

The Effect of Learning on Heart Rate and Behavior of  
European Starlings (*Sturnus vulgaris*)

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## Abstract

Five wild-caught European starlings (*Sturnus vulgaris*) were exposed to a learning task in order to determine whether heart rate (HR) and behavior responses to the learning are similar to changes seen during chronic stress conditions. Birds performed a task in a closed economy that required them to discriminate between images of opposite convexity (one concave and one convex) based on shading cues, a task that a previous unpublished study (Qadri et al., 2010) demonstrated that starlings can learn. Once the starlings learned the task, we changed the task in three ways: (1) we manipulated the angle and shape of the image; (2) we altered the availability of the task; (3) and we reversed the positive stimulus. During each of these changes we measured the effects of having to adapt previously established behaviors and learn new ones. Learning the discrimination task did not appear to chronically stress the starlings. They decreased their HR, rather than the predicted stress-induced increase, in response to the training and original discrimination treatments. However, HR increased when the task became unavailable and decreased upon its return, showing that the birds were capable of mounting a physiological response to a condition perceived as stressful. Behaviors measured included the accuracy of responses, the number of trials/min, and the number of perch hops/min, the later serving as a measure of activity within the testing section of the cage. Birds decreased the number of trials/min in the first day of the discrimination task and reversal treatment. The

number of trials/min increased through the initial learning period of these treatments, as did the success rate, suggesting that starlings may try to conserve energy expenditure when they have less access to food. This is also supported by the decrease in perch hops/min when the task was unavailable, and the subsequent increase upon its return. Overall, these results suggest that learning per se is not a stressor for wild animals.

## 1. Introduction

Learning to cope with a constantly changing environment is essential to an organism's health and survival. Stress is a term used to describe unpredictable conditions or events that threaten an organism's homeostasis and trigger physiological and behavioral responses (Wingfield and Romero, 2000). Catecholamines and glucocorticoids are released in response to acute stress in birds, and are shown to affect the cardiovascular system by increasing heart rate and blood pressure (Sapolsky and Romero, 2000). Corticosterone (CORT) is the main type of glucocorticoid released by the hypothalamus-pituitary-adrenal pathway in birds, and helps maintain sustained energy availability in response to stress (Wingfield and Romero, 2000). Long-term activation of this pathway, however, is known as chronic stress, and has been shown to increase overall CORT production in birds, but attenuate the response to an acute stressor (Rich and Romero, 2005; Strohlic and Romero, 2008).

Cardiovascular consequences of chronic stress include increased risk for the development of cardiovascular disease and hypertension (e.g. Sapolsky and Share, 1994, Rupp 1999). In European Starlings (*Sturnus vulgaris*), chronic stress has been shown to alter heart rate (HR) by increasing daytime baseline HR, but decreasing the HR response to acute stressors (Cyr et al, 2008). These studies show a clear negative effect of exposure to chronic stress on a bird's

cardiovascular health, allowing changes in heart rate to serve as an indication of whether an animal perceives an event as stressful.

Recent studies have suggested that a bird's response to laboratory stressors may differ from their response to conditions that they may face in the wild (Romero and Remage-Healy, 2000). The way an animal perceives its environment is extremely important to their survival, as they must be able to constantly evaluate their surroundings to adapt their old behavioral responses and learn new behaviors to new conditions. Studies have examined the effect of changing environmental conditions on eliciting an avian stress response (e.g. Romero et al 2000; Wingfield et al., 1998), but none have explicitly tested whether actual learning is the critical stressor in these situations.

Studying an animal's perception of the visual images in its environment is a reliable way to investigate the effect of learning independently of other environmental factors (D'earth and Stone, 1998; Neuringer, 2004). Many species of birds have displayed the ability to discriminate between visual images based on number of stimuli, as well as more complex cues such as dimension, shading, and shape ([Burkhalter](#) and [Cuénod](#), 1978, Alonso, 1998; Cavato and Cook, 2006). Birds also display similar reactions to objects and pictures of the objects, validating tasks using pictures to represent bird's reactions to objects (Bovet and Vauclair, 2000). Pigeons are able to transfer specific characteristics of an object to unfamiliar stimuli, showing the ability to encode details and use memory to change behavior (Emmertson and Delius, 1993). Shading is an extremely important cue in the perception of an object's shape, and the ability to discriminate objects by their shading has been documented in humans, mammals, and birds (Tomonaga, 1998). The importance of shading is also seen in the evolution of countershading by many prey, in which they display coloring that eliminates shading information from the sun that aids predators in detecting prey (Ruxton et al., 2004). While the majority of research on shading

perception in birds uses pigeons as the study species (e.g. reference), a recent experiment showed that European starlings were also able to successfully use shading information in discrimination tasks (unpublished, Qadri, 2010).

Though the effect of stress on learning hasn't directly been studied in birds, CORT has been implicated in affecting both learning ability and cognition in many other taxa (e.g. Shors et al, 1992; Saldanha et al., 2000, Vita et al., 2007). CORT implants in developing seabird chicks negatively affected performance on visual association and spatial tasks involving locating food sources, as well as speed of learning acquisition (Kitaysky et al., 2003). However, in Zebra finches bred for high baseline CORT, only spatial memory and acquisition speed were affected (Hodgson, 2007). Some studies suggest that mild increases in CORT improve cognitive performance, as moderate CORT increases in Mountain Chickadees were found to improve spatial memory and retrieval compared to control birds (Pravosudov, 2003). Clearly, more studies need to be done to understand the relationship between learning and stress in birds.

This study expands upon previous research showing successful visual discrimination based on shading cues by European starlings to examine the physiological and behavioral effects of learning. The response of HR to the training and the acquisition of visual discrimination tasks in a closed economy system were measured to determine if starlings perceived learning as a stressor. Once the starlings were successful at the original task, we began a series of manipulations to alter the type of visual image or the availability of the task in order to determine whether the process of learning to change established behaviors was also perceived as stressful. Our primary hypothesis was that the adaptation and acquisition of new behaviors in response to environmental change would be perceived as stressful for the birds and result in an increase in HR during the learning tasks.

## 2. Materials and Methods

### 2.1 *Animals*

We chose European starlings (*Sturnus vulgaris*) as our study species because of the extensive research published on this species' response to acute and chronic stress in both laboratory and natural conditions (e.g. Astheimer et al., 1991; Wingfield and Romero, 2001; Rich and Romero, 2005). Starlings have also shown the ability to distinguish the convexity of objects based on shading cues, validating the use of visual image discrimination to study the effects of learning (unpublished data, Qadri 2010). Previous experiments have also successfully used implanted heart rate monitors in starlings while maintaining normal motion and flight activity in the birds (Nephew et al, 2003; Cyr et al, 2008).

