EMG Analysis of Octopus Arms' Muscles

by

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ABSTRACT

The unparalleled motion and manipulation abilities of an octopus have intrigued engineers and biologists for many years. How can an octopus having no bones transform its arms from a soft state to a one stiff enough to catch and even kill prey? The octopus arm is a muscular hydrostat that enables these manipulations in and through its arm. The arm is a tightly packed array of muscle groups namely longitudinal, transverse and oblique. The orientation of these muscle fibers aids the octopus in achieving core movements like shortening, bending, twisting and elongation as hypothesized previously. Through localized electromyography (EMG) recordings of the longitudinal and transverse muscles of *Octopus bimaculoides* quantitatively the roles of these muscle layers will be confirmed. Five EMG electrode probes were inserted into the longitudinal and transverse muscle layers of an amputated octopus arm. One into the axial nerve cord to electrically stimulate the arm for movements. The experiments were conducted with the amputated arm submerged in sea water with surrounded cameras to record the movement, all housed in a Faraday cage. The findings of this research could possibly lead to the development of soft actuators built out of soft materials for applications in minimally invasive surgery, search-and-rescue operations, and wearable prosthetics.

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INTRODUCTION

1.1 Bio-Inspired Robotics

Bio-inspired robotics is the field of robotics that is inspired by biological systems in nature. The functioning of nature is always inspiring and provides abundant solutions to enhance technology. It is also a fact that nature uses far less energy than humans do - It is at least four times more efficient than current technology. Researchers have always been curious about the biomechanics of the animals and have tried to understand and imitate them for solving complex human problems.

The bio-inspiration in this research is an octopus, ultimately to engineer a soft robotic arm manipulator. An octopus has tremendous attention due to many of its ability to quickly conform and adapt their body and behavior as per requirement. Hence, it is aimed at transferring the working principles from soft-bodied living organisms to robotics [1]. The effective and risk-free adaptation of soft robots have been widely explored in relation to manipulation activities [2], also understanding difficult operating conditions. For example, soft grippers have been adopted in natural, mysterious environments such as the deep sea, to grasp unfamiliar objects with variable and irregular shapes [3]. A soft robot inspired by the human hand is shown in fig.1, which is capable to conform and adapt to the curves with a smooth grip during handshakes. Softness with extreme flexibility in these cases is exploited to improve and simplify grasping tasks by reducing control complexity: the high precision required by rigid grippers and manipulators is here entrusted to the intrinsic compliance of soft systems. Bioinspiration and soft robotics would therefore prove useful and effective to achieve what was not possible previously.

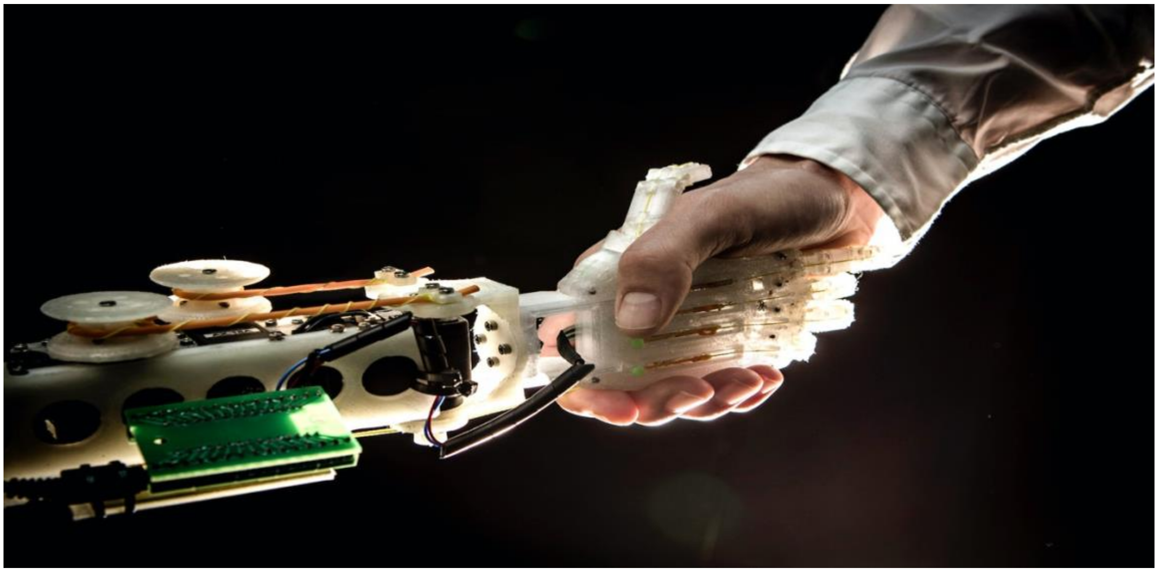

Fig.1 Bioinspired robotic hand [28]

On the other hand, it is not a trivial task. The research of bio-inspired soft robots is always inter-disciplinary, combining the efforts of biologists, mechanical engineers, electrical engineers and statisticians. In the beginning of soft robot development, researching the movements of animals is a significant process. That will be beneficial to control the robots more accurately by replicating their natural biomechanics. However, the observation of the movement of the octopus's arms is very difficult because the octopus's arms do not have the joints, and it is very difficult to describe the movement. Hence, this research explains the muscular system by analyzing EMG signals which allows engineers to interpret which muscle is active to achieve specific movement.

1.2 Why Octopus?

Octopuses are soft-bodied mollusks with eight arms, each of which is lined with suckers along the oral surface. Each arm is thicker at its proximal base and tapers uniformly towards its distal tip [2]. The arms and suckers constitute most of the body mass and account for most of the neurons and muscles; most of their behaviors depend upon these appendages [3]. The arms and suckers are multi-functional; collectively they are used for locomotion, grasping, pushing, pulling, wrapping, twisting, and chemo-tactile sensing. Behaviorally, the arms are used for crawling, walking, disruptive camouflage, signaling, mimicry, capturing prey as in fig. 2, fighting, and mating [4,5].

Fig. 2 Octopus using its arm to prey on a live crab [29]

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Furthermore, the octopus has sophisticated motor, sensory and cognitive capabilities [8]. Since there are no rigid structures in an octopus arm (i.e., no joints), octopuses have more degrees of freedom than other animals. The end goal is to engineer robots with bioinspired capabilities that permit adaptive and flexible interactions with the natural environment [9]. However, before being able to achieve the end-goal, there are a lot of difficulties, for example: materials of soft robotics, motion control, complexities in the arm as it is naturally designed with different muscle groups - where the same material cannot be used! As for the motion-control, there are many hypothesized suggestions for the activations of muscles for distinct movements. However, involving the complexity of research, it is rather difficult to finish, but still attracts a lot of scientists' and engineers' interest. This research strives to present experimentally determined data for different arm movements of an octopus. It is a unique approach than those done earlier, as the approach in this research is based on studying the individual EMG activity in each of the transverse and longitudnal muscle layers while electrically stimulating the octopus arm.

CHAPTER 2

OCTOPUS ARM STRUCTURE AND MECHANISM

The arms of the octopus belong to cephalopods. Like all cephalopods, the octopus lacks a rigid skeleton to support its body. The octopus arm consist of a tightly packed three-dimensional array of muscle fibers. As such they lack the rigid skeletal elements that characterize skeletal support in many vertebrates. The arms of octopuses also lack the fluid-filled cavities that provide a type of support termed a “hydrostatic skeleton” that is common in many invertebrate animals. The musculature of the arms of octopus not only generates the forces required for movement, deformation and changes in stiffness, it also provides the required support. This type of support system is termed a “muscular hydrostat” and is also observed in the tongues of mammals and lizards, in other parts of the bodies of cephalopods and other mollusks, and in the trunk of an elephant [11].

