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The effects of Corticosterone on Neuronal Migration in Zebra Finches

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by

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Abstract

Developmental stress has been known to have detrimental effects on zebra finches. Previous research has shown that early stress can affect behaviors such as song and brain structures such as the HVC. However, little is known about how developmental stress affects cell migration in areas of the brain such as the HVC and the hippocampus. This current study used 16 zebra finches in a between subject's design to measure cell count differences in the HVC and the hippocampus. Corticosterone treatment was used as a form developmental stress. Specifically, total, round and fusiform cells were measured across three treatment conditions. The treatment conditions consisted of control, vehicle, and corticosterone groups. Results revealed a significant difference was found between the vehicle and corticosterone conditions in the total cell count in the HVC, however, when sex was added into the analyses there were no longer significant differences between conditions. Furthermore, no significant differences existed between treatment conditions in any of the cell counts in the hippocampus. These results suggest that further research needs to be completed to assess when cell migration is impacted by developmental stress.

The effects of Corticosterone on Neuronal Migration in Zebra Finches

In order for stress to be recognized, a situation has to be interpreted either as being novel, unpredictable, or an individual must feel as if they lack control over a situation (Mason, 1968). Effects of stress on humans can be quite detrimental. Stress can lead to stunted growth, disease, cognitive disruption, mental health problems and ultimately an increased mortality rate (Hostinar & Gunnar, 2013). Once a situation has been recognized as stressful by the brain, certain mechanisms are triggered in response. Lupien, Maheu, Tu, Fiocco and Schramek (2007) explain that these mechanisms result in the release of glucocorticoids from the adrenal cortex. In humans the main glucocorticoid released is cortisol. The release of cortisol is often beneficial in the short term to enable coping. However, chronic cortisol elevation can be detrimental.

Cortisol impacts several regions within the brain. Specifically, the hippocampus, amygdala, and frontal lobe all contain a high number of glucocorticoid receptors. These regions are important for learning and memory (Lupien et al., 2007). Respectively, the hippocampus plays a role in declarative and spatial memory, the amygdala plays a role in fear processing as well as in processing emotional memories, and the frontal lobe plays a role in working memory (Scoville & Milner, 1957; Lupien et al., 2007; Young, Sahakian, Robbins & Cowen, 1999). Lupien et al. (2007) identified that stressed individuals were associated with having smaller hippocampus volume. Interestingly, individuals diagnosed with mental health disorders have been found to have smaller hippocampal volumes in comparison to the average person (Fuchs, 2007). Evidently, stress can have adverse effects on both behavior and brain structure.

In contrast to humans, the main glucocorticoid released in many other animals is corticosterone (Lupien et al., 2007). Non-human animals react to a variety of stressors in the environment, including those that reflect the quality, supply and availability of nutrition

(Honarmand, Thompson, Schatton, Kipper, & Scharff, 2015). Similar to humans, high levels of glucocorticoids can have profound effects on other species. In a study that induced stress among songbirds, Buchanan, Leitner, Spencer, Goldsmith, and Catchpole (2004) found that song complexity was reduced. Furthermore, several brain regions were reduced in size as a result of elevated corticosterone levels. Evidently, excessive stress affects humans and other species in similar ways.

Songbirds have been used in research to observe the effects corticosterone has on the brain. Specifically, zebra finches are often used because of the similarities between song learning and human speech development. Doupe & Kuhl (1999) explain that both humans and songbirds have a critical period in which they must learn vocalization. In early stages of life, both species rely heavily on adults to first observe than mimic vocalization. This leads to initial vocalization being very basic, which gradually overtime becomes more complex. In both groups, learning is guided by innate predispositions that allow for learning to occur (Coene, Schuawers, Gillis, Rooryck & Govaerts, 2011; Glaze & Troyer, 2013). These innate predispositions are located in specific regions within the brain. However, one primary difference between humans and zebra finches is that only male zebra finches develop specific neural mechanisms in relation to vocalization. Additionally, the length of time it takes for the brain to become fully functional is much quicker among zebra finches in comparison to other species such as humans. One example of this is how zebra finches develop neural mechanisms responsible for vocalization much quicker than humans (Janta & Margoliash, 1999).

Among zebra finches the sensitive period generally lasts for 90 days which can be split into two phases. The first phase of song learning is the sensory phase. The sensory phase lasts from approximately day 25 to day 65 (Roper & Zann, 2006). The second phase of learning is the

sensorimotor phase. The sensorimotor phase overlaps with the sensory phase and lasts from approximately day 30 to day 90 (Janta & Margoliash, 1999). These two phases are responsible for the rapid growth of the brain. These two phases are a period when neurogenesis is extremely important. Neurogenesis is a time in which nervous tissue develops and important regions in the brain begin to expand and differentiate (Pytte et al., 2012). The process of neurogenesis has multiple steps that include but are not limited to the birth of neurons and their journey into existing circuitry (Barnea & Pravosudov, 2011). Specifically, nearly 18,000 neurons are formed between post hatch day (PHD) 20 and 64 (Nordeen & Nordeen, 1988). Once the sensory and sensorimotor phases are completed all learning becomes crystalized. Zebra finches maintain a specific song throughout their lifetime which males will use to attract a mate throughout the reproductive period. Although there are many different types of cells in the brain, for this study, round cells and fusiform cells will be identified. Round cells are those that have finished migrating and have begun the process of differentiation, in contrast, fusiform cells have not yet begun differentiation as they are still in the process of migrating (Alward, Madison, Parker, Balthazart and Ball, 2016).

