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The difference a day makes: Breeding remodels hearing, hormones and behavior in female Cope's gray treefrogs (*Hyla chrysoscelis*)

Megan D. Gall[a,1], Mark A. Bee[b,c], Alexander T. Baugh[d,*,1]

[a] *Department of Biology, Vassar College, 124 Raymond Ave., Poughkeepsie, NY 12604, USA*
[b] *Department of Ecology, Evolution, and Behavior, University of Minnesota - Twin Cities, 1479 Gortner Ave, St. Paul, MN 55108, USA*
[c] *Graduate Program in Neuroscience, University of Minnesota - Twin Cities, 321 Church Street SE, Minneapolis, MN 55455, USA*
[d] *Department of Biology, Swarthmore College, 500 College Avenue, Swarthmore, PA 19081, USA*

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ABSTRACT

In seasonal breeders, there are behavioral, endocrine, and neural adaptations that promote the sexual receptivity of females and tune their sensory systems to detect and discriminate among advertising males and to successfully copulate. What happens immediately after this key life history event is unclear, but this transitional moment offers a window into the mechanisms that remodel sexual phenotypes. In this study of wild female Cope's gray treefrogs (*Hyla chrysoscelis*), we tested the hypothesis that oviposition results in a suite of coordinated changes in the sexual phenotype. Specifically, we predicted that sexual receptivity and discrimination behaviors would decline along with circulating concentrations of steroid hormones (corticosterone, estradiol, testosterone) and auditory sensitivity to the acoustic frequencies emphasized in male advertisement calls. We conducted these trait measurements before and after oviposition (ca. 24-h period). There was a 100% decrease in behavioral responsiveness after oviposition, and the concentrations of all three steroids plummeted during this brief window of time, especially testosterone. Moreover, higher concentrations of corticosterone—an important component of the endocrine stress response—were associated with longer response latencies, suggesting that adrenal hormones should be considered in future studies on the hormonal basis of mate choice. Counter to our prediction, auditory sensitivity increased following oviposition, and the amplitude of the auditory brainstem response was influenced by concentrations of estradiol. In pre-oviposition females auditory sensitivity diminished with increasing estradiol concentrations, while sensitivity increased with increasing estradiol concentrations in post-oviposition females, suggesting non-linear estrogenic modulation of peripheral auditory neural recruitment. Overall, our results indicate that there is considerable remodeling of behavioral output following oviposition that co-occurs with changes in both endocrine and sensory physiology.

1. Introduction

For animals that have transient windows of reproductive readiness, sexual receptivity and its underlying mechanisms may shift rapidly and dramatically. For vertebrates that rely on their auditory system during mate choice, these mechanisms may include the tuning and sensitivity of the auditory system and the endocrine systems that modulate activity in those pathways and other sexually relevant brain areas (Schmidt, 1984; Hillery, 1984; Sisneros, 2009; Miranda and Wilczynski, 2009; Caras et al., 2010). Such dynamic and coordinated shifts in physiology may also be reflected at a finer scale within a reproductive bout as females approach the critical stage of oviposition (Lynch et al., 2005). Hence, female sexual selection on male traits may vary both across and within female reproductive bouts (Baugh and Ryan, 2009; Bastien et al., 2018). For most ectothermic vertebrates, the act of breeding involves a quick life history transition from social to non-social. In female anuran amphibians (frogs and toads), this transition often occurs over the course of a single day. Therefore, the natural life history contrast between pre-oviposition and post-oviposition frogs offers an opportunity to evaluate the mechanisms that underlie this shift and how they are coordinated (Wilczynski and Lynch, 2011).

One major class of mechanisms to examine is the gonadal and adrenal steroid hormones because they are known to modulate vertebrate reproductive behavior. For example, in many songbird species, estradiol (E_2) implants elevate the frequency of copulation solicitation displays in females (reviewed in Maney and Pinaud, 2011). In

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[*] Corresponding author at: Swarthmore College, 500 College Avenue, Martin Biological Laboratory, Swarthmore, PA 19081, USA.
E-mail address: abaugh1@swarthmore.edu (A.T. Baugh).
[1] Co-first authors.

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amphibians, similar patterns are present. For example, female túngara frogs [*Physalaemus* (= *Engystomops*) *pustulosus*] are most receptive to male advertisement calls when found in amplexus (Lynch et al., 2005), which coincides with females having elevated concentrations of progesterone (PROG) and E_2, both of which decline after mating (Lynch and Wilczynski, 2005; Lynch et al., 2005). Experimentally elevating E_2 in female túngara frogs using E_2 injections or human chorionic gonadotropin injections (hCG; Chakraborty and Burmeister, 2009; Lynch et al., 2005) increases female receptivity (approach toward mating calls, phonotaxis) prior to copulation. The same effect was shown in female American toads (*Anaxyrus americanus*) injected with hCG (Schmidt, 1984). In female gray treefrogs, injections with PROG and prostaglandins also increase receptivity compared to vehicle controls (*Hyla versicolor*, Gordon and Gerhardt, 2009; *Hyla chrysoscelis*, Ward et al., 2015), and females with higher concentrations of PROG and E_2 are more receptive (Gordon and Gerhardt, 2009).

Complementing the gonadal steroid studies, recent evidence has also shown that adrenal/interrenal hormones such as glucocorticoids modulate aspects of female mate choice across a range of vertebrate taxa, including mammals (DeVries et al., 1996), birds (Woodgate et al., 2010), reptiles (Vitousek, 2009; Vitousek and Romero, 2013), and amphibians (Davis and Leary, 2015). In the green treefrog, *Hyla cinerea*, experimentally elevated concentrations of corticosterone (CORT) reduced the species-typical preference for higher call rates (Davis and Leary, 2015). Because the gonadal and adrenal/interrenal steroids might interact antagonistically (reviewed in Toufexis et al., 2014), it may be informative to evaluate the contributions of both the HPG and HPA/I axes when investigating hormonal mechanisms of mate choice.

Such transient behavioral receptivity indicates that the nervous system is rapidly changing as well. For acoustically communicating species, this shift likely involves changes in how the female auditory system is processing sound. In frogs, fishes and birds, peripheral and central auditory processing exhibit seasonal or socially induced changes (Hillery, 1984; Sisneros and Bass, 2003; Velez et al., 2015; Gall and Wilczynski, 2015) that are associated with changes in reproductive state. This seasonal and socially-modulated plasticity is thought to be influenced by changes in steroid hormones (reviewed in Wilczynski and Lynch, 2011).

Indeed, there is growing evidence that steroid hormones modulate both peripheral and central auditory processing (Sisneros et al., 2004; Lynch and Wilczynski, 2008; Maney and Pinaud, 2011; Rohmann and Bass, 2011; Caras, 2013; Forlano et al., 2015). In general, estradiol has been shown to increase auditory sensitivity (Remage-Healey et al., 2013), although high levels of circulating estradiol appear to inhibit auditory function in some cases (Caras et al., 2010). Less work has investigated the relationship between circulating androgens and auditory processing in birds and frogs, while little is known about the role of glucocorticoid hormones in modulating auditory function in non-mammals. We also know little about the extent to which the auditory system may be plastic in the transition from a reproductive to a non-reproductive state, as most work has focused either on the transition from a non-reproductive to reproductive state or on longer-scale seasonal changes.

