Abstract

The purpose of this experiment is to see how acetylcholine (ACH), epinephrine (EPI), and atropine (ATR) alter heart rate in 3-and 5-day-old chick embryos. The study's goal is to determine when neurotransmitter receptors for these substances become functional throughout the two highlighted stages of development. Both 3-day and 5-day chick embryos provide different concentrations of ACH and EPI (1 µmol/L, 10 µmol/L, and 100 µmol/L), but just one concentration of ATR (100 µmol/L) is used. Zen software, a cell counter, a pipette, a pair of tweezers, scissors and a Zeiss Stemi305 stereomicroscope are used to measure heart rate. To ensure accuracy, the experiment is carried out with three technical replicates for each treatment. Chick saline is used as the control. In 3-day embryos, the heartbeat is consistent across all neurotransmitter concentrations. This suggests that there is minimal receptor functionality at this age. 5-day embryos exhibit a decrease in heart rate with ACH and an increase in heart rate with EPI and ATR. For example, ACH reduces heart rate from 54.86 BPM to 43.43 BPM, while EPI raises it from 50.25 BPM to 62.25 BPM. These results show that neurotransmitter receptors for ACH, EPI, and ATR become functional in chick embryos after the third day of development. The study enhances our understanding of embryonic heart development and can drive future research in the LIFESCI 3L03 course, allowing for more investigation of cardiovascular functions in model organisms.

Introduction

Model organisms are organisms that are used in the field of science to study experimental designs and help scientists understand general biological processes (Leonelli & Ankeny, 2013). Model organisms are non-human organisms, and their benefits include ease of maintenance, low cost, fast reproduction, and genetic similarity to humans (Leonelli & Ankeny, 2013). The chick embryo is a widely used model organism used to study cardiovascular development and evolutionary biology (Vilches-Moure, 2019). One of the key advantages of using chick embryos for research is their ability to be compared to the well-known

developmental stages known as the Hamburger-Hamilton stages (Vilches-Moure, 2019). The Hamburger-Hamilton staging system, established in 1951, is a widely used reference for studying chick embryo development (Doty, 2011). It divides the 46 distinct phases of embryonic development into various stages, from a fertilized egg to a fully formed chick (Doty, 2011). Chick embryos are valuable for studying the cardiovascular system because their heart development provides easy-to-obtain knowledge about vertebrates (Doty, 2011). Epinephrine is a drug or a hormone that increases heart rate and blood pressure. It is commonly used to treat heart problems and asthma attacks (Chipps, 2024). Acetylcholine is a neurotransmitter that slows heart rate and stimulates nerve cells to regulate heart rate, heart contractions, and more (*Acetylcholine*, 2022).

Previous research on the heart development of chick embryos helps provide valuable knowledge to current studies about neurotransmitter regulation during the early stages. The chick embryo's cardiovascular system develops rapidly, transitioning from a simple tubular structure into a functional heart with distinct features (Vilches-Moure, 2019). This makes it an ideal model for studying heart development and the mechanisms influencing early cardiac function. Acetylcholine lowers heart rate by activating muscarinic receptors, whereas epinephrine raises heart rate by boosting adrenergic receptors (Coraboeuf et al., 1970) (Kroese et al., 2004). Atropine raises the heart rate by inhibiting the function of acetylcholine on muscarinic receptors (Chiba et al., 2004). A study by Claudio Stern demonstrated how certain signals that control development and activity affect the development of the heart in chick embryos. This study showed that the roles of specific genes and proteins impact the development of the cardiac system (Stern, 2005). These processes are key to maintaining healthy heart health (Stern, 2005). Understanding how heart rates are impacted by external factors aids in the understanding of embryonic development. A study conducted in 2015 by Gordan et al. examined the role of certain receptors. They monitored how neurotransmitters such as ACH and EPI play a role in receptor reactions and heart rate. The heart rate can be increased by epinephrine binding to β-adrenergic receptors and decreased by acetylcholine activating muscarinic receptors (Gordan, et al., 2015).

