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Effect of the Acute Stress Response on Foraging Behavior in Mountain White-Crowned Sparrows, *Zonotrichia Leucophrys*

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Effect of the Acute Stress Response on Foraging Behavior in Mountain White-crowned
Sparrows, *Zonotrichia leucophrys*

A Thesis Presented

By

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Abstract

Free-living vertebrates likely encounter many stressors throughout their lifetime, from fighting off a predator to coping with unpredictable weather. As a result, vertebrates will mount an acute response to the stressors. Here, we outline previous research conducted in behavioral endocrinology and stress physiology as it relates to our research. We then discuss our study with white-crowned sparrows (*Zonotrichia leucophrys*) in Tioga Pass Meadow, in which we examined how the acute stress response affects foraging behavior 24 hours after a stressor. In birds that underwent a stress series, we found there to be a significant 57% decrease in foraging behavior 24 hours following the stressor. Additionally, we found no significant difference in foraging of unstressed birds during this same time frame. Our findings suggest that the acute stress response in this species causes a reduction in foraging activity 24 hours following a stressful event.

Chapter 1: A Review of Hormonal Effects in Vertebrates

Hormones influence a range of effects from phenotypic differences in populations to variations in behavior for a single organism. As such, they serve as interesting points of study for biologists. These bioregulatory compounds, usually in the form of peptides or steroids, are typically secreted into the blood from a site of synthesis in order to affect sites of action (target tissues) (reviewed in Ketterson and Nolan, Jr., 1999). Simply put, hormones are biological signaling molecules that can affect physiology and behavior. They are critical in achieving the behavioral characteristics of various physiological states the organism undergoes (Landys et al., 2006). More importantly, they often produce their effects during crucial periods in development and offer organismal systems sequential and periodic activation throughout its lifetime (Ketterson and Nolan, Jr., 1999). They are capable of a wide range of effects due to their ability to permit, inhibit, and enhance expression of traits (reviewed in Ketterson and Nolan, Jr., 1999). As a result, they can influence many processes for the organism, including reproduction, digestion, and growth.

Due to their broad field of influence, hormones have the capability to affect various aspects of the vertebrate's life. Testosterone, for example, is a steroid hormone that is important in male sexual development, and has been the subject of many studies in relation to

hormonal effects on physiology. In male dark-eyed juncos (*Junco hyemalis*), researchers have found that manipulated levels of plasma testosterone had various phenotypic consequences, such as alterations in mass and body fat (Ketterson and Nolen, Jr., 1992). Additionally, a study with tree lizards (*Urosaurus ornatus*) found that permanent differentiation of territorial phenotypes is achieved via hormonal mechanisms that happen during their early development (Moore et al., 1998). Phenotypes that are endocrine-mediated, such as the ones mentioned above, are thought to be the result of alterations in responsiveness of receptors on target tissues (reviewed in Ketterson and Nolan, Jr., 1999). Studies such as these demonstrate how hormones play integral roles in achieving specific phenotypic ends for the organism.

Hormones and Behavior

Researchers have also spent a great deal of time examining how hormones can affect and cause variations in behavior, an area called behavioral endocrinology. As with phenotypic influences, hormones' diverse capabilities can lead to various behaviors. Testosterone-treated male lizards (*Sceloporus jarrovi*) were seen more frequently and these more visible ones were less likely to survive in one study (Marler and Moore, 1988), demonstrating how hormones can play a critical role in survival through the behavioral response. Variations in testosterone have also been related to variation in the mating system of birds (reviewed in Ketterson and Nolan, Jr., 1999). These examples demonstrate how alterations in just one hormone (testosterone) can effect survival and reproduction in organisms. With oxytocin (OT), a peptide hormone that is important for social bonding and milk letdown in mammals, it was found that mice with knocked-out OT failed to recognize their familiar conspecifics despite normal learning abilities (Ferguson et al., 2001). In juvenile brown trout (*Salmo*

trutta), growth hormone injections led to a reduced antipredator behavioral response when confronted with a trout predator (Johnsson et al., 1996). For yet other hormones, the gonadotropic follicle-stimulating hormone (FSH) and luteinizing hormone (LH), researchers have found that they can enhance and suppress several physiological, morphological, and behavioral traits in side-blotched lizards (*Uta stansburiana*) (Mills et al., 2008). Moreover, hormone-induced transcription may even cause information to be transmitted via newly synthesized neurotransmitters to new locations, resulting in previously unexpressed behavior (Ketterson and Nolan, Jr., 1999). As demonstrated through the aforementioned studies, in addition to innumerable more, hormones can affect an incredible breadth of behaviors.