In the present study of Cope's gray treefrog (*Hyla chrysoscelis*), we tested the general hypothesis that three features of the sexual phenotype of females—circulating hormone levels, sensitivity of the peripheral auditory system to the spectral content of advertisement calls, and behavioral receptivity to male advertisement calls—are remodeled in a coordinated manner across a rapid life history transition from reproductive readiness (pre-oviposition) to post-breeding (post-oviposition), which occurs over the course of a single day. By taking advantage of this natural transition and comparing sexual phenotypes before and after oviposition, we have a window into the plasticity of sexual behavior and its mechanisms. We tested the following four specific predictions. First, sexual receptivity in females will be markedly transient—females will suddenly enter a behaviorally refractory period following oviposition. We predicted that positive phonotaxis toward male advertisement calls would decline precipitously following oviposition, presumably because of a lack of any functional value of locating and approaching an advertising male. Second, coincident with a reduction in behavioral responsiveness will be declines in plasma gonadal and interrenal hormone concentrations (corticosterone, CORT; 17-beta estradiol, E_2; and testosterone, T), as secretion rates are thought to decrease during a refractory period between breeding events and because the eggs themselves likely serve as a major source of gonadal steroids (Lynch and Wilczynski, 2005; Lynch et al., 2005; Chakraborty and Burmeister, 2009; Narayan et al., 2010). Third, coincident with reductions in behavior and hormones, peripheral auditory sensitivity to the acoustic properties of male advertisement calls will decline following oviposition. To test this we used measures of auditory evoked potentials (AEPs), specifically, the auditory brainstem response (ABR), and we predicted higher ABR thresholds and smaller ABR amplitudes following oviposition. Fourth, changes in auditory sensitivity and behavior are correlated with circulating steroid hormones. Specifically, lower concentrations of E_2 and T and higher concentrations of CORT will be associated with reduced behavioral receptivity and auditory sensitivity following oviposition. Hence, the first and second predictions which have been borne out in other species, represent confirmatory predictions in the present system, whereas the third and fourth predictions represent novel tests in a vertebrate system.

2. Materials and methods

2.1. Animals and experimental design

This study was carried out on the St. Paul campus of the University of Minnesota. In June 2017 we collected mating pairs of the western genetic lineage of Cope's gray treefrog (Ptacek et al., 1994) from wetlands located in the Carver Park Reserve (Carver County, MN), the Crow-Hassan Park Reserve (Hennepin County, MN), and the Hyland Lake Park Reserve (Hennepin County, MN). Pairs found in amplexus were collected and placed in small plastic containers in the field and brought to the lab. Thus, all females in the present study were collected in a reproductively ready state. All pairs except the ‘no chill’ group were then maintained at approximately 4 °C until the following day, when they were tested or permitted to oviposit. The ‘no chill’ group was sampled for plasma hormones immediately upon return from the field (Fig. 1).

We used a mixed within- and between-subjects design in which each female was tested in one of two reproductive states, either *prior* to oviposition (‘pre-oviposition’) or *after* oviposition (‘post-oviposition’); post-oviposition females were allowed to oviposit during a 24-hour period at 20 °C in individual tanks with water (Fig. 1). Frogs were randomly assigned to one of these two states and were further assigned randomly to one of two experimental groups or one of two control groups. We tested a total of 128 frogs and no frogs were used in more than one reproductive state or group. The two experimental groups included females tested for behavior in pre-oviposition (N = 21) or post-oviposition (N = 20) states; and females tested for AEPs in pre-oviposition (N = 13) or post-oviposition (N = 13) states (Fig. 1). All experimental females were held at 4 °C following collection. We used two control groups to control for the potential effects of holding at 4 °C and handling during phonotaxis and AEP measurements and thus to estimate the effects of these methods on plasma hormones. In one control group, the ‘no chill’ females were not held at 4 °C and not tested for behavior or AEPs but were sampled for plasma hormones in the pre-oviposition state (Fig. 1, N = 25). Although we have not previously observed any effect of this holding procedure on behavior or hormone concentrations in the closely-related eastern gray treefrog (*H. versicolor*; Bastien et al., 2018), this ‘no chill’ group allowed us to evaluate the potential effect that this holding procedure had on plasma hormones in our study species. In the second control group, females were not tested

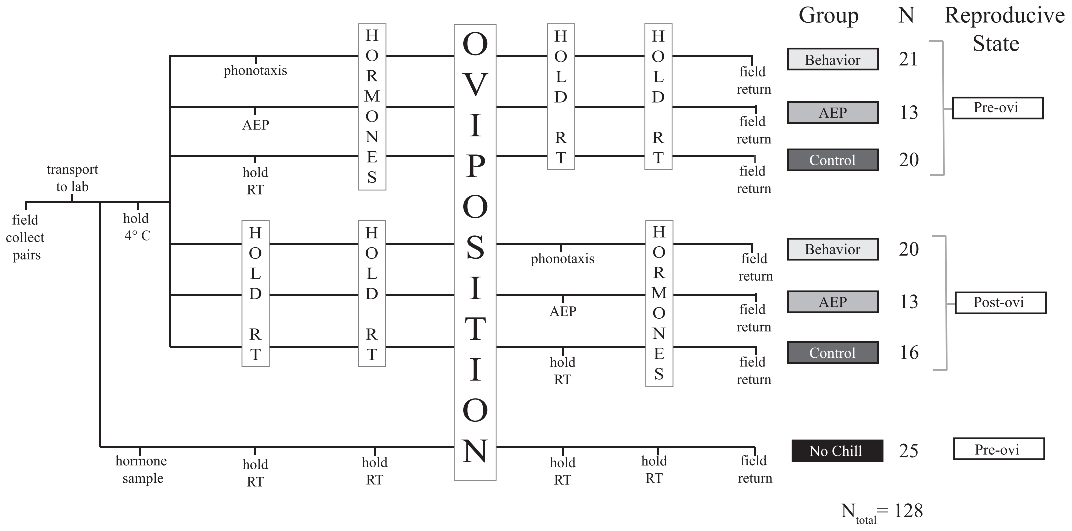

Fig. 1. Experimental design. There were two reproductive states (pre-oviposition and post-oviposition). Within each state, there were two experimental groups: (1) plasma hormones and behavior were measured (Behavior), and (2) plasma hormones and AEPs were measured (AEP). Additionally, for both states there was a control group wherein only plasma samples were collected (Control). Lastly, there was a 'no chill' control group that was not held at 4 °C but was sampled for hormones in the pre-oviposition state. Therefore, the following comparisons and inferences were permitted: (i) pre-oviposition control versus 'no chill': the effect of holding at 4 °C on pre-oviposition hormones; (ii) pre-oviposition control versus post-oviposition control: the effect of oviposition on hormones in the absence of any handling; (iii) pre-oviposition Behavior versus post-oviposition Behavior: the effect of oviposition on behavior, controlling for behavioral handling and holding at 4 °C; (iv) pre-oviposition AEP versus post-oviposition AEP: the effect of oviposition on AEPs, controlling for AEP handling and holding at 4 °C. A total of 128 females were used and no female was used in more than one reproductive state or group. RT: room temperature.

for behavior or AEPs but were held at 4 °C and sampled for plasma hormones in either the pre-oviposition (N = 20) or post-oviposition (N = 16) state.

Testing our hypotheses involved an experimental design trade-off: because the act of ovipositing inevitably takes time, the pre-oviposition and post-oviposition females differed not only in whether or not they had oviposited, but also in the duration of time they were held since capture in the field before being tested (i.e., a time confound). If we were to control for total time held, pre-oviposition females would have to be held for an additional 24 h at 4 °C to delay oviposition. Therefore, we chose to control for time at 4 °C, rather than total time held in the lab, because we have previously observed that females held for more than about 3 days at 4 °C tend to exhibit reduced behavioral receptivity (Bee, personal observation). Thus, post-oviposition females were tested for the three phenotypic attributes (behavior, hormones, AEP) following an additional 24-hour post-capture period compared to pre-oviposition females. We assumed any differences between pre-oviposition and post-oviposition females would be dominated by the change in reproductive status, not the additional day of age/holding.

Approximately 20 to 40 min prior to behavioral or auditory testing or control group blood sampling, we allowed pre-oviposition pairs to reach body temperatures of 20 ± 2 °C in an incubator. Pairs resumed amplexus during this period but did not begin ovipositing. Prior to testing, females were separated from their male mate and tested individually. After testing, pre-oviposition females were reunited with their male mate in a plastic terrarium (L × W × H, cm; 32 × 18 × 22) filled to a depth of 8 cm of aged tap water at one end and allowed to oviposit in order to confirm that they had normal clutch sizes at the time of testing. Females assigned to the post-oviposition state were also warmed to 20 ± 2 °C with their mates and allowed to resume amplexus and oviposit during a 24-hour period before they were tested for behavior, AEPs, or bled if in the control group. All blood sampling for the experimental groups took place immediately following the behavioral or AEP testing procedures.

