



Elevated stress hormone diminishes the strength of female preferences for acoustic signals in the green treefrog



A. Gabriell Davis, Christopher J. Leary*

University of Mississippi, Department of Biology, Oxford, MS 38677, USA

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ABSTRACT

Mate selection can be stressful; time spent searching for mates can increase predation risk and/or decrease food consumption, resulting in elevated stress hormone levels. Both high predation risk and low food availability are often associated with increased variation in mate choice by females, but it is not clear whether stress hormone levels contribute to such variation in female behavior. We examined how the stress hormone corticosterone (CORT) affects female preferences for acoustic signals in the green treefrog, *Hyla cinerea*. Specifically, we assessed whether CORT administration affects female preferences for call rate – an acoustic feature that is typically under directional selection via mate choice by females in most anurans and other species that communicate using acoustic signals. Using a dual speaker playback paradigm, we show that females that were administered higher doses of CORT were less likely to choose male advertisement calls broadcast at high rates. Neither CORT dose nor level was related to the latency of female phonotactic responses, suggesting that elevated CORT does not influence the motivation to mate. Results were also not related to circulating sex steroids (i.e., progesterone, androgens or estradiol) that have traditionally been the focus of studies examining the hormonal basis for variation in female mate choice. Our results thus indicate that elevated CORT levels decrease the strength of female preferences for acoustic signals.

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Introduction

Females often exhibit variation in mate choice and mating preferences that can alter the strength of sexual selection and the evolution of elaborate male traits (Jennions and Petrie, 1997). While a comprehensive understanding of the physiological basis for this variation is lacking, circulating hormone levels appear to play a pivotal role. Sex steroids, in particular, have been the focus of most studies examining the hormonal basis for such variation with elevated levels often causing a reduction in female choosiness (Adkins-Regan, 2005; Gordon and Gerhardt, 2009; Lynch et al., 2006; McGlothlin et al., 2004). Elevated sex steroids, however, are unlikely to account for the variation in female mate choice observed under stressful environmental conditions. For example, increased predation risk and decreased food availability are often associated with increased variation in female mate choice (Cotton et al., 2006; Johnson and Basolo, 2003) and both factors are well known to stimulate the neuroendocrine stress axis and suppress sex steroid production (Wingfield and Sapolsky, 2003). The association between environmental stressors and mate choice behavior by females suggests that circulating stress hormones play a central role in mediating such behavior.

Consistent with this hypothesis, corticosterone (CORT) levels have been shown to be inversely correlated with choosiness (the number of mates assessed) in female Galápagos marine iguanas, *Amblyrhynchus cristatus* (Vitousek and Romero, 2013). Such findings link circulating CORT levels to variation in female choosiness observed in this species during harsh environmental conditions (i.e., during droughts when food is scarce and circulating CORT levels are high, Vitousek, 2009). However, it is unclear whether elevated CORT levels are causally associated with variation in female choosiness or whether a reduction in choosiness affects selection on any particular aspect of the male phenotype in this species (i.e., whether elevated CORT level affects female mate choice or preferences, see Jennions and Petrie, 1997).

To understand the role of stress hormones in mate choice, we examined how CORT administration influences female preferences for call rate in female green treefrogs, *Hyla cinerea*. Variation in this call property is likely to be a major factor contributing male mating success in this species because females typically show strong preferences for calls produced at high rates (Gerhardt, 1987), as do females of many other species that attract mates with acoustic signals (Gerhardt and Huber, 2002). Despite strong directional selection on call rate in most anurans (Gerhardt and Huber, 2002), preferences for this call property are known to vary within and among females (Murphy and Gerhardt, 2000). Here, we show that elevated CORT levels contribute to such variation in female preferences.

* Corresponding author.

E-mail address: cjleary@olemiss.edu (C.J. Leary).

