Senior Thesis for the Department of Biology (2018)

The Effects of Enrichment on the Behavior and Physiology of Captive Starlings (*Sturnus Vulgaris*)

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1. Abstract

Undomesticated animals are prone to developing abnormal behaviors in captivity, such as stereotypies, but the physiological stress profiles of these animals can vary widely. Animal caretakers often implement environment enrichment to attenuate behavioral abnormalities and stress pathologies. This study implements an intermittent enrichment protocol and examines its effect on the behavior and stress physiology of eight starlings in long-term captivity. Originally, the starlings were housed in individual bird cages that were attached to experimental apparatuses. Enrichment was provided using an aviary approximately 9x larger in volume than a home-cage, first containing familiar objects, then containing novel perches and toys for enrichment purposes. Two groups of four starlings spent three hours in the aviary twice a week, providing social enrichment. Blood was sampled at regular intervals to determine HPA axis functioning using a three-part measurement (baseline, stress-induced, and negative feedback) related to physiological stress. Additionally, the birds were video recorded weekly in their home-cages and in the aviary in order to examine behavioral effects. We found that the intermittent enrichment protocol significantly changed the behavior of the starlings but did not significantly affect their CORT profiles, with social-based enrichment having a more positive impact that toy-based enrichment.

2. Introduction

Captivity is widely believed to be a chronically stressful condition for birds due to the absence of important abiotic stimuli, restricted foraging opportunities, and abnormal social interaction (Morgan and Tromberg, 2007). This has the potential to negatively impact an animal's health (Romero, 2004) and affect (Bateson & Matheson, 2007). Therefore, it is important to measure the amount of stress an animal experiences in captivity so that the stress can be alleviated and life quality can be improved.

To accomplish this, zoos and laboratories often implement enrichment programs. Enrichment attempts to mimic important abiotic stimuli, provide foraging opportunities, or mimic conspecific social interaction to rectify impoverished conditions (Rosier & Langkilde, 2011). Enrichment styles that address different problems may have differing success at improving the welfare of the animal (Newberry, 1995). In order to assess whether or not the enrichment procedure is improving the quality of life of the animal, rigorous scientific investigations need to be conducted as the enrichment is implemented. Unfortunately, measuring the effect of captivity on an animal's stress and welfare is complicated and difficult.

While the effects of captivity can be measured in many ways, there are two common methods. Physiologically, one can quantify corticosterone (CORT) secretion. Behaviorally, one can assess the presence and amount of stereotypic behaviors. Corticosterone, the analog to mammalian cortisol, is the glucocorticoid produced by the hypothalamic-pituitary-adrenal (HPA) axis in birds in response to stressors, both acute and chronic (Romero & Wingfield, 2016). How the release of CORT is modulated by the HPA axis is often used as an indicator of stress. For example, the ability of the animal to mount a CORT response after enduring a stressor and to downregulate that response is considered a measure of the animal's ability to regulate the HPA

axis (Romero &Wingfield, 2016). The latter measure, stereotypic behavior, is a compulsive, repetitive behavior with no apparent goal performed by animals in captivity (Mason, 1991). Stereotypic development is directly correlated with poor captive conditions (Schoenecker, Heller, and Freimanis, 2000; Novak et al, 2006; Wurbel and Stauffacher, 1997) and a change in the prevalence of stereotypic behavior is often used as an indicator of an animal's welfare (Broom, 1991).

Although both measures are used to assess the quality of captive life, the relationship between stereotypies and CORT remains inconsistent. Studies looking at CORT metabolites in feces have found a direct correlation between stereotypies and CORT (e.g., Owen & Lane, 2006; Costa et al, 2016; Wielebnowski et al, 2002) or no clear relationship between the two (e.g., de Almeida, Palme, & Moreira, 2018; Gusset, 2005). Plasma CORT studies are similarly mixed, with many revealing no correlation between stereotypic behavior and CORT (e.g., Schapiro et al, 1993; Rosier & Landkilde, 2011). Less common measures of CORT, like feather CORT, have also been used to show the alleviation of stress by enrichment (e.g., Fairhurst et al, 2011). The inconsistent relationship between CORT and stereotypic behavior suggests that the relationship between "stressful" behavior and stress physiology remains to be clarified. While enrichment is often focused on reducing stereotypic behaviors (Mason et al, 2007), looking at changes in physiology can provide a completely different perspective as to the effectiveness of the enrichment at improving an animal's life quality.

In this study, eight wild-caught European starlings (*Sturnus vulgaris)* in long-term singly-housed captivity were introduced to an enrichment aviary. This enabled the birds to spend time with each other in the aviary twice a week and spend the rest of the time in their normal home cages. This type of intermittent, out-of-cage enrichment is, to our knowledge, a new approach, as

opposed to enrichment that is available in an animal's home environment, usually for long periods of time. Additionally, the birds were exposed to an empty aviary before enrichment objects were added so that the effect of social enrichment can be teased apart from the effect of physical enrichment objects. At each stage, behavior and plasma CORT profiles were assessed to provide a complete picture of the effect of the enrichment protocol on stress.

