INSECT-MACHINE INTERFACING

by

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DEDICATION

To my parents, Timoteo and Martha Melano.

TABLE OF CONTENTS

ABSTRACT

A terrestrial robotic electrophysiology platform has been developed that can hold a moth (*Manduca sexta*), record signals from its brain or muscles, and use these signals to control the rotation of the robot. All signal processing (electrophysiology, spike detection, and robotic control) was performed onboard the robot with custom designed electronic circuits. Wireless telemetry allowed remote communication with the robot. In this study, we interfaced directionally-sensitive visual neurons and pleurodorsal steering muscles of the mesothorax with the robot and used the spike rate of these signals to control its rotation, thereby emulating the classical optomotor response known from studies of the fly visual system. The interfacing of insect and machine can contribute to our understanding of the neurobiological processes underlying behavior and also suggest promising advancements in biosensors and human brain-machine interfaces.

CHAPTER 1

Introduction

This dissertation describes a system developed to interface an insect and a machine, such that bioelectric signals from the insect are interpreted by a central processing unit and used to control a terrestrial robot. The insect chosen for this study was the large hawkmoth *Manduca sexta*. Its large size (up to 2.75 inches in length) and well-mapped neural and flight muscle system makes it an excellent candidate for electrophysiological studies of the brain or muscle, or both. Our system was developed to be controlled by either responses from one or more neurons (via an extracellular tungsten electrode) or a muscle (via a thin copper wire electrode). The robot carried an electrophysiology printed circuit board (PCB) with three extracellular amplifier channels and a microcontroller. This system was able to detect spikes, calculate the spike rate of bioelectric signals and send binary commands to the robot. The moth-machine hybrid was largely developed and tested in a cylindrical arena of similar nature to those used for classical insect optokinetic studies. The novelty of this system is its potential use for closed-loop studies of neural systems while also providing mechanosensory feedback related to turning. Projected as an application in the future design of robotics, the present system contributes to the area of study of biosensors and brain-machine interfacing.

1.1 Biology and Control Theory

Brains remain the most complex, and least understood, computing systems. The most complex of brains, that of ourselves, underlies personalities, behaviors, thoughts, memories and social interactions. Yet, because of ethical limitations it is essentially off limits for experimental manipulation. Also, its enormous complexity raises numerous challenges to our understanding: from relatively simple sensorimotor integrative functions to the most complex functions of memory and intellect. In order to better understand the general mechanisms that apply to brains, both human and animal, we are obliged to use non-human model systems. This is on the assumption that there has been one evolutionary origin of all nervous systems, implying commonalities of neuronal function across animal phyla (Denes et al., 2007). Invasive experiments, such as those that penetrate the brain to record electrical and/or chemical signals, cannot of course be performed in humans. However, these types of experiments are important because insights gained from these can help explain the mechanisms that control psychophysical behaviors. Our understanding of how nerve cells and nervous systems work are based on knowledge gained from such invasive experiments. Studies using animal model systems can contribute to therapies for human diseases in cases where neuronal mechanisms are dysfunctional.

In the field of neuroethology, which is a discipline of neuroscience, the ultimate goal is to understand the neural basis that underlies the generation and control of behaviors, ranging from sensory perception and central processing, to motor outputs. Investigations in this field usually are based on identifiable, quantifiable and stereotypical behaviors displayed by an animal. To investigate these neural mechanisms, one can either begin by looking at the neuroanatomy and physiology to infer how the neuronal architecture might lead to behaviors or one can design behavioral experiments that can be used to infer qualities of the neural pathways without actually resolving them as structures. However, the most informative methods employ a combination of anatomical, behavioral, electrophysiological, pharmacological or ablation techniques (including genetic modifications of brains or neurons in animals amenable to these approaches).

Needless to say, even those experiments that attempt to combine any of these techniques are likely to be limited. For example, a trivial phenomenon such as mechanical vibrations can present huge challenges in electrophysiological recording. This is because intra- and extracellular recordings are sensitive to vibrations and most preparations require the experimental animal to be fixed in place, semi-mobile and constrained on an anti-vibration table. Although such preparations usually do not interfere with primary sensory areas, and are therefore suited for single cell studies of primary sensory systems, they leave much to be desired when it comes to relating the responses of single neurons to a natural behavior, even at the level of multijoint movement. Few behaviors remain intact under such constrained conditions, an example being the jamming avoidance responses of electric fish (Heiligenberg, 1991). In general, physical restriction of the animal preparation will present problems, because the animal and its sensory neurons may respond to stimuli differently than they would in the unconstrained animal (Hitschfeld et al., 2009).

As an animal navigates in an environment, its behavior is controlled and coordinated by sensory-motor loops between the perception of stimuli, their processing by the central nervous system (including integration of the ambient context of environmental conditions and previous experience), the execution of motor actions, and sensory feedback to the brain about those actions. While empirical models based on experimental data are a useful basis for investigations of neural information cascades, mathematical models require precise identification and rigorous definition of vague concepts and can therefore lead to an understanding of the dynamics of the neural system in question. A subfield of engineering that can be usefully applied for creating models of neural sensory motor loops is called Control Theory. Control Theory has a framework for approaching problems that break complex systems into individual components, including feedback, and analyzing how these affect the dynamics of the system as a whole (Kuo, 1991).

At its most rudimentary level, Control Theory begins with conceptualizing a system as a black box with inputs and outputs. The types of inputs the system responds to and the form of the outputs it generates are directly related to what is inside the black box. To get a basic understanding of what is inside this black box, one can first investigate the response of the system to changing conditions (also known as transient analysis) and constant conditions (also known at the steady-state response; Kuo, 1991). When the input to the system is suddenly changed from one state to another, the output will also change over a transitory time-period before settling to a steady response pattern. Given a single system, one can begin to imagine that many systems can be linked, with the outputs of one system becoming the inputs of others. Furthermore, information from one system can serve as feedback to others. An entire system of interconnected components can be modeled given knowledge of the individual parts.

Phenomenologically speaking, living organisms are also systems with inputs, outputs and many computational networks between. Organisms with visual, olfactory, or other sensory systems receive input from their environment and form internal representations of their world. Any sort of action, for example a motor behavior, that is released by these sensory inputs can be considered as a response to these particular inputs and can thus serve to analyze the organism's input-output relation. Everything that happens in the transformation between information from the physical environment into sensory percepts and the corresponding behavior is analogous to a black box in Control Theory. Perhaps at the highest level of the analysis of biological systems are human psychophysical studies. In these studies, the inner workings of the human brain are probed with controlled sensory inputs while the participants are asked to respond according to what they perceive. Neuroethological studies of whole animal preparations, while not delving into what the animal "thinks," usually rely on a preparation, in which specific patterns of stimuli reliably elicit behaviors. These studies nonetheless investigate the inner computations of these reflexive behaviors.