The octopus features an extensive nervous system, including the brain, an axial nerve cord in each arm, and the intrabronchial commissure, a ring-like structure connecting all the axial nerve cords [12]. Within the arms, the central axial nerve cord is accompanied by four peripheral intramuscular nerve cords and nerves that are associated with each sucker. In regard to the internal structure of the octopus's arm, the work by Yoram Gutfreund [13], we learn that the histological cross-sectional areas of the octopus’s arm are similar, no matter what kind of octopus. In fig.3, the nerve cord (AN) is in the center of the arm. Then, the muscle group surrounding the nerve cord and looking like a “X”, is the transverse muscle (T) group. The four bigger muscles besides the transverse muscle group are longitudinal muscle groups. In addition to the longitudinal muscle groups, there are multiple smaller layers, which are the oblique muscle groups. The longitudinal muscle groups are the main muscle groups observed in this study. The suckers on the octopus’ arms are attached along the oral side of the arm as seen in fig. 4.

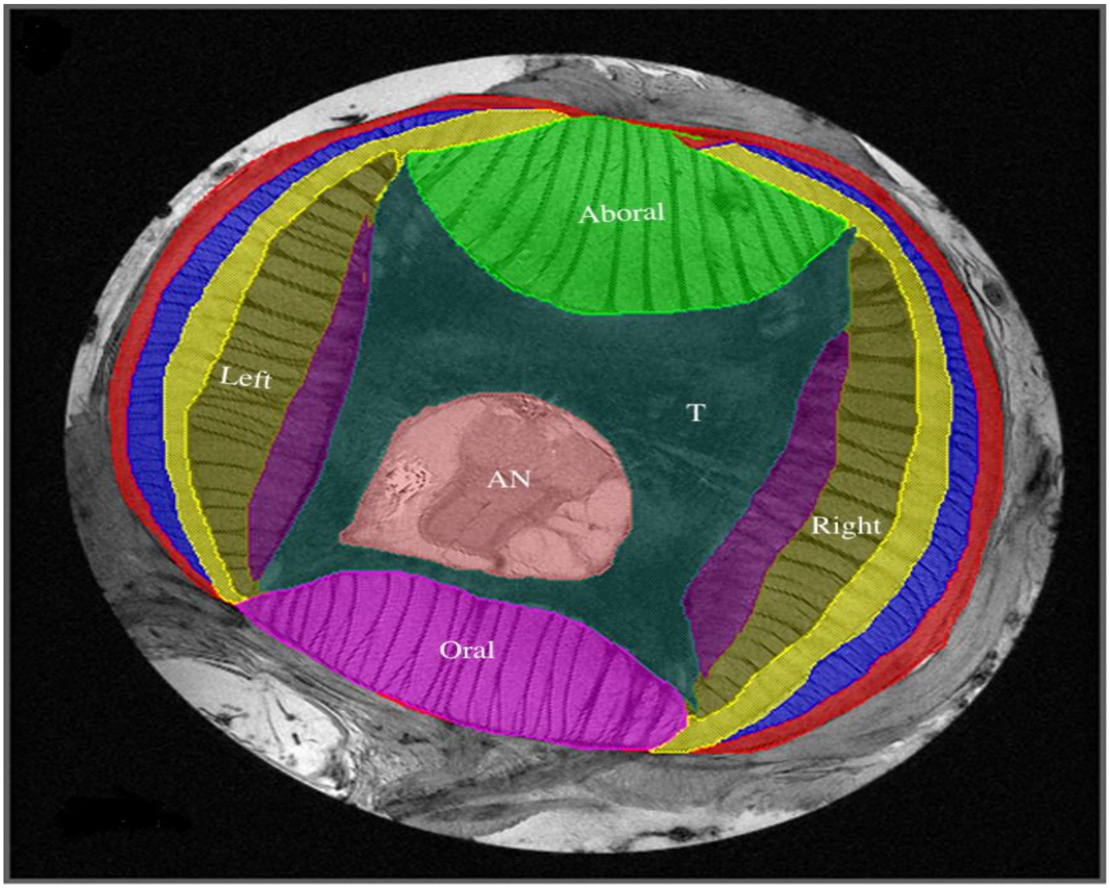

Fig. 3 In the center of octopus's arm is the axial nerve cord (AN). Surrounding the axial cord is the transverse muscle (T). The longitudinal muscles include Aboral, Oral, Left and Right [36]

The muscle mechanism in an octopus-arm is what powers the flexibility in the octopus's arm that results in a bend in any direction, elongation, shortening and twisting either clockwise or counter-clockwise [14]. The tissue in the octopus arm cannot be filled with gas or liquid, so the total volume of the octopus arm cannot change. If one muscle group increases in one dimension, the other two dimensions of the octopus arm will decrease. For example, if it elongates the arm in y direction, the cross section in x and z plane must decrease. The basic principle of function is straightforward. Since the volume of the octopus arm is essentially constant, a decrease in one dimension must result in an increase in another dimension. The other main point is that the transverse and longitudinal muscle groups have been hypothesized to be antagonistic muscles.

Fig. 4 *Octopus bimaculoides* from an oral view [30]

The axial nerve cord runs longitudinally down the center of the octopus arm and is surrounded by an extensive mass of transverse muscle [15]. Until now, the muscle group functionalities have been hypothesized and not confirmed using experimental results. The elongation of the octopus arm has been hypothesized to be caused by the contraction of the transverse muscle group, while shortening of the octopus arm is through the contraction of the longitudinal muscle groups. Twisting is proposed to be achieved by the oblique muscles. The bending movement has been proposed to occur when one side of the longitudinal muscle group contracts, and the transverse muscle group also contracts simultaneously to resist the force generated by the longitudinal muscle group. For the bending point and the bending direction, they are dependent on which muscle groups are stiffening [14]. The longitudinal and transverse muscle groups are proposed to be crucial for all types arm movements (e.g., extension, shortening, bending, reaching.) Hence, it is the major focus of study in this research.

The hypotheses being tested in this research is that bending, which is one of the important arm movements, requires selective contraction of the longitudinal muscle on the side of the arm to which it intends to bend, the muscle which represents the inside radius of the bend. Elongation of the arm is a result of contraction of the transverse muscle, as the study in [35] shows that the muscle fibers run perpendicular to the arm. Shortening of the arm is caused by contraction of the longitudinal muscle, as its muscle fibers run parallel to the arm from base to distal tip [35]. This research is aimed at testing these hypotheses using EMG analysis of the transverse and longitudinal muscle layers along with the motion analysis of the arm.

2.1 Focus on Longitudinal and Transverse Muscles

In this research the focus is only on the longitudinal and transverse muscle layers for the following reasons:

1. The majority of movements in an octopus arm are caused by the biomechanics of the longitudinal and transverse muscle layers.
2. Both these muscle layers are significantly larger in size compared to the oblique muscle layer.
3. During the experiment process of inserting EMG electrode probes, it is more likely to place the probes precisely into the transverse and longitudinal layers. Hence, we can determine the accurate contributions of these muscles in an octopus arm to achieve core movements like shortening, elongation and bending.

CHAPTER 3

ELECTROMYOGRAPHY

Electromyography (EMG) refers to collecting the electric signal from muscles, which is controlled by the nervous system and produced during muscle contraction. The signal represents the anatomical and physiological properties of muscles. It is the electrophysiological signal which can represent the neuro-muscular activity during movement [16].

There are two different methods for recording EMG. One is attaching the EMG sensor on the skin and collecting the EMG signal from the skin surface. This method is called surface EMG (sEMG) [17]. The other method is inserting the needles with electrodes into the muscle groups, that is called “Intramuscular EMG”. Collecting the EMG signal can allow people to understand which muscle is active during particular movements or actions.

3.1 Surface EMG (sEMG)

Surface EMG is widely used in numerous applications because it is non-invasive and available to conduct by personnel without professional knowledge [18]. The myoelectric activity appears on the surface of the skin as electric potentials with limited bandwidth, from 15 to 400 Hz, and with very small amplitude, from some micro to a few milli volts peak-to-peak, depending on the intensity of muscle contraction. Very sensitive instruments are then required for the detection, amplification, conditioning and digitization of surface EMG.