We predicted that the implementation of the enrichment aviary would alleviate stereotypies, thereby creating a healthier CORT profile. Although the relationship between social enrichment and the HPA axis is dependent on an animal's hierarchical rank and the population density (Creel et al, 2012), the gregarious nature of starlings (Bateson & Asher, 2010) led us to predict that the reduction in stress would be starker when social enrichment was added than when toy enrichment was added. The results of this study will help identify useful tools to improve captivity for wild starlings, reveal the efficacy of intermittent enrichment, and illuminate the relationship between behavioral and physiological stress.

3.1 Overview

To test the response of starlings to social and object-based enrichment, 8 captive starlings were used (4 females and 4 males). All starlings had been captive for at least five years. All birds were housed individually in experimental chambers for psychological experiments before and during the testing period on a 12/12 light cycle. Hyperkeratosis made it impossible to fit bands around the birds' legs, so birds were differentiated by marking their feet with different colors of non-toxic nail polish (Emosa Nail Polish, Amazon). All experiments were conducted with the approval of the Tufts Institutional Animal Care and Use Committee.

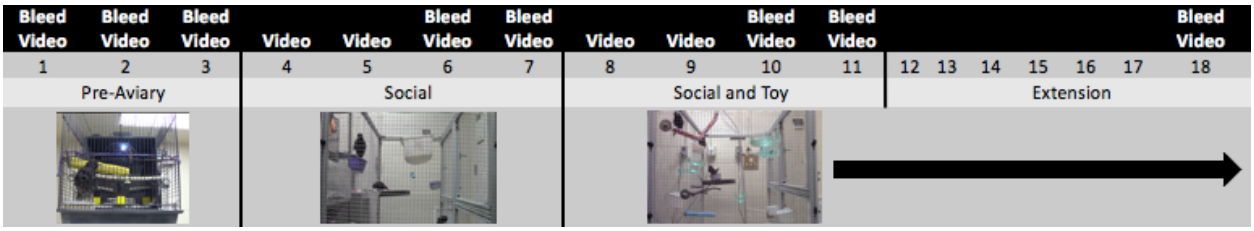

Figure 3.1 The above timeline outlines the time course of the experiment. The top row indicates when blood and video samples were taken. The second row indicates the week numbers. The third row indicates the phase that encompassed those weeks. The fourth row provides photographs illustrating the differences between the phases.

Three sets of blood samples were taken prior to introducing the enrichment (Pre-Enrichment Phase; Figure 3.1). These samples would serve as the pre-enrichment control levels to which the other samples will be compared. Additionally, the behavior of each individual bird in their home cage was recorded during this Pre-Enrichment Phase.

After the Pre-Enrichment Phase was completed, the birds were introduced to the Social Phase of the enrichment aviary (Figure 3.1). The enrichment aviary was a 56in x 47in cage enclosed in chicken wire which was wheeled into the birds' home room while it was in use. For the Social Phase, the enrichment aviary contained food dishes, water dishes, and perches identical to those in the birds' home cages. The birds were separated into two cohort groups based on length of captivity. The birds of each cohort would be placed into the aviary at the same time. Henceforth the two cohort groups will be referred to as Group 1 and Group 2. Group 1 was placed in the enrichment aviary from 7am-10am on Mondays and Wednesdays. Group 2 was placed in the enrichment aviary from 7am-10am on Tuesdays and Thursdays. This schedule was maintained throughout entirety of the experiment.

During the Social Phase, blood samples were taken each week to assess physiological changes due to the aviary. Group 2 was sampled on Mondays and Group 1 was sampled on Tuesdays such that the birds being sampled were in their home cages while the other cohort was

in the aviary. During the first half of the Social Phase, the birds were videotaped each week for the first fifteen minutes they spent in the aviary on Wednesdays for Group 1 and Thursdays for Group 2. During the second half of the Social Phase, the birds were videotaped for fifteen minutes in their home cages on Wednesdays for Group 1 and Thursdays for Group 2.

After the four weeks of Social Phase, the Social+Toy Phase of the enrichment aviary was introduced (Figure 3.1). For the Social+Toy Phase, the enrichment aviary contained food dishes, water dishes, and perches identical to those in the birds' home cages as well as novel enrichment objects: bird baths (1), branch perches (2), a colorful rope (3), swings (4), two foraging toys (5), and a UV lamp (6) as shown in Figure 3.2. These enrichment objects were chosen because they were popular bird toys marketed at pet stores. The toys provide outlets for natural behaviors (like the bird baths, branches, and UV light) or provide new and engaging activities (like the colorful rope and swings).