1.2 Examples of Experimental Approaches in Biological Control Theory

To further illustrate the application of systems analysis to organisms, the physicist Werner Reichardt was one of the first scientists to apply systems analysis to the phenomenology of the insect's response to visual motion in its environment (Flynn, 1999). As director of a Max Planck Institute, Werner Reichardt and his colleagues pioneered systems analysis, using modern physics approaches, to study the relationship between defined visual stimuli and quantifiable behavior of flies (Poggio, 1993). His initial and famous work leading to the optomotor "motion detection circuit" was done in collaboration with Bernard Hassenstein. Together the two developed a mathematical model that largely predicted the way in which insects compensate their velocity to involuntary disturbances in their desired trajectory (Hassenstein and Reichardt, 1951). These experiments were carried out by gluing a beetle to a stick and placing it inside a rotatable drum. Rotating the drum caused the insect to mistakenly perceive self-motion in the opposite direction. Whenever this occurred the insect's response was to compensate the undesired motion stimulus by walking in the direction that would negate this perceived change in trajectory. The mathematical model to explain insect visuo-motor control was based on a delay-and-compare operation of light intensity signals as measured by two neighboring photoreceptors: the signal of one photoreceptor was delayed with a low pass filter and compared by multiplication with a non-delayed signal of the other (Borst, 2000). In order to get a fully directional model, first, two of these delay-and-compare operations were paired in a mirror-symmetric fashion. Next the outputs of these operations were subtracted.

In the analysis of a system there are four main items with which to be concerned: namely, a description of the type of inputs, the type of outputs, the mathematical operation of the system and the scalars that scale the mathematical operation. These can form a sound understanding of the system's dynamics. Using the Reichardt model as an example, the input would be the light intensities from the visual environment, the output would be the direction of turn (left or right), the mathematical operation would be the delay-and-compare mechanisms, and the numerical values would be the cut-off frequencies assigned to the low pass filters as well as the distance between the photoreceptors. Scientists are usually confronted with a situation in which the input and output are observable but the mathematical operation and scalars - the inner computations - are not, much like the way it is with living organisms. In order to approach a solution the scientist will isolate as well as possible the individual components within the black box, and apply carefully designed patterns of inputs and observe the outputs. If the scientist has designed the inputs carefully and on a sound hypothesis, the results of the experiment will allow the scientist to formulate a model with numerical constants. The observer will know the model is successful when the results closely match the observed data and withstand future experiments that attempt to disprove the model. Furthermore, a successful model will lead to unforeseen predictions and insights.

It is the isolation of a particular component within a system that can be most difficult. The structure of a central nervous system is microscopic, entangled and fragile. Because of its complexity and vulnerability to destruction by physical probing, the individual components of a neural control system can be complicated to decipher and difficult to isolate. While isolating the computational components within an organism is difficult, it is not impossible. Investigations into the fly's optomotor response eventually gave way to discovering a set of nerve cells, named lobula plate tangential cells, whose neural response correlates with the optomotor behavior (Collett and Blest, 1966; Bishop and Keehn, 1967; Dvorak et al., 1975). Visual motion that covers large portions of the visual field modulates the activity of these neurons. Furthermore, the response of these neurons fit well with Reichardt's correlation model thus strongly suggesting that these cells participate in the optomotor behavior that was also observed in flies.

In order to observe the electrophysiological response of these cells, the fly must be securely fixed in place with its head tilted forward. Any sort of mechanical disturbances must be strictly avoided since they make recording from these cells difficult and highly unlikely. Studies on these cells represent the isolation of an important computational step within the overall visual flight control of the fly. Much is now known about the properties of these motion detection cells, such as signal gain adaptation (Maddess and Laughlin, 1985) and their spatiotemporal receptive fields (Weber et al., 2010). Although these results are important, most of what is known about these cells was gathered while the insect was restrained. To put this condition in terms of Control Theory, the results of studies on lobula plate tangential cells were gathered under open-loop conditions, i.e. the output from the neuron in question is not involved in the control of the insect's behavioral nor are its inputs equivalent to those under normal behavioral circumstances. While studies under open-loop conditions are important, they provide a limited perspective if the goal is to understand how the cell responds when the dynamics of their inputs are naturalistic or if the goal is to understand the neural basis of natural behavior.

Control Theory provides a framework for documenting the dynamics of systems with mathematical operations. Closed-loop models can be predicted from the response of components while they are static, i.e. under open-loop conditions. One example is the modeling results of *Drosophila* flight that integrates the dynamics of the visual system and the aerodynamics of the wings and body (Reiser et al., 2005). The advantage of closed-loop models is that they can be quite complex and integrate several input and output lines, while also including many intermediary feed-forward and feed-back processing steps. Ultimately, accurate modeling of a system makes a strong statement about the level of understanding of its internal workings.

1.3 Brain-Machine Interfacing

A very important advance in the field of medical devices is the creation of brain-machine interfaces (BMI; also known as brain-computer interfaces). In brain-machine interfaces, neural signals that represent a subject's particular intention or mental task are acquired, processed and interpreted by a computer. This information is then used to control a machine (e.g. an artificial limb). Born of the extensive knowledge of basic neural coding principles developed by neuroscientists and the advances in computers, mathematics and robotics, brain-machine interfaces promise new directions in the augmentation of human abilities as well as significantly improving the lives of physically disabled people. The device being controlled by the brain can either be a real machine, like a wheelchair or a robotic limb, or it can be a virtual machine, like an alphabetic communication software or a web browser. By providing an interaction link with the outside, people suffering from motor disabilities due to traumatic lesions to the spinal cord, stroke, and degenerative neuromuscular diseases will be able to regain many of their abilities that were lost. Likewise, sensory functions can be restored by interfacing with the brain (e.g. hearing aids based on cochlear implants).

There are many variations of neural signals that are currently being used as sources for control signals. The neural signals available are intra-cortical multi-unit activity, electrocorticogram, and electroencephalogram. The electroencephalogram, which is acquired by electrodes placed on the surface of the scalp, is the best non-invasive signal available at the moment. The other signals at the very least require a craniotomy. Electrocorticograms are signals recorded by a grid of electrodes placed on the surface of the cortex, which offers an improvement of signal quality over the electroencephalogram whose signals must travel through the skull. The signal with the highest spatial resolution is the intra-cortical signal, which is produced from thin, sharp electrodes that penetrate into the brain. This renders this method of signal acquisition the most invasive and most hazardous.

Two main branches of BMI fall under those that utilize EEG and those that utilize intracortical recordings (Millán et al., 2010). Both have their advantages and disadvantages, and their respective advantages are sufficient enough to continue research in these two directions. The advantage of EEG is that it is not invasive and the signal at the surface of the scalp is good enough to be used for machine control. The disadvantage is that the signal quality and information transmission rate is quite low, which is mainly due to the sampling of neural ensembles having nothing to do with the task at hand and the signal degradation caused by bone and tissue between the brain and the electrode (Niedermeyer and Silva, 2005). The main advantage of intracortical recordings is that they provide highly localized and specific neural information. The main disadvantage with intracortical recordings is that they require a highly invasive procedure that carries a risk of infection and permanent tissue damage (Lebedev and Nicolelis, 2006). Scarring around the electrode and tissue movement may also cause the intracortical signal to degrade. Ultimately these two methods will probably be applied according to individual circumstances and therefore research in these areas is necessary and important.

