

Insect–Machine Interface Based Neurocybernetics

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Abstract—We present details of a novel bioelectric interface formed by placing microfabricated probes into insect during metamorphic growth cycles. The inserted microprobes emerge with the insect where the development of tissue around the electronics during the pupal development allows mechanically stable and electrically reliable structures coupled to the insect. Remarkably, the insects do not react adversely or otherwise to the inserted electronics in the pupae stage, as is true when the electrodes are inserted in adult stages. We report on the electrical and mechanical characteristics of this novel bioelectronic interface, which we believe would be adopted by many investigators trying to investigate biological behavior in insects with negligible or minimal traumatic effect encountered when probes are inserted in adult stages. This novel insect–machine interface also allows for hybrid insect–machine platforms for further studies. As an application, we demonstrate our first results toward navigation of flight in moths. When instrumented with equipment to gather information for environmental sensing, such insects potentially can assist man to monitor the ecosystems that we share with them for sustainability. The simplicity of the optimized surgical procedure we invented allows for batch insertions to the insect for automatic and mass production of such hybrid insect–machine platforms. Therefore, our bioelectronic interface and hybrid insect–machine platform enables multidisciplinary scientific and engineering studies not only to investigate the details of insect behavioral physiology but also to control it.

Index Terms—Bioelectric, cyborgs, flight control, implantable electrodes, insects, metamorphosis, neural implants, neuromuscular.

I. INTRODUCTION

HIGHLY miniaturized CMOS electronics on micromachined probes has enabled complex brain–machine interfaces (BMI) [1]. Developments in microprobe technology have shifted the notion of vertebrate implantable neuromotor prosthetics from science fiction to reality [2], [3]. This technology

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Color versions of one or more of the figures in this paper are available online at <http://ieeexplore.ieee.org>.

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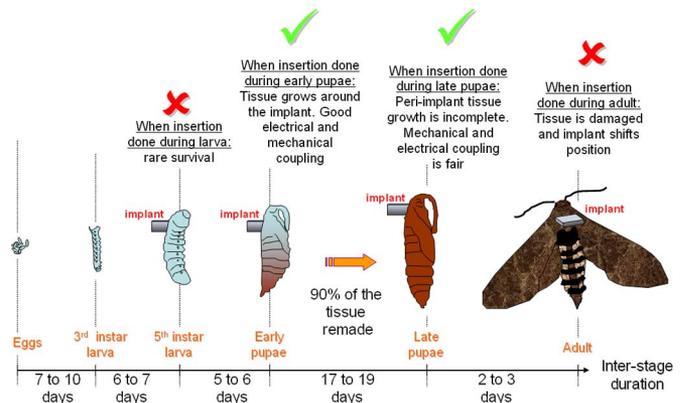


Fig. 1. Life span of *Manduca sexta* during the metamorphic development and the results of device insertions at various stages of metamorphosis.

provides techniques and tools to understand and generate robust electronically controlled muscle movement. Restoring impaired motor function has been possible in vertebrates such as rabbits, cats, and monkeys by controlling the motor function using either external operator commands or the output of the subject's own brain [4], [5]. Remarkably, these systems are already at the size scale ($\sim 5 \text{ mm}^3$) to fit on an insect to build insect–machine interfaces (IMI). IMI provides neural engineers an access to the neuromuscular system of insect to study and control its sensory and behavioral physiology. Previous attempts at IMI for neural studies has posed many challenges [6], [7]. Superficially or externally attached interface payloads have the potential to be rejected both physiologically and behaviorally, since they are perceived as foreign objects by the insect. Payloads also can move because of inertial and stress loading, introducing tissue damage and experimental variability. Moreover, the surgery required to accurately place electronics onto or into adult tissue is challenging because the insect tissue and organ feature dimensions are comparable to the electronic neural or muscular probe dimensions. The exoskeleton is covered with the readily shedable scales and piles limiting the surgical innervations. The hard outer cuticle is often difficult to penetrate without significant collateral tissue and probe damage.