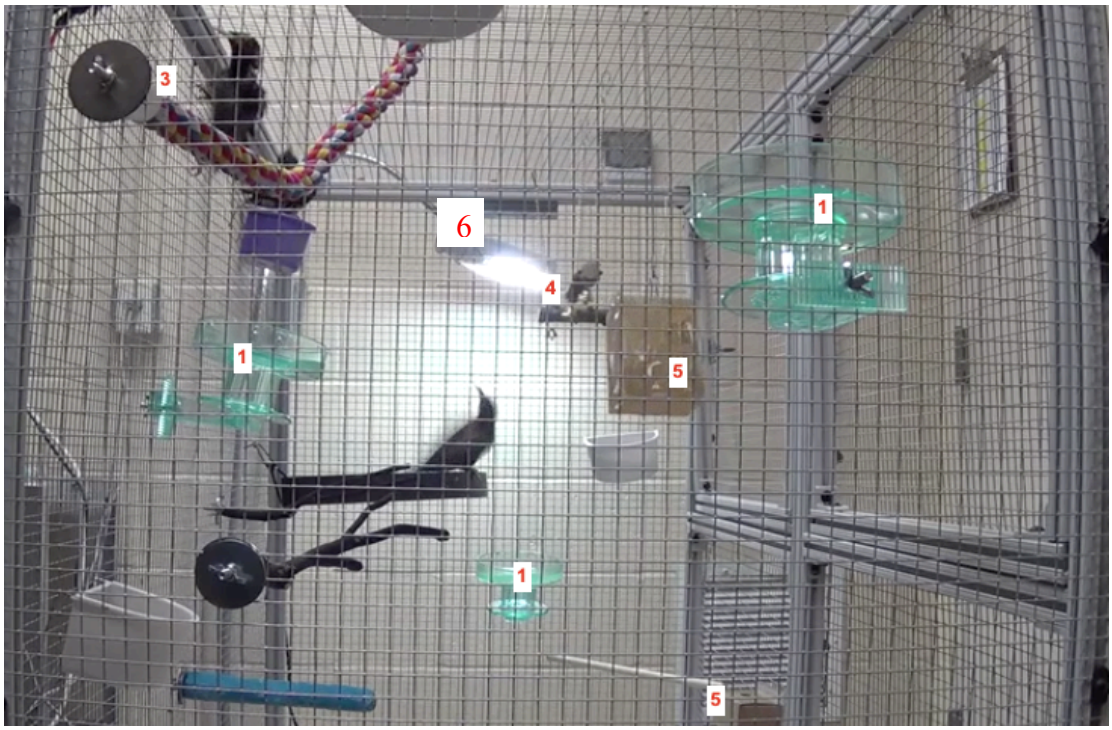

Figure 3.2 The above is a photograph of the enrichment aviary during the Social+Toy phase. The different novel enrichment objects are labeled: birds baths (1), branch perches (2), colorful rope (3), swings (4), foraging toys (5), and a UV lamp (6).

The birds were placed in the aviary following the same procedure as the Social Phase. The birds were also videotaped and plasma samples were taken following the same procedure as the Social Phase.

After the four weeks of the Social+Toy Phase, the birds entered the Extension Phase (Figure 3.1) in which they were not videotaped and no plasma samples were taken for seven weeks. During this time, no changes were made to the enrichment aviary or protocol from the Social+Toy Phase. After seven weeks, additional physiological and behavioral samples were taken.

3.2 Behavioral Monitoring

The birds were videotaped in their home cages at eight time points to assess changes in home cage behavior between the Pre-Enrichment, Social, Social+Toy, and Extension Phases. Home cage recordings occurred on Wednesdays for Group 2 and Thursdays for Group 1. The birds were also videotaped inside the enrichment aviary at nine time points to assess changes in aviary behavior between the Social, Social+Toy, and Extension Phases. These recordings occurred on Wednesdays for Group 1 and Thursdays for Group 2.

The night before videotaping, camcorders were placed in front of the birds' home cages and one was placed in front of the aviary. At 7:00am the following morning, the camcorders were turned on and recording began. The recording ended at 7:15am. If a camera malfunction interfered with the recording, videos were not assessed if they were under five minutes in length.

Once all video recordings were collected, the videos were given randomly generated names so they would be blinded to the behavioral scorer. All videos were coded by the same individual (SB). The scorer watched each video and recorded when each bird began performing a

certain behavior (Table 1) and when it stopped. All behavioral changes were recorded for the full length of each video.

3.3 Blood Sampling

Blood samples were taken at eight time points to assess changes in HPA axis functioning between the Pre-Enrichment, Social, Social+Toy, and Extension Phases. Sampling occurred on Mondays for Group 2 and Tuesdays for Group 1. All blood samples were taken from the alar vein and collected in heparinized capillary tubes.