While the field of BMI has progressed tremendously since the first demonstration that cortical activity could be used to control a robotic manipulator (Chapin et al., 1999), many problems must still be solved before BMIs are brought to clinical trials and are marketed. There are major hurdles that the field of BMI must tackle (Lebedev and Nicolelis, 2006). There are issues such as obtaining long-term recordings from hundreds to thousands of neurons, developing computationally efficient algorithms that translate neural activity into real-world actions, and learning how to best take advantage of the brain's ability to learn to control extracorporeal objects.

It is generally assumed that human BMIs will be composed of an implantable component that will wirelessly transmit neural information transcutaneously to another component that will do most of the heavy computing and machine control. Within the scope of computationally efficient algorithms exists the drive to compress the size of data extracted from the brain in order maximize the information transmitted from within the body. Whether a BMI is detecting multi-unit activity, or a single neuronal unit, the question of how to process and compress neural activity on a single electrode is an important issue. Of particular interest has been the issue of fast and efficient detection of spikes (Obeid and Wolf, 2004; Watkins et al., 2004).

1.4 Biosensors for Robots

For many years scientists and engineers have been investigating methods for using insects as sensors. Insects are capable of being trained via classical conditioning methods to exhibit behavioral changes in response to odors that have no natural relevance to an insect's survival (Rains et al., 2008). Many studies have demonstrated that honey bees, moths and wasps can be trained to exhibit behavioral responses, such as a foraging response or a proboscis extension response, to volatiles of explosives when the odor is associated with a food reward.

Insects sensory systems present several advantages over man-made electronic sensors. For instance, insects have been shown to be more sensitive and energy efficient than current man-made sensors. In a study that compared the olfactory sensitivity limits of the wasp, as measured by their antennating search behavior, and an electronic nose found that the wasp's response limit was at least 74 times more sensitive (Rains et al., 2004). Another study that compared the energy cost of information transfer of a fly photoreceptor model to a silicon photoreceptor circuit model determined that energetic costs of the silicon model were approximately 10 times higher (Abshire and Andreou, 2002). Therefore, besides using an entire insect organism and its behavior as the indicator of a target signal source, scientists have utilized insect primary sensory afferents as the sensor while excluding the rest of the body. Such is the case with the antennae of the male silk moth. The male silk moth antennae are extremely sensitive to particular, species-specific pheromones, being able to detect the presence of only a few molecules. The neurons in the antennae continue to respond to pheromone when they have been amputated from the moth. Therefore, by interfacing amputated male silk moth antennae to a robot, it has been demonstrated by scientists that robots programmed to interpret the electrical activity of these "biological sensors" can also track pheromone plumes (Kuwana et al., 1995).

1.5 Guide to Thesis

The primary purpose of Chapter Two is to build a foundation for understanding the two papers appended to the end of this dissertation. I give an overview of what is known about visual modulation of neural activity within the hawkmoth *Manduca sexta*, the model system used in this study. I begin by describing neurons in the visual areas of the moth's brain that respond to motion, and continue with neurons further downstream that display similar properties. I then describe how this activity manifests itself in EMG recordings of the muscles involved in the steering of flight. Through this description I demonstrate evidence for the flow of information from early processing centers, to midstream processing centers and finally to the behavioral output.

Next I discuss a critical component at the interface of spiking neurons and machines. As we were developing our robotic system it became obvious that our initial spike detection system needed to be capable of adjusting itself to the changes a neural signal undergoes during robotic motion. I include an account on the properties of neural signals as a necessary prerequisite to understanding the logic behind choosing an appropriate spike detection method. To give context to my contribution to the field of spike detection, I review prominent spike detection methods used to enhance the neural signal and facilitate the detection of spikes.

In Chapter Three, Appendix A, and Appendix B, I present two papers that contain my major contributions. In the first paper I describe the robot that I helped develop. I also present data that demonstrates proof that I was able to control this robot with neural and muscular signals. Although there are now several publications in the scientific literature on the interfacing of primate brains to machines, this was the first time a brain signal from an insect was used to control a machine. Furthermore, I present data that shows that EMGs from a steering muscle were used to control the robot. The implications of these results are many. Firstly, this work represents a technological development in the direction of creating mobile electrophysiological platforms that essentially give a neural system a synthetic body with which to behave. Secondly, this work further supports the pre-existing idea of using the sensory system of insects as biosensors for robots and machines.

In the second paper I present the adaptive spike detection system that we appended to our initial spike detector. During my experiments I continuously found that motion of the robot was detrimental to maintaining the amplitude of the neural signal. But in many of the cases where motion caused the neural signal to decrease in amplitude, there were still spikes that could have been detected if the spike detection threshold was quickly lowered. Therefore the system was composed of hardware and software that automatically adjusted the spike detection threshold according to the maximum height of the neural signal. This adaptive spike detector belongs to a new class of spike detection systems that are hardware-based and that are well suited for brain-machine interfaces. This adaptive spike detection system also represents an unexpected outcome of my project. By solving the challenges with interfacing an insect brain to a machine, we developed a spike detection system that can be applied to human brain-machine interfaces.

Finally, in Chapter Four I include three studies that can be performed with the system described in Appendix A.

CHAPTER 2

Background

2.1 Neurobiology of *Manduca sexta*

Although this species is well known as a model system for olfaction from over 100 publications by J. G. Hildebrand and collaborators (three examples are: Vickers et al., 2001; Christensen and Hildebrand, 1987; Homberg et al., 1991), there is also a body of work that has focused on the visual system of this and closely related species of hawkmoths. In fact, the first recordings of visual motion sensitive neurons were performed in the late 1960s using the privet hawkmoth *Sphinx ligustri* (Collett and Blest, 1966), and the neuroanatomy of the moth optic lobes was published a few years later (Strausfeld and Blest, 1970). The behavioral repertoire of the moth appears quite sophisticated to us, at least in comparison to insects such as houseflies and even dragon flies, which mainly employ ballistic trajectories. Diurnal hawkmoths can learn to discriminate colors after two trial learning, a performance that surpasses that of honey bees (Kelber, 1996). Visual control of flight includes the ability to hover and feed from flowers while maintaining station: making corrective maneuvers to compensate movements of the nectar source, in front of which the animal is hovering (Kern and Varju, 1998). Investigations into the neural basis of this extraordinarily precise compensatory behavior have led to electrophysiological and morphological descriptions of looming neurons (Wicklein and Strausfeld, 2000) and wide-field motion-sensitive neurons tuned to vertical and horizontal motion (Wicklein and Varju, 1999). The response of these wide-field motion-sensitive neurons is marked by an increase in spike rate in the preferred direction, and a decrease from the spontaneous spike rate in the opposite direction, or increases and decreases in firing in response to expanding or contracting visual stimuli (see also: Collett and Blest, 1966; Wicklein and Varju, 1999). Information about visual motion that has been computed in the brain's optic lobes is relayed by descending neurons to motor circuits in the thorax that control direct muscles involved in modulating the attitude and vault of the wings, and thus steer flight direction (Rind, 1983; Kern, 1998).