Five wild European starlings were caught in winter 2009 in eastern Massachusetts. They were immediately brought to Tufts University (Medford, MA, USA) and housed in an outdoor aviary. During this time, water and Purina Mills Start and Grow<sup>®</sup> were provided *ad libitum*. Water remained *ad libitum* throughout the entire experiment. All birds underwent surgery to receive implanted heart rate transmitters, and fully recovered before the experiment began (see below). These starlings were also previously used in an experiment studying heart rate and stress, but were returned to the outdoor aviary for over two months before the current experiment began. Two of the subjects were female and three were male. Previous experiments have shown no difference in the stress response between sexes in captive starlings (Nephew and Romero, 2003). The birds were brought from the outdoor aviary and allowed to acclimate to indoor individual cages for over a week before the experiment began. Birds were kept on a 12L: 12D light:dark cycle, with lights turned on at 7:30am and turned off at 7:30pm. The Association for

Assessment of Laboratory Animal Care guidelines were adhered to during all experiments and were approved by the Tufts University Institutional Animal Care and Use Committee.

### *2.2 Heart-Rate Transmitter Implantation*

A complete description of the implantation procedure can be found in Nephew and Romero (2003), but will be summarized below. Starlings were anesthetized by a mixed injection to the pectoral muscle of ketamine (30mg/kg) and xylazine (10mg/kg). ECG leads surrounded by flexible polyurethane material were inserted into an incision in the abdomen, threaded under the skin, and sutured to muscles under the skin in the neck and pygostyle. The transmitter (4g, dimensions 20mm x 10mm x 10mm) was placed in the abdominal cavity, which was then sutured shut. All closed incisions were treated with antibiotic ointment. Birds were given six days to recover in indoor individual cages.

### *2.3 Heart-Rate Measurements*

The heart rate transmitters emit a signal picked up by a receiver attached to the cage. The receiver sends the data to a computer in an adjacent room, allowing data to be collected without disturbing the birds and affecting their HR. We used Dataquest Advanced Research Technology Gold 4.0 (St. Paul, MN) software to continuously record ECG signals and compute the average heart rate per minute every 30 seconds.

HR data were recorded when the birds were undergoing new types of training or visual discrimination tasks. Whenever possible, measurements were taken for seventy-five minutes after introduction of the novel training or task. Average HR per minute was determined over fifteen minute intervals. On days when there were no task manipulations, heart rate measurements were taken over a seventy-five minute period corresponding to the same time of day.

## *2.4 Apparatus*

The cage used to house the starlings in this experiment was the same used in a previous experiment testing the ability of starlings to use shading in visual discrimination tasks (unpublished data, Qadri 2010). One side of a metal-wired cage (dimensions: 35.5cm x 46.0cm x 34.5cm) with a plastic bottom was removed and attached to an open plastic opaque box (dimensions: 35.5cm x 35.5cm x 30cm) so the bird could move freely between the two areas. The cage contained the water dish and 1.75cm thick perch. The box served as the testing area, and the side opposite the opening of the cage was made of clear plexiglass. Behind the plexiglass was a Dell 1908 FPt LCD monitor which displayed the visual stimuli. The monitor remained on during the entire “day” when the lights were on, and automatically turned off at “night” when the lights turned off, but the computers remained running throughout the entire experiment. The testing area also contained three 13cm perches, one in the center of the area, and one on each side. Perches had to be depressed for 300ms in order for the computer to register a perch selection to prevent triggering by unintentional movement. In front of each side perch was a trough to catch food pellets released from feeders (Phidgets. Inc) located outside of the cage.

The tubes connecting the feeders to the troughs were narrow and were clogged easily, so BioServe® dustless precision pellets were used throughout this experiment. When moved to the testing apparatus, subjects were given these pellets mixed in equal amounts with their previous food during the acclimation period. The amount of pellets was increased daily until after a few days, the subjects were eating only the new pellets. Two birds were unable to adjust to the new pellets, and were removed from the study and replaced with two other starlings.

## *2.5 Discrimination Task and Training*

### *2.5a Visual Stimuli*

The stimuli, training and original visual discrimination task are the same as the one used in a pilot experiment on the ability of starlings to discriminate convexity based on shading cues, and is summarized by Qadri et al., (unpublished, 2010). The visual stimuli presented were blue or peach continuous surfaces, light from above with a ripple in the middle showing concavity or convexity. Lighting originated from either the rear-left, rear-right, front-left and front-right to create different shadows on the images. The shapes were all the same size and had a camera angle of 25° or 35° in respect to the image.

### *2.5b Central Perch Training*

The training period conditioned the starlings to associate perching with a food reward. Initially, the side perches were removed so only the center perch was present. The birds first learned to associate triggering the center perch for 300ms with a warning signal (a large white circle in the center of the screen) before the visual discrimination task could appear. The signal was displayed for 15s, and if the center perch remained inactivated, would disappear for 2 seconds before reappearing. If a starling activated the center perch when the warning signal was not displayed, it would remain hidden until the bird moved from the perch. When the starling perched when the warning signal was displayed, a food pellet was released. The side the pellet was dispensed from was random. The computer program was able to track each starling's progress and success at correct perch activation. After a high success rate (~85%), was reached, indicating adequate conditioning, the bird could move to the next training phase.

Starting with this initial training with the central perch, the starlings were moved to a closed economy. The only food available was from the successful completion of the task and the task was available throughout the daylight hours. Weight was monitored every other day during the training period and bi-weekly during the experimental period to ensure body weight was

maintained. Amount of food received was also monitored continuously throughout the experiment to ensure the subjects were receiving enough food.

### *2.6c Side Perch Training*

One side perch (native perch) was placed into the testing area. The side was determined randomly, with a right side native perch for 3 birds, and a left side native perch for two birds. This phase of training required the starling to successfully activate the center perch in order for the monitor to display the visual stimuli above the native perch. The native perch had to be depressed while the stimulus was displayed in order for a food pellet to be released. The positive stimulus was counterbalanced between birds and native perch side to eliminate bias towards a particular shape. Once the birds had adequate success with the native perch, it was removed and replaced by the opposite-side (secondary) perch. The birds went through the same training with this perch, with the visual stimulus appearing on the side as the secondary perch. Once the birds had been successfully trained to use the apparatus they were given a day of free feeding, where the monitor remained off for the entire day and food was provided *ad libitum*. On this day, all three perches were placed into the testing area.

### *2.7 Discrimination Task*

The discrimination task began immediately following the training period. During a single trial, a visual stimulus was displayed on the computer monitor next to each side perch. One stimulus was positive, and had the same convexity of the stimulus used during the training period. When the perch in front of this stimulus was triggered, a food reward was released into the trough on the same side. The stimulus of the opposite convexity was the negative stimulus, and triggering the perch underneath this image resulted in a timeout of the discrimination apparatus for 15 seconds, when no warning signal was displayed. A choice between the stimuli

had to be made within 20 seconds of their appearance, or the trial would be repeated. The two types of stimuli were presented in a randomly distributed order, but in equal numbers throughout each block.