Hormones' Relationship to Allostasis

Through their influence on the organism's physiology, particularly as it relates to behavior, hormones can serve as important means in ensuring allostasis for the vertebrate. This is the process of achieving internal stability during change, whether due to environmental conditions or life-history demands (Sterling and Eyer, 1988; Landys et al., 2006). Hormonal secretion can be stimulated by external or internal cues (reviewed in Ketterson and Nolan, Jr., 1999). These cues, if possibly disrupting allostasis, could result in the release of hormones to combat this change. Allostasis is different than homeostasis, which is a process through which variables are kept within a given physiological set point (Landys et al., 2006). Whereas this framework is more rigid, allostasis implies that there could be multiple stable states for the organism. More specifically, the allostatic model considers anticipating need and making crucial adjustments in advance (Sterling and Eyer, 1988).

Hormones play critical roles in the organism's management of its allostatic load through multiple ways. Namely, hormones are selected in response to both internal and external stimuli, they can have numerous targets and effects, and their complexity of action can cause variation in response (Ketterson and Nolan, Jr., 1992). Once the hormone is released, it will travel to the site containing the proper receptor where it can effect change in the organism. These targets can be local or distant from the source of secretion and can utilize the circulatory system as a means of getting from one place to another (reviewed in Ketterson and Nolan, Jr., 1999). Depending on the hormone, it will bind to a particular kind of receptor. Peptide hormones typically bind to receptors in cell membranes because proteins are not lipid-soluble and they act via second messengers (reviewed in Ketterson and Nolan, Jr., 1999). Steroid hormones, which are lipid-soluble, tend to bind to intracellular receptors to act as transcription factors in order to initiate cellular transcription (reviewed in Ketterson and Nolan, Jr., 1999). Hormones and their receptors interact through multiple capacities, which vary depending on factors such as location and timing, multiple different receptors responding to the same hormone, and transcription factors that lead different cells to transcribe different genes in response to the same hormone (reviewed in Ketterson and Nolan, Jr., 1999). Targets may require that the hormone be converted into a particular form before it is active or the affinity of receptors at different targets could dissociate the response (Ketterson and Nolan, 1992; reviewed in Ketterson and Nolan, 1999). In these ways, hormones can create a multitude of effects depending on how they are specifically interacting with their receptors. Once the hormone-receptor complex is created, a cascade results that can carry each physiological response to the next until the desired effect is achieved for the organism.

Glucocorticoids and Behavior

Particularly there has been an increase in the study of glucocorticoid hormones and their influence on behavior. Glucocorticoids (GCs) come in the form of either cortisol or corticosterone. GCs are glucocorticosteroids, meaning they are lipid-soluble and capable of passing through the plasma membrane in order to affect intracellular receptors. They are essential for regulating behavior in addition to coordinating metabolic, cardiovascular, immune and neuroendocrine activities in the body (Uchoa et al., 2014). GCs have been suggested to be important mediators of allostasis due to their ability to allow for rapid readjustment of vertebrate physiology and behavior when necessary (reviewed in Landys et al., 2006). Paramount among this is that GCs' regulation of physiology and behavior, at seasonal baseline levels, can keep systems within the organism operational without inducing a facultative emergency response (Landys et al., 2006).

As a result of the incredible implications within the study of GCs, much research has been done to explore a variety of physiological and behavioral responses to GCs. Research has found that acute glucocorticoid production is linked with achievement of the animal's behavioral goal (Haller et al., 1998). In a study with humans, the findings suggest that higher endogenous cortisol levels may enhance memory for patients battling arachnophobia (Lass-Hennemann and Michael, 2014). Furthermore, there is also evidence that glucocorticoids can be responsible for aggressive behavior. Researchers found that corticosterone applied into the brains of rats stimulated aggressive behavior rapidly (Mikics et al., 2004). Moreover, a study with humans examined how cortisol levels correspond to conduct disorder (CD) in boys. It was concluded that boys with CD had higher levels of cortisol than those without and, additionally, there were higher cortisol levels for boys with an aggressive form of CD than

those without (van Bokhoven et al., 2005). Overall, researchers from this study determined that adolescent boys with chronic reactive aggression seem to have more active stress response systems than their counterparts (van Bokhoven et al., 2005). In bird species, research with song sparrows has shown that high levels of CORT in males may suppress territorial behavior (Wingfield and Silverin, 1986).

