Effects of Paternal Deprivation on Nestling Zebra Finches (*Taeniopygia guttata*)

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Abstract: Early interactions between parents and offspring have been shown to influence behavior development in avian offspring, which links to their fitness as adults. In bi-parental species, previous research has looked at what developmental alterations have occurred when the mother is removed from the nest during the developmental stage. By looking at the behaviors of the offspring when the father is removed during the nestling stage, we can better understand where the developmental alterations are stemming from. In this study, zebra finches were assigned to control and paternal removal groups, and from post-hatch day 1 through 18, we quantified important parent-offspring interactions that occurred during 1-hour long video recordings inside nests. We are still in the process of quantifying behaviors from all 19 nests, but we expect that the mother will attempt to compensate for the lack of paternal care in the paternal removal group, but not be fully successful. As a result, offspring will have fewer interactions with a parent in the paternal removal group. Determining the effects of paternal deprivation during the nestling stage will allow us to further understand the importance of bi-parental care during development.

Keywords: Zebra Finch, Paternal Deprivation, Developmental Stress, Stress Physiology, Nesting Behavior

Introduction

Early interactions between parent and offspring are critical for normal stress physiology and development in birds (Ogawa et al. 1993). Studies have supported that these interactions influence social behavior development in offspring (Lundberg et al. 2017). In addition, parental care is often tied to the facilitation of offspring performance behaviors, such as increased immune responses, that link to fitness as adults (Klug and Bonsall 2014). Researchers in previous studies have concluded that parental care also leads to an increased vulnerability to chronic stress in adult offspring through developmental stress (Zhang et al. 2013). Regulation of stress responses is critical for the survival of a vertebrate to be at an ideal level, and abnormal responses can be detrimental to the health of an animal (Yeh et al. 2013).

The hypothalamic-pituitary-adrenal axis (HPA axis)—which is a system of feedback interactions between the hypothalamus, pituitary gland, and adrenal glands—moderates responses to tense situations (Sapolsky et al. 2000). Secretion of stress hormones, known as glucocorticoids (GCs), occurs through the activation of the HPA axis and deploys energy to meet metabolic requests from acute stressors (Crino et al. 2017). Studies on corticosterone (CORT), a dominant GC, conclude there to be a correlation between CORT and effects on behaviors in parents and reduction of care given to offspring (Crino et al. 2017). Exposure to elevated levels of CORT during the developmental stage has sustained impacts on offspring physiology and behavior, such as immune and reproductive responses, learning ability, and survival (Crino et al. 2017).

Previous avian studies have revealed that nestlings left unattended for longer periods of time during the nestling stage have higher baseline CORT levels (Rensel et al. 2010). In studies using zebra finches (*Taeniopygia guttata*), deprivation in maternal presence throughout the nestling period of offspring resulted in higher CORT levels in response to isolation present in adult zebra finches (Banerjee et al. 2011). Results from these studies support the hypothesis that the HPA axis enters into a long-term hyper-responsive

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stage when young offspring become isolated from one parent (Banerjee et al. 2011). Adult female zebra finches that are isolated from their mate maintain higher levels of CORT than females who are not isolated due to separation-induced stress on the experimental female (Crino et al. 2017, Remage-Healey et al. 2003). However, previous studies have not quantified the baseline parental levels of CORT and the behaviors of parents in treatment groups. Furthermore, since the alteration of parental provisioning occurs with maternal figures containing higher CORT levels, isolating mothers from their mates may additionally result in secondary effects on offspring development (Silverin 1990).

The HPA axis in uniparental mammalian species may be susceptible to changes in social environments (Banerjee et al. 2011). The quantification of both the baseline CORT levels in mothers and parental care behavior is necessary due to this study and other studies suggesting the interactions occurring between parents and offspring mold the development of the HPA axis through variations in CORT levels (Banerjee et al. 2011, Rensel et al. 2010). The quantification of CORT levels will be beneficial in establishing a starting point for large-scale analyses of the stress of parent-deprived zebra finches (Yeh et al. 2013). Researchers in previous studies have analyzed the effects of removing the mother from the nest of offspring, but they have not been able to determine the impacts of offspring behavior resulting from a lack of paternal interaction (Banerjee et al 2011).

In this study, zebra finches are used as test subjects to evaluate the effects of the removal of the father during the nestling stage of development to the behavioral alterations in adult offspring. Zebra finches are an avian species widely researched in areas of neurobehavioral studies due to their capabilities of easily developing song and understanding avian speech through social interactions (Spierings and ten Cate 2016). This species is beneficial to studying brain plasticity through stress-induced situations due to the size reduction of the HVC, the song control nucleus, following early instances of stress (Shahbazi 2012). This species is socially monogamous and is bi-parental, with both parents providing equal amounts to offspring care (Zann 1996). In this study, zebra finches were placed into a control and experimental group, where the father was removed at hatching of the last hatchling, and offspring were observed for behavioral adjustments and changes in CORT levels. We predict that the earlier removal of a father from the nest of zebra finch hatchlings, the more responsive the HPA axis will be—due to the rise in CORT levels—and more developmental alterations will occur later on in the adult offspring. If the results support our hypothesis, this information could lead to further research to determine how paternal deprivation during developmental stages affects other species.

