Wild European Starlings (*Sturnus vulgaris*) Adjust to Captivity with Sustained Sympathetic Nervous System Drive and a Reduced Fight-or-Flight Response

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ABSTRACT

Although research on wild species typically involves capture, handling, and some degree of captivity, few studies examine how these actions affect and/or alter the animal's underlying stress physiology. Furthermore, we poorly understand the immediate changes that occur as wild animals adjust to captive conditions. Most studies to date have investigated relatively long-term changes in the glucocorticoid response to an acute stressor, but immediate changes in the fight-or-flight response are relatively understudied in wild-caught species. In this study, we investigated changes to the cardiovascular stress response during the first 10 d of captivity of freshly captured wild European starlings (*Sturnus vulgaris*). We demonstrated that (1) baseline heart rate (HR) remains elevated for several days following transport into captivity, (2) the normal balance between sympathetic nervous system (SNS) and parasympathetic nervous system regulation of HR is disrupted, with the SNS exerting relatively greater control over baseline HR for the first days of captivity, and (3) the HR response to startle, a mild stressor, becomes significantly reduced compared to that of starlings maintained in captivity for several months and remains below the control response for at least 10 d. These data are the first to show that successive acute stressors and introduction to a captive setting significantly alter the physiology and responsiveness of the cardiovascular stress response system.

Introduction

Capture and handling are important aspects of field research in wild animals, and often researchers bring these animals into captivity to conduct studies in a more controlled environment. Little is known about the effects of such prolonged anthropogenic activities (i.e., capture, handling, captivity) on the stress physiology of wild species. It has been suggested that bringing animals into a captive environment can cause chronic stress (Morgan and Tromborg 2007), but the exact nature of the alterations of the underlying stress physiology is poorly understood.

Although it is impossible to know exactly how an animal perceives a net or trap or being held by a human hand, we can assume that the resulting stress response is similar to that of a serious predation attempt. Two main pathways comprise the acute stress response. One pathway is the hormonal response mediated through glucocorticoids and is more often studied in wild species. During an acute stress response, the adrenal gland secretes glucocorticoids (GCs) via signaling from the hypothalamic-pituitary-adrenal axis (HPA). Among a diverse range of trapping techniques and a wide range of species, an animal typically begins mounting a GC response as soon as they are caught, before human interaction (Kock et al. 1987; Romero and Romero 2002; Romero and Reed 2005; Lynn and Porter 2008). In addition, increasing GC concentrations as a result of capture and human handling are consistently seen in birds (Wingfield et al. 1992; Astheimer et al. 1995; Davidson et al. 1997; Romero et al. 2008), reptiles (Cash et al. 1997; Moore et al. 2000), and mammals (Kenagy and Place 2000; Reeder et al. 2004; Schutz et al. 2006).

In the case of capture and handling, if the animal is released, and thus "escapes predation," the response mounted would be considered an acute stress response that promotes short-term survival (Sapolsky et al. 2000). However, when either an acute stressor persists or many acute stress responses are mounted consecutively, the short-term physiological or behavioral changes crucial for alleviating or ameliorating the acute stressor can lead to pathological conditions (Wingfield and Romero 2001; McEwen 2005), such as alterations in the acute GC response via changes in the sensitivity of the HPA axis (Rich and Romero 2005). Captivity alone creates a situation in which a barrage of unfamiliar environmental factors can trigger stress responses (Morgan and Tromborg 2007). Capture, handling, transport, and captivity can elevate baseline GC concentrations (Coddington and Cree 1995; Davidson et al. 1997; Nilsson et al. 2008) and alter how the HPA axis functions (Romero and Wingfield 1999). Therefore, capture, handling, transport, and captivity potentially create a situation in which the consecutive and protracted series of acute stress responses leads to chronic stress.