In order to solve the issues with external attachment of control and recording electronics to the insects, we have developed a novel implant insertion pathway based on metamorphic development. During metamorphic growth, holometabolic insects transform from larva (or nymph) into an adult insect passing through a pupal stage (Fig. 1). During these steps, insects undergo an extensive programmed degeneration and remodeling to transform from a slow crawling caterpillar to the adult with many complex behavioral capabilities [8], [9]. It has been shown that, insects can survive extreme parabolic surgeries performed during the pupal stage, where surgery related wounds are rapidly

repaired through histogenesis [10], [11]. In light of this fact, we have developed a surgical procedure in which an electronic payload is inserted into the insect at the early stages of metamorphosis. The adoption of the payload by the body, as the developing tissue forms around it, not only ensures a secure attachment to the insect, but also enables a highly predictable electronic interface to the insect's sensorial, neural, or muscular systems. We call this procedure Early Metamorphosis Insertion Technology (EMIT).

EMIT procedure allows for reliable IMI and hybrid bioelectronic structures where CMOS devices and MEMS structures are coupled with insects natural sensors and actuators. These systems can lead to the solution of many engineering challenges by understanding and manipulating the communication and automatic control systems of insects. One of such cybernetic challenge is to control insect locomotion to use them to carry electronic payloads. Animal populations have been utilized by man for a wide range of tasks as early as 15 000 BC [12], [13], the foremost being as beasts of burden [14], [15]. Although insects possess a much higher muscle force to body mass ratio relative to the large mammals, their control for directed locomotion, to benefit from their muscle power, has not been exploited reliably and reproducibly. Navigation of large animals historically has been facilitated by man-made devices such as horseshoes, reins, and blinders. Pathways to utilize insects for their load carrying capacities have not been established until now, because the navigation technology needed for small sizes has not existed. Moreover, manufacturing insect-scale transportable payloads has been an issue. With continued miniaturization of information sensing, computing, and processing technologies, it now seems likely that insects could be navigated as mobile information gatherers. Therefore, our specific methodology has broad implications not only for studying the insect-machine interface but also for the future use of navigated insects as environmental sentinels. When instrumented with equipment to gather information for environmental sensing, such insects potentially can assist man to monitor the ecosystems that we share with them.

II. SELECTION OF MUSCLE GROUPS TO STUDY LOCOMOTION CONTROL THROUGH EMIT

For this study, we utilized EMIT for neuromuscular recording and actuation of the tobacco hawkmoth *Manduca sexta*. *Manduca sexta* has a pupal stage duration of three weeks and an adult lifetime of two to three weeks. With a typical wingspan of 10 cm, it can carry up to 1 grams of payload and can fly miles without stopping [16], [17]. In order to control flight with embedded electronics, we chose to directly actuate the muscle groups responsible for flight, which are located in the dorsal-thorax of the moths. Occupying most of the space in thorax, flight muscles alone can comprise as much as 65% of the total body mass [18]. Because of their relatively larger volume compared to the microprobes, these muscles are easy to locate on the pupae during insertion surgery.

The basic wing beat of *Manduca sexta* is realized by the morphological arrangement of the main flight powering dorso-

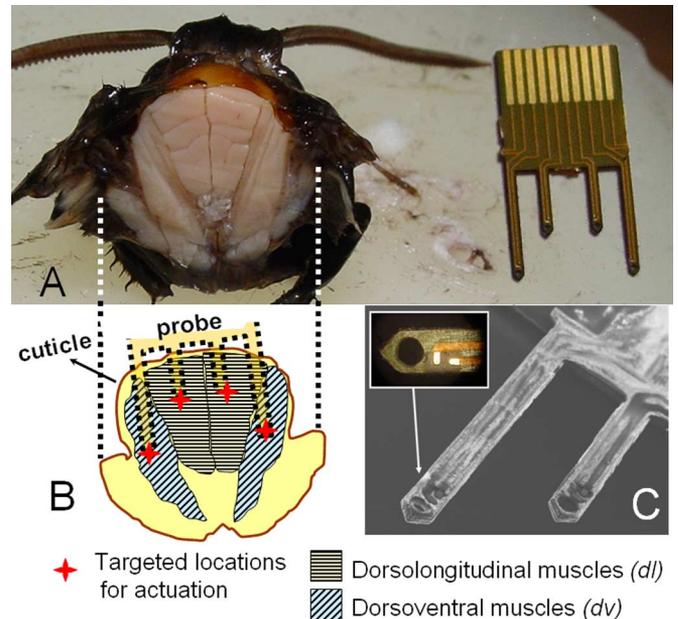


Fig. 2. (A) Cross section and (B) illustrated diagram of the flight muscles powering the up- and downstroke of *Manduca sexta* wings. The tips of the flexible probe in (A) target the flight powering muscles *dl* and *dv* (B). SEM image of the flexible-probe tip with expanded image of the ground and actuation pads can be seen in (C). The hole at the tip is opened for muscle growth.

