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Electromagnetic Levitation Platform for Wireless Study of Insect Flight Neurophysiology

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Abstract— **An electromagnetic levitation platform for use in a light emitting diode (LED) arena based virtual reality environment was developed for wireless recording of neural and neuromuscular signals from the flight related muscle groups in *Manduca sexta.* The platform incorporates the use of Early Metamorphosis Insertion Technology to implant recording electrodes into the flight muscles of late stage pupal moths. Analysis of the insects' response to changes in the LED arena rotation direction indicate that this setup could be used to perform a variety of flight behavior studies during yaw maneuvers.**

I. Introduction

Many methods for studying how neural systems function during natural insect flight involve the insertion of electrodes with attached electronic systems into the insects [1]. These systems have been faced with the challenge of avoiding disturbance of the insect's natural flight mechanisms as implanted electrodes and attached telemetry systems can shift due to body movement and also be rejected physiologically by the insect. The procedure of Early Metamorphosis Insertion Technology (EMIT) reduces the difficulty in addressing these challenges by implanting electrodes and recording systems during the rebuilding of the tissue system in holometamorphic insects [2-11].

The EMIT technique for establishing compatible recording setups on the insects can be combined with a controlled visual environment to provide a more robust setup for studying the neurophysiological behavior of insects during flight. Understanding the coordination between muscle groups would also allow for enhanced stimulation strategies for more accurate control of insect biobots enabled by EMIT [2-11].

Presenting a controlled visual environment to tethered insects has been the most common tool for studying the neurophysiology of complex locomotory behaviors. A common method for establishing virtual-reality environments that induce insect flight maneuvers have been to use cylindrical arenas composed of dynamic light emitting diodes (LED) [12,13]. Tethering of smaller insects, such as Drosophila, in such platforms have been achieved by attaching a metal pin to the dorsal thorax of the insect and placing the pin under a permanent magnet [14,15] where the magnetic forces are enough to balance the weight of the insect. Application of this method for larger insect models like *Manduca sexta* has been impractical due to its heavier weight (3-4g). We have addressed these shortcomings of earlier setups in a novel way by designing an electromagnetically levitated tethering frame that wirelessly records electromyograms from *Manduca sexta* during yaw maneuvers. It is worth noting that *Manduca sexta* is one of the important model animals serving as a model system for hormonal control of development and metamorphosis, and neurophysiology of flight dynamics [3,16,17,18].

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II. Materials and Methods

A. Levitation Platform and LED Arena

In order to provide a setup that could wirelessly record electrophysiological signals from an insect unhindered by tethering wires, a levitation platform was constructed using electromagnets to balance a tethering mechanism (Fig.1). An array of electromagnets are positioned in the base platform in such a way as to provide a magnetic force that could support the levitation of an external magnet placed in the center. This external magnet was fastened to the base

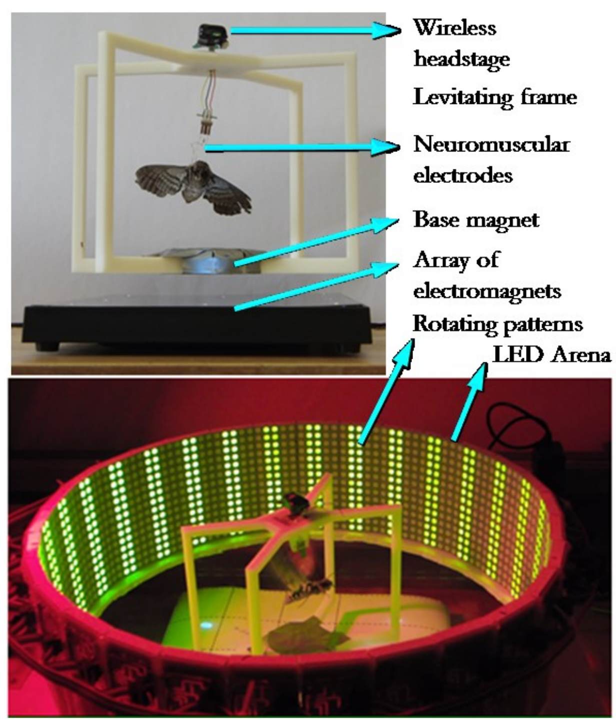

Figure 1. (Top) Tethering frame with wireless headstage levitated by electromagnets in a base platform. (Bottom) The levitation platform in the LED arena with rotating vertical LED patterns [19].

