Organized Caudal Photoreceptors in the Medicinal Leech

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Organized Caudal Photoreceptors in the Medicinal Leech

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Abstract

Visual systems are essential to an organism’s survival. There are a wide range of photosensory organs across the animal kingdom, varying by means of structure, complexity, and the way in which they transduce information. Regardless of variation, visual systems provide the organism with information regarding external stimuli based on how light interacts with surrounding matter. The medicinal leech, *Hirudo verbena*, is one of the many important model organisms of neurobiology as this segmented annelid possesses a relatively simple nervous system and a rudimentary visual system. The leech also exhibits a restricted range of quantifiable overt behaviors and is capable of adapting to environmental challenges. Consisting of thirty-two segments, the first four segments of the animal are coalesced to form the head, and the last seven segments are combined to form the tail. The photoreceptive organs of the head and body have been established through past research (Kretz et al., 1976), five bilateral pairs of photoreceptive eye cups are present in the cephalic region, and seven bilateral pairs of photoreceptive sensilla are present in each body segment. The photoreceptive organs of the caudal region have yet to be clarified. Behavioral studies have indicated that the animal is responsive, engaging in escape behavior when UV light is shone on the caudal portion (Jellies, 2014). Further, a study done in 2019 isolated the caudal ganglion and its peripheral nerves subjecting the solitary nervous tissue to light stimulation (Spivey 2019). The study concluded that the isolated caudal ganglion exhibit S cell responses to light between 632 nm - 372 nm (Spivey, 2019) in a manner directly comparable to the response seen in body wall sensilla (Jellies, 2014). As it has been established that the tail sucker contains photoreceptive cells, the aim of this study is to establish the presence of photoreceptive organs, such as sensilla, in the tail, and describe their distribution. *Hirudo verbena* leeches were dissected, and caudal nervous tissue stained with DiI. A confocal
microscope was used to view the neuronal structures of the tail sucker. Presumptive photoreceptive sensilla were located. These sensillar organs appeared to be organized in a line of three at the distal ends of eight different nerves branching from the hind brain, for a total of twenty-four caudal sensilla. We have suggested some possible explanations for the caudal sensilla structure and organization.

**Introduction**

The ability of an organism to detect and respond to external stimuli is crucial for survival. There is a plethora of means by which various organisms transduce stimuli into neuronal input, related to the transduction of energies, processing of information, and the logic circuitry in nervous systems. Specifically, visual systems all transduce electromagnetic energy into some form of neurological signal, yet among species they vary greatly. Despite this variance, each visual system provides the animal with information regarding light and the ways that light interacts with matter in the environment. When considering the fundamentals of neurobiology, it is important to first understand these sensory organs to ultimately evaluate how the information is transduced to inform behavior.