At each of these time points, a series of blood samples was taken starting at 7:30am. Baseline (BL) samples taken within 3 minutes of entering the room were considered representative of a bird's baseline CORT concentration as it takes more than three minutes for CORT to enter the bloodstream after a response (Romero and Reed, 2005). If the sample was unable to be taken within three minutes, this was noted. Birds were then placed in a cloth bag for 30 minutes, after which a blood sample (stress-induced; SI) was taken. After the SI sample was taken, 1 mg/kg dexamethasone (DEX; Phoenix Pharmaceuticals, Inc., St. Joseph, MO, USA), a synthetic glucocorticoid, was injected intramuscularly to assess the potency of the negative feedback (NF) in the HPA axis. Following the injection, birds were placed back in their home cages. Birds were sampled again 90 minutes after the DEX injection. Samples were stored on ice until processing which occurred no more than 5 hours after sampling. Centrifugation at ~1200g for 10 minutes (Centrific Model 225, Fisher Scientific, Pittsburgh, PA, USA) was used to separate plasma, which was frozen at -20˚C until assay.

3.4 Corticosterone Assays

A radioimmunoassay (RIA) was performed to measure the CORT concentrations in each plasma sample (Wingfield et al., 1992). Plasma sample volumes were 17.4±6.1μL. The steroids were extracted using dichloromethane, then dried under N_2 gas. Once the drying process was complete, the samples were rehydrated with 550 μL phosphate-buffer saline with gelatin. A standard curve was created and the RIA was run using the antibody B3-163 (Esoterix, Calabasas Hills, CA, USA). Assay sensitivity was 1 ng/ml; baseline and negative feedback samples were often below the level of detection and therefore assigned this floor value (83 samples total). The inter and intra-assay CVs were 21.4% and 5.1% respectively.

3.5 Data Analysis

All statistical analyses were conducted in RStudio (RStudio Team, 2015). Prior to conducting any linear mixed effects models, the data were tested for heteroscedasticity by graphing the residuals on a QQ line plot and visually assessing fit. If the data was a poor fit, it was log-transformed and retested.

3.5.1 Group and Sex Effects

The effect of group and sex in the data sets were tested to see if these factors impacted the behavioral and hormonal results. This was accomplished using a linear mixed effects model, with bird as a random effect and group and sex as main effects (nlme package; Pinheiro et al, 2018), and the “anova” function (anova function; Chambers & Hasties, 1992). There was no main effect of group or sex in the behavioral and hormonal data ($p>0.1926$). There were also no

interaction effects for group or sex in the behavioral or hormonal data ($p>0.2981$). Therefore, sex and group were ignored in the rest of the data analysis.

3.5.2 Behavioral Data

Data analysis focused on three behaviors – perching, preening, and stereotypies – which formed 78.4% of all observed behavior. Videos shorter than five minutes were excluded from the analysis. Time spent performing each behavior was analyzed as a percent of the total available video time.

A linear mixed effects model (nlme package; Pinheiro et al, 2018) was used to test the effect of location (home or aviary) and phase (Pre-Aviary, Social, Social+Toy, or Extension) on proportion time spent performing a behavior with bird as a random effect. A separate linear mixed effects model was performing for each of the three behaviors; perching, preening, and stereotypies. If the "anova" function (anova function; Chambers & Hasties, 1992) revealed a significant difference between variables, the "summary" function (summary function; Chambers & Hastie, 1992) was used as a post-hoc test to investigate where the differences occurred.

3.5.3 Hormone Data

For baseline (BL) and stress-induced (SI) bleeds, the amount of CORT in the samples were quantified. For negative feedback (NF) bleeds, the percent decrease in CORT from the SI bleed to the NF bleed was analyzed to test the efficacy of the negative feedback response.

A linear mixed effects model (nlme package; Pinheiro et al, 2018) was used to test the effect of phase (Pre-Aviary, Social, Social+Toy, or Extension) on CORT. A separate linear mixed effects model was performed for each of the three bleeds; BL, SI, and NF. If the "anova"

function (anova function; Chambers & Hastie, 1992) revealed a significant difference between variables, the "summary" function (summary function; Chambers & Hastie, 1992) was used to investigate where the differences occurred.