Throughout the 90-day sensitive period crucial neural song mechanisms are developed among male zebra finches. Of particular importance is the motor pathway, which consists of the HVC (proper name) and the robust nucleus of the arcopallium (RA). These brain structures are responsible for both learning song in the sensory and sensorimotor phases, as well as production of song in the sensorimotor phase (Vu, Mazurek & Kuo, 1994; Yu & Margoliash, 1996). Rapid growth of the HVC throughout the sensitive period suggests that the HVC is responsible for both learning and producing song (Buchanan et al., 2004). Bottjer, Miesner and Arnold (1986) explain that from PHD 12 to 35 the HVC volume doubles in size. Many of these neurons in the HVC

project towards the RA. The pathway between HVC and RA is thought to sustain song production. Interestingly, this pathway is almost entirely shaped by song learning in the sensory and sensorimotor stages (Sohrabji, Nordeen & Nordeen, 1993). The 90-day sensitive period is a time when zebra finches are most affected by stress. Stress experienced during the sensitive period can lead to reduced song length, complexity (Spencer, Buchanan, Goldsmith & Catchpole, 2003), and HVC volume (Nowicki, Peters & Podos, 1998; Buchanen et al., 2004; Honarmand et al., 2015).

The *Developmental Stress Hypothesis* posits that features related to song may be honest indicators of quality among males. These features of song may reflect the stress experienced in early development. In early development, many different physiological systems can be simultaneously altered by stress (Spencer & MacDougall-Shackleton, 2011). Among zebra finches, early stress is often induced by food restriction or corticosterone treatment. This early stress affects song complexity in adulthood (Spencer et al., 2003). Song complexity is related to the quality within a species because it can indicate the amount of stressors in the early environment. Additionally, song complexity is also related to how an individual coped with stressors in the early environment. Stress experienced throughout any stage of development is likely to contribute to differences in the adult phenotype (Spencer & MacDougall-Shackleton, 2011). Why stress can lead to significant neural differences among songbirds is yet unknown, though it is known that developmental stress can directly influence brain structures in both size and density (Buchanan et al., 2004; Honarmand et al., 2015).

Spencer et al. (2003) conducted a study to assess the impact of developmental stress on birds. Broods were randomly assigned to parents to ensure any effects had a purely environmental cause. In the study adult zebra finches were bred into either a control condition or

one of two treatment conditions. The treatment conditions consisted of stress induced by either food restrictions or corticosterone treatment. In contrast, the control condition was raised in an environment absent of any stressors. Birds were treated from PHD five to 35, upon which any stressors were removed from treatment conditions. Song was than measured after crystallization occurred (anywhere from PHD 100 to 200). Compared to treatment birds, control birds were significantly heavier by day 18. Interestingly, blood work revealed that birds in the food restriction condition did not have significantly elevated corticosterone levels; although they experienced similar song defects. Both treatment conditions had a significant effect on the length of song. However, no significant difference was found between the two conditions. Furthermore, the number of syllables and peak frequency of song were also affected. These findings provided support for the Developmental Stress Hypothesis as they revealed that stress experienced in the environment as well as elevated glucocorticoid levels can have an impact on song. However, one limitation of this paper was that researchers failed to address the impact either treatment conditions had on structures such as the hippocampus or the HVC.

Buchanan et al. (2004) conducted a study to assess the impact developmental stress has on the brain. Clutches were randomly assigned to parents to ensure any effects had a purely environmental cause. In the study adult zebra finches were bred into either a control condition or one of two treatment conditions. The treatment conditions consisted of stress induced by either food restrictions during feeding or corticosterone treatment to chicks. Birds were treated from PHD five to 35, upon which any stressors were removed from treatment conditions. From PHD 35 to 60 the offspring were then separated from parents by wire mesh (offspring could still see and hear parents). Birds were then transferred into a sex specific aviary until they were sacrificed around PHD 215. Researchers found that foster siblings raised by foster parents were

significantly more likely to be similar to one another in certain aspects. The HVC and RA had fewer differences in comparison to individuals in other nests. This similarity led to the idea that early environment had a strong influence on brain development. Specifically, HVC size was found to be significantly smaller for birds who experienced stress in early development. These findings provide an explanation for the limitations in the Spencer et al. (2003) study. While Spencer et al. (2003) found that developmental stressors may affect song; Buchanan et al. (2004) provided direct insight as to what led to these differences. These findings reveal that a variety of stressors may lead to changes in HVC. Furthermore, that HVC is particularly likely to be affected by stress in comparison to other brain areas. However, one limitation of this paper was that researchers were unable to explain how changes in the HVC occurred.