longitudinal (*dl*) and the dorsoventral (*dv*) muscles and their interaction with the wing articulation geometry [Figs. 2(A) and (B)] [19]. During straight flight, the *dl* and *dv* muscles contract approximately 180° out of phase. During turning, *dv* on the turning direction side is activated earlier with respect to other side, causing a reduction of thrust in that direction [20]. Contraction of the other smaller muscles, attached directly into axillary sclerites, twist and rotate the wings to increase steering precision. The normal wing beats for turning and tilting can be achieved by stimulating the *dl* and *dv* muscles, either solely, or in combination with other smaller flight muscle groups [20]. Therefore, we chose to implant in the *dl* and *dv* muscles to achieve IMI-based insect flight direction.

III. FABRICATION OF FLEXIBLE MICROPROBES FOR RECORDING AND ACTUATION

In order to electrically excite the flight muscle groups at the targeted locations, polyimide thin-film-based flexible microprobes [Fig. 3(A)] were designed and fabricated following standard micromachining procedures for flexible circuit technology. Copper layer (18 μm thick) was deposited on 100-μm-thick Kapton polyimide substrate. Copper traces were coated with 20 μm of liquid photo imageable (LPI) soldermask for insulation, except for the locations of the excitation/recording pads. Electroless nickel and immersion gold (ENIG) layer (3 μm thick) was deposited on the pads for biocompatibility. Each actuation pad is 75 × 75 μm² and each polyimide probe has a width of 400 μm [Fig. 2(C)] to space two actuation pads across the probe width. The distance between the probe arms on

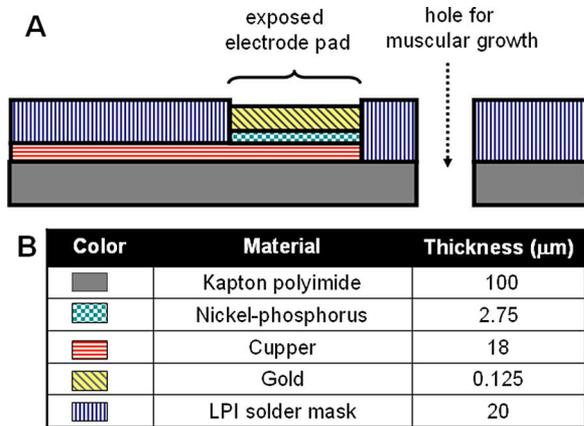


Fig. 3. (A) Cross section and (B) description of the layers used in the fabrication of the flexible probe.

each side was 1.5 mm based on the anatomical characteristics of the *dl* and *dv* muscles.

Polyimide was chosen as the base material because its mechanical flexibility could be matched to the flexural rigidity of the flight muscles. Furthermore, polyimide is a proven biocompatible material for long-term chronic neuroprosthetic applications [21]. Insulated microprobes were electrically active only at the tips with gold-coated electrode sites. Gold was chosen for biocompatibility to muscle tissue [22]. We provided orifices (200 μm diameter) at the probe tips for the muscles to grow through, thereby creating biomechanical anchors [Fig. 2(C)]. Part of the probe designed to remain outside the insect body for electrical connections. For tethered muscle excitation experiments, a flexible cable was connected to the external probe body, where the cable was used both for signal delivery and for mechanical suspension [Fig. 7(A)]. Tethered wires give experimental design flexibility and can be replaced with radio frequency controlled microsystems for specific applications.

IV. NOVEL SURGICAL IMPLANTATION PROCEDURE

Manduca sexta were obtained for surgery from the Boyce Thompson Institute, where they were reared on artificial diet under a 17:7 h light/dark cycle regimen at 26 $^{\circ}\text{C}$ and $\sim 60\%$ humidity. Both chronological and morphological criteria were used to record the developmental stage for the probe insertion. Different visual patterns on the pupal wings and thorax indicate the stage of the metamorphic growth.