Medicinal leeches are one important model organism for neurobiology as they have a relatively simple nervous system making dissection and manipulation more practical, a limited behavioral repertoire with many overt behaviors that can be quantified, and yet they are complex enough that their nervous systems evidence adaptation to complex environmental challenges. The medicinal leech of the *Hirudo* genus is a segmented annelid consisting of thirty-two segments. Their rudimentary visual system consists of two types of photoreceptive organs. These simple organs have been described as the eyecups, and sensilla, each of which contain photoreceptive cells, among other cell types (Whitman, 1886; Mann, 1961; Laverack, 1968;
Kretz et al., 1976). These photoreceptors are phaosomal neurons that respond with a membrane depolarization to light stimulation and project axons centrally (Lasansky and Fuortes, 1969; Fioravanti & Fuortes, 1972, Kretz et al., 1976). Segments one through four of the animals are coalesced during embryogenesis to form the anterior brain. Five eyecups are organized in bilateral pairs in two longitudinal rows within the dorsal surface of the head region (Kretz et al., 1976). Figure 1 from Kretz et al. (1976) depicts the five bilateral eyecups of the cephalic photosensory system. The photoreceptive sensilla are similarly organized into bilateral pairs of seven, with fourteen in each body segment of the organism (Kretz et al., 1976). Figures 2 and 3 from Kretz et al. (1976) illustrate the layout of photoreceptive sensilla within the leech body wall. Comparable to the anterior brain, the posterior brain of the leech is formed during embryogenesis by the fusion of the last seven segments. The photoreceptive anatomy of the tail region, however, has yet to be elucidated. A study done in 2019 isolated the caudal ganglion and its peripheral nerves, and subjected the solitary nervous tissue to light stimulation (Spivey, 2019). The study concluded that the isolated caudal ganglia exhibit S cell responses to light between 632 nm - 372 nm (Spivey, 2019) in a manner directly comparable to the response seen in body wall sensilla (Jellies, 2014). Through this study (Spivey, 2019), it is evident that the tail sucker alone can be responsive to electromagnetic radiation, and thus must contain photoreceptive cells. What was entirely unknown is whether the sensillar homologues retained any of their body wall organization during the formation of the fused sucker, whether individual neurons became distributed throughout the sucker, or whether they had remodeled into entirely new structures as is seen in the fusion of anterior segments in the head. Thus, the number, organization and regional distribution of photoreceptors remains to be determined.
When considering the current knowledge of the anatomical orientation of photoreceptor cells, we know that they are present within the eyecups and body wall sensilla. Photoreceptor cells are not known to be dispersed independently within the body wall (Kretz et al., 1976). Based on this, our assumption is that the photoreceptors of the tail sucker are assembled in sensilla. However, if the anatomical orientation of sensilla in the tail remains consistent with that in the body segments, the tail would contain fourteen sensilla for each of the seven merged segments. This would mean that there is a total of ninety-eight sensilla present in the tail sucker alone. The aim of this study is to first determine the presence of photoreceptive sensilla in the tail sucker of *Hirudo verbana*, then based on their presence, estimate the number of photoreceptive sensilla and describe their regional/spatial distribution.

Small medicinal leeches, *Hirudo verbana*, were anesthetized in ice, then carefully dissected to reveal the nervous system, paying special attention to the caudal sucker and brain. Individual tail suckers were isolated and pinned out in a petri dish. Dil stain was injected into the hind brain of the animal and after chemical fixation allowed weeks to spread throughout the peripheral nerves of the tail. The tail suckers were then viewed under a confocal microscope to evaluate for homologues of body wall sensilla. These sensilla have characteristic anatomical profiles and these features were used to develop a search strategy.

**Materials and Methods**

**Dissection**

Medicinal leeches, *Hirudo verbana*, were the subject of dissection. The average length of each leech ranged from about 2.5cm-7.5cm. Prior to dissection the leeches were placed in a small glass jar containing artificial pond water. The jar and leech were then set in a bucket of ice for approximately ten minutes as a means to anesthetize the animal. The leech was then removed
from the jar and pinned into a wax dish with a layer of chilled ringer solution. Care was taken to avoid damaging the caudal sucker of the animal. Dissection scissors were used to make an incision through the integument of the animal along the dorsal midline. As the concern of the experiment is the tail sucker of the animal, the incision was made from approximately segment eight to twenty-one, so that the hind brain was exposed. The body wall of the animal was then reflected out and pinned to the side exposing the internal workings of the animal. The connective tissue and bowels of the organism were removed with fine tipped forceps to reveal the nervous system. At about the nineteenth segment, the posterior portion of the leech was removed and placed into a petri dish. Five micro pins were placed around the furthest periphery of the tail sucker to pull the tissue taut. This damage was unavoidable, but care was taken to place pins in locations that were as distal as possible. After dissection and pinning were completed, the tissue was submerged in fixative (4% paraformaldehyde in 0.1M PO₄– pH 7.4 and 1:1000 Hoechst solution- C₂₅H₂₄N₆O) and placed in the refrigerator for 24-48 hours. Hoechst stain binds DNA labeling all cell nuclei (Whiteside et al., 1997).