### *2.8 Correction Procedures*

During acquisition of the visual discrimination task, bias towards the native perch was seen in two of the starlings. One bird displayed this bias during the training phase, and went through two additional days of side perch training until it was eliminated. The other bird showed this bias during the acquisition of the discrimination task, and a correction procedure was implemented. When the bird chose the negative stimulus while the correction procedure was running, the trial would repeat until the correct choice was made. The bird that displayed this bias received this procedure half a day before the bias was eliminated.

There were a few minor instances of other events that may have temporarily affected the training and learning acquisition. The food delivery tubes occasionally became clogged, and no food reward was delivered from that side, decreasing the effectiveness of the condition period. Also, feces would build up underneath the central perch, and if not removed, would prevent the perch from being depressed enough to be recognized by the computer. All of the starling's progress was continuously monitored, and problems were fixed immediately after detection to ensure these factors had no significant impact on the bird's discrimination ability.

### *2.9 Manipulation of Task*

#### *2.9a. Probe Trials*

When a high success rate of discrimination was sustained (accuracy above 85%), we changed the appearance of the two stimuli by varying the shape of the image and camera angle

between  $5^\circ$  and  $85^\circ$ . Previous studies have indicated that starlings have been shown to be able to successfully discriminate between stimuli with these novel camera angles and shapes, though accuracy decreased as the change in angle increased (unpublished, Qadri 2010). During probe trials, novel stimuli were dispersed randomly throughout the experimental period, appearing with the same frequency as the original stimuli. Probe trials spanned 2 hours, and were used on days where only the original discrimination task was given. Only four birds reached the probe treatment.

### *2.9b Availability of the Discrimination Task*

The discrimination task was made temporarily unavailable to investigate how the birds reacted to being unable to access the task and food reward. When the lights turned on, the monitor remained off, though water was still provided *ad libitum*. After seven hours, the monitor was turned on and the warning signal displayed, and the birds could activate the perches and perform the discrimination tasks. Only four birds underwent this treatment.

### *2.9c Reversal of the Discrimination Task*

Each starling had been trained to have the positive stimulus correlated to either a convex or concave shape. In this manipulation, the convexity of the positive and negative stimulus was reversed. In order to obtain a food reward, the bird would have to extinguish its previous stimulus-convexity association, and learn a new association between the opposite shape and the food reward. This reversal was permanent for the remainder of the discrimination task. Only four birds reached the reversal treatment.

### *2.10 Behavior*

The computer program recorded different behaviors relating to the starling's use of the discrimination apparatus to provide a more detailed analysis of their reaction to learning. All behavioral measures were recorded concurrently with HR. The behaviors measured were overall accuracy in the discrimination task, the number of trials per minute, and the number of center perch hops per minute. Accuracy provided information on the birds' success at the different tasks and how quickly they were able to learn the discrimination task. The number of trials per minute recorded the average number of completed trials and was a measure of how hard the birds were working at the task. The minimum time for a single trial was estimated to be five seconds, allowing for time for the starling to depress the center perch, choose a side perch, consume the reward and for the next warning signal to appear. Since there was no time-out for a correct response, a bird could continuously work if its accuracy was 100%, making the maximum trials per minute twelve. Perch hops per minute measured the number of times the center perch was depressed, even if the trial was not completed. This served as a way to measure general activity, as a starling flying or hopping around its cage would likely land onto the center perch and activate it.

### *2.11 Data Analysis*

All data were analyzed in Prism (ver. 5.0). We used repeated measures, two-way ANOVAs with post hoc tests to determine if there was an effect of treatment on HR and behavior over time. Data were partitioned by type and day of treatment: training, original discrimination, probe, task availability and reversal. Each measurement was taken either 15min, 45min or 75min after stimulus presentation, to distinguish between immediate and delayed changes in HR or behavior. For the probe treatment, task availability, and reversal, HR and behavioral responses

were compared to control measurements taken at the same time of day but the day before the new treatment began.

We could only collect behavioral data when the discrimination task was active, so that certain treatments (training and unavailability of discrimination task) did not have behavioral data. Since one bird never reached a success rate of 85% in the original discrimination task, values for HR were taken on the last day of the experiment for this measurement. Graphed data are displayed as the mean of the measurements for all five birds  $\pm$  standard error.

### 3. Results

#### 3.1 Training

There was a significant effect of training (Fig. 1) on heart rate, which significantly decreased as training commenced (center perch only), started to recover when training moved to the native perch, and then nearly fully recovered when training was completed (effect of training:  $F(3,12)=3.67, p=.02$ ). There was no effect of time since stimulus presentation:  $F(2,12)=.06, p=.494$ ), nor was there an interaction between time since stimulus presentation and treatment on heart rate (training\*time since stimulus presentation:  $F(6,36)=.23, p=.96$ ).

#### 3.2 Original Discrimination Task

There was a significant effect of the original discrimination task (Fig. 2) on heart rate, which progressively decreased over the consecutive days of the discrimination task (effect of discrimination:  $F(3,12)=3.79, p=.02$ ). There was no effect of time since stimulus presentation (i.e. onset of exposure to the discrimination task) on heart rate (effect of time since stimulus presentation:  $F(2,12)=.05, p=.95$ ), or interaction between treatment day and time since stimulus

presentation on heart rate (discrimination\*time since stimulus presentation :  $F(6,36)=.28$   $p=.94$ ).

There was also a significant effect of the original discrimination task on accuracy, which increased during the discrimination period (effect of discrimination:  $F(3,12)=15.70$ ,  $p<.0001$ ).

There was no effect of time since stimulus presentation on accuracy ( $F(2,12)=.07$   $p=.93$ ), nor an interaction between discrimination day and time since stimulus presentation on accuracy (discrimination\*time since stimulus presentation:  $F(6,36)=.98$   $p=.45$ ). The time since a stimulus presentation in the task had a significant effect on trials/min, which decreased as the bird was exposed to a new task longer (effect of time since stimulus presentation:  $F(2,12)=5.99$ ,  $p=.02$ ).

There was no effect of the discrimination task on trials/min or an interaction between the original discrimination task and time since stimulus presentation (effect of discrimination:  $F(3,12)=1.20$   $p=.33$ , discrimination\*time since stimulus presentation :  $F(6,36)=.45$   $p=.84$ ). There was also a significant effect of the original discrimination task on perch hops/min, which increased over the acquisition of the original discrimination period, but decreased when the bird finally learned the task (effect of discrimination:  $F(3,12)=3.93$ ,  $p=.02$ ). There was no effect of time since stimulus presentation on perch hops/min, or interaction of the discrimination treatment and time since stimulus presentation on perch hops /min (effect of time since stimulus presentation :  $F(2,12)=1.60$ ,  $p=.24$ , discrimination\*time since stimulus presentation:  $F(6,36)=1.51$   $p=.20$ ).

