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Social Signals Influence Hormones Independently of Calling Behavior in the Treefrog (*Hyla cinerea*)

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Social signals play an important role in regulating hormone–behavior relationships. In anurans (frogs and toads), acoustic signals are an essential aspect of reproductive behavior; however, the physiological consequences of receiving social signals has remained largely undescribed. Each night for 5, 10, or 20 days, we presented acoustically isolated male treefrogs with a conspecific mating chorus, an array of tones, or no sound. We recorded calling rate of individuals throughout the experiment and collected blood before and after treatment. Days of stimulus exposure had no effect on any dependent measure. Acoustic treatment influenced steroid levels; testosterone, dihydrotestosterone, and corticosterone increased only in the group exposed to the chorus. Chorus-exposed males also showed an increase in stimulus-evoked calling. We found no correlation between androgens and calling within each treatment group. In addition, noncallers in the chorus group had higher levels of androgens than males in the tone or no sound groups. Further, chorus-exposed males with zero, low, or high rate of calling had similar levels of androgens. These data indicate that social signals increase circulating androgens independently of calling behavior. Elevated corticosterone associated with chorus reception did not inhibit calling behavior, and corticosterone showed no correlation with androgen levels.

Key Words: **communication; androgens; corticosterone; amphibian; reproductive behavior.**

In many species, hormone–behavior relationships are regulated by proximate cues. Successful reproductive behavior depends on appropriate expression of behaviors associated with male–male competition and courtship, as well as the production of gametes. By using predictive cues, such as photoperiod, to stimulate gonadal recrudescence, individuals can coordinate the physiological, behavioral, and neural changes necessary for the timely expression of reproductive behavior (Wingfield, 1983). Supplementary cues, such as storm events, food availability, and social signals, allow for additional facilitation and/or inhibition of reproductive readiness, so that reproductive investment is maximized. The role of social signals as supplementary cues is of particular interest because reproductive (and competitive) behavior is inherently social. Therefore, in order to understand the physiological and behavioral events accompanying reproductive behavior, we need to understand the contribution of social cues and how these cues relate to simultaneous changes in behavior and hormones.

Social interactions have been shown to influence sex steroids (Macrides, Bartke, and Dalterio, 1975; Propper and Moore, 1991; Chu, Burmeister, and Wilczynski, 1998), immune function (Klein, Hairston, DeVries, and Nelson, 1997), neurobiology (Francis, Soma, and Fernald, 1993; Hartman and Crews, 1996; Tai, Schiml, Li, and Rissman, 1997; Tramontin, Wingfield, and Brenowitz, 1999), and reproductive physiology (Brzoska and Obert, 1980; Delville, Sulon, Hendrick, and Balthazart, 1984; Cheng, 1986; McComb, 1987; Gudermuth, Butler, and Johnston, 1992; Rissman, 1992). Many studies have investigated facilitation by intersexual signals; however, intrasexual signals are also important and may inhibit (Francis, Soma, and Fernald, 1993) or facilitate (Cheng, 1986, 1992) reproductive physiology. Although there has been a long history of studies of social influences on physiology, many issues about this phenomenon have remained unresolved. Unlike steroid hormone effects, which can be illuminated by removal and replacement of the

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hormone, social interactions are inherently confounding: individuals interact. Social experiences are characterized by many distinct but interdependent elements, including the sensory experience of the social (communication) signals of the partner, the behavioral response of the subject, and the subjects' sensory reception of his/her own social signals. In addition, hormone levels are reciprocally related to the expression of social behavior (both reproductive and competitive), and hormones affect sensory systems involved in the reception of social cues (Yovanof and Feng, 1983; Penna, Capranica, and Somers, 1992). In a few model systems, the relative roles of the components of a social experience have been examined and characterized (e.g., Cheng, 1992). However, in many cases, it is unclear which components are necessary to affect a physiological response.