As the first step of the EMIT procedure, we visually mapped the target probe location for flight muscles using the exocuticle surface topology. The pupa has an external (exo-) and internal (epi-) cuticle layers developing during metamorphic growth. The exocuticle is secreted by the epidermal cells of the larva and goes through a complete transformation to form the hard shell around the pupa. This hard shell is shed during the emergence of adult insect. Although both layers are penetrated through into the flight muscles, the microimplants are anchored to the body for mechanical stability at the epicuticle. This layer cannot be observed until the emergence of the adult insect from pupa (eclosion). However, the body parts such as wings, antenna, and

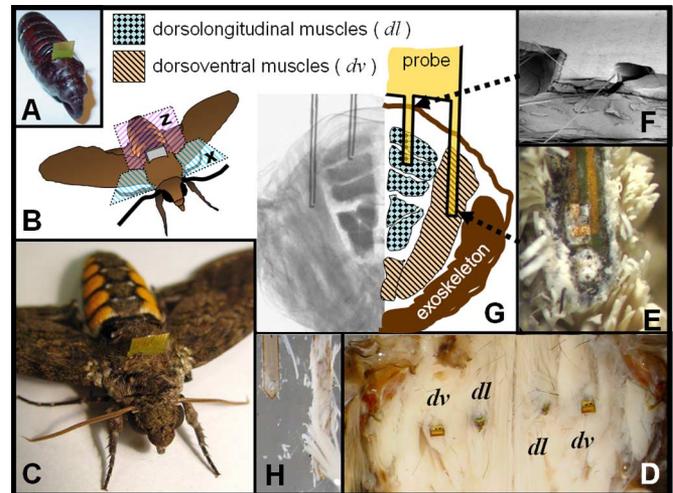


Fig. 4. (A) Pupal stage insertion of probes and (C) successful emergence. (B) Postexperimental dissections were performed in transverse (z) and frontal (x) planes. Probes embedded in *dl* and *dv* muscle groups can be seen in frontal dissection facing the dorsal direction. (G) The X-ray image of the thorax shows probe localization to the targeted muscle groups with an explanatory schematic of thoracic flight muscles. (E) Muscle growth around the probe tip and (F) natural sealing at the cuticle indicate integration by the body. (H) The removed tissue with the probe when it was extracted from an adult insect, *dl* probe on the left and *dv* probe on the right.

regions of the thorax (pro-, meso-, and metathorax) can be differentiated visually by means of conformational overlays and intersegmental folds of the exocuticle. Some of these exocuticle features are linear depressions that serve as mechanical stress points where the exocuticle fractures. After fracture, the exocuticle is shed by means of peristaltic and partial rotary movements of the abdomen. The surface features on the mesothoracic exocuticle provide target localization for insertions in the pupal flight muscles. Although the size of the insect larva changes dramatically before molting, the pupal stage growth is negligible because of sclerotization of the inner and outer cuticle. Therefore, shifting of the targeted location in the body secondary to metamorphic growth is not a concern.

For the surgical insertions, we anesthetized the insect pupae at various stages of metamorphic development with cold treatment (4 $^{\circ}\text{C}$) for 10–15 min. Subsequently, four mesothoracic incisions ($2 \times 0.5 \text{ mm}^2$ for each probe tip) were created with a scalpel both on the epicuticle and exocuticle of each insect, to match the probe tip spacing. The microprobe was pushed into the muscle tissue through these incisions. The insects with inserted microprobes payloads were supported from the left and right body sides for at least 36 hours to prevent pupae rotatory movement, which could shift the probes before sealing occurred. No adhesive was used to seal the incisions. Instead, the cuticle was allowed to heal naturally, creating a biological seal. Artificial glues were found to adhere the exocuticle to the epicuticle and prevent successful eclosion.

