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Initial transference of wild birds to captivity alters stress physiology

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ABSTRACT

Maintaining wild animals in captivity has long been used for conservation and research. While often suggested that captivity causes chronic stress, impacts on the underlying stress physiology are poorly understood. We used wild-caught chukar (Alectoris chukar) as a model avian species to assess how the initial 10 days of captivity alters the corticosterone (CORT) secretory pathway. In the first few days of captivity, birds lost weight, had lower hematocrit and demonstrated changes in CORT concentrations. Both baseline and restraint-stress-induced CORT concentrations decreased by days 3–5 of captivity and remained significantly lower throughout the 10 days although stress-induced concentrations began to recover by day 9. To delineate potential mechanisms underlying these CORT changes, we evaluated alterations to the hypothalamic–pituitary–adrenal (HPA) axis. Although chukar appear to be resistant to arginine vasotocin's (AVT) effects on CORT release, adrenocorticotropin hormone (ACTH) stimulated CORT release; however, ACTH stimulation did not differ during the 10 days of captivity. In contrast, negative feedback axis sensitivity, as determined by both dexamethasone suppression as well as endogenous negative feedback, decreased by day 5 but was regained by day 9. In addition, the combined stressors of capture and long distance transport eliminated the animals' ability to mount an acute CORT response on the day following the move. Therefore, introduction into captivity appeared to shift the chukar into a temporary state of chronic stress that began to recover within 9 days. The duration of these alterations likely varies due to differences in capture techniques, transport distance, and species studied.

1. Introduction

Bringing wild species into captivity is an important aspect of conservation as well as a way for researchers to conduct studies with wild animals in a more controlled environment. Although it has been suggested that transfer of wild animals into a captive environment can cause chronic stress (Morgan and Tromborg, 2007), the exact nature of the alterations in the underlying stress physiology is poorly understood.

Chronic stress is normally defined in relation to acute stress. During an acute stress response, the adaptive physiological reactions to a noxious stimulus (Sapolsky et al., 2000), the animal will initiate two main pathways. The first is the fight-or-flight response mediated through catecholamine release by the sympathetic nervous system (Axelrod and Reisine, 1984). The second is the slower hormonal response mediated through glucocorticoids. In birds, during an acute stress response, the adrenal gland secretes corticosterone (CORT, the typical avian glucocorticoid) via the hypothalamic–pituitary–adrenal axis (HPA). This axis begins with stimulation of the hypothalamus, which secretes arginine vasotocin (AVT) and corticotropin releasing factor (CRF) to stimulate the pituitary; the pituitary then secretes adrenocorticotropin hormone (ACTH), which stimulates the adrenal to release CORT. Negative feedback then quickly ensures that CORT release is shut off after a stressor ceases (Dallman and Bhatnagar, 2001; Sapolsky et al., 2000).

As seen with a diverse range of trapping techniques in a wide range of species, an animal typically begins mounting a glucocorticoid response immediately upon capture and prior to human interaction (Kock et al., 1987; Lynn and Porter, 2008; Romero and Reed, 2005; Romero and Romero, 2002). In addition, increasing glucocorticoid concentrations as a result of capture and human handling is consistently seen in birds (Astheimer et al., 1995; Davidson et al., 1997; Romero et al., 2008; Wingfield et al., 1992), reptiles (Cash et al., 1997; Moore et al., 2000), and mammals (Kenagy and Place, 2000; Reeder et al., 2004; Romero et al., 2008; Schutz et al., 2006).

The above studies illustrate the acute stress response, a physiological response believed to promote short-term survival (Sapolsky et al., 2000). However, persistence of an acute stressor or exposure to consecutive acute stress responses alters the short-term physiological and/or behavioral changes crucial for alleviating or ameliorating the acute stressor and can lead to pathological conditions (McEwen, 2005; Wingfield and Romero, 2001). The process of bringing animals into captivity exposes individuals to both persistent stressors and consecutive acute stress responses. For example, capture, handling and transport of wild animals can cause multiple stress responses (Coddington and Cree, 1995; Davidson et al.,

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1997; Nilsson et al., 2008). In addition, a captive environment itself contains various stressors (Morgan and Tromborg, 2007) such as lack of adequate stimulation in housing (Young, 2003), crowding in cages (Nephew and Romero, 2003) or inappropriate social housing (Rogovin et al., 2003).

Considering these factors, we expect a high potential for wild-caught animals to become chronically stressed when placed in captivity. As seen in both free-living and wild-caught animals, experimentally-induced chronic stress alters both the HPA axis (Cyr and Romero, 2007; Rich and Romero, 2005) as well as the fight-or-flight response (Cyr et al., in press). Bringing wild birds into captivity causes similar changes to chronic stress; the sympathetic nervous system drive to the heart is initially increased and the fight-or-flight response dampened for up to 10 days (Dickens and Romero, in press). In addition, captivity alters the HPA axis in such a way that the level of the axis (i.e. hypothalamus, pituitary, or adrenal) that limits that maximal secretion of CORT changes during captivity as compared to free-living individuals (Romero and Wingfield, 1999). However, how and when these changes in the HPA axis are established during the initial phases of captivity (i.e. within the first few days) is not currently known.

