



4-16-2019

The Presence Of Photoreceptor Sensilla In The Posterior Sucker Of Medicinal Leeches Inferred Through Functional Visual Response.

Claire Spivey
Western Michigan University, claire.e.spivey@gmail.com

Follow this and additional works at: https://scholarworks.wmich.edu/honors_theses

 Part of the Biochemistry Commons

Recommended Citation

Spivey, Claire, "The Presence Of Photoreceptor Sensilla In The Posterior Sucker Of Medicinal Leeches Inferred Through Functional Visual Response." (2019). *Honors Theses*. 3133.
https://scholarworks.wmich.edu/honors_theses/3133

This Honors Thesis-Open Access is brought to you for free and open access by the Lee Honors College at ScholarWorks at WMU. It has been accepted for inclusion in Honors Theses by an authorized administrator of ScholarWorks at WMU. For more information, please contact wmu-scholarworks@wmich.edu.



The Presence of Photoreceptors in The Posterior
Sucker of *Hirudo Verbana* Inferred Through Functional Visual Response.

Claire Spivey

Western Michigan University

2019

Thesis Committee: John Jellies and Robert Eversole

Abstract

Medicinal leeches gather visual input from both its eyecups and photoreceptors across its body surface; each of its 21 midbody segments possesses 14 photoreceptive sensilla. It has been previously established that the posterior sucker is composed of seven body segments that are fused during embryogenesis. Similarly, four body segments fuse to create the anterior brain. There are five bilateral pairs of eyes located on the dorsal surface of the leech's head and seven bilateral pairs of sensilla distributed over the surface of each midbody segment (Kretz et al., 1975). If no sensilla were lost during the fusion of the seven body segments that make up the posterior sucker, it would possess in the order of one-quarter of the receptors in the entire body. It has been observed that leeches have different responses to ultraviolet light stimulation on the anterior and posterior portion of their bodies suggesting that leeches are responsive to stimuli at both their head and tail (Jellies, 2013). The presence of photoreceptors on the tail sucker could contribute to the difference in the observed behaviors. There have been few studies on the hindbrain and tail sucker of the Medicinal Leech, however, there are known pathways in which the hindbrain and anterior brain communicate that play a role in rhythmic and mesenteric movements like crawling and swimming (Baader et al., 1997). In 10 trials, this study isolated the caudal region of the leech through partial ablation of the connective nerve between ganglion eleven and twelve and division of the body wall between body segment twelve and thirteen, then tested visual responses with and without the hindbrain isolated. Extracellular recordings were analyzed for S-cell response then standardized and compared. All 10 trials showed S-cell response to light stimuli while the hindbrain was isolated suggesting the tail sucker possesses sensilla.

Introduction

The fundamental purpose of neurobiology is to understand how the nervous system senses its external and internal environments, evaluates that input, and generates a behavior from it. Leeches relatively simple nervous systems and large ganglia make them a practical and commonly used model for neurobiological studies. This simplicity allows us to select a behavior and deduce how the neuron and its interconnections produce its observed behavior (Kristan et. al., 2005). Leeches, like many other animals, orient their positions in three-dimensional space using light and gravity (Jellies, 2014). This allows them to navigate their environment and gain access to potential food sources while also protecting themselves from predation. There are numerous studies on the leeches mutually exclusive behaviors such as swimming, crawling, mating, and feeding trying to decipher how the leeches decide their mode of action. In previous studies from 2013, it was established that leeches have unique avoidance and escape responses to ultraviolet light dependent on if the stimulation is presented anterior or posterior suggesting leeches respond to visual input from both the head and tail (Jellies, 2013). While the photoreceptors in the eyecups of the leech sensilla in the body wall have been characterized, the posterior sucker has not despite its possible photoreceptive density and possible role in avoidance and escape of ultraviolet light.

This study focused on light stimulation of the posterior half of the leech attempting to prove the presence of photoreceptors in the tail sucker. Leeches consist of 32 body segments, four of which are fused to create the anterior head. Similarly, seven body segments are fused to form the tail sucker. When considering a single segment of the 21 midbody segments has 14 photoreceptive sensilla (Kretz et al., 1976), there are a possible 98 photoreceptive sensilla in the tail sucker. The anatomy of the hindbrain was characterized in a previous study that focused on the hindbrain's

role in crawling, it contains 14 neuromeres that project into the tail sucker (Baader et al., 1997). In the body wall photoreceptors and touch, receptors excite the interneuron S cell which synapses on the L motor neurons which shortens longitudinal muscles in individual body segments (Sahley et al., 1994).