of an acrylonitrile butadiene styrene (ABS) rapid-prototyped frame constructed by a fused-deposition modeling machine. The frame base covered an area of 14.5×9.0 cm^2 with a height of 12 cm. The weight of the frame was 195 g. The tethering wires attached to the top of the frame supported the weight of a *Manduca sexta* (3-4g). This set up restricts the movement of the attached insect to yaw maneuvers only. The levitation frame was suspended within an LED arena to stimulate yaw maneuvers on the insect. The arena was constructed of 60 panels each composed of an array of 5×7 individual LEDs. The LED pattern consisted of segmented vertical line pairs that could rotate either clockwise (cw) or counterclockwise (ccw). A Microchip (Chandler, AZ) PIC18F4520 microcontroller was programmed to control both the rotational direction and speed of the LED pattern. Manduca sexta, like Drosphila, orient their turning direction to match the rotational direction of a revolving static LED pattern [12]. The overall diameter of the circular array of LED panels was 15 cm. For inducing yaw maneuvers in this study, the rotational speed was set to 7.3 degrees per second.

B. *Wireless Recording of EMG Signals*

The tethering wires were connected to a Triangle BioSystems International ™ (Durham, NC) (TBSI) W5 FI wireless head stage transmitter unit positioned on top of the levitation frame (Fig.1) [20]. The transmission range of this head stage unit was 4 m. The head stage was powered by an integrated re-chargeable battery. The signals recorded from the insect passed to this head stage unit which sent the signal to a W-series data acquisition (DAQ) base station. This base station was connected to a computer that had TBSI's Neuroware software. The Neuroware software was used to acquire single-channel electromyogram (EMG) recordings from the dorsoventral muscle group; the muscle group responsible for wing upstroke. The EMG captured signal by the Neuroware software was processed in Mathematica (Champaign, IL).

C. *Preparation of Electrode Board*

A 0.5×1.0 cm^2 printed circuit board (PCB) was used to connect the three electrodes inserted in the insect to the connector for the wireless head stage unit (Fig.2). Two of these were 3 cm long active electrodes made of coated and annealed stainless steel wire (0.28 cm coated, 0.02 cm bare). The third was the ground electrode (0.7 cm stainless steel) connected to the PCB through a 4 cm long litz wire.

D. *Electrode Insertion to Manduca sexta Pupae*

The active electrode insertions were performed following EMIT while the insects were in the late pupal stage [3]; 4 to 7 days before emergence (Fig.2). The pupae were placed in the refrigerator at 4 °C for 6 hours for cold treatment before electrode insertion. The two active electrodes on the PCB were inserted approximately 5 mm into the mesothorax, targeting the dorsoventral muscle group. After the insects emerged as adult moths, the ground electrode on the PCB was inserted at a 3-4 mm depth into the previously prepared window on the dorsal abdomen (Fig.2).

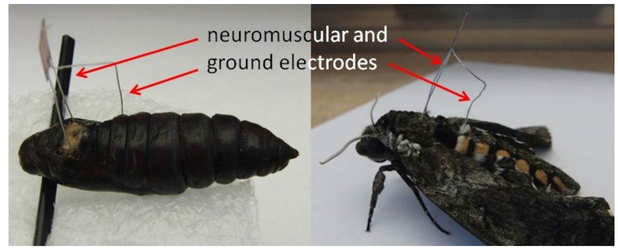

Figure 2. (Top) Late pupal insertion using EMIT insertion technique. (Bottom) Moth with EMIT electrode insertion after emergence.