Although previous studies have looked at how acetylcholine and epinephrine affect heart development in chick embryos, measuring the time and analyzing beats per minute (BPM) to identify precise differences in how the drugs affect heart rate is not fully understood. While acetylcholine and adrenaline's effects on chick embryo heart rate have been investigated previously by other LIFESCI 3L03 students, this study aims to confirm and expand on those results. This study is driven by the hypothesis that states that the heart tissue of chick embryos does not have developed or operational acetylcholine (ACH) and adrenaline (EPI) receptors until after the third day of development. Based on earlier research, the chick embryo's nervous system, which controls heart rate through neurotransmitters like acetylcholine (ACH) and epinephrine (EPI), is not fully developed before this stage. In 5-day-old chick embryos, the receptors are matured. This should lead to a stronger response to these neurotransmitters compared to the 3-day-old chick embryo. This experiment focuses on how ACH and EPI affect the heart pulse in 3- and 5-day-old chick embryos. The goal is to see how receptors vary for different ages with different drug administrations. To build on previous LIFESCI 3L03 investigations, heartbeat responses were assessed utilizing three technical replicates per concentration.

Methods and Materials

Windowing Process

The experiment began by windowing the egg, which is a process that involves drilling a small portion of the shell with tweezers to drain the albumen. Once 2-3 mL of the albumen was drained, a larger hole was cut using scissors on the top of the eggshell to view the embryo. Fertilized eggs were then observed under a Zeiss Stemi305 Stereomicroscope. Through windowing, a clearer view of the embryo was achieved.

Dilutions & Administration

Serial dilutions were used to prepare the various EPI and ACH concentrations. The ACH stock solution originally had a concentration of 1000 µmol/L, which was diluted ten times to

produce a 100 µmol/L solution (Refer to **Table I**). This was diluted again to produce 10 µmol/L and 1 µmol/L solutions. The same procedure was used for epinephrine (Refer to **Table II**). Every substance was administered separately. The lowest concentration (1 µmol/L) was tested first, and the greatest concentration (100 µmol/L) was tested over time. Limiting the amount of medication given to each embryo prevented the overstimulation of embryonic sensors.

Monitoring Heart Rate

Heart rate was monitored by counting the number of heartbeats over a 20-second interval using a cell counter. This was done for three technical replicates for both administrations at each dilution. It was viewed using a Zeiss Stemi305 Stereomicroscope and Zen Software for a clearer observation. This result was multiplied by three and helped achieve a result for every minute to get beats per minute (BPM). Only a single concentration of atropine was used. Chick saline was utilized as the control during the heart rate measurements. Due to limited fertilized eggs, tests weren't done on three biological replicates. Some embryos were explanted into chick saline when in ovo measurements were not possible. Data from other experiments provided results.

Results: Text

The purpose of this experiment was to examine how differing levels of acetylcholine (ACH), epinephrine (EPI), and atropine (ATR) altered the heartbeat in 3- and 5-day-old chick embryos. ACH, EPI, and ATR were administered to 3-day and 5-day chick embryos at varying dosages as part of the experimental approach to evaluate their effects on their heartbeat. First, ACH was administered to measure its impact on the heartbeat**. Figure 1** reveals that the heartbeat was consistent in 3-day embryos throughout concentrations. ACH administration decreased the beats per minute (BPM) for the 5-day embryos. The beats per minute decreased from 54.86 BPM in the control group to 51.57 BPM at 1 µmol/L, 47.52 BPM at 10 µmol/L, and 43.43 BPM at 100 µmol/L. Then EPI was administered to measure its impact on the heartbeat. **Figure 2** illustrates that the heart rates of 3-day embryos varied very little.