### *3.3 Probe Treatment*

The probe treatment (Fig. 3) had no significant effects on heart rate (effect of probe:  $F(2,9)=1.75$ ,  $p=.20$ , effect of time since stimulus presentation (i.e. exposure to probe):  $F(2,9)=.06$   $p=.939$ , probe\*time since stimulus presentation:  $F(4,18)=.09$   $p=.99$ ), accuracy (effect of probe:  $F(2,9)=1.36$ ,  $p=.28$ , effect of time since stimulus presentation:  $F(2,9)=.07$ ,  $p=.93$ , probe\*time since stimulus presentation :  $F(4,18)=.87$ ,  $p=.50$ ), number of trials/min (effect of

probe:  $F(2,9)=.03, p=.97$ , effect of time since stimulus presentation:  $F(2,9)=1.16 p=.37$ ,  
 probe\*time since stimulus presentation:  $F(4,18)=1.53 p=.23$ ), or perch hops/min (effect of probe:  
 $F(2,9)=2.48, p=.11$ , effect of time since stimulus presentation:  $F(2,9)=.93 p=.43$  probe\*time  
 since stimulus presentation:  $F(4,18)=2.0 p=.14$ ).

### *3.4 Availability of Discrimination Task*

There was an effect of the availability of the discrimination task (Fig. 4) on heart rate (effect of availability of discrimination task:  $F(3,6)=5.42 p=.02$ ), as heart rate increased when the task became unavailable, and decreased when it became available again, though it remained elevated compared to the control measurement. There was no effect of time since stimulus presentation ( $F(2,6)=.04 p=.97$ , nor in the interaction between the availability of the discrimination task and time since stimulus presentation ( $F(6,18)=.25 p=.90$ ). There were no effects or interactions of the availability of the discrimination task and time since stimulus presentation on accuracy (effect of availability:  $F(1,6)=.74, p=.42$ ,  $F(2,6)=.36 p=.71$ , effect of time since stimulus presentation :  $F(2,6)=.05 p=.95$ ), number of trials/min (effect of availability:  $F(1,6)=.45, p=.53$ , effect of time since stimulus presentation:  $F(2,6)=1.26 p=.35$ , availability\*time since stimulus presentation :  $F(2,6)=1.11 p=.39$ ), or on the number of perch hops/minute (effect of availability:  $F(1,6)=.01, p=.91$ , availability\*time since stimulus presentation:  $F(2,6)=1.91 p=.23$ ). However, there was a nearly significant effect of the time since stimulus presentation for the availability of the discrimination task on number of perch hops/min, as the number decreased after the unavailability, but then increased compared to the control as the task remained available for a longer period of time (effect of time since stimulus presentation:  $F(2,6)=4.25 p=.07$ ).

### *3.5 Reversal Treatment*

There were no effects of the reversal treatment (Fig. 5) on heart rate (effect of reversal:  $F(2,9)=1.36, p=.28$ , effect of time since stimulus presentation:  $F(2,9)=.30, p=.75$ , reversal\*time since stimulus presentation:  $F(4,18)=.73, p=.59$ ), but both accuracy ( $F(2,9)=29.18, p<.0001$ ) and the number of trials/min ( $F(2,9)=3.68, p=.05$ ) decreased from the control period to the first day of the reversal treatment and then increased from the first day to the second day of the reversal treatment. There was no effect of time since stimulus presentation ( $F(2,9)=.09, p=.91$  and  $F(2,9)=.85, p=.46$ ) or interaction between the reversal treatment and time since stimulus presentation ( $F(4,18)=.40, p=.80$  and  $F(4,18)=.07, p=.99$ ) on accuracy and number of trials/min, respectively. Furthermore, reversal had no effect on the number of perch hops/min (effect of reversal:  $F(2,9)=.41, p=.67$ , effect of time since stimulus presentation:  $F(2,9)=1.57, p=.36$ , reversal\*time since stimulus presentation :  $F(4,18)=.77, p=.56$ ).

### 3.6 Comparison of initial HR across the experiment

Initial HR (i.e. the controls) did not change over the course of the four experimental treatments (effect of treatment:  $F(4,9)=.60, p=.66$ , effect of time since stimulus presentation:  $F(2,9)=.002, p=.99$ , reversal\*time since stimulus presentation:  $F(8,36)=.31, p=.96$ ).

## 4. Discussion

Our lab has shown in previous studies that exposure to chronic stress decreases the HR response to acute stress, but increases baseline daytime HR in European starlings (Cyr et al., 2008). This study tested the hypothesis that European starlings would perceive the acquisition of new learning tasks as stressful, which would result in an acute increase in HR. Furthermore, based upon the earlier work, the persistent learning that occurred in the closed economy of this

study was predicted to result in a long-term increase in HR. In contrast, the data presented here indicate that HR decreased through the initial days of each treatment period when learning was first taking place. In addition, there was no significant difference in the HR across the study, i.e. between the first days of every subsequent treatment. Both the acute and chronic HR responses thus contradict our prediction that learning would elicit the same effect on HR seen under chronic stress conditions. Furthermore, the decrease in HR was accompanied by a decrease in activity in response to a sudden change in their environment. Both the original discrimination task and the reversal treatment resulted in an acute decrease in trials/min. However, the birds increased their activity during the learning acquisition period, but had fewer perches/min once the task was learned.

These results suggest that learning by itself does not induce either acute or chronic stress in starlings. The four-week experimental period in this study was longer than most previous experiments that have induced chronic stress in passerine birds (e.g. Astheimer et al., 1991; Rich and Romero, 2005). If learning was perceived to be stressful, we would expect to see a decrease in the HR response to subsequent treatments because previous studies showed chronic stress both attenuates the HR response to acute stressors and increases in daytime resting HR (Cyr et al., 2008). We did not see either of these changes. In fact, we saw the opposite, as HR decreased over the training period (Fig. 1) and during the discrimination task (Fig. 2a), indicating that the birds in our study were not chronically stressed. The increased HR when the visual discrimination task became unavailable (Fig. 4a) further supports this conclusion. The birds were still able to mount an increased HR response to a novel condition (removal of their food source) with a subsequent decrease in HR following the removal of the stressor (the return of the discrimination task), consistent with the normal starling response in HR after exposure to an acute stressor (Nephew et al., 2003).

However, no HR response was seen during either the probe or reversal treatments periods (Fig. 3a and Fig. 5a), which suggests that the manipulation of a previously learned task had less of an effect on starlings than the original learning task and training period. The bird's accuracy during the probe treatment did not change significantly from the original discrimination task (Fig. 3b), indicating that the birds were easily able to transfer previously learned skills to these new images. The degree of novelty has been found to be an important factor in the magnitude of an animal's stress response to a change in its environment (Romero et al., 1995). Consequently, the change in camera angle or shape used as probes in this study did not appear to be significant enough to be perceived as novel by the starlings. This may not be surprising because changes in angles and shape are well within the bird's experience. During normal flight, wild starlings would see objects in their environment in constantly changing angles and shapes.