In treefrogs and many anurans (Wells, 1977, 1988), acoustic signals are the primary signal used to mediate both intra- and intersexual communication. Males gather in aggregations and produce an advertisement call that attracts females and regulates intermale spacing (Wilczynski and Brenowitz, 1988; Brenowitz, 1989). As in a lek, females are attracted to aggregations of displaying males. Therefore, participation in a chorus is critical to reproductive success, and the presence of other calling males is an important social cue. Recordings of advertisement calls evoke calling from males in the laboratory and the field, which allows us to present an effective stimulus in the absence of confounding conspecifics. In addition, the behavioral (Gerhardt, 1974, 1988) and neurophysiological (Magela-Simmons, Moss, and Daniel, 1985; Moss and Magela-Simmons, 1986; Mudry and Capranica, 1987; Allison and Wilczynski, 1991; Allison, 1992) response of male treefrogs to conspecific calls is well characterized, and the behavioral response to advertisement calls is easily quantifiable (i.e., we can simply count calls).

We investigated the effects of social signals on steroid secretion in the green treefrog (*Hyla cinerea*), a model which allows for simultaneous consideration of the reciprocal relationships between sensory stimulation, individual behavior, and circulating hormones. We examine three (not mutually exclusive) models of the relationship between social signals, androgens, and calling (Fig. 1): simultaneous effects of social signals on calling and androgens could be mediated independently by social signals, or changes in one could be secondarily mediated by the other; all three models could also apply. Each night for 5, 10, or 20 days, we presented acoustically isolated male treefrogs with a conspecific mating chorus, an array of tones, or no sound. We determined the effect of social signals on calling behavior and circulating steroids (testosterone, dihydrotestosterone, and corticosterone). In addition, we investigated the response of corticosterone (CORT) to social stimulation and the relationships between CORT, androgens, and calling behavior.

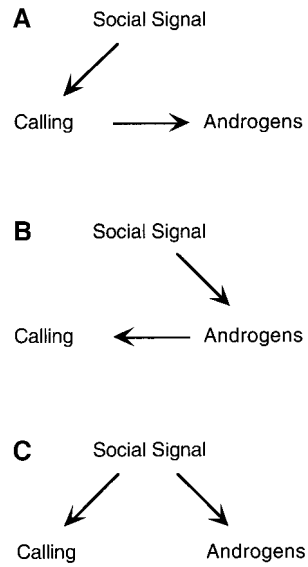

FIG. 1. Social signals could influence calling and androgens in one of three ways. (A) Social signals indirectly affect androgens through changes in calling. (B) Social signals directly affect androgens, which secondarily increase calling. (C) Social signals independently affect androgens and calling. Additional possibilities in which combinations of the three models apply simultaneously are not shown here.

METHODS

Experimental Design

Breeding male *H. cinerea* were purchased from Charles Sullivan Co. (Nashville, TN). During the first 22 days of laboratory housing, trunk blood was collected on days 1, 12, and 22 postarrival in order to determine initial hormone levels. Before the experiment began, subjects were housed in groups under a 14:10 h light:dark cycle and fed crickets every 2–3 days. Initially, the males chorused robustly in the laboratory, and many individuals continued to call sporadically throughout this period of group housing. Behavior of the animals during this time was not systematically documented.

The experiment began 71 days after arrival in the

laboratory. We housed males individually in acoustic chambers, which had two compartments. In the lower housing compartment (internal dimensions: 14 × 14 × 20 cm), the animal was provided with a water dish and artificial foliage. The housing compartment was separated from an upper compartment by a screen, on which a speaker (RadioShack 277-1008C), microphone, and 1.4-W fluorescent light were positioned. Temperature (24–31°C) and light cycle (14:10 h light: dark) were similar to summer breeding conditions. Every 3 days, we removed subjects from their chambers to be fed crickets.

Each night for 5, 10, or 20 days, we presented a total of 72 males with a conspecific chorus, an array of tones, or no sound. The stimulus played for 5 h from 21:30 to 02:30 h (stimulus onset 1–1.5 h after lights out), which is typical of a breeding chorus. We monitored calling behavior of individuals with a computer that received input from the chamber microphones, using software written for the study. Because we were limited by the number of acoustic chambers ($n = 21$), we were unable to run all subjects simultaneously. Instead, we used a relay design in which a smaller number of individuals from each treatment group were run until completion. As a result, the date of experiment onset differed among individuals.

