The Effects of Different Colors on the Sound Spectrum on *Caenorhabditis elegans* Chemotaxis Toward NaCl or Water

Abstract

The aim of this experiment was to determine how different arrangements of frequencies in the form of brown, pink, white, and blue noise affect *Caenorhabditis elegans* chemotaxis toward NaCl. Many people, such as students, utilize these forms of background noise to study and sleep. It would be interesting to determine if any variations of background noise cause a change in *C. elegans* behavior. *C elegans* is commonly used as a model organism, because of the genetic similarities between it and humans. Using that knowledge, this experiment's results may be used to study the more complex human brain's response to background noise. It was hypothesized that brown noise will have the greatest effect on *C. elegans* chemotaxis and that the worms will display a positive chemotaxis index, toward NaCl. This hypothesis was not supported, as there was no significant difference between each of the variables. However, there is room to expand upon the experiment, as it was found that noise that highlighted lower frequencies correlated to a positive chemotaxis index, and noise that highlighted higher frequencies correlated to a negative chemotaxis index.

Introduction

In a previous experiment, researchers tested the effects of specific frequencies on N2, CB1338, and the JT6924 strains. They observed the effects of these frequencies on worms with normal neurons, deficient in nonciliated neurons and 2 ciliated neurons, and deficient in *daf-19* and *daf-12*, which cause the worm to reach the adult phase with deficiencies in developmental pathways, respective to each strain (Holbrook and Mortimer). It was found that the N2 strain only changed behavior in response to 23 Hz, the lowest frequency applied. Worms under silent treatment showed significantly higher numbers of reversals, but a significantly lower distance. The CB1338 strain showed significant differences in 23-111 Hz, an increase in reversals at 23 Hz and a decrease in reversals at the other frequencies. The JT6924 strain showed no changes in behavior for the 23 Hz treatment, but 23-111 Hz showed a significant reduction in the number of reversals (Holbrook and Mortimer). It was established that *C. elegans* was able to sense vibrations, and change its behavior for lower and higher frequencies. Holbrook and Mortimer suggested that future studies may be done to study sensory pathways of vibrational sensitivity to determine differences between sustained vibration, short term vibration and touch.

In another experiment, researchers discovered that although *C. elegans* is an invertebrate, and the majority of invertebrates insensitive to sound, *C. elegans* is responsive to airborne sound of frequencies reaching the kHz range (Iliff et al.). The worm sensed sound when the sound waves vibrated the skin which acts as a sound pressure to displacement transducer, which triggers the activation of sound-sensitive neurons attached to the skin (Iliff et al.), and thus phonotaxis behavior occurs. Phonotaxis is the movement of an organism in accordance with a sound stimulus. The researchers hypothesized that the worms might exhibit aversive behavior to sound, as some of their predators produce audible sounds. They found that when sound (2 s, 1 kHz, 80 dB)was delivered to the head, reversals were triggered. Likewise, when sound was aimed at the tail, forward movement was triggered (Iliff et al.). To test if the worms were being affected by air-borne or substrate-borne vibrations, the scientists measured sound-evoked substrate vibrations through laser Doppler vibrometry. They detected minimal substrate vibrations (Iliff et al.). They also tested the mec-4(e1611) mutant worms, which have degenerated touch receptor neurons. The mutant worms exhibited normal phonotaxis behavior, showing that airborne vibrations did not play a role in the original experiment (Iliff et al.). This experiment urges more scientists to study the evolution of auditory sensation, in the scope of convergent evolution. Iliff challenges the previously held conception that only vertebrates and a few insects are capable of hearing.

These experiments engage a question: what happens if C. elegans is exposed to substrate-borne sound of a mixture of frequencies? There are usually a multitude of sounds in a natural environment, so it would be interesting to observe the effects of sound, in mixtures which highlight certain ends of the frequency spectrum. This experiment engages this thought process. In the future, scientists could be able to study which frequencies most closely resemble a natural environment, and study the effects of arrays of frequencies on the much more complex human brain. Since many people use these background noises to study or sleep, perhaps eventually scientists could study how exactly these sounds impact the brain. This information could be harnessed to facilitate greater efficiency and productivity, if found to be different than a control group.