All frogs were measured for body mass before and after oviposition (to the nearest 0.01 g) and were measured for two estimates of body length (snout-vent length, SVL; tibia-fibula length, TFL) using calipers (to the nearest 0.01 mm). Body mass correlated positively and strongly with both SVL and TFL in both the pre-oviposition and post-oviposition states (R^2 range: 0.50–0.74), but the correlation was nominally higher using TFL. Thus, residual body mass (RBM) was calculated from the TFL versus body mass regression. We also recorded the date and time of collection.

2.2. Stimuli and behavioral testing

Each female assigned to the behavior group was tested in two behavior trials. In a *recognition test*, a single speaker broadcast a synthetic *H. chrysoscelis* call that was generated using custom-written software in MATLAB to have acoustic properties close to the average values of calls recorded at 20 °C in local populations (Ward et al., 2013). Each pulse in this "standard call" was created by adding two phase-locked sinusoids with frequencies (and relative amplitudes) of 1250 Hz (−11 dB) and 2500 Hz (0 dB). Thirty identical pulses were concatenated to create an average-length call (50 pulses/s, 50% pulse duty cycle, 20-ms pulse period, 10-ms pulse duration, 3.1-ms pulse rise time, 5.4-ms fall time). The call was broadcast at a rate of 11 calls/min. In a *discrimination test*, one speaker broadcast the standard call and the opposite speaker broadcast a synthetic "alternative call" designed to simulate the advertisement call of a closely related species, the eastern gray treefrog (*Hyla versicolor*). The alternative call had the same frequency spectrum and call rate as the standard call, but a slower pulse rate of 20 pulses/s. The pulse duty cycle of the slow pulse-rate alternative was maintained at 50% (50-ms pulse period, 25-ms pulse duration), and values of pulse rise and fall times were equivalent to those in the standard call in terms of their proportions of pulse duration. Females of *H. chrysoscelis* strongly discriminate against this slower pulse repetition rate (Bush et al., 2002; Ward et al., 2013; Lee et al., 2017; Tanner et al., 2017). Stimulus files were prepared for these playback tests that included each stimulus in a leading and lagging context (i.e. whether the first call broadcast was the standard or the alternative call). For each female we randomly determined whether the recognition test or discrimination test would be performed first or second, which speaker broadcast the stimuli, and which speaker broadcast a leading versus lagging stimulus. All calls were broadcast at 85 dB SPL at 1 m, which approximates a natural call amplitude in this species (Gerhardt, 1975). We used a

Larson Davis Model 831 sound-level meter (Larson Davis Inc., Depew, NY) to calibrate the sound pressure levels (SPL re 20 μPa; LCF_{max}) of each experimental stimulus prior to testing each day. Testing was carried out in a rectangular arena (L × W, cm; 240 × 125) constructed from hardware cloth covered in black fabric and located on the floor of a sound-attenuating chamber outfitted with acoustic tiles on walls and ceiling and carpeted with low pile carpet (L × W × H, cm, internal dimensions: 295 × 275 × 195; Industrial Acoustics Company, Winchester, United Kingdom). Playbacks were performed using a PC with Adobe Audition (version 1.5; San Jose, CA) connected to an external sound card (Firewire 410; M-Audio Inc., Cumberland, RI), amplifier (XLS 1000; Crown Audio Inc., Elkhart, IN), and two satellite speakers (Mod 1; Orb Audio, NY, NY). Speakers were centered at opposite ends of the arena along its long axis and placed 5 cm from the arena walls to permit females to walk behind the speaker. A response zone semi-circle (10 cm radius) centered around each speaker was outlined with reflective tape on the arena floor.

For each trial, the female was placed in an acoustically transparent release cage located at the center of the arena (origin) and 1 m from each speaker. We broadcast the stimuli for 1 min prior to releasing the female by remotely lifting the lid of the release cage. During each trial we recorded her latency to exit the origin (“origin latency”), her latency to enter a response zone (“response latency”), and, in the discrimination test, which stimulus was chosen, if any (“choice”). Females were given up to 5 min to enter a response zone; typical response latencies in this species are on the order of 70–90 s. Females were not re-tested if they failed to respond within 5 min. We recorded the ambient temperature in the chamber after each trial (20–21 °C). In total we tested 41 females for behavior ($N_{pre} = 21$; $N_{post} = 20$).

2.3. Auditory evoked potentials (AEPs)

We used measures of the auditory brainstem response (ABR) to assess changes in the peripheral sensitivity of the auditory system. The ABR is a form of AEP that reflects the synchronous activity of auditory nerve fibers and can be used as a minimally-invasive physiological measure of peripheral auditory sensitivity. In total we successfully measured auditory evoked potentials from 26 females ($N_{pre} = 13$; $N_{post} = 13$). All experiments were conducted in a small acoustic chamber (MAC-3, Industrial Acoustics Company; L × W × H, cm, internal dimensions: 81.3 × 61 × 61) lined with 5-cm pyramidal acoustic foam. The temperature in the chamber was monitored by a digital thermometer that was placed adjacent to the animal and maintained at 20–21 °C.

As stimuli, we used brief tone bursts designed to assess the sensitivity of the peripheral auditory system to the two spectral components of the male advertisement call. Each tone burst was constructed by adding two phase-locked sinusoids (starting phase of 0°) with frequencies (and relative amplitudes) of 1250 Hz (−11 dB) and 2500 Hz (0 dB). These frequencies and relative amplitudes are near the population mean of the two spectral components of *Hyla chrysoscelis* calls, which are effective at eliciting positive phonotaxis in pre-oviposition females (e.g., Ward et al., 2013). Each tone burst was 5 ms in duration and was shaped with a 1 ms Blackman onset/offset ramp. In behavior tests, pre-oviposition females exhibit positive phonotaxis to advertisement calls with pulses as short as 2 ms (Schul and Bush, 2002). Hence, the tone bursts used here were designed to mimic the spectral content and duration of behaviorally relevant pulses in advertisement calls while also containing stimulus features (e.g., short duration signals) effective at eliciting ABRs. Tone bursts were presented at amplitudes of 90–70 SPL in 10 dB steps and from 65 to 45 dB SPL in 5 dB steps at a rate of 31.1 stimuli s^{-1}. Two sets of 400 repetitions were presented at each stimulus amplitude. Additionally, broadband clicks were presented at 100 dB SPL every 10–20 min to monitor the physiological stability of the subject.

All stimuli were generated in SiGenRP and presentation was coordinated with BioSigRP (Tucker Davis Technologies [TDT], Alachua, FL). The stimuli were passed to a TDT RP2 digital processor (sampling rate = 48.8 kHz), attenuated with a TDT PA5 programmable attenuator, and amplified by a Crown XLS 202 amplifier before presentation through an Orb Mod 1 speaker. The speaker was placed inside of a copper mesh Faraday cage that was connected to the building ground. Prior to the experiments we calibrated the frequency response of the speaker by playing 5-s tones in the frequency range relevant for communication signals and recording the speaker output with a Larson Davis System 824 sound-level meter. The amplitude of each stimulus was subsequently adjusted using a TDT PA5 programmable attenuator.

Females were immobilized with an intramuscular injection of succinylcholine chloride in frog ringer's solution (mean ± S.D. = 3.4 ± 0.8 μg/g). After injection females were placed on an acoustically transparent pedestal that was 30 cm from the speaker. The female was loosely draped with moist surgical gauze to facilitate cutaneous respiration. We applied a 2.5% lidocaine solution to the head of the female and then inserted three subdermal needle electrodes (Grass F-E2; West Warwick, RI) that included a reference electrode adjacent to the left tympanum, an inverting electrode at the apex of the skull between the two eyes, and a ground electrode adjacent to the right tympanum. The impedance between the electrodes was maintained between 1 and 5 kΩ, although impedance was usually below 3 kΩ.

The electrodes fed into a TDT RA4LI low-impedance headstage and TDT RA4PA pre-amp (Gain = 20). Digitized responses were then passed through a fiber optic cable to a TDT RZ5 biological signal processor and then a computer running BioSigRP software. All responses were high-pass filtered at 10 Hz, low-pass filtered at 3 kHz, and notch-filtered at 60 Hz. Responses were analyzed offline for ABR amplitude (Schrode et al., 2014; Schrode and Bee, 2015; Supplemental materials S1) in Praat version 6.0.40 (Wong and Gall, 2015; Boersma and Weenink, 2018). Auditory thresholds were determined by two trained observers using the visual detection method (Beatini et al., 2018).

