A Miniature Animal-Computer Interface for Use with Free-Flying Moths

Dwight Springthorpe, II

A thesis submitted to the faculty of the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Master of Science in the Department of Biomedical Engineering.

Chapel Hill
2010

Approved By:

Robert Dennis, PhD

Mark Tommerdahl, PhD

Tyson Hedrick, PhD

ABSTRACT

Dwight Springthorpe, II: A Miniature Animal-Computer Interface for Use with Free-Flying Moths
(Under the direction of Robert Dennis, PhD)

Although the neurophysiological basis of insect flight control has been studied extensively and successfully in animals attached to rigid tethers, these conditions disrupt the natural feedback between the subject's intentions, sensory input, and motor output. Understanding how individual control algorithms are integrated at a behavioral level requires acquisition and modification of biopotentials in completely untethered, free-flying animals. Herein, I present and test a miniaturized animal-computer interface for use with freely-flying *Manduca sexta* hawkmoths. This device is capable of simultaneously acquiring two independent biopotential signals, applying electrical neuromuscular stimulation, and correlating collected and applied signals with behavioral data from high-speed videography. Application of this device may offer substantial insight into how insects fly and, by replicating these mechanisms, facilitate wider application of micro air vehicles through improved flight efficiency, stability, and maneuverability.

ACKNOWLEDGEMENTS

Ty: Thank you for the opportunities you have provided and for your mentorship.

Bob: Thank you for your guidance.

Mark: Thank you for your instruction and your perspective.

Iuliu: Your assistance with this project was invaluable.

Thanks to ONR, AFOSR, and NSF for funding.

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LIST OF ABBREVIATIONS AND SYMBOLS

Unit Prefixes

Symbol	*Value*
n	$x10^{-9}$
μ	$x10^{-6}$
m	$x10^{-3}$
c	$x10^{-1}$
k	$x10^{3}$
M	$x10^{6}$

Units

Symbol	*Name*
g	Gram
s	Second
m	Meter
Hz	Hertz
V	Volts
A	Amps
Ah	Amp-Hour
Ω	Ohm
B	Byte (8 bits)
cc	Cubic Centimeter
^{o}C	Degrees Celsius

Abbreviations

AC	Alternating Current
ADC	Analog-to-Digital Converter
DAC	Digital-to-Analog Converter
DC	Direct Current
DLM	Dorsal Longitudinal Muscle
EMG	Electromyogram
I/O	Input/Output
LED	Light-Emitting Diode
M. sexta	*Manduca sexta*
PCB	Printed Circuit Board
RAM	Random-Access Memory
SPI	Serial Peripheral Interface
SRAM	Serial Random Access Memory

Chapter 1

INTRODUCTION

1.1 Motivation

Human-engineered devices tend to exhibit a high degree of specialization. A specific combination of gears will transfer and multiply rotational force in a predictable and repeatable fashion but cannot usually serve another function. This is generally desirable because it allows engineers to easily decompose machines into functional units. If the torque through a gearbox needs to be increased, the gear ratios may be adjusted without fundamentally changing the character of the device. While multi-purpose devices can be engineered or improvised, they are less efficient than an equivalent purpose-built component or device. Human engineering increases efficiency and precision at the cost of versatility.

Animals, however, tend to have multi-purposed components. Muscle, a single functional unit, serves almost every mechanical actuation need from pumping blood to running, climbing, swimming, and flapping. Evolution constantly adapts to changing requirements without changing the fundamental nature of any component between subsequent steps.

This is especially evident in insect flight. Human-engineered aircraft have distinct lift, thrust, and orientation control systems while flying animals have only flapping wings.

Position, orientation, velocity and acceleration are controlled by a single, highly actuated mechanism which demonstrates a remarkable aptitude for both stability and rapid flight maneuvers [1]. Despite remarkable progress [2], robust insect-scale air vehicles continue to elude complete replication.

The control mechanisms and algorithms used to control insect flight are critical to their widespread success. Insects, using information from distributed sensory systems, constantly modify their motor output to maintain stability and navigate through complex environments. Although the neurophysiological basis of these controls has been extensively studied in constrained subjects [3], only recent technological developments allow similar experiments in freely-flying animals where the natural flight control mechanisms, including sensory and motor feedback, are still intact.

An adaptable, miniaturized animal-computer interface may permit verification of models generated from tethered or theoretical experiments and could offer insight into how individual neurosensory and neuromuscular systems are integrated at a behavioral level. Replicating these mechanisms in small-scale air vehicles through bio-inspired design approaches could facilitate wider application through improved flight efficiency, stability and maneuverability.

1.2 Background:

Electrical techniques, first studied over 200 years ago (Galvani, 1786), have long been used to study biological systems. It was first noted by Hitzig and Fritsch (1870), that electrical stimulation to the cerebrum of a dog could elicit involuntary muscular contractions. Subsequent experiments over the next eighty years, including those by Caton (1875), Krause (1911), and Penfield and Rasmussen (1952), determined that electrical signals are used to systematically control muscle activation and conduct sensory information. Current electrophysiological techniques permit extremely detailed studies of bioelectrical systems ranging from single neurons to mapping electrical propagation within whole organs [4-6].

Modern electrophysiological techniques, as applied by Pringle and Roeder [7,8], first contributed to insect flight by enabling identification of the difference between synchronous and asynchronous flight muscles; these findings were critical to understanding how small insects' wingbeat can exceed their maximum neural excitation frequency. Experiments by Kammer identified direction control mechanisms in hawkmoths by correlating asymmetric muscle activation with behavioral changes in rigidly-tethered subjects [9-13].

Although valuable, these experiments highly constrained the subjects due to precision electrical equipment, delicate electrodes, or a need for careful control of applied stimuli. As insects naturally rely on the constant action of an intention-modulated feedback loop between sensory input and mechanical output [14-16], understanding how individual controls are

integrated within the animal and related to the animal's behavior requires a method which restores or emulates free flight while still permitting neurophysiological measurements.

Some have restored aspects of natural motor-sensory integration by using adaptive feedback techniques, such as a display that reacts according to detected inputs from a tethered subject [17, 18]. These have proven to be an excellent mechanism for studying neurosensory and neuromuscular feedback for individual systems. While these experiments restore some aspects of free flight and have produced a number of control models, these experiments represent true free flight only as well as it is understood and modeled. Additionally, tethered subjects can exhibit unnatural behavior or introduce artifacts which may disrupt the investigation [19, 20]. To test experimentally- and theoretically-generated models of insect flight, true free-flight electrophysiological experiments are required.

Though appropriate techniques have existed for over fifty years, only recent advances in miniaturized electronics permit installation of adequate neurophysiological instrumentation on free-flying insects. Fischer and Kutsch first used a small, transistor-based amplifier and radio transmitter to study muscle timing in relation to behavior in free flying locusts [21-26]. Increasingly sophisticated insect-portable electromyogram-acquisition devices have since been applied with promising results [27-30]. By coupling information reported by these biotelemetry systems with biomechanical and behavioral data from high-speed videography, researchers can investigate neuromuscular flight control on both within-wingbeat and longer time scales [31, 32].

Free-flight experiments can be supplemented with neurophysiological stimulation. Relatively simple stimulation methods are capable of inducing complex behavioral changes in insects [33-36]. These techniques, coupled with a device which can monitor neurophysiological activation, could be useful for evoking specific behaviors and measuring activation or response. A combination acquisition-stimulation device could implement motor activation re-writing techniques, similar to those used by Sponberg and Full [37], to modify motor activation patterns in running cockroaches. These experiments could offer insight into flight control algorithms and neural plasticity.