**Staining**

The fixative solution was rinsed from the petri dish and replaced with PBS saline solution. A small glass needle attached to a syringe with plastic tubing was used in the staining process. 10mg of Carbocyanine dye (DiI) (1,1′,dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate) diluted with 100 µl of DMSO and 50 µl of 100% ETOH was taken up into the needle largely by capillary action. The needle was then used to penetrate the hind brain of the leech and inject a small bolus of DiI. DiI is a lipophilic, fluorescent stain that works as a retrograde and anterograde tracer of the nervous system (Honig & Hume, 1989). The stain rapidly diffuses into cell membranes and can be used to trace neuronal branches. DiI has also
been found as a successful neuronal label in fixed tissues (Godement et al., 1987), within formaldehyde fixed tissues, the dye retains its ability to diffuse through plasma membranes and progress along axons. After staining, the tail was left to sit in the dark, at room temperature, for weeks to months submerged in saline solution. Periodic saline solution changes were made to prevent drying of the tissue and mold growth. A total of twenty-five leeches were dissected and stained through this process for the purpose of this study.

**Microscopic Evaluation**

To evaluate the tail under the confocal microscope, the saline solution was removed, and the tail was submerged in 50% glycerol solution for approximately one hour. The suspension was removed and replaced with 90% glycerol solution and allowed to sit for one hour. All pins were removed from the tissue. The tail was removed from the petri dish and placed on a microscope slide. Rubber cement was used to seal the edges of the coverslip, prevent further drying, and stabilize preparation on the confocal stage.

**Results**

To determine the presence of photoreceptive sensilla in the tail sucker, known sensillar structures in the body wall were used for comparison. Body wall sensilla are organs with round photosensory cells and numerous rod-shaped sensory cells with hair like filiform processes projecting from the central region beyond the overlying tissue (Mann, 1961; Kretz et al., 1976; Derosa & Friesen, 1981). In each body segment of the leech, the seven bilateral sensilla are connected through an auxiliary nerve branch to a medial ganglion within the nerve cord (Kretz et al., 1976). In this study, I first applied the anatomical tracing techniques to body wall segments to provide a baseline of sensillar structures revealed. Through the use of a confocal microscope, we were able to identify body wall sensilla stained with DiI. Figures 4 and 5 depict body wall
sensillum 1 (S1) through the view of a confocal microscope. As described by Mann (1961) the body wall sensilla appears as a cluster of numerous round and elongated sensory cells among epidermal cells and other cell types. Filiform like hair projections emanate from the center of the sensillum which may protrude beyond the body wall 50 μm more or less (Derosa & Friesen, 1981). Similar to the body wall ganglia, in the tail of the leech, nerves branch from the hind brain fanning out in a radial manner throughout the circular shaped sucker as seen in figure 6. To be considered a successful preparation, the DiI propagated from the hind brain throughout the nerve branches, clear to the edges of the sucker. Of the twenty-five leeches dissected and stained, ten were considered successful preparations upon microscopic examination. Using the confocal microscope to follow the nerve branches, sensillar organs were identified within the tail (figures 7 and 8). As we continued to evaluate additional tail dissections, more sensilla were identified, and an anatomical pattern emerged. When viewing the tail sucker as a whole (figure 6), the sensilla appear to be organized along the distal portions of eight nerve branches, in lines of three, nearing the outer edge of the tail sucker. When considering the lines of three, it was found that the sensilla at furthest periphery of the sucker was consistently larger (about two times) than the two subsequent sensilla. These most distal sensilla also seemed to consist of two lobes, consistent with a fusion of two or more subsidiary structures.

**Discussion**

It has been established that the medicinal leech was receiving visual input from the tail sucker (Spivey, 2019), however information about the presence and distribution of photoreceptive organs was still lacking. As predicted, presumptive photoreceptive sensilla were located in the tail, however the number and distribution are unlike that which is found in the body wall. Each body wall segment contains fourteen sensilla (Kretz et al., 1976) (Figures 2 and
If the nervous tissue anatomy remained consistent, it would be expected that fourteen sensilla would be present in each of the combined seven segments joined to make up the tail sucker. Instead, based on our microscopic evidence, there appears to be eight sets of three sensilla for a total of twenty-four sensilla. Each sensilla appears as a cluster of large round cells amongst epidermal cells and other cell types. Elongated rod-shaped cells are found in the center with filipodia like hair cells protruding from the center of the cluster. It is important to note that because the body of the animal overlays part of the tail sucker, the view of this portion is obstructed. More sensilla may be present beneath this covered portion, however not visualized under the confocal microscope. The body obstruction of the tail sucker can be seen in figure 7.