The advantage of surface electrodes is that they are non-invasive, and the patient need not be anesthetized before placing the electrode. The operation is simple and painless. However, the disadvantage of surface EMG is recording the signal from several muscle groups at the same time, which is also called ’cross-talk’ [19]. Hence, it is not an appropriate method for measuring the activity of a specific muscle. In addition, considering the size of the surface EMG electrode, which is quite evident in fig. 5, this is not suitable for the octopus arm.

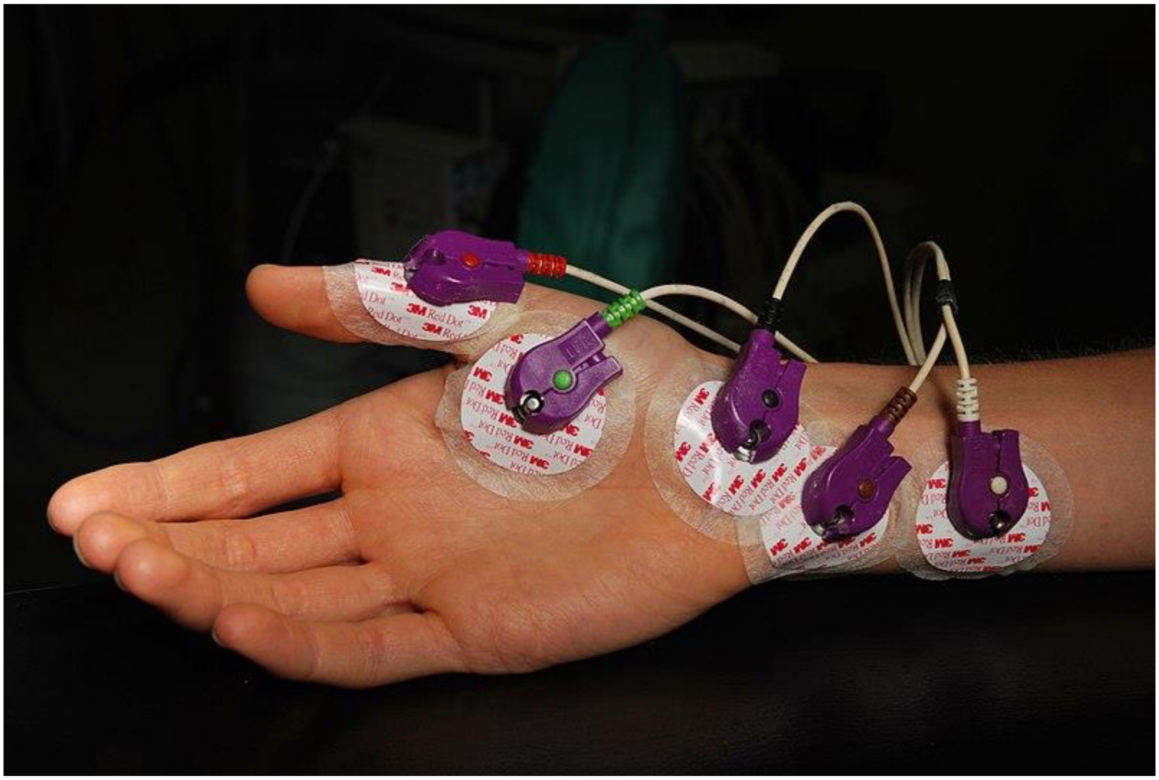

Fig. 5 Surface EMG (sEMG) [31]

3.2 Intramuscular EMG

The EMG method used in this research is intramuscular. The electrode for the intramuscular EMG is a needle and fine wire, as shown in Fig. 6 and described in Table 1. To perform intramuscular EMG, a needle electrode or a needle containing two fine-wire electrodes is placed within the muscle of interest. The advantages of fine wire EMG are extremely sensitive, available to record single muscle activity, access to deep musculature, and low concern of crosstalk. However, there are several disadvantages. The fine wire EMG is invasive, as the fine wire is being inserted into the muscle. Once the fine wire is inserted, it is difficult to reposition [20].

Fig. 6 Intramuscular EMG probe manufactured by microprobes [29]

Table 1. Specifications of Microprobe EMT-2-30 [27]

EMG Hook Probes (Microprobe EMT-2-30)	
Wire specifications:	
Material:	304 Stainless Steel
Size:	50-micron diameter
Insulation:	
Material:	H-PN Red bifilar insulation
	Biocompatible Polyurethane
Electrode Tip Exposure:	0.5mm
Method of Insertion:	27-gauge hypodermic needle

3.3 Noise in EMG Signal

EMG signal recording is not quite an easy process, there is always a possibility of error. While it is impossible to be a 100% free from noise, we can certainly foresee the possibilities and minimize the noise to the best extent possible. During the experiments the different sources of noise in the EMG signal could be due to the following reasons:

a) Inherent Noise in the Electrode

For recording the EMG, the non-invasive electrodes are applied to the skin of the subject. For recording purposes, electrodes made of silver/silver chloride (10 × 1 mm) have been found to give adequate signal-to-noise ratio and are electrically very steady. For this reason, they are widely used as surface electrodes [21].

b) Movement Artifact

Movement of the cable connecting the electrode to the amplifier and the interface between the detection surface of the electrode and the skin creates motion artifacts. Muscle fibers generate electric activity whenever muscles are active. EMG signals are recorded by placing electrodes close to the muscle groups.

c) Electromagnetic Noise

Electromagnetic sources from the environment superimpose the unwanted signal, or cancel the signal being recorded from a muscle. The surface of the body is continuously inundated with electric and magnetic radiation, which could be the source of electromagnetic noise.

d) Cross Talk

An undesired EMG signal from a muscle group that is not targeted for data collection is called “crosstalk”. Crosstalk contaminates the signal and can cause an incorrect interpretation of the signal information [22].

e) Internal Noise

Anatomical, biochemical and physiological factors take place due to the number of muscle fibers per unit, depth and location of active fibers, and amount of tissue. These factors are called internal noise and directly affect EMG signal quality.

Now since the researchers are aware of the possibilities of noise in the signal, it is quick to rectify, which helps save a lot of time during the experiment.

CHAPTER 4

EXPERIMENTAL PROCEDURE

The experiment section is one of the most important stages of this research. BIRTH lab had dedicated over two years to design the experimental protocol to collect the EMG signals, not only to make the experiment successful, but also follow the ethics.

4.1 Ethics Statement

The Institutional Animal Care and Use Committee (IACUC) at Arizona State University (ASU) oversees any laboratory using animals for research, in order to ensure human care and use of animals in accordance with all pertinent laws and regulations of the land. The husbandry, anesthesia, and experimental protocols used in this study were developed under the supervision of Arizona State University veterinary staff, referencing the EU Directive 2010/63/EU and published guidelines for cephalopod use. The proposed work has been approved by ASU IACUC for use.

4.2 Animal Husbandry

The *Octopus bimaculoides* is referred to as the California two-spot octopus due to its distinctive ocelli. Each of them weighs anywhere from 150g. At the order request placed by the laboratory, these octopuses are collected from the coast of southern California by Aquatic Research Consultants and shipped overnight to the vivarium at ASU. It is allowed to adapt to captivity for a week's time before the experiments are performed. The staff members of ASU's Department of Animal Care and Technologies in the vivarium care for the octopus. The octopus was fed a diet of live fiddler crabs, alternating with frozen shrimp, with a day of fasting in between [13]. During this process of interaction with the octopus, if they lay eggs, it is noted. Otherwise, the sex is always confirmed after the completion of experiments through dissection of the reproductive organs within the mantle cavity. In the raceway, each octopus was housed in individual 30 L tanks containing several lego parts and pipes for hiding and enrichment.

4.3 Why *Octopus bimaculoides*?

As mentioned in section 4.2, *Octopus bimaculoides* is also referred to as bimac or California two-spot octopus due to its distinctive ocelli. This specie was chosen for experiments in this study due to its presence along the coast of California, which makes it easier in terms of logistics to ASU. The bimac is used to being on top and under water and this helps in terms of the experiment setup. They are of relatively good size, ideal for this study. One of the important features of the bimac is that they do well in captivity. Hence, bimac was chosen as the specimen for our experiments.