The wide variety of responses that result from GC secretion lies in part due to the ability of GCs to interact with different types of receptors. They may produce concentration-dependent effects for the organism due to binding different—and specific—receptor populations (Landys et al., 2006). There are three GC receptor types that can lead to these various effects. Low-affinity GC receptors (GRs) and high-affinity mineralcorticoid receptors (MR) are genomic receptors (Landys et al., 2006). Based on studies thus far with these receptors, the MR is most fully occupied at low plasma GC concentrations (under normal conditions) and the GR requires higher GC levels, like those seen during stress or diurnal rhythm (Reul and Kloebe, 1985). There is also the non-genomic membrane-associated receptor (mGR), which plays an important role in GC binding. Whereas food intake and energy metabolism are transduced using the GR, locomotor activity seems to be regulated using the mGR (Landys et al., 2006). It is worth keeping in mind, however, that GC secretion and subsequent binding to genomic receptors results in alterations to gene transcription and protein synthesis and thus their actions are not immediate (Landys et al., 2006). This is different than the response mediated through nongenomic receptors, which can involve GC binding to mGR receptors and initiating rapid physiological and, in turn, behavioral changes (Haller et al., 1998).

Glucocorticoids' Relationship to Stress

An important application of the information surrounding hormones like the glucocorticoids is their relationship to stress. Stressors can threaten the vertebrate's homeostasis, or the maintenance of internal stability. These modes of stress can come in forms like unpredictable weather changes and fighting with members of the same or other species. To combat stress and prevent disruption of homeostasis, the organism initiates an acute stress response. The acute stress response that takes place in vertebrates is characterized by the actions of the hypothalamic-pituitary-adrenal cortex (HPA) axis. Following signals by tropic hormones released from the hypothalamus and the anterior pituitary, the adrenal cortex releases a glucocorticoid. This glucocorticoid is either cortisol or corticosterone, both of which are abbreviated CORT. Cortisol is released in fish and most mammals whereas corticosterone is released in other animal groups, namely birds, reptiles, and amphibians (Romero and Butler, 2007). Upon release into the blood plasma, CORT travels to receptors on various tissues to affect the physiology and behavior of the organism. Previous studies strongly implicate that GCs aid in the support of energetic demands happening in this stress response (reviewed in Landys et al., 2006). The physiological and behavioral changes that result from the acute stress response are in an effort to help the vertebrate survive the stressor and maintain internal equilibrium. To do this, GCs suppress non-essential life processes—namely, reproductive function—at high levels in order to funnel the energy toward survival (Landys et al., 2006). More specifically, a stress response is a set of behavioral and physiological coping mechanisms which are exhibited during an emergency stage of being (reviewed in Landys et al., 2006). The stress response causes changes in vertebrate physiology, including increasing blood glucose and energy in

exercising muscles and decreasing reproduction (Romero & Butler, 2007; Sapolsky et al., 2000). Effects like increasing blood glucose and energy in muscles can help the organism fight the stressor, if applicable, or fly the scene of stress. Through decreasing reproduction, energy can be funneled into these other activities that increase locomotion. These effects all serve to aid the organism in surviving the stressor.

Another way to consider the use of GCs in ensuring the stress response for the organism is its role in allostasis maintenance. The condition of being “stressed out” is a major form of allostatic load and causes elevation in cortisol levels as a result (McEwen, 2008). In other terms, and as previous studies have shown, GCs have a number of behavioral and physiological effects that promote short term fitness (Wingfield and Kitaysky, 2002). However, in order to ensure there are no long-term deleterious effects on fitness, some species have been found to suppress adrenocortical activity when confronted with prolonged food shortages (Kitaysky et al., 2001a). To prevent a long-term (chronic) stress response, which can be harmful to the organism’s fitness, there is a negative feedback loop. The acute stress response system relies on the hypothalamus to release corticotropin-releasing hormone (CRH), which in turn stimulates the anterior pituitary to secrete adrenocorticotropin (ACTH), which finally triggers the adrenal cortex to release CORT. Once an accumulation of GCs has developed, negative feedback inhibits release of CRH and ACTH. Due to the rapid feedback response, many lines of evidence have suggested that this process is mediated through nongenomic mechanisms (Borski, 2000). An earlier study with white-crowned sparrows supported this evidence, as researchers thought that the rapid effect CORT administrations had on behavior was likely acting through nongenomic means as well (Breuner et al., 1998).

