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Article

Cathodal galvanotaxis: the effect of voltage on the distribution of *Tetrahymena pyriformis*

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SUMMARY

***Tetrahymena pyriformis* are unicellular eukaryotes with thousands of hair-like structures called cilia on the surface of their bodies that aid in cell motility and food consumption. The similarities between their cilia and that of humans, particularly in the human respiratory and olfactory track, are why they are model organisms for investigating cell movement and cilia functionality. Further research may help scientists better understand how cilia are affected by common ciliopathies, genetic disorders caused by the abnormal formation or function of cilia. The objective of this experiment was to investigate the effect of voltage on the distribution of *T. pyriformis* across a capillary tube. *T. pyriformis*-filled capillary tubes were connected to a power supply for one minute. We calculated the percent distributions at the anode and cathode of the capillary tube by counting *T. pyriformis* with a hemocytometer. Our results indicate that cathodal galvanotaxis is induced at 4V and that, despite increases in voltage from 4–30V, the percent distribution of *T. pyriformis* at the cathode remained constant at approximately 80%. These data suggest that while calcium and potassium voltage-gated ion channels are mediated by graded potentials and are triggered at specific thresholds, further increasing voltage above that threshold had little effect.**

INTRODUCTION

Galvanotaxis refers to the movement or innate behavioral response of cells toward an electrical stimulus. Cell galvanotaxis, particularly migration patterns and migration speeds, is often investigated and applied to research in cell signaling, tissue regeneration, and microcellular robotics (1). In this experiment, we used *Tetrahymena pyriformis*, a unicellular eukaryote 30–50 µm in size, because supplying voltage through its surrounding conductive media induces quick behavioral responses that can be readily quantified. *T. pyriformis* possess hundreds of cilia—fine, hair-like projections—along their membranes. Studying their function can therefore allow us to gain insight into the ciliary linings found in the respiratory and olfactory tracts of mammals (2, 3). These repeating cilia units are *T. pyriformis*'s defining structures responsible for efficient cell motility (i.e., the independent movement of an organism). The interaction of electrical current with potassium and calcium channels on cilia membranes results in cell movement towards the negative, or cathode, end of an electrolytic cell (4). More specifically, the direction and speed of propulsion are dependent on the influx of potassium and calcium ions into the cell and, in turn, the oscillation of rows of cilia at specific frequencies (5). Thus, galvanotaxis is a simple method used to assay overall cell movement and to better understand cilia functionality. Research in this field may provide insight into cilia functionality and could potentially help treat common ciliopathies, such as Bardet–Biedl syndrome (BBS) and Alström syndrome (ALMS). Both BBS and ALMS are characterized by vision and hearing impairment, which arise due to mutations in proteins implicated in ciliary function (6).

Because cilia are vital to cellular mobility, it should be noted that several factors can cause deciliation (the loss of cilia) in *T. pyriformis*. Specific temperatures, calcium inhibitors, cytoskeleton inhibitors, protein synthesis inhibitors, and various types of media can cause deciliation, rendering *T. pyriformis* immobile and unable to feed themselves. However, the process in which galvanotaxis affects the movement and direction of cilia movement is unlike the process of cell deciliation.

The media solution in which *T. pyriformis* are subcultured is an electrolyte, a conductive medium that allows voltage to be supplied through the solution. When voltage is supplied through the medium, a potential difference between intercellular and extracellular potential is generated across the *T. pyriformis* cellular membrane. A higher voltage is applied and as this potential difference increases, the cell becomes hyperpolarized at the anode, which in turn triggers voltage-gated potassium channels to open and release potassium ions from the cell (**Figure 1**) (5). As a result, the cilia on this side of the *T. pyriformis* beat faster. On the side of *T. pyriformis* facing the cathode, depolarization causes voltage-gated calcium channels to open, after which the difference