Honarmand et al. (2015) conducted a study to assess whether developmental stress has an impact on neurogenesis within HVC. In the study adult zebra finches were bred into either a control condition or a treatment condition. The control condition consisted of birds being fed high quality food. In contrast, the treatment condition consisted of babies being fed low quality food. The young birds were injected with a marker on two separate occasions. Initially, fledglings were injected daily from PHD 14 to 18. For this injection BrdU was used, in order to identify how many neurons were recruited to the HVC following birth at the fledging stage. This was followed up by an injection at PHD 35. For this injection EdU was used, in order to assess how many cells were being divided in the ventricular zone. After the final injection birds were then sacrificed. Researchers found that treatment had a negative effect on neurogenesis. Specifically, at PHD 35 the HVCs of males in the low quality condition contained significantly fewer neurons born in the fledging stage. The fledging stage was also a period when the birds in the treatment condition had a lower weight in comparison to control birds. However, the

influence of the treatment condition on HVC neurons born from day 14 to 18 was found to be short lasting. By PHD 35 the region of the ventricular zone that supplied neurons to the HVC showed no statistical differences between the two conditions. These findings provide a partial explanation for the limitations in the Buchanan et al. (2004) study. While Buchanan et al. (2004) found that developmental stressors may affect HVC size; Honarmand et al. (2015) specifically address at which the sensitive period the HVC is affected. However, one limitation of this paper was that researchers were unable to fully explain how stressors affect the different stages of neurogenesis.

Past research has identified that stressors may affect song (Spencer et al., 2003) and HVC size (Buchanan et al., 2004). However, other than Honarmand et al. (2015), there has been no research completed so far as to understand what causes the HVC to be smaller. Current gaps exist in the literature in regards to which stage of neurogenesis in the HVC is most vulnerable to corticosterone. Additionally, no research to date has yet been completed that examines the relationship between developmental stress and hippocampus size among zebra finches. This is an important gap to consider because explaining the biological mechanisms that result in a reduced hippocampus and HVC size can specifically highlight how stressors affect the brain. Although the HVC is the primary area of focus for this study, the hippocampus will be present in many of the sections containing the HVC and will therefore also be analyzed.

This study seeks to examine the effects of corticosterone on cell migration. The study uses oral corticosterone administration to induce early developmental stress. The hypothesis for this study is that zebra finches exposed to corticosterone treatment in early periods of the developmental stage will have a smaller hippocampus and HVC in comparison to control birds. Specifically, there will be fewer cells throughout the hippocampus and HVC during the

sensorimotor stage due to apoptosis. Previously observed effects of stress on HVC result from apoptosis rather than reduced migration. 16 zebra finches experienced either a control or a treatment condition. In the control condition eight zebra finches were raised by parents with no interruption. In the first treatment condition four birds were exposed to a vehicle (peanut oil). In the second treatment condition four birds were exposed to the vehicle mixed with 0.20 mg of corticosterone. The effects of corticosterone on neurogenesis throughout the sensorimotor stage were examined by analyzing cell counts in the hippocampus and HVC under a microscope.

Materials and Methods

Subjects and Housing

In this experiment sixteen zebra finches were used. The zebra finches were bred at the Advanced Facility for Avian Research Western University from October 2015 to February 2016. To begin the breeding process, 12 males and 12 females were selected and housed in individual cages. Each cage received beta chips, grit, a cuttlebone, water, food and egg food. Additionally, each cage was supplied with a plastic nesting box, cotton, wool, straw and paper for nest building. The room containing the birds maintained a 14:10 hour light:dark cycle and the average room temperature was 24ºC.

Experimental Design

A between-subjects design was used for this experiment. The independent variable consisted of one treatment and two control groups. Once the first chick in a cage hatched the entire nest was assigned to one of the conditions. The chicks in the treatment condition were pipetted peanut oil infused with corticosterone into their open beaks. In comparison, the two control groups consisted of a peanut oil group and an undisturbed group. In the peanut oil group, chicks were pipetted with the same amount of liquid as the treatment group to control for

possible stress experienced during the interaction. The other control group consisted of absolutely no interaction with chick to counter any possible effects of contact. The dependent variable was how many cells migrated from the ventricular zone to the hippocampus and HVC during the sensorimotor stage.

Experimental Treatment

The zebra finches in each cage typically laid an egg each day and after an average of eight eggs began to incubate. Before the experiment began a fellow researcher mixed 270 Ml 70 mL of 0.2 mg/mL corticosterone solution. This was done by dissolving 53.2 mg of corticosterone in 5 mL of acetone, which was then added to 266 mL of peanut oil. The combined solution was then stirred until the acetone evaporated. Both the corticosterone and the peanut oil conditions began ten days after the chick hatched. The corticosterone condition consisted of 50 µL of 0.2 mg/mL corticosterone being pipetted into the open beak to chicks for a period 20 days. The peanut oil condition consisted of 50 µL of peanut oil being pipetted into the open beak for a period of 20 days. In contrast, the no treatment condition was left alone for 30 days.

Perfusion and Brain Removal

On day thirty, the zebra finches were sacrificed using isoflurane inhalation. Upon sacrifice birds were drained of blood using PBS, followed by 4% phosphate-buffered paraformaldehyde to stiffen brain tissue. The brains were then removed, and each chick was dissected in order to identify sex by observing the testes. After the brains were removed the fixation process began by storing them in 4% phosphate-buffered paraformaldehyde for 24 hours and then transferring them to sucrose for 48 hours. The brains were then dried and frozen using dry ice. They were kept in a -80ºC freezer until every brain was collected. Once all brains were collected they were then individually fixated and sliced in the coronal plane at 40 µm thickness.