4. Results

4.1 Behavioral results

4.1.1 Location effects

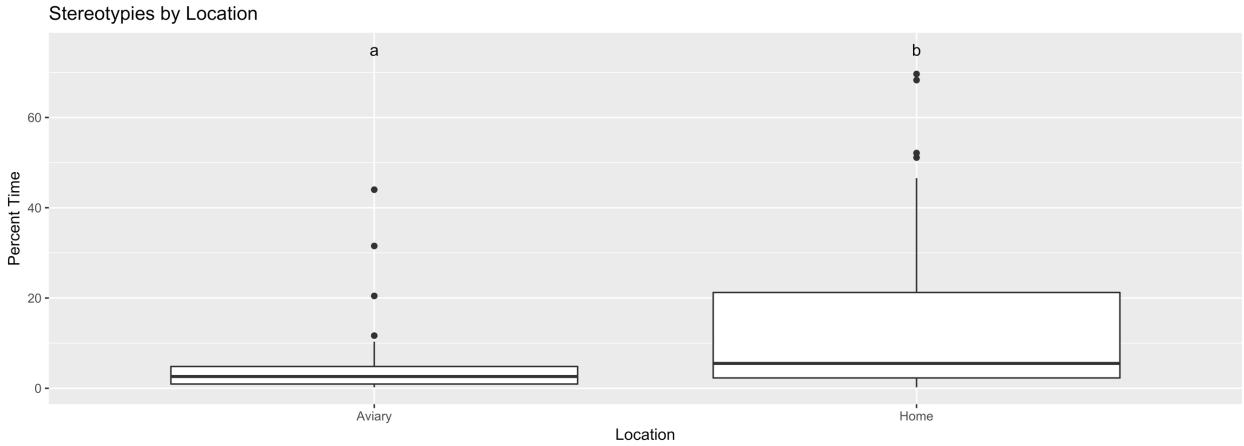

Figure 4.1 The figure above shows percent time spent performing stereotypic behavior on the y-axis and the location where behavior was recorded on the x-axis. Significance is designated by letters above the box plots.

Figure 4.1 depicts the differences in behavior between the home cage and the aviary, showing a significant effect of location on stereotypic behavior. The linear mixed effects models revealed no significant difference in time spent perching ($F_{1,44} = 0.16$, p=0.69) or preening ($F_{1,43} = 0.08$, p=0.78) between the home cage and the enrichment aviary. The linear mixed effects model did reveal a significant difference in in time spent performing stereotypies between the home cage and the enrichment aviary ($F_{1,42} = 11.27$, p=0.002).

4.1.2 Phase effects

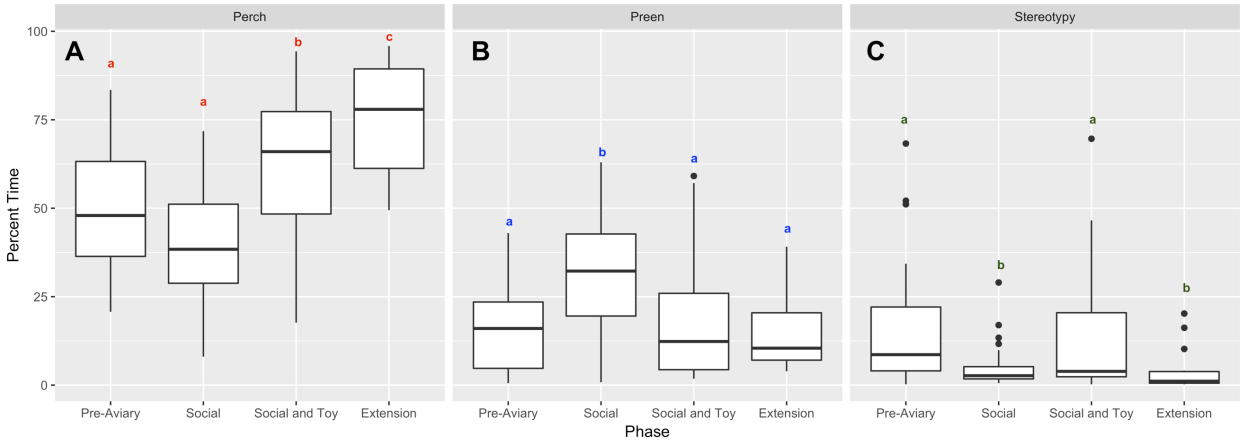

Figure 4.2 The figure above shows percent time spent performing behavior, combined across the home cage and the aviary, on the y-axis and phase on the x-axis. 4.1A shows the percent time spent perching by the birds in each phase of the experiment with significant differences designated by red letters. 4.1B shows the percent time spent preening by the birds in each phase with significant differences designated by blue letters. 4.1C shows the percent time spent performing stereotypic behavior in each phase with significant differences designated by green letters.

Figure 4.2 depicts the changes in behavior across the phases, showing possibly dramatic changes in stereotypies and preening behavior. The linear mixed effects model revealed significant differences in perching behavior ($F_{3,44}$=25.19, p<0.0001), preening behavior ($F_{3,43}$=5.59, p=0.0025), and stereotypic behavior ($F_{3,42}$=3.45, p=0.025) between phases. When considered separately, the amount of time the birds spent performing stereotypies in the aviary did not significantly differ across phases ($F_{2,14}$=3.30, p=0.067) but did differ for stereotypies performed in the home cages ($F_{3,19}$=3.42, p=0.038).

4.1.2.1 Perching

Figure 4.2A depicts the changes in perching behavior across the phases, with the Social+Toy and Extension phases showing an effect. Post-hoc tests revealed an increase in perching behavior between the Pre-Aviary phase and the Extension phase (p<0.0001) and the Pre-Aviary phase and the Social+Toy phase (p=0.013). There was no significant difference in

perching behavior between the Pre-Aviary phase and the Social phase ($p=0.092$). There was also a significant increase in perching behavior between the Social phase and the Social+Toy phase ($p<0.0001$) and the Social phase and the Extension phase ($p<0.0001$). There was a significant increase in perching behavior between the Social+Toy phase and the Extension phase ($p=0.01$).