To investigate the immediate effects of capture, transport, and short-term captivity, we used wild-caught chukar (Alectoris chukar), a desert-adapted gallinaceous bird, as a model species. We tested the functioning of the HPA axis in three ways in order to quantify the changes in stress physiology that occur within the first 10 days of captivity. First, we assessed the change in natural CORT release in response to restraint, a common protocol used to elicit an endogenous maximal CORT response (Wingfield, 1994). Second, we used a series of injections similar to those employed by Rich and Romero (2005) to test the sensitivity of various sites within the HPA axis. We used exogenous AVT to test pituitary sensitivity and exogenous ACTH to test adrenal sensitivity. Third, we assessed the efficacy of negative feedback using two protocols. We tested endogenous negative feedback by monitoring the cessation of CORT release once restraint had ended, and we tested the capacity of negative feedback by injecting dexamethasone (DEX), a synthetic glucocorticoid, to artificially shut off the axis.

We also extended the study in three ways. First, we attempted to delineate the role of transport in the response to captivity by varying the transportation distance. If transportation itself an important factor in captivity-induced changes, then we would expect more profound changes as the transportation distance increased. Second, we measured changes in body weight during the 10 days of captivity since chronic stress typically causes weight loss (Konkle et al., 2003). Finally, we measured hematocrit, the ratio of packed volume of red blood cells to total blood volume in a microhematocrit capillary tube. Although hematocrit has been used to indicate general body condition (Howlett et al., 2002; Sanchez-Guzman et al., 2004) several studies have noted that this is a poor application of the measurement (Cuervo et al., 2007; Dawson and Bortolotti, 1997) and, therefore, we employed it as a measurement of hydration (Vleck and Vleck, 2002) or to indicate behavioral changes during captivity.

Overall, we predict that short-term captivity will cause chronic stress such that we will observe changes in the stress response, the HPA sensitivity, weight, and hematocrit levels.

2. Materials and methods

2.1. Study species and trapping techniques

Chukar are desert-adapted ground birds of the steep, arid mountains of southwestern Eurasia. Chukar were introduced to North America beginning in the early 1900s. For this study, we captured individuals from the wild at the China Lake Naval Air Weapons Station (CLNAWS) near Ridgecrest, CA (117°37′W, 36°3′N) from mid-July until late August in 2006 and 2007. Birds used in the transport study as well as the dose response analysis were captured in September 2005 and August 2006, respectively, and immediately transported to a captive facility at Idaho State University. We trapped chukar at "wildlife guzzlers", man-made rainwater catchment systems, by fitting a trap across the entrance such that once a chukar entered to drink, the only exit was through a wire funnel tunnel leading to a wire cage (Delehanty et al., 2004). Trapped animals were provided with water and shade and were removed from the cage within 3 h.

2.2. Dose response—long-term captives

We determined dose response curves for each of our injected hormones using long-term captives maintained at the Idaho State University captive facility for 9 months. Upon removal from the aviaries, we immediately injected individuals intramuscularly with a given dosage of drug. Following injection, we placed the birds into opaque bags and sampled at 15, 30, 60, and 90 min post-injection of DEX and at 15 and 30 min post-injection of ACTH or AVT. We injected AVT (Sigma Chemical Co., St. Louis) at 1.25 μg/kg ($n = 7$), 2.5 μg/kg ($n = 8$), and 5 μg/kg ($n = 8$); porcine ACTH (Sigma) at 25 IU/kg ($n = 5$), 50 IU/kg ($n = 7$), and 100 IU/kg ($n = 6$); and DEX (Pheonix Pharmaceuticals, St. Joseph, MO) at 25 μg/kg ($n = 5$),100 μg/kg ($n = 5$), 1000 μg/kg ($n = 6$). In this captive group, we also tested a protocol in which we injected DEX, took blood samples every 30 min for 90 min and then injected either AVT ($n = 8$) or ACTH ($n = 9$).

2.3. Injection protocol and controls

Since animal welfare concerns prevented us from following the protocol described above, we derived a 60-min injection series. In this series, a baseline sample was obtained within 3 min and then the birds were placed in opaque bags and sampled after 15 min to obtain peak stress-induced CORT (time was determined by preliminary studies). Following these samples, some individuals were either injected intramuscularly with DEX to stimulate negative feedback or saline (SAL) and then sampled at 45 min (30 min after DEX injection). Following this sample, we injected the individuals intramuscularly with either ACTH to stimulate the adrenal gland or saline, and sampled at 60 min (15 min after ACTH injection). As controls, we used naive individuals immediately upon capture from the wild in order to avoid potential effects of captivity in the following groups: DEX → ACTH ($n = 12$), DEX → SAL ($n = 7$), SAL → ACTH ($n = 8$), and saline → saline ($n = 9$).

2.4. Long-distance transport experiment

In 2005, we captured chukar from the wild. Some individuals were sampled immediately ($n = 16$; see restraint protocol below) and all were immediately placed in transport pens for 2–4 days. The animals were then loaded onto a covered truck bed and driven 1270 km to the Idaho State University captive facility (outdoor aviaries). The day after arrival, individuals ($n = 7$) were removed from the aviary and sampled for baseline (sample taken in under 3 min from approach of pen) and restraint-stress-induced CORT, at 15, 30, 60 and 90 min. As a control, individuals ($n = 13$) that had been captured and transported one year prior were also sampled from the Idaho State University aviary.

2.5. Sample collection—short-term captives

All individuals were captured from the wild and immediately brought into a captive facility comprised of five shade cloth covered pens (120 cm × 240 cm × 140 cm) kept in an outdoor but