Immunohistochemistry

To analyze the impact conditions had on cell migration throughout the sensorimotor stage, immunohistochemistry was performed on all brain sections. Doublecortin (DCX) was used to identify migrating cells in the hippocampus and HVC. To begin this process, floating sections were washed two times for five minutes in 0.1M PBS, pH 7.5. Endogenous peroxidases were blocked using 0.5% H_2O_2 in PBS for 15 minutes, which was followed by the sections being washed three times for five minutes in 0.1M PBS, pH 7.5. Sections were then incubated in 10% normal horse serum (NHS) in 0.3% PBS/T for one hour. Afterwards, sections were then drained of NHS and incubated with stock DCX (C-18) at 1:250 in 0.3% PBS/T for a period of 24 hours at 4C. The following day sections were washed two times for five minutes in 0.1% PBS/T, which was followed by a one-hour incubation with biotinylated Horse Anti-Goat IgG, 1:400 in 0.3% PBS/T. Afterwards, sections were washed three times for five minutes in 0.1% PBS/T which was followed by a one-hour incubation with avadin-biotin horseratdish-peroxidase complex (Vectastain ABC, Elite Kit) 1:200 in 0.3% PBS/T. Sections were then washed three times for five minutes in 0.1% PBS/T and visualized using 15ml diamniobenzidine (SIGMAFAST DAB, Sigma-Aldrich) for one minute, upon which sections were immediately washed in PBS. Sections were then mounted on HistoBond slides and were serially dried in ethanol and xylene for five minutes before being cover slipped using Permount.

Microscopy and Statistical Analysis

Once immunohistochemistry was completed for DCX brains were then placed under the microscope for analysis. Pictures were taken under 20x zoom with a computer software program (Scope). Five pictures of the hippocampus and five pictures of the HVC were taken for every brain. Once all photos were taken, round and fusiform cells were counted (Figure 1.).

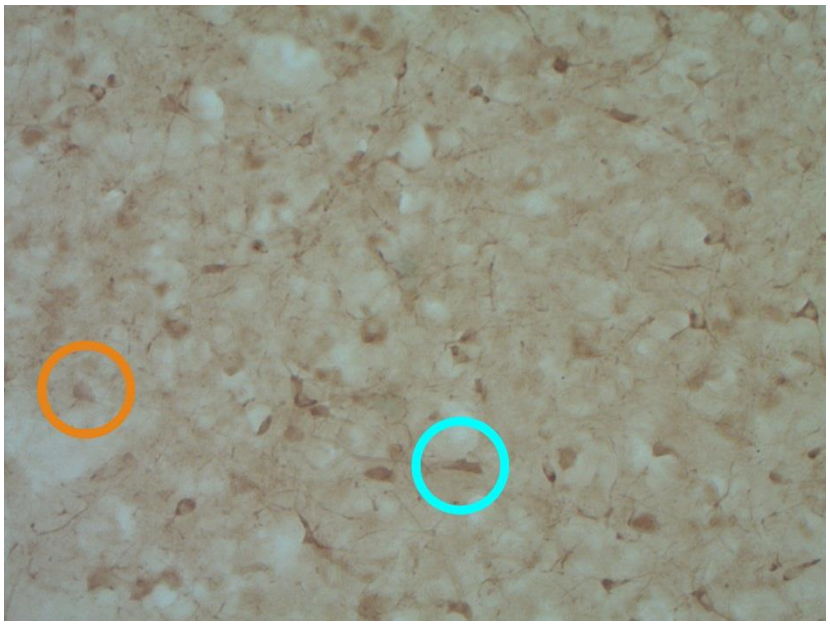

Figure 1. A section of the HVC. The cyan circle is highlighting a fusiform cell meanwhile the orange circle is highlighting a round cell.

Round cells were used because they are new neurons that have migrated to their final resting site. These neurons are stable and have begun the process of differentiation. In contrast, fusiform cells were used because they were still migrating. These neurons have not yet begun the process of differentiation. Once all cells were counted round cells and fusiform cells were added together to produce a total cell count. Round, fusiform, and total cell counts were then all averaged for each brain. These numbers were then analyzed with a one-way ANOVA and Tukey's post hoc using SPSS.

Results

A one-way between subject's ANOVA was conducted to compare the effect of developmental stress on cell counts in the control, vehicle, and corticosterone conditions. For each condition total, round, and fusiform cell averages were analyzed for both the hippocampus and HVC. No significant differences were found in the amount of total cells in hippocampus at the $p<.05$ level across the three conditions $F(2,16)=0.86$, $p=0.446$ (Figure 2.).

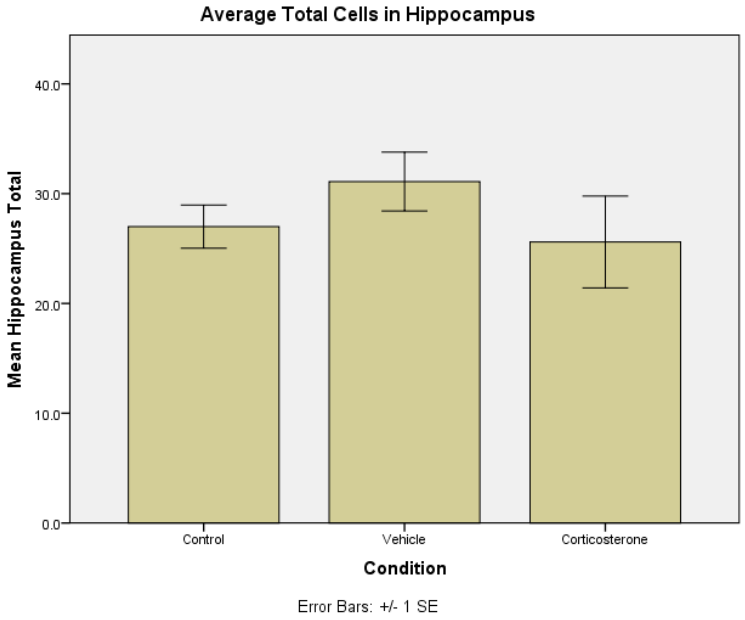

Figure 2. The average number of total cells (fusiform + round) across the three conditions (mean + SE), n=16.