S cells are excited by light and touch, this experiment used S cell responses to determine the presence of photoreceptors in the tail sucker. The S cell synapses with its two neighboring ganglia to create what is referred to as the fast conducting system (FCS). The FCS has the largest and fastest conduction velocity in the ventral nerve cord. The S cell synapses on L motor neurons in the body wall which shorten longitudinal muscles and while the S cell cannot produce a behavior alone, it contributes to the modification and control of complex behaviors. S cells are related to predatory escape through their observed roles in dishabituation and sensitization (Sahley et al., 1994). It was also previously established that S cells play a role in maintaining orientation in three-dimensional space in lieu of statocysts (Jellies, 2014). The potential 98 sensilla from the tail sucker would contribute a quarter of the sensilla in the body wall, the tail suckers subsequent influence on the S cell would be more than any single body segment.

The experiments described in this paper aimed to prove the presence of photoreceptors in the tail sucker of *Hirudo Verbana*. Testing was done on the isolated posterior of the leech using ablating techniques to sever the body wall between the twelfth and thirteenth body segment and of the connective nerve cord between ganglion eleven and twelve (figure 1). The data collected contains ten trials of dissection that compare characteristic responses to light while the peripheral nervous system of the body fully intact and while only the caudal ganglion's peripheral nerves were intact. Each dissection was followed by severing the peripheral nerves of the hindbrain to explore the

possibility of photoreceptors or S cell stimulation of the nervous system itself. Recordings of S-cell activity were measured using extracellular suction electrodes and stimulated with normalized red, green, blue, ultraviolet, followed by a wave stimulus from a pipette and white light.

Materials/Methods

Leeches

The *Hirudo Verbana* leeches used in this study were isolated from a breeding colony in the lab that originated and is maintained by Niagara Leeches (Cheyenne, WY, USA). The leeches were maintained at room temperature (20-25° Celsius) with weekly artificial pond water changes [Full strength Instant Ocean sea salt (Spectrum Brands Inc., Madison, WI, USA) diluted 1:100 with purified water]. The leeches used in these experiments ranged from 6-10 centimeters in length and fasted through the duration of the experiments. This is to prevent the possibility of photophobia some species of leech exhibit when satiated (Gee, 1912; Herter 1936; Kretz et al., 1976).

Specimen Preparation

Prior to testing leeches were isolated in glass jars filled with fresh pond water. The specimen was then placed on ice for approximately five minutes until anesthetized. The leech was then pinned, avoiding putting any pins directly into the sucker, into a dissection plate filled with cold Ringer's solution (pH 7.4, 115 mM NaCl, 4 mM KCl, 1.8 mM CaCl₂, 1.5 mM MgCl₂, 10 mM D-glucose, 4.6 mM Tris malate, 5.4 mM Tris base) (Jellies, 2014). The entire nervous system was exposed with an incision down the dorsal midline making sure to leave peripheral nerves intact. The body wall was pinned open for access to the peripheral nerves in later testing and incisions were made to the body wall around the tail sucker to expose the maximum possible area of the dorsal surface

of the tail sucker. The body wall was partially ablated between the twelfth and thirteenth body segment and the connective nerve was ablated between the eleventh and twelfth body segment (figure 1).