One of the pairs of mirror symmetric neurons identified in the ventral nerve chord of *Manduca sexta* has dendritic arborizations that originate in the protocerebrum of the brain and whose bushy terminals are found in the meso- and meta-thoracic ganglia, as well as the abdominal ganglia (Rind, 1983). Such neurons are crucial in our understanding of what kind of data is relayed to motor neurons and muscles. And while the present study focuses more on muscle responses, such premotor elements will provide important targets for future research. These neurons demonstrate responses to bilateral receptive fields and are thus similar to binocularly receptive motion sensitive tangential cells in the lobula plate of flies (Hausen, 1982). The behavioral response induced by visual stimuli, which has been identified in flies and hawkmoths, is strongly suggestive of the optokinetic response typifying human eye movements. A separate study of *Macroglossum stellatarum*, a relative of *Manduca sexta*, identified a wide range of descending neurons that respond to various orientations to wide-field motion stimuli (Kern, 1998). Given the moth's need for quick reaction to dynamic stimuli (e.g. visual motion, wind) it is not surprising that the moth's control of flight muscles transduces visual information almost directly, with very few synaptic delays, between the retina and the motor neuron (Land and Collett, 1974).

Studies on the activity of muscles during tethered flight have revealed that a particular set of three muscles, the third axillary pleurodorsal muscles of the second segment of the thorax, are correlated with the amount of retraction of the moth's wings (promotion or remotion; Kammer, 1971). Insect flight is an orchestration of the mechanical structures of the thorax and the muscles contained therein. Two systems play a major role in flight, the indirect flight muscles, whose role is to generate power, and the direct flight muscles, which are involved in steering and of which the third axillary muscles belong to. The fine directional control of flight in insects stems from the direct flight muscles that anchor to the thorax and attach to cuticular structures that form the base of the wing. The direct flight system is a complex system of muscles, cuticular specializations and ligaments at the wing hinge that allow the variation of wing path, supination, pronation, remotion and promotion (Kammer, 1971). Evidence also suggests that the abdomen acts as a rudder during flight, thus adding further neuromuscular components in the control of moth flight (Gray et al., 2002; Mavoori et al., 2004).

The third axillary sclerite and its accompanying muscles are an important component of wing control. These muscles are attached at various points on the sclerite and are anchored anteriorly on the episternum and epimeron such that contraction of these muscles cause the wing to retract. The third axillary muscular system is composed of three distinct bundles. The larger bundle, the II PDu, is attached most anteriorly on the episternum while the two smaller bundles, the II PDm and II PDu, are attached on the dorsal area of the epimeron (nomenclature after Wendler et al., 1993) where II indicates the second thoracic segment, i.e. the mesothorax, the PD is pleurodorsal and u, m, l indicate upper, middle, and lower, respectively). Each unit of the II PD muscle is innervated by one motor neuron (Rheuben and Kammer, 1987). What makes the third axillary muscle group most intriguing is the fact that the middle unit, II PDm, is spontaneously active in the quiescent animal. Furthermore, it has been shown that this activity in the quiescent animal is modulated by visual stimuli that represents yaw, roll, and forward thrust (Wendler et al., 1993).

2.2 Properties of the Neural Signal

Measuring neural activity is performed with a conductive material placed in living tissue. The conductive material, which is commonly a sharp metal electrode or glass capillary, is connected to an electronic circuit that amplifies the electrical potential changes within the extracellular field of the electrode tip. The typical action potential signal recorded by the amplifier will have a characteristic fast deflection followed by slower recovery period toward the DC baseline. Neural spike widths are between 0.3 ms to 3ms while the peak-to-peak amplitude can vary between 50 μV to 500 μV (Rogers and Harris, 2004). Although, neural spikes can be smaller than 50 μV and can occasionally be larger than 500 μV (W. Gronenberg, personal communication). The distance between the source of the signal and the electrode tip can affect the size and shape of the neural spike. The type of biomass between the electrode and the cell can also have an effect on the size and shape of the action potential. The frequency at which neurons can spike varies but is certainly limited by the cell's refractory period, which is greater than 1 ms for primate cells (Lewicki, 1998).

It is also common for there to be neural spikes from different sources within the electrode tip's volume of sensitivity. Usually they are distinguishable by shape and size, but often enough these spike signals fire within the refractory period and overlap. The average rate of overlap for x neurons with spike duration of Δt seconds and with an average firing frequency of y is given by $y^2 \Delta t(x-1)$ (Chandra and Optican, 1997). The resulting superposition of waveforms affects the geometrical attribute of the signal, including the peak-to-peak amplitude of the signal.

Besides the occasional neural spike, a normal recording from a brain will also contain background noise. Large levels of background noise often prohibit measuring neural activity. Background noise, at its best, contains weak signals that originate from distant neural and muscle activity that exists globally in the extracellular medium. Other non-biological sources may enter and pollute the electrophysiological signal. These sources are largely electromagnetic signals that come from power lines, motors, lights, computers, etc. High levels of noise pollution, more accurately measured as the signal-to-noise ratio (i.e. SNR), can greatly handicap the ability to extract any useful information from the neural signal.

2.3 Methods of Spike Detection

Spike detection is the correct identification of neural spiking events in time. The process of spike detection can be implemented in software, hardware or a combination of the two. There are several issues that must be considered in selecting which method to employ. These issues include the nature of the neural signal, the computational costs of the spike detection algorithm and, most importantly, the application for which it is intended. Traditionally, spike detection was a method that enabled the study of the temporal properties of extracellular neural recordings (Kim and Kim, 2003). With the increasing access of computers, many mathematical techniques have been proposed to automate and increase the robustness and reliability of spike detection (Lewicki, 1998). Overall, many software based methods for spike detection implement variations of methods such as wavelet transforms (Hulata et al., 2002; Yang and Shamma, 1988; Kim and Kim, 2003), statistical based thresholding (Chandra and Optican, 1997; Yang and Shamma, 1988; Snider and Bonds, 1998; Kaneko et al., 1999), and the use of the non-linear energy operator (Kim and Kim, 2000; Mukhopadhyay and Ray, 1998). These methods are typically used as a way to enhance the neural signal and suppress the background noise. But more recently, efforts have been directed toward spike detectors within the framework of brain-machine interfacing (Harrison, 2003; Obeid and Wolf, 2004; Rogers and Harris, 2004; Watkins et al., 2004).

