Nicotine's Affect on Developing Chicken Embryo's

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Introduction:

This experiment will further examine the development of the brain in chicken embryo's during the first five days of development. Development of the brain during these initial days is critical. This experiment will closely look at the relationship of a well known teratogens affect on the development of the brain, specifically the growth rate. Teratogens affect on developing embryo's is an active area of research today and studies are being done to determine what the negative affects may be. Many processes are affected when a developing embryo is in the presence of a teratogen. Affects from certain teratogens can show a loss of gene expression in developing embyro's which can result in an underdeveloped fore and mid brain (Blader et al. 1998). This study hopes to further the knowledge in this field by testing the harmful and commonly used teratogen, nicotine.

Nicotine is a well known teratogen that has been known to cause neural abnormalities in developing embryos. Unfortunately, one in four women continue to smoke during pregnancy, possibly unaware of the effects that nicotine can have on the development of their baby. Studies have shown that the effects of nicotine on pregnant females can lead to birth defects as severe as missing limbs or digits in developing embryos. This may be due to an effect that nicotine has on protooncogene, which ultimately leads to cell loss. The protooncogene, c-*fos*, when over-expressed results in apoptosis. The affects of smoking during pregnancy has shown that even prenatal exposure to c-*fos* can result in apoptosis and ultimately cell loss (Slotkin et al. 1998). More specifically the cell loss has been noticed in the developing brains of embryo's. However, studies have shown that this cell loss is recoverable later in development and ultimately cells can "catch-up" in their growth.

In the presence of nicotine, the critical early stages of development are hindered by cell loss and due to this, the size and development of the embryo can be affected specifically in brain growth and heart strength. I expect the results to reflect previous research and studies on nicotine's affect on developing embryo's. However, my dose will be far less than many previous experiments. I will use a 150 micron dose of liquid nicotine which is mixed with 150 microns of methanol alcohol. In this study we tested the hypothesis that a modest dose of 150 microns of nicotine solution will show a hindrance on overall development and cell loss will be noticed; however, larger doses may be needed to show significant results. My access to more nicotine is limited due to other research groups sharing the same nicotine solution.

Materials and Methods:

The Nicotine Solution will be made prior to the experiment and administered equally to each of the experimental group embryo's. The Nicotine dose will be 150 microns of Sigma nicotine solution. The dose will be the drug standard and will be 1 mg/mL + or - %5 in methanol. 8 eggs will be tried in each experiment; however due to varying viability 5-6 six will most likely be able to be used. 3 eggs will be used for the control, and 3 eggs will be used as the experimental nicotine group. All Petri dishes and weigh boats will be weighed prior to the experiment. After adding the chick embryo's to the trays each will again be weighed to find the weight of the embryo. The Nicotine dosage for the experimental group will be a set amount which will be determined by the average weight of a chicken embryo.

Prior to the experiment forceps and plastic egg trays in Petri dishes will be brought to each lab bench. Before removing the eggs for our experiment all benches will be sterilized. Our hands will be sterilized before touching the eggs. The eggs will be sterilized with 70% ethanol and let to dry wide end down in a tray to rotate the embryo. After the

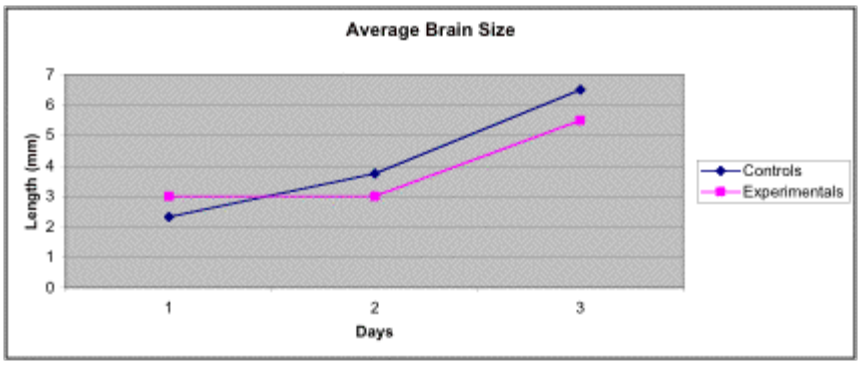
eggs dry they will be cracked carefully with the forceps at the wide end of the egg. The tips of the forceps will then be used to peel away the open shell. Small bits shell will be removed until all the air space in the egg is exposed. The egg will then be placed over the plastic tray and turned open end down. A small hole will then be poked into the small end of the egg very carefully. The egg will fall into the tray and be quickly covered by the Petri dish lid.

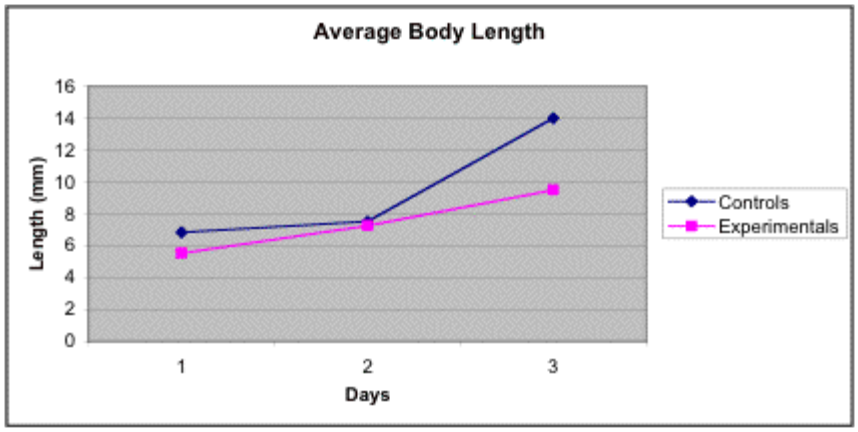
The embryos will be kept in an incubator at 37 degrees Celsius for 6 days. Each day they will be observed under a dissecting microscope. Specific measurements will be taken on the brain and heart. The size of the brain will be recorded and also measured relative to other developing organs and limbs. The pulse will be measured each day in beats per minute. Movies taken in the ICUC will help to measure the strength of the heart. The size of contractions will be measured using this method. Additionally, the total area of brain growth will be recorded using the Image J program. By tracing the area around the brain I will be able to determine the area of the brain and then convert the total number of pixels into mm2.

