

# Sensory Feedback and its Role in the Maintenance of Continuous Swimming

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## Social Impact

Rhythmic behaviors are present in practically all species, ranging from breathing to walking. The mechanisms of these behaviors across species have common underlying neural features in terms of intrinsic properties, in particular the way that sensory feedback influences behavior. The leech is an appropriate model organism for examining rhythmic behaviors due to the few behaviors that they engage in and their relatively simple nervous system. This research takes steps towards better understanding how sensory feedback can modulate rhythmic behavior, specifically continuous swimming, in leeches. Research on organisms such as leeches can help to explain general mechanisms of behavior that may be difficult to recognize in more complex animals, such as humans.

“Sensory feedback from the body plays an important role in generating rhythmic locomotion”



## Biography

Saša Jusufović is a fourth-year biology and pre-veterinary student originally from Bosina-Hercegovina. When not in school, she lives with her family in Virginia Beach, Virginia, where she attended Princess Anne High School. She currently conducts independent research in the Friesen Lab, performing neurophysiology and behavior experiments on leeches. Her main focus in research is examining the role of sensory feedback in maintaining swimming in leeches as well as the effects of sensory feedback on various swim parameters. Saša received a small research and travel grant through the College of Arts and Sciences towards her research for the spring of 2011. Outside of the lab, Saša enjoys volunteering at the Wildlife Center of Virginia as a member of the treatment team.

# Abstract

Virtually all species exhibit rhythmic behaviors such as walking, chewing, swimming, and breathing. These behaviors have many common underlying neural features in terms of intrinsic properties, specifically the way that sensory feedback influences motor activity. Leeches are excellent model systems for understanding neural mechanisms underlying rhythmic behavior because they exhibit relatively few behaviors and their nervous system is relatively simple compared to other species. Research on organisms such as leeches can help explain general principles of behavior that are conserved or analogous across species, but are too difficult to recognize in more complex animals. The goal of the proposed experiments is to determine how sensory feedback affects various parameters of rhythmic swimming behavior, such as cycle period and swim duration. In most species examined, the patterned neural activity that drives rhythmic movements can occur in the isolated nervous system without the presence of skin, muscle, and other non-neural tissues. However, certain aspects of such “fictive” behavior are altered when compared to the normal animal, indicating that sensory feedback from the body plays an important role in generating rhythmic locomotion. This study investigates the changes in cycle period and swim duration as a function of the amount and region of sensory feedback in the leech, *Hirudo verbana*. After collecting and analyzing the experimental data, it was concluded that the removal of sensory feedback significantly shortens swim duration and increases cycle period. It was also determined that regional differences in sensory feedback affect these swim parameters; particularly, anterior sensory input plays a greater role in maintaining swimming than posterior sensory input. Results suggest that the interaction between the amount and location of sensory feedback plays a role in the maintenance of continuous swimming.

## Introduction

One important goal in neuroscience is to understand how the nervous system and the body interact to produce efficient locomotion. Invertebrates provide a good model for studying connections between the nervous system and behavior, which makes it possible to understand the neural mechanisms in more complex animals. Many neural structures and mechanisms are conserved from relatively simple animals to more complex animals. Swimming in an invertebrate such as the leech, *Hirudo verbana*, is an excellent model system to aid in understanding the neural mechanisms underlying rhythmic behavior. *H. verbana* has a relatively simple and accessible nervous system and exhibits only a few behaviors, such as swimming and crawling (Delcomyn, 1998; Kiehn & Kjaerulff, 1998). This study focuses on the effect of sensory feedback in altering swim maintenance (the processes that sustain swimming behavior). The leech nervous system consists of 21 midbody ganglia (labeled M1-M21) and a brain at each end of the nerve cord. Sensory input is conveyed to the nerve cord through nerve roots extending from each ganglion that innervate the muscles in the body wall (Kristan et al., 2005). While sensory feedback is important for efficient locomotion, the isolated leech nervous system, which receives no sensory input, is capable of generating rhythmic output. This is due to a network of neurons present in the leech nervous system called the central pattern generator (CPG), which controls the correct timing of motor neuron outputs and, therefore,