4.1.2.2 Preening

Figure 4.2B depicts the changes in preening behavior across the phases, with the Social phase showing an effect. Post-hoc tests revealed an increase in preening behavior between the Pre-Aviary phase and the Social phase ($p=0.009$), but no significant difference between the Pre-Aviary phase, the Social+Toy phase ($p=0.79$), or the Extension phase ($p=0.93$). There was also a significant decrease in preening behavior between the Social phase and the Social+Toy phase ($p=0.0027$) and the Social phase and the Extension phase ($p=0.0009$). There was no significant change in preening behavior between the Social+Toy phase and the Extension phase ($p=0.64$)

4.1.2.3 Stereotypies

Figure 4.1C depicts the changes in stereotypic behavior across the phases, revealing significant changes at each phase. Post-hoc tests revealed a significant decrease in stereotypic behavior between the Pre-Aviary phase and the Social phase ($p=0.030$) followed by a significant increase between the Social phase and the Social+Toy phase ($p=0.022$). There was also a significant decrease in stereotypic behavior between the Social+Toy phase and the Extension phase ($p=0.028$). There was a significant decrease in stereotypic behavior from the Pre-Aviary phase to the Extension phase ($p=0.040$). There was no significant change from the Pre-Aviary

phase to the Social+Toy phase (p=0.68) or from the Social phase to the Extension phase (p=0.99).

When considered separately, stereotypic behavior in the home cage decreased from the Pre-Aviary phase to the Social phase (p=0.018), increased from the Social phase to the Social+Toy phase (p=0.020), and did not change significantly from the Social+Toy phase to the Extension phase (p=0.090). Stereotypic behavior in the home cage did not differ significantly from the Pre-Aviary phase to the Social+Toy phase (p=0.946) or the Extension phase (p=0.080). Stereotypic behavior in the home cage did not differ between the Social phase and the Extension phase (p=0.614) (Figure 4.1).

4.2 Corticosterone results

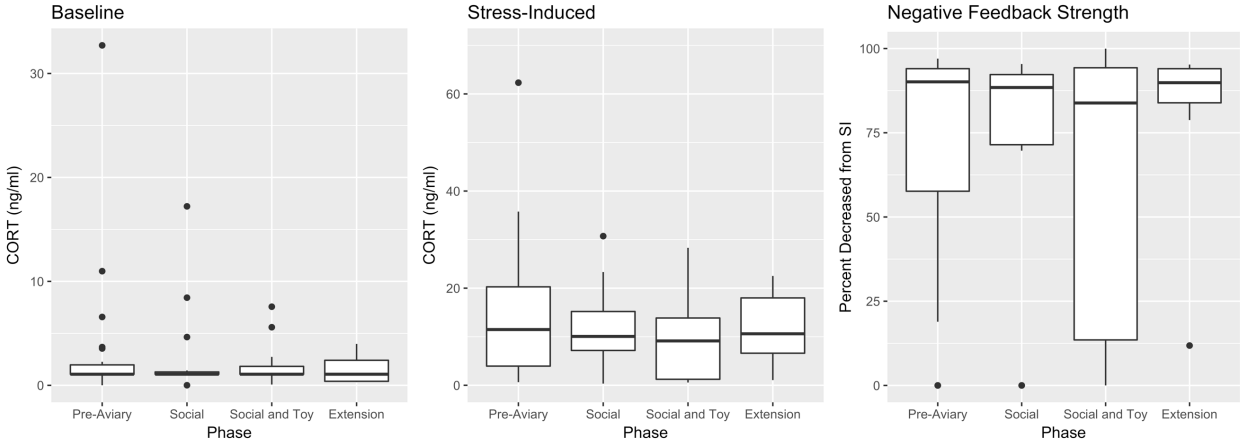

Figure 4.3 The above shows three boxplots; the baseline and stress-induced bleeds have corticosterone concentration for plasma sample in ng/mL on the y-axis. Note the differing y-axis scales on the baseline and stress-induced bleeds. The negative feedback bleed has the percent CORT decreased from the stress-induced bleed to the negative feedback bleed on the y-axis. Each plot has phase on the x-axis. There were no significant changes across phase for the three bleeds.

Figure 4.3 depicts the changes in HPA axis functioning across each phase of the experiment. The linear mixed effect models for the baseline ($F_{3,21}$= 1.05, p=0.32) and stress-induced ($F_{3,20}$=0.36, p=0.78) CORT revealed no significant changes in plasma CORT between

phases. The linear mixed effect model for the negative feedback bleeds ($F_{3,20}$=0.85, p=0.48) revealed no significant changes in negative feedback efficacy between phases.

5. Discussion

5.1 Behavior

The starlings' behavior did not change between the home cage and the aviary except in regards to stereotypies (Figure 4.1), which were more prominent in the home cage. Since enrichment targets stereotypic presentation (Mason et al, 1991), it was expected that the starlings would show fewer stereotypies in the aviary during enrichment than in their un-enriched home environment. However, significant changes in stereotypic behavior across phase appear to be driven by changes occurring within the home cage, implying that the effects of enrichment were apparent even when the individual was not currently experiencing the enrichment. This is a promising result for the efficacy of intermittent enrichment.