V. OPTIMAL PUPAL STAGE FOR INSERTIONS

At the end of the pupal stage, the cuticle cracked along the line formed by the surgical incisions and insects eclosed

TABLE I
OBSERVED SURVIVAL RATES OF THE SURGERIES FOR DIFFERENT STAGES
OF INSERTIONS

Insertion Stage	Number of insertions	Survival rate
Larva	10	0%
Pupae	20	95%
Adult	10	80%

naturally [Fig. 4(C)] with a successful emergence rate of 90%, where all of the moths had fully inflated wings. The optimized time for surgical insertion was found to be seven days before emergence (Table I). With insertions earlier than seven days, the fluidity of the muscle precursor tissue prevented adequate sealing around the insert and decreased the survival rate by leaking out. When inserted later, very close to eclosion, the preformation of epicuticle and muscle fibers led to incomplete attachment and inefficient anchoring of the insert to the tissue. In this case, the tethered implants detached easily during the experiments. The insertion process also suffered from buckling of the flexible microprobe during insertion into the stiffer muscle tissue. Moreover, failure of the bioelectrical interface was more frequent with these late inserted probes because of similar failure mechanisms observed with the adult inserted microprobes.

VI. POSTEMERGENCE ANALYSIS OF BIOCOMPATIBILITY AND TARGET LOCALIZATION

With the emerged adults, possible probe compatibility failures were inspected as described below in a chronic timeline to improve the reliability of implantation technology. Postexperimental dissections [Fig. 4(C)] were performed on the implanted insects and the tissue response to the persistent presence of the probes was inspected. No inflammation or tissue disturbance was observed at the site of the dissection [Figs. 4(D) and (E)] in any of the insects. Postexperimental X-ray and computed tomography (CT) imaging were also performed to assess the success rate of the microprobe geometry for localizing the targeted *dl* and *dv* muscles. We measured a success rate of 98% [Fig. 4(G)] with more than 100 insertions. The few placement errors that occurred resulted from bending of the probe in the muscle tissue during the insertion process.

Adverse reactions of the tissue to the microprobe insertion were avoided, because the microprobes were inserted to the soft gel-like pupal muscle tissue, prior to the formation of flight muscle fibers. The body flexure-induced strain mismatch between the probe and muscle tissue was also minimized because of the flexibility of the probe. Force applied to the insect via the tether cable was sustained successfully at the hardened cuticle-microprobe interface as a result of the rigid matrix formed by the cross-linking between the cuticular protein molecules around the implant. Fig. 4 (F) shows the healing of the cuticle through formation of these proteins around the probe at the insertion points. Tissue adhesion to the microprobe and growth of muscle tissue through the holes at the tip further improved mechanical anchoring [Fig. 4(E)]. When the probes were extracted, a considerable amount of tissue was removed with the pupae-inserted

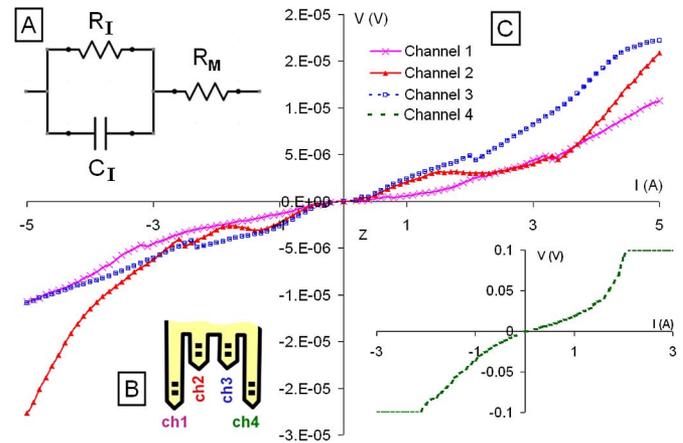


Fig. 5. (C) The I - V curves of each electrical pad and (A) a simple RC network modeling the muscle between the pads. The pad diagram is given in (B). In this example, Channel 4 (shown separately in fourth quadrant) has poor bioelectrical coupling.

probes indicating good anchoring [Fig. 4(H)]. In contrast, when probes were removed from moths in which probes had been implanted in the adult stage, very little tissue remained on the probe, indicating a weaker biomechanical interface and highlighting a key advantage of early pupae insertion.

