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THE EFFECTS OF VISUAL COLOR STIMULI ON ZEBRA FINCH BEHAVIOR AND  
STRESS RESPONSE

An Honors Thesis

Submitted in Partial Fulfillment of the  
Requirements for Graduation with  
Undergraduate Research Honors

Georgia State University

2014

by

Bao Chau Ly

Committee:



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Dr. Laura L. Carruth, Honors Thesis Director

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Dr. Sarah Cook, Honors College Associate Dean

December 16, 2014

Date

THE EFFECTS OF VISUAL COLOR STIMULI ON ZEBRA FINCH BEHAVIOR AND  
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by

BAO CHAU LY

Under the Direction of Laura L. Carruth, Ph.D

ABSTRACT

The Australian Zebra Finch, *Taeniopygia guttata*, is a common vertebrate model for understanding behavioral, neurological, and physiological changes across the life span. The goal of this study was to determine if color in the environment can act as a stimulus and activate the zebra finch stress response. Zebra finches are diurnal and have color vision. Their plumage coloration is sexually dimorphic and they show behavioral changes to color; females prefer males with redder beaks, and both sexes show individual color preferences for materials in nest building. This experiment was conducted to test whether or not a novel color in the environment can elicit a stress response. A colored poster board was introduced to the adult zebra finches' habitat, and behavioral changes were measured immediately and then again after twenty four hours. In addition, plasma corticosterone (CORT), the main avian stress hormone, concentrations were measured twenty four hours after introduction of the color stimulus. The introduction of the color stimuli resulted in immediate behavioral changes in the birds and increased activity was observed with the addition of green, blue, and red stimuli and decreased activity with the addition of yellow. However, after twenty four hours there were no changes in behavior or plasma CORT levels for any of the colors. These findings suggest that zebra finches

show varied behavioral responses to novel stimuli based on color differences and that these changes are temporary.

INDEX WORDS: Zebra Finch, Color Stimuli, Visual Stressor, Behavioral Change, Corticosterone, CORT

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An Honors Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of

Bachelors of Biological Sciences  
in the College of Arts and Sciences  
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Georgia State University  
August 2013

## DEDICATION

I dedicate the thesis to my parents, Man and Linh Ly, for having been an exemplary support system in my academic career, and as a result, helped me realized my predilection for science.



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## TABLE OF CONTENTS

DEDICATION	vii
ACKNOWLEDGEMENTS	viii
LIST OF TABLES	x
CHAPTER	
1. INTRODUCTION	1
2. METHODS	5
Animal Subjects	5
Color Exposure	5
Behavioral Observations	6
Plasma CORT Concentration	6
Radioimmunoassay	6
Statistical Analysis	7
3. RESULTS	8
CORT Assay	8

Behavioral data	9
4. DISCUSSION	
5. DISCUSSION	10
6. CONCLUSIONS	13
7. FUTURE DIRECTIVES	14
REFERENCES	17

## LIST OF TABLES

Table 1. Plasma CORT levels before the addition of colored poster boards and 24 hours after the addition of colored poster boards.....	8
Table 2. Qualitative descriptions of posture and activity after the addition of color and control stimuli to each cage.....	10

## INTRODUCTION

Australian zebra finches (*Taeniopygia guttata*) are highly social birds endemic to the grasslands of Australia (Zann, 1996). Due to their sociability, accessibility, domestication, and ease of maintenance, they are a common song bird. As with all birds, color is an important visual cue for social interactions (Etman et al., 2001). This study was designed to test whether or not a novel color in the environment can elicit a stress response as measured by changes in behavior and increase in plasma stress hormone levels.