On the day of experiment onset, males were weighed, snout–vent length was measured, and blood was collected by heart puncture (41 successful heart punctures). At the end of acoustic exposure, we weighed males a second time and collected testes and trunk blood (70 samples collected) following rapid decapitation. We centrifuged blood promptly and stored plasma at −20°C. Plasma volume collected from heart puncture ranged from 10 to 93 μl and from 18 to 100 μl for trunk-collected plasma. We weighed testes and calculated gonadosomatic index (GSI) as testes mass / body mass. One subject died during the experiment.

The conspecific chorus stimulus was a 12-min recording of a naturally breeding population. Using computer software (SoundEdit 16, Macromedia, Inc., San Francisco, CA), we created the alternate acoustic stimulus by replacing every frog call of the chorus stimulus with a pure tone of the same duration and approximate amplitude as the call it replaced. As a result, the overall patterns of sounds of the two stimuli were identical; however, the tones had no amplitude modulation. Based on the known sensitivity range of *H. cinerea* (Magela-Simmons *et al.*, 1985; Moss and Magela-Simmons, 1986; Mudry and Capranica, 1987), we selected tones randomly from 200 to 500, 1000 to 2600, and 3800 to 5000 Hz. This range is within the hearing range of the species, but excludes tones that are known to be important in conspecific communication (Gerhardt, 1974; Gerhardt and Mudry, 1980). For subjects in the no-sound group, the speakers were powered, but received no input.

A computer monitored calling behavior continuously throughout the experiment, recording the number of calls produced in each 15-min period. The system was not designed to discriminate between advertisement and aggressive calls. We defined two classes of calling behavior. Stimulus-evoked calling rate was defined as the mean number of calls per hour produced during the stimulus period (21:30–02:30 h). Spontaneous calling rate was defined as the mean number of calls per hour produced in the absence of the acoustic stimulus (02:30–21:30 h). We analyzed both mean call rate and log-transformed call data (see below). For cases in which call rate was zero, animals were assigned a log-transformed score of −1.

The research presented here was described in Animal Research Protocol No. 05962101C1 approved by the Institutional Animal Care and Use Committee of the University of Texas at Austin.

Radioimmunoassay

Radioimmonoassay (RIA) procedures followed previously published methods (Wingfield and Farner, 1975; Schwabl, 1992). Briefly, we measured plasma levels of steroids by RIA following separation on chromatographic columns. Prior to assays, we added ^{3}H-labeled steroid (approximately 2000 cpm) to each sample for recovery determinations. We extracted plasma steroids with 2 × 4 ml anhydrous diethyl ether using Extrelut (EM Science, Gibbstown, NJ). We then dried and resuspended extracts in 10% ethyl acetate in isooctane. We purified the extracts on short celite: propylene glycol:ethylene glycol columns with a celite:water:glycol trap. We eluted steroid fractions by increasing concentrations of ethyl acetate in isooctane as follows: 1.5 ml 10% for DHT, 2.0 ml 20% for T, and 2.5 ml 50% for CORT. Finally, we assayed dried eluates in duplicate, and separation of bound and free counts was achieved by the addition of dextran-coated charcoal. We processed water blanks and known amounts through the entire procedure. We adjusted results for recovery and expressed results as nanograms per milliliter of plasma.

Recoveries of ^{3}H-labeled steroids after extraction and chromatography were 74% for T, 69% for DHT, and 63% for CORT. All samples of the experimental

subjects were run in a single assay, and plasma collected from subjects after arrival in the lab was run in a second assay. Intraassay variations were 4.5% (T), 7.7% (DHT), and 10.7% (CORT). Interassay variation was not calculated since steroid levels were not compared across assays. The antiserum for androgens (T and DHT) was purchased from Wien Laboratories, Inc. (Succasunna, NJ) and for CORT from Endocrine Sciences (Tarzana, CA). The antiserum for T/DHT has 59.5% cross-reactivity with 5α-DHT, 48.5% with T, 15.5% with δ-5-androsten-3β,17β-diol, and less than or equal to 5.0% for other steroids. The antiserum for CORT has 57.8% cross-reactivity with progesterone (P), 43.7% with deoxycorticosterone, 16.3% with 20α-hydroxyprogesterone and less than or equal to 5.2% for other steroids. The high cross-reactivity with P is not a concern because CORT was separated from P and partially purified on diatomaceous earth chromatographic columns prior to assay. The lower limits of detection for each assay, expressed as nanograms per milliliter of plasma, were 0.24 (T), 0.36 (DHT), and 0.37 (CORT).