Taking the home cage and the aviary together, the behavior of the starlings changed across phases (Figure 4.2). From the Pre-Aviary to the Social Phase, preening increased and stereotypic behavior decreased while perching stayed at a similar level. While the decrease in stereotypic behavior is widely considered to be a positive effect of enrichment, it is harder to assign affect to the increase in preening. An increase in preening has been associated with negative outcomes, such as increased feather damage (Owen & Lane, 2006; Costa et al, 2016), or negative events, such as removal of enrichment objects (Assis et al, 2016; de Almeida, Plame, & Moreira, 2018). However, preening is not always related to feather-damaging behavior (Cussen & Mench, 2015) and can be a socially facilitated behavior in many bird species (e.g., Palestis & Burger, 1998; Birke, 1974). Because the increase in preening is associated with a decrease in

stereotypies and no negative change in health or feather quality was noted throughout the experiment, it is possible that the increase in preening in this case is indicative of an improvement in conditions.

From the Social Phase to the Social+Toy Phase, preening decreased and stereotypic behavior increased to Pre-Aviary levels, while perching increased. The increase in stereotypic behavior during the Social+Toy Phase was unexpected and may be due to neophobic effects. The starlings had never seen the physical enrichment objects prior to this phase and a fear of new enrichment objects has been recorded in this and other species (e.g., Feenders, Klause, & Bateson, 2011; Meehan & Mench, 2002). Additionally, the decrease in preening (if preening is interpreted as a positive behavior) indicates a worsening of captive conditions during this phase. The increase in perching behavior may indicate a reduction in activity which, like preening, is difficult to assign affect to. Increases in activity in response to enrichment has generally been considered a positive effect (e.g., Assis et al, 2016; de Almeida, Palme, & Moreira, 2018) but in other studies, increased restfulness has been touted as a positive outcome (e.g., Laurence et al, 2015). Because of the increase in stereotypic behavior, it appears that the Social+Toy Phase may have negatively affected the birds.

From the Social+Toy Phase through the Extension Phase, stereotypic behavior decreased to Social Phase levels while perching increased. Preening behavior did not change. The decrease in stereotypic behavior after prolonged exposure to the enrichment objects is further indication that the initial increase was due to neophobia. The increase in perching behavior and the lack of change in preening behavior complicate the results and may indicate that there are still aspects of the aviary set-up or enrichment objects that are causing the birds some amount of stress (although, again, the affect behind these two behaviors remains unclear). Although these

enrichment objects are popular and often implemented to provide new behavioral outlets for captive birds, it appears that even long-term, these enrichment objects are not effective. Overall, because stereotypy levels decreased to that of the Social Phase, we conclude that the physical enrichment objects were not detrimental to the effectiveness of the enrichment aviary after initial neophobic responses subsided but also did not add to its effectiveness as compared to just social enrichment.

5.2 Corticosterone profile

Unlike behavior, the CORT profiles of the starlings did not change significantly across phase (Figure 4.3). Baseline CORT levels remained constant throughout the experiment. Stress-induced CORT levels were increased from baseline levels but did not differ across phase, showing that the birds were able to mount a CORT response at each phase of the experiment. Additionally, negative feedback efficacy remained similar across phases, indicating that the birds were able to reduce their CORT responses from maximal levels at each phase.

Similar to many other studies in birds and in mammals, a relationship between stereotypic presentation and CORT among this population of starlings was not found (e.g., Schapiro et al, 1993; Rosier & Landkilde, 2011; de Almeida, Palme, & Moreira, 2018; Gusset, 2005). It is possible that measuring plasma CORT via capture-stress protocols provides information that is not on the correct scale to detect changes in CORT across phases. The plasma CORT measurements were not taken directly after the enrichment protocol – that is, they were taken asynchronously from the enrichment protocol so that the CORT measurements represented the birds' general responses to a capture-stress scenario during each phase and not responses to the enrichment itself. In this way, the plasma CORT measurements represent a broader picture of

the animals' stress physiology similar to the information provided by feather and fecal CORT measurements. Interestingly, these other integrated CORT levels (like feathers in Fairhurst et al, 2011; or feces in Owen & Lane, 2006; Costa et al, 2016; Wielebnowski et al, 2002) have found that enrichment effects stress physiology.

Additionally, it is important to note that at no point during the enrichment phases did the birds exhibit unhealthy CORT profiles, such as the inability to mount a negative feedback response or extremely high baseline concentrations, indicating that the enrichment implemented was at least not detrimental to the physiological stress of the starlings.

5.3 Limitations

This study was limited in two major ways. Firstly, only eight birds were used for the experiment. While small sample sizes are a common limitation of studies involving captive animal stress and enrichment (due to the lack of individuals in captivity in zoos), the small number of subjects is certainly worth mentioning when considering the applicability and reliability of the results. Secondly, because of the small sample size, all starlings went through the enrichment phases in the same order (Social Phase first, followed by Social+Toy, followed by Extension) which means that the confounding effect of order or time cannot be eliminated or ignored. It is possible that the changes in behavior observed were due to the order or time course of aviary exposure.