No significant differences were found in the amount of round cells in hippocampus at the $p<.05$ level across the three conditions $F(2,16)=0.93$, $p=0.421$ (Figure 3.).

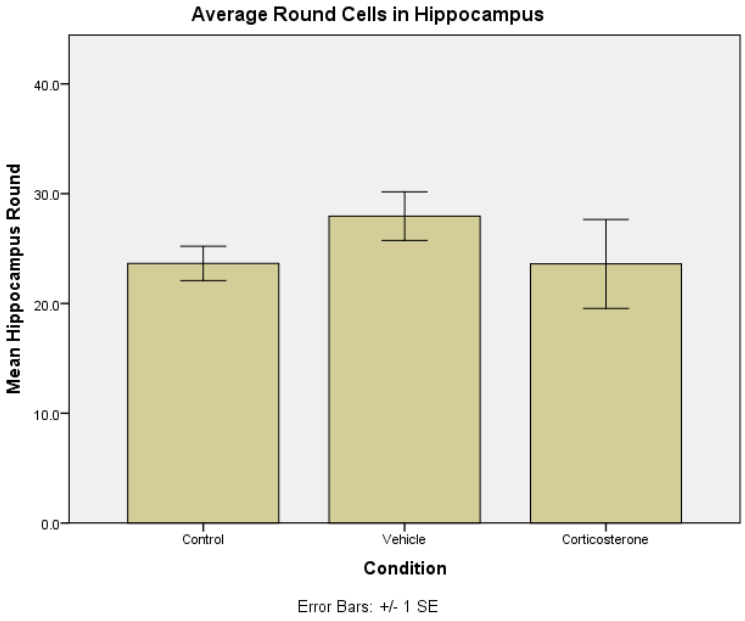

Figure 3. The average number of round cells across the three conditions (mean + SE), n=16.

No significant differences were found in the amount of fusiform cells in hippocampus at the $p<.05$ level across the three conditions $F(2,16)=0.65$, $p=0.537$ (Figure 4.).

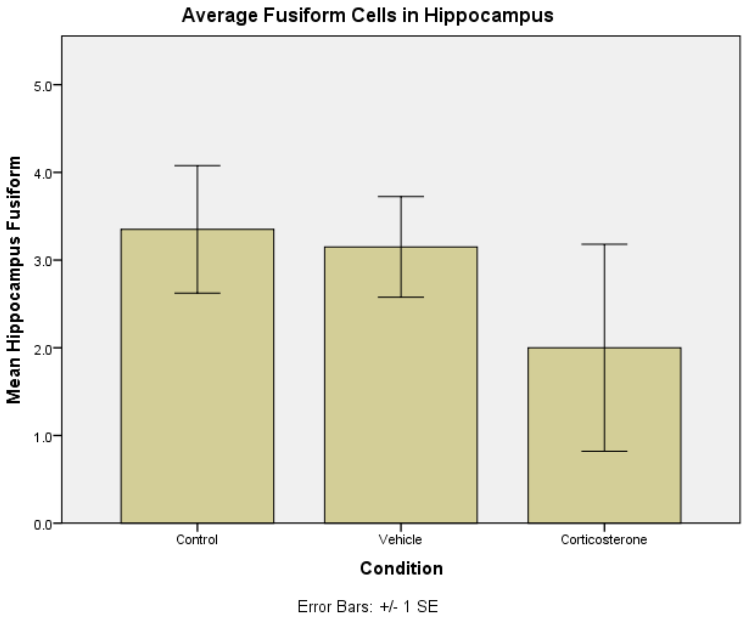

Figure 4. The average number of fusiform cells across the three conditions (mean + SE), n=16.

A significant difference was found in the amount of total cells in HVC at the $p<.05$ level across the three conditions $F(2,16)=4.94$, $p=0.025$ (Figure 5.).

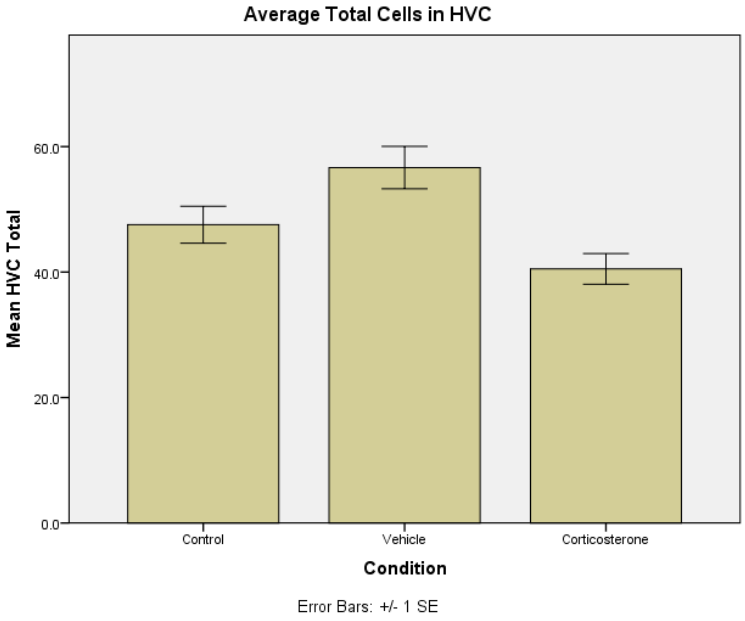

Figure 5. The average number of total cells across the three conditions (mean + SE), n=16.