Material and methods

We hand-captured gravid female *H. cinerea* in amplexus from the University of Mississippi Field Station (Lafayette County, MS, USA) during the 2012 and 2013 breeding seasons. We then separated females from amplexant males and either did not treat them or immediately injected them intraperitoneally with 100 μ l saline only or with 4, 8 or 16 μ g of CORT in 100 μ l saline vehicle (see Leary et al., 2006). We then conducted phonotaxis tests 60–100 min after treatment between 2200–0100 h. We prepared CORT injections by dissolving crystalline CORT (Steraloids, Inc., Newport, Rhode Island, USA) in ethanol, evaporating off the ethanol and subsequently resuspending the sample in saline. Aliquots were frozen in Eppendorf tubes until needed. We randomly assigned hormone doses to females.

We assessed female preferences for call rate using dual-speaker phonotaxis tests (as in Leary et al., 2006). Stimuli used in playback experiments were acquired from a recording of an isolated advertisement call from a single individual male *H. cinerea* with vocal parameters that approximated the mean values for the study population (e.g., dominant carrier frequencies = 800 and 2400 Hz, call duration = 140 ms). We created two continuous loop recordings of the call that varied in intercall duration using Raven software (Cornell Bioacoustics Laboratory); calls were broadcast at an amplitude of 95 dB SPL (see Gerhardt, 1987) from SME-AFS Amplified Field Speakers (Saul Mineroff Electronics, Inc.) connected to Marantz PMD222 recorders during phonotaxis tests. One speaker broadcast the advertisement call at a rate of 2.5 calls/s (i.e., possessing a 325 ms intercall interval) and the other speaker broadcast the call at a rate of 1.2 calls/s (i.e., possessing an 830 ms intercall interval), well within the natural range of call rate for this species (Gerhardt, 1987). Thus, calls were identical except the rate at which they were broadcast. Stimulus amplitude was checked prior to each phonotaxis trial using a sound pressure level meter (Ivie Technologies Inc., model IE-45, fast weighting setting). To control for side biases, we alternated stimuli between speakers across successive trials. Positive phonotactic scores included female orientation, approach, and direct contact with the speaker. We also recorded the latency of phonotactic responses (the time elapsed from when the funnel was raised until the female selected a speaker) for each female during phonotaxis trials and used this measure as an estimate of a female's motivation to mate.

We obtained approximately 100 μ l of blood within 5 min from female frogs via cardiac puncture (using a 28 gauge heparinized sterile needle) immediately following phonotaxis trials. Blood samples were kept on ice until they were returned to the lab (<6 h) and centrifuged for 12 min at 3000 rpm. Plasma was stored at -20 °C until it was assayed for steroid hormones (details provided in Supplementary information). We marked females on the venter using a portable tattoo device (Tattoo-A-Pet, Fort Lauderdale, FL, USA) prior to release at the site of capture following phonotaxis trials to prevent resampling the same individuals in future phonotaxis tests. We used binomial tests to assess whether untreated females showed a preference for the faster call rate and to test whether the probability of choosing the call broadcast at the higher rate differed among treatment groups. We used the probability of choosing the high call rate in untreated females as our expected value and the proportion of females choosing the high call rate in each of the treatment groups as the observed values. We used one-way ANOVA with Tukey's post-hoc comparisons to determine whether treatment groups differed with respect to plasma hormone concentrations and linear regression to determine if hormone level was related to the latency of phonotactic responses. We used eta squared for effect size estimates in all ANOVAs.

Scientific collecting permits were provided by the Mississippi Department of Wildlife, Fisheries and Parks. All procedures were approved by the University of Mississippi Animal Care and Use Committee (protocol #12-014).

Results

Untreated controls showed a strong preference (when compared to random choice) for the call broadcast at a higher versus lower rate (binomial test, $p = 0.03$; Fig. 1A). Females administered saline only and 4 μ g CORT did not differ from the untreated control group in the probability of choosing the call broadcast at a higher rate (binomial tests, $p \geq 0.22$; Fig. 1A). However, females administered 8 or 16 μ g of CORT showed a significant decrease in the probability of selecting the call broadcast at a higher rate (binomial tests, $p \leq 0.03$; Fig. 1A).