Methods

While the entire study investigates both the behaviors of parents and offspring and CORT levels in offspring, this specific project focuses mainly on coding behaviors between the parents and the offspring from post-hatch days 1-18. Zebra finches were randomly assigned to one of two treatment groups. The control group consisted of nests of zebra finches with both parents present (n=12). In the paternal removal group, the father in a nest was removed at the hatching of the youngest offspring (n=5). Each nest contained between 1-5 hatchlings.

Adult zebra finches were obtained from a breeding colony at Oklahoma State University-Stillwater. Parents and the hatchlings were housed indoors in a temperature-controlled aviary with an ambient temperature ranging from 20-22.7°C, a humidity of 20-50%, and a light:dark cycle of 14:10h. Subjects were contained in separate nesting boxes (12Wx12Dx15H cm) attached to the side of stainless steel nest cages (45Wx45Dx40H cm) with food, water, perches, and nesting material (shredded paper and cotton balls) in each cage (Crino et al. 2017, Alger et al. 2011). The nesting boxes were checked daily to monitor for hatchlings.

Once hatched, we recorded parental behaviors in every nest due to previous studies indicating that the development of the HPA-axis relies on stimulation between offspring and their mothers (Banerjee et al. 2011, Lovic et al. 2001, Rensel et al 2010). To quantify parent-offspring interactions, videos were taken every 2 days from post-hatch day (PHD) 1 until PHD 18. Beginning on PHD 1 of the last hatchling in the nest (hatch day=PHD 0) until PHD 18, nest cameras were set up inside the nest cages, facing the entrances of the nest boxes. Recordings only occurred for increments of one hour due to restrictions on tape size for each camera. To correct for behavioral differences due to time of day, recordings occurred during hours 1300 and 1600 using a Hawk Eye Nature Cam X00018UD9X. To correct for possible disturbance-induced behavioral changes, we did not begin scoring behaviors until two minutes into recording and stopped one minute before the end of each video, as cameras are manually-operated. The behaviors recorded consisted of nest attendance, visiting, allopreening, brooding, and feeding and were scored using Behavioral Observation Research Interactive Software (BORIS), as seen in Figure 1 (Friard and Gamba 2016).

Code	Type	Description
nest attendance	State event	adult: complete entrance into the nest
clumping with partner	State event	adult: time spent clumping with partner in nest
allopreening nestlings	State event	adult: grooming nestlings
feeding nestlings	Point event	adult: feeds nestlings
brooding nestlings	State event	adult: time on top of nestlings
nest visit	Point event	adult: incomplete nest visit (peaking)

Figure 1: Above is an ethogram of parental behaviors, including nest attendance, allopreening nestlings, feeding nestlings, brooding nestlings, and nest visits.

Progress To Date

We have recordings of 19 nests, but we are still quantifying the behaviors in each video. With further observation, we predict mothers will attempt to compensate for the loss of paternal care by increasing their care, as seen in Figure 2. However, we predict females will not be able to fully compensate for the lack of paternal care, and as a result, offspring will have fewer interactions with a parent in male removal groups. The reduction in parental care is expected to impact the social behavior, learning ability, and stress of offspring. This study is a subset of a larger study, and once video behaviors are fully quantified during the nestling and fledgling stages, we will quantify CORT levels in blood samples collected from mothers and offspring to identify the physiological consequences of paternal deprivation on the stress physiology of zebra finches.

Discussion

This behavioral data will be used to test for any treatment differences and to relate variation in paternal care to possible effects on stress physiology and behavior in adult offspring. However, video quality varies with each camera used, which made reliably scoring every interaction difficult. In addition, nesting material inhibited the camera view of each interaction in some videos, or the birds hit the camera and moved it out of the standard placement. Due to the subjects

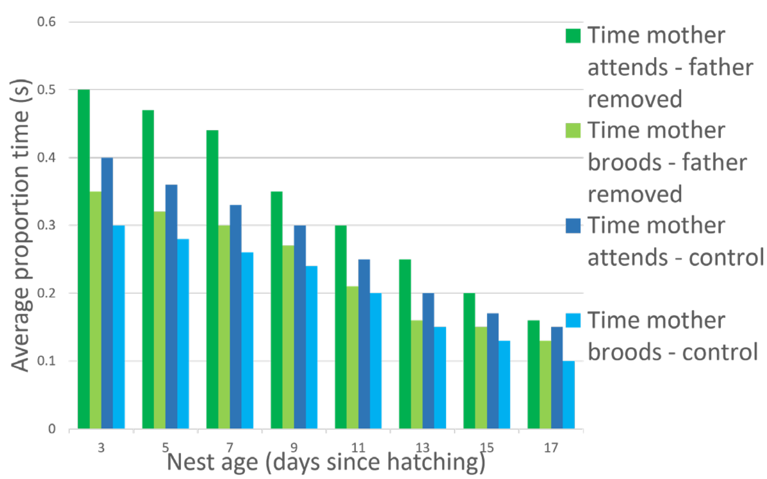

Figure 2: This graph represents predicted results of the average proportion time females spend attending to the nest and brooding over 8 recording periods.

being tested in a laboratory setting and not in nature, other factors will possibly affect the stress levels of offspring. This research is a continuation of previous research on parental deprivation during the developmental state of hatchlings. Once scorings are finished, we will run ANOVAs on the data to distinguish whether the behaviors are different between treatments. We hope that determining the short- and long-term effects of paternal deprivation on offspring will increase our understanding of the importance of bi-parental care and create paths for further research to determine how paternal deprivation affects other species.

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