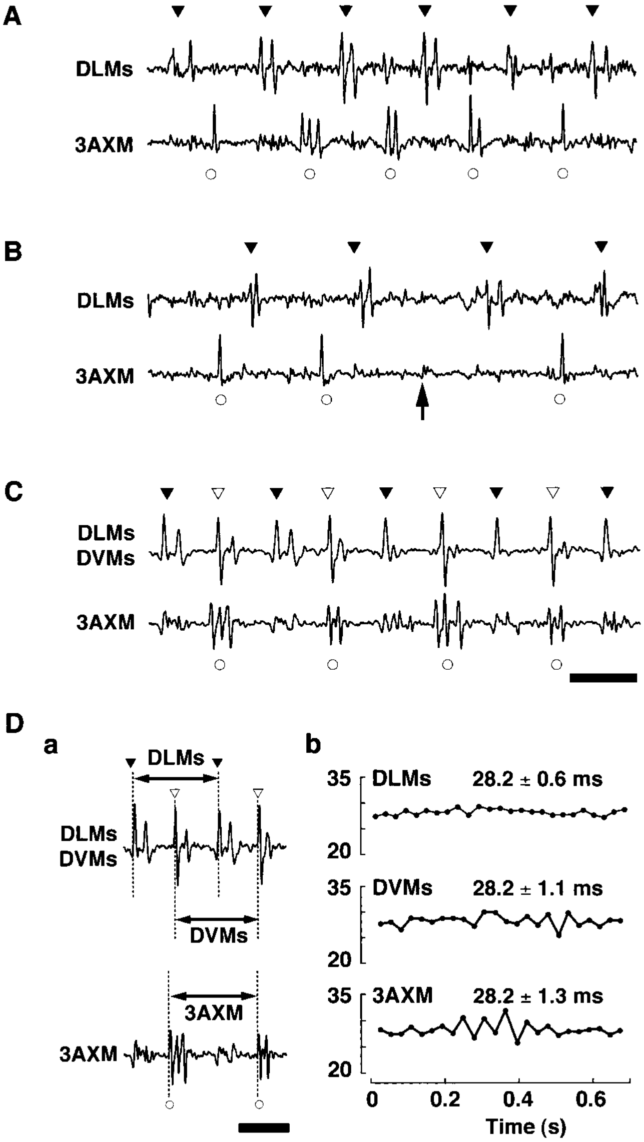

Fig. 2. Recordings from the 3AXM and the indirect muscles (the DLMs and the DVMs) during free flight. (A, B) Simultaneous recordings of the DLMs (solid arrowheads) and the 3AXM (open circles). Recording was made only from the 3AXM and the DLMs. No distinct spike of the 3AXMs was observed at an arrow in (B). (C) Simultaneous recording from the DLMs (solid arrowheads), the DVMs (open arrowheads) and the 3AXM (open circles). Scale bar, 20 ms. (D) Comparison of the cycle length of muscle activities. (a) Definition of the cycle length. Intervals shown as arrows indicate the cycle length of each muscle, see materials and methods. Definitions of symbols are the same as (C). Scale bar, 20 ms. (b) Cycle length of each muscle activities during free flight for 0.7 s. Mean±SD is shown in each graph. Since each muscle was activated once in each wingstroke, mean values were the same; while cycle length of the 3AXM was variable compared to that of the DLMs.