Zebra finches are commonly used in studies that examine changes in physiological functioning, such as those associated with acute (short-term as measured in seconds to minutes) and chronic stress or long-term stress (Griffith and Buchanan, 2010; Perfito, 2010). The two main adrenal stress hormones, or glucocorticoids, corticosterone (CORT) and cortisol, are secreted in response to stress. Corticosterone is the main stress hormone in birds and rodents while cortisol is the primary stress hormone in primates and fishes (Palme et al., 2005). When an animal is stressed (either by a negative or positive stressor) the hypothalamic-pituitary-adrenocortical (HPA) feedback system is activated, stimulating the hypothalamus to release corticotropin releasing hormone (CRH), which acts on the anterior pituitary gland to release adrenocorticotropic hormone (ACTH), signaling the adrenal cortex to synthesize and secrete glucocorticoids (Dallman et al., 2004; Malisch et al., 2007). For zebra finches, stressors can include environmental stimuli, such as predator exposure, food shortage, social isolation, and physical stressors, such as human handling or temperature changes (Eraud et al., 2007; McGraw et al., 2011). Circulating CORT increases gluconeogenesis or the availability of circulating glucose as an energy source, lowers metabolic rate, and increases peripheral blood flow (Delaunay et al., 1997; Zimmerman et al., 1989). However, continual long term exposure to

elevated circulating plasma CORT levels may have deleterious chronic effects on the physiology homeostasis of an organism. These effects include prolonged mobilization of energetic resources causing growth suppression, continual breakdown of proteins resulting in muscle wastage, changes in physical features due to immunocompetence, and impaired cognitive and behavioral functions due to neuronal death (Axelrod and Reisine, 1984; Bremner, 1999; Johnson et al., 1992).

Zebra finches are a sexually dimorphic species, with the sexes differing in two distinct ways, behaviorally and in their plumage and bill coloration. Males have redder bills, black and white-striped collar feathers and orange cheek patches, while female plumage is typically muted brown and their beaks are orange. In addition, males sing a courtship song that females cannot sing and they learn this song from a male tutor, usually their father (Deregnaucourt and Gahr, 2013). Zebra finches have a complex process of mate selection that is driven by female mate choice. Females prefer males that sing a more complex song and who have redder bills, (Griffith and Buchanan, 2010). Zebra finches are also a diurnal species and have excellent color vision that allows them to assess the coloration of their mates as well as the environment (Bowmaker et al., 1997; Maddocks et al., 2001). They also have been shown to display behavioral changes based on color (Bolund et al., 2007; Hunt et al., 1997). When male zebra finches are exposed to the steroid hormone testosterone for short periods, their bills become redder and they exhibit higher levels of aggression and become more dominant (Ardia et al., 2010).

There are many studies that have examined the influence of color on animal behavior and physiology and various colors and hues may have significant effects on the growth and development of vertebrates. For example, yellow light lowers the stress-induced cortisol response in rainbow trout (*Oncorhynchus mykiss*), and fish reared under yellow light showed a

decreased stress response (Heydarnejad et al., 2013). A different species of fish, the Nile tilapia was reported to prefer yellow light as well, however after seven days of confinement, stressed fish demonstrated a decrease in ventilation frequency (the higher the frequency, the more stressed the fish is) when exposed to blue light (Maia and Volpato, 2013). In humans environment color also plays a significant role in stress perception. Research by Kutchma and colleagues (2003) demonstrated that when test subjects were kept in red rooms, they reported a higher stress perception than subjects in white or green rooms.

As with all vertebrates, the zebra finch HPA axis is highly evolutionarily conserved and can be experimentally manipulated (Shahbazi et al., 2011). Although there have been no published studies on how and if environmental colors can act as a stressor for birds, zebra finches are well documented to show complex color mediated behavior. Females prefer male conspecifics wearing red leg bands to orange leg bands, and disfavored males with light green leg bands (Burley et al., 1982); this color preference was found to be inhibited by an ultraviolet light filter (Hunt et al., 1997). Zebra finches also show color preferences which vary between individuals—for nest building, Sargent found that birds preferred brown over green (Sargent, 1965) while later studies showed preferences for green to brown. Individual color preferences for nest building materials vary; some prefer brown, while others prefer green or blue (Muth et al., 2013).