Hypothesis

I hypothesized that brown noise would have the greatest effect, meaning the most significantly different results, on *C. elegans* chemotaxis. I also hypothesized that *C. elegans* would have an greater chemotaxic preference toward NaCl.

Materials and Methods

The materials used in this project were: 4 bottles of Nematode Growth Medium (NGM) agar, microwave, 36 small sterile petri dishes, lighter, hot hands, hot pad, 1 bottle of Luria broth, *Escherichia coli* OP50 stock plate, sterile inoculating loop, sterile L spreader, p-200 micropipette with tips, p-20 micropipette with tips, p-10 micropipette with tips, metal spatula, scale, weigh boat, 100 mL media bottle, 70% ethanol, 10% bleach, dissecting microscope, *C. elegans* N2 stock plate, S Basal solution, conical tubes, centrifuge, 0.005 M NaCl solution, 1% sodium azide, dH_2O, sharpie, speaker, laptop, timer, alcohol burner, 30-35°C incubator, Ambient incubator.

4 bottles of NGM agar were melted by loosening the cap and microwaving each individually for 15-second intervals until the agar was completely melted. Between intervals, the bottles were swirled. While the agar was being melted, 36 plates were labeled. After it was fully melted, the agar bottle was placed onto a hot pad to cool. When the bottle was cool to the touch, hot hands were used to pick it up. The cap was unscrewed, then the mouth of the agar bottle was flamed with a lighter. All 36 labeled plates were filled ⅔ full of NGM agar. The plates were left to cool. When they were solidified, the plates were transferred to the refrigerator.

0.03 g of NaCl (for a 0.005M NaCl solution) was measured out onto a weigh boat on a scale, using a metal spatula. The NaCl was transferred into a 100 mL media bottle. dH_2O was added up to 100 mL total solution. The bottle was swirled forcefully to mix the NaCl and dH_2O together. The bottle was labeled. The bottle was placed into the storage bin.

A bottle of Luria broth was labeled The mouth of the Luria broth was flamed. Using the sterile loop, a single colony was picked from the *E. coli* OP50 stock plate. The loop with *E. coli* was placed inside the bottle and swirled around to dislodge the bacteria from the loop. The cultures were placed into an incubator set at 30-35°C for 2 days.

5 of the NGM agar plates were labeled to include information about the *E. coli* cultures The mouth of the Luria broth containing *E. coli* OP50 was flamed. Using a p-200 micropipette, 50 µL of the broth was pipetted onto the center of each plate. Using an L spreader, the *E. coli* was spread to create a slightly larger lawn, about 1 cm from the edge of each plate. The 5 *E. coli* seeded plates were placed into the 30-35°C incubator.

26 plates (without *E.coli*) were split into 4 even quadrants by drawing a centered cross with a sharpie. A circle with a 0.5 cm radius was drawn into the middle of each plate, centered around the cross intersection. 4 points were drawn equidistant from one another, with one per quadrant, 2 cm from the origin. The top left and bottom right points were labeled as "C," and the top right and bottom left points were labeled as "T." These 26 plates were put into the refrigerator.

E. coli plates were labeled to include information concerning *C. elegans*. A *C. elegans* N2 stock plate was observed to make sure that the worms were alive. A metal spatula was sterilized by dipping it in ethanol and then holding it over a lit alcohol burner to burn off the ethanol. The spatula was cooled for about 30 seconds. A lively area was selected, and a chunk was cut out of it using the sterilized spatula to create a square with dimensions of the edge of the spatula. 2 chunks were transferred to each *E. coli* OP50 seeded plate by flipping them so the side with the worms was touching the surface of agar without *E. coli*. The plates were transferred to an incubator set at an Ambient temperature and left alone for 4 days.

To wash the worms, 2 mL of S Basal was pipetted onto one of the *C. elegans* plates with a p-1000 micropipette. The plate was tilted to ensure that the worms were washed from the plate surface and transferred to the S Basal. 1 mL of the worm and S Basal solution was pipetted into a microcentrifuge tube labeled "W." A counterbalance was created by pipetting 1 mL of H_2O into a microcentrifuge tube labeled "H_2O." Both tubes were placed into the microcentrifuge on opposite sides. The microcentrifuge was turned on for 10 seconds. The S Basal was pipetted off of the worm tube's contents, being careful not to disturb the pellet formed during centrifugation. 1 mL of S Basal was added to the tube. The tube was placed back into the microcentrifuge and centrifuged for 10 seconds. The S Basal was pipetted off of the worm tube's contents. This was repeated 4 times. Most of the S Basal was pipetted off of the worm tube's contents, leaving about 100 µL of worm and buffer solution. The pellet was broken up and mixed by swirling it around with a micropipette tip, then taking up fluid and expelling it multiple times. The tube was observed under a dissecting microscope to confirm the presence of worms.