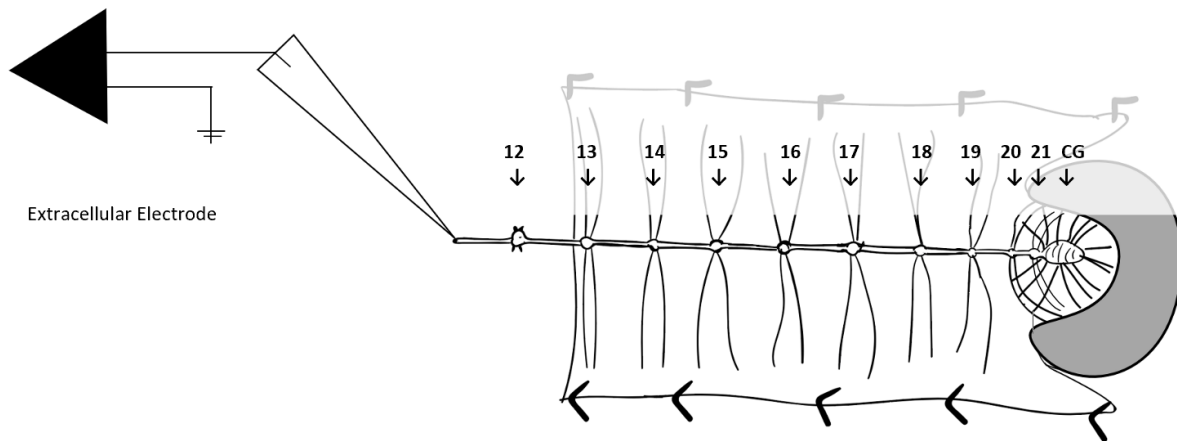


Figure 1. Partial Ablation of the body wall and connective nerve with skin pinned outward and the maximum possible area of the dorsal side of tail sucker exposed (grey). The ganglion of body segment 12 is isolated from the body wall and its peripheral nerves have been severed. Body segments 13-21 each have a single ganglion with four peripheral nerves projecting into the body wall. The caudal ganglion (CG) projecting its 14 neuromeres into the tail sucker. Recording extracellular suction electrode is applied to the cut end of the connective nerve.

Stimuli

In a previous study, light wands were constructed and tested for wavelength and photon emission. The red wand peaked at 632 nm emitting $2.25-2.75 \times 10^{15}$ photons $\text{cm}^{-2} \text{s}^{-1}$, green peaked at 513 nm emitting $3.17-3.5 \times 10^{15}$ photons $\text{cm}^{-2} \text{s}^{-1}$, blue peaked 455 nm emitting $2.53-3.26 \times 10^{15}$ photons $\text{cm}^{-2} \text{s}^{-1}$, ultraviolet peaked at 372 nm emitting $2.20-2.75 \times 10^{15}$ photons $\text{cm}^{-2} \text{s}^{-1}$ (Jellies, 2014). These light wands were used to test visual response along with a white light and touch stimuli. Each trial consisted of two rounds of stimulations from the normalized light wands

in the order red, green, blue, and ultraviolet followed by a touch stimulation and white light stimulation. Following the second round of stimulations, a third stimulation of ultraviolet light was administered. Each normalized stimulation was regulated by the recording system (see electrophysiology) to consist of three consecutive light impulses lasting 2 seconds with 2 seconds between them. White light stimulation was emitted from a dissection light and monitored using a 2-mm fiber optic coupled to a phototransistor (Jellies and Kueh, 2012). Touch stimuli consisted of three consecutive drops of water from a pipet with approximately two seconds between drops.

Electrophysiology

Recordings were captured using an ADI PowerLab 4/35 (ADInstruments, Colorado Springs, CO) set at 10 Hz on an Apple Mac Mini (Apple, Cupertino, CA). Using an external suction electrode sealed to the severed end of the nerve cord, recordings were amplified using an A-M systems model 1700 differential amplifier (A-M Systems, Sequim, WA) with filters from 10 Hz to 10,000 Hz and gain set to 1,000× (Jellies, 2014).

Isolating the Caudal Ganglion

Each dissection involved three separate tests. The first test was on the initial preparation with the peripheral nerves of the midbody ganglia intact. The second test was collected while all peripheral nerves were severed from the 9 midbody ganglia, leaving only the caudal ganglion's 14 peripheral nerves intact. The final test was used as a control with all peripheral nerves severed. If the control test showed S cell responses, there would either be a peripheral nerve that was not entirely severed or the nervous system itself may have contained photoreceptors. However, when the peripheral

nerves were successfully detached there was no observed S cell activity suggesting there are no photoreceptors on the nervous system itself.

Data Analysis

All data was analyzed manually using LabChart 8 Reader (ADInstruments) on a Microsoft Surface Book (Microsoft, Redmond, WA). Data collected from 10 leeches was standardized according to the maximum latency period and frequency in the first second after stimulation. The standardized data and latency periods were averaged and compared (figure 3 & 4).

Results

In 100% of trials, the isolated caudal ganglion exhibited S-cell responses and the isolated nervous system showed no response to light stimuli. In the standardized data, the average white light response of the initial dissections showed an average 22% decrease in the frequency of response (Figure 3). Trials of the isolated tail sucker ranged from a 51% decrease to 12% increase in relative frequency to white light stimuli when compared to the intact preparation. Of these, 8 of the 10 trials showed below a 50% decrease in response frequency. When the entire peripheral nervous system was intact, and the tail sucker was isolated S cells were responsive to wavelengths of light between 632 nm - 372 nm with the similar response frequencies to ultraviolet, blue, and green light and less, sometimes no response to red light. In 4 of the ten trials, the isolated peripheral nerves of the hindbrain showed at least one instance of no response to red light stimuli. The intact preparation and isolated tail sucker preparation had similar latency periods with an average ratio of .91:1 (intact; isolated tail sucker). In all trials, the isolated tail sucker exhibited more phasic character

with higher frequency responses initially, but fewer signals 1 second after stimulation in comparison to the fully intact peripheral nervous system.