The other stress response pathway, activation of the sym-

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pathetic nervous system (SNS) or fight-or-flight response, is relatively understudied in wild-caught species as compared to the hormonal response. This fast, immediately initiated pathway is mediated by the SNS release of catecholamines (epinephrine [E] and norepinephrine [NE]; reviewed by Axelrod and Reisine [1984]). Initiation of the fight-or-flight response allows for a quick reaction to the stressor and includes elevation in heart rate (HR), increased blood pressure, and mobilization of energy sources. However, the same aspects of a sympathetic response that allows a quick short-term response, if chronically stimulated, may have serious implications for long-term effects on cardiovascular health since excess catecholamines can lead to hypertension, myocardial infarction, increased cardiac output, and arrhythmias (Rupp 1999). Chronic stress causes significant changes in cardiac function in wild-caught European starlings (*Sturnus vulgaris*), predominantly through alterations in the parasympathetic nervous system's control over heart rate (Cyr et al. 2009). In addition, since the fight-or-flight response is the animal's first line of defense to a stressor, the ability to mount an appropriate response is crucial to survival.

Because of the difficulties of studying the fight-or-flight response in the field, and even in wild-caught captive animals, no studies to date have determined how capture, handling, and captivity affect this pathway of the stress response. Our goal was to assess the initial changes to the fight-or-flight response during the initial 10 d after introduction into a captive setting using wild-caught starlings. We approached this goal with three main questions: (1) Does baseline HR change while animals are introduced to captivity? (2) Does the normal balance between parasympathetic nervous system (PNS) and SNS regulation of baseline HR change as the birds adjust to captivity? and (3) Does the HR response of captive animals to a mild stressor (startle) change during the adjustment period compared to the response of individuals that have been in captivity for many months?

Methods

Birds

All starlings were captured from the wild during the winter in eastern Massachusetts and immediately brought into captivity. Within 6 h of capture, we implanted each bird with heart rate transmitters (see below) and placed them into individual cages in a room maintained on a light cycle of 12L : 12D. These newly captive birds were designated the "new" group. Although ideally our controls would have been free-living starlings, this was impossible using our current heart rate transmitters. Instead, the control group consisted of individuals that had resided in captivity, an outdoor communal aviary, for 10–14 mo. After implantation, the control individuals were placed in individual cages and maintained on a 12L : 12D light cycle. Starlings were a mix of males and females because previous research indicates that there are no sex differences in the HR responses to stress in this species (Nephew and Romero 2003; Nephew et al. 2003, 2005). All experiments complied with Association for Assessment of Laboratory Animal Care guidelines and were approved by the Tufts Institutional Animal Care and Use Committee.

Transmitter Implantation

Implantation of the heart rate transmitters followed the protocol presented by Nephew et al. (2003). Briefly, either immediately after capture and transport to Tufts (new) or after birds had been in captivity for about 1 yr (control), individuals were implanted with a 4.0-g, Data Sciences International transmitter (model TA10EA-F20, DSI, St. Paul, MN). Each bird was anesthetized with an intramuscular injection of ketamine and xylazine. The two electrocardiogram (ECG) leads with exposed ends were secured at both the neck region and the back region, and the body of the transmitter was placed in the abdominal cavity. Antibiotic ointment was applied to all incision sites. Some birds were given Ketofen to relieve any pain associated with the surgery. Recordings began immediately after the birds were returned to the cage to recover from the surgery; however, we restricted the data analysis to readings made a few hours after anesthetic recovery. Every individual demonstrated a full range of motion and flight ability within 24 h of surgery.

Baseline HR and Activity Measurements

Each implanted transmitter connected to a computer running Dataquest Advanced Research Technology (A.R.T., St. Paul, MN) Gold software via a receiver plate on the side each implanted starlings' cage. We monitored HR every 2 h, for 3-min intervals, over a 24-h period to acquire baseline HR measurements for the first 10 d of captivity. However, technical issues with the transmitters prevented collection of ECG traces that were sufficiently clean for analysis at every recorded period. When the target period was not analyzable, we instead analyzed the better of the ECG traces collected at the sampling periods immediately before or immediately after the target time period. Consequently, for statistical purposes, all analyses were grouped at the middle of the 6-h period surrounding the assigned time period. We also analyzed the waveform information to calculate the heart rate variability (HRV). As described by Cyr et al. (2009), HRV is an analysis tool that allows a distinction between relative sympathetic nervous system (SNS) and parasympathetic nervous system (PNS) control over the HR. Daily changes in HRV were assessed for the first 4 d after surgery and also at the end of the 10 d of captivity/postsurgery. The software program also recorded each individual's activity by evaluating the change in signal strength over time as the animal moved in relation to antennae. These data were recorded for 3 min every 2 h as a relative unitless measure expressed as the average across the 3 min (Cyr et al. 2008). We did not include certain time periods in the analysis because they either coincided with the startle stress test, were recorded during feeding, or were not recorded by the program for unknown reasons.