1.3 Thesis Overview

I present my attempt to develop a miniaturized animal computer interface which permits the acquisition of and modification to various biopotentials in a completely untethered, minimally encumbered insect capable of complex flight maneuvers. Chapter 2 details the prototype system's design. I present tests, conducted in contexts similar to intended experiments, in Chapter 3. And in Chapter 4, I propose additional applications of the current system, and possible design improvements.

Chapter 2

SYSTEM DESIGN

2.1 Specifications and Requirements

Free-flight neurophysiological experiments will require a small and lightweight system capable of adapting to a variety of experimental conditions, extracting general biopotential signals, and applying experimentally-relevant electrical stimulation to neural, sensory, and muscular tissues. While appropriate electronic equipment has long existed on a bench-top scale, the physical size and carrying capacity of suitable subjects impose a number of limits on the system. The specific requirements and constraints are as follows:

- Ability to simultaneously amplify and sample at least two independent biopotentials at 1000 samples per second per channel.

- Acquired data must be stored locally or transmitted in real time. If stored locally, the system must be able to accommodate at least 8 seconds of continuous data from both channels at 1000 samples/s.

- Must be able to apply electrical stimulation, initiated by either external command or according to pre-programmed parameters, to the subject with at least two independent channels.

- Capable of at least 15 minutes of stand-alone operation.

- A mechanism which permits correlation of the collected data or applied stimulation with biomechanical data collected from high-speed videography.

- Subject-portable mass.

- Physical size and placement which minimally interferes with the animal's normal range of motion and behavior.

2.2 Experimental Subjects

The first and most critical component of a system to study neurophysiological activation in free-flying insects is the insect itself. A suitable subject should be capable of complex flight maneuvers, carrying the instrumentation without excessively disrupted flight behavior, and being cultivated in a laboratory setting.

In this regard, *Manduca sexta,* or the Carolina Sphinx, is a nearly ideal model for insect flight. Widespread use in many fields of research means that domestic cultivation

techniques and basic physiological information are well established. Their feeding behavior, similar to that of hummingbirds, inclines them to perform a wide variety of aerial maneuvers, including prolonged, stable hovering. Additionally, they are relatively large insects; typical specimens have a body mass of 1.5 to 2.2 g and a wingspan of approximately 10 cm. Their size and dietary habits permit them to carry a significant payload; individuals have been observed consuming several hundred milligrams of fluid while still flying competently.

M. sexta's physical characteristics and capabilities impose the majority of constraints on the system. Overall system mass must be less than 750 mg. Its large wing stroke limits both the location and size of system. For instance, placing the system on the dorsal thoracic surface limits the system width to no more than 10 mm.

2.3 Design Implementation

Figure 2.1 shows a diagram of the system architecture. Biopotentials are amplified and passively filtered by two independent instrumentation amplifier systems. Signals are acquired by an analog-to-digital converter (ADC) onboard the system's microcontroller. The microcontroller controls sampling and stimulation characteristics. It also controls user and peripheral interfaces, including high-speed camera synchronization and the serial RAM used for local data storage. A light emitting diode (LED) and phototransistor enable simple communication and correlation of biopotential data with high-speed camera recordings. Data transfer and system programming is accomplished through a wired serial interface.

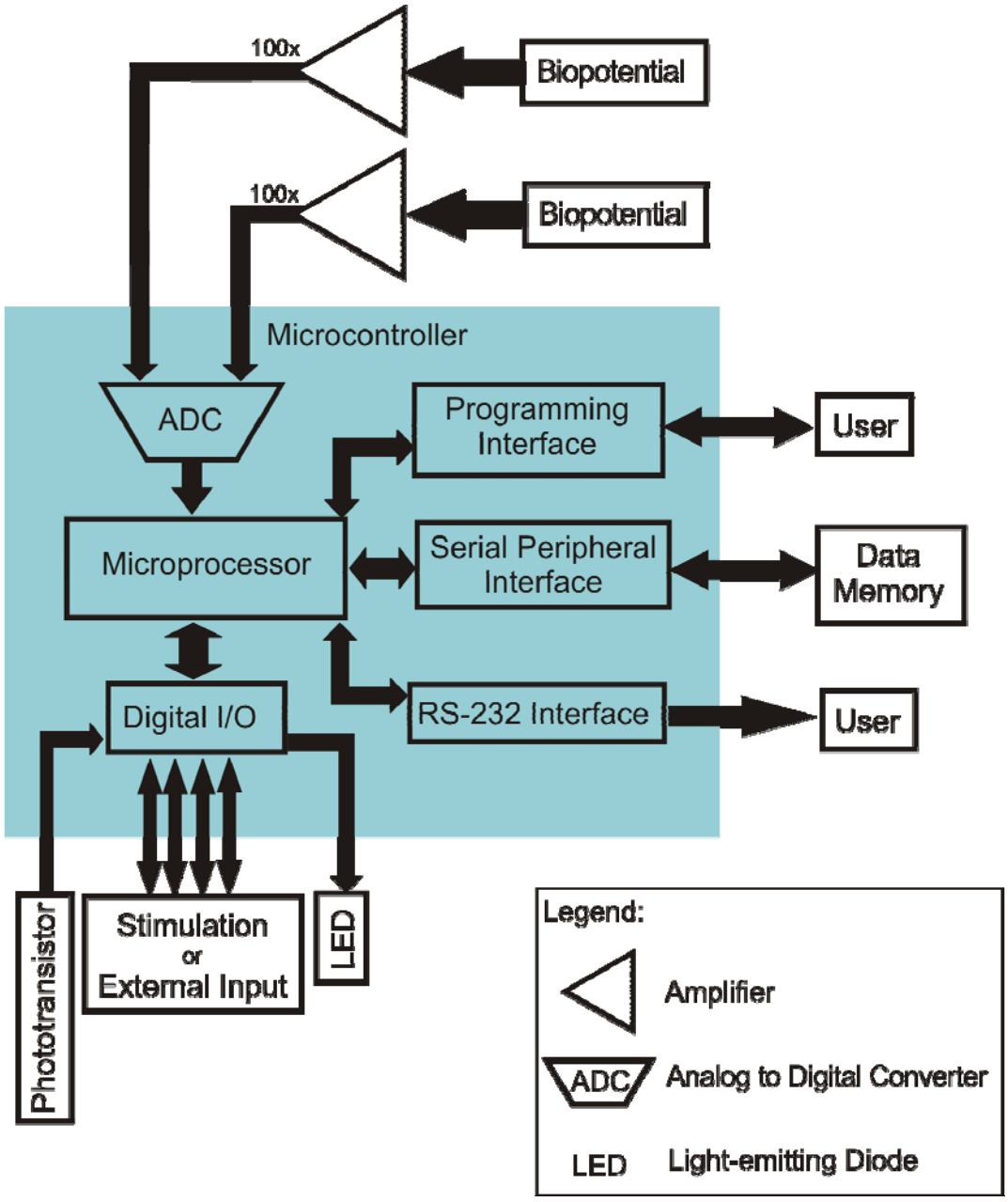


Figure 2.1: Block diagram representing the major architectural features of the moth-portable animal-computer interface. Arrows denote the flow of information. Blocks within the shaded area represent functions integrated into the Microchip PIC16LF1936 microcontroller.

2.3.1 Amplification and Acquisition

Electromyograms (EMGs) from the main flight muscles of *M. sexta*, collected with an intramuscular electrode (impedance on the order of 1 kΩ) have a typical amplitude and bandwidth of 1-10 mV and 100-500 Hz respectively [38]. These characteristics and the limited, single-ended supply voltage available from moth-portable batteries require the amplifier to have an input impedance on the order of at least 100kΩ, and a gain which maximizes single strength while keeping the signal within +/- 1.5 V.