Chemotaxis assays were prepared by obtaining 5 plates that were divided into a cross. Using a p-10 micropipette, 1 µL of the 0.005M NaCl solution was pipetted onto each "T" site. 1 µL of dH_2O was pipetted onto each "C" site. 1 µL of 1% sodium azide was pipetted onto each "T" and "C" site. 2 µL of the *C. elegans* and S Basal solution was pipetted onto the area inside the circle where the cross' lines intersected. The plates were placed on top of a speaker. For the control the speaker was silent. For each of the other variables, the speaker was connected to a laptop playing one of the variables of noise. Chemotaxis ran for 60 minutes. After the timer was finished, the noise was turned off and the plates were placed into the refrigerator. This procedure was repeated for each variable on separate days.

To maintain safety with the microorganisms used, *C. elegans* and *E. coli,* all materials were sterilized with 70% ethanol, 10% bleach, or fire. Lab tables were cleaned with 70% ethanol before and after any procedures.

Results

While counting the worms at each site, qualitative data was collected as well as quantitative data. For the silence variable, many worms were observed to have stayed clumped together at the origin (*Figure 1.1*), which can explain the high standard deviation. Also, as shown in multiple images of different variables (*Figures 1.2* and *1.3*), most worms did not reach the point at which the solution was pipetted onto, rather coming close, therefore showing decision making.

The chemotaxis index (CI)I of each replicate was calculated, and then the mean CI of each variable was calculated (*Figures 1.5 and 1.6*). The standard deviation of these values was also calculated. This is represented graphically in *Figure 1.4*.

A one-way ANOVA was performed to compare the effect of different colors of noise on *C. elegans* chemotaxis towards NaCl or water (*Figure 1.7*). The XLMiner Analysis ToolPak extension on Google Sheets was used. The ANOVA calculated a P-value of 0.708 between the groups.

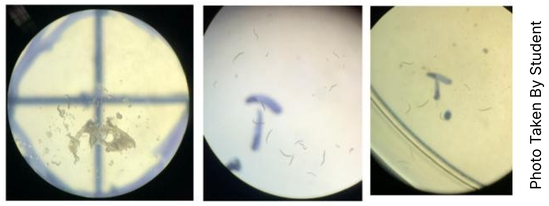


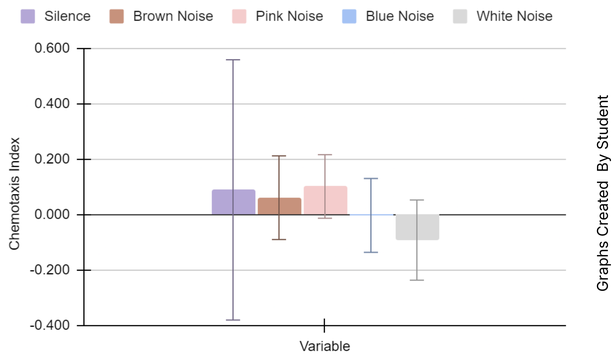
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Figure 1.1: Control Origin

Figure 1.2: T-site of White Noise Plate 1

Figure 1.3: T-site of Brown Noise Plate 5

Figure 1.4: Mean Chemotaxis Indexes Towards NaCl with Different "Colors" of Background Noise with Standard Deviation Error Bars



Graphs Created By Student

Figure 1.5: Chemotaxis Indexes of Each Replicate

	Chemotaxis Index				
Replicate	Silence	Brown	Pink	Blue	White
1	-0.161	0.249	-0.048	-0.205	-0.188
2	0.138	-0.116	0.212	-0.053	-0.243
3	-0.444	0.168	0.219	0.143	-0.140
4	0.096	-0.053	0.088	0.031	0.005
5	0.818	0.058	0.037	0.070	0.108