VII. PREACTUATION INSPECTION OF THE BIOELECTRICAL INTERFACE

Around 10% of 80 tested pads on late-pupae-inserted probes were not able to actuate the flight mechanism due to failed interface between the gold actuation pad and the muscle tissue due to currently unknown failure mechanism. These failed pads can easily be identified via preexperiment I - V curve measurements. Typical I - V curves of satisfactory coupling and a failed probe can be seen in Fig. 5(C). I - V measurements were performed on a visually quiescent moth using Keithley-4200 oscilloscope. The interface between the probe pads and actuated muscle fibers can be modeled with a simplified equivalent circuit [Fig. 5(A)]. Here, R_M denotes the resistance of intra- and extracellular fluids of muscle fibers, whereas R_I and C_I are the resistance and capacitance at the metal-tissue interface. Other elements such as the resistance of the metal trace and the capacitance of the insulator between the metal trace of the electrode and the conducting interstitial fluid were omitted since they are negligible. Lines were fitted to the I - V curves at dc to give the approximate addition of R_I and R_M . The obtained resistivity sum from this analysis (ρ ; see Table II) for the satisfactory probe-tissue interface is in good agreement with the reported skeletal muscle resistivity (300–500 Ω -cm) [23]–[25] and the metal-tissue interface resistance measured in 0.9% saline solution (75 Ω -cm). Failed probes, however, give abnormally reduced resistivity values, as indicated in Table II.

The implanted probes can also be used to record electrophysiological signals during natural flight to study the locomotive behavior. Further feedback studies of insect muscle can be conducted to optimize the flight control. In this study, the recording

TABLE II
CALCULATED RESISTIVITIES BETWEEN PROBE PADS

Channel	Trendline	ρ (ohm·cm)
Ch1	$x=516,804y$	484
Ch2	$x=303,739y$	285
Ch3	$x=354,742y$	332
Ch4	$X=22.9y$	0.021

Lines were fitted to the I-V curves in Figure 5 to calculate the resistivities. Channel 4 has poor electrical coupling.

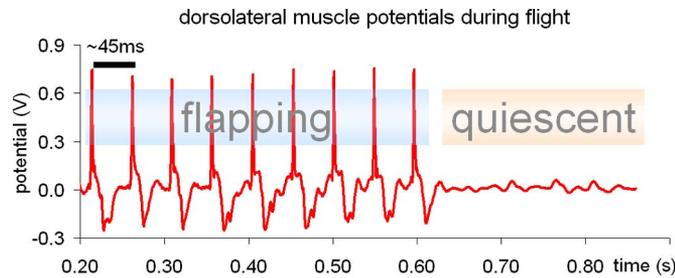


Fig. 6. Actuation probes were also used to record muscle potentials as an indication of efficient tissue–probe coupling. Potentials recorded from the dorso-longitudinal muscles (multiple-cell recording with 50–100 times amplification) have spiking frequency similar to wing-flapping rate (~ 23 Hz). Observed spikes disappeared immediately when wing flapping stopped.

capability was used solely to assess the success of bioelectrical coupling (Fig. 6).

VIII. ACTUATION OF THORACIC FLIGHT MUSCLES

Navigating and domesticating insects such as *Manduca sexta* require the ability to elicit reproducible flight maneuvers. Different stimulation schemes need to be developed for each maneuver and these maneuvers should be combined to obtain more precise and complex locomotion behavior. As a starting point, to evoke a yawing maneuver, flight muscles *dl* and *dv* of *Manduca sexta* were stimulated by applying phased electrical pulses via the indwelling probes, while motor nerve activity and wing movement were monitored (Fig. 7). When the moth was resting in a quiescent state, stimulation of *dl* muscle on one side of the insect caused the wing on that side to move downward, whereas stimulation of the *dv* muscle caused the wing to move upward. The depression and elevation of the wing persisted as long as stimulation was continued and both bilateral and unilateral wing actuation were demonstrated reproducibly. Following cessation of stimulation, the wing rebounded passively to its resting position as a result of the elasticity of the thorax [Figs. 7(B)–(D)]. Flight stopped immediately when these two muscles contracted simultaneously with high frequency pulses (70–100 Hz). The wing actuation and direction of flight can be best seen in movie format [26].