While the decrease in HR during the original discrimination task could be attributed to the increasing accuracy (Fig. 2a) and the resulting increase in the food reward, this can't explain the effect of the reversal treatment. Though accuracy initially plummeted during reversal (Fig. 5a), there was no HR response. This indicates that HR is not strictly coupled to accuracy. Similarly, access to food was unlikely to explain changes in HR. All starlings' weight and amount of food received was continuously monitored throughout the experiment to ensure the birds were maintaining body weight. Body weight did not change for any starling throughout the experiment (data not shown). It's likely that a body mass threshold must be reached before animals mount a physiological response to a decrease in food availability (Wingfield and Romero, 2001). Since the animals mass never significantly fell, and accuracy increased as the treatment continued, its possible the reversal treatment wasn't perceived as a significant source of stress.

Interestingly, the number of trials per minute during the reversal treatment and original discrimination task followed the same pattern as the accuracy (Fig. 5c). Both decreased during the initial day of treatment, but increased on the second day. Food restriction has many effects on a bird's physiology and behavior, including causing a decrease in energy expenditure and activity, increasing baseline CORT levels, and decreasing stress-induced CORT concentrations (Bautista et al., 1998; Strohlic and Romero, 2007). While the decrease in food availability was relatively short and mild, the change in behavior may indicate it was perceived as stressful enough to elicit a behavioral response. The birds showed a significant decrease in trials per minute during the acquisition of the original discrimination task (Fig. 2c), and a nearly significant decrease of perch hops per min when the discrimination task was unavailable (Fig. 4c), where food access was reduced or eliminated. When the discrimination task was made available again, the number perch hops per min. increased (Fig. 4c), indicating an increase in activity, but not trials performed, with the increase of access to food. The contrast between behavior and HR, where the behavioral responses suggest that the learning tasks were stressful but the HR responses do not, fit with earlier data components of the stress response (i.e. behavior and HR) can be decoupled for mild stressors (Nephew et al., 2003)

Though our results indicate that the European starlings were not chronically stressed by our learning tasks, our findings support many studies on foraging theory and cognitive effects of reward-based learning. The physiological effects of cognitive challenges have been studied mostly in mammals. For example, in dwarf goats HR increased initially during the learning of a visual discrimination task, but decreased as subsequent tasks were presented, indicating adaptation to the task (Langbein et al., 2004). Another study found that goats continued to perform the discrimination task even when the reward (water) was made freely available (Langbein, 2009). This behavior, known as contrafreeloading, is thought to occur in animals

because it invokes a sense of control over the environment and the positive emotions involved in receiving a reward (Hagen and Broom, 2004). Our results support this idea. The constant availability, and thus constant ability to work for a food in a closed economy, would be associated with gaining a sense of control over the environment and likely contributed to the decrease in HR seen over the training and discrimination treatments. Contrafreeloading has been shown to increase as the difficulty of the task increases, which could explain why our subjects had an increase in the amount of trials per minute during the reversal treatment, when the discrimination task become more complicated (Inglis et al., 1996).

One surprising finding was the lack of an increase in the number trials/min when the availability of the task was returned to the subjects after being removed (Fig. 4c). Since accuracy did not suffer, it is unlikely that the birds “forgot” how to use the apparatus. One likely explanation is that starlings may have become more cautious about performing a task when food availability was decreased (due to a low success rate at the discrimination task/availability of the task itself), and responded by optimizing energy expenditure by decreasing the amount of trials and increasing their resting time (Bautista et al., 1998). Our subjects decreased the amount of trials per minute over the first 75 minutes of being subjected to the original discrimination task (Fig. 2c) when accuracy was low, as well as the first day of the reversal treatment, when accuracy was extremely low (Fig. 5c). This is supported by previous studies on optimal foraging in starlings, which hypothesize decreased motivation to work for a reward when the task was perceived as being hard in favor for minimizing energy usage (Bautista et al., 2001).

In conclusion, our results suggest that learning during changing environmental conditions did not induce changes in HR or behavior inconsistent with starlings under chronic stress conditions. They also suggest that the predictable nature of the discrimination task and the

constant ability to work for food (similar to foraging) may be a major contributing factor in the physiological and behavioral changes seen in this study.

Wild starlings do not appear to perceive learning in response to changing environmental conditions as stressful, and may have evolved ways of coping without mounting a full stress response. Future experiments looking at CORT concentrations, as well as larger sample size would help validate this hypothesis, and help us gain a more complete idea of how animals interact with their environment.