Statistics

Changes in plasma steroid levels were assessed with repeated-measures analysis of variance (ANOVA) for each steroid separately. Group differences in mean calling rate were determined using the nonparametric Kruskal–Wallis ANOVA. We used Pearson's correlation to examine relationships between variables; when we used call rate in correlational analyses, we used log-transformed call rate data. Finally, because T and DHT were highly correlated, we used multivariate analysis of variance (MANOVA) when investigating the interaction between call rate and androgen changes. In all cases, follow-up analyses were only performed after finding significance with the full statistical model. Significance was determined at an alpha level of 0.05.

RESULTS

Hormones

Androgen levels did not change during the first 22 days in the laboratory, but pretreatment androgen levels did differ according to day of experiment onset (T: $r = -0.34$, $P = 0.03$; DHT: $r = -0.38$, $P = 0.015$), indicating a decline over time in the laboratory (Fig. 2). CORT levels were low and stable during the first 22 days in the lab and did not differ with date of experiment onset ($r = -0.07$, $P = 0.67$). Although individuals differed in the date of experiment onset, and therefore in initial androgen levels, repeated-measures analyses allowed us to determine the stimulus-specific changes in steroid levels.

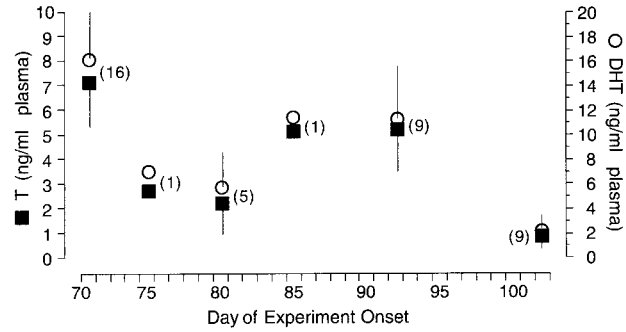

FIG. 2. Mean (SEM) pretreatment levels of T (■) and DHT (○) according to day of experiment onset. Day of experiment onset is expressed as day since arrival in the lab (i.e., day 71 was first day of experiment). Subject number is indicated in parentheses.

There was no effect of acoustic treatment on gonadosomatic index [$F(2, 68) = 0.57$, $P = 0.57$] and no effect of days of stimulus exposure on GSI or steroid levels. Further, GSI did not correlate with plasma steroids. For each steroid, we found a significant interaction between acoustic treatment and pretreatment versus posttreatment steroid change [T: $F(2, 37) = 6.76$, $P = 0.003$; DHT: $F(2, 37) = 6.09$, $P = 0.005$; CORT: $F(2, 37) = 5.98$, $P = 0.006$] (Fig. 3). Follow-up analyses showed that exposure to the conspecific chorus significantly increased circulating T [$F(1, 11) = 12.02$, $P = 0.005$], DHT [$F(1, 11) = 9.86$, $P = 0.009$], and CORT [$F(1, 11) = 19.79$, $P = 0.001$]. In contrast, exposure to tones or no sound had no effect on steroid levels. Table 1 shows the absolute steroid levels before and after acoustic treatment for all individuals in the experiment. T and DHT were highly correlated with one another ($r = 0.96$, $P < 0.001$), but CORT did not correlate with either androgen (T: $r = 0.16$, $P = 0.17$; DHT: r = 0.19, P = 0.13).

Behavior

Levels of spontaneous calling did not differ between groups. Only males receiving the chorus increased their calling rate during the stimulus presentation, suggesting that increases in calling rate were stimulus-specific (Fig. 4A). Stimulus-evoked calling varied with acoustic stimulus [$\chi^2(2) = 31.3$, $P < 0.0001$]. Stim-