E. *Prerecording EMG with Oscilloscope*

The electrode PCB needed to be connected to the TBSI wireless head stage unit through a prepared Flat Flexible Cable (FCC) connector. Before head stage connection, EMG data was collected from the head stage adapter PCB using an oscilloscope in order to verify the reliability of the signals obtained from the inserted electrodes. A neural amplifier (AM Systems Model 3500) was connected to the three-mini plug connectors on the head stage adapter PCB. The neural amplifier was set to a gain of 100, a high pass cut-off frequency of 1 Hz, and a low pass cut-off frequency of 20 kHz. The EMG signal from the dorsoventral muscle group was collected during both moth resting (quiescent) phases and active wing flapping.

F. *Wireless EMG Recording in LED Arena*

Once oscilloscope EMG recordings verified a working electrode implant setup, wireless EMG recordings were taken. The goal was to demonstrate that our platform could wirelessly record EMG signals from indirect flight muscles as the moth responding to LED patterns inducing yaw maneuvers.

As the insect was suspended in the LED arena, the lights were turned on and the rotational speed of the LED pattern was set to 9.0 degrees per second. The moths were observed as they responded and turned in the direction that matched the rotational direction of the LED pattern. As these visual stimuli were applied to the insect, EMG signals were recorded with the wireless head stage unit (Fig. 1).

G. *Yaw Maneuver Video Analysis*

Video recordings were captured with a Casio EX-FH25 digital camera from multiple angles at a frame rate of 30 fps to observe how the insects responded to the LED stimuli over time. A typical demonstration can be found in [16].

Still frames were extracted from the videos to analyze the moths' response using Matlab (Natick, MA). Every tenth frame was extracted from the video for analysis. In each frame, the angle of the moth's body with respect to a stationary point on the arena wall was calculated (Fig.3). The moth body angle (θ) was calculated using two points with one point in the center of the thorax and the other in the center of the moth's head. This measurement gave the moth's displacement in degrees from a starting angle of zero over time. The moth's displacement angle was compared to the position of the LED pattern over time with Mathematica.

Figure 3. The moth's displacement angle (θ) was measured over time in response to the angular displacement of the LED pattern (γ) [16].

The displacement angle (γ) of a representative LED vertical pattern was measured in each frame a moth body angle was measured.

III. Results and Discussion

Fig. 4 shows representative data of a trial to compare the moth's flight direction to the rotation of the LED pattern in the arena with a constant angular velocity of 9 degrees per second. Changes in the slope direction indicate moments in time when the rotational direction of the LED pattern reversed. Moths, typically, followed the direction of the LED pattern but only after a brief period of delay. This is due to the rotational inertia that the moth has to overcome to stop the rotation of the frame and change flight directions. This duration was in the order of 5 to 10 seconds and is a function of physical factors such as the diameter of the arena, the rotational velocity of light patterns and the weight of the frame. Overall, this setup allowed the insect to demonstrate a dynamic flight response to changes in the surrounding LED array pattern. The time delay between the change in the optic flow and the insect's actual turn provides a new experimental paradigm to understand the visiomotor system of *Manduca sexta* and model how optic flow drives the flight control muscles.

A representative oscilloscope EMG data set collected from the dorsoventral muscle group before and during wing flapping is given in Fig.5. As can be seen during the quiescent phase, the recorded voltage shows a flat baseline. When the insect begins flapping its wings, the EMG signal appears in spikes that has a frequency of 15 to 20 Hz. The purpose of the oscilloscope measurements were to verify the reliability of the implanted electrodes before the insect was placed in the LED area.

