

# Minimal Effects When Acute Nicotine Exposure Introduced to 3 Day Old Chick Embryos

Amy Langevin  
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## Introduction

Thousands of mothers smoke cigarettes daily while pregnant, sometimes without concern for the effect that it might be having on the developing embryo within them. Nicotine is what is known as a teratogen, which is an agent that perturbs normal developmental processes, particularly morphogenic movements in a developing embryo (Morris, 2007). Common teratogens include alcohol and other drugs such as cocaine. Due to the focus on the affects that these agents have on unborn babies, nicotine exposure is usually overlooked, and receive little attention because it doesn't seem as extreme. In fact, cocaine exposure to developing embryos is subject to four times the number of studies per year as nicotine (Slotkin, 1998).

There have been few studies showing the affects that nicotine has on developing embryos, so it is still unclear the exact effect that cigarette smoking has on developing embryos inside of a smoking mother. It once was only thought to cause respiratory disorders in the newborn child, but now is being studied for affects on the cardiovascular and nervous systems (Slotkin, 2003). Experiments have been carried out which test the effect of nicotine on rats and it has been found that, "embryos treated with nicotine in 3-6  $\mu\text{M}$  were smaller... and most of the embryos had an open neural tube in the anterior brain regions (Reese, 2005).

Due to the fact that there is little research done on the effects that nicotine exposure has on heart strength of a developing embryo, I completed an experiment that tested the effects that nicotine has on the heart of a three day old chick embryo. In the experiment, chick embryos were extracted from their shells and observed in a Petri dish. Some were injected with a nicotine solution while others were not. Each day the heart strength of the developing embryos (with measurements beginning at three days) were measured. This part of the experiment that measured heart strength was done by measuring the millimeters of distance the heart expanded in one full beat. The strength of the heart was also tested through measuring the beat of each heart per minute.

Before the experiment was completed that was aimed to test the affects that nicotine would have on a developing

chick, I believed that at the end of the ten days, the strength of the hearts in the nicotine injected embryos would be weaker than those of the healthy developing embryos. The embryos injected with the nicotine solution would have a slower heartbeat count than those without the nicotine solution, and the embryos injected with nicotine would have a heartbeat that doesn't expand as greatly as those without the nicotine solution.

In this study, we explanted the chick embryos to find that there is no direct correlation between the nicotine that we injected into the embryos and the heart strength of the embryos.

## Materials and Methods

Before the effects of nicotine in developing chick embryos could be observed, the embryos needed to be explanted from their eggs, so that their development could be observed within the ten day time frame that the experiment was taking place. This was a careful process that needed to be done precisely so that the chick embryo was not damaged or infected by the transfer. Tools that would be needed in the experiment were collected first, so that when the explanting began there was not too much moving about around the desk tops which could cause dust or other particles to infect the exposed embryo. The materials needed included one pair of forceps, one paper egg tray, and one pie tin. The lab bench where we would be working with our embryos, needed to be cleaned using paper towels and 70% ethyl alcohol as to sterilize it. The egg that was to be opened up was disinfected using a paper towel moistened with 70% EtOH as well. The egg was placed wide end down in the egg tray not only to dry but also to allow the egg to rotate so the embryo was facing the egg's narrow end, away from the air space that formed in the wide end of the egg (Morris, 2007).

The wide end of the egg was then cracked with the forceps. This step was done over a sterilized weigh boat that was "glued" to a Petri dish using wax. This step was also done very gently, as to not push any shell fragments into the egg and also to make sure that the edges of the hole were as smooth. This way, the egg was not torn as it slipped out the hole. The forceps were then used to remove small bits of shell and expose the air space.

The egg was then flipped with the air space pointing down into the sterile fixed weigh-boat, and the shell membrane was pierced at the small side with the point of the forceps. This displaced the air space to the small end of the egg. When the hole was larger using more careful precision with the forceps, the yolk dropped out into the weigh boat, leaving the yolk and chick embryo exposed (Armstrong et al, 1994).

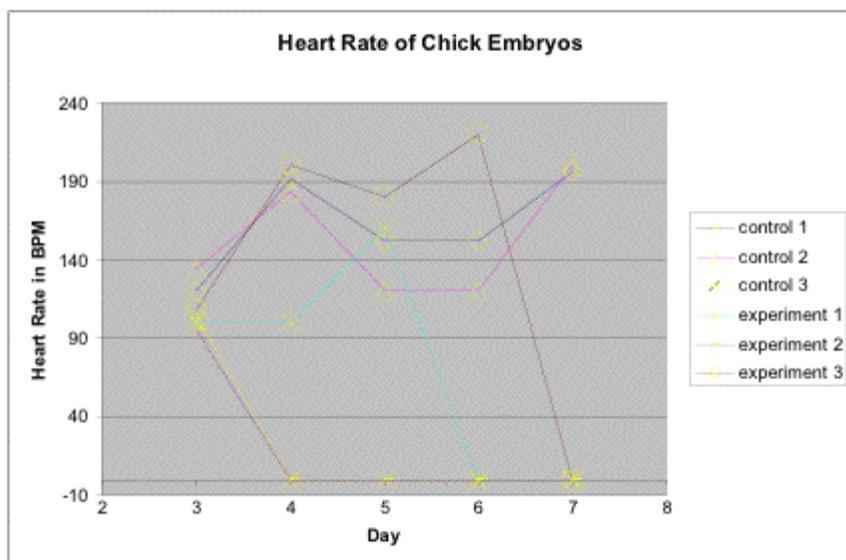
We attempted to extract 6 eggs for each trial in sterile weigh boats, using that method of extraction. Once all of

the embryos for the experiment were extracted, some embryos were designated “Control,” some “Experimental.” The nicotine solution that we bought was: Sigma, Nicotine, 1.0 mg/mL +/- 5% in methanol, drug standard, catalog #N5511. Each “Experimental” chick embryo was injected with 150 microliters of this solution which contained nicotine and methanol. This concentration was equivalent to what would be an acute nicotine exposure (Slotkin, 1998). Because the experiment was testing only the effects of nicotine, not nicotine plus methanol, we injected with “Control” embryos with 150 microliters of methanol.