2.3.1 The Wavelet Transform

The wavelet transform is a linear transform that appears many times in the spike detection literature. Unlike the Fourier transform, which loses temporal information once a signal is transformed into the frequency domain, the wavelet transform is able to preserve temporal information while elucidating the spectral properties of a signal. The general equation of the wavelet transform is as follows: $W(a, b) =$

$\int_{-\infty}^{\infty} f(t)\frac{1}{\sqrt{a}}\psi_{a,b}(t)dt$, where $\psi_{a,b}(t) = \psi(\frac{t-b}{a})$. The most relevant components are ψ, a and b. The function ψ is called the mother wavelet, so called because of its short length and because it is the adjustable template used when transforming the signal into the temporal-spectral domain. There are several templates for the wavelet transform available, for instance the Coiflet mother wavelet, or the Haar mother wavelet. The mother wavelet is chosen based on the problem at hand. The scale parameter a is a scalar that dilates or stretches the mother wavelet along the time axis. When performing the transform the original signal is projected onto several wavelets of various scales. These scales are created on a logarithmic base chosen by the user. For example, if the base scale is 3 then the first scale is 3, the next scale is 9, the next scale is 27, and so forth. If 2 is chosen, then the scales used will be 2, 4, 8, 16 and so forth. Therefore the scalars always take the values of a^i, where $i = 1, 2, 3, ...$. The base wavelet stretches at higher scalar values in the same manner that a function will expand or contract when its input variable is scaled. Out of convenience, 2 is the most common scalar base. The parameter b is the translation parameter and determines the time location of the wavelet along the signal being processed. It is also worth mentioning that by virtue of being a linear transform, an inverse wavelet transform can be used to reconstruct the original waveform.

The wavelet transform is a popular method to emphasize neural spikes and suppress background noise since neural spikes are short, high frequency pulses that lend themselves to wavelet transform analysis. Hulata *et al.* corroborates this statement because, according to them, neural spikes are localized functions analogous to the wavelet bases (Hulata et al., 2002). Furthermore, when the SNR is low and the statistical properties of the background noise and the neural spikes are similar, the wavelet transform can be more effective as a preprocessor than traditional filtering techniques, e.g. band pass filtering (Kim and Kim, 2003).

According to Yang and Shamma (1988), the wavelet transform is indeed a powerful method of detecting spikes because of the spike-like characteristics of the transform bases, i.e. the bases formed by the wavelets. In their study they performed a wavelet transform on neural signals and applied a threshold to select those regions of the transformed region that correspond to neural spikes. After thresholding the wavelet-domain signal, they performed an inverse transform to reconstruct the denoised neural signal. Next they thresholded the signal again and perform spike sorting.

In an attempt to detect spikes at very low SNR (SNR = 1.5-3), Kim and Kim (2003) designed an algorithm that multiplied the output coefficients of wavelet scales. In this method, the base scale, a, was 2^i. The first scale chosen was the one that produced the largest coefficient. The subsequent two scales that were chosen were the two adjacent smaller scales. For example, if the coefficient of 2^4 produced the largest coefficient, then the two other scalars that were chosen were

2^3 and 2^2. The coefficients from these three scales were then multiplied and passed through a threshold.

Although the wavelet transform allows the simultaneous display of spectral and temporal information, it can fall short in the case of detecting overlapping spikes. The explanation for this is that larger values of a, i.e. the scaling factor that determines the width of the wavelet, increase the width of the wavelet. This is turn increases the probability that two overlapping spikes will fall within the time window of the wavelet.

This problem is related to the Uncertainty Principle, which states that the exact momentum and the position of a particle cannot be known simultaneously. The analogy to signals is that the instantaneous frequency at an instantaneous point in time cannot be known. What can be known are the band of frequencies that existed during a period of time. To be specific, this principle applies to signals by the following equation: $\Delta\omega\Delta t \leq 2\pi$.

To address the problem of overlapping spikes, Hulata et al. (2002) used a mathematical technique, called Wavelet Packets Decomposition (WPD), which is an expanded version of the wavelet transform, and created an optimal set of basis vectors that best highlighted the characteristic features of neural spikes. The wavelet transform divides the whole frequency domain with one wavelet for each scale. Each wavelet, which can also be seen as a filter, has a bandwidth of $\Delta\omega = \frac{\pi}{aT_s}$, where a is the scale and T_s is the signal sampling period. Each bandwidth begins at 0 hertz, i.e. at DC.

WPD is different in that each scale is used to create multiple bandwidths that cover the entire frequency domain. In other words, each scale, which has a bandwidth of $\Delta\omega$ divides the frequency domain $\frac{2\pi}{\Delta\omega}$ times, thus forming filters of equal bandwidth that cover the frequency domain from 0 to $\Delta\omega$, from $\Delta\omega$ to $2\Delta\omega$, from $2\Delta\omega$ to $3\Delta\omega$ and so forth. Therefore each scale is used to cover the frequency domain into blocks of $[j\Delta\omega, (j+1)\Delta\omega]$, where $j = 0, 1, ... \frac{2\pi}{\Delta\omega}$.

The WPD creates overlapping divisions of bandwidths since each scale creates several blocks that cover the entire frequency domain. In order to create an optimal, non-overlapping set of bases filters, Hulata et al. (2002) utilized a “best basis algorithm” method to select the smallest number of bandwidths that best spanned the neural signal.

2.3.2 The Nonlinear Energy Operator

The Nonlinear Energy Operator (NEO) is another neural spike emphasis algorithm. This method exploits the instantaneous rise in amplitude and frequency of the neural spike. The formula that forms the basis of this energy analysis is: $\Psi[x(t)] = (\frac{dx(t)}{dt})^2 - x(t)(\frac{d^2x(t)}{dt^2})$. By looking at the kinetic equations of a simple harmonic oscillator it can be shown that the output of the NEO is proportional to the amplitude and frequency squared of the signal (Maragos et al., 1993). A simple harmonic oscillator with only a spring and mass can be described with the equation $m\frac{d^2x(t)}{dt^2} + kx = 0$, where m is the mass and k is the spring constant. A solution for this second-order differential equation is $x(t) = Acos(\omega_0 t + \theta)$, where A is the amplitude of the oscillatory motion, ω_0 is the frequency of the system, and θ is the phase of the oscillation. The constant ω_0 can be determined from the mass (m) and the spring constant (k) and is equal to $\sqrt{\frac{k}{m}}$. When the NEO is applied to $x(t)$ the following result develops: $\Psi[Acos(\omega_0 t + \theta)] = (A\omega_0)^2$. Therefore, the output of the operator is proportional to the square of the amplitude and frequency of the oscillation (Maragos et al., 1993).