4.4 Experiment Day Preparation

The first step is to clean up the experiment surface with 10% bleach water and prepare the tools for experiment, including tweezers, rulers, scalpel, and probes. Researchers bring the octopus with 4 liters of sea water from the Life Science Building to the laboratory. Then, fill the 12 x 6 x 4.5 inches container with 2.5 L of salt water from the octopus tank. After that, the researchers move the container to the scale and zero the scale. Next, the octopus is put into the container to measure the mass. This step is only done when the octopus has full arms. Generally, the mass of the octopus is from 150 grams to 220 grams. The researchers also prepare the probes in this section, and the needles are marked with sharpie pen to distinguish which probes would go into which muscle layer (during dissection this helps identify if the probe was in the intended location or elsewhere.)

4.5 Anesthesia and Euthanasia Protocols

After discussion and guidance from ASU's clinical veterinarian, the below mentioned anesthesia and euthanasia protocols were established. Animals are initially allowed to acclimate themselves to the environment, minimum of 1 week prior to an anesthesia event. The animal was then transported from its 30 L housing tank to a 12" x 6" x 6" experimental container with 2–2.5 L saltwater, depending on the animal's size. 5mL of ethanol was then dispensed into the experimental container at 0.25% increments every 1 minute until a maximum ethanol concentration of 1.4% was reached.

Throughout the process, the animal's breathing cycle was monitored after every two increments of ethanol to ensure a stable breathing rate. After the 1.4% of ethanol is dispensed, we test the animal for reactions (to check if it is anesthetized) in two ways: (a). pinch the octopus to check for any reactions to pain, or (b). flip the mantle upside down. Generally, the octopus reorients its mantle straight while it is active, and if it didn't - it is successfully under anesthesia. On average, it took 15 mins to achieve anesthesia.

According to the protocol, we only amputate 4-5 arms because with fewer than 4 arms, the octopus struggles to survive. Hence, on the last experiment day, we experimented with 2 arms - giving us a maximum of 5 arms from one octopus.

The octopus will be euthanized by the overdose of ethanol, if we have dissected four arms or the octopus is in bad condition. To achieve euthanasia, the ethanol concentration will be increased to 10% in the experimental container, until the animal cease to breathe. The corpses are saved in the freezer to later be dissected to determine its sex.

It is also to be noted that all the liquid involved during the experiment is discarded via the biohazard waste protocol at ASU.

4.6 Arm Amputation

Amputating the octopus arm is the next step in the experimental procedure. The octopus arm will be isolated and placed with suckers facing down onto a plastic ruler. Using two forceps, researchers slightly stretch the area of amputation (proximal end of the arm) and amputate the arm using a scalpel. Usually, the first right and left arms cannot be dissected because those functions are fetching the prey. Hence, any of the other arms are the better choices. After the amputation, it needs a week's gap before the next experiment. After arm amputation, the amputated arm is transported out of water and prepared to insert the probes. The octopus is also now transported back to the vivarium.

4.7 Inserting Probes

The probes used are from Microprobes, which is the model EMT2-30 sub-cutaneous bipolar hook electrode that can collect the EMG signal in an unstable environment. A 27-gauge hypodermic needle used to insert two 50-micron diameter stainless steel wires into the desired muscle group.

Inserting probes into the octopus arm is the most challenging process that needs experience and practice. Researcher holds the arm with one hand and inserts the probes with another. Ideally the five probes are expected to be in the five different muscle groups. If inserted to the undesired muscle group, it will affect the result (for this reason, dissection is a very crucial role.) In the experiment, there are two ways to insert the probes to the longitudinal muscle groups, transverse, right, left, oral, and aboral muscle groups. First, insert the probes from the amputation (proximal) end. The inserting distance is around 25 mm. Then, the hypodermic needle is withdrawn, leaving the flexible electrode wires securely fixed in the muscle groups. However, often the wires confine certain movements of the arm.

Second way is to insert the probes from the side of the octopus arm. The position is at the proximal of the octopus arm, which is around 50 mm from the point of amputation. This method can avoid confining the movement of the octopus arm by the wires. However, this method is rather difficult to insert the probes to the desired positions precisely. The function of the probes inserting to the longitudinal and transverse muscle group is to detect the EMG signal individually for all the muscle layers; therefore, the positions of the probes are very important. After inserting the probes to the desired muscle groups, all the wires of the probes will be taped together by the electrical tape to restrict them and prevent wire entanglement.

After probing individual muscles layers, there is one probe which should be inserted in the axial nerve cord to trigger the movements. To trigger the movements, the axial nerve is important.

After inserting the probes, the researcher will pull the skin of the proximal end of the arm and clamp it using forceps. Then, the forceps are mounted on the mounting structure specially designed for this experiment. For security, the probes are taped on the mounting structure. Finally, the octopus arm, probes and mounting structure are moved to the experimental container (12” x 6” x 6”). The experimental container is filled with 4 liters of the salt water and a ground wire is set inside the container. The container with an amputated arm hanging in salt water is seen in fig. 7. The probes are connected to the Intan board and signal generator for electrical stimulation, which is further explained down in this chapter.

4.8 Post Experiment Dissection

The post experiment dissection is a significant step in the experiment procedure. Once the octopus arm from the experimental container is transferred for dissection, the researchers start from the end to slice the arm to discover the locations of probes and confirm its position with respect to intended location. This step is vital because it will affect how the authors interpret the experiment results. The problem on the electrode end is color coded to distinguish which is which. After that, the probes are removed from the octopus arm to pack it safely.

In brief, while summarizing chapter 4 a typical experiment day steps are as follows:

i. Cleaning up the experiment surface with 10% bleach water.
ii. Bringing the octopus from vivarium with 2/3rd of the container full of water.
iii. Fill the 12” x 6” x 4.5” container with 2.5 L of salt water from the octopus tank.
iv. Put the container on scale and zero the scale.
v. Transfer the octopus in the container to measure the mass of the octopus (this step is only done once when the octopus has all arms.)
vi. Anesthesia process with breathing cycle reading 3 times between every 2 alcohol dispensation cycles.
vii. Verify if the octopus is sedated by:
    a) poking the animal and observing no reaction from the animal.
    b) testing to see if the animal would self-adjust to sit upright or relax its arm when disturbed.
    c) observe discoloration of the animal skin as an indication for sedation. The skin has a vivid color during normal state and a discolored skin during sedation.
viii. Have bipolar probes out and ready, and needles marked with sharpie to desired insert length.
ix. Isolate desired arm, place arm with suckers facing down onto a plastic ruler, using two forceps to stretch the area of amputation (proximal end of the arm), and amputate the arm using a scalpel.
x. Transfer amputated arm with plastic ruler out of water.
xi. Hold the arm with one hand and insert the probes with another for measuring the EMG signals.
xii. Tape the probes together using electrical tape to restrict them and prevent entanglement of the wires.
xiii. Pull the skin of the proximal end of the arm and clamp it using forceps.
xiv. Mount the forceps on the mounting structure.
xv. Secure the probes by taping them to the mounting structure.

xvi. Fill the experimental container (12” x 6” x 6”) with salt water and put a ground wire in the container.

xvii. Put the mounting structure in the experimental container (12” x 6” x 6”) containing salt water.

xviii. Connect the probes to the Intan Headstage and the voltage supply probes to the signal generator.

Fig. 7 In this figure, it shows more details for the experiment. For example, there is the red LED light at the corner to show the active status of the electrical stimulus input. Once the electrical stimulus stops, the LED light will be turned off. The LED can assist the authors to associate the EMG signals with the movements. In addition, the light under the container is to illuminate the experiment, or researchers cannot see anything in the video. Furthermore, this figure shows how the octopus arm bends during the electrical stimulus [29].

4.9 Intan Technology

The EMG recording electronic devices are RHD USB RHD2216 Arduino Shield, which is also used for this experiment. The hardware is as seen in fig. 8. RHD USB Interface is to record electrophysiology signals. It allows users to monitor biopotential signals (typically EMG or ECG) from two low-noise amplifier channels using the RHD2216 digital electrophysiology chip from Intan Technologies. RHD Recording Headstages are optimized to collect the EMG signal where the electrodes insert. They can support sampling rates from 1 kS/s to 30 kS/s. There are 16 bipolar channels to record EMG signals.