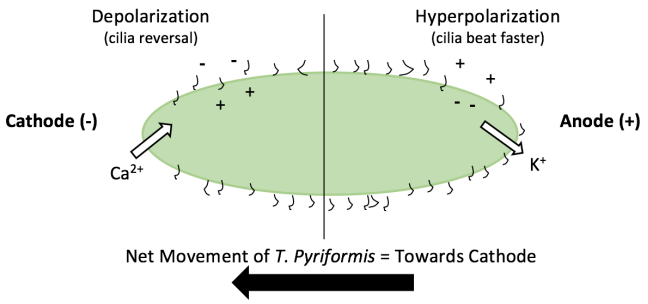

Figure 1. Diagram of the mechanism underlying *T. pyriformis* cathodal galvanotaxis. Depolarization due to influx of calcium ions aligns the cell towards the cathode and hyperpolarization due to outflux of potassium ions in *T. pyriformis* increases cilia beating rate.

in electrochemical gradient causes calcium ions to flood into the cell. As a result, these cilia beat in reverse. The net effect of depolarization at the cathode and hyperpolarization at the anode results in *T. pyriformis* swimming towards the cathode. If cells are orientated in the opposite direction or perpendicular to the electrical fields, the torque generated from the electrical fields immediately causes the cells to realign themselves to face the cathode (7).

Previous studies have confirmed cathodal galvanotaxis of *T. pyriformis* at 5V, but the extent to which voltage affects cell distribution remains unclear (7). Thus, the goal of this experiment was to determine how increasing voltage would affect the distribution of *T. pyriformis* across a capillary tube, with the distance travelled used as a proxy measurement for the net effect of each subset of cilia beating in a different direction. We hypothesized that because calcium channels affect the direction of cell movement and potassium channels affect the rate at which cilia beat, the ion channels will behave in a graded manner; increasing voltage would increase the magnitude of depolarization and hyperpolarization of ions in the membrane and, thus, increase the distribution of *T. pyriformis* at the cathode end of the tube. Our results reveal that galvanotaxis is induced at 4V and that an increase of voltage beyond 4V yields a distribution of approximately 80% at the cathode.

RESULTS

To determine the effect of voltage on the *T. pyriformis* distribution, *T. pyriformis* were exposed to 0V-30V for one minute. By comparing the cell distribution in the anodes and cathodes of capillary tubes, we determined that increasing the voltage supplied through the *T. pyriformis* solution resulted in galvanotaxis at 4V or greater (**Figure 2**; $p<0.0125$, two-tailed t-test with Bonferroni correction). We also found that the average normalized percentage of cell distribution at the cathode or anode across the different voltages was similar between treatment groups at 0 and 2V, which both show about a 50% distribution of cells at each end of the capillary tube ($p>0.05$, two-tailed t-test). Cell distributions for treatment groups at 10, 20 and 30V were also similar, with roughly 80% of cells localized toward the cathode in each case ($p>0.05$, two-tailed t-test). The distribution of the treatment group at 4V is statistically different from the distribution at 10 and 30V ($p<0.05$, two-tailed t-test). Thus, our results reveal that galvanotaxis was induced at 4V and that further increases in voltage has little effect on cell distribution.