rhythmic movements. Although rhythmic patterns driven by the CPG can occur without sensory feedback in the isolated nerve cord, these patterns are not enough for efficient locomotion, as shown by the smaller intersegmental phase lags in isolated nerve cord preparations when compared to intact animals (Pearce & Friesen, 1984). Intersegmental phase lags remain nearly constant with respect to cycle periods (the inverse of swim frequency), which results in the maintenance of a single wavelength per cycle in intact leeches swimming at any speed. The smaller phase lags seen in isolated nerve cords would not produce one body wave per cycle. This decrease in intersegmental phase lags due to removal of the body wall shows that sensory inputs are crucial for effective swimming in the leech.

The importance of sensory feedback was further demonstrated in experiments where the nerve cord was severed between M10-M11 in otherwise intact leeches. Despite the absence of neural communication between the two ends of the leech, coordination was observed between the ends during swimming activity which could only have originated from sensory inputs from the body wall (Yu et al., 1999). However, phase lags on either side of the lesion were increased and more variable than in the intact animals. While sensory feedback alone is capable of generating intersegmental coordination without the nerve cord, these results suggest that both sensory feedback and the nervous system are required for efficient locomotion in the leech.

It is not well understood how brief sensory input leads to prolonged behavioral responses, such as swim-maintenance (Friesen et al., 2011). It is known that a transient stimulus can elicit a prolonged behavioral swim response in the leech and that the intensity of an initiating stimulus is not correlated with the subsequent swim duration (Brodfuehrer & Friesen, 1986; Friesen et al., 2011; Mullins et al., 2011). This shows that there are independent swim-initiation and swim-maintenance systems in the leech. Cycle period, or swim frequency, and swim durations are the parameters of swim maintenance considered in this study. In isolated nerve cord preparations, swimming is always episodic, meaning that it occurs in discrete bouts. However, swim episodes are longer when the head brain is removed, showing that the head brain has an inhibitory effect on swim maintenance. Preliminary data in this lab suggested that continuous swimming, or swimming for prolonged periods without stopping, occurs in the intact leech, but only when the head brain is detached. This study seeks to determine the extent to which sensory input modifies swim maintenance responses to demonstrate the importance of sensory feedback in locomotion.

## Materials and Methods

Adult leeches of specie *Hirudo verbana* were used to carry out the experiments. Leeches were kept in an aquarium in a temperature-controlled room on a 12-hour light/12-hour dark cycle at 18- 21°C. Leeches were pinned onto a wax-bottomed dish filled with cold saline, anesthetized, and small incisions were made in the body wall to either locally denervate selected body segments or to disconnect the head brain from the rest of the nerve cord. To remove sensory feedback, the body wall was cut open from the dorsal side, pinned open to expose the underlying ganglia, and the nerve roots exiting the desired ganglia were cut away from the desired segments of the body wall. This method was used to remove 1/3 or 2/3 of the sensory feedback from the anterior or posterior end of the ganglia and 1/3 or less from the middle of the ganglia. Cutting the nerve roots also removed motor neuron input to the body wall. The body wall was sewed shut to prevent damage to the nerve cord. To suspend the leech, threads were placed in denervated regions either through each sucker, through the anterior sucker and either side of a posterior segment or on either side of both a posterior and anterior segment (Figure 1). Leeches were allowed to recover for about 5 minutes. After recovery, the leech was suspended in a trough filled with saline in order to prevent damage to the nerve cord.

Swim parameters for nine types of preparations were compared (Figure 2). Control leeches had all sensory feedback intact except Segment 17 and Segment 4, which were denervated in order to suspend the leech. In these control animals, the entire nerve cord was intact (head brain



**Figure 1. Photograph of suspended leech.** In this example (M2-T), the head brain has been removed and all segments receive sensory input, except for one posterior segment, which was denervated to allow threads to be attached to the leech. Black lines were drawn into the figure to illustrate location of threads, which are otherwise difficult to detect in this picture.