Since zebra finches have been demonstrated to show robust behavioral changes in response to color, it is important to understand if the colors can be a stressor. Different environmental conditions may cause activation of the HPA axis and stress response. Furthermore, the use of color in housing conditions may also be used to facilitate the stress axis activation, providing a more physiologically relevant alternative to current methodologies.

For this study, we recorded changes in behavior and plasma CORT in zebra finches after exposure to different colors in the environment for 24 hours. We found that there were no significant changes in behavior or CORT plasma concentrations before or after the introduction of the color stimulus.



## MATERIALS and METHODS

### **Animal Subjects:**

For this study, 5 male and 5 female zebra finches were used. Prior to starting the experiment subjects were housed in large flight cages (60 3/8" W x 30 3/8" D x 78 1/4"H) with multiple families and mixed age cohorts and then transferred to stackable cages (38 5/16"W x 20 3/8"D x 20"H) and separated upon sex for the duration of the study. All subjects had identifiable color band that were of no specific designation. Red bands were avoided. All subjects were adults of 12-24 months of age. All of the birds were healthy; however, female Brown 42 had an on-going eye infection that was continuously treated with veterinarian-grade tropical antibiotic. Birds were kept under controlled environmental conditions (temperature 21.1–23.8 °C, humidity 50–65%, constant light schedule of 12L: 12D, lights on at 08:00 with standard fluorescent lighting) at the Georgia State University animal care facility in the Petit Science Center. All finches received a mixed seed (Fort-Diet Pro Health Finch, Kaytee Products, Wisconsin) diet, water, grit and cuttlefish bone (calcium) ad libitum. All animal procedures were approved by the Georgia State University Institutional Use and Animal Care Committee.

### **Color Exposure:**

The subjects were exposed to five monotone colors (blue, yellow, green, red with white as the control) during the experimental period. A monotone color board (Roselle Vibrant Art Construction Paper, Assorted) of 18''x 24'' was taped on the back wall and a 9''x 12'' color board was taped on the side wall. The subjects were exposed to the stimuli for 24 h.

**Behavioral Observations:**

Acute behaviors were recorded before placement of stimulus, immediately after, and 24 h post-exposure. Behaviors, such as perch hopping, body positioning relative to stimulus and beak-opening were qualitatively compared within each trial.

**Plasma CORT Concentration:**

CORT was analyzed in blood plasma. Baseline blood samples were collected on day 1. After exposing the subjects to stimulus for 24 h, experimental blood samples were collected. Blood was collected followed the protocol of Newman et al., 2010. Briefly, blood samples were collected from the alar wing vein by puncturing the vein using a 26-gauge needle and blood was then collected into heparinized microhematocrit tubes. The blood collection occurred within 3 min after capture at the same time each day (around 1100 h EST) for each bird to prevent fluctuation in CORT level due to handling stress. Blood samples were kept on ice until centrifuged. After centrifuging blood, plasma was collected and stored at -20°C until analysis. A 10 day-rest period was given between each trial to allow sufficient blood replenishment. CORT level was analyzed with 2 tail-T test.

**Radioimmunoassay:**

Corticosterone level measurement followed the procedure of Shahbazi et al., 2014. Total plasma CORT concentrations were measured using a Corticosterone Radioimmunoassay (RIA) kit (Corticosterone 125I RIA kit, catalog # 07 – 120102; MP Biomedicals LLC, Solon, OH, for rat plasma). To validate the assay, pooled zebra finch plasma was run with pooled rat and hamster plasma. The standard curve was matched to the zebra finch CORT concentrations range

(by adding 6.25ng and 12.5ng through serial dilution of 25ng standard to increase sensitivity). Once this was established, a dilution series (1:2 98%, 1:4 102%, and 1:6 115%) of zebra finch plasma was run to get linearity and obtained the overall recovery. In addition to the two (low and high concentrations of CORT) controls that were provided by the kit, pools of zebra finch, rat (control for assay), and hamster (in house control) plasma were used as extra controls for each assay run. Recovery rate was 98% and detection limit was 6.25-1000 ng / ml. Intra-assay & inter-assay coefficient variations were 4.4% and 14.6%, respectively.