Free CORT, Bound CORT, and CBGs

There are three particularly important players in the vertebrate stress response: free CORT, bound CORT, and corticosteroid-binding globulins (CBGs). Free CORT is not bound to CBGs and as a result is free to interact with tissue receptors to affect behavior and physiology. Bound CORT is attached to CBG and cannot bind to receptors. CBGs are glycoproteins with one binding site for its corticosteroid hormone (Westphal, 1983). They are used as a transport mechanism for water-insoluble steroid hormones, such as CORT (Malisch and Breuner, 2010). Though the free CORT is the hormone configuration that is able to interact with and affect tissues, the bound CORT also serves an imperative role for the vertebrate. This bound complex can act as a reservoir of “inactive” hormone, which can be mobilized if the organism should need it (Malisch and Breuner, 2010). Not only can CBGs act in a way to buffer the actions of CORT, but they also influence the disposition of CORT (Bright, 1995). CBG-binding may also regulate the availability of free CORT to affect tissues and alter its clearance rates from vertebrate systems (Breuner and Orchinik, 2002a). Importantly, GC actions can be regulated through the mechanics of receptor binding as well (Romero and Butler, 2007). The previously mentioned receptors on target tissues can be local, adjacent to, or distant from the source of secretion (reviewed in Ketterson and Nolan, Jr., 1999), which can in turn affect GC action. These receptors can also act differently from each other to affect sensitivity to the stressor (Breuner and Orchinik, 2002b). In house sparrows (*Passer domesticus*), for example, when CORT levels are highest, intracellular GC receptors are at their highest concentration and membrane GC receptors are at their lowest (Breuner and Orchinik, 2002b).

Chapter 2: Research Study: Effect of Acute Stress on Foraging Behavior

Introduction

Conventionally it is assumed that a stressor causes a transient acute hormonal stress response that dissipates after several hours. In the majority of vertebrates, GC levels increase between 4- and 10-fold within minutes of stressor confrontation (Breuner et al., 2008). Baseline CORT levels are expected to rise initially, but by 24 hours following the stressor, presumably these high CORT levels return to baseline. However, recent research has found that CORT levels remain elevated 24 hours following an acute stressor in mountain white-crowned sparrows (Malisch et al., 2010a). Perhaps more importantly, it's also been found that CBG levels are low 24 hours following the stressor, at the same time that there is an elevation in CORT levels (Malisch et al., 2010a). This translates to a high level of free CORT circulating in the blood that is available to bind to receptors on target tissues and affect physiology and behavior of the organism. Previous research in Japanese quail (*Coturnix japonica*) is consistent with the white-crowned study as researchers found a 30% reduction in CBG and 250% increase in free CORT 24 hours following an acute stress handling protocol (Malisch et al., 2010b).

In our experiment, we were interested in examining the effect of the acute stress response on foraging behavior in mountain white-crowned sparrows (*Zonotrichia leucophrys*) 24 hours following a stressor. Our prediction for this study came from a careful consideration of previous studies' findings. First and most broadly, previous research has suggested there is a positive correlation between plasma GC concentration and locomotive behavior (Landys et al., 2006). Elevated levels of GCs, as seen through the acute stress response, would then suggest greater levels of locomotion. It has also been established that a component of the

emergency life history stage is a redirection of everyday behavior to increased foraging and elevated gluconeogenesis (Wingfield and Kitaysky, 2002).

Additionally, earlier research conducted on white-crowned sparrows saw that an increase in CORT levels may have led to an increase in foraging activity (Breuner and Hahn, 2003). In Macaroni penguins (*Eudyptes chrysolophus*), it was found that higher levels of CORT were associated with significantly higher levels of foraging and diving activity (Crossin et al., 2012). Moreover, rats who had undergone adrenalectomy and removal of GCs displayed a reduction in food intake (Dallman et al., 2003). Research with pelagic seabird (*Diomedea exulans*) found that pre-trip CORT levels were positively correlated with distance traveled and maximum range at sea during foraging trips (Angelier et al., 2007a). Especially interesting is a study that found increased plasma CORT levels caused red-eyed vireos (*Vireo olivaceus*) to visit a food bowl with meal worms more frequently compared to control treated birds (Lõhmus et al., 2006). Lastly, researchers found an increased stress response at nightfall in fish (*Acipenser medirostris*), when their activity period is starting, could be linked to increased swimming activity and therefore increased foraging behavior (Lankford et al., 2003). Overall, considering the stress response is activated in an effort to increase energy spent performing “fight or flight” actions, an increase in food intake would be expected to compensate for energy expenditure. We, therefore, predicted to see an increase in foraging behavior in the birds that were stressed 24 hours previously.