Post hoc comparisons using the Tukey HSD test indicated that the mean score for the vehicle condition ($M=56.65$, $SD=6.76$) was significantly different than the corticosterone condition

(*M*=40.50, *SD*=4.89), *t*(16)=3.13, *p*= 0.020. Specifically, birds in the vehicle condition had on average a higher number of total cells in the HVC when compared to the corticosterone condition. However, the control condition (*M*=47.55, *SD*=8.31) did not significantly differ from the vehicle *t*(16)=2.04, *p*=0.143 and corticosterone *t*(16)=1.58, *p*=0.290 conditions. No significant differences were found in the amount of total cells in HVC at the p<.05 level across the three conditions when sex was included in the ANOVA *F*(2,16)=1.70, *p*=0.22. No significant differences were found in the amount of round cells in HVC at the p<.05 level across the three conditions *F*(2,16)=3.43, *p*=0.064 (Figure 6.).

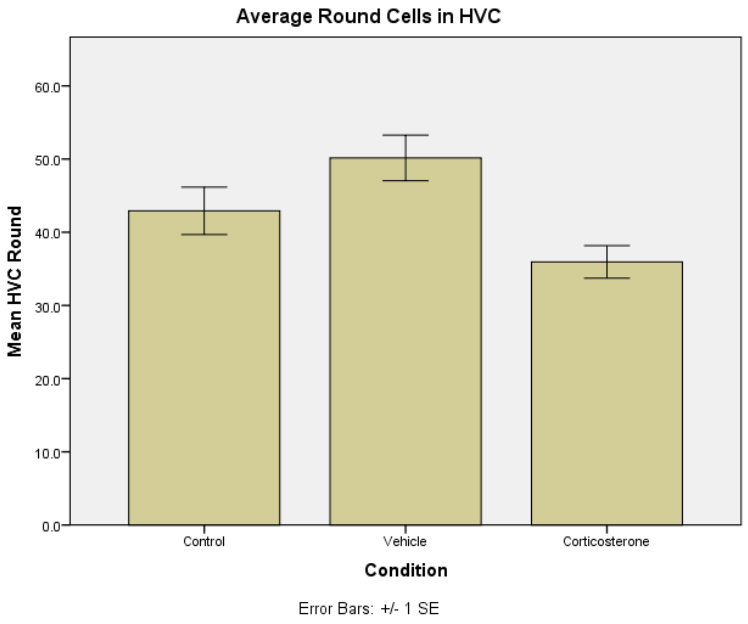

Figure 6. The average number of fusiform cells across the three conditions (mean + SE), n=16.

However, since the results revealed marginally significant findings, a post hoc comparison using the Tukey HSD test was run which indicated that the mean score for the vehicle condition (*M*=50.15, *SD*=6.22) was marginally significantly different than the corticosterone condition (*M*=35.95, *SD*=4.44), *t*(16)=2.62, *p*= 0.520. Specifically, birds in the vehicle condition had on average a higher number of total cells in the HVC when compared to the corticosterone condition. However, the control condition (*M*=42.93, *SD*=9.18) did not significantly differ from

the vehicle $t(16)=1.54$, $p=0.306$ and corticosterone $t(16)=1.48$, $p=0.330$ conditions. No significant differences were found in the amount of fusiform cells in HVC at the $p<.05$ level across the three conditions $F(2,16)=1.75$, $p=0.213$ (Figure 7.).

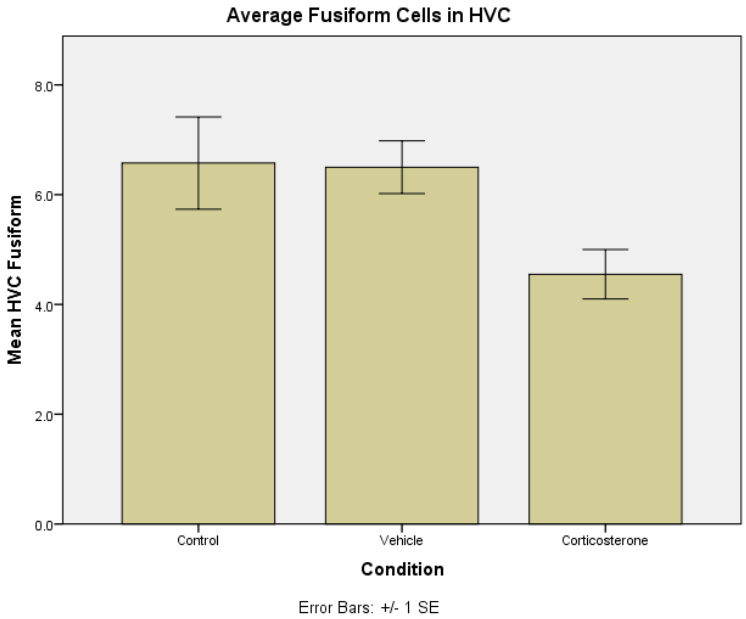

Figure 7. The average number of fusiform cells across the three conditions (mean + SE), n=16.