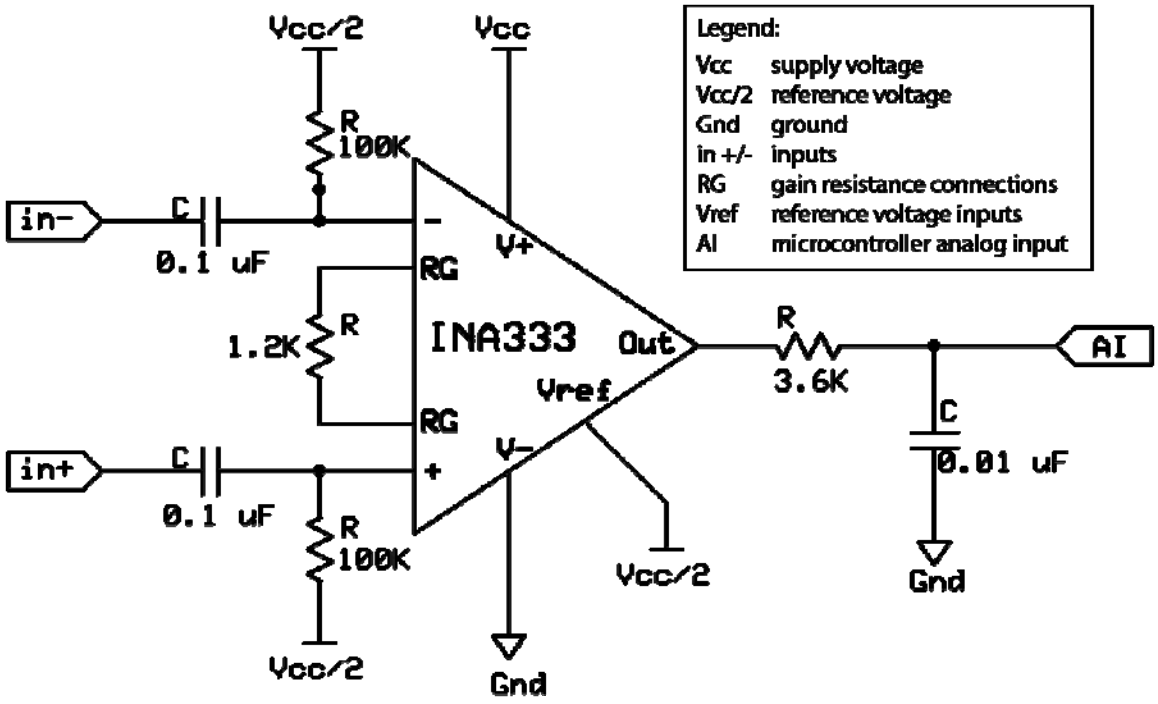


Figure 2.2: Single-channel biopotential amplifier and passive filter. Two identical systems are used for dual-channel biopotential acquisition. AC-coupled inputs are differentially amplified by a precision instrumentation amplifier (TI INA333). The reference voltage, Vcc/2, is generated by a voltage follower shared between both amplifier channels.

Figure 2.2 shows a circuit diagram for one of the two identical amplifier channels included in this design. Input signals are AC-coupled to the input of an INA333 instrumentation amplifier (Texas Instruments: Dallas, TX) configured for a gain of 100 using an external resistor. Gain may be adjusted by replacing this resistance. Single-supply operation is supported by a reference voltage, shared between the two amplification channels, supplied by an OPA330 (Texas Instruments: Dallas, TX) configured to follow either half of the supply voltage or a voltage supplied by the microcontroller's on-board digital-to-analog converter (DAC). The amplified output is passed through a passive filter with a knee frequency of 4 kHz.

The output of the amplifier is supplied directly to the microcontroller's on-board, 14-channel, 10-bit ADC and sampled at a software-selectable rate (typically 1000 samples/second). The ADC can be configured to operate in either 8- or 10-bit modes with resolutions of approximately 12 mV and 3 mV respectively. Because the 8-bit mode permits more data to be collected and simplifies data storage and retrieval, I have chosen to employ it exclusively in this system. If additional precision is needed, however, one or both channels can be configured in software to operate in 10-bit mode at the cost of data capacity but with no changes to the system's hardware.

2.3.2 Microcontroller and Data Storage

Adapting the system to a variety of experimental conditions may require modification of sampling rates, stimulation characteristics, user and data interfaces, or integration of new architectural components. In this respect, a microcontroller is an ideal system core. In

addition to general programmability, many recent microcontrollers include support for common peripheral interface standards, multi-purpose and software-selectable pin assignments, and onboard ADC and DAC in small-scale packages.

A PIC16LF1936 (Microchip: Chandler, AZ), operating on an internal system clock, controls the acquisition and storage of biopotential data, electrical stimulation, and all input/output and device interface functions. This microcontroller provides a number of on-board functions including up to fourteen analog inputs, digital to analog channels, programmable system clock, and built-in support for several communication protocols, including SPI and RS-232. The program memory and computational power is sufficient for a variety of acquisition, stimulation, and communication routines programmed in PIC-C (Custom Computing Services: Brookfield, WI).

Data storage is augmented by a 23K256 serial RAM (Microchip: Chandler, AZ) which provides approximately 32 kB of memory. Assuming a 2000 Hz, 8-bit data acquisition rate, this is sufficient for 16 seconds of continuous operation. Since animal behavior can often be unpredictable, my design uses a circular data writing scheme where, when the memory capacity of the RAM is exceeded, the system begins writing over the previously-recoded data starting from the oldest address. As the available high-speed camera configuration typically operates with a four-to-six second circular recording, this sixteen second window is suitable.

Using currently unimplemented microcontroller pins, the system can be expanded to meet future requirements. Additional devices, such as accelerometers or gyroscopes, could also be controlled by the microcontroller. Although the current processor speed of 2 MHz should be sufficient to execute on-board filtering or simultaneous two-channel sampling rates up to 1.5 kHz, the system clock can be increased in software up to the hardware limit of 20 MHz without requiring any changes to the system's hardware if more processing capability is required; power consumption, however, will increase with clock speed.

2.3.3 Communication

Both wired and wireless communication systems are employed by the current system. The user can send simple commands, such as 'start,' and 'stop,' using a hand-held light to trigger the photo-transistor. The system's LED can send simple status and synchronization messages to the user and observing cameras. Stored data and programming changes are transmitted to and from the system via wired serial interface which can be attached while the animal is at rest.

To index collected biopotentials with high-speed camera data and avoid data aliasing, the system's visible-light LED flashes the current memory address using a four-bit binary encoding scheme. This occurs once per second to ensure that multiple indications are visible during the four-second video window. While a single observation of the indication sequence is sufficient to correlate the recorded data with the high-speed camera images, this method increases the reliability of successful correlation even if the LED becomes obscured during the part of the experiment.

After the collected data have been uploaded from the system to a computer, the user correlates high-speed video data with biopotential data by finding complete address indications in the video. Error in this correlation can be reduced to within +/- 1 video frame for video framerates up to and including 1000 frames per second.

2.3.4 Stimulation

Up to four independent channels of electrical stimulation can be applied to the animal by the microcontroller's digital outputs. The stimulation can be configured for either uni- or bipolar stimulation by referencing the output to system or analog grounds respectively. Both modes are sufficient to activate the principle flight muscles when applied through invasive electrodes. Stimulation duration and frequency can be modulated and activated by internal programming, external triggering through the system's photo-transistor, or by an external digital signal.

