Quantification of the Electric Potential in Zebrafish Hearts

05/06/16

Abstract

In this paper, the electric potential in zebrafish hearts is explored in varying depth following the procedures given by Dr. Fenton's lab. Various techniques are illustrated as they relate to the procedures performed, including an overview of heart dissection, circuitry, and related equipment involved. A brief overview of the difficulties encountered is also discussed. Finally, an analysis of data retrieved is given alongside an account of potential experimental errors.

Introduction

The intention of the experiment was to find a suitable technique to measure the electric potential in the zebrafish heart via an electrocardiogram (ECG) using only low cost materials. The experiment was inspired by research done by Lin et al [1] and Dr. Fenton's lab. Both experiments explored EKG recordings and optical mapping, however, for the purposes of this report, only the electrical potential properties were assessed.

As explained [1], the zebrafish was chosen for numerous reasons. The species has numerous mammalian-like cardiac properties and similarities to human electrical activity. Heart rates, action potentials, phase durations, and susceptibility within the organism are in close approximation with those of mammals. As well, it's genome has been fully mapped – leading to an incredible amount of data archived for any one structure within the fish. Finally, it's size and fecundity was notably alluring – especially within the constraints of this experiment's budget and technologies.

The first goal of the experiment was to minimize costs of the experiment using available technologies within the Physics of Living Systems lab. Convenient access to personal smart phones and applications were also acquired, although these costs were not considered. Cost minimization included construction of heart cages and circuit boards - which went through numerous trials. Secondly, a live zebrafish heart was dissected and the potential difference across it was measured via the circuitry and smartphone application.

Methods

After thorough research on the procedures already performed, the group set out to create a viable circuit using diagrams obtained from a previous and related experiment designed to analyze the electrical signals propagating through cockroach specimens [Fig.1]. After reconstruction of the circuit board, tests were done on human muscles to validate functionality of electrodes, wiring, and iPhone applications.

In tandem, a mechanism for holding the heart in place was also designed and created – using stray materials around the lab, and held together with silicon glue. The first trap consisted of the bottom half of a plastic liquid container with three holes punctured in its sides. The two near the top were for the positive and negative wires, while the one near the base was for the ground.

Before serious attempts were made at collecting and recording clear data, the procedure for taking out the zebrafish heart was learned and subsequently practiced. First, a zebrafish was selected from the population and immediately placed within an ice bath to shut down its nervous system. Great difficulty was encountered with dealing with the actual geometry of the specimen due to its delicate structure and scale (~1 mm^2). The fish was properly prepared and dissected.

The heart was then placed within the premade trap and flooded with Tyrode solution [1]. Electrodes were places on adjacent sides of the heart, spaced a small distance away (less than 1 mm). These electrodes, along with the ground wire, were then connected to the circuit along with a battery. Feedback was recorded via the "Backyard Brains" application on iPhone. However, the group found too much noise in the system to obtain a reliable and analyzable signal.

A new trap was constructed with a larger height and diameter. The second prototype failed due to a leakage in the side of the apparatus which was fixed in the next model. To minimize future noise in observations, the circuit was rebuilt and tested using a signal generator. The third dissection was a success, and similar procedures were followed until new feedback was recorded. This signal had less noise and appeared periodic in structure. However, between cyclic oscillations, large spurts of chaotic noise were peppered within. These findings were better than the initial test readings, but still reasonably troubling.

The following week the experiment was tried again using a combination of equipment from the group's design and from Dr. Fenton's lab. The first goal was to record a zebrafish EKG using only the machines from Dr. Fenton's lab. After several failed attempts finally a clear reading was created in MATLAB and recorded. The next step was to use the chamber created by the lab and the Backyard Brains app on the phone to acquire a recording. The results were similar to the ones from the previous week but the periodic spike that represented the contractions of the heart was slightly more pronounced.

Conclusions

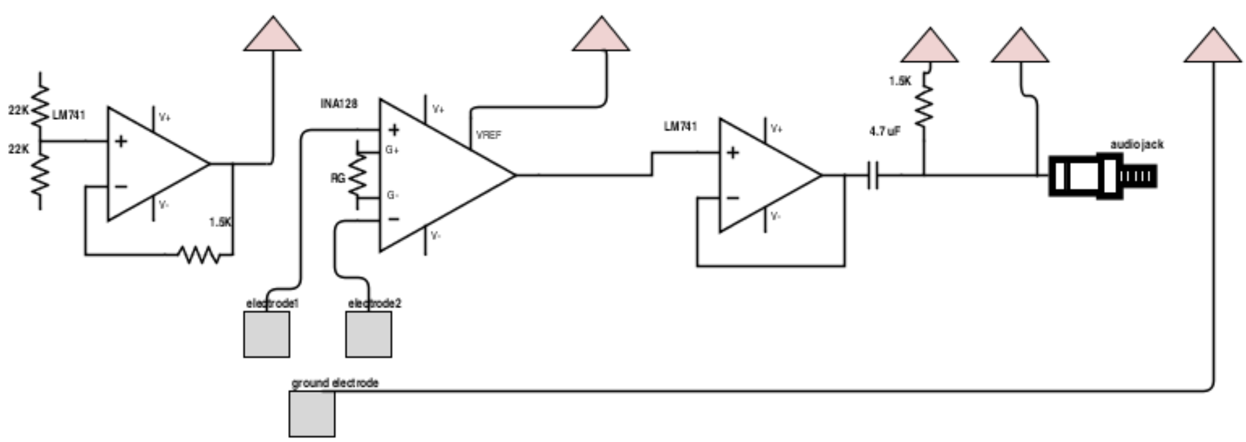
Overall the experiment was a moderate success and it was demonstrated that it is possible to take a zebrafish heart EKG using only elementary materials. The materials designed were acceptable to record a basic EKG but in order to attain a clear one more work will need to be done on the circuit and in researching the Backyard Brains app. In conclusion, the experiment explored the possibility of creating a lost cost apparatus to record a zebrafish heart EKG and succeed in creating basic groundwork for future work to be done.

References

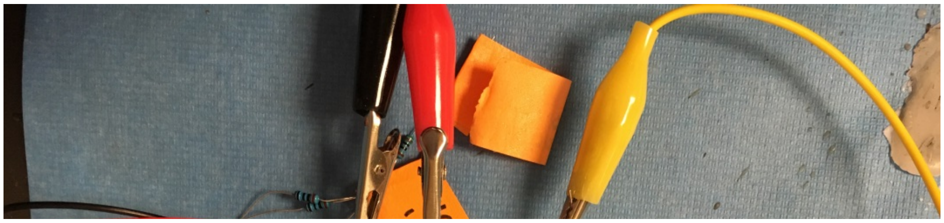
[1] E. Lin, C. Craig, M. Lamothe, M. Sarunic, M. Beg, G. Tibbits. *Construction and use of a zebrafish heart voltage and calcium optical mapping system, with integrated electrocardiogram and programmable electrical stimulation*. American Journal of Physiology, 2015.

Figures

[1]



The Final Circuit



Zebrafish Heart in the first chamber