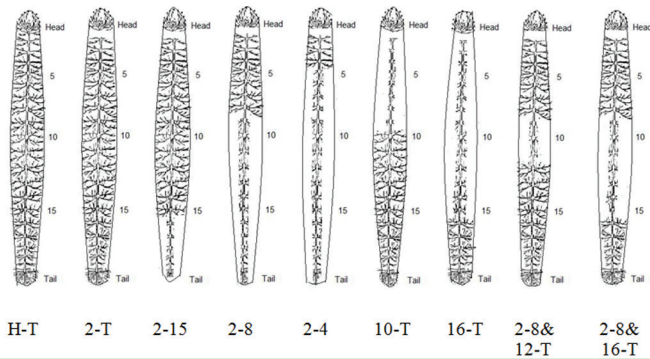
to tail brain, or H-T) for the initial experiments. Following completion of the first set of experiments, the head brain was detached by cutting between M1 and M2 and the experiment was repeated. The remaining preparations had the head brain detached (M2-T) and received sensory input in only some segments, with segments 2-T, 2-15, 2-8, 2-4, 10-T, 16-T, 2-8 & 12-T, or 2-8 & 16-T innervated. Each preparation type used four or five leeches.

After suspending a leech in the trough, it was observed for 30 minutes and recorded swimming using the program PixeLink Capture OEM and a high-speed camera. Videos were taken at either 30 frames per second (fps) for 150 seconds as the standard capture values to analyze cycle periods or at 8 fps for 4800 seconds (10 minute duration) for evidence of prolonged swimming. If the leech swam for the entire 30 minutes, it was scored as swimming continuously. For preparations that did not swim for the entire 30 minutes, the average swim duration from all swim episodes that occurred during that time frame was determined. ImageJ was used to analyze cycle periods by defining a reference point during the swim and counting the number of frames that captured that point during each swim cycle. GraphPad Prism 5 was used to carry out statistical data analysis.

## Results

### Removal of Head Brain

The control leeches swam for an average duration of  $4.1 \pm 2.0$  minutes (mean  $\pm$  standard error)(Figure 4). None of these leeches swam continuously (Figure 3). The average cycle period was  $0.50 \pm 0.032$  seconds (Figure 5). When the input from the head brain was removed by cutting between M1 and M2, all of the leeches swam continuously ( $n=4$ ). The average cycle period for these M2-T leeches was  $0.63 \pm 0.04$  seconds. These data show that with sensory feedback intact and without inhibitory inputs from the head brain, the suspended leech will maintain swimming for prolonged periods of time.

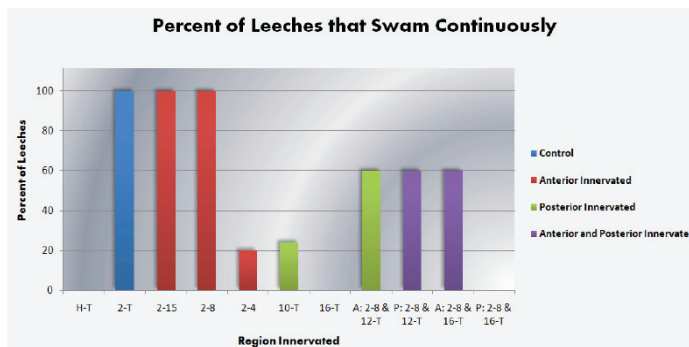


**Figure 2. Depictions of regions innervated.** The leech nerve cord runs down the length of the body with nerves exiting to innervate the body wall. Areas where nerves remain in the diagram indicate innervated segments. Although the head brain was technically innervated in all preparations, it had no communication with the remaining nervous system due to the lesion between M1 and M2 in all but the H-T preparations.

### Removal of Anterior Sensory Input, Posterior Innervated

The effect of sensory feedback on swim durations and cycle periods was studied by denervating the anterior third of the body so that only ganglia M10-T received sensory input. Following this procedure, only one out of four leeches exhibited continuous swimming. The remaining three leeches swam for an average duration of  $1.4 \pm 0.1$  minutes. The average cycle period of all four leeches was  $0.54 \pm 0.01$  seconds.