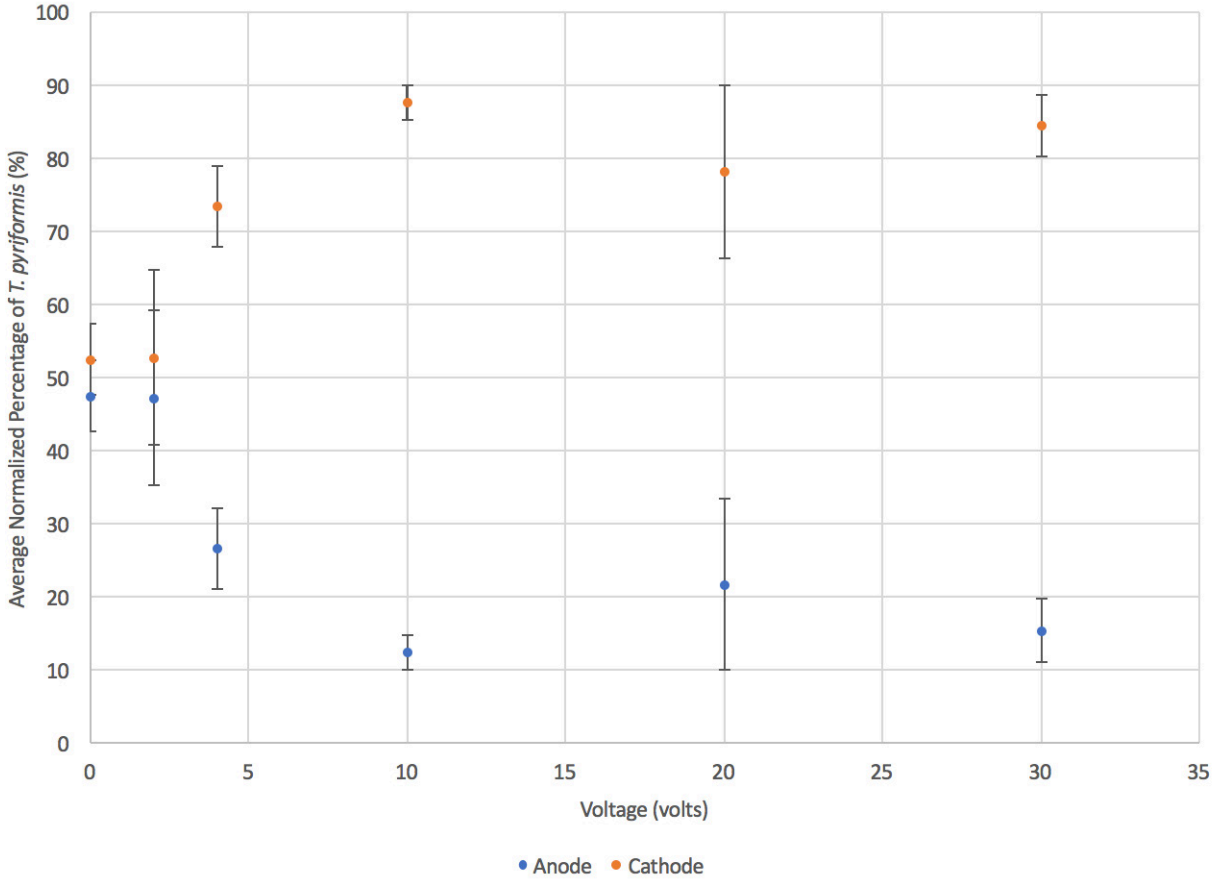

Figure 2. Distribution of *T. pyriformis* in the anode and cathode of the capillary tube at different voltages. The average normalized percentage of *T. pyriformis* in the anode and cathode of the capillary tube when induced to 0-30V for one minute (n=5-6) was plotted. Error bars represent the average absolute deviation value for the five trials at different voltages. The distribution of *T. pyriformis* in the anodes and cathodes was significantly different at 4 to 30 volts ($p<0.0125$, two-tailed t-test with Bonferroni correction). The cell distribution was similar across 0 and 2V ($p = 0.964$) but was significantly different between 0V or 2V and 4, 10, 20 and 30V ($p<0.05$, two tailed t-test).

DISCUSSION

We found that the distribution of *T. pyriformis* at both the anode and the cathode was approximately 50% for groups treated with 0V and 2V. This finding indicates that galvanotaxis only occurs when *T. pyriformis* is exposed to a voltage of 4V or greater. The fact that average normalized percentage of *T. pyriformis* in the cathode was ~50% at 0V and 2V, ~75% at 4V, and ~80 % at 10-30V supports our hypothesis that there is a linear increase in the percentage of *T. pyriformis* that migrate to the cathode as the voltage increased. Closer examination of the data suggests that the average normalized percentage of *T. pyriformis* in the cathode plateaued to 80% during galvanotaxis, supported by the constant percentage of *T. pyriformis* at the cathode despite the wide range of voltage experimented with.

The hypothesis that *T. pyriformis* distribution at the cathode would increase in response to an increase in voltage was based on the concept of graded potentials, where an increase in stimulus results in a greater change in membrane potential (8). When the threshold, or change in membrane potential, for voltage-gated ion channels is met, the channels are open 50% of the time. However, at lower and higher voltages, the decreases and increases in membrane potential affect the amount of time the channels are open as well as the speed at which ions travel through the channels. We speculate that the constant distribution of *T. pyriformis* at the cathode when exposed to 10-30V is because the increase in voltage has no effect on the magnitude of response, and thus biological behavior, that occurs as a result of depolarization in the cell. Voltage-gated ion channels are mediated by graded potentials and are activated at specific thresholds. Consequently, if this threshold is not met, ions will still flow through some channels, but not enough for galvanotaxis to occur (8). Our data suggests that beyond 10V, the same response, or same distribution of cells, occurs. When examined under the microscope, a clear change in swimming movement is observed specifically when voltage is increased from 2 to 4V, demonstrating the point at which enough ions are passing through the channels to cause cathodal galvanotaxis.