Figure 1.6: Means and Standard Deviations of Figure 1.5

Variable	Mean CI	STDEV
Silence	0.089	0.469
Brown	0.061	0.151
Pink	0.102	0.115
Blue	-0.003	0.133
White	-0.092	0.145

Figure 1.7: Single-Factor Analysis of Variance of Figure 1.5

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.127	4	0.032	0.540	0.708	2.866
Within Groups	1.179	20	0.059			
Total	1.307	24				

Conclusion

There was no significant difference between the silence, brown, pink, blue, and white noise variables. This is because the P-value calculated by the ANOVA was 0.708, which is greater than the alpha value of 0.05. The hypothesis was that brown noise will have the greatest effect on *C. elegans* chemotaxis and that the worms will have a chemotaxic preference toward NaCl. The part that brown noise would have the greatest effect on *C. elegans* chemotaxis, was not supported since none of the variables displayed a significant difference to one another. The second part was also not supported, as the blue and white noise variables had negative CI values.

Looking upon *Figure 1.6*, each variable did have different results, although they were not significant. The highest mean positive CI, and the largest absolute value CI, was with pink noise, with a mean CI of 0.102. Blue noise had a mean CI incredibly close to 0, which was -0.003. White noise had the highest negative CI, of -0.092. Each variable had a CI value much closer to 0 than 1, meaning that the worms were mostly evenly distributed between NaCl and water, with slight preference.

Though not significantly different, the graphical data in *Figure 1.4* suggests that a negative dB/octave slope is correlated to a positive CI value, and a positive dB/octave slope is correlated to a negative CI value. Brown noise has a slope of -6.02 dB/octave (*Figure 2.1*). Pink noise has a slope of −3.01 dB/octave (*Figure 2.1*). Blue noise has a slope of +3.01 dB/octave (*Figure 2.1*). White noise has an even power spectrum slope (*Figure 2.1)*. Furthermore, the noise that included lower frequencies (brown, pink, and white) had higher CI values than blue noise, which highlighted higher frequencies. This shows that although the hypothesis was not supported, that brown noise will have the highest CI value, the reasoning behind it shows some correlation. Previous experiments have shown that *C. elegans* displayed the most statistically significant behavior at 23 Hz, the lowest frequency of the range that those scientists tested (Holbrook and Mortimer).

Potential errors involved in this experiment stem from inexperience. As shown in *Figure 1.4*, the silence variable had significantly greater standard deviation bars due to the great amount of worms clumped together at the origin, shown in *Figure 1.1*. This was the first time the worms were washed, and the pellet was not broken up as thoroughly as in the other tests. When pipetting 1 µL of various solutions onto the chemotaxis plates, a droplet of the substance stuck to the outer edge of the micropipette tip by adhesion many times. It was shaken off by tapping the tip lightly against the interior of the solution's container. However, this could have led to unequal amounts of NaCl solution, dH_2O, or sodium azide within the chemotaxis plates.

To expand on this experiment, an experiment could be designed to test the effect of substrate-borne vibration, found by localizing the area where the sound was played. In this experiment, sound surrounded the plates, so the worms had no choice but to move somewhere while being constantly in the vicinity of sound. In a localized sound experiment, a rod would be placed into the agar, and sound would be played. The data recorded would be the movement of *C. elegans* toward or away from the rod. The same variables would be used. This would test the worms' aversion to each variable rather than their altered decisions toward or away from a known chemoattractant when surrounded by each variable.

Another possible way to expand upon this experiment would be to test it with the CB1338 and JT6924 strains, from Holbrook and Mortimer's research, to determine if different types of neurons heavily impact the perception of a mixture, rather than a singular frequency.

In the future, perhaps an experiment could be conducted to test human preference, and if there is any boosted activity. *C. elegans* is not able to articulate human experience, so this proposed experiment could deal with how the noise makes people feel, which could impact behavior.

Figure 2.1: Various dB/octave Distributions of Noise Variables

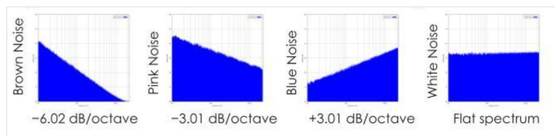


Image Taken From Wikipedia

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