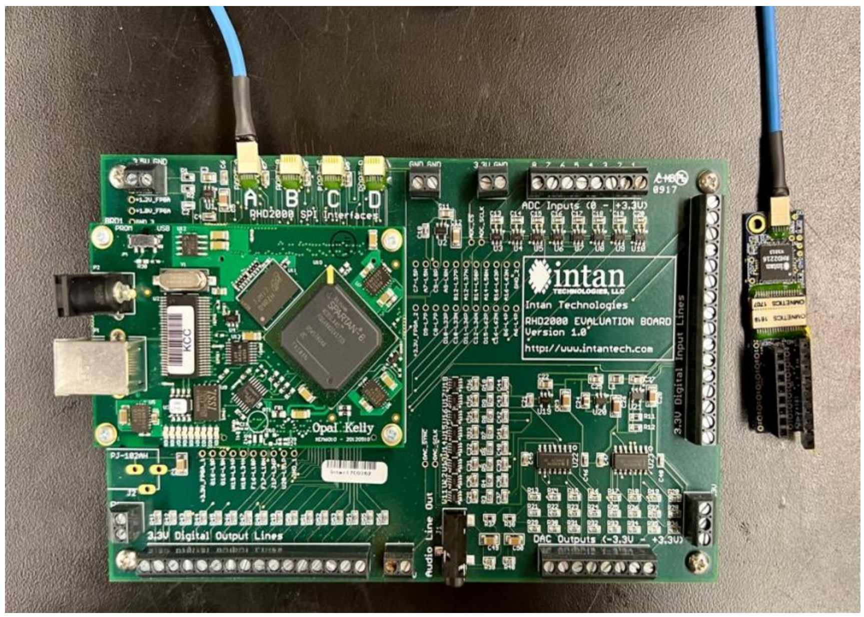

Fig. 8 Intan technology - RHD USB Interface [29]

The primary function of the shield is to stream digitized electrophysiology signals from the RHD2216 chip to the host computer. Data can be sent through the Arduino's USB serial port and can also be sent as analog voltage signals through the on-board two-channel DAC. Additionally, the shield can perform basic activity detection, in which a digital output signal is toggled when a channel increases or decreases in activity. The detection threshold is controlled with an on-board slide potentiometer, allowing the user to adjust the sensitivity of the activity detection in real-time.

The capabilities of the RHD USB board in are:

- Supports all Intan_RHD headstages with sampling rates from 1 kS/s to 30 kS/s.
- Software supports *in situ* electrode impedance measurement.
- Eight analog input ports with 0 to +3.3V range (no negative voltages allowed).
- Eight analog output ports with ±3.3V range can reconstruct selected amplifier channels.
- 16 digital input ports accept 2.5V, 3.3V, or 5V signals.
- 16 3.3V digital output ports.
- Analog and digital inputs are recorded in sync with amplifier waveforms; may be used to trigger automated episodic recording sessions.

4.10 Intel RealSense

Intel RealSense D400 series depth cameras calculate depth by the stereo-based algorithms. The important advantage of Intel RealSense is to connect multiple cameras and record one specific scene. In the experiment, two Intel RealSense cameras are set up in the Faraday cage. These devices are compact, we can see it in fig. 9, and fits well to one of the corners of the experiment cage.

The authors referred to the document from Intel to learn how to synchronize the two Intel Realsense cameras to capture at the exact same time and rate [18]. The components to work this camera are the breadboard, sync cables, resistors, capacitors, and some wires. There are nine connector ports on the cameras and only fifth and ninth connector ports should be inserted into the sync wires. In the software, Intel Realsense Viewer, the one Intel Realsense must be the master and the other should be the slave. In addition, closing the auto-exposure can avoid the error of frame per second (fps). The precision of the synchronization is checked by recording the clock time to see the difference between two cameras. They are sometimes different, but the difference is lower than 0.04 second. In Intel RealSense Viewer, it only records the RGB videos.

Fig. 9 Realsense D400 depth camera [29]

4.11 Experiment Setting

As discussed in chapter 3, noise is undesirable in any signal recording circumstance. However, it is all around us - all the electronic devices in the laboratory may cause background noise. It is necessary that we try to best eradicate such interference. Hence, the researchers have created a Faraday Cage to isolate the octopus arm experiment from the surroundings. For example, by covering the experiment space by the aluminum foil and bundling all the probes together. The aluminum foil walls are to create a Faraday cage like environment to decrease the influence of external electromagnetic fields. The bundling of probes is to ensure the electrical flow is the same amongst all probes.

A Faraday cage operates because an external electrical field causes the electric charges within the cage's conducting material to be distributed so that they cancel the field's effect in the cage's interior. Faraday cages cannot block stable or slowly varying magnetic fields, such as the Earth’s magnetic field, however they shield the interior from external electromagnetic radiation if the conductor is thick enough and any holes are significantly smaller than the wavelength of the radiation.

4.12 Electrical Stimulation

In the experiment, the stimulus is to trigger the octopus arms to bend, shorten, or elongate. In addition, the stimulus should be recognized easily, and not affect the EMG signal. The stimulus is not assumed to make the octopus arms have a specific motion. It is impossible to assign the motion by stimulus. The goal is to trigger the motions and observe the EMG signal from each muscle group of the octopus arm after the stimulus.

Electrical stimulus is the main way to stimulate the octopus arm, because it is easy to distinguish in the signal. The signal will be applied to the nerve cord (axial) by the probes, which is connected to a signal generator. The signal can be designed by the different levels of frequency, voltage, duty cycle and types of waves. Most of the time, the higher voltage is seen to trigger a larger motion of the octopus arm; however, it may have some harmful impact. For example, the octopus arm might be less sensitive after the high voltage electrical stimulus. But if the voltage is too low, it cannot be discovered in the EMG signal. The voltage ranges used in this experiment are 12V, 16V, and 20 V and the frequency is around 50 Hz. There are three wave forms which have been tested at the early stages of the research. However, based on the Fast-Fourier Transform, the possibility of the sine wave was removed. After the filtering process, the EMG signal was hard to distinguish. For the sine wave and square wave, they will charge and build up the electricity in the muscle - by which the arm can reach saturation very fast. Hence, the biphasic wave is a highly recommended stimulus method, because it allows the octopus arm to receive a break, which means that it will not charge the electricity in the muscle as the square wave and sine wave would. Using biphasic waves also discharges the arm and brings it back to normal conditions. Furthermore, during the EMG signal process, because the biphasic signal presents around 50% wave train, the authors can observe that period and look for the EMG signal related to the active muscle. Based on these two reasons, the biphasic wave is the main stimulus method that was used in the experiment.