2.4. Blood collection

Immediately following body measurements in the ‘no chill’ and control groups (Fig. 1), or after behavior and AEP testing in the experimental groups, we collected blood by cardiac puncture, a technique that has been used successfully in other North American treefrogs (Gordon and Gerhardt, 2009; Davis and Leary, 2015; Bastien et al., 2018). We rapidly (< 5 min) collected blood (ca. 50 μL) using a 30-gauge insulin syringe (BD Micro-fine U-100, 0.3 mL) pre-rinsed with heparin. Using this technique in *H. versicolor* previously, Bastien et al. (2018) demonstrated that this method accurately captures plasma CORT concentrations without any elevation due to handling. We then centrifuged whole blood (7500 RPM for 10 min; Eppendorf 5418 at 8 °C) and stored the plasma fraction at −20 °C for 3 weeks and then shipped the samples on dry ice to Swarthmore College where they were stored at −80 °C for 6 months until assayed.

2.5. Plasma steroid validations

Prior to processing our experimental samples, we validated our extraction and quantification methods for each steroid. Using pools of plasma collected from the experimental samples, we evaluated parallelism, determined the optimal dilution factor (1:40 for CORT; 1:30 for E_2; 1:15 for T), and estimated the percent recovery. Parallelism was conducted by serially diluting plasma pools. We found that serial dilutions were parallel to the standard curve for each hormone (CORT: Δ slope = 0.02, SE = 0.21, $t_9 = 0.11$, p = 0.91, Supplemental materials S2; E_2: Δ slope = 1.06, SE = 6.71, $t_7 = 0.15$, p = 0.88, Supplemental materials S3; T: Δ slope = 0.16, SE = 1.58, $t_8 = 0.10$, p = 0.92, Supplemental materials S4). For recovery determination, we first stripped endogenous steroids from plasma pools and then spiked them with a known concentration of each commercial steroid (supplied by kit). We followed the stripping methods from Delehanty et al. (2015) by adding dextran-coated activated charcoal to plasma pools ($70\,\mathrm{mg\,mL^{-1}}$), then vortexing and incubating at 37 °C for 4 h. We then centrifuged three times and spiked the supernatant using purified steroids (supplied by kit; N = 9 replicates per steroid) at concentrations that approximated the average levels found in the parallelism component: CORT: $10{,}000\,\mathrm{pg\,mL^{-1}}$; E_2: $800\,\mathrm{pg\,mL^{-1}}$; and T: $10{,}000\,\mathrm{pg\,mL^{-1}}$. We then extracted and processed these stripped/spiked samples, as well as a stripped/unspiked sample (to determine background concentration), using the same procedures applied to the experimental samples. The stripped/spiked samples were added to each plate (N = 2–3 per plate) of experimental samples in order to estimate intra- and inter-assay coefficients of variation (CV). The intra- and inter-assay CVs, respectively, were 14.8% and 23.9% for CORT; 12.1% and 0.8% for E_2; and 9.6% and 10.0% for T. Recovery efficiencies were consistent for all three hormones (mean ± SE): CORT: 74.9 ± 5.9%; E2:76.4 ± 3.6%; T: 76.2 ± 2.5%. Neither control nor experimental sample concentrations were adjusted to account for this average recovery.

2.6. Steroid extraction and reconstitution

We used a liquid diethyl ether extraction method that has proven effective for small volumes of plasma and results in high recoveries (Baugh et al., 2012), including in frogs (Baugh et al., 2018). Our validations indicated that small volumes of plasma were sufficient to precisely quantify each hormone in this species (CORT: 5 μL; E_2: 8 μL; T: 10 μL). Plasma was vortexed prior to subsampling and then added to borosilicate vials. Next, 200 μL of RO water was added to each vial in order to increase the aqueous volume for ease of decanting. We then added 2 mL of diethyl-ether to each vial and thoroughly vortexed and the aqueous layer was frozen in a dry ice and methanol slurry. The organic layer was decanted to a new borosilicate vial and the aqueous layer was allowed to thaw; this extraction process was repeated a second time. The ether extracts were then dried using a Speedvac centrifuge at 37 °C (Thermo Fisher Savant Speedvac SPD1010) and resuspended in assay buffer (supplied by kit) and allowed to reconstitute overnight at 4 °C.

2.7. Enzyme immunoassays

We estimated steroid concentrations using commercial EIA kits (DetectX® kits, Arbor Assays, Ann Arbor, MI) for plasma corticosterone (Cat. No. K014, Donkey anti-Sheep IgG), 17-beta estradiol (Cat. No. KB30, Donkey anti-Sheep IgG), and testosterone (Cat. No. K032, Goat anti-Rabbit IgG). Reconstituted samples and kit reagents were allowed to reach room temperature prior to use and samples were vortexed prior to plating. Samples were randomly assigned to wells and assayed in duplicate along with blanks, standards, stripped samples, and stripped/spiked samples. Samples were assayed following manufacturer instructions. Briefly, 50 μL of sample or standard for the CORT and T plates and 100 μL for the E_2 plates were plated into wells along with conjugate and antibody. Plates were then placed on an orbital shaker (500 RPM) at room temperature for 1 h (CORT) or 2 h (E_2, T) and then washed four times with wash buffer (supplied by kit). Substrate was then added and the plate was incubated at room temperature for 30 min without shaking. The reaction was stopped and optical densities were read at 450 nm on a $\mathrm{Versa_{max}}$ microplate reader with SoftMax Pro software using a four-parameter curve fitting equation (Molecular Devices, Sunnyvale CA). Intra- and inter-assay coefficients of variation (CV) for the assays were estimated by including three stripped/spiked samples per plate, thereby incorporating cumulative technical error during extraction and assaying (see Baugh et al., 2018). We accepted the average of duplicate wells unless a CV between duplicates exceeded 15%, in which case the sample was re-assayed until this criterion was met. The assays have detection limits and sensitivities, respectively, of $16.9\,\mathrm{pg\,mL^{-1}}$ and $18.6\,\mathrm{pg\,mL^{-1}}$ for CORT, $2.05\,\mathrm{pg\,mL^{-1}}$ and $2.21\,\mathrm{pg\,mL^{-1}}$ for E_2, and $9.92\,\mathrm{pg\,mL^{-1}}$ and $30.6\,\mathrm{pg\,mL^{-1}}$ for T. The cross-reactivity of the antiserum for each kit is as follows: CORT: 100% for corticosterone, 12.3% for desoxycorticosterone, 0.62% for aldosterone, 0.38% for cortisol; E_2: 100% for E_2, 3.2% for estrone sulfate, and 2.5% for estrone; T: 100% for T, 56.8% for 5a-dihydrotestosterone (hereafter DHT), and 0.27% for androstenedione. The high cross reactivity of the T antibody for DHT could indicate that our T estimates may better represent testosterone plus DHT (i.e. 'androgens'). However, two recent studies using high performance liquid chromatography-mass spectrometry showed that plasma DHT concentrations are undetectable in closely related eastern gray treefrog females (*Hyla versicolor*; Bastien et al., 2018) and female túngara frogs (*Physalaemus pustulosus*; Baugh et al., 2018). Therefore, we hereafter provisionally refer to these estimates as testosterone.

2.8. Statistics

We used paired *t*-tests to analyze the within-subject results from behavioral tests and Pearson's correlations for the hormone-behavior correlations. Parametric assumptions were met in most statistical analyses, and we report the non-parametric alternatives and corrections in instances where assumptions were violated. We used separate general linear models to evaluate the effects of two between-subjects factors, group (control, behavior, AEP) and reproductive state (pre-oviposition and post-oviposition), on hormone concentrations, which were $\log_{10}$ transformed to improve normality. Effect sizes (eta-squared and Cohen's d) were calculated for main effects, interactions and pairwise comparisons.