Although this method does not allow stimulation amplitude to be changed, work by Mavoori, Bozkurt, and others has found that these changes are not necessary to evoke behavioral changes from *M. sexta* [30,36,39]. Furthermore, digital stimulation is faster than using the microcontroller's DAC and is simpler to implement.

2.3.5 Battery

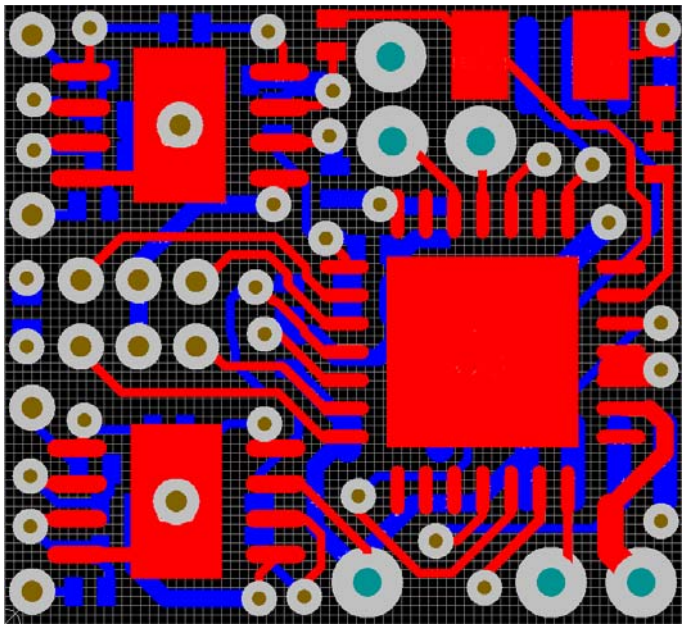
Power to the untethered system is provided by a light-weight, rechargeable, 3V lithium ion battery (SY-103, 5.5 mAh; Sanyo: Moriguchi, Japan). This is soldered to the chip to save weight but is replaceable. This battery offers between 20 and 30 minutes of operation depending on the demands placed on the system. The battery is recharged using the same connection used during serial data transfer.

2.3.6 Construction

Figure 2.3(a) shows the printed circuit board (PCB) layout for the system. The layout, designed by Tia Research SRL (Tulcea, Romania), uses four layers to reduce size. Active circuit components are all small outline, surface-mounted packages. Passive components are in 0201 packages. This reduces size and weight while still permitting conventional assembly techniques. The board, shown assembled in Figure 2.3(b), has a footprint of 9.5 mm by 8.7 mm. Boards were fabricated by Beta Layout Ltd. (Shannon, Ireland) from 1.7mm thick FR4 and assembled by hand.

The system's total mass, excluding accessory electrodes, is 674 mg; this is within the determined carrying capacity of the *M. sexta*. The small size of the system permits several placements, including on the dorsal thoracic surface, which do not interfere with the animal's normal wing stroke.

(a)



(b)

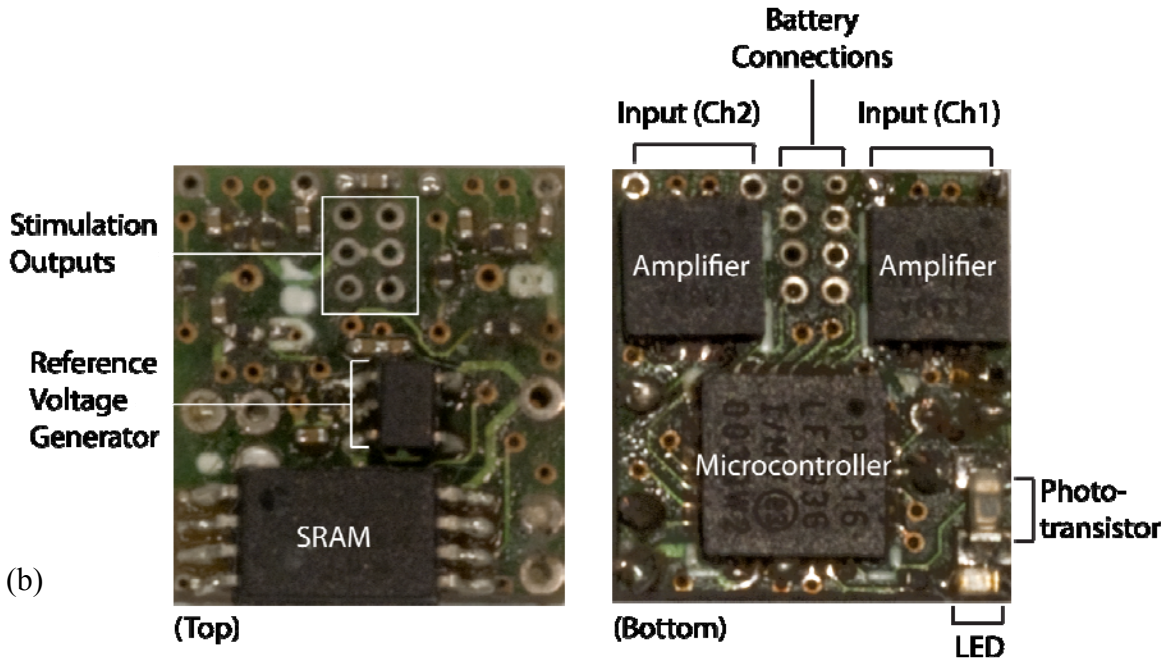


Figure 2.3: Physical design and layout of system (a) PCB layout. The top layer (red) connects to the microcontroller, amplifiers, LED, and phototransistors. The bottom layer (blue) connects to the SRAM, reference voltage generator, and most passive components. Not shown: interior supply and grounding layers. (b) Photographs of assembled system. Actual size of system: 9.5 mm x 8.7 mm.

Chapter 3

TESTING AND RESULTS

3.1 Introduction

To evaluate the system's suitability and performance, I attached it to several moths and engaged them in a variety of experimental conditions. Tests inside a small enclosure were used to compare results and performance to previous tests with long, flexible tethers. Expanded flight volume tests, not previously possible with available equipment, were used to assess the application of the system to correlating EMGs with longer-timescale behavior. Finally, simple stimulation techniques were tested to assess the system's ability to evoke simple behaviors.

3.2 Methods

3.2.1 Subjects

Male and female adult *M. sexta* moths, between four and ten days old were obtained from established laboratory colonies (University of Washington, Seattle, or University of North Carolina, Chapel Hill). Moths ranged in mass from 1.5 g to 2.1 g. Prior to implantation, moths were inspected for intact wings, good general health and competent

flight ability. Moths with developmental defects or that failed to demonstrate good flying ability were excluded from testing.

3.2.2 Electrodes

A common electrode design (shown in Figure 3.1a), intended for use in the dorsal longitudinal muscles (DLM), was used for all acquisition and stimulation tests. Electrodes consisted of a pair of 0.1 mm diameter tungsten wires attached to a plastic structure and signal wires with conductive epoxy. Tungsten electrodes penetrated approximately 1.0 mm to 1.5 mm into the subject's muscle tissue.