Methods

To carry out this experiment, white-crowned sparrows were habituated to 14 potter-feeding stations set in Tioga Pass Meadow, just East of Yosemite National Park, from May 17th, 2014 to June 21st, 2014. Potter stations were locked in the open conformation and

supplied with millet, the preferred seed of the mountain white-crowned sparrow, for two weeks prior to any stress application. Each feeding station was located in a different tree cluster around the meadow and named based on either location or historical name (Appendix, Figure 1 and Table 1).

Species and Sex of Interest:

The species of interest for this experiment was the *Zonotrichia leucophrys*, a migratory songbird that breeds at high elevations and which has been studied in Tioga Pass Meadow for over 35 years. Though males and females were caught and banded during the first two weeks, only males were ultimately stressed in the stress batches, so as to reduce the amount of time females were held away from potential eggs and nestlings at the nest.

Banding:

During the two weeks prior to experimental manipulations, stations were occasionally unlocked (trapping configuration) so birds could be caught and banded with three plastic color bands and a USGS aluminum service band. These bands would help with later identification of the birds on video. Measurements of flattened wing chord, tarsus, keel, cloacal protuberance, fat, and body mass were also taken at the time of banding.

Stress Series:

Stress series were performed in five-day installments. In the locked open conformation, the 14 stations were videotaped for the three days leading up to the stress day. On the fourth day, the stations were placed into the unlocked (trapping) configuration. Acute stress was induced by capturing the white-crowned sparrow, holding him in a cloth bag and taking blood samples immediately after capture (baseline), 15 minutes post-capture and 30 minutes post-capture. The fifth day mirrored the first three, with the station resuming the

locked open position and being taped for foraging activity. Foraging behavior was ultimately quantified by analyzing video recordings of the white-crowned sparrows at the 14 potter stations and calculating the time that each bird spent foraging in and around the stations. This was repeated three times, resulting in three batches of stress series and 200 hours of video.

Data analysis:

All taping was performed with Veho MUVI cameras. Due to camera battery life, the feeding stations could only be taped for 1 hour and 15 minutes at a time (i.e. station 3% was taped each of the three days prior to and one day following a stressor application for approximately 75 minutes, totaling around 300 minutes of footage for those five days of study). Average foraging on days 1-3 was compared to foraging time on day 5 with a paired t-test. Foraging time was adjusted for total tape length, however the results were almost identical to raw foraging time, therefore raw tape time is reported for simplicity. Some birds were stressed in more than one batch and for these birds only the data from their first capture and stress was used in the analysis. Additionally, only birds that were taped on at least one pre-stress day (day 1-3) were included in the final analysis.

Results

During the study, 27 birds were caught and banded and 13 of those underwent stress series (Table 1). Birds were not stressed twice in a row (e.g. in both batch 1 and 2) until sufficient time had passed (at least 10 days) (Table 1). Each batch represents a five-day series of data collection.

Table 1. List of target birds (denoted by their color band sequence) stressed and in which stress batches they participated (n=13).

Bird	Batch 1	Batch 2	Batch 3
KYGS	X		X
GROS	X		
GSMO	X		
RWGS	X		
YGGs	X		
DSRM		X	X
YMKS		X	
DRMS		X	X
OGWS		X	X
OWGS			X
ODKS			X
GMYS			X
WMDS			X

Of the 13 birds that were captured and underwent stress series, nine of them provided pre- and post-stress foraging data to be analyzed. Approximately 200 hours of video were examined and the total amount of foraging time (time spent foraging in and around the feeding station) was calculated for each bird on each day. Statistical analysis performed on these data of interest showed a significant decrease of 57% in foraging behavior post-stress compared to pre-stress (paired t-test, $p=0.0249$, $df=8$, Figure 1).