We had two dependent variables in our auditory evoked potential models, ABR thresholds and ABR amplitude, which were analyzed separately. ABR amplitudes were log-transformed to improve normality. We used mixed models (PROC MIXED) in SAS v. 9.3 to analyze our AEP data. In the ABR threshold model the between-subjects factors were reproductive state (pre-oviposition vs. post-oviposition), each of the $\log_{10}$ hormone levels (CORT, E_2, T) and the two-way interactions of reproductive state and each hormone level. The ABR amplitude model was similar, but also included a within-subjects factor of stimulus amplitude, and in this model frog identity was included as a random factor. Interactions that were not significant were removed from the model. Significant interaction effects that included continuous variables were explored using the solutions option in PROC Mixed. Cohen's d was calculated for the effect of oviposition, using the residual covariance parameter to estimate standard deviation. Due to the use of restricted maximum likelihood and an autoregressive covariance structure, we did not calculate effect sizes for continuous variables or variables with more than one level.

3. Results

3.1. Behavior

The purpose of our behavioral experiments was to determine the extent to which females suddenly become behaviorally unresponsive to male advertisement calls following oviposition. We found strong support for our prediction that the act of ovipositing would be associated with reduction in female sexual receptivity. One-hundred percent of pre-oviposition females (N = 21) exhibited robust positive phonotaxis in both the recognition and discrimination trials, and in every trial females entered the response zone for the standard call. Pre-oviposition females did not exhibit differences in mean (± SE) latencies between the recognition and discrimination tests (origin latency: recognition = 14.52 ± 3.3 s; discrimination = 17.8 ± 6.9 s, $t_{20} = 0.42$, p = 0.68, Cohen's d = 0.17; response latency: recognition = 77.0 ± 7.2 s; discrimination = 73.0 ± 9.5 s, $t_{20} = 0.44$, p = 0.66, Cohen's d = 0.10; paired *t*-tests). Latencies were somewhat longer, however, on the first compared to the second trial (origin latency: first trial = 22.8 ± 7.0 s; second trial = 9.5 ± 2.2 s, t_{20} = 2.03, p = 0.06, Cohen's d = 0.26; response latency: first trial = 85.7 ± 8.9 s; second trial = 64.4 ± 7.1 s, t_{20} = 2.71, p = 0.01, Cohen's d = 0.38; paired *t*-tests; pooled recognition and discrimination trials). This result highlights the value of randomizing the order of behavioral tests. Because behavior did not differ between recognition and discrimination tests, below we report the average latencies (within females) between these two test types. In contrast to the pre-oviposition females, none of the post-oviposition females exhibited phonotaxis (i.e. N = 0 of 20); in fact post-oviposition females exited the origin in only 20% of trials (8/40), and were motionless in the remaining 80% of trials. Moreover, latencies to leave the origin were lower in the pre-oviposition compared to post-oviposition state (mean ± SE: pre-oviposition = 16.1 ± 5.4 s, N = 21; post-oviposition = 201.3 ± 29.8 s; N = 8; recognition and discrimination trials combined).

As a supplemental question, we evaluated whether cardiac puncture methods interfere with subsequent behavior testing. In a separate group of pre-oviposition females not evaluated in the main study (N = 10), we found that females unanimously exhibited robust positive phonotaxis toward the standard call when tested immediately (< 2 min) following cardiac puncture. Although this is not relevant in the current design—because all females in the main study were tested *prior* to cardiac puncture—it is useful to know that future studies could potentially employ experimental designs that balance the order of behavior testing and hormone sampling without major consequences of the procedure on female phonotaxis. Average latencies to the response zone, however, were approximately twice as long compared to females not bled prior to pre-oviposition behavior testing (mean ± SE: bled before testing: 123.2 ± 18.3 s, N = 10; not bled before testing: 64.0 ± 32.7 s, N = 21, first trials only; $t_{11.8}$ = 2.45, p = 0.03, Cohen's d = 0.52, correction for unequal variances).

3.2. Hormones

A major purpose of our plasma hormone measures was to determine the extent to which concentrations were lower following oviposition. We found strong support for our prediction that plasma hormone concentrations would be lower after oviposition in concert with abolished behavioral receptivity. Moreover, we found that the largest and most consistent differences between the reproductive states were in the concentrations of the two gonadal steroids, especially testosterone. However, we also identified a negative correlation between circulating levels of corticosterone and behavioral response latencies in pre-oviposition females.

3.3. Corticosterone

Overall, concentrations of CORT were highly variable among all frogs, spanning more than two orders of magnitude (mean ± SE: 19.1 ± 2.6 ng mL^{-1}; range: 0.2–120 ng mL^{-1}; all groups pooled, N = 128) and varied significantly across reproductive states and groups ($F_{5,101}$ = 6.19, p < 0.0001, eta-squared = 0.23). Concentrations of CORT were substantially lower following oviposition (Fig. 2a). This effect was observed in each of the three groups (behavior, AEP, control) and was of similar magnitude, as indicated by a significant main effect of reproductive state ($F_{1,101}$ = 18.8, p < 0.0001, eta-squared = 0.16) and no significant interaction between reproductive state and group ($F_{2,101}$ = 1.1, p = 0.33, eta-squared = 0.02). There was, however, a main effect of group ($F_{2,101}$ = 4.2, p = 0.02, eta-squared = 0.08), with AEP tested females experiencing elevated CORT compared to the behavior group (p = 0.018; Sidak-corrected for multiple comparisons; all other post-hoc tests were not significant, p > 0.1). This result was not surprising given that the AEP group experienced the most intense handling and restraint period, which presumably induced a stronger endocrine stress response (time elapsed between beginning of procedures to the completion of the cardiac puncture for the pre-oviposition state; mean, range: pre-oviposition control = 45, 24–74 min; pre-oviposition behavior = 56, 25–178 min; pre-oviposition AEP = 148, 100–200 min). There were no significant correlations between CORT and any of the biometric variables (TFL, mass, RBM) or date and time of testing in either the pre-oviposition or post-oviposition states (all p > 0.10). Holding females at 4 °C prior to testing did not affect concentrations of plasma CORT (mean ± SE (CV), ng mL^{-1}; no chill: 20.4 ± 5.1 (123%); pre-oviposition control: 17.9 ± 5.7 (142%); t_{43} = 0.33, p = 0.75, Cohen's d = 0.09).

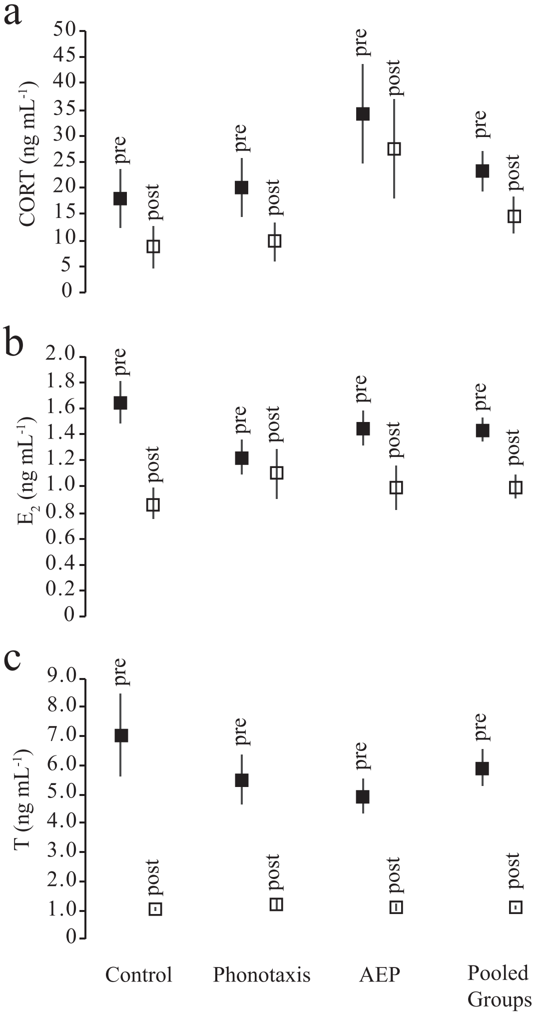

Fig. 2. (a) Mean corticosterone (CORT) (ng mL^{-1}) ± SEM in the pre-oviposition and post-oviposition states. (b) Mean estradiol (E_2) (pg mL^{-1}) ± SEM in the pre-oviposition and post-oviposition states. (c) Mean testosterone (T) (ng mL^{-1}) ± SEM in the pre-oviposition and post-oviposition states. All three groups are also pooled to depict the main effect of reproductive state.