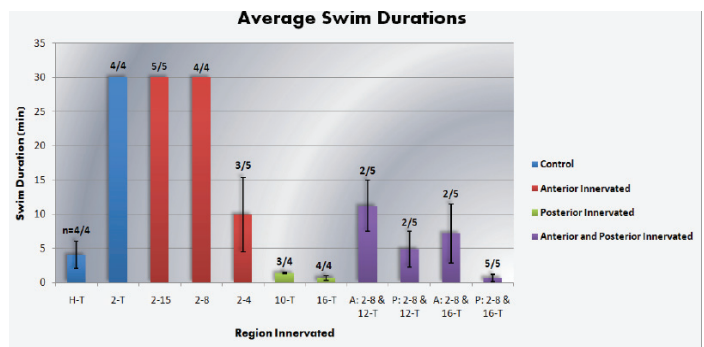
After cutting the nerves in the anterior two-thirds of the leech so that only body segments M16-T were innervated, none of the leeches swam continuously. These leeches ( $n = 4$ ) had an average swim duration of  $0.71 \pm 0.34$  minutes with an average cycle period of  $0.63 \pm 0.04$  seconds. These data show that sensory input from the posterior end is not sufficient to maintain continuous swimming.



**Figure 3. Percentage of leeches that swam continuously within each group.** "A" refers to only the anterior end swimming and "P" refers to both the posterior and anterior end swimming.

### Removal of Middle Sensory Input, Anterior and Posterior Innervated

Different amounts of sensory input from the middle of the body wall were removed to study the effect of middle sensory input. For the first group, input from M9-M11 was removed, leaving Segments 2-8 and 12-T innervated. Three out of five leeches swam continuously. In these preparations, sometimes the entire animal swam, while other times only the anterior end swam and the posterior end was still. For this reason, separate swim durations for the anterior and the posterior ends were calculated. The average swim du-



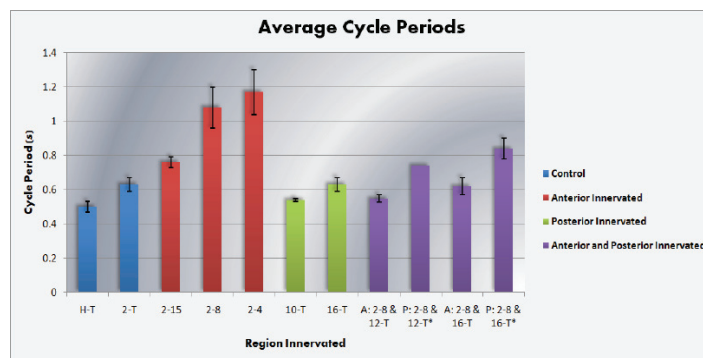
**Figure 4. Comparison of average swim durations with respect to the innervated regions.** Fractions above bars denote number of leeches out of the total leeches that were used to calculate the averages. In preparations with leeches that scored as swimming continuously as well as episodically, the leeches scored as swimming continuously were not used to calculate the averages.

ration (excluding leeches that swim continuously) for the anterior end was  $11.0 \pm 3.8$  minutes while the average swim duration for the posterior end was  $4.9 \pm 2.6$  minutes. Both ends exhibited an average cycle period of  $0.55 \pm 0.02$  seconds when both ends were swimming, showing that the ends were phase-locked when they swam at the same time (as opposed to swimming simultaneously with independent frequencies). Only one swim episode (4 cycles recorded) occurred where the anterior end was swimming without the posterior end. These limited data show that the average cycle period of the anterior end was  $0.74 \pm 0.00$  seconds.