Discussion

The hypothesis that corticosterone administration during early development will result in fewer cells in the hippocampus was not supported by the results of this study. Results from the study demonstrate that the three conditions did not contribute to a statistically significant difference in the number of total, round, and fusiform cells in the hippocampus. The hypothesis that corticosterone will result in fewer cells in the HVC was partially supported by the results of this study. A statistically significant difference was found between the three conditions in the total count cells in HVC, with further analyses revealing that the difference existed in the average number of cells between the vehicle and corticosterone conditions. Furthermore, a marginally statistically significant difference was found between the three conditions in the round cell count in the HVC, with further analyses revealing that the difference exited in the

average number of cells between the vehicle and corticosterone conditions. No statistically significant difference was found between the three conditions in fusiform cell count in the HVC. However, when further analyses including sex were run, total and round cell counts in the HVC were no longer significantly different. This suggests that sex may have been an influencing factor rather than the condition experienced.

Based on the current literature there is a gap in terms of how developmental stress affects the size and growth of the hippocampus among zebra finches. In contrast, past research identifies that developmental stress is detrimental to the size and growth of HVC among zebra finches (Spencer et al., 2003; Buchanan et al., 2004; Honarmand et al., 2015). This study was able to replicate and explain findings by Honarmand et al. (2015) because a difference between conditions only existed between the total numbers of cells. Furthermore, the difference existed between two treatment groups rather than a control and a treatment group. This study was hoping to achieve a statistically significant difference between the control and corticosterone conditions in the number of fusiform cells in the HVC. This would have identified that developmental stress does in fact limit the migration of cells in the sensorimotor stage. Although these results were not found to be statistically significant, a future study which addresses some of the limitations of the current study may still find these results.

One explanation for why no statistically significant differences were found between the control and corticosterone conditions was that the birds were sacrificed at PHD 30. In the Honarmand et al. (2015) study the researchers observed that HVC size and volume was statistically different between birds at PHD 17. However, these results were not replicated at PHD 35 between their conditions. Specifically, at PHD 17 control and treatment conditions had statistically different volumes of HVC, however, by PHD 35 these differences disappeared and

were no longer statistically significant. Although Kim and Devoogd (1989) suggest that neurogenesis rapidly develops in the first 30 days post hatch, perhaps birds who are developmentally stressed redirect growth to specific brain structures rather than physical structures. This may explain why developmentally stressed birds were found to have similar HVC volumes upon sacrifice. Furthermore, Honarmand et al. (2015) describe that effects of developmental stress are long lasting and may be expressed later on in life, which may also be an explanation for why this current study only found partially statistically significant results.

Although Area X was not one of the sections being examined in this immediate study, several sections contained this brain region. Wada, Newman, Hall, Soma and MacDougall-Shackleton (2014) conducted a study on adult sparrows where they found very few cells in Area X. The sections were also studied under the microscope and were found to contain a very high concentration of both round and fusiform cells (Figure 8.) in comparison to areas such as the HVC (Figure 9.) or the hippocampus (Figure 10.).

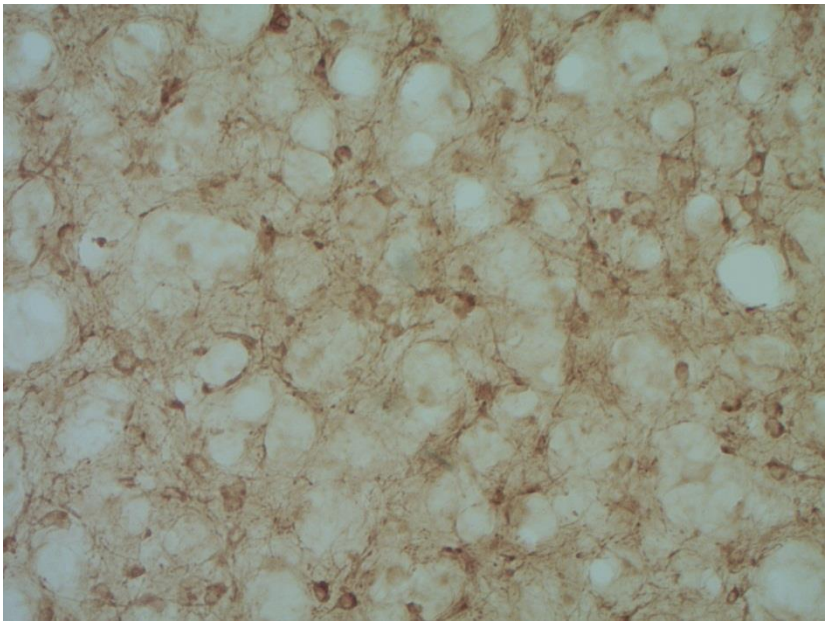

Figure 8. Cells in Area X.