Plasma samples were acquired from 24 of the 45 females used in phonotaxis trials; mean body length and mass of gravid females were 5.3 cm and 8.86 g and, thus, it was difficult to obtain adequate blood from all females. The highest dose of CORT resulted in circulating CORT levels (mean = 80.1 ng/ml, range = 52.4–110.0 ng/ml) that approximated the natural upper levels known for this species (95 ng/ml, Leary unpubl. data). Circulating levels of CORT differed significantly across the 5 treatment groups ($F_{4, 19} = 6.36$, $p = 0.002$, $\eta^2 = 0.57$); the 16 μ g CORT group had significantly higher CORT levels than all other treatment groups (Tukey's post hoc test, $p < 0.02$) but there were no detectable differences in circulating CORT levels in pairwise contrasts for the other treatment groups (Tukey's post hoc tests, $p \geq 0.49$; Fig. 1B). In contrast, there were no detectable differences among treatment groups with respect to circulating levels of P ($F_{4, 19} = 1.80$, $p = 0.17$, $\eta^2 = 0.27$), DHT ($F_{4, 19} = 1.35$, $p = 0.29$, $\eta^2 = 0.22$), T ($F_{4, 19} = 1.15$, $p = 0.36$, $\eta^2 = 0.19$), or E2 ($F_{4, 19} = 1.13$, $p = 0.37$, $\eta^2 = 0.19$).

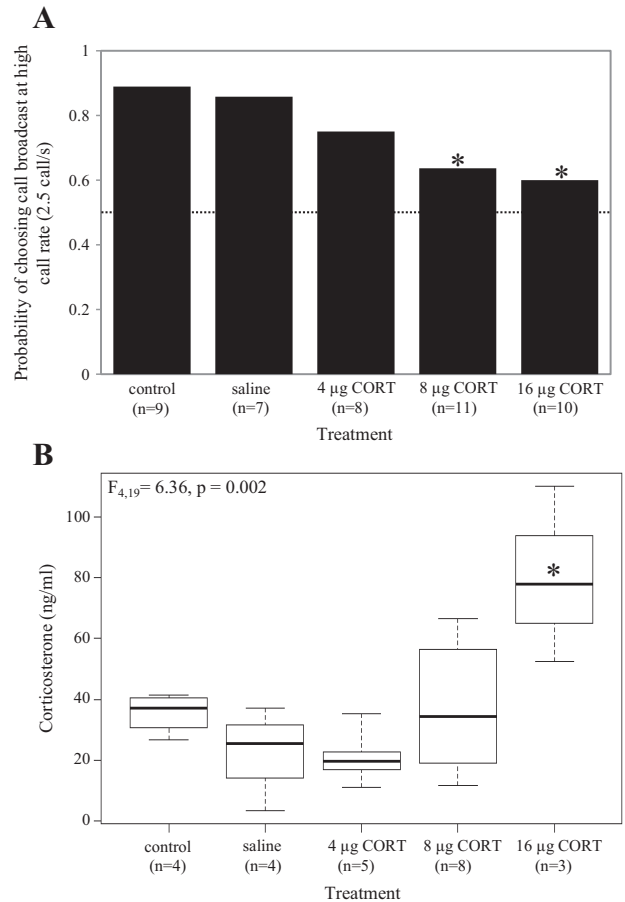


Fig. 1. A) The probability of female *Hyla cinerea* choosing the call broadcast at the higher rate (2.5 calls/s) over the lower rate (1.2 calls/s) following no treatment, treatment with saline vehicle only, or treatment with 4, 8, or 16 μ g of corticosterone (CORT) in saline. Dotted line reflects random choice by females. B) Circulating CORT levels for females in each treatment group. Boxes represent 75th (upper) and 25th (lower) percentiles, with means (solid horizontal lines), and maximum and minimum values (whiskers).