The control group averaged 38.96 BPM, whereas the experimental groups ranged from 36.25 BPM at 1 µmol/L, 36.63 BPM at 10 µmol/L, and 37.63 BPM at 100 µmol/L. The 5-day embryos' beats per minute increased. It went from 50.25 BPM in the control to 53.38 BPM at 1 µmol/L, 56.13 BPM at 10 µmol/L, and 62.25 BPM at 100 µmol/L. Finally, a single dose of ATR was administered to measure its impact on the BPM. **Figure 3** shows how the control group's average heart rate for the 3-day embryos was 34.25 BPM, but it rose to 34.88 BPM when it received 100 µmol/L of atropine. The control group's heart rate in 5-day embryos was 51.67 BPM on average; however, after being exposed to atropine, the heart rate rose to 53.57 BPM. These findings indicate that atropine did not affect the heart rates of 3-day embryos. The 5-day embryos were impacted, which could be due to their more mature receptive systems. The results showed that the 3-day embryos' heart rates were consistent for all ACH, EPI, and ATR concentrations. This indicates that there is little to no reaction to these neurotransmitters in the embryos likely due to underdevelopment.

Results: Figures

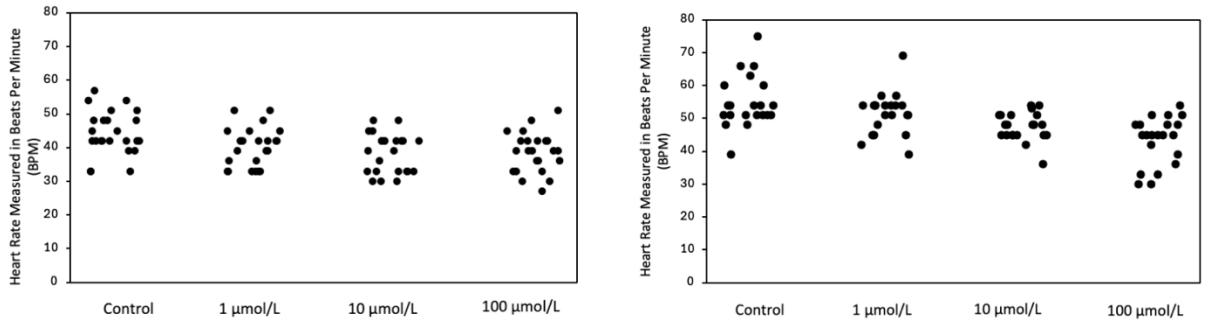

Figure 1: Dot Plot of the Impact of Acetylcholine (ACH) on Chick Embryo Heartbeat Over Various Concentrations. 2 dot plots showing the heart rate (BPM) of 3-day (left image) and 5-day (right image) chick embryos after administration of ACH (1 µmol/L, 10 µmol/L, and 100 µmol/L) and a control (chick saline). The heart rate decreases slightly in the 3-day embryo, and it decreases a lot (as administration increases) in the 5-day embryo.

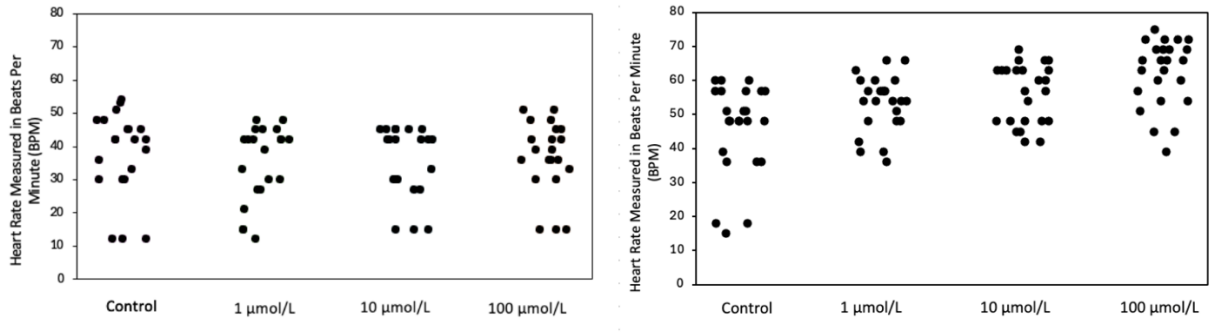

Figure 2: Dot Plot of the Impact of Epinephrine (EPI) on Chick Embryo Heartbeat Over Various Concentrations. 2 dot plots showing the heart rate (BPM) of 3-day (left image) and 5-day (right image) chick embryos after administration of EPI (1 µmol/L, 10 µmol/L, and 100 µmol/L) and a control (chick saline). The heart rate shows minimal change in the 3-day embryo, and it increases (as administration increases) in the 5-day embryo.