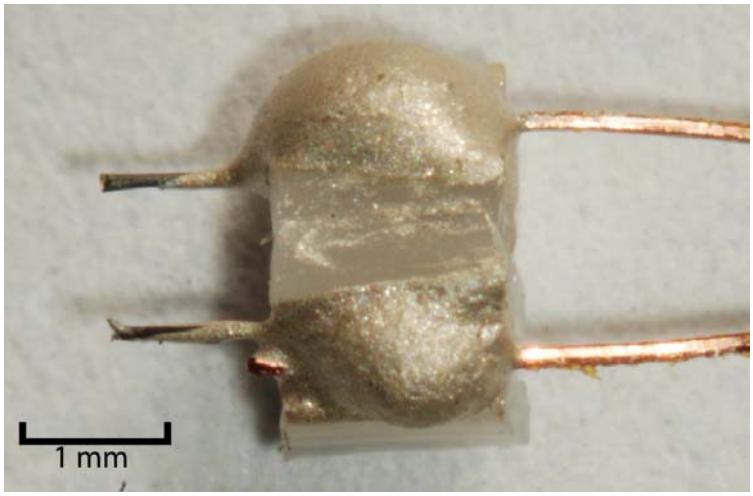
3.2.3 Implantation and Attachment

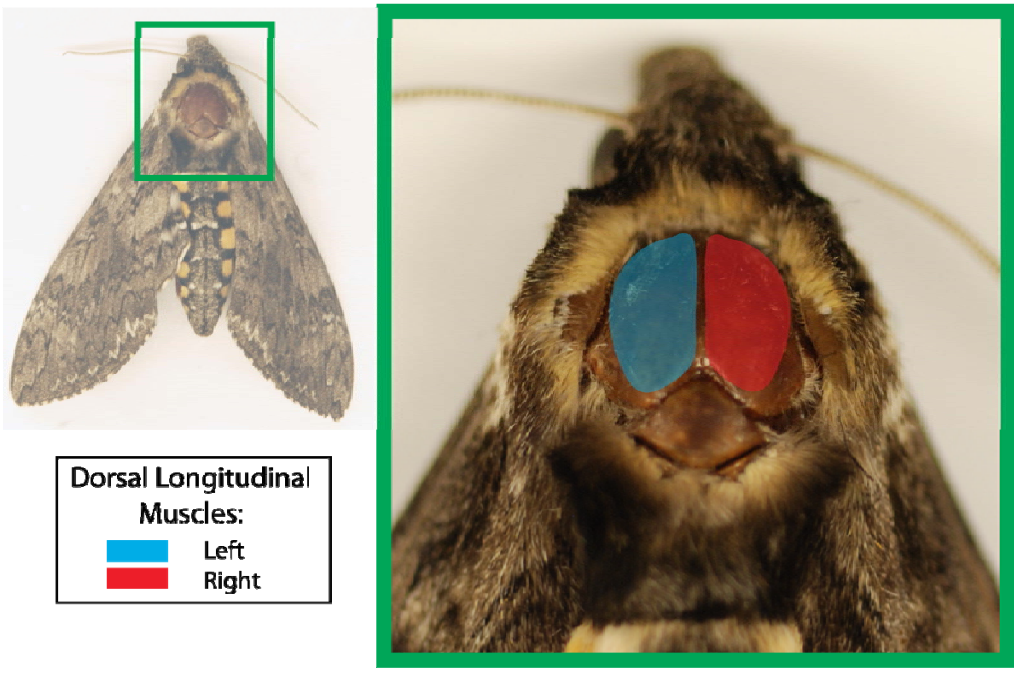
The DLM was selected for all experiments because its large size (7 mm x 3 mm) and location on the dorsal thorax permit electrode attachment without interfering with the normal wing stroke. Additionally, the function of this muscle in flight is well known so EMGs or stimulation may be easily correlated with observations [40].

Moths were anesthetized in a small, air-tight chamber by applying a dose of Flynap (0.11 cc per gram of body mass; Carolina Biological: Burlington, NC) for three to seven minutes until unresponsive. Moths were transferred to a metal table, chilled to 5-6 °C, and restrained with padded clamps over their wings. Scales covering the dorsal surface of the thorax were removed using compressed air and forceps.

Electrode pairs were inserted by hand through the denuded cuticle into the DLM tissue and secured in place with cyanoacrylate adhesive. Additional adhesive secured the electronics and battery to the posterior and anterior dorsal thoracic surfaces, respectively. After implantation, moths were removed from the plate and allowed to recover in fresh air for at least one hour prior to testing. A recuperated moth, prepared for testing, is shown in Figure 3.2.

(a)





(b)

Figure 3.1: Electrode implantation. (a) Electrode pair for use with a DLM. Two pairs are required for bilateral DLM acquisition. (b) Location of DLM on *M. sexta* subject prepared for implantation.

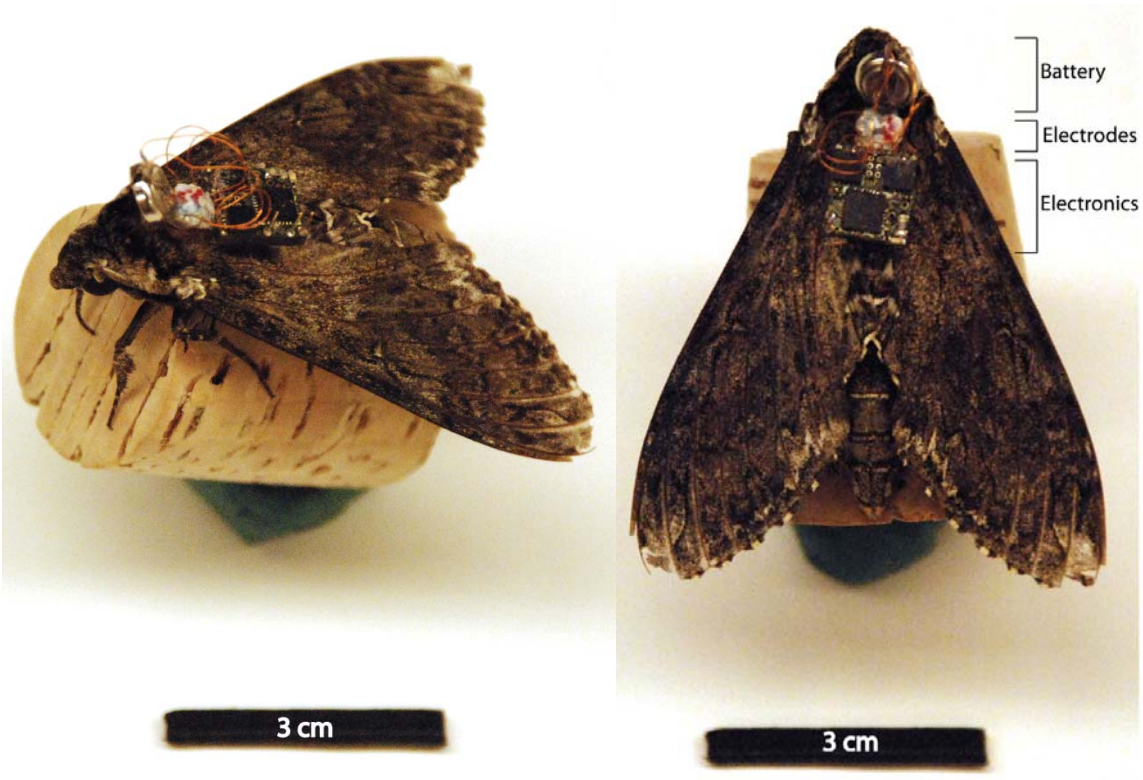


Figure 3.2: A moth prepared for free-flight tests.

3.3 Results/Testing:

3.3.1 Small enclosure testing

System-equipped moths were placed on a perch inside an enclosed transparent chamber with a volume of approximately one cubic meter. Moths were recorded by a set of three high-speed video cameras (two Phantom v7.1 and one Phantom v5.1; Vision Research: Wayne NJ) operating at 1000 frames per second and a shutter duration of 990 μs. The moth was illuminated in the near infra-red (760 nm) with a set of eight custom high-intensity LED lamps.