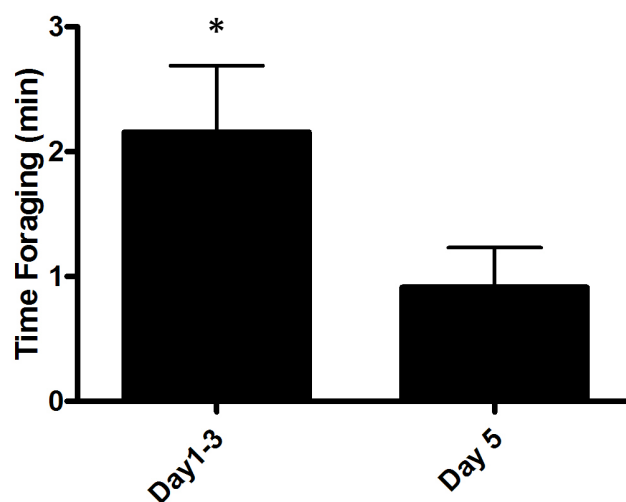


Figure 1. Mean (\pm SE) time target (stressed) birds spent foraging pre-stress and post-stress (n=9).

Time unstressed birds spent foraging was also collected for analysis and days 1-3 and day 5 averages were compared (Figure 2). Birds that foraged but were not caught or stressed did not change their foraging behavior on the same days (paired t-test, $p=0.3927$, $df=20$, Figure 2).

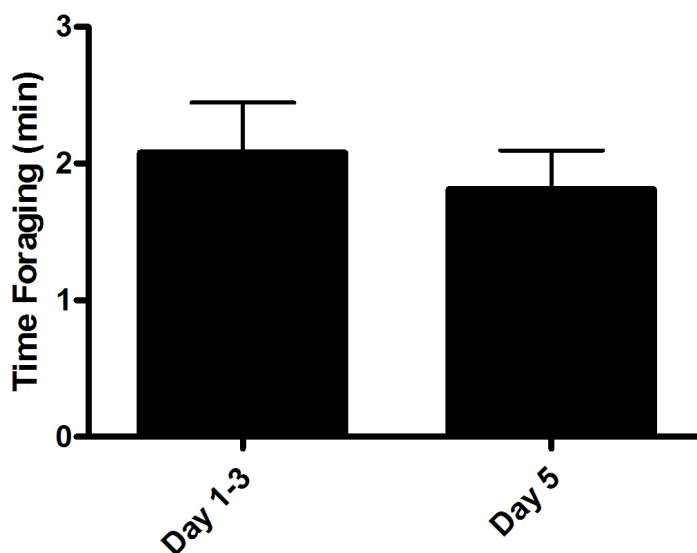


Figure 2. Mean (\pm SE) time each unstressed bird spent foraging during the stress batch (n=21).

Stressed birds' activity was quantified to observe which stations were foraged at pre-stress, which station they were captured and stressed at, and which stations were foraged at post-stress (Table 2). Within these results, three birds foraged post-stress at the same station they had been stressed at 24 hours previously and six birds kept foraging at the same stations pre- and post-stress, regardless of which station they were stressed at (Table 2).

Birds	Pre-Stress Stations (Batch 1)	Post-Stress Stations (Batch 1)	Pre-Stress Stations (Batch 2)	Post-Stress Stations (Batch 2)	Pre-Stress Stations (Batch 3)	Post-Stress Stations (Batch 3)
KYGS	3% Yosemite View	Yosemite View			Yosemite View, 3%, Ranger	3%, Yosemite View
GSMO	Paul Bunion	Paul Bunion				
RWGS	SWW	SWW				
DSRM			120 Pines, LPI, Butt Rain	120 Pines, Butt Rain	120 Pines, Gaylor, Butt Rain, Porsche	120 Pines
YMKS			Butt Rain, Porsche	Butt Rain, Porsche		
DRMS			Ranger, 120 Pines, Yosemite View, Gaylor	Ranger, 120 Pines, Yosemite View, Gaylor	120 Pines, Gaylor, Butt Rain, Ranger, Yosemite View	120 Pines, Ranger
OGWS			Ranger	Ranger	Ranger	Ranger
ODKS					Creek View, Butt Rain	Creek View
GMYS					Butt Rain, Porsche	Creek View

Table 2. List of birds (denoted by their color band sequence) stressed in each batch (stress series) and which traps they foraged at pre-stress and post-stress, as well as what trap they were caught and stressed at (n=9).

