The Effect Of Nicotine on Zebrafish Embryo Development

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Abstract

The purpose of the experiment is to observe the effect of nicotine on zebrafish embryological development. If nicotine effects the development of zebrafish embryos then the zebrafish will develop birth defects and/or will die before being fully developed because nicotine causes induced paralysis of the embryo causing it not to move leading to certain motoneurons and/or muscles in the embryo to not be developed (Svoboda, 2014, 9). The method used to analyze the hypothesis was through an experiment since it includes repetition of nicotine exposure so the results can be observed first hand and allows to collect a sufficient amount of data to prove or disprove our hypothesis. The results should conclude that zebrafish exposed to nicotine had embryos that either died before adulthood or end up being abnormally formed due to the effects nicotine has on neurological systems especially in the brain. This proved to be true since higher amounts of nicotine exposure lead to less zebrafish embryos fully developing and hatching. What helps in this study is that zebrafish and humans carry the same neuron of nicotinic acetylcholine receptors or nAChRs which “activates that neuron” and ions will enter the cell (Svoboda, 2014, 8). In human children, nicotine exposure before birth can connect with endogenous acetylcholine receptors which disrupts neurological development and causes abnormalities because of the disrupting of the timing of neurotrophic actions (Wickström 2007). This means the underdevelopment of zebrafish embryos can be directly related to human development.

Intro

Zebrafish embryos are an excellent form of study objects because the embryos have quick development, allowing for collection of data in a shorter amount of time (Svoboda,

2014,6). Additionally, they require little maintenance so they will not need to be closely monitored (Svoboda, 2014, 6). Choosing to study the effects of nicotine on development was easy since vaping, which contains nicotine, has become known as a very popular pastime especially in teens and young adults. This study could show to the general population that things like vaping are detrimental to later development of fetuses.

"Nicotine could disrupt endocrine targets" in zebrafish (Kanungo, Cuevas, Guo, et. al., 2012) which means that GHRH (Growth hormone-releasing hormone) and GH (Growth Hormone) could be inhibited from interacting with the pituitary gland, hypothalamus, muscles, bones, tissues, and body cells which would lead to the underdevelopment of embryos. Other prior research on effects of nicotine on zebrafish shows that higher doses of nicotine lead to less movement (Petzold, Balciunas, Sivasubbu, et. al., 2009). This means that the nAChRs spinal motoneurons are activated by nicotine when they are entered into the embryo, allowing the nicotine to transport to the motoneurons and inducing paralysis of the embryo because they cannot further bend or move their muscles while in the embryonic stage (Svoboda, 2014, 9).

Since the effects of nicotine on already hatched and developed zebrafish is understood, this leads to an additional question: does the amount of nicotine a zebrafish embryo is exposed to have an effect on development? If the embryos are exposed to a higher concentration of nicotine, then they will be less developed leading to less embryos hatched because of underdevelopment of motoneurons and muscle tissues (Svoboda, 2014, 9). The method chosen to analyze the hypothesis was through an experiment. It includes repetition of nicotine exposure, so it allows to observe the results first hand and be able to collect a sufficient amount of data to prove or disprove our hypothesis. If properly executed, some zebrafish will not be fully developed

meaning they will be born with birth defects and/or some embryos will hatch later during the experiment. This experiment is testing the effect of nicotine on zebrafish embryo development.

Materials & Methods

The safety concerns for this experiment were to wear gloves while handling the equipment. The materials needed to perform this experiment were: 1 bottle each of stock solutions of Nicotine and specifically for our group: a bottle of 0.01 mg/mL solution and 0.2 mg/mL solution. A beaker for dead embryos and liquid disposal, 1 Sharpie to label each bottle of stock nicotine solution, 1 per group plus extras of large bore transfer pipette, minimum bore, 1.5 mm for transferring eggs to observation container and manipulating them in the container), and 1 per group of transfer pipettes was also needed. To place the embryos and solutions in, 1 multi-well plates and a 28.50℃ Incubator was needed. Finally, there needed to be 1 dissecting and compound microscope to observe the embryo development each day.