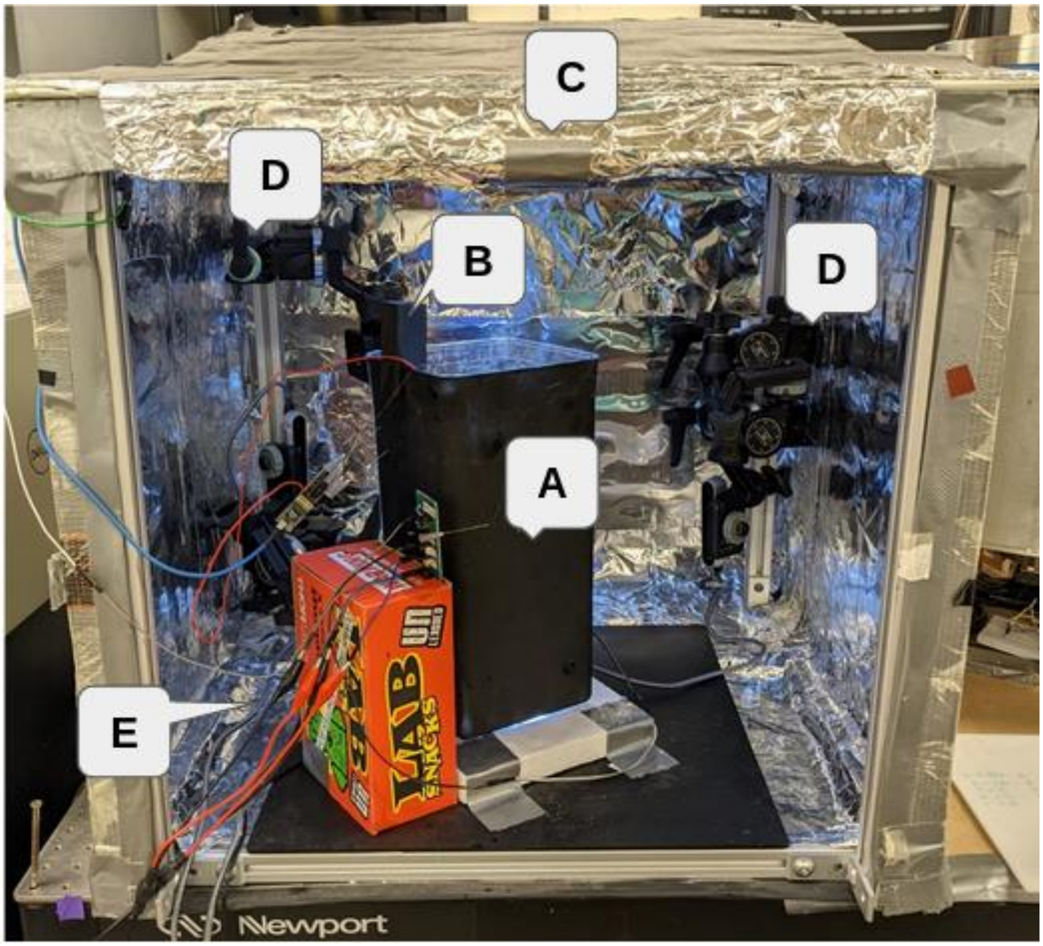

Fig. 10 Components of the experimental setup [29]

The components in fig. 10 are as follows:

A. Exp. Tank w/ Salt Water

B. Amputated arm, inserted probes, mounted in exp. tank

C. Faraday Cage

D. Intel RealSense Cameras

E. Wiring (Oscilloscope, Intan Board, Ground, Signal Generator)

4.13 Signal Generator and Oscilloscope

A signal generator or function generator is an electronic test equipment or software used to generate different types of electrical waveforms over a wide range of frequencies. Some of the most common waveforms produced by the function generator are the sine wave, square wave, triangular wave, and sawtooth shapes. These waveforms can be either repetitive or single shot (which requires an internal or external trigger source). Integrated circuits used to generate waveforms may also be described as function generator ICs.

In addition to producing sine waves, function generators may typically produce other repetitive waveforms including sawtooth and triangular waveforms, square waves, and pulses. Another feature included on many function generators is the ability to add a DC offset.

In this research we specifically use a biphasic waveform for reasons mentioned in section 4.11. The biphasic type describes a two-phase Doppler waveform with an extension of the reverse flow phase during the whole stimulation process. This helps the discharge of energy from the octopus arm after each trial in an experiment.

Once the stimulation procedure is completed and all data is collected for that experiment day, the following steps are to be followed:

- Remove the arm from mounting structure with probes intact
- Staring from the distal end begin to slice the arm to discover the location of probes and confirm consistency with intended location
- All probes are removed after dissection from the proximal end after confirming probe locations
- The arm remains are put into a petri dish and put inside a freezer
- The entire experiment surface is sanitized and cleaned. All the apparatus and equipment are put back in place.

Fig. 11 SIGLENT SDG1000X function generator [29]

In this experiment, the signal generator used is a SIGLENT SDG1000X, as seen in fig. 11. It is a series of dual-channel function/arbitrary waveform generators with specifications that include up to 60 MHz maximum bandwidth, 150 MSa/s sampling rate and 14-bit vertical resolution. The proprietary EasyPulse technique helps to solve the weaknesses inherent in traditional DDS generators when generating pulse waveforms, and the special square generator can generate square waveforms up to 60 MHz in frequency with low jitter. With these advantages, the SDG1032X waveform generator can provide a variety of high-fidelity / low jitter signals.

Oscilloscope:

The function generators are often supported by an oscilloscope for human understanding. An oscilloscope (informally called a scope) is a type of electronic test instrument that graphically displays varying electrical voltages as a two-dimensional plot of one or more signals as a function of time. The main purposes are to display repetitive or single waveforms on the screen that would otherwise occur too briefly to be perceived by the human eye. The displayed waveform can then be analyzed for properties such as amplitude, frequency, rise time, time interval, distortion, and others. Originally, calculation of these values required manually measuring the waveform against the scales built into the screen of the instrument. Modern digital instruments may calculate and display these properties directly. The general-purpose oscilloscope used in this experiment is a RIGOL DS1054Z, shown in fig. 12.

Fig. 12 RIGOL DS1054Z oscilloscope [29]

RIGOL DS1054Z is a digital oscilloscope of 50 MHz with 4 Channel, featuring 1 GS/s, 24 Mpts. Oscilloscopes are often used in the research, sciences, medicine, engineering, automotive and the telecommunications industry. General-purpose instruments are used for maintenance of electronic equipment and laboratory work. Special-purpose oscilloscopes may be used to analyze an automotive ignition system or to display the waveform of the heartbeat as an electrocardiogram, for instance.

Signal Processing and Video Analysis

This step is performed to analyze the information recorded in the muscle groups. However, before analyzing the data, we need to process it as there is a lot of noise surrounding the experiment. It is rather difficult to study the signal without cleaning it. Therefore, all the signals should be filtered and smoothed. The method to recognize the EMG signal is by comparing two values obtained using the bipolar probes, i.e., values of without and with movement for each individual muscle.

There are several steps involved in this process of analyzing the EMG signal, they are explained below.

5.1 Reading EMG Signal on MATLAB

All the collected signals are converted to analog signals via an Intan processor. This step is to separate the files into several trails representing different muscle groups. Furthermore, recording the start time and stop time of each stimulus is helpful in analyzing the signal in the following sections. In this section, the notch filter is applied to get rid of the 60 Hz noise.

The Intan Technologies website provides several versions of codes for users to read the signal from the Intan recording system, for example, codes in MATLAB and Python. At BIRTH Lab, the researchers decided to analyze the EMG signal with MATLAB. Users can base their codes provided by Intan Technologies to develop their own solutions, which means the codes can be made compatible to the need of the researcher. The developed code includes an added section of calculation for moving average, integration, normalization. These calculations are useful for signal analysis.

There are three different saving formats for the files that are saved. The researchers can select to save the signals into separate files. Moreover, they can choose to save the input signal or not. For these two options, the file format is ".dat". If the researchers want to save all the data into one file, the format is ". rhd". Both ".dat" and ".rhd" can be used in the MATLAB codes. However, the ".dat" format with input signal is more recommended because it includes more information than others. That can help users to match the EMG signal with input signal when analyzing the signal data. The method of reading signal data is according to "RHD2000 Data Files Formats" [13].

5.2 Fast Fourier Transform

The Fast Fourier Transform (FFT) is to convert the signal from the time domain into the frequency domain. Before doing the FFT, the sampling rate should be checked. In the experiment, the sampling rate has been set to 30kHz, which means one signal wave has 30000 data points. After the signal is transformed to the frequency domain, there are several specific frequencies. One is 60 Hz which is from the environment, the other is the frequency from electrical stimulus.

5.3 Notch Filter

A notch filter is applied in the signal processing code. Where low-pass filters attenuate all signals above a specified frequency, notch filters remove only a narrow band of frequencies. Notch filters pass the frequency components below and above the notch frequency. The fact that notch filters pass high frequencies leads to their strongest attribute: they usually cause little phase lag at the gain crossover frequency, assuming the notch frequency is well above that. Notch filters can be useful on the command for a fixed-frequency noise source such as that from line frequency (50 or 60 Hz) noise. Notch filters are also used to remove resonances from the system. Both notch and low-pass filters can cure resonance; notch filters do so while creating less phase lag.