Movement of the perch or gentle pressure to the abdomen encouraged the moths to leave the perch and fly through the volume in a self-selected manner while EMGs were logged by the system. After moths demonstrated competent initiation and control of flight, they were recaptured and recording was disabled. Subjects were allowed to rest on a perch while EMG data was uploaded to a computer and the battery recharged. A representative trial, showing left and right DLM EMGs correlated with a flight initiation is detailed in Figure 3.3.

Tested moths carried the system with little difficulty. Moths that were allowed to feed within the previous twelve hours exhibited greater difficulty in flying; this is likely due to the additional mass of fluid, which can be several hundred milligrams, in their digestive system. EMG quality was similar to that obtained in earlier experiments using similar electrodes wired to an external amplifier with long, flexible wires. The moth-portable system appears less susceptible to ambient electronic noise due to reduced lead wire length.

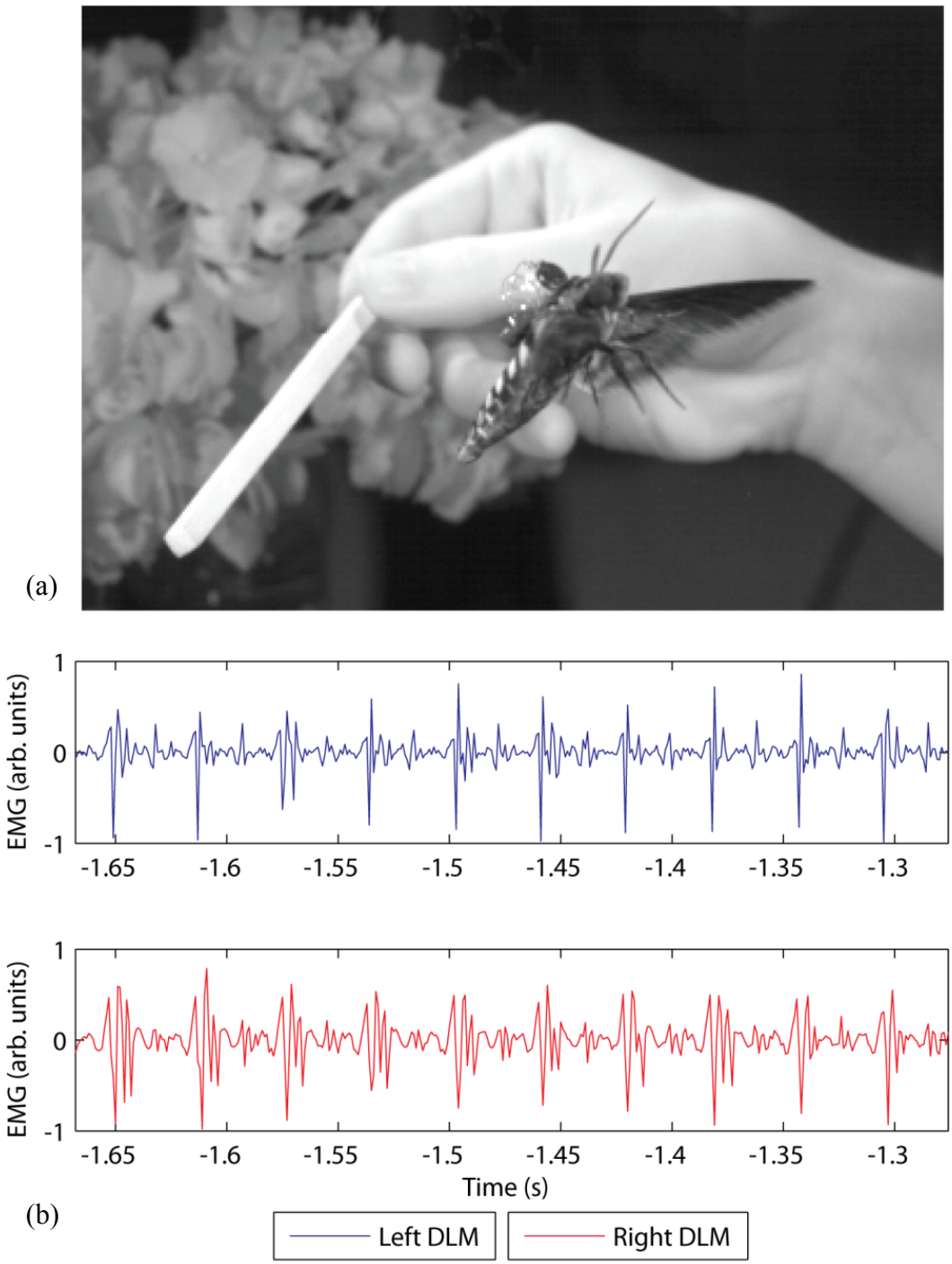


Figure 3.3: Small-volume flight test results (a) System-equipped moth in flight as viewed by one of the three recording cameras. (b) DLM EMGs, processed using a fourth-order, zero-lag, digital Butterworth filter (100 Hz – 500 Hz bandpass), from a segment of the flight (bottom). The subject took flight from the perch and flew for approximately 1.5 s before leaving the camera-observed area and landing on the interior of the enclosure. As minor differences in electrode placement changes EMG signal intensity, the signals shown have been normalized with respect to the maximum of the absolute value of the signal over the duration of interest.

3.3.2 Unrestricted Flight

Intended experiments, including flight mazes and in-flight perturbations, preclude the use of flexible tethers. To test my system's applicability to larger flight volumes and longer-duration recordings, I repeated the previous experiment in a much larger volume and for a longer duration.

Moths, prepared as described in section 3.2, were placed on a perch in an open indoor area. Three high speed video cameras (as used in the previous section), operating at 200 frames per second and a shutter duration of 4900 μs, recorded a volume of approximately six cubic meters. No additional illumination was used. Moths were coaxed to begin flight by moving the perch or with gentle pressure to the abdomen. The subject was allowed to fly in a self-directed manner until it left the observed area. Subjects were then recaptured and recording was stopped.

Figure 3.4 shows collected left and right DLM EMGs correlated with the moth's position within the recorded volume, as determined by three-dimensional triangulation [41]. These results verify the applicability of my system to experimental conditions which were not previously possible with available laboratory equipment.