There have been numerous studies that highlight the effectiveness of the NEO processor as a computationally light spike enhancer. Mukhopadhyay and Ray (1998) studied the ability of the NEO to accentuate spikes of various temporal widths and found that even at large widths the NEO was able to give low false positives and false negatives. Furthermore, they compared the NEO to other processors and found that the NEO's gain in spike signal versus background noise was greater than the other algorithms. Kim and Kim (2000) made the case for the NEO as a spike enhancer at very low SNR (SNR $\approx$ 1.0). In their study they demonstrated the tremendous gain in SNR when the NEO was employed. For instance, when the input SNR was 1.4, the emphasis was such that the gain in SNR was 10. When the input SNR was

2.0, the output SNR was 80. This boost in SNR allowed an 80 percent probability of detecting a spike when the SNR was only 1.1. Kim and Kim (2000) did make the point that this algorithm may not perform well when the signal has both low SNR and when the background noise and the spike being detected have similar spectral properties. But as Mukhopadhyay and Ray (1998) state, this algorithm is particularly attractive because of its low computational complexity and its usability in the real-time and simultaneous processing of multiple channels.

2.3.3 Thresholding based on Statistical Distributions of the Signal

The wavelet transform and NEO preprocessing methods effectively suppress noise and enhance neural spikes. Nonetheless, a threshold must still be applied to the processed signal in order to correctly identify spiking events. There have been many different thresholding strategies for appropriately selecting parts of the raw signal that contain a spiking event of interest. For instance in the Wavelet and NEO studies by Kim and Kim (2000, 2003) the threshold was set to a level that correctly identified a segment of 20 action potentials and simultaneously minimized the number of false positives. In the study by Mukhopadhyay and Ray (1998) they set the threshold manually to a scaled value of the mean of the NEO processed signal.

A more mathematical approach to setting a threshold to detect spikes is one in which the threshold is set based on the statistical properties of the signal. Early in the field of spike detection it was noted that neural signals, as well as the background noise, could be separated based on histograms of their amplitude distributions (Heetderks, 1978). Furthermore, these distributions seemed to exhibit a normal, i.e. Gaussian, distribution. Since then, many studies have used this finding to set thresholds for detecting neural events. For instance, Bankman and Menkes (1992) developed an algorithm that automatically separated the background noise from spiking segments in order to obtain an accurate estimate of the background noise distribution and set an appropriate threshold that avoids false positives. In their study on the application of the wavelet transform as a spike enhancing algorithm, Yang and Shamma (1988) assumed that the noise was independently and identically distributed with common Gaussian distribution and then applied a non-linear threshold scheme that effectively removed segments of the signal less than 4.1 standard deviations above the mean.

2.3.4 Spike Detection for Brain-Machine Interfaces

The need for spike detection can be driven by different applications and therefore different demands. In the case of purely scientific studies, spike detection methods can afford to be computationally intense since processing does not need to be done in real-time and can therefore be performed off-line. In fact, some algorithms are specifically designed to be performed off-line. This is clearly not appropriate for methods that require information about the neural signal in real time. According to Kim and McNames (2007) there are clinical applications where neurosurgeons prefer to detect the most dominant spike. In situations where a neurosurgeon is performing electrophysiology on a human patient, it is critical that the spike detection algorithm achieve accurate and immediate results. But given that physical space is not a constraint in the surgery room setting and also that computational resources are no longer issues, achieving real-time and adaptable spike detection in the medical setting is not a difficult challenge.

With the advent of brain-machine interfacing, there are many new constraints that pose interesting challenges for real-time spike detection. In human brain-machine interfaces, the ultimate goal is to achieve an implantable computer that amplifies neural signals and transmits information about these neural signals to the machine that is to be controlled (Obeid et al., 2003). Under these circumstances space, power consumption and bandwidth for transmitting digital information are highly constrained (Obeid et al., 2003).

Rather than transmitting the entire neural record, an effective spike detection algorithm will enable a massive compression of data transmitted from inside the body by communicating only spike shapes and their timing. This further enables the ability to record from more electrodes and the probability of recording from more neurons.

In response to these challenges, new solutions that address the specific needs of human brain-machine interfaces have been proposed. The creation of new analog Very Large Scale Integrated (VLSI) circuits for spike detection may provide key solutions posed by the constraints of implantable brain-machine interfaces. The strength of these devices is that they are ultra-low consumers of power, which maximizes the life of a power supply and minimizes heat dissipation. There have been at least two hardware solutions that have been proposed as prototypes for feasible spike detectors for brain-machine interfaces.

The first one was composed of two low-pass filters, a subtraction stage and an adjustable-threshold comparator (Rogers and Harris, 2004). In this detector, the raw signal was passed to both low-pass filters. One filter had a high cut-off frequency that was meant to remove high frequency noise and the other had a low cut-off frequency that removed all high frequency content and created a local average. The difference of the two signals was then passed to the threshold detector that emitted a digital pulse to indicate when the signal was above the threshold. The design was meant to provide robust spike detection even in situations of high frequency noise and low frequency shifting baselines. According to a simulated analysis, their chip only consumed $1\mu W$ of power and achieved 90 percent true positives at SNR = 5.

The other hardware design was based on the concept of setting a detection threshold at a multiple of the standard deviation of the background noise (Harrison, 2003; Watkins et al., 2004). This circuit had two main components, a feedback circuit used to determine the value of the raw signal's standard deviation and another component that compared the raw signal to a multiple of the standard deviation. This circuit assumed that the background noise was Gaussian, although the authors do note that the t-distribution more closely resembled the histogram of the raw signal. But according to the authors, the performance of the circuit was not affected by this difference.

These two designs certainly present a new direction in the field of spike detection since the spike detection algorithms were implemented in hardware rather than being implemented in software.

CHAPTER 3

Present Study

3.1 Introduction

From 1952 to 1955, Werner Reichardt (see Chapter 1) working with Bernhardt Hassenstein at the Fritz-Haber-Institute of the Max-Planck-Gesellschaft in Berlin developed mathematical models that describe the behavioral response of the beetle *Chlorophanus* to visual motion (Poggio, 1993). For these experiments, the researchers placed the experimental subject inside a rotating optokinetic drum. When the drum was rotated, the perceived motion induced the insect to attempt to correct (or compensate) this by moving in the direction of the rotation (Reichardt, 1962). The results of this collaboration led to what is commonly known as the Hassenstein-Reichardt model for motion detection (Hassenstein and Reichardt, 1956). This model had a tremendous influence on the study of motion vision in invertebrates as well as vertebrates (Borst, 2000).

The story of how the Hassentein-Reichardt came about is an excellent example of methodical and rigorous science that was born of a simple experimental setup, i.e. a beetle in a rotating drum. The motion detection "model" laid the foundation for many predictions about how motion is processed in visual animals, and also led to the discovery of the motion sensitive neurons in the fly (Dvorak et al., 1975). Given the scientific and historical importance of conducting initial experiments in the rotating drum, it seemed appropriate that the initial experiments on the moth-robot hybrid, the first of its kind, be conducted in an equivalent set up.