HR and HRV Analysis

HR and ECG waveforms for HRV analysis were collected using Data Sciences International Dataquest A.R.T. Gold 4.0 software package. These data were then uploaded into PONEMAH Physiology Platform (Data Sciences International, Valley View, OH), a software program designed to mark the waveform of the continuous ECG signals and calculate the time interval between R waves in the QRS complex. HRV was calculated from the variability between these R-R intervals. As described by Cyr et al. (2009), our validation study examined changes in HRV relative to HR during both baseline and restraint stress after injection of saline (control), atropine (blocked PNS drive), or propranolol (blocked SNS drive). Similar to what has been shown in the mammalian literature (overview in Cyr et al. 2009), this study demonstrated that the saline controls at baseline matched both HR and HRV for PNS drive, while during restraint stress, the saline controls matched the HR and HRV for SNS drive.

Startle Response

Birds were exposed to a mild startle stress (short, loud noise) in the morning of the second (36 h), fifth (88 h), and tenth (228 h) day after surgery. HR was calculated every 30 s for 10 min before and 20 min after the startle stressor. For each individual, a baseline HR value was calculated as an average HR across 5 min worth of HR values during the time in which the animals were still completely undisturbed (i.e., 10–5 min before startle). Each individual's HR values for the 5 min before the startle (i.e., 5–0 min before startle) and for 20 min poststartle were subtracted from their individual baseline. The HR measurement of the 30 s immediately following the startle stressor represented the peak HR. We also determined the overall elevation in HR following the startle by calculating the integrated HR for the first 3 min after the startle.

Data Analysis

We analyzed baseline HR and HRV by group across time periods using a Proc Mixed model (SAS version 9.1, 2003). We also ran post hoc tests within each group for both HR and HRV looking for a difference in least squared means in order to determine how HR and HRV changed over time. For the activity data, we separated each individual's activity for day and night hours and analyzed the groups with a paired *t*-test. For the startle, we compared the peak and integrated response for both groups by day postcapture to the corresponding control group for that day postsurgery using Student's *t*-test. If variances were not equal, as determined by a Levene's test at $P < 0.05$, then the data were transformed by using the natural log and the analysis rerun.

Results

Baseline HR

These data had a significant time × group interaction ($F_{9,88} = 5.35$, $P < 0.0001$; Fig. 1*A*). When we analyzed the groups separately, we found a significant time effect for the newly captive group ($F_{9,40} = 6.89$, $P < 0.0001$) but not the control group. The time period at which the recovery baseline HR stabilized was determined by evaluating at which time period there were no later values significantly different (using pairwise post hoc tests). For the group of newly captive starlings, baseline HR was initially elevated but steadily declined until 30 h, when subsequent HR recordings were statistically indistinguishable (using pairwise post hoc tests).

Baseline HRV

These data had a significant time × group interaction ($F_{9,88} = 4.53$, $P < 0.0001$; Fig. 1*B*). When we analyzed the groups separately, we found a significant time effect for both the new captives ($F_{10,40} = 13.18$, $P < 0.0001$) and controls ($F_{10,47} = 8.33$, $P < 0.0001$). In the new group, baseline HRV was reduced initially and steadily increased until 48 h postcapture, when subsequent HR recordings were statistically indistinguishable (using pairwise post hoc tests). Although we found a time