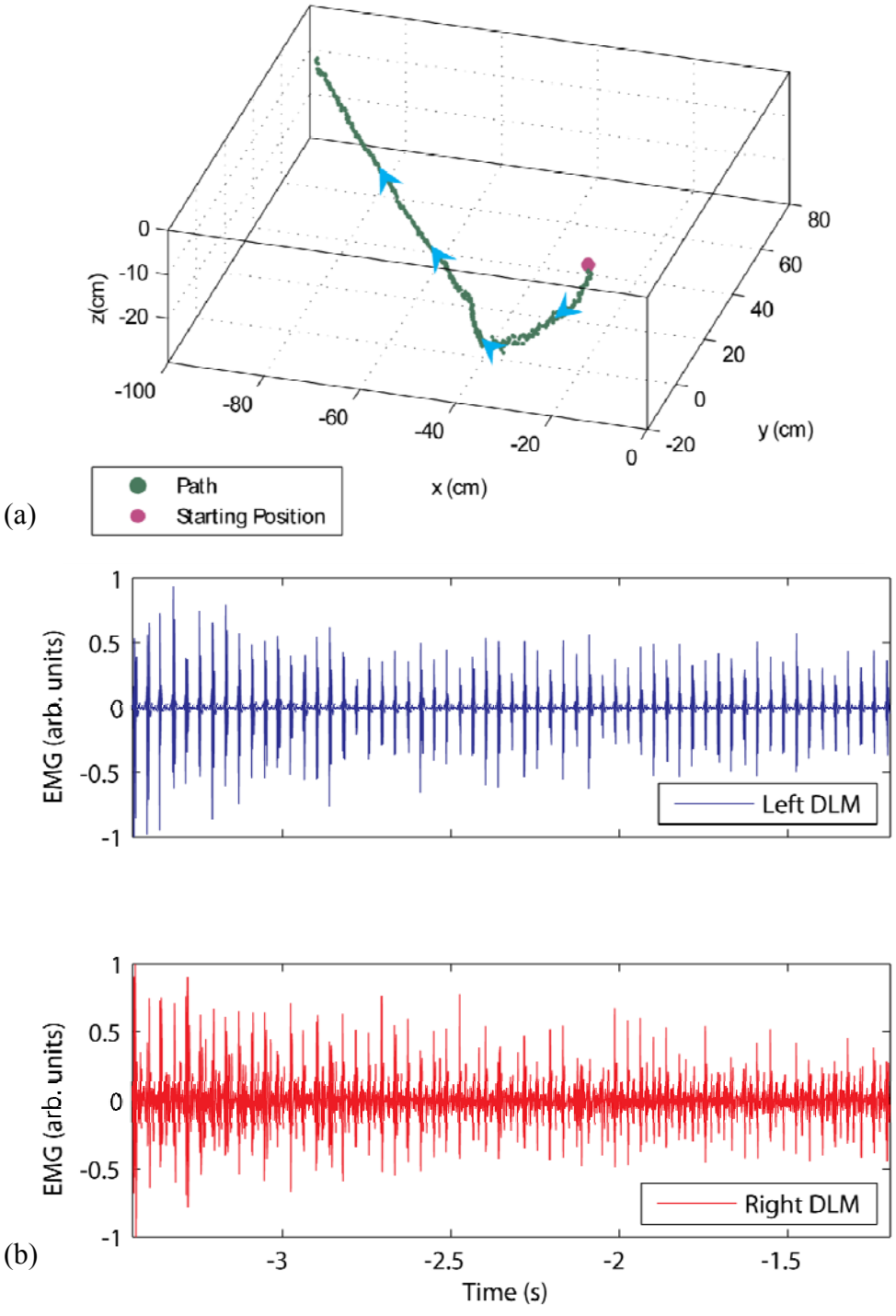


Figure 3.4: Expanded-volume flight test results. (a) Moth's path through open flight area as determined by three-dimensional triangulation. Left and right DLM EMGs, processed using a fourth-order, zero-lag, digital Butterworth filter (100 Hz - 500 Hz bandpass) corresponding to this flight are shown in (b). The moth initiated flight from a hand-held perch in an open room and flew for approximately 3 s before leaving the recorded volume. EMGs have been normalized with respect to the maximum of the absolute value for the signal over the region of interest. Note: The right electrode became damaged during the moth's flight but it was possible to recover the signal after filtering due to the low noise of the system.

3.3.3 Stimulation

I tested stimulation capabilities by tethering subjects to a fixed rod with cyanoacrylate adhesive and placing electrodes in the left and right DLMs. Intramuscular stimulation was supplied identically by two of the system's stimulation channels. Wing flexion in response to applied stimulation was measured using three high-speed video cameras (as described in Section 3.3.1; 1000 frames per second, 990 μs exposure, near-infrared illumination used). Three-dimensional triangulation was used to track the position of the left and right wing tips relative to the animal's thorax. Stimulation was recorded by splitting the system's outputs into a computer data acquisition system (USB-6251; National Instruments: Austin, TX). The set-up is diagramed in Figure 3.5.

A stimulation protocol and resulting wing flexion are shown in Figure 3.6. Bipolar, rather than unipolar, stimulation was used because it reduces damage to the subject's tissue and is more biologically relevant [29,42]. It was possible to elicit a variety of flapping frequencies from the moth, including driving the muscle to tetanus with stimulation frequencies above approximately 40 Hz. Typical flapping was not produced by this stimulation method because the moth was neither in the appropriate posture nor was the upstroke muscles, required for normal flapping, stimulated.

Stimulation as tested may be useful to produce aerial stumbles without transferring energy to the animal as would other perturbation methods, such as a projectile or a gust of air. By directing stimulation to the animal's nervous system, it may also be possible to evoke different behaviors, such as postural and directional changes [36,39], or overwrite natural muscle activation patterns [37,43].

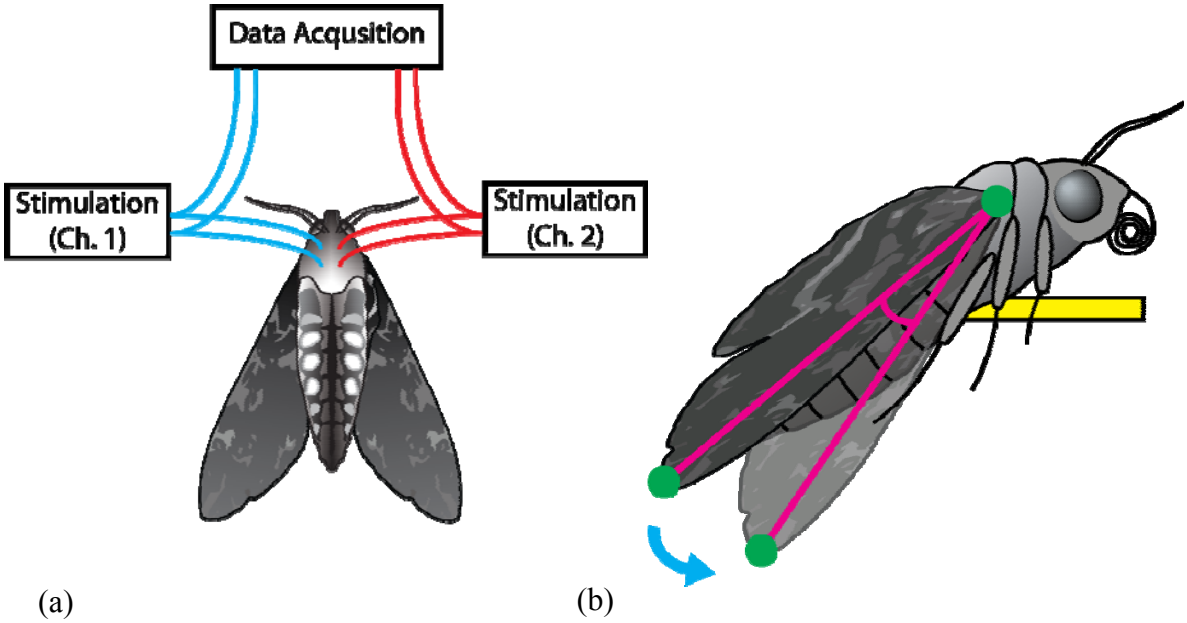


Figure 3.5: Stimulation test set-up. (a) Diagram of electrical connections to moth for stimulation experiments. Two electrode pairs were inserted into the left and right DLM. To improve wing visability and ease attachment of additional electrical connections, the system was not attached to the moth in this test. (b) Illustration indicating attachment of moth to rigid tether (yellow), points tracked (green) using three-dimensional triangulation to determine wing deflection angle (magenta), and typical wing motion in response to DLM stimulation (cyan).