The embryos were set in an incubator at 37 degrees Celsius for ten days. Each day they will be observed underneath a dissecting microscope at either a magnification of 1 or .8, depending on the size of the embryo at any given time. Each day, they live and thriving embryos were taken into the ICUC, a specialized computer lab that allows for pictures and videos to be taken of the embryos. When not under the dissecting scope, the embryo’s heart rate was measured in beats per minute. After videos we able to be taken, it was possible to measure the distance that the heart contracted with each beat, and by slowing down the frames on the movies, it was possible to describe the strength of the heart in the difference in millimeters.

## Results

None of the embryos made it past day seven. In the two sets of trials done, there only were three overall “Control” embryos with quantifiable data and three “Experimental” embryos with quantifiable data. The rest of the eggs did not make it past night three. Figure One shows the heart rate of the chick embryos from day three when the measuring began, up to the day that the last embryo died, which turned out to be day seven.



**Figure One: The heart rate of the chick embryos in beats per minute was recorded each day, as to see if there was a difference in the heart rate of the embryo's injected with the nicotine solution versus the heart rates of the chick embryo's that were not injected with the nicotine solution.**

The "Control 1" chick began with a heart rate of 120 beats per minute and by day two it had increased to 192 bpm (beats per minute), day three it lowered to 152 bpm, and by day seven it was up again at 196 bpm. The "Control 2" chick had a starting heart rate of 134 beats per minute. At day two it had increased to 184 bpm, day five lowered to 120 bpm, and by day seven it was up again to 200 bpm. The "Control 3" chick began with a heart rate of 104 bpm and died after the third day.

The "Experimental 1" chick began with a heart rate of 100 bpm and the heart rate remained here until day five, when it shot to 160 bpm before death. The "Experimental 2" Chick had a heart rate of 96 on day three, and shortly after. The "Experimental 3" chick began with a heart rate of 108 beats per minute on day three, which increased to 200 bpm on day four, lowered too 180 bpm on day five, and on its last day living (day six) had a heart rate of 220 bpm.

Measuring heart strength was measured with one "Control" chick and one "Experimental" chick. On day three the heart strength of the "Control" chick was measured at .57 meaning that when the heart was fully expanded it measured at 1.07 mm. and when it was at it smallest is was .5mm. Figures 2.1 and 2.2. show this visually.

Heart Strength was easier to measure in the "Experimental" chick that was chosen because it lived longer. On day three, the heart strength was measured at 1.17 mm. because it was 1.6 mm. when fully expanded and .43 mm. when at its smallest. Figures 3.1 and 3.2 show this visually. On day four, the heart strength was 1.66 mm. because it was .94 mm. when the heart was at its smallest and 2.6 mm when the heart was at its largest. Figures 4.1 and 4.2 show this visually. The last day where the "Experimental" chick could be measured for heart strength was day five, when it had a heart strength of .44 mm. because the heart size as 1.1 mm. when it was at its smallest and 1.54 mm. when it was at its largest. Figures 5.1 and 5.2 show this embryo visually.

## Discussion

After completing the experiment, no clear conclusions could be drawn about the affect that nicotine has on a developing chick embryo. The results did not indicate that cigarette smoking, or the injection of nicotine into the developing chick embryos is directly injurious to embryonic development because of the delivery of nicotine alone. Many of the “Experimental” chicks lived longer than “Control” chicks did. According to a study done by Theodore Slotkin at Duke University, these results may be because “for many drugs, including nicotine... the placenta provides significant fetal protection by metabolizing a portion of the drug by introducing a phase delay between the maternal and fetal circulation. Thus, episodic [nicotine] delivery produces less penetration into the fetal compartment than does continuous exposure” (Slotkin, 1998). There is a chance that because our embryos were only exposed to one injection of nicotine, that this might not have been enough of a concentration to do any significant damage on the embryo. A further experiment would need to be done to test if there were effects with long term smoking throughout pregnancy rather than just a single acute exposure to the drug.

In order to obtain more accurate results, a second experiment would need to be completed, in which there were more control embryos and more experimental embryos. This way, there could be more chances to take video footage in the ICUC of the beating hearts, so that measuring heart strength might bring forth legitimate and plentiful quantifiable data.

The results of the experiment were certainly not expected. I believed that nicotine exposure would weaken the heart of the developing chick embryo, and cause for an overall weaker cardiovascular system. Although the data did not go against my original hypothesis, this hypothesis was refuted due to lack to significant evidence. Most experiments done that test the effects that nicotine has on a developing organism focus on nervous system and brain development, and the affect that nicotine has on these parts. Future experiments should be completed that test longer term nicotine exposure on chick embryos past the first ten days of development. Instead of injecting the chick with one bout of the nicotine solution, a small amount should be added each day, in order to test what daily exposure to the drug might have on the fetus.

## References

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