**Statistical Analysis:**

All data were analyzed using IBM SPSS Statistics for Windows, version 19.0 (SPSS Inc, Chicago, IL). First, data were examined for assumptions of parametric statistical test but no assumptions were violated. We used one-tailed test in our statistical analysis because of our directional hypothesis. Statistical significance was accepted at  $P < 0.025$ .

## RESULTS

### CORT Assay:

Baseline CORT was established by assaying blood collected 24 h prior to the addition of colored poster boards. Experimental plasma CORT levels (24 h CORT) was established by assaying blood collected from the subjects 24 h after the addition of the colored poster boards. Due to the small size of the birds, it is not possible to collect two blood samples within 24 hours of each other. Male and female plasma CORT levels were analyzed after exposure to the colors yellow, red, blue, and white; male and female plasma CORT levels were combined for green color. There were no significant differences ( $P < 0.025$ ) found in any of the baseline CORT when compared to the 24 h CORT levels.

Table 1. Plasma CORT levels before the addition of colored poster boards and 24 hours after the addition of colored poster boards.

Poster board Color	Sex	Baseline CORT (Mean $\pm$ SD)	<i>n</i>	24 h CORT (Mean $\pm$ SD)	<i>n</i>	<i>P</i>
Yellow	Male	6.05 $\pm$ 4.61	4	4.05 $\pm$ 4.10	4	0.570
Yellow	Female	7.33 $\pm$ 7.28	4	5.76 $\pm$ 6.02	4	0.694
Red	Male	6.32 $\pm$ 6.76	4	8.20 $\pm$ 10.81	4	0.592
Red	Female	6.31 $\pm$ 4.86	3	14.97 $\pm$ 15.26	3	0.290
White	Male	2.81 $\pm$ 1.73	4	7.01 $\pm$ 6.90	4	0.242
White	Female	3.63 $\pm$ 0.20	3	7.73 $\pm$ 5.27	3	0.322
Blue	Male	7.43 $\pm$ 3.91	5	7.05 $\pm$ 4.09	3	0.900
Blue	Female	13.67 $\pm$ 8.20	5	11.33 $\pm$ 5.75	5	0.632
Green	Combined	19.75 $\pm$ 10.08	7	18.85 $\pm$ 10.46	7	0.726

### Behavior Data:

After the color stimulus was added to the cages that contained the experimental birds, we observed immediate changes in behavior during the 10-min observation period. These changes will be quantified as part of a future study; qualitative assessment was performed for the current study. There were no observed differences in behavior of the experimental birds 24 h after exposure to color stimulus. As predicted, there were no observed behavioral changes after exposure to the control and the color stimuli. These findings are summarized in Table 2.

Table 2. Qualitative descriptions of posture and activity after the addition of color and control stimuli to each cage.

	Male	Female
<b>White (control)</b>		
<b>Posture</b>	No changes in open beak/feather frilling noted	No changes in open beak/feather frilling noted
<b>Activity</b>	No changes in movement or feeding behavior noted	No changes in movement or feeding behavior noted
<b>Red</b>		
<b>Posture</b>	3 out of 5 birds open beak/feather frilling for the first 3 minutes	No changes in posture noted
<b>Activity</b>	increased generalized hopping activity, no feeding behavior for the first 10 minutes	increased generalized hopping activity, no feeding behavior for the first 10 minutes
<b>Green</b>		
<b>Posture</b>	2 out of 5 birds open beak/feather frilled for the first 2 minutes	1 out of 5 birds showed signs of threatened/stress for the first 2 minutes
<b>Activity</b>	increased hopping movement towards the stimuli for 10 minutes	increased hopping towards and away from stimuli for 10 minutes
<b>Yellow</b>		
<b>Posture</b>	1 out of 5 birds showed threatened behavior for the first 2 minutes	No changes in posture noted
<b>Activity</b>	some decreased movement	some decreased movement
<b>Blue</b>		
<b>Posture</b>	3/5 birds open beak/feather frilling for the first 3 minutes	No change
<b>Activity</b>	No changes	generally hopped away from stimulus