The latency of phonotactic responses (i.e., time elapsed to select a speaker) also did not differ among treatment groups ($F_{4, 40} = 1.48, p = 0.23, \eta^2 = 0.12$) and was not related to circulating levels of any of the measured hormones (Table 1).

Discussion

Female green treefrogs that were administered higher doses of CORT showed a significant reduction in the probability of choosing calls broadcast at high rates. These results were best explained by circulating CORT levels; there was no evidence that variation in female choice across treatments was related to elevated levels of sex steroids. Our results thus suggest that elevated glucocorticoid levels can decrease the strength of directional selection on male vocal traits by reducing the strength of female preferences.

The mechanism by which CORT alters female mate choice is currently unclear. Heightened CORT levels can, however, induce early oviposition in lizards (Radder et al., 2008). If elevated CORT levels initiate similar responses in female treefrogs, they may become less choosy as the time to oviposition approaches (i.e., when greater costs associated with stronger preferences are expected, Jennions and Petrie, 1997). However, in our study there was no significant relationship between the latency of phonotactic responses and treatment or circulating CORT levels, suggesting that CORT did not influence the motivation to mate.

An alternative explanation for the effects of CORT on female preferences described in the current study is that circulating CORT level alters neural selectivity for acoustic signals. For example, auditory neural selectivity for various call properties in anurans arises from the interplay between inhibition and excitation, and small changes in their strength and/or time course can result in dramatic changes in neural tuning (Edwards et al., 2007; Leary et al., 2008). If CORT attenuates inhibition in auditory neurons, as it does in some rat hippocampal neurons (Maggio and Segal, 2009), calls produced at slow rates could elicit behavioral phonotaxis because cells would be closer to threshold for spiking. While glucocorticoid receptors are distributed throughout many key auditory processing regions of the anuran brain (i.e., the torus semicircularis, Yao et al., 2008) CORT effects on auditory selectivity remain unknown. Intracellular neurophysiological recording techniques used to deliver pharmacological agents to focal neurons (Rose et al., 2013) could be modified to examine the effects of CORT on auditory neural selectivity.

Regardless of the mechanisms involved, a CORT-mediated reduction in the strength of female preferences may be advantageous under stressful environmental conditions, particularly when females have already invested in the development of eggs and the time to oviposition is approaching. Under such circumstances, it may better to mate with an “unattractive” male (despite potential indirect costs) than it is to run the risks associated with increased exposure to the stressor. For example, a reduction in the strength of mate preferences may be advantageous when predation risk is high.

To date, most studies have focused on how sex steroid levels affect mate choice. Elevated sex steroid levels appear to reduce female choosiness by increasing the motivation to mate given that elevated sex steroids often increase neural selectivity (e.g., fish, Sisneros et al., 2004; birds, Remage-Healey et al., 2010). Alternatively, the administration of

sex steroids could result in elevated glucocorticoid levels (Leary and Knapp, 2014) that contribute to variation in mate choice behavior. Future studies should incorporate measures of both sex steroids and glucocorticoids to examine this hypothesis.

In summary, despite considerable evidence linking environmental stressors to variation in mate choice by females and the well-known links between environmental stressors and glucocorticoid production, there has been little assessment of the potential role of circulating glucocorticoids in mediating variation in female mate choice. Our results indicate that circulating glucocorticoid levels can play an important role in mediating this variation.

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Appendix A. Supplementary

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.yhbeh.2015.01.005>.

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Table 1

Linear regression results for relationship between hormone level and the latency of phonotactic responses in *Hyla cinerea* ($n = 24$).

Hormone	F-statistic	r ² value	p-Value
Corticosterone	1.13	0.006	0.72
Progesterone	0.09	0.004	0.76
Dihydrotestosterone	0.001	3.23e−05	0.98
Testosterone	1.45e−05	6.62e−07	0.99
Estradiol	0.53	0.02	0.47

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