On day 1, rinsed embryos were obtained from the teacher. While one partner, placed the alive embryos in each well, the other created the nicotine stock solutions and labeled them with the names and class hour. One jar of 0.01 mg/mL and one jar of 0.2 mg/mL nicotine solutions were created. The exact numbers of live embryos on student data sheet were recorded. And the dead embryos were discarded. Then the embryos were divided so there are approximately 10 embryos in each well. The plates were labeled on the student

Figure 1. Zebrafish embryos on Day 1

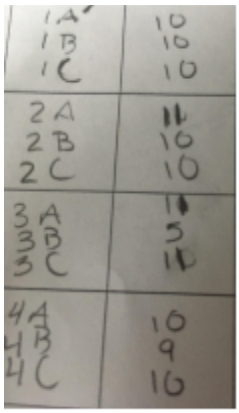


Figure 2. Recorded number of embryos in each well on Day 1

data sheet to help keep track after each day of observation. Out of the 16 wells, 8 wells were controls, 4 wells with 0.01 mg/mL of nicotine solution, and 4 wells with 0.2 mg/mL of nicotine solution. The 8 control wells were filled with 1 mL of Instant Ocean/Embryo Media solution using the transfer pipette. The remaining wells were filled with the appropriate nicotine stock solutions. Once all of solutions were placed in the wells, observation of our embryos took place under the dissecting microscope and recorded observations on student data sheet. To finish the day, each plate was placed in the 28.5OC incubator overnight.

On day 2, the plates were removed from the incubator. The dead embryos were then removed from the plate using the disposable pipette and squirted dead embryos into waste beaker. The remaining embryos and hatched fish were counted and recorded in data table. Next, the control solutions and the nicotine stock solutions were removed from each well of the plate. The control solution and the nicotine stock solutions were replaced with the appropriate fresh nicotine stock solution using a clean pipette each time. After, each plate was placed under the dissecting microscope and observations were recorded on student data sheet. Any developmental markers and abnormalities were noted and described. Once switching the solutions and taking observations were finished, the plate was returned to the appropriate 28.5℃ incubator.

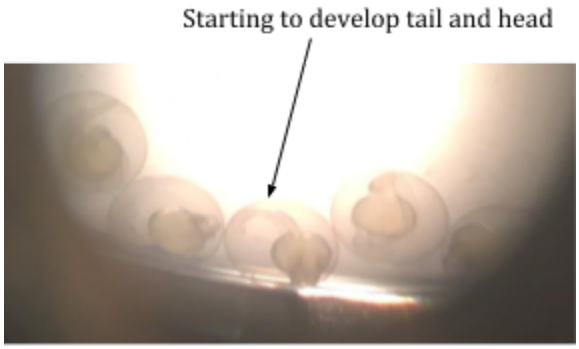


Figure 3. Zebrafish embryos on Day 2

On day 3, day 4, and day 5 day 2 work and observations was repeated and recorded all data to finalize the data for the experiment (University of Wisconsin-Milwaukee, 2003 revised 2018, 37-38).