Furthermore, the fact that cell distribution for the treatment group at 4V is significantly different from the distribution at 10V and 30V may be indicative of the threshold of voltage-gated potassium channels not being met. The voltage-gated calcium and potassium channels likely have different thresholds, and it may be possible that at 4V, the triggering of voltage-gated calcium channels causes the cells to orient their position towards the cathode. Without the triggering of the voltage-gated potassium channels, it is possible that *T. pyriformis* do not move as quickly towards the cathode since their cilia do not beat quite as fast.

The major source of uncertainty in our experimental approach was the addition of one drop of iodine solution to fix the 25µL of *T. pyriformis* in the anode or cathode. We estimated the volume of one drop to be approximately 50 µL; however, since we did not use a micropipette to be precise in measuring the amount of iodine, there was a large amount of uncertainty in cell counts, especially because of the large volume of iodine added relative to the volume of *T. pyriformis*. This variability likely explains much of the high percent average absolute deviation (AAD) seen in our data and could easily be eliminated with the use of a micropipette to add a smaller volume of iodine.

Another source of uncertainty that may have affected the distribution of *T. pyriformis* across the capillary tube is the heat generated from the increased voltage. When handling the power supply, we noticed that the machine itself felt warmer at 30V than at 2V, and a change in temperature likely occurred in the capillary tube as voltage increased. An IR thermometer could be used to measure the change in temperature through the capillary tubes and, thus, help gauge the significance of temperature as an uncertainty. Increased temperature tends to increase ciliary movement by causing an increase in binding affinity between actin and myosin. Increased ciliary movement tends to lead to more rigorous eating habits but could also result in faster movement along the capillary tube (9).

To better examine whether *T. pyriformis* galvanotaxis can vary with voltage, a similar experiment could be conducted specifically in the voltage range of 2V to 10V. This would allow one to confirm whether increasing voltage leads to a step-like increase in *T. pyriformis* distribution, as well as provide insight into whether this phenomenon is due to the triggering of calcium-gated ion channels before potassium-gated ion channels.

Moreover, to fully confirm that *T. pyriformis* galvanotaxis occurred as a result of voltage-gated ion channels, the experiment should have been performed with calcium and potassium ion channel blockers, such as cadmium chloride and tetraethylammonium (TEA) chloride, respectively. If our theory is correct, blockage of these channels would decrease *T. pyriformis* cathodal distribution. However, the alternative result would suggest that *T. pyriformis* have intracellular signaling pathways that detect electrical stimuli and can instruct cilia to propel themselves towards the cathode.

Despite the uncertainty in cell counts and in the effects of temperature, our data suggests *that T. pyriformis* will begin to perform cathodal galvanotaxis when we apply a potential difference of approximately 4V across a capillary tube. Further increase in voltage during galvanotaxis caused the distribution of *T. pyriformis* at the cathode to remain at approximately 80%.

METHODS

T. pyriformis Growth and Maintenance

T. pyriformis used for this experiment were subcultured by adding two drops of concentrated *T. pyriformis* in axenic proteose peptone medium (Carolina Biological Supply Company) into the *Tetrahymena* subculture medium (Carolina Biological Supply Company 13-2315), after which *T. pyriformis* were grown in an incubator at 27°C for 2 days.