Measurement of body kinematics

Video images recorded on videotapes (NTSC) were converted

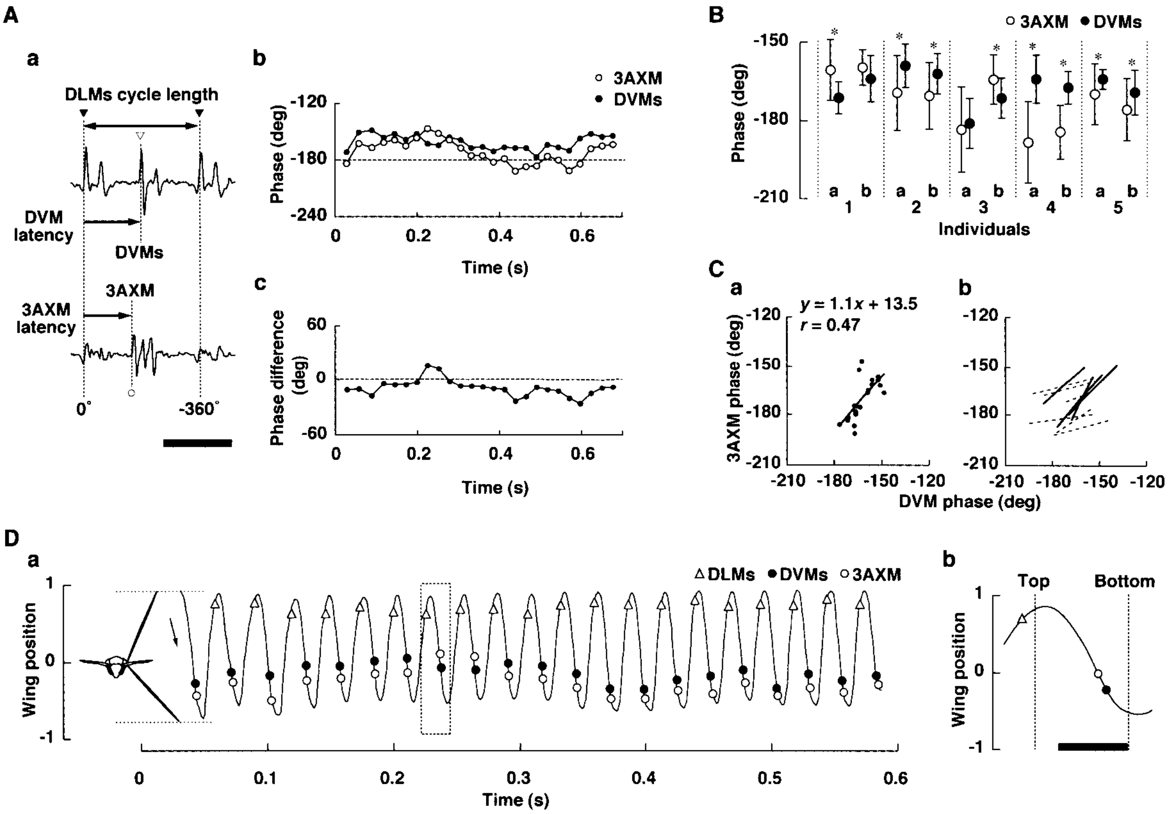

Fig. 3. Comparison of phase of the 3AXM activities to phase of the DVMs and wingstrokes. (A) Phase of the 3AXM and the DVMs relative to the cycle length of the DLMs during free flight. (a) Definition of phase of each muscle. Latencies of the 3AXMs and the DVMs are the intervals between onset of the DLM activities and successive activities of the DVMs and the 3AXM. (b) Changes of the 3AXM phase (open circles) and the DVM phase (solid circles) during free flight for 0.7 s. Phase of 180° (dotted line) indicates a muscle fired at a half of cycle length of the DLMs. (c) Subtraction of the 3AXM phase from the DVM phase. Phase difference of 0° (dotted line) indicates activities of both the 3AXM and the DVM were synchronized. Negative values indicate phase delay of the 3AXM activities compared to the DLMs. (B) Comparison of the mean phase of the 3AXM (open circles) and the DVMs (solid circles) in all 10 trials performed by 5 moths. Identification of individuals (1–5) and trials (a, b) correspond to table 1. Each plot indicates mean value±SD. Asterisks indicate statistically significant differences in mean phase between the 3AXM and the DVMs (a paired *t*-test, $p<0.05$). (C) Relationship between the change of the 3AXM phase and the DVM phase. (a) A scatter and regression line of the DVM phase vs. the 3AXM phase during the same trial as (A). An equation of the regression line fitted by a least square method and correlation coefficient (*r*) was described. (b) Relationships between the 3AXM phase and the DVM phase of all 10 trials. Solid regression lines indicate statistical significance ($p<0.05$) whereas dotted lines are not significant. (D) Wing movements and timing of onset of the DLMs (open triangle), the DVMs (solid circles) and the 3AXM (open circles) during the same flight as (A). Each wing position, normalized by wing length, is a coordinate along the *z* axis (vertical axis relative to the body axis) in the body-axis-fixed coordinate system. An arrow indicates the direction of a downstroke. The area surrounded by a dotted square is the most phase advance of the 3AXM is expanded in (b). (b) Timing of muscle activities and each peak of a wingstroke (top of an upstroke and bottom of a downstroke). Peaks of strokes were defined as the minimum points of velocities of wingstrokes. Scale bar, 10 ms.