3.4. Estradiol

Overall, concentrations of E_2 were less variable among frogs than CORT, spanning one order of magnitude (mean ± SE: 1.2 ± 0.07 ng mL^{-1}; range: 0.36–4.0 ng mL^{-1}; see Fig. 2b), but still varied significantly in the omnibus test of the model ($F_{5,101}$ = 5.06, p = 0.0003, eta-squared = 0.20). Like CORT, concentrations of E_2 were substantially lower following oviposition (Fig. 2b). This effect was observed in each of the three groups (behavior, AEP, control) and was of similar magnitude, which is reflected by a significant main effect of reproductive state ($F_{1,101}$ = 21.0, p = 0.00001, eta-squared = 0.17) but no main effect of group ($F_{2,101}$ = 0.27, p = 0.76, eta-squared = 0.005) and no interaction between reproductive state and group ($F_{2,101}$ = 2.2, p = 0.12, eta-squared = 0.04). There was a trend toward a smaller effect following oviposition in E_2 in the behavior group compared to the decline in CORT and T. There were no significant correlations between E_2 and any of the biometric variables (TFL, mass, RBM) or date and time of testing in either of the reproductive states (all $p > 0.10$; Pearson's correlations). Holding females at 4 °C prior to testing did not affect concentrations of plasma E_2 (mean ± SE (CV), ng mL^{-1}; no chill: 1.83 ± 0.21 (56%); pre-oviposition control: 1.65 ± 0.17 (45%); t_{43} = 0.67, p = 0.50, Cohen's d = 0.19).

3.5. *Testosterone*

The plasma sample for one female (pre-oviposition behavior) was insufficient in volume to measure all three steroids and thus we chose not to measure T, making the sample size for analyses of T and behavior N = 20. Overall, concentrations of T were highly variable among frogs, spanning more than two orders of magnitude (mean ± SE: 3.6 ± 0.4 ng mL^{-1}; range: 0.09–26.1 ng mL^{-1}) and varied significantly in the omnibus test of the model ($F_{5,100}$ = 34.4, $p < 0.0001$, eta-squared = 0.63). Concentrations of T were substantially lower following oviposition (Fig. 2c). This effect was observed in each of the three groups (behavior, AEP, control) and was of similar magnitude, which is consistent with a significant main effect of reproductive state ($F_{1,100}$ = 168.4, $p < 0.0001$, eta-squared = 0.41) but no main effect of group ($F_{2,100}$ = 0.20, p = 0.82, eta-squared = 0.004) and no interaction between reproductive state and group ($F_{2,100}$ = 0.04, p = 0.96, eta-squared = 0.0007). Compared to the other two steroids, the difference in T levels between the pre-oviposition and post-oviposition states was the most dramatic, and the range of T concentrations after oviposition was markedly compressed (i.e., variances decrease for T only; Fig. 2). There were no significant correlations between T and any of the biometric variables (TFL, mass, RBM) or date and time of testing in either the pre-oviposition or post-oviposition states (all $p > 0.05$; Pearson's correlations). Holding females at 4 °C prior to testing reduced concentrations of plasma T (mean ± SE (CV), ng mL^{-1}; no chill: 26.2 ± 3.4 (64%); pre-oviposition control: 7.03 ± 1.44 (91%); t_{42} = 4.78, $p < 0.0001$, Cohen's d = 0.23).

3.6. *Correlations between hormones*

There was a strong positive correlation between E_2 and T in both the pre-oviposition and post-oviposition states (pooled groups: pre-oviposition: r = 0.74, $p < 0.0001$, N = 55; post-oviposition: r = 0.47, p = 0.001, N = 51; Supplemental materials S5). The other two pairwise correlations between hormones were not significant in either reproductive state in the pooled dataset (all $p > 0.1$). There were, however, positive correlations between CORT and the other two steroids in the control group but only positive trends in the behavior and AEP groups (see Supplemental materials S6).

3.7. *Hormones and behavior*

A second purpose of our hormone and behavior measures was to determine the relationship between these two levels of the phenotype. We found positive correlations between the mean (per female) phonotaxis latencies (recognition and discrimination trials) and CORT concentrations. Females with higher CORT concentrations were slower to exit the origin (ρ = 0.57, p = 0.007, N = 21) and slower to enter a response zone in the recognition test (r = 0.44, p = 0.045, N = 21; Fig. 3). There were no significant relationships between latencies and E_2 or T (all $p > 0.1$).

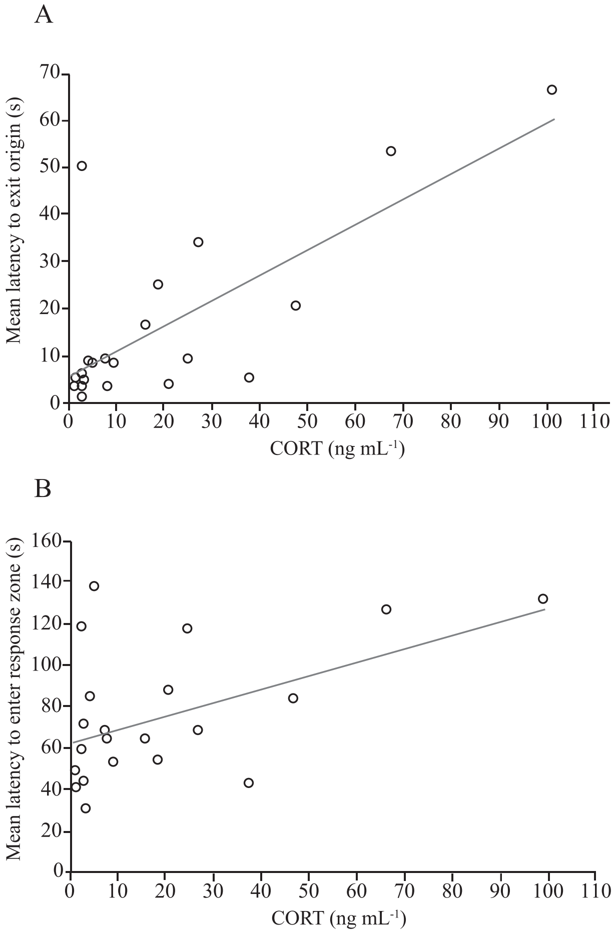

Fig. 3. Correlations of corticosterone (CORT) concentrations and the mean (per female) latency to exit the origin (A) and enter the response zone (B). Untransformed hormone concentrations are depicted and statistical analyses were performed on $\log_{10}$ transformed values.

3.8. *Auditory evoked potentials*

The objectives of our measures of AEPs were to (1) evaluate the extent to which reduced behavioral receptivity to advertisement calls after oviposition was correlated with reduced sensitivity of the peripheral auditory system to frequencies emphasized in calls, and (2) determine whether changes in auditory sensitivity (if found) were correlated with changes in circulating hormone profiles that occur in conjunction with oviposition. In contrast to our predictions, however, we found strong evidence that auditory sensitivity was greater following oviposition, and we found little evidence that these changes were strongly correlated with changes in circulating hormone levels. Thresholds for post-oviposition females (mean ± SE: 53.9 ± 1.68 dB SPL) were > 6 dB lower than the thresholds of pre-oviposition females (mean ± SE: 60.5 ± 1.78 dB SPL) and this difference was significant (reproductive state: $F_{1,21}$ = 6.45, p = 0.02, Cohen's d = 0.89, Fig. 4a). However, this difference in threshold did not appear to be driven by hormones, as their effects in the model were not significant ($F_{1,21} < 2.85$, $p > 0.11$, Fig. 5a–c). One pre-oviposition animal had a threshold of 85 dB SPL, which was approximately 25 dB above the average threshold. When this individual was removed from our analysis the results were qualitatively similar; thresholds remained significantly influenced by reproductive state ($F_{1,20}$ = 5.2, p = 0.03, Cohen's d = 0.67) but not by any of the hormones ($F_{1,20} < 1.79$, $p > 0.19$, Fig. 5a–c).