The most distal sensilla, when considering the groups of three, is consistently about twice as large as compared to the other two sensilla in the trio (Figure 8). We have suggested that this size difference reflects the remodeling of the tissues during sucker formation involving a great expansion of the ventral midline tissues, effectively pushing the ventral sensilla outward toward the edge of the sucker. When considering body wall sensilla, it is known that S1 and S2 sensilla remain in a larger size class as compared to S3-S7 (Derosa & Friesen, 1981). It is possible that the most distal sensilla are homologous to body wall S1/S2, and the more proximal sensilla are homologous to S3-S7. An additional feature is that the larger distal tail sensilla appear as two lobes, these sensilla might actually represent two or more sensilla that were coalesced together during the tissue migrations and condensations of embryogenesis. Simply identifying the presence of sensilla and their general anatomical orientation is a good first step. Further research should be conducted to provide a more in depth understanding of the tail sensillum structure.

A general biological theme is that form follows function. With this in mind, previous studies displaying the functional responses of leeches to electromagnetic stimuli may provide
insight into the structure of the organs that detect it. The seven bilateral body wall sensilla are
distributed laterally, dorsal to ventral in orientation (Derosa & Friesen, 1981). In other words,
body wall sensilla S1 and S2 are oriented most ventral, and S6 and S7 most dorsal. In a study
conducted comparing dorsal ventral response to UV light (Jellies, 2014) it was found that ventral
presentation of UV light evoked a stronger response as compared to dorsal. When the UV light
was presented to the ventral side of the animal, almost always that response was to invert,
ultimately minimizing ventral exposure to UV radiation, even if this meant placing the dorsal
surface at direct exposure (Jellies, 2014). It was deduced from this experiment that ventral
sensilla are more equipped to detect UV light. In another study conducted with UV light
stimulation (Jellies, 2014), it was found that leeches respond to both cephalic and caudal, dorsal
presentations of UV light with different variations of escape behavior. Dorsal presentation of UV
light at the head elicits the animal to release the anterior sucker and retract the body. UV light
presented dorsally at the tail also evokes the animal to release the anterior sucker, however
subsequently the animal extends, stretching the head out and away from the stimulus. Through
these studies it has been established that ventral sensilla are most sensitive to UV exposure, and
dorsal exposure to UV light at both the head and tail regions elicits specific escape responses.
Considering these behavioral responses, the hypothesis that the larger caudal sensilla oriented at
the periphery may be body wall S1/S2 homologs pushed distally to the edge of the tail sucker by
expanding midline tissues during embryogenesis, would be supported. In this scenario, UV
sensitive ventral sensilla would be present dorsal side of the tail sucker, and may account for the
strong escape response seen upon dorsal UV light stimulation of the tail sucker. These studies
evaluating how leeches respond to light may provide some insight into the structure of caudal
sensilla, however further research should be conducted to conclude on the true composition of caudal sensillum.

Figure 1: From Kretz et al. (1976) displaying the caudal region of *H. medicinalis*. The right side of the image depicts the five bilateral eyecups and their optic nerves.
Figure 2: From Kretz et al. (1976) showing the seven bilateral sensilla of each body segment as well as each segmental ganglion.

Figure 3: From Kretz et al. (1976) showing H. medicinalis anterior and posterior root neuronal branches from the ganglion. Segmental sensilla S1 – S7 displayed.
Figure 4: Z stack of body wall sensillum S1 displayed in Texas red under a confocal microscope. The image was taken from the dorsal perspective showing the filipodia like hair cells protruding upwards. Hoecst nuclear stain is displayed in blue.

Figure 5: Body wall sensillum S1 displayed in Texas red under a confocal microscope. Hoecst stain displayed in blue
Figure 6: Dorsal view of the entire tail sucker. Nervous tissue depicted in Texas red under a confocal microscope. Long nerve branches fanning out radially from the hind brain to the edges of the sucker. Small bulb like structures, near the edges of the neuronal branches, in lines of three – suspected sensillar organs. The body of the animal extends from its attachment at the center of the sucker to the right of the photo, partially obstructing the view of the tail sucker.
Figure 7: Tail sucker sensillum displayed in Texas red under a confocal microscope. Hoecst nuclear stain displayed in blue/purple.

Figure 8: Three tail sucker sensilla. Left to right, distal to proximal respectively.
References