## References

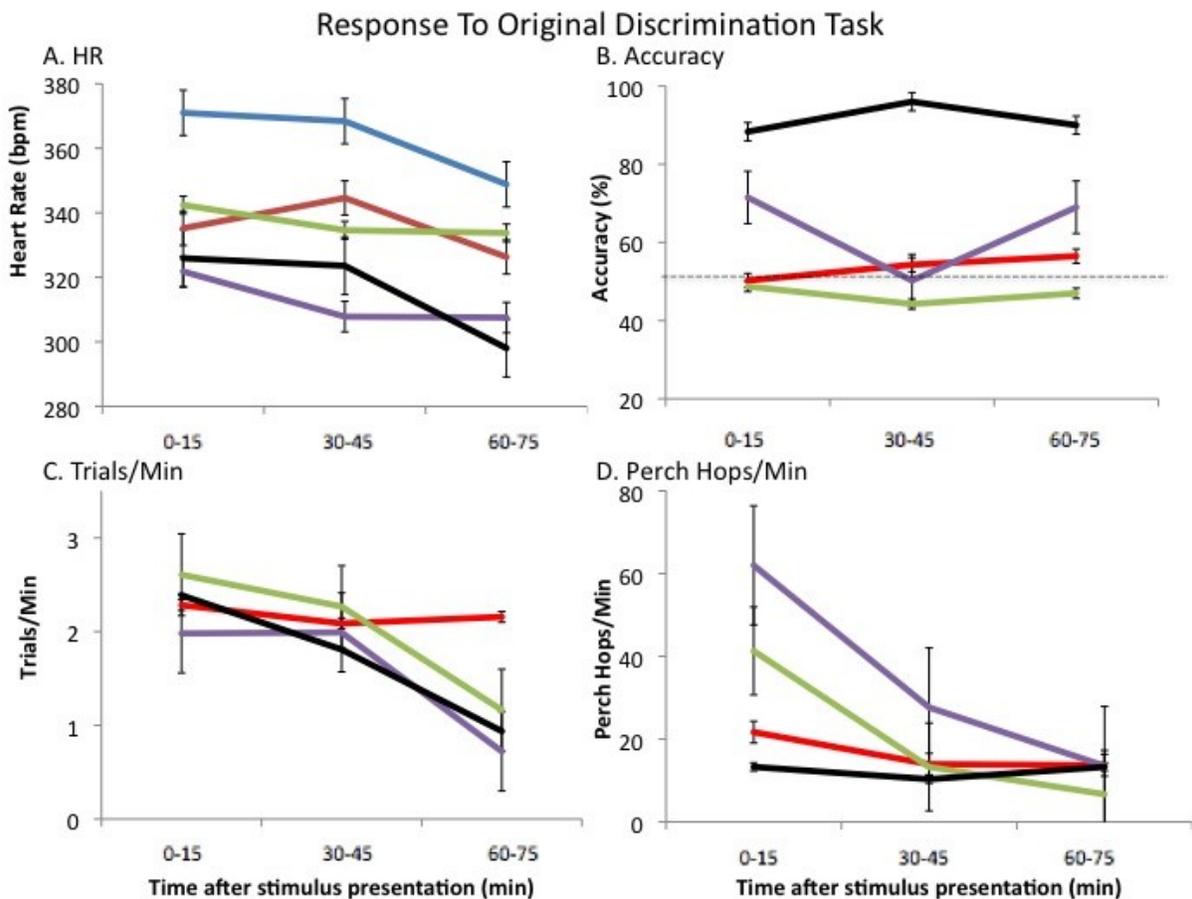
1. Alonso, Y. (1998). Lateralization of visual guided behavior during feeding in zebra finches (*Taeniopygia guttata*). *Behavioral processes*, 43, 257-263
2. Astheimer, L. B., Buttermer, W. A. & Wingfield, J.C. (1992). Interactions of Corticosterone with Feeding, Activity and Metabolism in Passerine Birds. *Ornis Scandinavica*, 23, 355-365.
3. Bautista, L.M., Tinbergen, J., Wiersma, P., & Kacelnik, A. (1998). Optimal Foraging and beyond: How Starlings Cope with Changes in Food Availability. *The American Naturalist*, 152, 543-561.
4. Bautista, L.M., Tinbergen, J., and Kacelnik, A. (2001). To walk or to fly? How birds choose among foraging modes. *Proceedings of the National Academy of Sciences of the United States of America*, 3, 1089-1094.
4. Bovet, D., and Vauclair, J. (2000). Picture recognition in animals and in humans: a review. *Behavioral Brain Research*, 109, 143-165
5. Burkhalter, A., and Cuenod, M. (1978). Changes in pattern discrimination learning induced by normal and commissurotomized pigeons. *Experimental Brain Research*, 31, 369-385

6. Cavoto, B., and Cook, R. (2006). The Contribution of Monocular Depth Cues to Scene Perception by Pigeons. *Psychological Science*, 17, 628-634
7. Cyr, N., Dickens, J.M., and Romero, L.M. (2008). Heart Rate and Heart Rate Variability Responses to acute and chronic stress in a wild-caught passerine bird. *Physiological Biochemical Zoology*, 82, 322-344
8. D'Eath, R.B., and Stone, R.J. (1999). Chickens use visual cues in social discrimination: an experiment with coloured lighting. *Appl. Ani Behav Sci*, 62, 233-242
9. Emmerton, J., and Delius, J. (1993). Beyond sensation: Visual cognition in pigeons. In Zeigler, H and Bischof, H (Eds.), *Vision, brain, and behavior in birds*. Cambridge, MA: MIT Press, pp 377-390.
10. Hagen, K., and Broom, D. (2004). Emotional reactions to learning in cattle. *Applied animal behaviour science*, 85, 203-213.
11. Hodgson, Z.G., Meddle, Z.G., S.L., Roberts, M.L., Buchanan, K.L., Evans, M.R., Metzdorf, R., Gahr, M, Healy, S.D. (2007). Spatial ability is impaired and hippocampal mineralocorticoid receptor mRNA expression reduced in zebra finches (*Taeniopygia guttata*) selected for acute high corticosterone response to stress. *Proceedings of the royal society of Biological Sciences*, 274, 239-245.
12. Inglis, I.R., Forkman, B., and Lazarus, J. (1997). Free food or earned food? A review and fuzzy model of contrafreeloading. *Animal Behaviour*, 53, 1171-1191.
13. Kitaysky, A.S., Kitaiskaia, E.V., Piatt, J.F., and Winfield, J.C. (2003). Benefits and costs of increased levels of corticosterone in seabird chicks. *Hormones and Behavior*, 43, 140-149.

14. Langbein, J., Nürnberg G., and Manteuffel, G. (2004). Visual discrimination learning in dwarf goats and associated changes in heart rate and heart rate variability, *Physiol. Behav.* 82, 601-609
15. Langbein, J., Siebert, K., and Nürnberg G. (2007). On the use of automated learning device by group-housed dwarf goats: Do goats seek cognitive challenges? *Applied Animal Behavioral Sciences*, 120, 150-158.
16. Nephew, B.C., & Romero, L.M. (2003). Behavioral, physiological, and endocrine responses of starlings to acute increases in density. *Hormones and Behavior*, 44, 222-232.
17. Nephew, B.C., Kahn, S., and Romero, L.M. (2003). Heart rate and behavior regulated independently of corticosterone following diverse acute stressors. *General and Comparative Endocrinology*, 133, 173-180.
18. Neuringer, A. (2004). Reinforced variability in animals and people: implications for adaptive action. *American Psychologist*, 59, 891-906.
19. Pravosudov, V., and Omanska, A. (2004). Prolonged moderate elevation of corticosterone does not affect hippocampal anatomy or cell proliferation rates in mountain chickadees (*Poecile gambeli*). *Journal of Neurobiology*, 62, 82-91
20. Rich, E. L., and Romero, L.M. (2005) Exposure to Chronic Stress Downregulates Corticosterone Responses to Acute Stressors. *AJP: Regulatory, Integrative and Comparative Physiology*, 288, 1628-1636.
21. Romero, L.M., Levine, S., and Sapolsky, R.M. (1995). Patterns of adrenocorticotropin secretagog release in response to social interactions and various degrees of novelty. *Psychoneuroendocrinology*, 20, 183-191.