In the second group, more sensory input was removed by cutting the nerves between M9 and M15. Three out of five of these leeches swam continuously. The average swim duration for those leeches that did not swim continuously was  $7.2 \pm 4.3$  minutes when only the anterior end swam and  $0.70 \pm 0.55$  minutes when both ends swam. These leeches also exhibited episodes where both the anterior and posterior were swimming at the same time or where only the anterior was swimming and the posterior was still. The average cycle period for both ends when both ends were swimming was  $0.62 \pm 0.05$  seconds; when the posterior end was not swimming, the anterior end had an average cycle period of  $0.84 \pm 0.06$  seconds. When both ends were swimming, the

ends were phase-locked. These data show that leeches with sensory input removed from the middle of the body sometimes swim continuously. Further, the lack of sensory feedback from the middle sometimes causes swimming in only the anterior half of the animal, despite the nervous system being intact. The posterior half was never observed swimming unless the anterior portion of the leech was swimming.

*Removal of Posterior Sensory Input, Anterior Innervated*  
 Posterior sensory input was removed by cutting the nerve roots from 1/3 of the posterior end of the leech so Segments 2-15 were innervated. All of the leeches ( $n = 4$ ) swam for 30 minutes with an average cycle period of  $0.76 \pm 0.03$  seconds. Sensory input from 2/3 of the posterior body wall was removed so that Segments 2-8 were innervated to determine whether continuous swimming would occur when only the anterior one-third was innervated. All leeches swam for 30 minutes with an average cycle period of  $1.1 \pm 0.12$  seconds. Sensory input was further reduced to Seg-



**Figure 5. Comparison of average cycle periods with respect to innervated regions, including error bars.**

ments 2-4 to test whether sensory feedback from only three ganglia would be sufficient to allow for continuous swimming. Of these leeches, one did not swim, three exhibited episodic swimming, and one swam continuously. The leeches with episodic swimming ( $n = 3/5$ ) swam for an average of  $10.0 \pm 5.4$  minutes with an average cycle period of  $1.20 \pm 0.13$  seconds. These data show that anterior sensory input is the most effective in maintaining continuous swimming.

### Statistical Analysis

Swim parameters in the three groups with a varying number of anterior segments innervated (2-15, 2-8, and 2-4) were compared. Swim durations and cycle periods data were significantly different between the three groups (one-way ANOVA,  $p = 0.015$ ,  $p = 0.0316$ , respectively). A similar analysis was performed on the groups with the posterior end or both ends innervated. Swim durations, but not cycle period data, were significantly different between groups with posterior region M10-T and M16-T innervated (Student's t-test,  $p < 0.0001$ ,  $p = 0.54$ , respectively). Swim duration and cycle period data for groups with both anterior and

posterior region innervated and middle region denervated were significantly different (one-way ANOVA,  $p = 0.0185$ ,  $p = 0.006$ , respectively; data for M2-M8 & M12-T both anterior and posterior ends swimming were disregarded since only one leech swam in this group). These results indicate that decreasing the number of segments innervated in any region decreases swim durations. Decreased sensory feedback also led to significantly increased cycle periods with the anterior ends and both ends innervated.

The above analysis indicates that the amount of innervation significantly affects swim duration and cycle period. The data suggest that the swim durations and cycle periods were related to the region innervated. Therefore, a comparison was made between groups with the posterior denervated, anterior denervated, and the middle denervated. Significant differences were found between all groups with swim durations being the longest with only the posterior end denervated, second longest with the middle denervated, and shortest with the anterior end denervated (one-way ANOVA,  $p < 0.0001$ ). Cycle periods were also found to be significantly different between the groups (one-way ANOVA,  $p = 0.0016$ ). They were longest in the posterior denervated groups. A comparison was made between the cycle period for groups with seven segments innervated on the anterior (2-8) or posterior (16-T) end. In the groups with only seven segments innervated, the leech with just the anterior end innervated was found to have swim durations and cycle periods significantly longer than the leech with just the posterior end innervated (Student's t-test,  $p < 0.0001$ ,  $p = 0.0129$ , respectively).