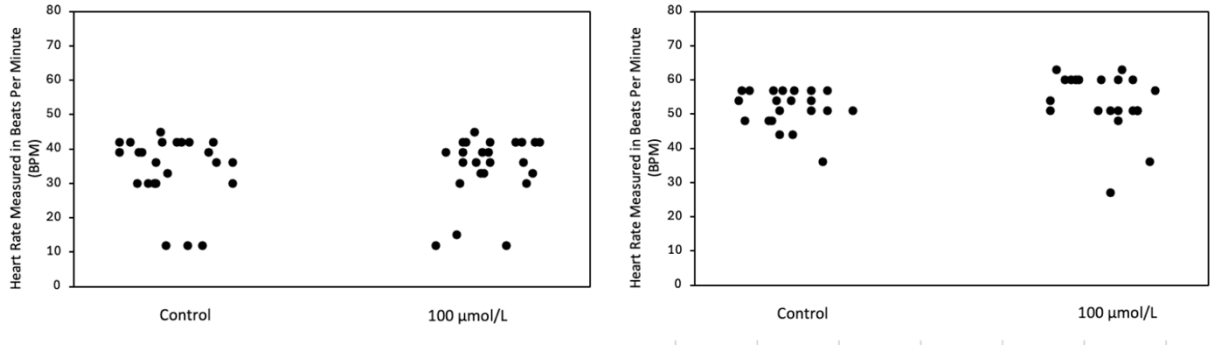

Figure 3: Dot Plot of the Impact of Atropine (ATR) on Chick Embryo Heartbeat Over Various Concentrations. 2 dot plots showing the heart rate (BPM) of 3-day (left image) and 5-day (right image) chick embryos after administration of ATR (1 µmol/L, 10 µmol/L, and 100 µmol/L) and a control (chick saline). The heart rate shows minimal change in the 3-day embryo, and it slightly increases change in the 5-day embryo.

Discussion

The findings of this experiment provide an understanding of the way the cardiac function (the heartbeat) of chick embryos react to acetylcholine (ACH), epinephrine (EPI), and atropine (ATR). These findings were conducted at the 3-day and 5-day stages of embryonic development in chicks. Numerous concentrations of neurotransmitters were used including 1, 10, and 100 μmol/L. A control solution consisting of chick saline was also used for comparison. The heartbeats for embryos that were three days old stayed primarily similar at all ACH, EPI, and ATR concentrations, suggesting little to no response to these neurotransmitters. The 5-day-old embryos showed more noticeable alterations, with EPI and ATR increasing the beats per minute (BPM) and ACH decreasing. The results of this study validate the initial hypothesis that chick embryos do not have fully functional neurotransmitter receptors for epinephrine and acetylcholine until after the third day of development. The absence of detectable variations in the heartbeat of the 3-day embryos at all doses implies that the neurotransmitter receptors are still developing.

The findings of this investigation align with previous research on the development of the chick embryo's heart and its relationship with neurotransmitters. According to Coraboeuf et al. (1970), acetylcholine interacts with muscarinic receptors to decrease heart rate function (Coraboeuf et al., 1970). This research is supported by observations made in 5-day embryos, which showed that heart rates decreased as ACH concentrations increased. Likewise, the increase in the heartbeats of the chick embryos after epinephrine administration shows how the experiment supports the work of Kroese et al. (2004). Kroese et al. (2004) states that epinephrine causes embryonic hearts' cardiac activity to increase by stimulating β-adrenergic receptors (Kroese et al., 2004). According to research by Chiba et al. (2004), atropine increases heart rate by obstructing the impact of acetylcholine on muscle receptors (Chiba et al., 2004). This stops the heartbeat from slowing down as it normally would. This fits with our findings, which showed that the cholinergic system becomes more operational as the embryos age. This is supported by the fact that the 5-day embryo that was affected by atropine displayed an