Discussion

We hypothesized that we would see an increase in foraging behavior in birds stressed 24 hours previously. However, we found a decrease in foraging activity for birds stressed 24 hours earlier. In fact, there was a significant 57% decrease in foraging post-stress (Figure 1). Moreover there was no significant decrease in the unstressed birds during these same days (Figure 2). Through examining which stations were being visited pre- and post-stress in stressed birds, we have seen that six of the nine birds kept foraging at the station(s) they had previously been visiting (Table 2). Interestingly, three birds foraged post-stress at the same station they had been captured and stressed at 24 hours before and of those three, one bird (KYGS; black-yellow-green-service banded male) started foraging at the station it was stressed at post-stress (Table 2), suggesting that trap aversion is not a likely explanation for our observed reduction in foraging behavior.

Of the 13 birds that were caught and stressed, 9 were included in the final analysis. The four excluded birds did not participate in pre-stress foraging (e.g. they did not forage on screen at the stations on days 1-3). Due to not being able to quantify their foraging behavior prior to their stress experience, they were excluded from the final analysis. However, this does not mean that these birds did not forage 24 hours post-stress; due to the camera battery life, the stations were not taped for an entire 24 hour day. Therefore, the four missing birds could have been demonstrating foraging behavior, but missed the critical time that marked exactly 24 hours post-stress, during which time taping was occurring.

Additionally, our experiment utilized potter-feeding stations as the means of capturing these white-crowned sparrows. We should consider that the stations were themselves triggering a lack of foraging in and around the station area (trap aversion), rather

than the actual stress experiment affecting foraging. However, we have many reasons to suggest this was not the case for our study. We had a total of 192 captures throughout the field season and, of these, 165 were recaptures. In other words, 86% of our captures were recaptures. Moreover, 24 out of the 27 birds banded were caught again. These results support the idea that the trap itself was not a tremendous deterrent to the birds. In fact, 10 of the 27 birds caught visited stations multiple times a day. For example, KSRO (black-service-red-orange banded female) was once caught three times in one day. If necessary, a way to further alleviate concerns of birds demonstrating decreased foraging behavior due to trap-aversion, this experiment model could be re-performed with the use of mist nets. However, alternations would need to be made to ensure that specific foraging behavior could be recorded via videotaping.

Several previous studies support the findings in this experiment. Research with Adélie penguins (*Pygoscelis adeliae*), for example, found that an increase in CORT levels decreased foraging time at sea (Thierry et al., 2014). A closely related study also with Adélie penguins found that penguins with elevated pre-trip CORT levels spent less time at sea and remained closer to their colony (Angelier et al., 2008). Interestingly, in dark-eyed juncos, researchers found that there were no differences in foraging behavior or food consumption despite changes in fat and muscle masses for birds given CORT implants (Gray et al., 1990). Also worthy of consideration are the factors that influence the early stages of the acute stress response, such as CRH and ACTH. The central effects of CRH in the stress response have been noted to inhibit foraging in order to support necessary survival behaviors (Crespi and Denver, 2005), for example. Finally, previous studies examined baseline CORT and

foraging whereas here we examined behavior 24 hours following an acute stressor, which may modulate foraging behavior through different mechanisms.

Chapter 3: Implications and Future Directions

In reflection of the research conducted, it is important to consider what implications our findings have in the areas of stress physiology and behavioral endocrinology. Our results suggest that 24 hours following a stressor, white-crowned sparrow males will decrease their foraging behavior at and around feeding stations. Many questions follow as to what this means for avian species that are faced with stressors in the wild. First off, as with previous research in this area of study, we make a crucial assumption that capture and handling create the same variation in individuals which more natural stressors would inspire (Breuner et al., 2008). Our results, if this assumption is accurate, imply that white-crowned sparrows would decrease their foraging behavior 24 hours after being presented with a naturally found stressor, such as unpredictable weather or predation. Future studies could examine this idea more closely by conducting an experiment exploring foraging behavior after an environmental stressor (for example, after a snow storm) to see if natural stresses can bring about results such as ours.