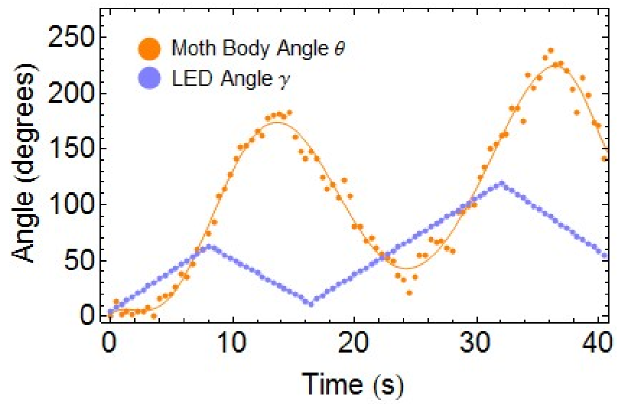

Figure 4: Representative three turns performed by a moth levitated within the LED arena. The LED angle shows the angular displacement of a single vertical pattern on the LED wall over time. The moth body angle was the angle formed by the moth's thorax and head with respect to a fixed point on the LED wall. The moth's displacement angle was fit to a 10th degree polynomial.

Fig.6 shows a wirelessly transmitted EMG recording from the same muscle group during yaw maneuvers in the levitated platform. As with the oscilloscope signal, EMG spikes occurred at 15 to 20 Hz during wing flapping and a flat baseline voltage was observed when the insect was quiescent.

IV. Conclusion

We demonstrated a novel tethering mechanism to study the yaw maneuver in larger flying insects such as *Manduca sexta*. This system was supported by a miniaturized wireless neural recorder for unrestricted measurements of EMG signals from indirect flight muscles. Further studies are in progress to acquire multi-channel EMG recordings and observe muscle coordination patterns between different muscle groups responsible for insect flight. These studies will be used to understand how moths coordinate their muscles during complex flight maneuvers and similar changes will be induced using implanted electronics to enable more accurate control of insect biobots [2-11].

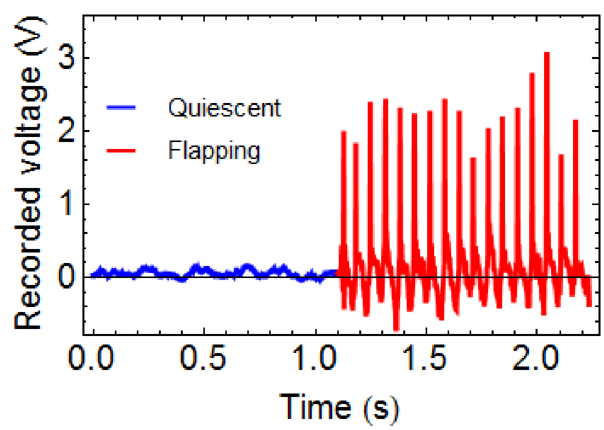

Figure 5. Representative oscilloscope data. The EMG signal caputured by the oscilloscope was first amplified by a factor of 100 and passed through a high pass filter of 1 Hz and a low pass filter of 20 kHz using an external neural amplifier.

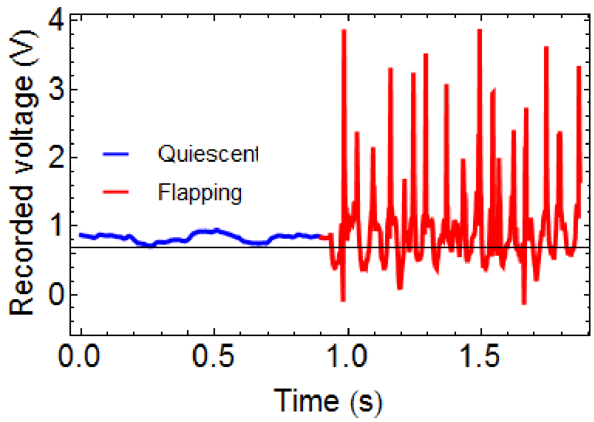

Figure 6. Representative Neuroware data. The EMG signal from the dorsoventral muscle group was transmitted wirelessly from the levitation frame to a computer with TBSI™ Neuroware software.

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