## DISCUSSION

There were no significant changes in plasma CORT levels after 24 h of exposure to any of the color stimuli. We were not able to determine if there were any changes in acute plasma CORT due to physical limitations of the subjects. To perform a second blood sampling without sufficient recovery time, i.e. 24 h, would place the birds under great health risk. CORT plasma levels for individual birds were assayed individually and sexes were analyzed separately. We hypothesized that the HPA axis would be activated (as determined by an elevation in plasma CORT) after exposure to the color stimuli; however, the results do not support this hypothesis. There were no major variations in individual bird responses to the colors, and the one outlier (a sick female bird) was excluded from the analysis.

Behavioral changes were noted immediately after the introduction of each color stimulus that were not observed with the control (white). However, after 24 h, the zebra finch behavior was comparable to baseline. This is consistent with the hypothesis that novel colors (stimuli) in the environment cause temporary behavioral and hormonal changes (we could not verify this). However, it is possible that the birds may still have been stressed 24 hours after color placement, and behavioral changes may have been too minor to measure. Chronic exposure to stressors may engender more long-term physiological changes, such as weight fluctuations, phenotypic coloration, and maximal growth size (e.g., Strohlic and Romero, 2008; Rosenthal et al., 2012).

The acute changes in behavior noted for the finches may be typical when novel stimuli are introduced. However, it is interesting to note that the subjects behaved differently when exposed to different colors. This suggests that zebra finches have psychophysiological association to colors of different wavelengths—similar to the way that color has been shown to affect mood and autonomic function of human beings (Sakuragi and Sugiyama, 2011).

It was expected that the addition of the white control would not cause a change in behavior or plasma CORT concentration. This indicates that color exposure alone was sufficient enough to affect behavioral change and that observed responses were not only due to the neophobia of having a novel object placed in their environments.

The most observable changes in behavior were noted during the first 10 min of exposure to the red color stimulus. Both male and female subjects stopped feeding, became fixated on the poster boards, and increased movement around their enclosure. Males showed an increase in stress-associated behaviors, such as opening of beak and feather frilling. This robust response is consistent with mating selection color preferences seen in the species. Female zebra finches have a predilection towards male finches with red beaks over other color variations, as the red body color tone acts as a marker for reproductive fitness. Vivid colors are correlated with high carotenoid circulation—an indirect measure of physiological health (Endler, 1983). Because carotenoids are acquired from diet, beak redness may be indicative of foraging ability, health and nutritional intake (Hill, 1992; Loranzo, 1994). Zebra finches metabolize carotenoids to keto-carotenoids, an energy-demanding metabolic process, to enrich the color of their beaks (McGraw and Toomey, 2010). Therefore, conspecifics with the most vibrant colors suggest reproductive fitness (Hill, 1996). Since strong color preferences for red evolved in the zebra finch, it is reasonable to expect behavioral expression to be displayed outside of the sexual context.

Further analysis is required to understand the variations in acute behavior to the introduction of yellow, green, and blue poster boards. Birds show diverse color preferences, which vary between conspecifics and species; for instance zebra finches show individual variations in color preference for materials used for nest building (Muth et al., 2013), and the kea (*Nestor notabilis*), an endemic parrot to New Zealand, show species wide color preferences in the color of food it

consumes (Weser and Ross, 2013). Currently, there are no published studies that explore the immediate behavior and physiological changes in zebra finches caused by colors in the environment. The future the data will be closely analyzed to extrapolate individual differences and variations in behaviors in response to changing the color stimuli.



## CONCLUSIONS

This study seeks to create a better understanding of the physiological changes caused by an environmental stimulus, such as color. The question of “*Can a novel color in the environment elicit a stress response as measured by changes in behavior and increase in plasma stress hormone levels?*” is addressed in this study. The results showed no significant changes in CORT plasma levels in experimental in comparison to basal levels after 24 h post-exposure to any of the colors, suggesting that color changes in the environment do not activate the HPA axis. However it is important to note that the best measurement would have been to assay CORT within 15 min of the initial color exposure. We were not able to do this because of limitations with taking a second blood sample from the birds after the baseline sample was taken, and therefore there may have been an initial elevation in CORT that was missed.