increase in heart rate. Although the results align with the hypothesis, there are many limitations to this experimental design. One limitation includes controlling the temperature. Minor variations could still happen even when the experiment's intended temperature is kept at 25°C. Since heart rate and temperature are directly correlated, even little temperature changes can make an impact. This limitation to precise results could be reduced by using a more accurate and consistent technology to keep the temperature at very consistent levels. Another limitation is the consistency of fertilized eggs. Due to the limited number of fertilized eggs, each group did not get to use three separate embryos for each experimental condition. Instead, certain groups spend more time finding fertilized eggs. Some groups do not have three data sets for each administration, which reduces the precision of the results. Monitoring embryo development through advanced technological devices may help reduce the loss of precise results.

In conclusion, this work is significant because it reveals important distinctions between the responses of 3-day and 5-day chick embryos to ACH, EPI, and ATR. The hypothesis that these neurotransmitter receptors are undeveloped at this stage is supported by the 3-day embryos' minimal change in heart rate across all doses of neurotransmitters. Furthermore, 5-day embryos showed distinct reactions. ACH lowers heart rate and ATR and EPI increase it. These findings indicate that between the third and fifth day of evolution, the chick embryos develop greater biological reactions.

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Appendix

Appendix A

Uploaded as separate Excel sheet.

Appendix B

Table I: Acetylcholine (ACH) Serial Dilutions. The successive dilutions of acetylcholine are shown in this table, along with the volumes (V_1, V_2), concentrations (C_1, C_2), and amount of diluent utilized.

C_1(µmol/L)	V_1 (mL)	C_2 (µmol/L)	V_2 (mL)	Volume of diluent (mL)
1000	1.5	100	15	13.5
100	1.5	10	15	13.5
10	1.5	1	15	13.5

Table II: Epinephrine (EPI) Serial Dilutions. The successive dilutions of epinephrine are shown in this table, along with the volumes (V_1, V_2), concentrations (C_1, C_2), and amount of diluent utilized.

C_1(µmol/L)	V_1 (mL)	C_2 (µmol/L)	V_2 (mL)	Volume of diluent (mL)
1000	1.5	100	15	13.5
100	1.5	10	15	13.5
10	1.5	1	15	13.5