Similarly to the first optomotor experiments, we introduce the proof of concept of our insect-machine hybrid in which a horizontal yaw motion stimulus was used to drive the biological system, i.e. a fixed moth, which in turn drove the robotic system to rotate around an insect-centered axis. This robotic system has the potential of studying the closed-loop control properties of the moth's nervous system at various points of computation. We believe that a growth in this highly interdisciplinary line of work can lead to advances in neuroethology, brain-machine interfaces for humans, and the use of insects as biological sensors for more intelligent robots and machines.

3.2 Appendix A

The first manuscript presents the initial results of an insect brain-machine interface. It describes the various components of the system as well as the experimental apparatus used to perform these initial experiments. First, the manuscript reviews the bioelectrical signals available in the hawkmoth *Manduca sexta* that can be used in a visually modulated insect-machine interface. Next, I describe the hardware and software involved in converting these bioelectrical signals into robotic motion. Lastly, I present open-loop and closed-loop results obtained with visual neurons and a flight steering muscle. I conclude by discussing the importance of including mechanosensory stimuli in studies of sensory integration and of studying neural systems under closed-loop conditions. I also present an argument for integrating insect sensory systems into machines as an alternative to developing synthetic systems that intend to emulate biology.

3.3 Appendix B

The second manuscript presents a spike detection system that automatically adjusts its detection threshold based on the height of the spiking signal. This adaptive system builds on a previously published spike detection system (Rogers and Harris, 2004). In the first section, I describe the problem that this new system attempts to solve, i.e. the loss of detected spikes due to a sudden decrease in spike height. I then describe the peak amplitude circuit and the spiking frequencies for which it was designed. I include that the microcontroller is used to adjust the digital potentiometer that determines the spike detection threshold. The data I provide shows that the peak amplitude circuit performs well and also that the entire system is able to adjust the detection threshold in response to a decreasing signal height. This system therefore is able to detect spikes as their amplitude degrades and can potentially increase the longevity of a brain-machine interface.

3.4 Relative Contributions

The author of this dissertation carried out the experiments and data analysis for the manuscripts in Appendices A and B. The electrophysiology board discussed in the manuscript in Appendix A was designed and constructed by Leslie Ortiz (Ortiz, 2006) with the assistance of Dr. Charles M. Higgins. The adaptive spike detection system discussed in Appendix B was designed by the author of this dissertation and Dr. Charles M. Higgins. The author wrote both manuscripts.

CHAPTER 4

Future Studies

Given the proof that bioelectrical signals can be used to control a mobile, terrestrial electrophysiological system (see Appendix A) it is necessary to consider the potential investigative paths. Afferent signals from primary sensory areas of the central nervous system, as well as efferent - meaning motor output - signals have been used to drive the robot. There now exists an instrument that can be used to investigate the closed-loop properties of neurons at every level within the behavioral control system. The following sections propose experiments that can immediately be performed with the given system. One suggested study is of the influence of vision and mechanical motion on the activity of a steering muscle. Another study is on the closed-loop properties of visual neurons, well founded in principles of Control Theory. The last proposed study is of the neural activity while a moth controls the robot with muscle signals generated during active tethered flight. These proposed investigations require little to no modifications of the robotic system described in this thesis.

4.1 The Influence of Mechanical Vibration and Visual Motion on the Activity of Pleurodorsal Muscles

In *Manduca sexta*, the third axillary muscles are involved in wing retraction (see Chapter Two for more information). One of the subunits is active and is modulated by sensory information even while the moth is at rest. The sensory information that influences the activity of this subunit is visual and tactile (Wendler et al., 1993). While it is unknown why this muscle is active during rest, it has been shown that it is active during flight behavior (Kammer, 1971). Given its involvement during flight and its unique property of being active while the moth is quiescent, this muscle provides a good opportunity to study the influence of mechanical and visual stimulation on a motor output under stationary conditions.

4.2 Closed Loop Properties of Wide Field Motion Sensitive Neurons

There have been many studies on the filtering properties of motion detection neurons (O'Carroll et al., 1996). These studies generally provide a motion stimulus while the response of the neuron is recorded. A very typical motion stimulus is composed of thin, alternating black and white stripes. To gain the full range of responses of a neuron to motion stimuli, the width of the stripes of the motion stimulus (spatial frequency) and the speed of the motion stimulus (temporal frequency) are incrementally varied and, at each step, the response of the neuron is recorded and quantified. To decrease the influence of transitory effects (e.g. adaptation) on data, the stimulus is delivered over a span of several seconds and the period of time during which the response demonstrates a steady response is used as the result.

Studies like these are similar to other attempts to characterize the basic input-output relationship of a system, which can be taken to be either a neuron or the entire organism. This strategy of characterizing a system is similar to the use of Control Theory, when describing a given system. The next step in analyzing a system is to determine its closed-loop properties.

Visual motion detection cells induce corrective effects by the organism. In other words, when presented with motion in a particular direction, the insect attempts to correct by turning in the same direction of the motion stimulus. It is from quantifying this response that the observer is able to conceptualize the optomotor control system as providing an error signal to the motor output system. Visual motion cells therefore transmit information about the magnitude of motion in the environment to an error correction system that subsequently corrects this error with a compensatory steering response. But how long does it take to correct for this error and which derivative of motion - velocity, acceleration, etc. - is most important in the control algorithm? These are the type of questions that can be answered when the steady-state errors of a system are analyzed (Savant, 1958).

In the analysis of the steady-state errors of a feedback control system, an input is applied and the closed-loop response is recorded. For example, let us assume that the system being tested is a horizontal motion sensitive neuron that is interfaced with the robot described in this thesis. The same cylindrical drum also provides the input. Suppose that an angular velocity of 1 degree per second is applied to the cylindrical drum. If the insect-robot machine is a perfect velocity compensator, then after a short amount of time the turning response will be in the same direction also at 1 degree per second. If the controller is not a perfect velocity compensator then there will be a steady-state error and the quantity of this error will be the difference between the desired output, which is equal to the input stimulus, and the actual output.

The type of input described in this last example is also called a step function, so called because of the step-like shape its values take when plotted versus time. The other basic driving function traditionally used in the analysis of the steady-state response of a feedback control system is the ramp function. This function starts at zero and linearly increases in value and therefore looks like a ramp when plotted versus time. The physical description of these driving functions can be anything, e.g. position, velocity, or acceleration. Interestingly, there exists a way of classifying a control system based on its steady-state response to these driving functions. The inputs are usually a step function in position, a step function in velocity (which is a ramp function in position), and a step function in acceleration (which is a ramp function in velocity). The classification of the system is based on its ability to track the input, i.e. whether or not there is a residual error between the input signal and the actual response.