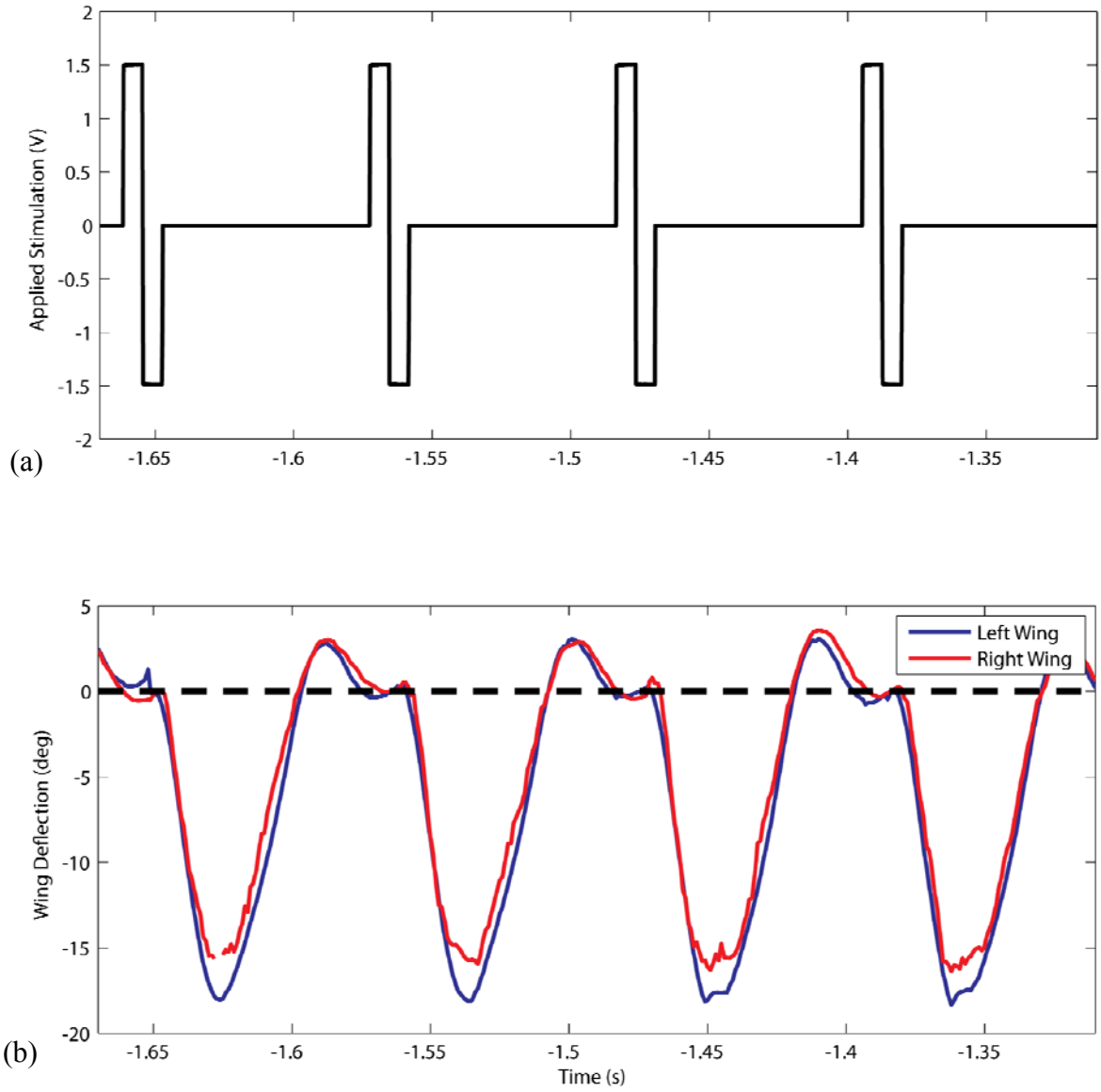


Figure 3.6: Stimulation test results. (a) Electrical stimulation applied to both left and right DLM. (b) Left (blue) and right (red) wing deflection.

Chapter 4

DISCUSSION

My moth-portable animal-computer interface meets all of the proposed design requirements: it is adaptable to variable experimental requirements through adjustable amplifier gain, general purpose I/O ports, programmable sampling, and stimulation modalities. The system's small size, light weight, and flexible interfaces will make it a valuable tool in insect flight research.

There are, however, a number of features that remain to be developed or tested, including improved electrodes and implementation of on-board signal processing. Some hardware modifications, necessitating fundamental changes to the system's architecture, would also improve functionality.

4.1 Further Testing with Current System

4.1.1 Improved Electrodes

Recent work indicates that relative timing of small, directly-actuating accessory muscles, such as the third axillary, may substantially characterize insect flight control [31]. The electrode design described earlier, however, is unsuitable for acquiring EMGs from muscles smaller than the DLM.

I am currently testing an alternate design, made by threading a small, uninsulated wire through the subject's exoskeleton and along the underside of the cuticle near the origin of the muscle of interest. This method, which is compatible with the current amplifier design, offers reduced weight and a lower profile. As similar techniques, are suitable for signal acquisition from and stimulation of the mesothoracic ganglion [30], this electrode design may be preferable for most applications. More intricate implantations performed during the moth's pupal stage could be used with my system and may enable a more intricate animal-computer interface [39,44,45].

4.1.2 On-board Computation and Signal Processing

As many insect muscles have only a small number of motor units [40], it may be reasonable for some experiments to record only muscle activation timing. The system possesses sufficient computational resources to execute on-line filtering of acquired biopotentials and should be capable of extracting the spike timing. By saving only the timing data, rather than the complete signal, existing storage structures could acquire much longer periods of continuous data.

Similar filtering and spike detection capabilities, coupled with neuromuscular stimulation, could permit motor activation modification experiments, such as those already conducted n cockroaches [46,47]. Such experiments may offer insight insect neural plasticity and biomechanical tuning of neuromuscular activation.

4.2 Architectural and Hardware Improvements

Alternate devices may be better choices for the system. Additional data memory could be supplied by several chips, such as the SST 25VF032B Flash RAM, which offer expanded storage while keeping an interface and package similar to that of the Microchip 23K256 currently in use. It may also be beneficial to integrate support for additional devices, such as gyroscopes or accelerometers, into the system. While the current implementation's circuit board cannot support it, these devices could be controlled without changing the current system architecture through the six analog/digital I/O pins which are currently unused.

I also found that the wired data interface, although functional, delayed experimentation. As soon as the desired behavior was demonstrated by the subject, the moth had to remain stationary for several minutes during data upload. As each rest requires a subsequent pre-flight warm-up before normal flight may resume, the animals quickly became exhausted. An improved version of this system, using a real-time radio link to transfer biopotentials to a computer data acquisition system would streamline experiments.

As the system's mass of approximately 700 mg is near the limit of moth-portability, it could also be improved by implementing some weight-reducing modifications. A thinner PCB composed of 0.4 mm thick FR4 should be possible and would reduce weight by an estimated 150 mg. This difference may reduce moth encumbrance or permit larger batteries, such as the Seiko MS920SE which allows the system to operate for up to 45 minutes, while

maintaining current system mass. Using wafer-level chip-scale packages where available may also facilitate weight reduction, although they will preclude many conventional assembly techniques.

4.3 Conclusions

The system, as described in Chapter 2, is a miniature biopotential recording and electrical stimulation device which can be carried by *M. sexta* in free flight. Tests to date, detailed in Chapter 3, have found that it is suitable for experiments requiring simultaneous monitoring of two principal flight muscles and correlation with biomechanical data obtained from high-speed videography. The electrical stimulation features are capable of inducing changes in behavior which are of experimental use.

Applying this device and more advanced future devices to experiments where the natural feedback between the subject's intentions, sensory input, and motor output remains intact may offer valuable insight into the neurophysiological basis of flight control at a behavioral level. In addition to explaining insect flight, this information, coupled with control models from tethered experiments, could help us build more general models of neural control. Such models are certain to offer insight into the basic structure of animal nervous systems and will drive both biomimetic and neural rehabilitation engineering.

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