22. Romero, L.M., Reed, J.M., and Wingfield, J.C. (2000). Effects of Weather on Corticosterone Responses in Wild Free-Living Passerine Birds. *General and Comparative Endocrinology*, 118, 113-122
23. Romero, L.M., and Remage-Healey, L. (2000). Daily and seasonal variation in response to stress in captive starlings (*Sturnus vulgaris*). *Gen Comp Endocrinol*, 119, 52-59
24. Rupp, H. (1999). Excess catecholamine syndrome: pathophysiology and therapy. *Annual New York Academy of Science*, 881-430-444
25. Ruxton, G., Speed, M., and Kelly, D. (2004). What, if anything, is the adaptive function of countershading? *Animal Behavior*, 68, 445-451
26. Saldanha, C., Schlinger, B., and Clayton, N. (2000). Rapid Effects of Corticosterone on Cache Recover in Mountain Chickadees (*Parus gambeli*). *Hormones and Behavior*, 37, 109-115.
27. Sapolsky, R.M., Romero, L.M., and Munck, A.U. (2000). How do Glucocorticoids Influence Stress Response? Integrating Permissive, Suppressive, Stimulatory, and Preparative Actions. *Endocrine Reviews*, 21, 55-89.
28. Sapolsky, R., and Share, L. (1994). Rank-related differences in cardiovascular function among wild baboons: Role of sensitivity to glucocorticoids. *Am J Primatol* 32: 261–270
29. Shors, T.J., Weiss, C, and Thompson, R.F. “Stress-induced facilitation of classical conditioning” *Science*, 24, 537-539
30. Strohlic, D., and Romero, L.M. (2007). The Effects of Chronic Psychological and Physical Stress on Feather Replacement in European Starlings (*Sturnus Vulgaris*). *Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology*, 149, 68-79.

31. Vita, B.m Fulvio, P., Gaspare, C., Marco, B., Giacomina, M., and Carla, C. (1997). Manipulations of Glucocorticoid Induced Stress Response May Differently Affect the Acquisition of a Reward-facilitated Spatial/Visual Learning Task.
32. Wingfield, J.C, Maney, D.L, Breuner, C.W, Jacobs, J.D, Lynn, S, Ramenofsky, M, and Richardson, R.D (1998 ). Ecological bases of hormone–behavior interactions: the ‘emergency life history stage’. *Integrated. Comparative Biology.* 38, 191–206
33. Wingfield, J.C., & Romero, L.M. (2000). Adrenocortical Responses to Stress and Their Modulates in Free-living Vertebrates. *Handbook of Physiology; Section 7: The Endocrine System; Volume IV: Coping with the Environment: Neural and Endocrine Mechanisms.* Ed. McEwen, B., S., & Goodman, H, M. New York: Oxford University, 211-243





### Response To Probe Treatment

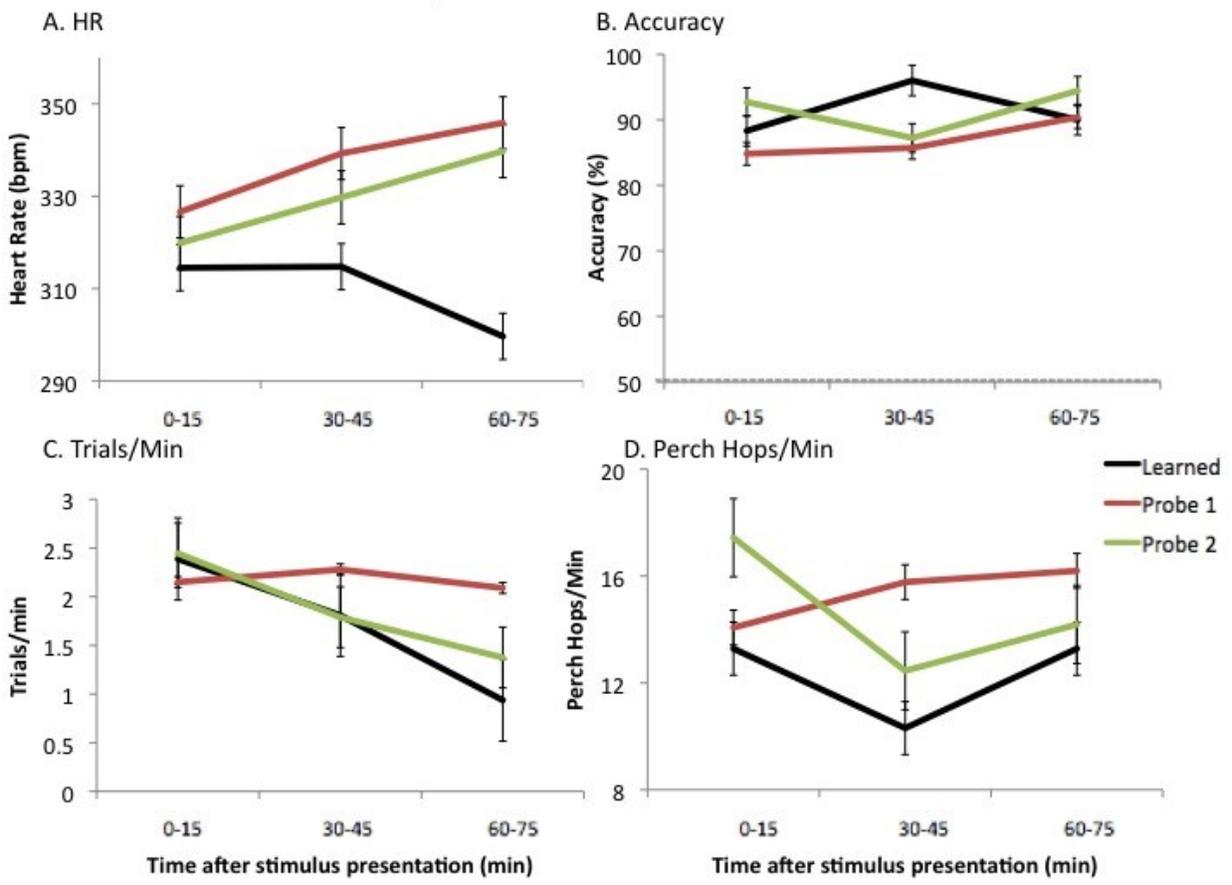


Figure 3. Measurements of heart rate (HR), accuracy, trials/min and perch hops/min taken