Similarly, the amplitude of the ABR was significantly related to reproductive state ($F_{1,20}$ = 18.6, $p < 0.001$, Cohen's d = 0.97,

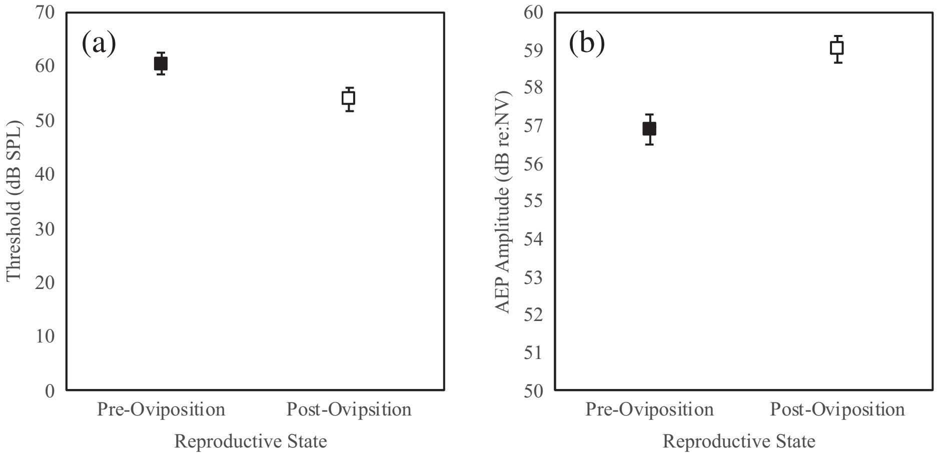

Fig. 4. ABR thresholds (a) and ABR amplitudes (b) to two-tone tone bursts with spectral content that reflects population means of *Hyla chrysoscelis* advertisement calls as a function of reproductive state. Values are LSMEANS ± S.E.

Fig. 4b), with post-oviposition animals having greater amplitude responses than pre-oviposition animals. ABR amplitude increased with stimulus amplitude ($F_{3,70} = 88.5$, $p < 0.001$) but was not influenced by the main effect of CORT ($F_{1,20} = 0.8$, $p = 0.37$), E_2 ($F_{1,20} = 1.95$, $p = 0.18$) or T ($F_{1,20} = 4.2$, $p = 0.053$, Fig. 5d, f). However, there was a significant interaction of E_2 and reproductive state on ABR amplitude ($F_{1,20} = 22.0$, $p < 0.001$, Fig. 5e). This interaction resulted from a significant decrease in ABR amplitude with increasing levels of E_2 in pre-oviposition animals (slope = -7.29 dB nV/ng mL^{-1}, $t_{20} = 3.3$, $p = 0.004$) and an increase in ABR amplitude with an increase in E_2 in post-oviposition animals (slope = 3.15 dB nV/ng mL^{-1}, $t_{20} = 2.49$, $p < 0.001$).

4. Discussion

We found a set of behavioral, endocrine, and auditory traits that rapidly shift during a single day as females of Cope's gray treefrog transition from a breeding to a non-breeding status. We found support for our prediction that female sexual receptivity would decline after oviposition. This rapid and complete reversal in behavioral responsiveness is in broad agreement with results from the one previous study of an anuran tested for phonotaxis behavior following oviposition. Lynch et al. (2005) also found a decline in behavioral receptivity in female túngara frogs; however, that study also showed that 20% of frogs remain receptive and exhibit positive phonotaxis following oviposition.

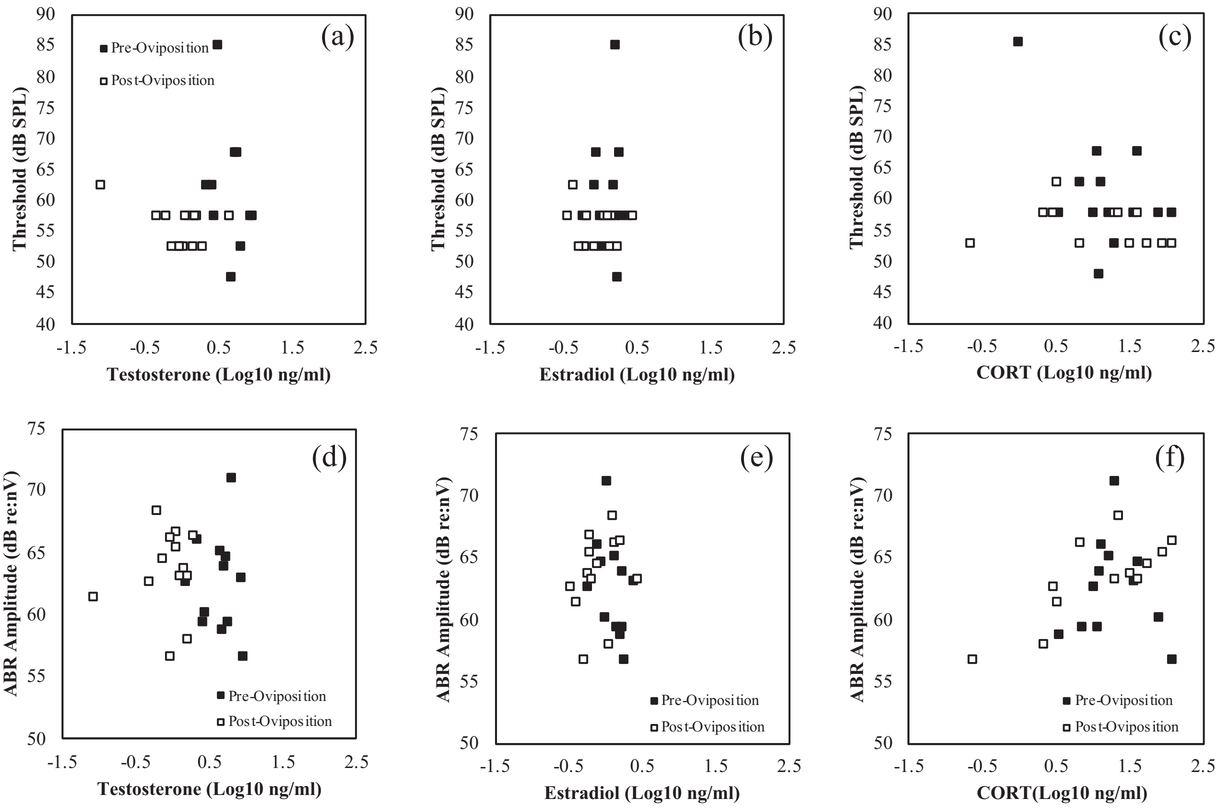

Fig. 5. ABR thresholds (a–c) and ABR amplitudes (d–f) to two-tone tonebursts with spectral content that reflects population means of *Hyla chrysoscelis* advertisement calls. ABR thresholds are plotted as a function of reproductive state and circulating levels of testosterone (a, d), estradiol (b, e) and corticosterone (c, f).

A novel finding in the present study was that peripheral auditory sensitivity increased following oviposition. We found that post-oviposition females had lower ABR thresholds and greater suprathreshold ABR amplitudes than those that had not yet oviposited. This is surprising given the magnitude and consistency of the behavioral change we found, from unanimous receptivity to unanimous unresponsiveness. It appears clear that the marked decrease in behavioral responsiveness to a male's advertisement call that occurs after oviposition is not driven by parallel decreases in peripheral auditory sensitivity to the frequencies emphasized in this vocalization. Unlike much of the previous work on reproductive status and peripheral auditory sensitivity, we did not find any relationship between hormone levels and auditory thresholds; we did, however, find that estradiol levels were negatively correlated with ABR amplitude at suprathreshold stimulus levels in the pre-oviposition state and positively correlated in the post-oviposition state. This suggests that the rapid increases in peripheral auditory sensitivity following oviposition are unlikely to be modulated by circulating levels of steroid hormones, although estradiol may be important in modulating auditory function within a reproductive state or over longer periods of time (e.g. seasonal). One functional hypothesis that could explain this relatively diminished auditory sensitivity during the height of reproductive readiness is that the frog's auditory system is protecting itself from the intense ambient sound pressure levels present in a breeding chorus. This remains to be tested, but it would be worth evaluating auditory sensitivity in both sexes, particularly because male anurans spend considerably more time in what are some of the loudest natural environments on the planet (Schwartz and Bee, 2013).