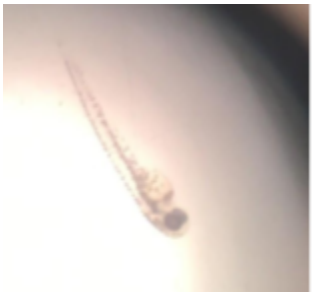


Figure 4. Zebrafish hatched on Day 3

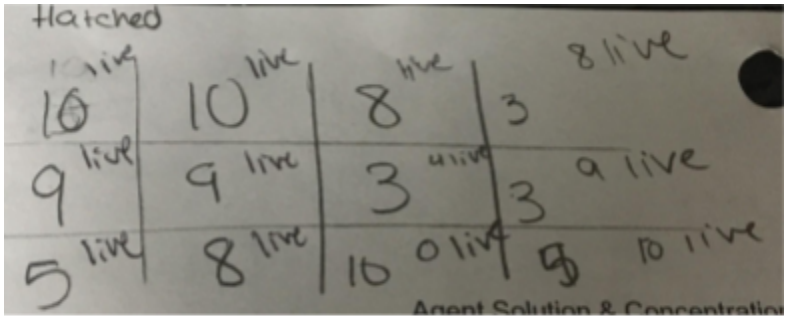
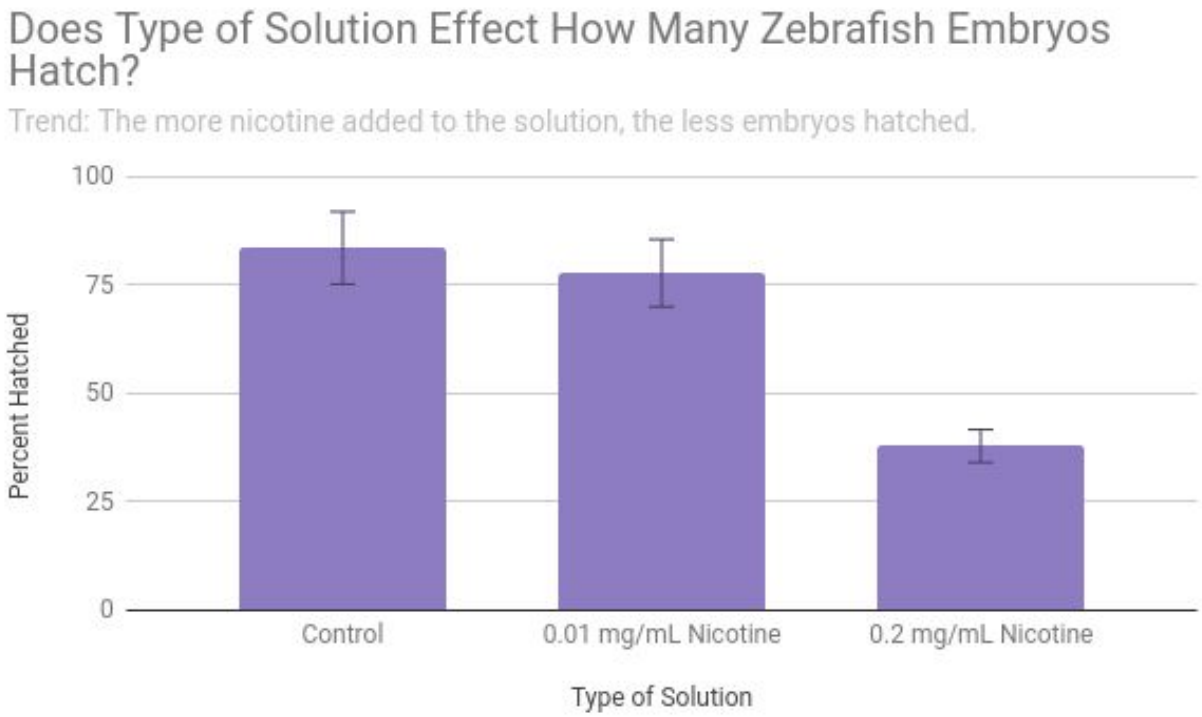


Figure 5. Observations of Zebrafish mortality and how many hatched on day 5 (final day).

Results & Data Representation

The independent variable of this experiment was the percentage of hatched zebrafish embryos and the dependent variable of the experiment was the amount of nicotine stock solution used on the embryos. The control group was zebrafish embryos with no nicotine solution placed in the wells and the experimental groups were zebrafish embryos that were placed in wells with 0.01 mg/mL of nicotine solution and embryos placed in wells with 0.2 mg/mL of nicotine solution. The sample size was 61 control embryos (6 wells) and 56 experimental embryos (6 wells) for a total of 117 zebrafish embryos (12 wells)

Graph 1. The data shows that there is a large gap in the amount of embryos that hatched in a solution with nicotine versus a control group. The trend represented by the data in the graph is that the more nicotine added to the solution, the less embryos hatched.