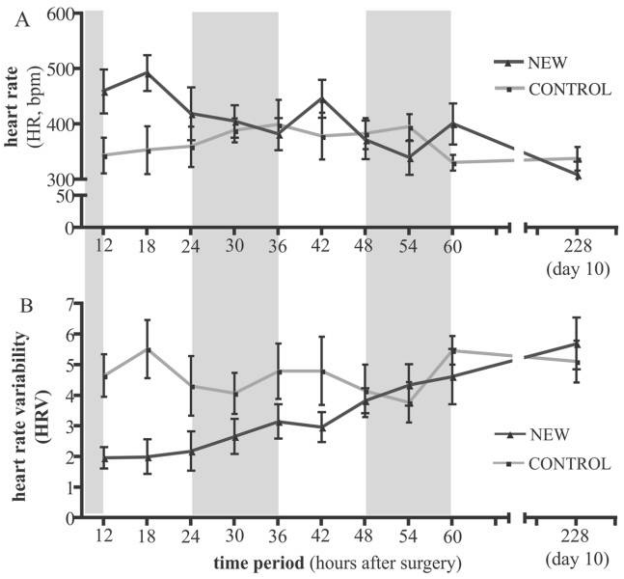

Figure 1. Heart rate (HR) and heart rate variability (HRV) for new birds after introduction into captivity and surgery and for control birds after surgery. Mean ± SE for (*A*) HR and (*B*) HRV at time periods postintroduction to captivity (new, $n = 7$) or postsurgery (control, $n = 7$). Shaded bars indicate nighttime hours. There was a significant interaction between the groups and time for HR ($F_{9,88} = 5.35$, $P < 0.0001$) and for HRV ($F_{9,88} = 4.53$, $P < 0.0001$) HR statistically stabilized at 30 h while HRV statistically stabilized at 48 h. Note that we show only the first 60 h and then the 228-h mark because HR and HRV did not change beyond this point.

effect for the long-term captive control group, there was no discernible pattern from post hoc analysis (i.e., the changes did not follow a linear pattern).

Activity

Activity of both groups during the day hours were 15 times higher than the activity during the night hours; however, day and night activity did not differ significantly between the two groups ($F_{2,14} = 0.17$, $P = 0.69$; Fig. 2*B*).

Startle Response

The startle response of the control birds did not vary for either peak or integrated measures between any postsurgery measurement periods (peak: $F_{2,5} = 0.4432$, $P = 0.67$; Fig. 4*A*; integrated: $F_{2,5} = 1.9445$, $P = 0.23$; Fig. 4*B*). By contrast, when compared to the controls, peak response (demonstrated in Figs. 3, 4*A*) was significantly lower in the newly captive birds at 36 h (df = 11, $P < .04$), 88 h (df = 11, $P < 0.004$), and 228 h (df = 11, $P < 0.01$). The integrated response over the 3-min period following the startle stressor (Fig. 4*B*) was similar to the peak response, being significantly lower in the newly captives at 36 h (df = 11, $P < 0.01$), 88 h (df = 12, $P < 0.02$), and 228 h (df = 10, $P < 0.002$).