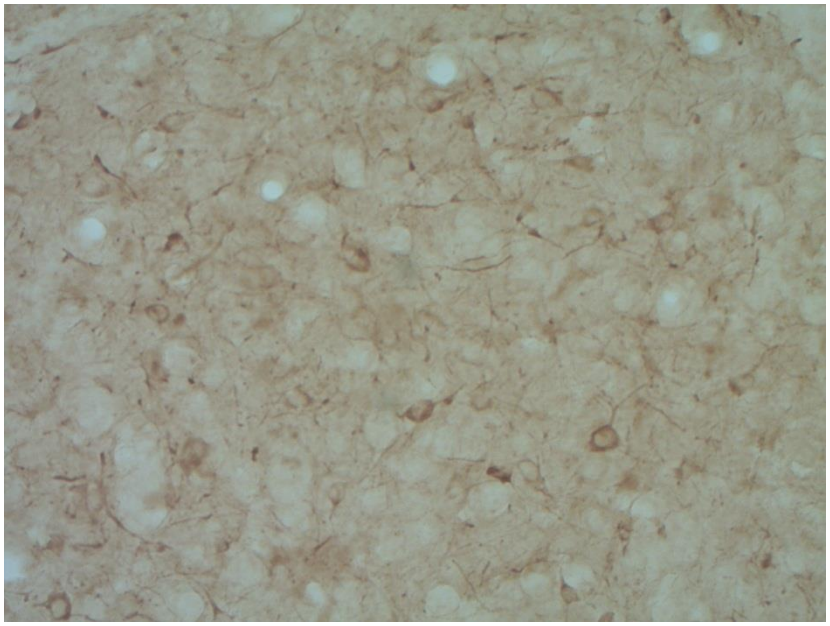

Figure 9. Cells in the HVC.

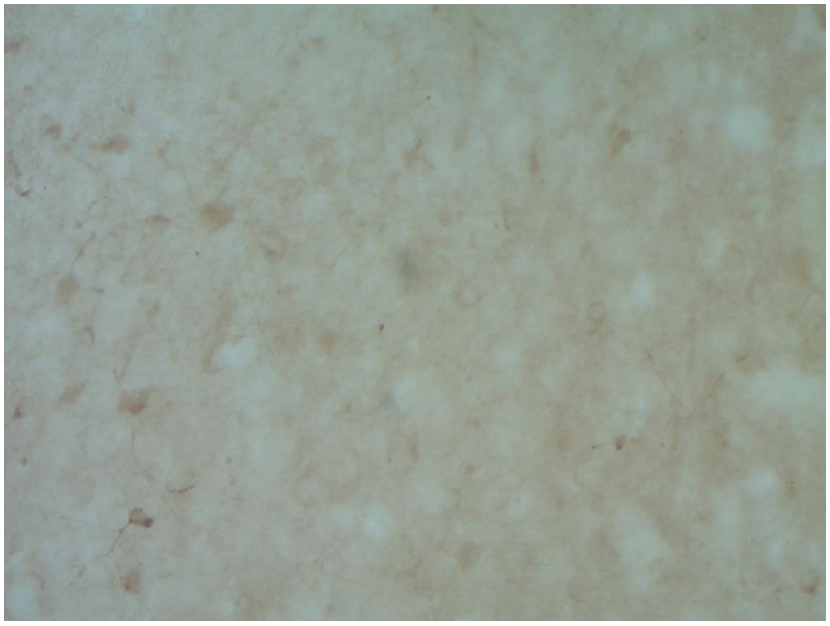

Figure 10. Cells in the hippocampus.

These findings are particularly interesting as it may suggest that neurogenesis occurs around 30 PHD among zebra finches. On the other hand, differences could also exist between sparrows and zebra finches in regards to how many cells project to Area X. Unfortunately, only two of the

sections contained Area X so there was not enough material to run an analysis between the cells in different conditions.

Limitations

In many experiments zebra finches have generally been bred before and have experience raising young. However, in this experiment, many of the birds were first time parents. Unfortunately, this may have been an additional developmental stress to chicks in every condition. Several birds from each condition were found with bumps on their skulls which suggests that their parents picked on them. Furthermore, another issue which may have affected the study was the small sample size. The control condition consisted of eight birds, meanwhile the vehicle and corticosterone condition consisted of four birds each. These small groups also contained uneven numbers of males and females. Specifically, the control group contained six males and two females, meanwhile the vehicle and corticosterone group contained three females and one male each. Due to the nature of the study, sex of the bird was left to complete chance as in almost every case sex is not identifiable until about PHD 35, meanwhile, the birds in this study were sacrificed PHD 30. Other studies strictly concentrate on male birds when studying areas such as the HVC, and they control for this issue by having large sample sizes insure an adequate number of males are born.

Future Research

Future research should focus on breeding much larger populations of chicks to ensure an adequate number of males are born. Furthermore, rather than picking a specific date in the sensorimotor phase to sacrifice, several points throughout the sensorimotor should be picked a few days apart to examine the differences in the number of cells migrating day by day. Since neurogenesis develops most rapidly in the first 30 PHD (Kim & Devoogd, 1989), it is

recommended that several days are selected before (such as 15, 20, 25, and 30) to directly measure how neurons migrate to areas such as the HVC. It is also strongly recommended that more than one developmental stressor be introduced, such as food restriction or the presence of a predator in a nearby cage. Additionally, future studies should observe how Area X differentiates between conditions throughout neurogenesis. Therefore, it would be opportune to slice several sections containing Area X and analyze the cell counts between conditions.

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