It is also known that food restriction and deprivation can activate the HPA axis and increase CORT levels, which in turn is thought to increase foraging rates (Breuner and Orchinik, 2002b). Our potter-feeding stations were seeded every day of the research season and nothing suggested the white-crowned sparrows were suffering from food shortages. It could be the case then that there was no observed increase in foraging behavior 24 hours following the stressor due to the birds having sufficient feeding opportunities. Furthermore, energy availability has been shown to play a role in regulating directionality of GC responses

(Astheimer et al., 1992). It was found that fasting white-crowned sparrows responded to CORT implants by foraging for longer and with greater intensity than their non-fasting counterparts (Astheimer et al., 1992). In this same light, limited and unpredictable food alone was found to be able to trigger significant changes in plasma CORT levels in mountain chickadees (*Poecile gambeli*) (Pravosudov et al., 2001). If the birds had sufficient energy stores, they may have felt less of a need to forage from the feeding stations post-stress. Moreover, previous research with GC implants in the study organism, the white-crowned sparrow, increased the activity range for the implanted birds around the breeding site (Breuner and Hahn, 2003). Additionally, a study conducted in black-legged kittiwake (*Rissa tridactyla*) discovered that CORT-implanted parents spent more time away from the nest and were perhaps foraging during this period (Kitaysky et al., 2001b). We were limited to 14 potter feeding stations and 14 video cameras. As such, we could not collect video data that encapsulated all foraging occurring 24 hours post-stress at all areas of our field site. Though we designed the study to allow our birds of interest to become habituated to the stations as feeding modules, there could have been target birds that foraged for longer amounts of time than in the three days pre-stress but did so away from the stations. In an ideal situation, this foraging would have been accounted for; the addition of a small camera to the birds, similar perhaps to a GoPro camera, would likely prove useful in filling in this gap of data for future studies. Additionally, and perhaps more simply, this experiment model could be used for a study with white-crowned sparrows in a lab setting so as to account for all foraging occurring 24 hours post-stress.

Additionally, another study with black-legged kittiwake suggested that birds in good condition, and especially ones with CORT implants, gained more mass than controls

(Angelier et al., 2007b). Moreover, researchers suggested that when these birds are faced with stressful conditions (i.e. environmental changes), birds in good condition could afford to increase foraging activity to provision for their young, while birds in poor condition would have to funnel their energy towards self-maintenance at the cost of their brood (Angelier et al., 2007b). Taking these findings into consideration, it is possible that our target birds varied in their degree of condition, with some who foraged more post-stress being in better condition than those who foraged less.

In order to extrapolate our findings to describe wild white-crowned sparrows' acute stress responses and their effect on foraging behavior, this experiment would need to undergo some supplemental work. Most importantly, it would be critical to perform the stress series on more birds in order to gather more data for analysis. A larger sample would provide a better, more fair assessment of the behavioral changes seen following an acute stress response.

Of equal importance would be to examine the physiological response following capture and handling through running corticosterone and CBG assays. Results from these analyses would aid tremendously in our understanding of the acute stress response and its effects on foraging behavior. A colleague of mine in Dr. Malisch's lab is currently working on a thesis that will include these hormonal analyses. Her work will examine how the birds we stressed (Chapter 2, Table 1) reacted physiologically to the stressors. From this thesis, we will hopefully be able to draw conclusions as to how a vertebrate's hormonal response to stress can predict whether or not they will forage at feeding stations 24 hours following the stressor. Furthermore, research conducted in the future will look at how foraging intensity (i.e. foraging rate) is affected by the acute stress response. Results from this research could

help further the findings presented in this manuscript through providing more information about vertebrate foraging behavior 24 hours post-stress.

Overall, the research presented here hopes to extend our current understanding of the acute stress response in vertebrates. In this ever-changing world, it is necessary to develop a strong knowledge base as to the effects of stress on living organisms. The findings from this study suggest that stressors have the potential to affect the organism for much longer than previously expected, and in ways that could be detrimental to its survival and fitness. It is with this in mind that we hope to see future works examine all the ways in which the acute stress response can affect physiology and behavior.

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Appendix



Figure 1. Layout of field site with positions of the 14 potter-feeding stations (denoted by blue X's) (scale marks 500 feet).

Table 1. List of the potter-feeding stations used (by name) and their GPS locations (n=14).

Potter feeding stations	GPS Location
3%	301561/4198492
Boundry	301545/4198414
Ranger	301641/4198427
Yosemite View	301611/4198481
120 Pines	301620/4198550
Gaylor	301581/4198574
Butt Rain	301599/4198609
LPI	301677/4198604
Porsche	301645/4198651
Creek View	301658/4198686
SWW	301656/4198725
50 Pines	301690/4198768
Patcholi Stink	301765/4198845
Paul Bunion	301677/4198895

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