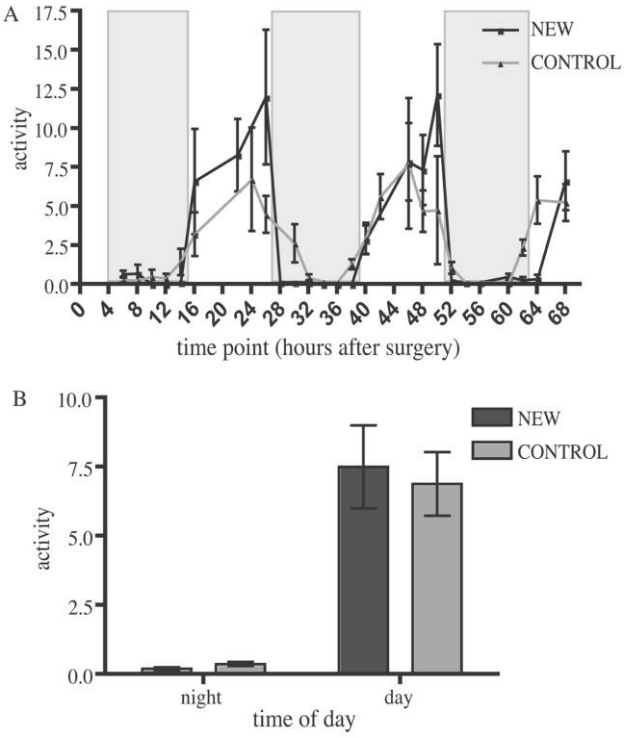

Figure 2. Activity data for first 3 d postsurgery/introduction to captivity. *A*, Average activity data for each time period ± SE for each group. Shaded bars indicate nighttime hours. Activity data are collected in arbitrary units of measurement, so no units are indicated for the *Y*-axis. *B*, Overall activity means ± SE for day versus night hours. Group patterns were not significantly different ($F_{2,14} = 0.17$, $P = 0.69$).

Discussion

The wild starlings in our study had an elevated baseline HR for the first 2 d and a corresponding decrease in HRV. HRV is a reliable measure of sympathetic drive in this species (Cyr et al. 2009). The decreased HRV indicates that the elevated HR is due to excessive drive from the SNS and not simply physical exertion by the individual. The activity data also confirm this observation because the new birds do not demonstrate a significantly higher degree of activity that would cause elevation in HR. A similar result is seen in turkeys in which human interaction and handling increases HR in turkeys being crated and transported (Prescott et al. 2000). Prescott et al. (2000) demonstrate that techniques with reduced physical demands on the animals but greater human interference cause a higher baseline HR during and after loading, whereas the technique that requires greater physical exertion by the animal but involves less human handling does not cause a sustained elevation in HR either during or after loading. Although these authors did not measure HRV, the observation that these animals had a higher baseline HR in the situation with reduced physical exertion implies that these animals have an increased SNS drive as a result of human handling. In addition, handling and bringing wild animals into captivity has been shown to increase SNS drive and potentially alter cholinergic tone (Davison et al. 1995), and transport alone can result in sustained elevation of catecholamines as determined by urinary analyses (Dehnhard 2007).

Ideally we would have assessed the HR and HRV values of individuals in the wild before captivity in order to determine whether the elevated HR and decreased HRV is simply representative of free-living individuals. Unfortunately, we have not been able to acquire these data for wild starlings, and although field measurements exist for several species, the size of the bird has a very strong correlation with the observed HR (Grubb 1983; Machida and Aohagi 2001), making the available studies incomparable to our own. For example, the Swainson thrush has a resting HR between 600 and 700 bpm (Bowlin et al. 2005) but is less than half of the size of a starling. Other studied birds include the barnacle goose, 1,700 g with an HR of <100 bpm (Ward et al. 2002), and the king penguin, 1,400 g with an HR of <100 bpm (Froget et al. 2004). We are unaware of any data comparing free-living and captive heart rates in the same species, which further limits this comparison. Therefore, we were restricted to using starlings that had adjusted to captivity (held for approximately 1 yr) as our controls. With this comparison, however, it appears that wild starlings require several days to adjust their HR and HRV to the steady, long-term captive range. In addition, the SNS-biased HR lasted almost a day longer than the elevation in HR. This implies that these individuals are exposed to elevated catecholamine concentrations for a period of time much