into DV signals by a DV converter (ADVC-100, Canopus, Japan) and captured onto a computer via IEEE1394 interface (resolution 720×480 pixels, aspect ratio of images was adjusted). Although these cameras could capture images for 1.363 s at 1000 frames s^{-1}, moths flew out of zone of the camera recordings in several trials. Therefore, we chose trials in which moths were captured by cameras for more than 0.5 s, and analyzed up to 0.7 s, even thought there were trials that lasted over 0.7 s. We analyzed 10 trials performed by 5 moths (two trials of each moth, see table 1). Consecutive location of the measuring points (thorax, abdomen and wing tips on both sides) were digitized 2 to 3 times and averaged for each trial manually by the NIH image software. Coordinates of these measuring points from two cameras were then integrated 3-dimensionally and body and wing kinematical parameters were calculated with Microsoft Excel. Smoothing was performed by linear regressions fitted locally to 3 (position of the wing tips) and 10 (position of the thorax and the abdomen) adjacent data points by a least square method. To make a body-fixed coordinate system (*x*: along to the body axis, *y*: transverse to the body axis, *z*: vertical to the body axis) by each frame, we calculated pitch, roll and yaw angles of the body. Since roll angle was not calculated from our measuring points, we estimated this from the inclination of the wing angle at the top of upstrokes between both sides. For comparison of the body kinematics to the muscle activities and wingstrokes, kinematical parameters during each stroke cycle (upstroke and down stroke) were averaged respectively.

Statistics

We used a paired *t*-test for comparison between mean values of parameters of muscle activities in each trial at a significance level of $p<0.05$. Correlations between parameters were calculated by a linear regression by a least square method at a significance level of $p<0.05$.

Table 1. Cycle length and phase of each muscle activity in 10 trials from 5 moths. Identification of moths (1–5), identification of trials (a, b), number of wingstrokes during each trials (strokes), mean±SD of cycle length and phase angle are described. Asterisks in phase indicate significant differences in phase between the 3AXM and DVMs (a paired *t*-test, $p<0.05$).

Moth ID	Trial (strokes)	Cycle length (ms)			Phase (deg)	
		3AXM	DLMs	DVMs	3AXM	DVMs
1	a (18)	27.4±1.2	27.4±0.4	27.3±0.5	–160.7±11.6*	–171.3±6.1*
	b (15)	27.2±1.0	27.2±0.5	27.2±0.9	–159.8±6.8	–164.1±8.9
2	a (24)	27.5±2.1	27.5±1.7	27.5±1.7	–169.4±14.3*	–159.1±8.2*
	b (21)	27.1±1.2	27.1±1.3	27.0±1.1	–170.6±12.8*	–162.2±7.7*
3	a (24)	26.9±2.7	26.8±2.4	26.9±2.3	–183.4±16.3	–181.0±9.6
	b (24)	28.2±1.3	28.2±0.6	28.2±1.1	–164.3±9.4*	–171.4±7.7*
4	a (23)	27.9±1.6	28.0±1.2	28.0±1.1	–188.3±15.6*	–164.1±9.2*
	b (23)	27.8±1.1	27.2±0.6	27.8±0.8	–184.3±10.3*	–167.4±6.2*
5	a (25)	26.6±1.2	26.6±1.1	26.6±1.0	–167.6±10.7*	–163.5±4.2*
	b (15)	26.6±1.1	26.3±0.9	26.2±1.0	–175.8±11.9*	–169.2±8.5*