Our finding of enhanced auditory sensitivity in sexually non-receptive females differs from previous work on reproductively-related auditory plasticity (Forlano et al., 2015; Wilczynski and Burmeister, 2016). However, our work was focused on the rapid transition from breeding to non-breeding status, while most previous work has focused on the transition from non-breeding to breeding status, either through artificially manipulating hormone levels (Caras et al., 2010) or by comparing wild caught individuals inside and outside of the breeding season (Sisneros and Bass, 2003; Gall et al., 2013; Velez et al., 2015). In these cases, reproductive animals typically have greater auditory sensitivity than non-reproductive animals. For instance, in the only study to examine seasonal changes in the auditory periphery in frogs, Zhang et al. (2012) found that Emei music frogs (*Babina daunchina*) had greater sensitivity (i.e. lower thresholds) in the breeding season than in the non-breeding season. Furthermore, estradiol level is typically positively associated with increases in sensitivity (Gall et al., 2013). However, high levels of circulating estradiol may lead to diminished auditory sensitivity, as is the case in white-crowned sparrows (Caras et al., 2010).

The decrease in sensitivity that we found is also quite different from findings in the central auditory system in anurans. Miranda and Wilczynski (2009) found that the auditory midbrain responses of unmated green treefrogs to band-limited noise were greater than those of recently mated females; however, they did not find a difference between the groups in their responses to advertisement calls. In their study post-mated females were collected in amplexus, while the gravid females were not in amplexus when collected. Both groups were then held for 3–6 days prior to testing. It is not clear whether the differences compared to our findings are a function of species, time course, or holding methods. Similarly, Hillery (1984) found that neurons in the auditory midbrains of eastern gray treefrogs had greater suprathreshold responses and lower thresholds to tonal stimuli during the breeding season compared with the non-breeding season. Finally, administering human chorionic gonadotropin or estradiol to non-breeding túngara frogs increases immediately early gene expression in the auditory midbrain (Lynch and Wilczynski, 2008; Chakraborty and Burmeister, 2015), suggesting enhanced processing of conspecific signals.

Our auditory processing results were especially surprising, as we also found that steroid hormones track the shifting behavior phenotype of females in different reproductive states. Concentrations of plasma CORT, E_2 and T declined after oviposition. A recent study of the eastern gray treefrog suggests that these declines are driven by a decrease in secretion and not merely an increase in clearance, as the concentrations of excreted metabolites of these steroids decline post-oviposition as well (Bastien et al., 2018). The decline in E_2 was predicted because of the role of this hormone in reproduction, including mating behavior and oviposition in female frogs, and because the eggs themselves likely serve as a major source of gonadal steroids (Lynch and Wilczynski, 2005; Lynch et al., 2005; Chakraborty and Burmeister, 2009). This decline also has been observed in eastern gray treefrogs (Bastien et al., 2018). CORT and T underwent similar absolute decreases in concentration in conjunction with oviposition (ca. 5–7 ng mL^{-1}), but T experienced a much larger relative change (T: −85%; CORT: −15%). The decline in T and its largely invariant concentrations following oviposition are particularly interesting. Testosterone can be converted to estradiol by aromatase at the target tissue (e.g. brain), and so it is possible that the concomitant declines in both T and E_2 result in a dramatic decrease in available E_2 in the brain. This is important because E_2 is known to play an important role in vertebrate audition. For example, estrogens can rapidly modulate acoustic processing and audiomotor integration in vertebrates (reviewed in Remage-Healey, 2012), and frogs are known to express estrogen receptors, as well as the estrogen synthesis enzyme aromatase, in neural pathways for audition (Kelley, 1980, 1981; Wu et al., 2003). Although we did not uncover any relationships between T and our other variables, the fact that our method of maintaining frogs at 4 °C prior to testing reduces circulating T dramatically suggests the relationship between T and other traits may be challenging to accurately assess in this system.

Although glucocorticoids are not traditionally considered reproductive hormones, it is well established in many mammalian species that a glucocorticoid surge during parturition is crucial for the initiation and maintenance of labor (Thorburn et al., 1977). Thus, the decline in CORT following oviposition in treefrogs is perhaps not surprising. The implications of having potentially elevated CORT during peak reproductive receptivity is unclear, especially because elevated glucocorticoids are thought to be antagonistic to sexual motivation (Husak and Moore, 2008; Bókony et al., 2009), including in amphibians where it can rapidly suppress sexual behavior (Moore and Orchinik, 1994; Rose et al., 1998)—a finding that is consistent with the longer phonotaxis latencies observed in the present study for female frogs with higher CORT. Two patterns are noteworthy here. First, lower CORT levels in the pre-oviposition state predict higher sexual receptivity yet post-oviposition females (which exhibit substantially lower CORT levels) are behaviorally non-receptive; it is important to note that the former represents a within-group correlation and the latter represents a between group effect. Although this is not technically a statistical interaction, it may indicate that glucocorticoids modulate sexual receptivity in a state-dependent manner, which in this case could be during peak gonadal steroid concentrations (pre-oviposition). Alternatively, or additionally, the modulating effect of glucocorticoids on behavior could be non-linear (e.g. moderate declines may elevate receptivity and large declines may abolish it). Second, given the substantial decline in CORT following oviposition in all groups, it might be that pre-oviposition concentrations represent a non-baseline (i.e. stress-induced) status. However, it appears that if pre-oviposition frogs were undergoing a stress-induced elevation in CORT, they were not at maximum levels because we found even more elevated CORT in the AEP group in both the pre-oviposition and post-oviposition states. This result suggests that there was still substantial dynamic range in the secretory capacity of the interrenal glands during pre-oviposition, and that females are sensitive to stressors such as handling in both the pre-oviposition and post-oviposition states (which was protracted for the AEP group). Further, the fact that pre-oviposition females with elevated CORT exhibited increased phonotaxis latencies suggests that these naturally varying levels of CORT are behaviorally relevant at the individual level and that the glucose mobilizing effects of elevated CORT does not merely stimulate higher locomotor activity during phonotaxis trials (Breuner et al., 1998). It further suggests that an endocrine stress response might modulate reproductive behavior in female vertebrates (Davis and Leary, 2015; reviewed in Husak and Moore, 2008). The transitional physiology of post-mating likely involves important energy reallocations, and glucocorticoids might play important roles in the metabolic shifts. For example, lower CORT levels could enable shifts away from gluconeogenesis by altering transcription at target cells (Gray et al., 1990; Hasselgren, 1999; Sapolsky et al., 2000; Oakley and Cidlowski, 2013), thereby enabling shifts in the energy storage and allocation required to recover from oviposition and prepare for another breeding attempt.

5. Conclusions

In female anurans, the rapid life history transition from reproductive readiness to post-breeding that occurs in conjunction with oviposition is associated with dramatic and rapid changes in sexual behavior, circulating steroid physiology, and the sensitivity of early stages of the ascending auditory system. Females become behaviorally unresponsive to male advertisement calls; exhibit substantial declines in circulating gonadal and adrenal steroids, especially testosterone; and, surprisingly, their peripheral auditory system becomes more sensitive to frequencies in the male advertisement call. Further, elevated circulating corticosterone appears to moderately suppress sexual receptivity, further supporting the idea that non-gonadal steroid hormones such as glucocorticoids should be considered when examining hormone-behavior relationships in vertebrate sexual behavior (Davis and Leary, 2015; Bastien et al., 2018). This major life history transition, and the effects it has on brain, plasma hormones, and behavior, may reflect more subtle fluctuations in acute reproductive condition within the pre-oviposition time window during which females execute mate choice. Future studies will need to address such intra-individual variance in mechanisms that give rise to temporally acute changes in behavior to better understand condition-dependent variation in receivers as a function of the constraints of reproductive readiness (Bastien et al., 2018; Baugh and Ryan, 2009).

Supplementary data to this article can be found online at https://doi.org/10.1016/j.yhbeh.2019.01.001.

Ethics

Animal collections were made under Special Permit 21947 from the State of Minnesota Department of Natural Resources. This study was approved by the Institutional Animal Care and Use Committee at the University of Minnesota (Protocol 1701-34456A, approved 3 March 2017).

Competing interests

We have no competing interests.

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