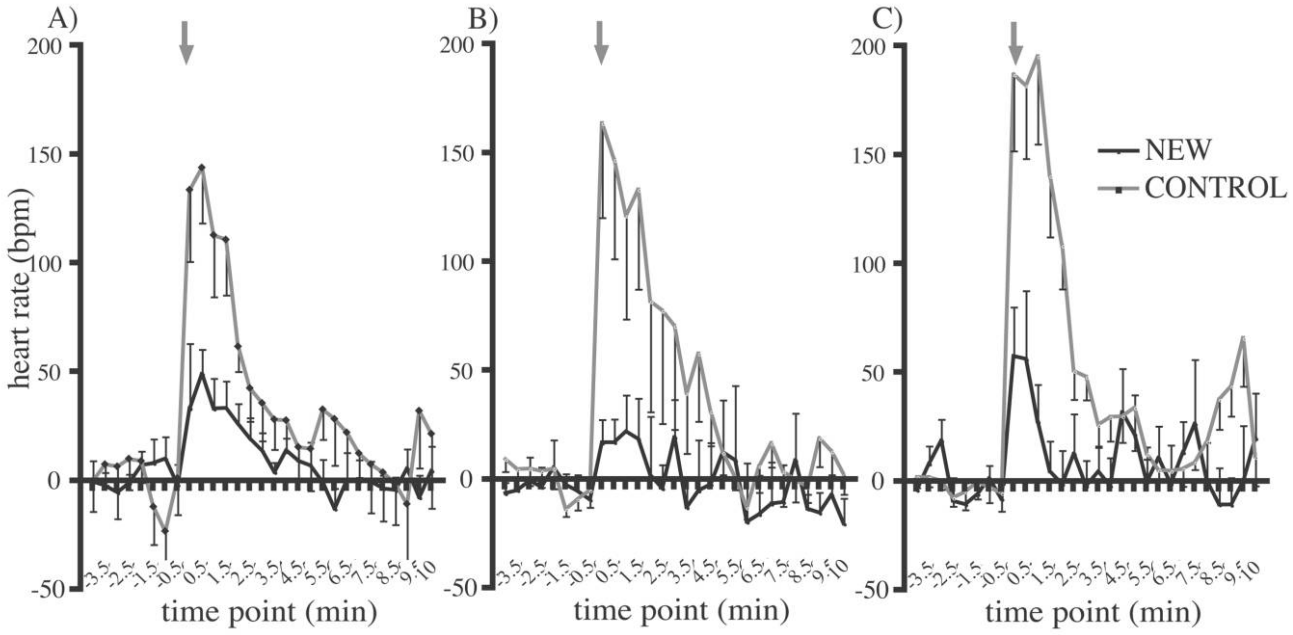

Figure 3. HR responses to a startle stressor. Startle response for wild-caught, newly captive individuals (new) and long-term captivity controls (controls) at (*A*) 36 h (new, $n = 5$; controls, $n = 7$), (*B*) 88 h (new, $n = 7$; controls, $n = 7$), or (*C*) 228 h (new, $n = 6$; controls, $n = 7$) after surgery. The arrow indicates the time period at which the startle was given.

longer than would occur in a fight-or-flight response situation. The mismatch in timing suggests that after the second day, the increased SNS drive no longer has an impact on HR. This response is similar to cardiovascular changes shown in this species after several weeks of chronic stress. In Cyr et al.'s (2009) study, increased SNS drive without elevations in HR were interpreted to occur as a result of lower catecholamine receptors on the heart—a mechanism that could explain these data as well. While a normal, short surge of epinephrine and norepinephrine may allow the animal to react quickly to a stressor in the wild, sustaining these stress-induced concentrations for any extended period can damage the cardiovascular system (O'Neill et al. 1997). Sustained high catecholamine concentrations in wild animals can also increase vulnerability to disease and changes in weather patterns (McAllum 1985; Griffin and Thomson 1998).

In addition to the sustained SNS drive, the successive anthropogenic stressors of capture, handling, and introduction to captivity virtually eliminated the animals' ability to mount a fight-or-flight response to the mild stressor. Although by 10 mo starlings have clearly reestablished a startle response typical of laboratory-bred animals (Rettig et al. 1986; Tovote et al. 2005), 10 d is not sufficient time. Once again, reduction of heart catecholamine receptors would be a potential mechanism to explain these data—even though there is increased SNS drive, the heart no longer responds to the signal. Further work is necessary to determine how long the attenuated response lasts. Interestingly, these results match those seen in obese humans and laboratory animals, in which the associated cardiac problems are linked to an increased SNS drive corresponding to a decreased responsiveness to stressful stimuli (Tentolouris et al. 2006).