RESULTS

General features of the 3AXM activities

First we compared the activities of the 3AXM relative to the DLMs and the DVMs. The 3AXM was activated with 1 to 4 spikes per each wingbeat cycle and the activities were alternately compared to the DLM activities (Fig. 2A). The 3AXM sometimes did not show distinct spikes during free flight, which were observed when the cycle length was extremely longer (Fig. 2B; Cycle length at this point of the DLMs was 32.3 ms whereas mean value of this moth was 27.9±3.5 ms). The silence of the 3AXM activities was also observed in tethered flight of *M. sexta* (Rheuben and Kammer, 1987). Simultaneous recording with the DLMs and the DVMs indicated that the 3AXMs were activated close to the phase of the DVMs (Fig. 2C). To know differences in modulation of timing of muscle activities, we compared cycle lengths of these three muscles. In Fig. 2Db (data from moth (3) – trial (b) in table 1), cycle length of the DLMs was relatively stable (28.2±0.6 ms) whereas activities of both the 3AXM and the DVMs varied occasionally, which are apparent in their higher standard deviations (3AXM, 1.3 ms; DVMs, 1.1 ms) and these trends were observed in most of trials (table 1). These differences in variation of cycle length indicate that timing of the 3AXM would be well modulated by each stroke compared to the DLMs.

Phase relationship

Second, we compared activities of the 3AXM and the DVM that showed similar activities in the previous section. We calculated phase of the 3AXM and the DVM activities relative to the cycle length of the DLMs (the 3AXM phase and the DVM phase, Fig. 3Aa). Fig. 3Ab shows phase of both two muscles during one of the flights (onset of a flight of moth (2) – trial (b) in table 1). Phase of the 3AXM was slightly later but close to the DVM phase. Subtraction of the 3AXM phase from the DVM phase indicated that the 3AXM was not always delayed from the DVMs but occasionally activated in advance (Fig. 3Ac, positive value). Comparisons between the mean phase value of both the 3AXM and the DVMs in all 10 trials (Fig. 3B, table 1) indicated that there were significant differences between both phases in 7 of 10 trials. 5 of 7 trials showed a delay of the mean value of the 3AXM phase, while 2 trials showed a phase advance. These results indicate that the mean phase of the 3AXM would be slightly later than the DVM phase, however considering the larger variation of the 3AXM phase (see standard deviation of the 3AXM phase in Fig. 3B and table 1), timing of the 3AXM activities were not restricted by prior activities of the DVMs. However, there was a positive correlation between the 3AXM phase and the DVM phase (Fig. 3Ca) and this correlation was observed in 4 of 10 trials (Fig. 3Cb). This relationship indicates that change of the 3AXM phase followed the DVM phase, however, the fact that 6 of 10 trials had no correlation might also reflect independent modulation of the 3AXM phase. This point will be discussed in the next section.

Fig. 3Da shows the timing of onset of each muscle's activities during stroke cycle. There was variability of wing positions at activities of the 3AXM and the DVMs, whereas all first spikes within 3AXM and the DVM activities were observed before the peak of upstroke (mean latencies: 3.9±1.0 ms, the 3AXM; 4.5 ms±1.0 ms, the DVMs; see Fig. 3Db) and the DLM activities were before downstroke (mean latency: 3.3±0.6 ms). These relationships indicate that the 3AXM activities would affect some properties of wing and body kinematics during upstroke.

Contribution to flight control

Finally, for understanding the functional role of the 3AXM activities during free flight maneuvers, we calculated parameters that reflect longitudinal control such as pitch, vertical and forward movements during the upstroke period. Since our recordings were restricted to muscles on one side, we focused analysis of flight maneuvers on longitudinal control. In this study, moths flew in the cage with lower flight speed (the maximal instantaneous flight speed per stroke cycle was less than 0.5 m s^{-1}) and moths flew forward and backward in a zone of camera recording. In such flight maneuvers, body pitch angle (Fig. 4A) was well controlled for longitudinal directional control and it had negative correlations with the 3AXM phase in 6 of 10 trials (Fig. 4B). These correlations were also observed with the phase differences between the DVMs and the 3AXMs in 6 trials (Fig. 4C). While only two trials showed positive or negative correlations with the DVM phase (Fig. 4D). These results indicate that phase delay of the 3AXM activity relative to the DLM cycle and the DVM phase were associated with increasing body pitch angle and phase advance of the 3AXM occurred when the body pitch angle was lower.