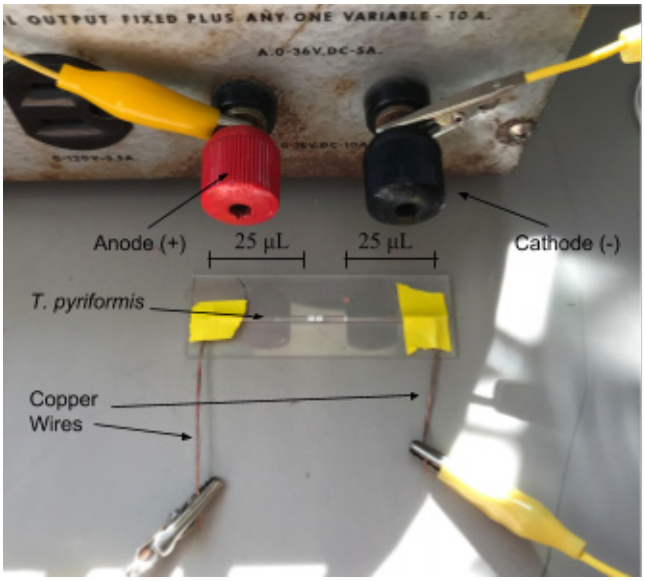

Figure 3. Experimental setup for galvanotaxis on *T. pyriformis* in an 86.5 µL micropipette capillary tube. After supplying the voltage for one minute, the anode and cathode (marked by 25 µL lines) ends of the tube were snapped off and counts of *T. pyriformis* were measured with a hemocytometer.

Experimental Apparatus Set-up

To determine the effect of voltage on the distribution of T. pyriformis, the Hampden voltage power supply was used to induce galvanotaxis while controlling current. Each 86.5 µL micropipette capillary tube used was snapped at the 25 µL end of the tube to generate a 61.5 µL capillary tube with markings for 25 µL at each end. Each capillary tube was inserted into a sterile 1 mL plastic pipette, and the pipette and tube were then filled with the *T. pyriformis* solution. While maintaining full volume in the capillary tube, the pipette was detached from the capillary tube. Copper leads were used to conduct electricity (**Figure 3**); the ends of the copper wires, approximately 4 inches long, were sanded to remove the paint layer and to allow for better conductivity. The capillary tube was then taped on a 1 inch x 3 inch glass slide, and the leads inserted into the ends of the tube were taped down, as well. Alligator clips attached the leads from the capillary tubes to the positive and negative ends of the power supply. A marker was used to indicate which end of the capillary tube was attached to the negative cathode end and the positive anode end of the voltage supply. Through preliminary testing, it was determined that one minute was sufficient for obtaining a significant difference in *T. pyriformis* distribution across the two ends of the tube. To measure distribution, the capillary tube was carefully snapped according to the 25 µL markings at each end of the tube. If the tubes were not precisely snapped or if there was an air bubble in the capillary tubes, the trial would be rerun in an effort to avoid uncertainty caused by the decreased amount of solution from the cathode or anode. The snapped pieces of the capillary tube were inserted into a pipette, and the solutions were squeezed out into two separate wells on a well plate. One drop of 1.5% iodine solution was dropped into each well to fix the *T. pyriformis*, and one drop of each mixed solution was counted on 9 mm x 9 mm x 0.1 mm hemocytometers to measure the distribution of *T. pyriformis* in the anode and cathode.

Experimental Procedure

To determine the exact voltage at which the distribution of *T. pyriformis* at the two ends of the tube were significantly different, voltage was varied at 0V, 2V, 4V, 10V, 20V, and 30V for final testing. Current was controlled at 5 amperes to avoid the uncertainty of the effects of current on *T. pyriformis* distribution. As determined by counting via a hemocytometer with a compound light microscope (100x), the cell density of *T. pyriformis* used for final testing was approximately 5.44 x 10^4 *T. pyriformis*/mL (dilution due to iodine is accounted for), and all data was collected in a single afternoon to reduce the uncertainty of increased cell density. The voltage was varied in 6 treatment groups, with 5 trials in each treatment group and 6 trials for the negative control. For the negative control, *T. pyriformis* was connected to the power supply at 0V for exactly one minute before the capillary tubes were snapped and the cell distribution at each end of the tube was measured with a hemocytometer.

Statistical Testing

The average number of *T. pyriformis* in the cathode and anode of all the runs were standardized to percentages so that statistical T-tests could be performed between the two ends of the tube to reveal the significance of a specific voltage on cell distribution across the capillary tube. Data was standardized so that the *T. pyriformis* count in cathode and anode added up to 100%; however, the anode piece and cathode piece snapped off does not account for total volume of *T. pyriformis* in the capillary tubes (**Figure 3**). T-tests were also performed between the treatment groups and the negative control to determine the significance of increased voltage on *T. pyriformis* distribution on the cathode end of the tube. Because of the large number of T-tests conducted, the Bonferroni correction was used to guard against false positives.

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