This resulting attenuation in the acute, quick fight-or-flight response would be extremely detrimental for survival in the wild. While we do not know how fast a normal fight-or-flight response will recover if the animals are released back to their natural habitat immediately after capture, these data suggest at least a temporary attenuation, which can have serious consequences. In procedures in which animals are exposed to extensive human interaction (e.g., translocation), researchers often list increased predation as a leading cause of death after release into the wild (Fischer and Lindenmayer 2000; Banks et al. 2002; Van Zant and Wooten 2003; Teixeira et al. 2007). A reduced fight-or-flight response in translocated individuals could underlie this increase in vulnerability to predation.

These data are also applicable to studies evaluating how animals adjust behaviorally to successive stressors. As seen in captive great tits (*Parus major*), excessive handling alters the individuals' behavior such that they are more easily captured when a human reaches into the cage (Oers and Carere 2007). While it is common to link this behavioral change to an alteration in perception of a "stressor," this alteration may instead, to some degree, reflect a physiological alteration in the fight-or-flight response due to those successive stressors (i.e., repeated handling or capture/transport/captivity). A reduced fight-or-flight response, possibly resulting from a decrease in receptor availability (Cyr et al. 2009), may underlie the observed

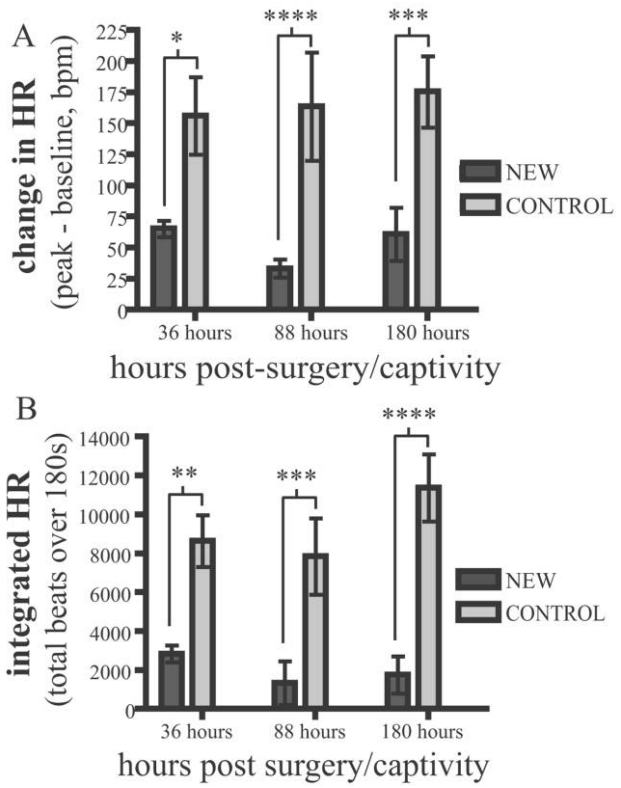

Figure 4. Comparison of peak and integrated HR responses to startle. From the data presented in Figure 3: (*A*) peak HR at startle was determined, and (*B*) integrated HR was calculated over 3 min. The control individuals maintained a nonsignificant change in either peak or integrated response after surgery, and so the experimental, newly captive birds were compared to the respective control group for each time frame. *One asterisk*, $P < 0.04$; *two asterisks*, $P < 0.02$; *three asterisks*, $P < 0.01$; and *four asterisks*, $P < 0.005$.

behavioral change that the birds no longer perceive handling as a stressor.

Studies investigating the effects of capture and captivity and chronic stress typically focus on the GC response; however, changes in GC may not reflect changes in HR and HR response (Nephew et al. 2003; Dickens et al. 2006). Although an ideal scenario to study the effects of chronic stress on HR changes in wild species would involve free-ranging animals, this is not possible as of yet. The acute stressors used in this study, however, fall well within the realm of what manipulated wild animals encounter. Therefore, we propose this study as a good model for what occurs within the cardiovascular stress physiology in wild birds exposed to chronic stress.

Although we could not continue this study beyond the 10 d of captivity, we can conclude that starlings captured from the wild and brought into captivity have a sustained SNS drive for the first 48 h of captivity and that the fight-or-flight response does not adjust to captivity by 10 d after capture. These data are the first to demonstrate that successive acute stressors and entrance into a captive setting have significant consequences on the cardiac responses to fight-or-flight events.

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