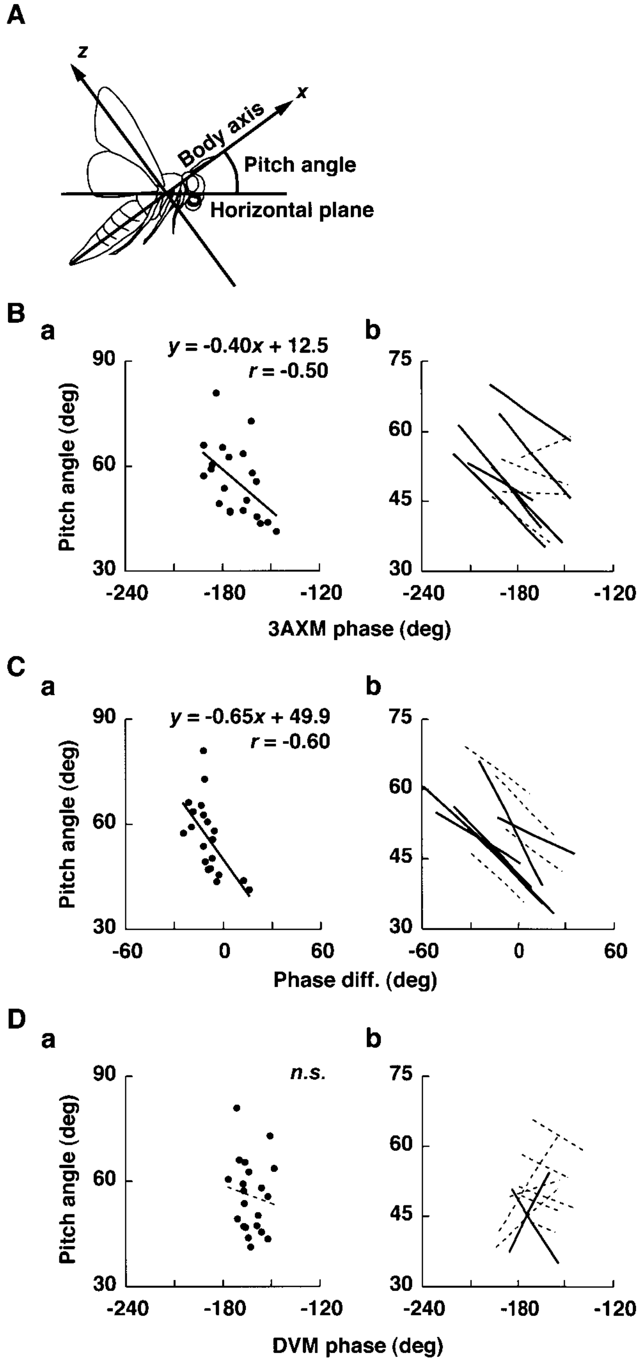

Fig. 4. Association of the 3AXM phase with body pitch angle. (A) Definition of body pitch angle. Pitch angle was defined as the angle between the body axis (x) and the horizontal plane. (B) Relationship between the 3AXM phase and pitch angle. (a) Scatter of the 3AXM vs. pitch angle during the same flight as Fig. 3. Pitch angle was averaged during each upstroke. An equation of the regression line fitted by a least square method is described. (b) Regression lines of the 3AXM phase vs. pitch angle in 10 trials. Solid regression lines indicate statistical significance ($p<0.05$) whereas dotted lines are not significant. These descriptions are also applied in the following graphs. (C) Scatter of the phase differences between the 3AXM and the DVM (the same data as Fig. 3Ab) vs. pitch angle during the flight (a) and regression lines of 10 trials (b). (D) Scatter of the phase of the DVMs vs. pitch angle during the flight (a) and regression lines of 10 trials (b). The dotted regression line in (a) is not statistically significant (*n.s.*, $p>0.5$).