When a unilateral stimulation pulse was applied to a tethered insect flying naturally in a straight direction, the most striking effect was a yawing maneuver toward the stimulated side. A backward shift of the wings occurred on the stimulated side, whereas the wings on the opposite side were drawn forward

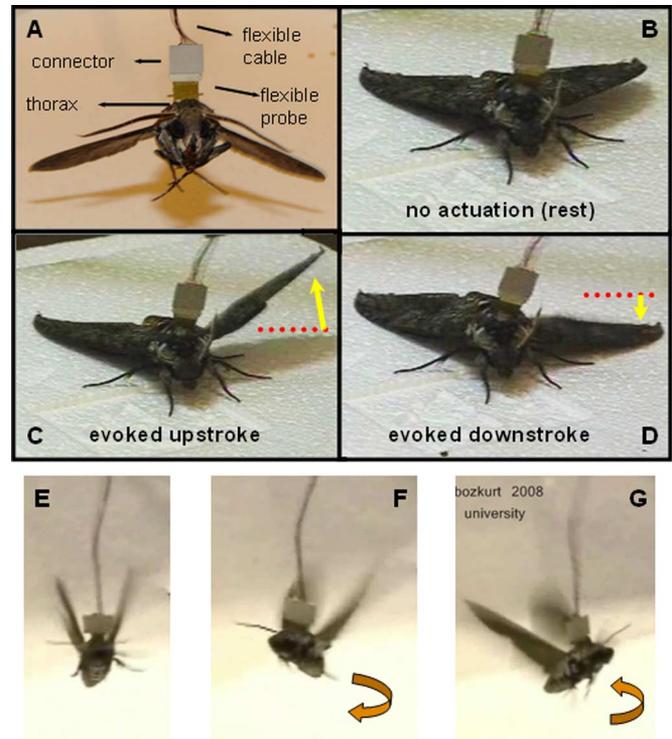


Fig. 7. The evoked up- and downstroke of a “single” wing (A–D) obtained by applying 5 V pulses to the indirect flight muscles (snapshots from the recorded movie). Under natural conditions, moths flap both wings together. Observed responses to pulse application to the wings during natural flight (E–G). Responses can be best seen in movie format (supporting online material).

[Figs. 7(E)–(G)]. When the *dl* and *dv* muscles were activated together, they opposed each other, stopping the wing flapping altogether. These initial results prove the concept of insect steering by applying external stimuli through implanted structures.

IX. DISCUSSION AND CONCLUSION

In this article, we demonstrated a novel neurotechnological pathway to integrate microelectronic sensing and actuation platforms on insects during metamorphosis to enable IMI. The metamorphic development not only provides an elegant and effective method of mechanically affixing artificial systems in or on an insect but also enables a reliable bioelectrical interface without any observable short-term adverse effect on insect flight behavior. Our experiments show that a reliable bioelectronic interface is realized for recording and controlling the motor function of insect muscles. As an application, we were able to demonstrate on-demand wing actuation and flight direction control using microprobes inserted through EMIT procedure, with an aim of insect navigation and domestication. For this study, excitation electrodes were directly embedded into muscle groups using a surgical method that could be completed in less than a minute. This process can be tailored for batch insertions enabling automated mass production. Moreover, the muscle tissue also develops around the inserted probes during metamorphosis, increasing the mechanical stability and integrity of the structure. Direct stimulation of brain and thoracic ganglia also should be possible with our methodology. However, stimulation of these

structures requires a more challenging and precise surgical operation during the pupal stage restricting the possibility of automated operation and diminishing mechanical integrity.

Insects demonstrate genetically programmed stereotypical behaviors triggered by environmental stimuli [27]. Therefore, as shown in this article, direct control of insect locomotion behavior through electronics is more straightforward than with larger animals (e.g., rats and monkeys). Based on the initial results presented here, more advanced electrical neuromuscular control strategies can be developed to instruct insects to navigate and to learn particular tasks using routine-operant conditioning techniques [28]. We have generated motor output by applying proprioceptive inputs directly to the peripheral neuromuscular systems. However, to stimulate behavioral responses, additional payloads can be implanted with our surgical procedure to provide exteroceptive inputs to the insect's chemical, mechanical, and visual receptors. These concepts have significant potential to train individual insects remotely to control their behavior. Hence, we validate a prototypical technology using insect muscle for controlled insect locomotion, which could lead to insect domestication as modern "beasts of burden," to carry information processing electronics and sensors. Moreover, electronics can be used for biological and environmental sensing by tapping into the sensory systems of the insects and using insects own natural receptors. Controlling motor function of invertebrates while also simultaneously recording from its natural sensors enables a vast number of applications for various scientific and engineering studies.

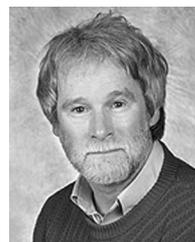
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