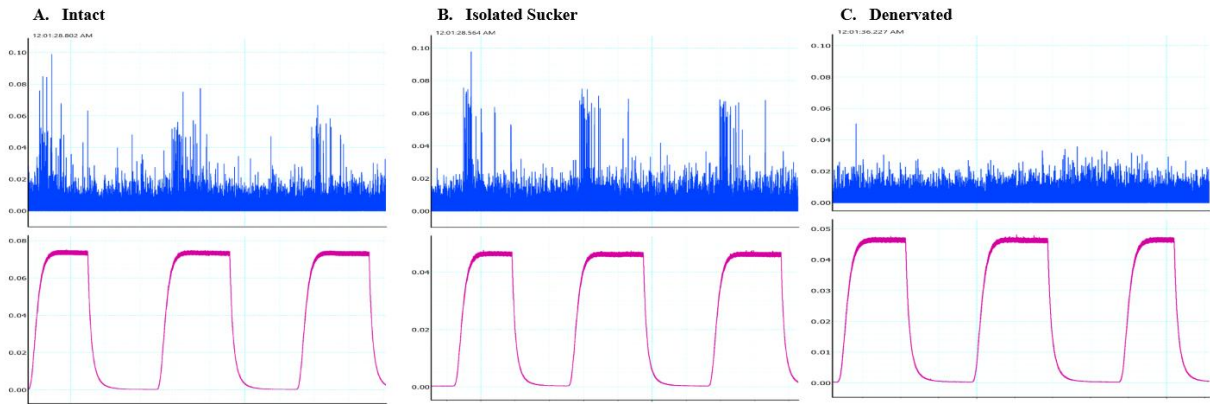


Figure 2. White light stimulation recorded on 11/15/2018, 6-second duration. A. Testing on the initial preparation with all peripheral nerves of the partial ablation intact. B. Results from isolated hindbrain. All peripheral nerves of body ganglia are detached and all neuromeres of the caudal ganglion are intact. C. All peripheral nerves are cut from both the midbody ganglia and caudal ganglia.

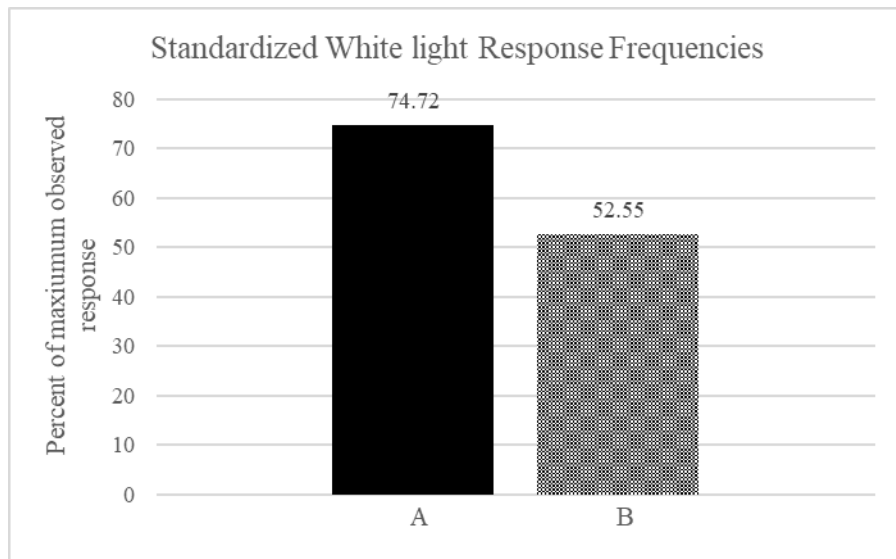


Figure 3. Comparison of response frequencies to white light as a function of the maximum observed response. A. All peripheral nerves of the body ganglia are attached. B. Only the peripheral nerves of the hindbrain are intact.