Appendix C

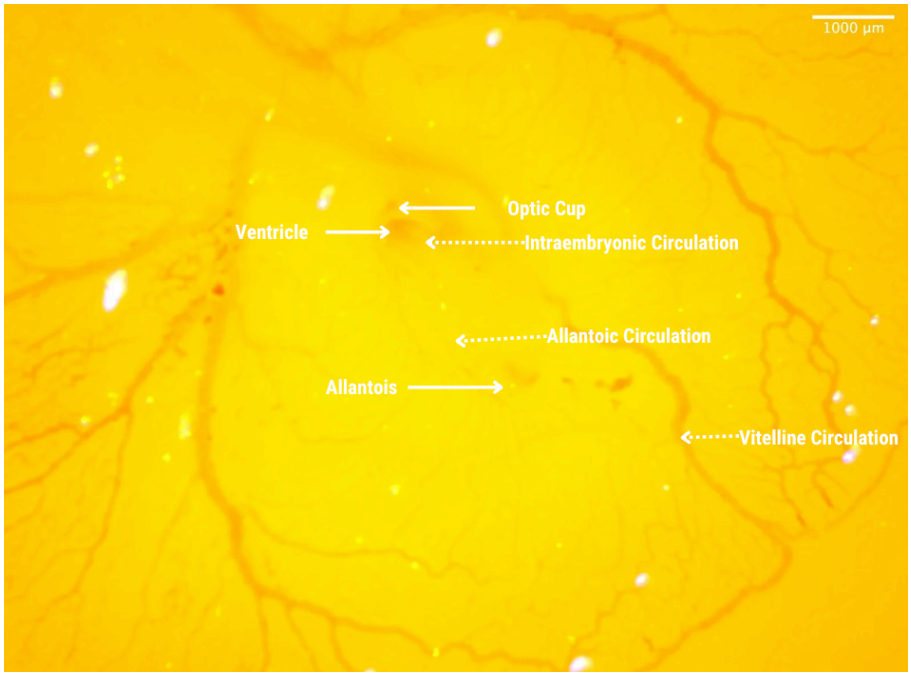

Figure 4: Detailed Image of a 3-Day Chick Embryo with Highlighted Structures at 10x Magnification. This image shows the 3-day chick embryo with visible anatomical structures. Arrows are used to highlight the structures including the ventricle, optic cup, intraembryonic circulation, allantoic circulation, allantois, and vitelline circulation.

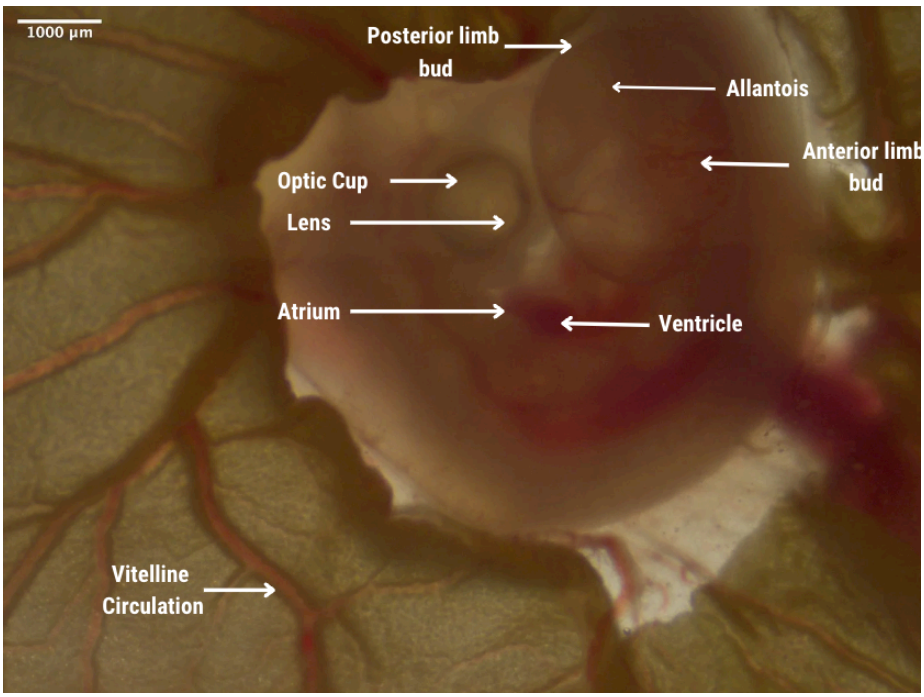

Figure 5: Detailed Image of a 5-Day Chick Embryo with Highlighted Structures at 10x Magnification. This image shows the 5-day chick embryo with visible anatomical structures. Arrows are used to highlight the structures including the anterior and posterior limb buds, allantois, optic cup, lens, atrium, ventricle, and vitelline circulation.

Appendix D

Table III: Heart Rates of 3-Day and 5-Day Embryos at 37°C and 15°C. This table compares the average heartbeat (beats per minute) of 3-day and 5-day chick embryos at 37°C and 15°C.

	Heart Rate (beats/min) at a nominal temperature of 37°C (average of technical replicates)	**Heart Rate (beats/min) at a nominal temperature of 15°C (average of technical replicates)**
3-day embryo	107.67	29.5
5-day embryo	184	65

Question 1:

The 3-day embryos showed a 72.6% decrease in heart rate, while the 5-day embryos showed a 64.67% decrease.

Question 2:

Yes, the group data does support the hypothesis due to high variations in heart rate across different temperatures.