**Sensory Transduction of Leech Neuronal Cells To Determine Their Receptive Field**

**ABSTRACT**

In this lab we seek to analyze mechanosensory properties, as well as deduce the possible location of a single neuron’s receptive field. Using a dissected medicinal Leech(*Hirudo verbena)*, a microelectrode was inserted into a the Segmental Ganglion, which hold three different types of mechanosensory neurons: T-cells, P-cells, and N-cells. Through stimulating internal areas of the leech’s body within the segmentations (annuli of the leech’s body), we induced cellular responses via applying physical pressure using different tools to mimic real-world stimuli. After recording from 4 different neurons, we hypothesized that the first cell we recorded from displayed characteristics of a P-type cell; whereas, the others elicited T-type responses. In all recordings, however, most of the activity resulted from applying a medium amount of force, using the plastic probe, to different annuli. After analyzing the recordings, we realized that all of our cells actually shows responses characteristic of T-type neurons. Not enough information was collected to **accurately** predict the location of the receptive fields first three T-type cells; however, the last recording was sufficient enough to allow us to deduce the most likely location for the cell’s receptive field. This means that stimuli in that area has the highest likelihood of eliciting a response from the cell.

**INTRODUCTION**

This study discusses an important characteristic of the nervous system in that it is capable of translating, and responding to physical stimuli. This process is called ‘sensory transduction, ‘and it is an evolutionary adaptation that provides animals the ability to survive in their environment. This transductatory process involves the stimulation of mechanoreceptors, each of which elicit activity should the stimuli occur within a specific area known as the cells ‘receptive field.’

There are numerous types of receptors responsible for the transduction of different types of stimulation. Sharks for example, have an ampullary electroreceptor which allows them to sense the bioelectric field given off by all organisms. These electric fields are extremely small (5 nV/cm); however, the ampullary electroreceptors have evolved as highly-sensitive receptor capable of responding to these fields.

In this experiment, we look at mechanoreceptors in the medicinal leech (*Hirudo* verbena) that respond to physically-applied forces. Using this organism is beneficial because of its natural segmentation which are termed ‘annuli.’ These segmentations provide a grid-like map, that allows for easy approximation of a neuron’s receptive field based on the weighted sum of activity elicited in different areas of the specimen.

A microelectrode was inserted into the Segmental Ganglion of the leech. Each ganglia contains three different types of mechanosensory neurons: T-cells, P-cells, and N-cells. These cell types encode specific types of mechanical stimulations. The T-type encode light touch (low force), the P-type encode pressure (medium force ), and the N-type respond to sharp pressure (high force) Through stimulating internal areas of the leech’s body within the segmentations (annuli of the­­­­­ leech’s body), we induced cellular responses via applying physical pressure using different tools to mimic real-world stimuli.

**METHODS**

The methods used in this experiment can be found in the “Introduction to System and Behavioral Neurobiology” manual, under the section titled “Weeks 2 and 3: Receptive Fields of Leech Mechanosensory Neurons. “

**RESULTS**

The results of this experiment show that applying mechanical stimulation to mechanoreceptors elicit characteristic responses of specific mechanosensory neurons. **Figure 1** shows that in applying a medium force to the ipsilateral wall of the specimen, the cells fire Action potentials upon applying the stimulus within the border of the cells receptive field. By then dividing the annuli into a grid, we found that specific areas induced much more activity than others (applied stimulus at point 3 and 5 in **Figure 1** induced no response; whereas, points 4 and 6 did), and that the induced activity exhibits rapidly adapting behavior. Similar behavior was seen in the different neurons depicted in **Figures 2, 3, 4a/b**, where upon stimulation at a certain point along the wall of the leech resulted in brief responses or no response.

The second cell recorded from was also likely a T-cell. This cell was located in the contralateral and rostral region of the ganglia. Rubbing along the contralateral side of the leech dissection, resulted in 2 action potentials; whereas, when specific points of the wall were stimulated, point 4 displayed induced a single action potential and point 3 induced nothing. Point 2, however, unexpectedly resulted in two hyperpolarizations.

The third cell recorded from was a T-cell located laterally along the center-contralateral region of the ganglia. Stimulation at point 1 (shown in **Figure 3**) did not invoke any activity; whereas, points 2 and 3 evoked single responses. Points 4 and 5 failed to induce activity due to the microelectrode losing contact with the neuron.

The final T-cell recorded from was located along the ipsilateral and caudal region of the ganglia. The neuronal responses to a medium amount of mechanical force resulted in a single spike at Point 2 (shown in **Figure 4a**) and no activity at points 1 and 2 through 9. This, however, changed as we stimulated more along the caudal segments of the specimen. As shown in **Figure 4b,** points 1,2, and 3 fired 2 Action potentials; whereas, at point 4, there was no response. The grid of stimulation on all points of the ipsilateral side of the dissection is shown in **Figure 4c**. The diagram shows areas of highest activity in hot red, areas of little response in light red, and the areas of no activity in a light-tan. The predicted receptive field for the cell is predicted to exist at large around section 3b on the grid (depicted by the green, dashed circle).

**DISCUSSION**

The results of the experiment show that our original prediction that the first cell we recorded from was a P-type cell, and the other three cells were t-type. Rather, all the cells responded to stimuli with the characteristics of t-type cells. In a study done by John E. Lewis, William B. Kristan Jr. on touch location of sensory neurons it was seen that t-type cells are most likely to exhibit rapidly adapting responses relative to that of P-type cells exhibit more tonic responses as long as stimulus is applied. Although we didn’t have any data on P-cells, the fast tonic responses to touch shown in the data means the neurons recorded from were most likely t-cells.

Many of the problems that resulted in the lack of data necessary to approximate the receptive field of the first three neurons, were due to the loss of contact between the microelectrode and the neuron being recorded from due to the sensitivity of the whole apparatus; if the table was jammed, or if we accidently applied too strong a force near the electrode, we’d lose contact with the neuron. Also, the microelectrodes we used had a low resistance ranging between 3.0 and 6.0 mV, which is well below what should be used to produce a large enough voltage change across the circuit such that we can truly observe the neural activity.

The data collected from the final neuron allowed us to approximate the receptive fields location based on the weighted sum of the induced activity at specific points. Had we continued to be in contact with this neuron, we would have attempted to stimulate the mechanosensory response at the area we approximated to determine how accurate we were. Also, we would have more accurately tested to see if these cells were t-type by stimulating the cells for a specific duration and seeing if the cell exhibited activity at the onset of the stimulus, adapted to the stimulus, then at the offset of the stimulus fired again.

**CITATIONS**

John E. Lewis, William B. Kristan Jr. (1998*). Representation of Touch Location by a Population of Leech Sensory Neurons*. Department of Biology, University of California, San Diego, La Jolla, CA.

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