DISCUSSION

3AXM activities during free flight

The upper unit of the 3AXMs in *M. sexta* has intermediate type muscle fibers and is innervated by a fast motor axon (Rheuben and Kammer, 1987). This unit acts as a wing retractor and is activated once or twice during a wingstroke (Kammer, 1971; Rheuben and Kammer, 1987; Wendler *et al.*, 1993). Kammer (1971) recorded from the 3AXM (the upper unit) during tethered flight of *M. sexta*, and reported that phase of the 3AXM activities is usually in phase or slightly before the depressor muscle activities (the DLMs and the subalar muscle) while in turning flight, the phase shifts and is in phase or slightly before the DVM activities. During this transition phase, the 3AXM activities are observed twice during a wingstroke. Therefore, Kammer concluded that the 3AXM retracts the wing during both upstroke and downstroke. Biphasic activities of two spikes during a wingstroke in response to visual stimuli in the 3AXM (upper units) were also recorded by Wendler *et al.* (1993). While our recordings of *A. convolvuli* indicated that activities of the 3AXM during free flight were observed once during a wingstroke (Fig. 2A, C), except for no spiking during extremely longer cycle length (Fig. 2B). The activities consisted of single or a burst of 2–4 spikes (Fig. 2A–C). Pleural activities that could be separated during a wingstroke were not observed since most of bursts of activities last for less than 7 ms (data not shown). The 3AXM phase was variable, however it was close to the DVM phase (Fig. 3A, B) and in advance of the onset of the upstroke (Fig. 3D). If we suppose that the maximum power output of a flight muscle is at 25% of phase of cyclic activities (Stevenson and Josephson, 1990), the maximum power would be generated approximately 7 ms after the onset of the 3AXM activities. Because the 3AXM activities were observed 3.9±1.0 ms before the onset of upstrokes, this latency would correspond to after the onset of an upstroke (Fig. 3Db). Therefore, the 3AXM activities during free flight would affect properties of the upstrokes. Regarding the differences in the 3AXM activities between *A. convolvuli* and *M. sexta*, one possible reason would be their differences in wingbeat frequency. The wingbeat frequency of *A. convolvuli* during free flight was approximately 36–37 Hz (=27−28 ms of cycle length, see table 1) while *M. sexta* during free flight is approximately 25 Hz (Willmott and Ellington, 1997), corresponding to 40 ms of cycle length. Under tethered conditions, wingbeat frequency is reduced as reported in locust flight (Kutsch and Stevenson, 1981). Wingbeat frequency of some recordings is less than 20 Hz (=50 ms of cycle length; Kammer, 1971). Longer cycle length is capable of producing effective maximum power output by pleural motor commands (Stevenson and Josephson, 1990), and longer trains of spikes within each cycle are observed in several moths that have lower wingbeat frequency such as saturniid moths (Kammer, 1967; Kammer and Nachtigall, 1973). The lower wingbeat frequency of *M. sexta* might be capable of producing the 3AXM activities twice during a wingstroke.

Controlling the timing of the 3AXM activities

Cycle length of both the 3AXM and the DVMs were more changeable than cycle length of the DLMs, which would indicate that timing of the 3AXMs and the DVMs were well modulated compared to that of the DLMs (Fig. 2Db). While the 3AXM phase was more variable (Fig. 3B, table1) than the DVM phase and associated with pitch angle (Fig. 4B, C). And the DVM activities seemed to have no constraint on the 3AXM activities (Fig. 3Ac). However, variation of the 3AXM phase was restricted around the DVM phase (Fig. 3Ab) and 4 of 10 trials showed positive correlation between phase of the 3AXM and DVMs (Fig. 3C). These results indicate that the motorneuron innervating the 3AXM would receive at least two types of motor commands: one is an independent command and the other is a relatively common command to the DVMs. The independent command might be close to sensory input via monosynaptic or several interneurons such as the A4I1 interneurons in locusts (Pflüger, 1984; Burrows and Pflüger, 1992). The A4I1 interneurons receive sensory input from wind sensitive hairs on the head and have direct pathway with motorneurons innervating the forewing M85, which is a homologous muscle with the 3AXMs in hawkmoths. The variability of the 3AXM phase might be occurred by such sensory pathways and symmetrical motor commands would modulate body pitch angle. While the common command would activate the 3AXM around the DVM phase. The changeable range of 3AXM phase would be restricted by this command that is associated with flight rhythm (such as motor commands for main depressors and elevator muscles). Because when a hawkmoth lands and the DLMs cease to fire, the 3AXM is more activated with a longer burst for folding the wing that is not observed during flight (Ando *et al.*, 2002). These common commands might be associated with the CPG or some sensory information that affect timing of wing elevation such as the tegula in locusts (Wolf and Pearson, 1988).