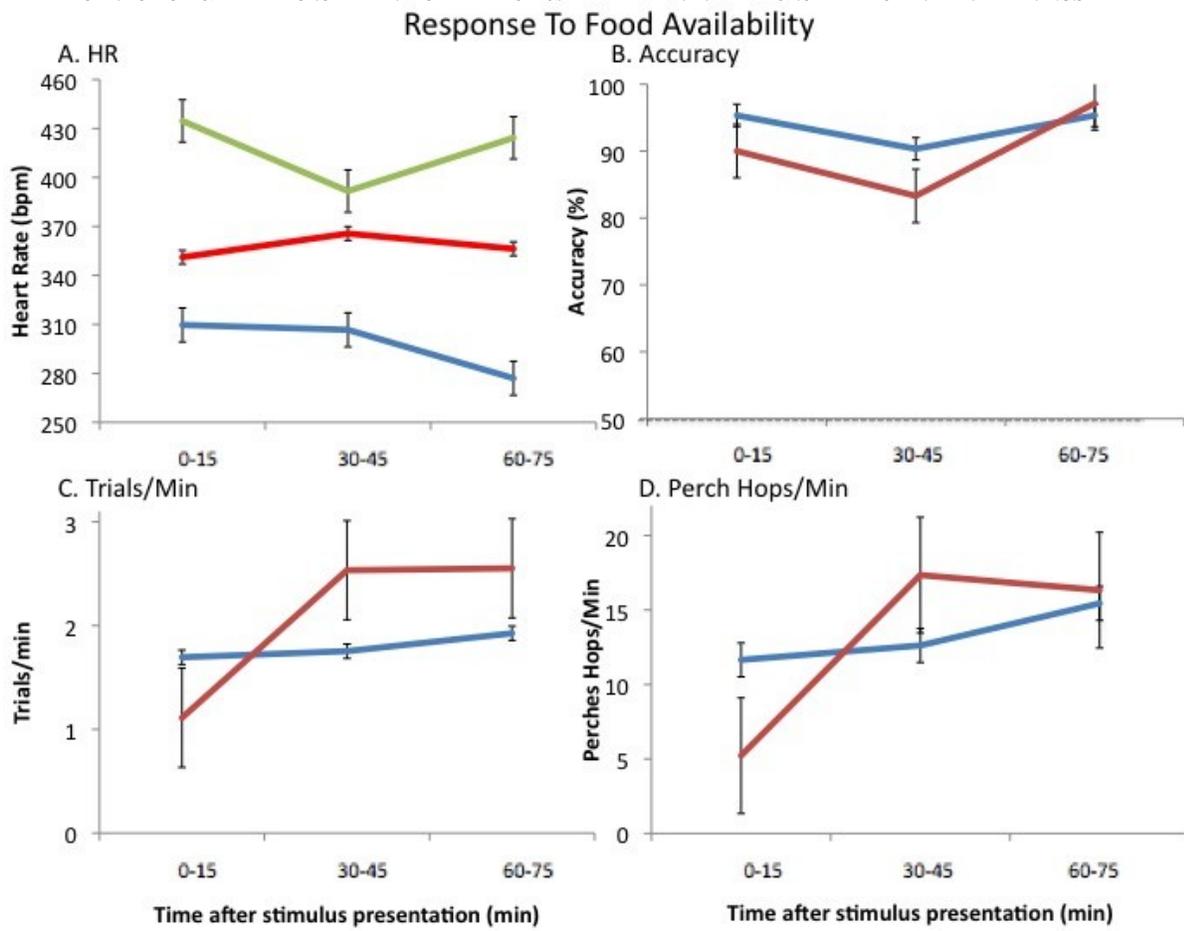




Figure 4. Measurements of heart rate (HR), accuracy, trials/min and perch hops/min taken immediately before the availability of the discrimination task was manipulated, during unavailability, and the initial period after the task became available again (return). The

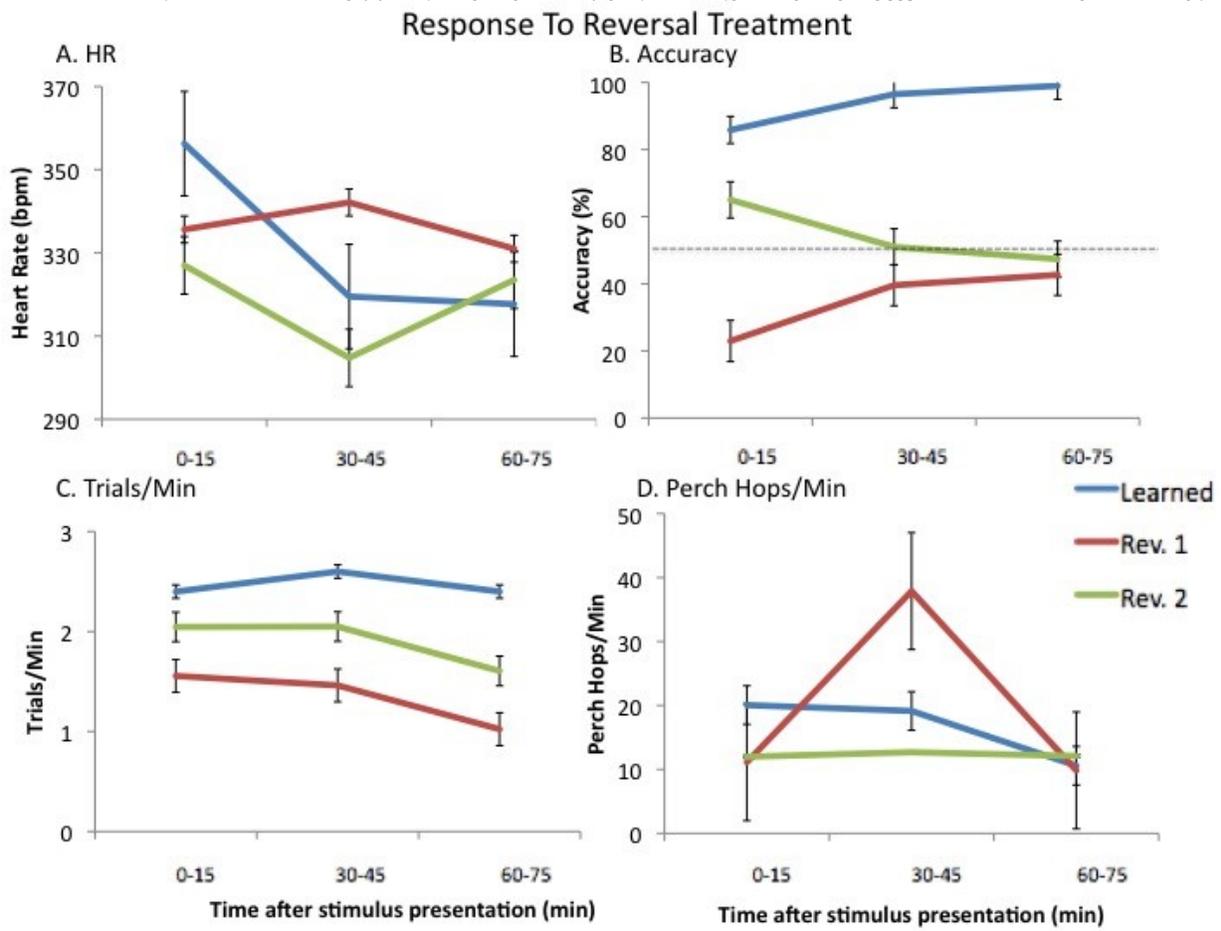




Figure 5. Measurements of heart rate (HR), accuracy, trials/min and perch hops/min taken immediately before (Learned) and the initial period during the first day (Rev. 1) and second day (Rev. 2) after presentation of the reversal treatment. The measurements are averaged ( $\pm$  standard error) between four birds. HR are averaged over a 15-minute span, and the average of the other measurements are taken during the same time. The dashed line in chart B represents accuracy if the birds are discriminating by chance at 50%. “Learned” measurements were taken before the reversal treatment when the bird was discriminating with 85% accuracy. Measurements taken on a day without a shift are taken within a 75-minute window.