The introduction of the color stimuli resulted in immediate behavioral changes in the birds: increased activity was observed with green, blue, and red poster boards; decreased activity was observed with yellow poster board. In addition, male birds consistently showed more aggressive behavior, i.e. beak opening and feathers frilling. These findings suggest that zebra finches show temporary sexually dimorphic behavioral responses to novel stimuli based on color differences. However, consistent with the lack of plasma CORT responses after the exposure of color stimulus was the lack of change in behavior after 24 h; birds were feeding and behaving as they were before the introduction of a stimulus.

## FUTURE DIRECTIVES

There are many directions this research can take. First, it is important to determine if there was an increase in plasma CORT that was missed by waiting 24 h before collecting blood. One possibility is to measure the baseline CORT 24 h before the color exposure instead of 15 min before the addition of the color stimulus. In addition, in order to further interpret acute behavioral changes, each individual must be assessed for differences in response to the addition of a novel colored poster board. Animals generally show differences in behavior and levels of neophobia when confronted with novel stimuli within a species, ranging from shy to bold (Verbeek et al., 1994; Wilson et al., 1993). Zebra finches may react differently to novel stimuli based on their genetic predisposition or personality—in studies of house mice (*Mus musculus*) Veenema et al. (2003) found that mice genetically selected for an active coping style are associated with low HPA reactivity, while those with a passive coping style show high HPA reactivity. Because of these differences in personalities, it is ideal to measure individual changes in behavior across specific individuals. Also, it has been shown that individuals consistently differ in their behavioral responses towards the same stressor in another songbird species great tit (*Parus major*) (Carere et al., 2005). Therefore, there is little concern that individual behavior would drastically change because they had previous experiences of color exposure.

Individual differences in HPA axis response based on personality may also have ramifications on the way in which data should be analyzed. In addition to assessing immediate behavioral changes to novel color stimuli, further analysis of physiological changes, such as CORT plasma levels, will be analyzed on an individual basis. This is done to elucidate any trends in physiological changes of individual birds reduce the standard deviation of results.

Measuring changes to biological markers in addition to CORT could be used to better understand the acute behavioral changes noted in response to color. The opening of beaks and frilling of feathers are characteristic signs of aggression in many avian species. Therefore future studies will not only assay CORT plasma levels but also levels of circulating androgenic hormones.

More will be done to investigate the differences in acute behavioral response to the addition of the novel stimuli of colored poster boards. As described above, for future studies, blood samples will be collected in a shorter period post-exposure. An alternative to venipuncture that was explored in other studies was observation of physiological markers of stressors, such as breathing rate or body temperature (Cabanac and Guillemette, 2001; Carere and van Oers).

Another question we will seek to resolve in future studies is: what effect would room color have on HPA activation and behavior? It is well established that room color can elicit changes in mood and behavior (e.g. Nelson, 1984; Yildirim K et al., 2011). One concern is that responses measured in this experiment may be due to stress typical of a novel object being introduced to the environment; a significant portion (25% of the entire surface area) of the birds' cage was covered with the colored poster board. However, housing the birds in a room of a different color may cause a behavioral response that is contingent on the color, and not on the presence of a novel subject.

An alternative to changing the color of the walls in the room would be to use colored lighting. Previous studies have investigated the effects of light colors on fish (Maia and Volpata, 2013; Heydarnejad et al., 2013) to establish ideal housing conditions. Similar studies could be applied to zebra finch to determine the effects of colored lights as a stress factor. A previous study found

that female zebra finches' mate preferences change in the absence of UV (Hunt et al., 1997). So, removing UV may induce behavioral responses that differ from the control.

Finally, if discovered that changing the color or light color of the room leads to changes in HPA activity, further study will be conducted to evaluate long term effects of being housed in rooms of different colors.

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