5.4 Bandpass Filter

A bandpass filter is an electronic device or circuit filter that allows signals between two specific frequencies to pass, but that discriminates against signals at other frequencies. The bandpass filter also optimizes the signal-to-noise ratio (sensitivity) of a signal.

The Passive Band Pass Filter can be used to isolate or filter out certain frequencies that lie within a particular band or range of frequencies. The cut-off frequency or fc point in a simple RC passive filter can be accurately controlled using just a single resistor in series with a non-polarized capacitor, and depending upon which way around they are connected, we have seen that either a Low Pass or a High Pass filter is obtained.

Filtering the signal is important, because the superfluous signal may hide some significant EMG signals when the noise in background dominates the EMG signal. According to Zullo in [25], the bandpass filter is set from 300 to 10000 Hz, and the fourth order Butterworth bandpass is chosen.

5.5 Rectification and Moving Average

The rectification means calculating the absolute value of the signal to eliminate the negative and positive position of the signals. If skipping this step, the average may be zero. The moving average is to smooth the signal. The window size is 5, which means calculating the average of every five data points. The moving average can prevent the wave from spiking.

5.6 Power of Signal

The amplitude of the processed signal was quantified by its Root Mean Square (RMS) value as given in equation 5.1 [26].

$$\text{RMS(i)} = \sqrt{\frac{1}{N}\sum_{n=1}^{N} y_i(n)^2} \tag{5.1}$$

Where yi(n) is the nth data of the rectified moving average and N is the total number of the rectified moving average.

5.7 Normalization

Normalization is another way to evaluate signal data. Because the resistances of each muscle group and the positions of each probe are different. Sometimes, the voltage values are varied. Therefore, normalizing data can standardize the signal and be compared by the ratio. However, calculating normalization should be careful, because there sometimes exist some unknown spikes, which are even higher than the stimulus in the signal. Those may affect the accuracy of the normalization.

5.8 Integration and Quantification

Integration is the method to show the energy of the signal intuitively. This method allows researchers to read that after the stimulus whether there exists the energy or not. The quantification is the sum of the integration results, but without the stimulus. Figure 13 represents the signal process stages from the raw signal to the integration.

In the final quantification of EMG data, the numbers are obtained in the following way:

The final EMG number is the integration of the area underneath the curve, as seen in fig.13. This is also mathematically expressed in Equation 5.2.

$$\text{EMG} = \int V \, dt_{emg} \tag{5.2}$$

The final Background number is the integration of the area underneath the curve, as seen in fig. 13. This is mathematically expressed in Equation 5.3:

$$\text{Background} = \int V \, dt_{background} \tag{5.3}$$

It is to be noted that in both the instances, according to Equation 5.4, we truncate the period such that the time frame of EMG signal is equal to the time frame of the Background signal, so that there is no bias in the final ratio reading which is given below.

$$t_{emg} = t_{background} \tag{5.4}$$

The ratio in Equation 5.5 is the final number that we compare to a threshold that is determined to judge and see if the muscle is active or inactive. Since, there are five muscle layers, there are five ratios that are generated for each trial.

$$\text{Ratio} = \frac{[\, EMG - Background \,]}{Background} \tag{5.5}$$

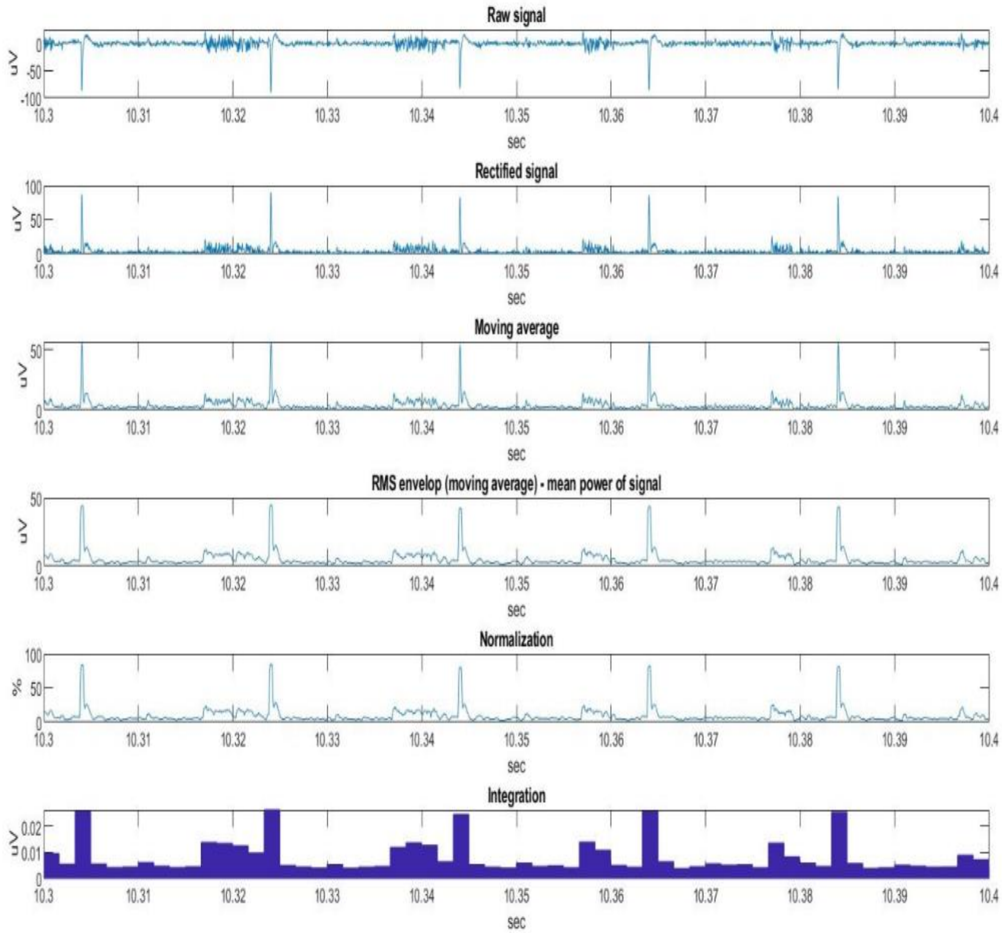

Fig. 13 Signal processing steps from raw data to integration [29]

5.9 Video Analysis

As discussed in chapter 4.9, while conducting the experiment there are two Intel RealSense cameras installed within the faraday cage. This is to record the movement of the octopus arm during electrical stimulation. In fig. 7, there is also a red LED light that is attached within the range of the camera to confirm that the electrical signal is being applied. In the procedure of this research, once the EMG analysis is completed, numeric values for EMG, Background and their respective Ratio is obtained. It is then important to establish what type of movements corresponds to the analyzed values. In order to help in understanding the type of movement, we go back to the video and look at it manually to discern the movement type.

The different types of movements that could occur are bending, twisting, elongation, and shortening. The recorded videos by Intel RealSense can only be viewed using a software called Intel RealSense Viewer SDK 2.0 which is downloadable from the official Intel RealSense website. Once the software is opened the file with '.bag' extension can be loaded into the viewer and the video plays. The video files are saved with time and date stamps so that it doesn't create confusions for different trials within the same experiment. Review process is relatively straightforward to see and understand what type of movement it is. However, it is important to note that in cases of bending, there can be different types. For example, if the bend is towards the octopus oral side – it is called oral-bending. If it is towards the right muscle side – it's called right- bending. Similarly, it could be aboral-bending or even left-bending. In some cases, there can be multiple movements happening at once or even multiple sequential movement, e.g., a twisting movement followed by elongation in the same experiment. However, the scope of this document is restricted to trials with only one movement.