### Discussion

Suspended intact leeches swim episodically with the head brain attached (H-T), but swim continuously with the head brain detached and almost all sensory feedback intact (2-T) (Figure 3, Figure 4). This continuous swimming is markedly different from the episodic swimming observed with the head brain removed in isolated preparations, indicating that sensory feedback provides excitatory input to the swim-maintenance system. These results are also consistent with previous conclusions that the head brain inhibits swim maintenance. Prevention of continuous swimming by the head brain is likely adaptive for the leech in order to prevent such an energetically costly behavior under normal circumstances. When sensory feedback was removed from the posterior end, nearly all of the leeches swam continuously, with the exception of two leeches when only three body segments (2-4) were innervated at the anterior end (Figure 3, Figure 4). It is remarkable that sensory feedback to only three anterior segments is often sufficient to elicit continuous swimming. Although all leeches with seven (2-8) or 14 (2-15) anterior segments innervated swam continuously, the equivalent number of segments innervated in the posterior end usually resulted in shorter, episodic swim episodes.

In most groups, the removal of sensory inputs led to decreased swim duration. As more sensory feedback from the posterior end and the middle segments was removed, the swim duration decreased (Figure 3, Figure 4). In addition, in the group where regions 2-8 and 16-T were innervated, the anterior and posterior ends of the leech were often uncoupled; the anterior end swam continuously in three out of five leeches while the posterior end never swam continuously. These results suggest that anterior sensory input plays a greater role in maintaining swimming than posterior sensory input. While ganglia are often described in the literature as being nearly identical, intrinsic differences between the ganglia have been observed. For example, anterior, middle and posterior ganglia have different intrinsic cycle periods (Friesen & Hocker, 2001). Further, ganglion 21 is smaller and has fewer neurons than the other ganglia (Mullins et al., 2011). There may be regional differences in the strength and quality of the sensory feedback itself. Stretch receptors in the body wall play an important role in generating and adjusting the swim rhythm by detecting tension changes along the body (Yu & Friesen, 2004); however, it is unclear if there are differences in the stretch receptors at different segments. If there are regional differences in these stretch receptors, then they may play a role in the differences in sensory feedback between the different groups observed in this study. Determining if and how such regional differences affected the results would be an interesting future study. Analysis of another swim-maintenance parameter, cycle periods, also showed that removal of sensory input decreased excitation to the swim-maintenance system. Cycle periods tended to increase, meaning that swim frequency decreased, with fewer segments innervated. Interestingly, when the middle region was denervated, the cycle periods were significantly longer when both ends were swimming than when only the anterior end was swimming. This suggests that the posterior sensory innervation increases cycle period. These results seem to be in contrast to the significantly smaller cycle periods for groups with the posterior end only innervated than those with the anterior end only innervated, which could be interpreted to suggest that excitatory drive to the maintenance system is stronger in the posterior end, at least in terms of cycle periods. This interpretation is also at odds with the swim duration analysis, which suggests that anterior sensory innervation most strongly excites the swim maintenance system. An alternate explanation for smaller cycle periods in the posterior end only innervation may be because the continuous swimming seen with anterior input weakens over time, while episodic swimming as seen with posterior input seems to be stronger. This could create average cycle periods that are longer for groups with continuous swimming than for groups with episodic swimming. To test this hypothesis, further experiments would have to be done with the same number of segments innervated in the anterior and posterior ends. The continuous swimming in

the anterior end-innervated preps could likely be stopped by mechanical inputs to the body wall (Kristan & Nusbaum, 1982). Then, the cycle periods at the beginning of swim episodes could be directly compared in the two conditions.

These results suggest that the interaction between the amount and location of sensory feedback plays a role in the maintenance of continuous swimming. Further experiments could explain the mechanisms behind these results. As leeches were only observed for 30 minutes, a future investigation could study how long leeches could maintain continuous swimming. Investigations into why the anterior sensory input is more effective for maintaining swimming than posterior sensory input and why cycle periods increase as sensory input is removed in most preparations would provide a greater understanding of the mechanisms that sustain swimming.

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**Appendix:** larger images of included figures