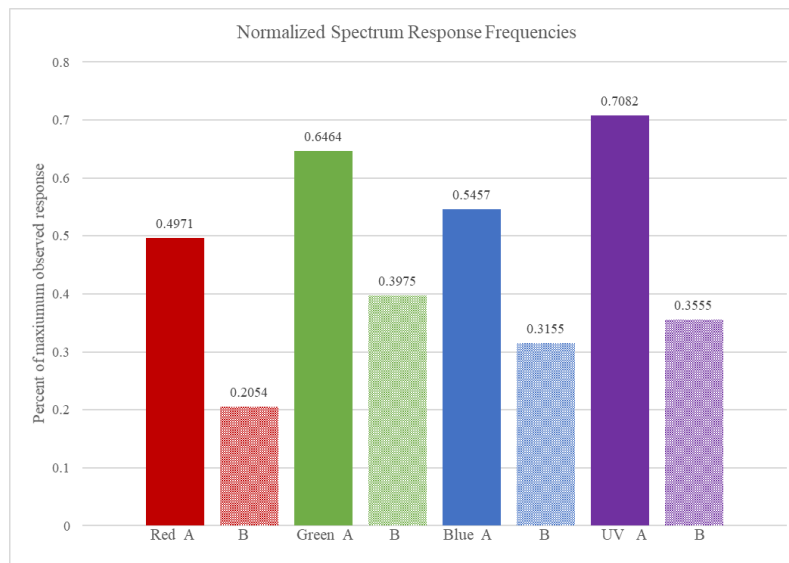


Figure 4. Normalized light response frequencies as a function of the maximum observed response. A. All peripheral nerves of the body ganglia are attached. B. Only the peripheral nerves of the hindbrain are intact.

Discussion

The medicinal leech is an aquatic predator that tends to avoid damage caused by direct ultraviolet radiation. It has been previously established that medicinal leeches have unique responses to ultraviolet light dependent on the area of its body the light is shined. Ultraviolet stimulus on the anterior region results in the leech withdrawing from the light by contracting their longitudinal muscles. The same stimulus applied to the posterior sucker causes the leech to first extend their body forward before contracting forward by relaxing longitudinal muscle while simultaneously contracting circular muscles. This is followed by another extension and crawl or swimming response (Jellies, 2013). The difference in observed response suggests photosensory input from both the tail and head contribute to avoidance and escape. The presence of photoreceptors in the

tail sucker likely aid in the hindbrain driven half of these behaviors by using the S cell as a labeled line allowing it to identify the location of stimulation on the body wall.

Prior to the condensation of the hindbrain's 7 ganglia, there is a possibility of 98 sensilla in the tail sucker. If no sensilla were lost during condensation, there would be one-quarter of the total sensilla of the body wall in the tail sucker alone. While more testing needs to be done to quantify the sensilla present in the tail sucker, this study proved that there is likely a relatively large density. The average decrease in response frequencies between intact body segment twelve and thirteen compared to the isolated tail sucker was 22%, suggesting the tail sucker input contributed more than the nine body segments.

Conclusion

The tail sucker and hindbrain of the leech have been linked to behavioral modification leading to escape and avoidance techniques through the S cell. This interneuron likely acts as a labeled line allowing for appropriate action when presented with the threat of damage by ultraviolet light or a shadow cast by potential predators and prey. The fusion of seven body segments to create the tail sucker and hindbrain along with the data collected in this experiment suggest there are photoreceptors in the tail sucker and, although more testing is required to quantify them, there is likely a significant number of them.

References

- Baader, A. P., & Bächtold, D. (1997). Temporal correlation between neuronal tail ganglion activity and locomotion in the leech, *Hirudo medicinalis*. *Invertebrate Neuroscience*, 2(4), 245-251. doi:10.1007/bf02211937
- Jellies, J. (2013). Detection and selective avoidance of near ultraviolet radiation by an aquatic annelid: The medicinal leech. *Journal of Experimental Biology*, 217(6), 974-985. doi: 10.1242/jeb.094243
- Jellies, J. (2014). Which way is up? Asymmetric spectral input along the dorsal-ventral axis influences postural responses in an amphibious annelid. *Journal of Comparative Physiology. a, Neuroethology, Sensory, Neural, and Behavioral Physiology*. 200: 923-38. doi: 10.1007/s00359-014-0935-x
- Kretz, J. R., Stent, G. S., & Kristan, W. B. (1976). Photosensory input pathways in the medicinal leech. *Journal of Comparative Physiology. a*, 106(1), 1-37. doi:10.1007/bf00606569
- Kretz, J. R., Stent, G. S., & Kristan, W. B. (1976). Photosensory input pathways in the medicinal leech. *Journal of Comparative Physiology ? A*, 106(1), 1-37. doi:10.1007/bf00606569
- Kristan, W. B., Calabrese, R. L., & Friesen, W. O. (2005). Neuronal control of leech behavior. *Progress in Neurobiology*, 76(5), 279-327. doi:10.1016/j.pneurobio.2005.09.004
- Nicholls, J. G., & Kuffler, S. W. (2012). *From Neuron to Brain*(5th ed.). Sunderland Mass.: Sinauer Associates.
- Sahley, C., Modney, B., Boulis, N., & Muller, K. (1994). The S cell: An interneuron essential for sensitization and full dishabituation of leech shortening. *The Journal of Neuroscience*, 14(11), 6715-6721. doi:10.1523/jneurosci.14-11-06715.1994