CHAPTER 6

Conclusion and Discussion

The octopus arm is composed of four different longitudinal muscle groups, left, right, oral and aboral muscle groups. Additionally, in the center of octopus arm structure is the axial nerve cord which is the function of triggering the motions. Surrounding the axial nerve is the transverse muscle layer. The longitudinal and transverse muscle groups play an important role in the biomechanics of different movements. For example, in a bending movement, if one longitudinal muscle group is stiffening, the octopus arm will bend toward that direction. This is supported by the biological research which is already hypothesized. A majority of movements in octopus arm is caused by the transverse and longitudinal muscles. Hence, they are the main focus of study here,

The purpose of the presented octopus project research is to determine the functionality of each muscle layer in an octopus. Electromyography (EMG) signals are quantitatively analyzed for complex motions of the electrically stimulated arm. It provides very subtle details which cannot be observed by the human eye, to understand the biomechanics of the arm and mimic it into a soft robot manipulator. The experiments procedures were successfully established and till date experiments were conducted on 13 octopus, out of which 8 were male and 5 females, with 28 anterior and 26 posterior arm distribution. So, the data is pretty even given the fact we only know the sex once we dissect the mantle.

There were several limitations to the study design presented here. Firstly, collecting the accurate EMG signal, and placing the EMG electrodes in the intended muscle layers. Since the octopus arm tapers downwards, it is difficult to insert the probes to a distance and still end up being in the intended muscle layer. Secondly, the freshly amputated arm loses its natural activeness after a period of time. It is to note that completing all the experimental procedures takes up significant amount of time, and when it is electrically stimulated, after around 16 trials the response of the arm beings to deteriorate. Thirdly, during intense movement of the arm during the experiment, it is possible that the probes could fall out of place or even slide into a different muscle layer. The successful setup of the experiment can increase the success rate of signal process.

In the future, researchers plan on conducting few more experiments to enlarge the date set. After the experiments are completed, the most significant task is to analyze the data and find similarities in trends. Working with biological data is not a trivial task. Often the trends may not match the hypothesis. This could be because of a lot of factors in the experiment. For instance, maybe the octopus arm is more active than the previous one for various reasons known and unknown; therefore, the intensity of activity during the electrical stimulus might not be the same. Researchers compare the signals from each muscle groups to determine if the muscle is active or not.

Another interesting future work is using the findings from analyzing the trends in the EMG muscle activity for specific movements in this research, to design and develop an artificial muscular hydrostat using soft materials. This design of a ‘soft robotic arm’ which is consisted of soft muscular structure, including a longitudinal actuator and transverse actuator would aid in the different combined contractions of the muscular structure causing controlled variations of stiffness of the soft robotic arm to achieve a majority of movements similar to an octopus arm.

In short, from conducting the experiment to dealing with signal processing and then post processing of signal data, there are a lot of details which should be concerned. After all of them, the data supported interpretation of the movement of the octopus arm can support the design and development of soft robotics arms.

REFERENCES

1. Soft Robotics: Trends, Applications and Challenges (Eds: C. Laschi, J. Rossiter, F. Iida, M. Cianchetti, L. Margheri), Springer International Publishing, Cham, 2017

2. J. Hughes, U. Culha, F. Giardina, F. Guenther, A. Rosendo, F. Iida, *Front. Robot. AI*, 2016, 3, 69.

3. Kier, W. M. & Stella, M. P. The arrangement and function of octopus arm musculature and connective tissue. *J. Morphol.* **268**, 831–843. https://doi.org/10.1002/jmor.10548 (2007)

4. Grasso, F. W. The octopus with two brains: How are distributed and central representations integrated in the octopus central nervous system? in *Cephalopod Cognition* (eds. Darmaillacq, A., Dickel, L. & Mather, J.) 94–12

5. Mather, J. A. How do octopuses use their arms? *J. Comp. Psychol.* **112**, 306–316.

6. Hanlon, R. T. & Messenger, J. B. *Cephalopod Behaviour*.

7. Feinstein, N., Nesher, N. & Hochner, B. Functional morphology of the neuromuscular system of the *Octopus vulgaris* arm. *Vie Milieu* **61**, 219–229

8. Kier, W. M. Hydrostatic skeletons and muscular hydrostats. in *Biomechanics (Structures and Systems): A Practical Approach.* (ed. Biewener, A. A.) 205–231

9. Kayvan Najarian, Robert Splinter, "Biomedical Signal and Image Processing second edition"

10. Henry Matzner, Yoram Gutfreund, and Binyamin Hochner, "Neuro-muscular System of the Flexible arm of the Octopus: Physiological Characterization"

11. Ian D. Walker, Darren M. Dawson, Tamar Flash, Frank W. Grasso, Roger T. Hanlon, Binyamin Hochner, William M. Kier, Christopher C. Pagano, Christopher D. Rahn, Qiming M. Zhang, "Continuum Robot Arms Inspired by Cephalopods"

12. Anders Grunnet-Jepsen, Paul Winer, Aki Takagi, John Sweetser, Kevin Zhao, Tri Khuong, Dan Nie, John Woodfill, "Using the Intel Realsense Depth cameras D4xx in Multi-Camera Configurations"

13. H. Bagheri, A. Hu, S. Cummings, C. Roy, R. Casleton, A. Wan, N. Erjavic, S. Berman, M. M. Peet, D. M. Aukes, X. He, S. C. Pratt, R. E. Fisher, H. Marvi, New insights on the control and function of octopus suckers. *Adv. Intell. Syst.* **2**, 1900154 (2020).

14. Ian D. Walker, Darren M. Dawson, Tamar Flash, Frank W. Grasso,Roger T. Hanlon, Binyamin Hochner, William M. Kier, Christopher C.Pagano, Christopher D. Rahn, Qiming M. Zhang, "Continuum Robot Arms Inspired by Cephalopods"

15. Nir Nesher, Guy Levy, Letizia Zullo, and Benyamin Hochner, "Octopus Motor Control"

16. William M. Kier "The Musculature of Coleoid Cephalopod Arms and Tentacles"

17. Asim Waris, Ernest Nlandu Kamavuakko, "Effect of threshold values on the combination of EMG time domain features: surface versus intramuscular EMG"

18. Stephanie Valentin and Rebeka R.Zsoldos, "Surface electromyography in animals: systemic review"

19. Scott Day, Important Factors in Surface EMG Measurement

20. Ian A.F. Stokes, Sharon M. Henry, Richard M. Single, Surface EMG electrodes do not accurately record from lumbar multifidus muscles

21. Thorsten Rudroff, Kinesiological Fine Wire EMG

22. Navarro X., Krueger T.B., Lago N., Micera S., Stieglitz T., Dario P. A critical review of interfaces with the peripheral nervous system for the control of neuroprostheses and hybrid bionic systems. *J. Peripher. Nerv. Syst.* 2005;**10**:229–258.

23. Gerdle B., Karlsson S., Day S., Djupsjöbacka M. Acquisition, Processing and Analysis of the Surface Electromyogram. In: Windhorst U., Johansson H., editors. *Modern Techniques in Neuroscience.* Springer Verlag; Berlin, Germany: 1999. pp. 705–755. Chapter 26.

24. https://intantech.com/files/Intan_RHD2000_data_file_formats.pdf

25. Zullo L., Fossati S.M., Benfenati F.,"Transmission of Sensory Responses in the Peripheral Nervous System of the Arm of Octopus Vulgaris"

26. C. Frigo, M. Ferrarin, W. Frasson, E. Pavan, R. Thorsen, "EMG signals detection and processing for on-line control of functional electrical stimulation"

27. https://microprobes.com/products/peripheral-electrodes/emg-hook

28. https://gummifactory.github.io/images/gummiarm_shake_close.jpg

29. https://birth.engineering.asu.edu

30. https://en.m.wikipedia.org/wiki/File:Octopus_tentacles.jpg

31. https://commons.wikimedia.org/wiki/File:Electromyographic_recording_at_adductor_pollicis_muscle_and_stimulation_of_the_ulnar_nerve.jpg

32. https://intantech.com/RHD_USB_interface_board.html

33. https://www.intelrealsense.com/depth-camera-d455/

34. https://siglentna.com/product/sdg2042x/

35. Kier, William M. "The musculature of coleoid cephalopod arms and tentacles." *Frontiers in cell and developmental biology* 4 (2016): 10.

36. Fisher Lab, ASU, https://web.asu.edu/fisher