5.4 Conclusions

Despite these limitations, this study has substantial implications for the use of enrichment for captive animals. This study revealed that intermittent enrichment, a less common form of

enrichment implementation, is effective at reducing behavioral signs of stress. Because trends in reduction of stereotypic behaviors occurred most strongly in the home cage, the effects of intermittent enrichment extended beyond the time in which the birds spent being enriched. Therefore, intermittent enrichment may be an important tool in captive contexts where enrichment cannot be implemented in the home cage or experienced constantly by the animals due to other constraints, either financial or experimental.

This study also revealed that social interaction may be more effective than physical enrichment objects at improving captive conditions for social birds such as starlings. Since the addition of enrichment objects initially caused increased behavioral stress which never decreased beyond the levels observed during the Social Phase, it does not appear that enrichment objects were effective at attenuating behavioral stress. It is important to consider the ecological needs of the species when implementing enrichment procedures; social behavior is highly important for European starlings as a mixed-flock, gregarious species (Bateson & Asher, 2010) while the physical enrichment objects may have been ineffective at mimicking the stimuli that a starling desires from its environment. This is also important in the context of consumer expenditures on popular pet toys – like the ones implemented in this experiment. While these types of toys have proved effective for parrots and nutcrackers (e.g., de Almeida, Palme, & Moreira, 2018; Assis et al, 2016; Fairhurst et al, 2011), they may not be applicable to other species of birds. In the case of the starlings, enrichment objects that mimic natural foraging behavior may be more successful at improving captive conditions, such as was effective in Japanese quail (Laurence et al, 2015).

It is also important to note that despite being wild-caught birds who have spent many years in captivity, stereotypic presentation was attenuated by enrichment procedures. Although it is possible for stereotypies to become engrained and non-responsive to enrichment (Marriner and

Drickamer, 1994), this study shows that enrichment interventions can bring positive outcomes to animals who have experienced captive conditions and developed long-term stereotypic behaviors. It is important to consider implementing and assessing the efficacy of enrichment procedures even if birds have been subjected to lengthy captivity.

Interestingly, behavioral changes were not mirrored by significant changes in stress physiology. While this is not a novel result, it reveals the importance of using more than one metric of stress to evaluate the effectiveness of enrichment. It is also worth questioning if stereotypies and corticosterone are indicative of the same "stress" or affect; after all, the lack of correlation between the two suggests that perhaps they are measuring different things. This study also looked at the plasma CORT profile of the birds asynchronously from the enrichment which provided a different perspective on how the enrichment effected the birds outside of the enrichment protocol. Future studies should compare behavioral, plasma CORT, fecal CORT, and feather CORT measurements in individuals undergoing enrichment to see how the stories told by these measurements compare.

Overall, because the intermittent enrichment protocol decreased signs of behavioral stress and did not adversely impact the CORT profiles of the birds, we conclude that the enrichment procedure improved the welfare of these starlings. Intermittent enrichment represents an important alternative for certain captive contexts and deserves exploration in different species and different implementations, especially if such studies continue to investigate the relationship between behavioral and physiological indicators of stress.

Table 1. Ethogram used during behavioral coding.

TYPE	BEHAVIOR	DESCRIPTION
Locomotor	Perch	Sitting still on perch.
	Hang on Cage	Hanging on the mesh of the cage.
	Fly	Flying un-repetitively.
	Walk	Walking un-repetitively.
Yawn	Yawn	Open-beak yawning.
Self-care	Eat	Eating.
	Drink	Drinking.
	Preen	Grooming own feathers.
	Bath	Sitting in water dish, flapping wings.
	Bill-wipe	Wiping both sides of bill against perch or another object after feeding.
Experiment	Work	Pecking at touch screen, flying from perch to perch.
	Trough look	Looking in the troughs for food without using experiment.
Social	Talk	Chirping, singing, to other birds or with other birds.
	Group bath	In sight of or with another bird, bathing in water dish.
	Touch	Touching other bird non-aggressively.
	Stare	Head held high, accentuated by crown feathers, indicates aggression.
	Open-bill threat	Bill slightly opened, staring at another bird, indicates aggression.
	Fight	Attacks another bird physically.
	Submissive bill-wipe	Wiping both sides of bill against perch or another object to indicate submission (not cleanliness).
Stereotypies	Head weave	Waves head back and forth repetitively.
	Flip	Back-flips or front-flips in place or off of perch or cage repetitively.
	Obsessive bill-wipe	Wiping both sides of bill against perch or another object for extended period of time.

	Feather pluck	Plucks out own feathers.
	Pacing	Repetitive route-tracing, such as hopping from perches in a particular order or flying from corner to corner.
Misc.	Floor scratch	Scratches paper at bottom of cage.
	Babble	Mimicking noises in the room or making random noises.
	Forage	Looking for food in substrate by scratching, pecking.

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