Contribution of the 3AXM activities for flight control

We focused on longitudinal control since we recorded the 3AXM activities relative to the DLMs and the DVMs on the ipsilateral side. The 3AXM phase had a correlation with body pitch angle (Fig. 4B): phase delay of the 3AXM was associated with increasing pitch angle. Therefore, the 3AXM phase would modulate appropriate upstroke movement of the wing for keeping the required body pitch angle though we could not clarify whether the 3AXM phase associated with generating pitch torque for active control. In contrast to the DVM phase that had few correlations with pitch angle (Fig. 4D), a motor command to the 3AXM for pitch control would be the independent motor command discussed in the previous section.

The phase difference between the 3AXM and the DVMs correlated with pitch angle (Fig. 4C), as did the 3AXM phase (Fig. 4B). We could not clarify which parameters, the 3AXM phase or the phase difference between the 3AXM and the DVMs, should be treated as an index of pitch angle. However, if we suppose that the 3AXM activities modulate some properties of upstrokes, the phase difference between the 3AXM and the DVM would be more important since interactions between wing retraction and wing elevation are thought to modulate wingstroke kinematics such as stroke plane. The pitch angle is an important kinematical parameter in insect flight since the stroke plane that determines force direction follows the pitch angle of the body, like the flight of helicopters (for a review, see Taylor, 2001). A similar correlation with muscle activities and flight direction was reported in locust free flight experiments with telemetry (Fischer and Kutsch, 1999). They reported that the phase of the direct elevator muscle activities is delayed in relation to the ascent angle that correlates with the body pitch angle during climbing flights. Therefore, flight muscles activated at just before the onset of the wing elevation would modulate upstroke properties and are important for the pitch angle. We could not identify wing kinematics associated with wing retraction and stroke plane, however, Willmott and Ellington (1997) reported in their free flight experiments of *M. sexta* that the body pitch angle decreased and stroke plane was retracted along the body axis in response to an increase of the wind speed (0−5.0 m s^{-1}). These changes of both pitch angle and wing retraction would suggest a contribution of the 3AXM activities to these kinematical changes. We have not identified whether asymmetrical activities of the 3AXM on both sides are associated with steering maneuvers as reported before in tethered experiments (Kammer, 1971; Wendler *et al.*, 1993). However, the correlation of the 3AXM phase with the pitch angle indicates that the 3AXM on both sides would also receive symmetrical motor command for longitudinal control as the middle unit of the 3AXMs in *M. sexta* at rest (Wendler *et al*., 1993).

Future direction of free flight experiments with telemetry

Variation of the 3AXM activities during free flight was less than the results under tethered conditions previously reported (Kammer, 1971; Rheuben and Kammer, 1987; Wendler *et al*., 1993). The flight maneuvers would be controlled by a slight change of the motor patterns and wing kinematics. We think that both tethered and free flight experiments would tell us the variability of the motor pattern. The tethered flight experiments are preferable to speculate on the internal mechanisms from sensory input and motor output that are identifiable. Free flight experiments seem to support tethered experimental findings, and they may also give us further information as to integration of sensory-motor pathways if we can clearly identify the motor output including body and wing kinematics. Presentation of identifiable sensory information such as visual, wind and attractants during free flight is also important for identification of sensory-motor pathways. Such stimuli could induce fairly simple flight maneuvers, which will be necessary for further progress in free flight experiments and also preferable for comparison among different experimental conditions.

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