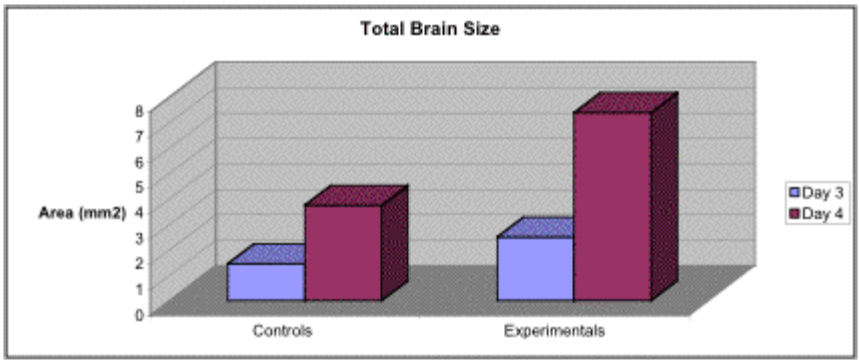
Results:

The total number of successful eggs used was changed and we were unable to use 6 eggs in each experiment. Instead the total number used was 4 or 5 eggs per experiment. Additionally, the nicotine dosage was changed to 100 microns of solution in the second experiment. Many of the eggs died during the experiment after only a few days of being exposed. The results reflect the third through fifth days of development.

In figure 1 and Table 1 the control doubled from the second to third day. The change was from a length of 7.5 mm to 14 mm. The experimental group average stayed consistent. The changes from day to day were 1.75 mm and 2.25 respectively. Figure 2 shows a peak of the control group just higher than that of the experimental group with a difference of 1 mm after five days. The control group showed more growth rate overall after three days, after starting out an average of 0.66 mm smaller and ending with a larger average brain size. Lastly the Figure 3 shows the greatest variance with the experimental group showing the largest growth rate on average. A significant difference was noticed between the third and fourth day averages. On the third day the experimental group had a slightly larger brain size on average, a difference of 1.04 mm2. However, after only one day the difference between the two groups was 3.64 mm2, with the experimental group having a much larger average brain size. In Figures 4 and 6 represent good images of two of the developing embryo’s. The brain size is easily measurable here and measurements were taken where the lines are drawn. However, Figures 5 and 7 show the difficulty of the positioning of the embryo’s and the trouble of measuring brain size from them.







Discussion:

The data we found in our experiment suggest that there may be a decreased amount of cell growth in the first five days of development. However, more data needs to be gathered to have reliable conclusions. Some of the data we found supported our hypothesis; however, other data was inconclusive due to a lack of healthy embryo's to test.

I found that there was conflicting data represented between Figure 3 and the rest of the Figures. In both Figures 1 and 2 there was a noticeable increase in the development of the control group over the development of the experimental group. In Figure 1 the measurement of body length was made in an anterior to posterior direction. This provided clear results which showed a greater growth rate of the control group. The largest difference in growth rate was noticed on day three. The control group doubled its total size on average while the experimental group showed no increased

growth rate. The experimental group stayed consistent with no sharp change on day three. This may suggest that there was a decrease in cell growth when the embryo's were in the presence of nicotine.

Again in Figure 2 the control group showed an increase in growth rate over the experimental group. The measurement for brain size was an anterior to posterior measurement as well. However, this change was less significant and it appears that further testing will need to be done to conclude any variance in brain size from these results.

When comparing Figures 2 and 3 a significant change is noticed. Figure 3 shows the most significant differences between the experimental and control groups. The experimental group showed a large increase in total brain area on the fourth day while the control group only showed a minor increase. The results in Figure 2 which show greater growth in the controls go against these findings. Furthermore, the results in Figure 3 go against previous literature and raise several questions. One concern was how to take consistent accurate results. Many times it was difficult to measure the area of the embryo's, because they were often not in ideal positioning. Many times the brain was covered by other parts of the surrounding embryo which made it difficult to see all parts of the brain. Figures 5 and 7 are good examples of this and show how it is not always easy to get an accurate measurement. Another issue can be answered by looking at the data base. Several of the control and experimental embryo's died on the fourth day. The difference can possibly be explained by the lack of having a larger sample to record data from. The embyros that survived in the experimental group may have been more resistant to the nicotine. The experimental embryo's that died should not be excluded, because they may represent a different affect from the nicotine that may have been a decrease in total brain growth. More embro's will need to be tested to determine a better average for the affect the nicotine solution has. The control group also needs to have more representative samples to create a better average as well.

More successful embryo's should be used, in both the control and experimental group, in order to have reliable results. Also, with more embryo's to test, it will increase the likelihood of having more successful measurements and an increase in total accuracy. More concrete results are needed and more research would have to be conducted to account for these shortcomings to the experiment. I believe the experiment is important to science as a whole and should be revisited with these corrections.

Sources:

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