There are four basic types of systems described in Control Theory. They are Type 0, Type 1, Type 2 and Type 3. They are called this based on the response to the various dynamic orders of motion, namely position, velocity, acceleration and the calculus derivative of acceleration. If a system response to a step function in position results in a steady-state error, then the system is said to be of Type 0. When a system's steady-state response to a step function in velocity results in a steady-state error, it is said to be of Type 1. Furthermore, a Type 1 system, while not able to perfectly compensate for a velocity step function, is able to compensate for a step function in position. A Type 2 system is one whose response will display a steady-state error to a step function in acceleration and whose response to a step function in velocity will be perfect. This logic extends to Type 3 systems, meaning that this type of system will display a constant steady-state error to a step function in the derivative of acceleration, but is able to track a step function in acceleration perfectly. The question, then, is what classification does a system with a horizontal motion detector fit under?

It has been shown that motion detection in flies is not purely a function of velocity and that the output of motion detectors is proportional to velocity only within a dynamic range of parameters (Egelhaaf and Reichardt, 1987). The nonlinearity of insect motion detectors also causes the output to be heavily influenced by the dynamics of the input (Borst, 2007). This illustrates the importance of studying the computation of these cells under more naturalistic dynamics. Unfortunately researchers are still not able to record from these neurons during flight. It is in these questions of closed-loop control that the present robotic system may offer a solution that comes closer to real closed-loop dynamics. Rather than using abstract and controlled stimuli, a closed-loop experiment will allow the system to dynamically adjust its input and perhaps uncover previously unseen dynamics. These cells are known as motion detectors but the dimension - such as velocity and acceleration - that is most relevant for the real closed-loop control of motion compensation is unknown.

Maintaining a single unit recording on the current robot system will be an issue, but there are ways around this problem. Electrophysiological recordings on a moving platform are most sensitive to sudden, jolting motion. The stochastic nature of the neural signal implies that although on average there is a mean firing rate, there are also brief periods of very high frequency bursts. Also, the robot was programmed to turn at three velocities: a large constant velocity, zero, or a large negative velocity. A robot control algorithm that controls the robot based on the instantaneous firing frequency and also commands the robot to make quick, large jumps in velocity will cause the robot to violently jitter. This undoubtedly makes the duration of neural recordings too short to run an experiment.

Two distinct solutions were developed to mitigate this issue. One was to place a low pass filter in the control algorithm to remove the high frequency velocity switching that caused the robot to jitter. This strategy removed the jitter, but the delay of the filter was very long and effectually caused the dynamics of the robot to overshadow the dynamics of the neural signal.

The other solution was to implement a ramping type function in the part of the control algorithm that set the robot's servo speed. Recalling the control algorithm described in Appendix A, in particular the threshold scheme, the spike count of the bioelectric signal was continuously compared to two user-defined thresholds, one was "high" and the other was "low." When the spike rate was above the high threshold, the robot was commanded to move in the preferred direction of the neuron. When the spike rate was between the high and low thresholds, the robot was commanded to stay still. When the spike rate was below the low threshold the robot was commanded to move in the anti-preferred direction.

In the ramping scheme, modulation of the robot's velocity was executed by incrementing or decrementing the servo velocity, depending on the direction of the turn command. When no turn was being commanded, the control algorithm incrementally brought the servo bit value toward zero (unless it was already at zero). Velocity was incremented or decremented away from zero during turn commands. Incrementing the velocity away from zero was caused by neural activity during preferred motion stimuli. Decrementing the velocity away from zero (i.e. increasing the value in the negative direction) was caused by neural activity during anti-preferred motion stimuli. The performance of the second solution (ramping) was not fully studied, but was likely to be superior to the first solution (low-pass filter). It is very likely that this second control scheme would eliminate the need for the low pass filter in the first solution, therefore allowing the dynamics of the biosignals to be visible in the behavior of the robot.

4.3 Robot Controlled by Flight Muscle Signals

There are several problems that prevent researchers from studying neural systems while an insect is in motion. One major challenge is that it is very difficult to record neural signals while an experimental insect is behaving. There does not exist equipment that an insect can carry and allow recordings from its brain. Despite this, scientists who study insect sensory integration and locomotion control have been able to find methods that provide sensory input that closely emulates the dynamics that come with natural behavior.

One approach was to create the visual input of a fly travelling within a visual scenery. The flight path of the insect was recorded with sensors and used to recreate what must have been its visual input (Schilstra and van Hateren, 1998). The visual stimulus generated was then presented to constrained insects while recording from their brains (Lindemann et al., 2003). While this study provides compelling reasons to believe that the recorded neural responses are probably similar to those while the insect is behaving, data collected from a behaving insect would be required to support or refute this assumption (Maimon et al., 2010).

A more multi-sensory approach to studying insect behavior involved a moth that was fixed in place while navigating in a virtual reality arena (Gray et al., 2002). The researchers were able to use the deflection of the moth's abdomen as a control signal and "convince" the moth to navigate through a virtual reality space. The investigators were also able to record from the moth's ventral nerve chord while it was "flying." Besides receiving visual information, the moth also received mechanosensory information, in the form of a wind stimulus, and olfactory information, in the form of pheromone puffs. This approach can shed light on neural computations in a "flying" insect.

The mobile electrophysiology platform described in this thesis could also be a platform for performing electrophysiology while a tethered moth is intentionally behaving. For this to happen there are two steps that must be taken. First, the robot must be programmed to interpret the spike patterns from flight muscles. Second, the moth must learn how to navigate the robot. There are also strong reasons to believe that this is possible. There are publications that demonstrate cases where an insect learned seemingly unnatural behaviors in order to control extracorporeal objects (Wolf et al., 1992; Heisenberg et al., 2001). The moth's task of learning to control the robot can be made simpler if the robot's control algorithm is based on the phasic EMG activity observed in steering (Wendler et al., 1993).

In the robotic system of this thesis there are three amplifiers available for recording. Two channels can be used to record steering muscle and power muscle activity. These two channels would be the information sources for the robot control system. The steering muscle signal can provide information about direction and the power muscle activity can provide information about speed. Once the moth learns to control the robot, the third amplifier, along with the electrode positioning system, will allow dorsal access to the cervical connective and the brain. This will allow investigations of neural activity during behavior, which also includes the mechanosensory feedback provided by forward and rotational motion.

4.4 Conclusion

The main motivation for building the mobile electrophysiological device described in this thesis was to create a system that allowed studies of neural systems while they are in closed-loop motion control. Any experiment that exploits the ability to combine closed-loop control and motion can now be performed. The primary neural pathway for this mobile electrophysiological system was the visual motion-flight control pathway. Introducing mechanical motion to studies of this pathway will likely bring surprising revelations, such as the influence of active flight on the gain of motion sensitive neurons (Maimon et al., 2010).

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APPENDIX A

A Robotic Platform for Studying Insect Neural Systems Under Closed-loop Conditions

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In preparation for submission to

Journal of Neuroscience Methods

APPENDIX B

Adaptive Spike Detection for Brain-Machine Interfaces

Timothy Melano and Charles M. Higgins

In preparation for submission to

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