The graph above shows the percentages of hatched zebrafish embryos in the control group and both amounts of nicotine exposure. The first bar shown was the combination of the two control columns (total of 6 wells). On final day of observation, the total number of embryos were counted. The two control columns were added together as well as the ratio of the total number of eggs in the wells on day 1 to the total number of eggs hatched on the final observation day. That number was multiplied by 100 to get a percentage of total eggs hatched over the course of the experiment. The second bar is from the wells that had 0.01 mg/mL of nicotine solution

placed in them and the third bar is from the wells that had 0.2 mg/mL of nicotine solution placed in them.

The procedure for calculating percentage from the control wells was done the same way on the experimental wells. However, the experimental columns were not added to together. The 0.01mg/mL calculation was separate from the 0.2 mg/mL calculation. The results showed that those exposed to nicotine had less hatched on the final day. This relates to the aim of the experiment in the way that nicotine has an effect on the growth and development of the embryo, slowing down the process of maturation and taking longer for the embryo to hatch.

Data Analysis of The Effect of Nicotine on Zebrafish Embryo Development

Group	Control	Experimental (0.01 g/mL & 0.1 g/mL nicotine stock solution)
Mean	83.33	56.00
SD	17.51	23.25
SEM	7.15	9.49
Sample Size	6	6

Table 1. Demonstrates the statistical analysis of the data

A GraphPad test was performed to determine if our results and observations taken were statistically significant or not. The data used was the percentage rate of eggs hatched in each group. ($p=0.0442$ meaning that our data is statistically significant). Statistically significant data results in a P value of 0.05 or lower. Being statistically significant means that your variables are directly related/correlated.

Discussion

As is shown in the first graph, the amount of embryos hatched decrease as nicotine exposure increased. This supports the hypothesis and proves that development of embryos are slowed when nicotine becomes present during the time of embryonic maturation. The limitations presented in this experiment were that all beginning numbers of embryos in each well were different. There was never the same number in all the wells, which allows for a greater percentage of error. Also, there were more control fish than the two nicotine experimental groups. With this being the case, if the same amount of control fish and experimental would have died, there would have been a greater difference in percentage of dead to alive fish in the nicotine exposed embryos.

The effect of nicotine on an embryo is detrimental to its growth and development. An observation taken during this experiment was that the nicotine exposed embryos tended to have a

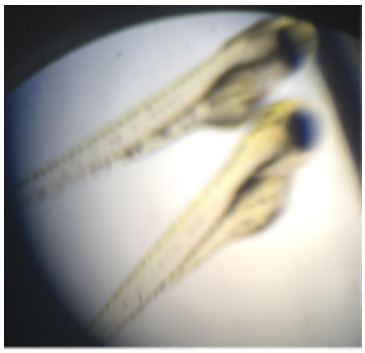


Figure 6. Control group typically hatched with a straight spine

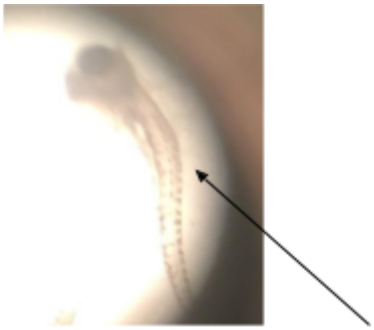


Figure 7. Embryos exposed to nicotine during development tended to hatch with a curved spine, scoliosis

curved spine, or scoliosis, once they hatched.

When nicotine is taken in by the body, it is quickly moved throughout all the body tissues (Gilchrist 2018). Smoking results in inflammation of the blood vessels which decreases necessary blood flow to the tissues (Gilchrist 2018). The body's blood to the intervertebral discs are damaged as well as collagen formation, one of the most necessary structural proteins of the discs (Gilchrist 2018). Smoking prevents certain, important nutrients from reaching the discs and will not allow adequate healing (Gilchrist 2018). "Theses processes causes discs to prematurely degenerate and become diseased"(Gilchrist 2018). This gives an explanation and supports the observed data presented within the experiment as to why some of the zebrafish that hatched and were exposed to nicotine as an embryo were born with a curved spine. Ultimately, the experiment performed proved our hypothesis that being exposed to nicotine and other drugs while in the womb will lead to possible birth and developmental defects.

Works Cited

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