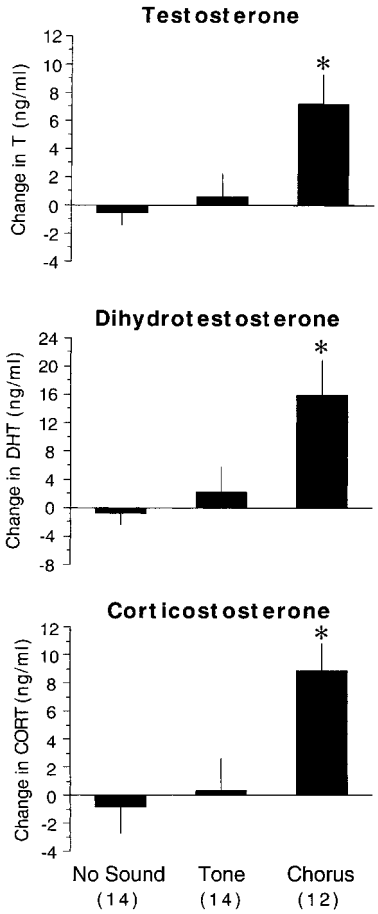

FIG. 3. Mean (SEM) change in plasma steroids following reception of acoustic stimulus (difference scores were taken between pre- and posttreatment values on each male and averaged). Asterisks denote significant pretreatment versus posttreatment change. Only subjects with pre- and posttreatment steroid levels are included. Subject number is indicated in parentheses.

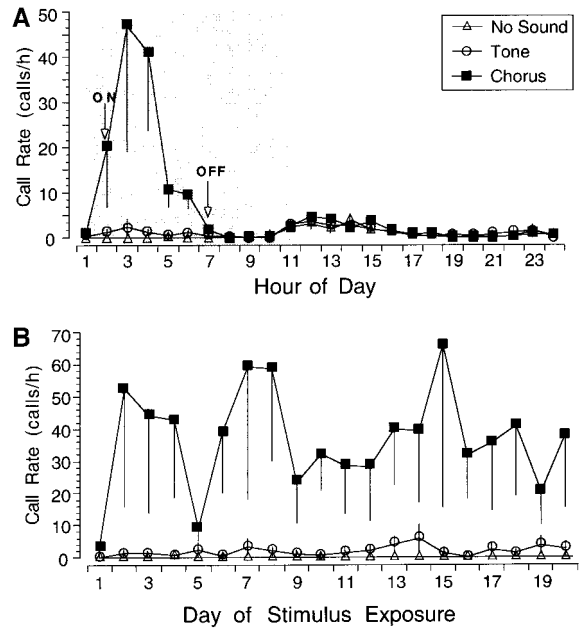

FIG. 4. Behavioral response of males to acoustic stimulus. (A) Mean (SEM) calling rate over the course of the day (hour 1 = 20:00–21:00 h). Shaded region indicates lights out. Stimulus period indicated by arrows. (B) Mean (SEM) calling rate throughout the experiment. Subject numbers varied with day: for days 1–5, there were 72 subjects; days 6–10, 48 subjects; and days 11–20, 23 subjects.

ulus-evoked calling increased as early as the second day, but did not appear to vary with the day of stimulus exposure (Fig. 4B). Although a few individuals called quite vigorously in response to the chorus, the levels of calling behavior did not approach those that would be observed in the field (personal observation). There were no significant correlations between calling rate and body size (SVL or mass) or gonadosomatic index.

Behavior–Hormone Relationships

Within each stimulus group, there were no significant correlations between plasma steroids (T, DHT, or CORT) and stimulus-evoked calling. The lack of a within-group

TABLE 1

Steroid Levels [Mean ng/ml Plasma (SEM)] of Experimental Subjects Pre- and Postacoustic Treatment and Males on Day 1 of Laboratory Housing

	No sound		Tone		Chorus		Laboratory
	Pre	Post	Pre	Post	Pre	Post	Day 1
N	14	24	14	24	13	22	9
T	2.2 (1.3)	3.0 (0.8)	6.8 (1.9)	5.7 (1.1)	5.0 (1.5)	11.7 (1.5)	10.0 (2.5)
DHT	4.5 (2.1)	7.0 (1.8)	15.3 (3.7)	12.9 (2.7)	11.9 (3.5)	26.4 (3.0)	16.9 (4.6)
CORT	2.4 (1.8)	3.0 (0.7)	7.2 (2.7)	5.6 (1.4)	1.3 (0.7)	7.8 (1.5)	3.4 (1.3)

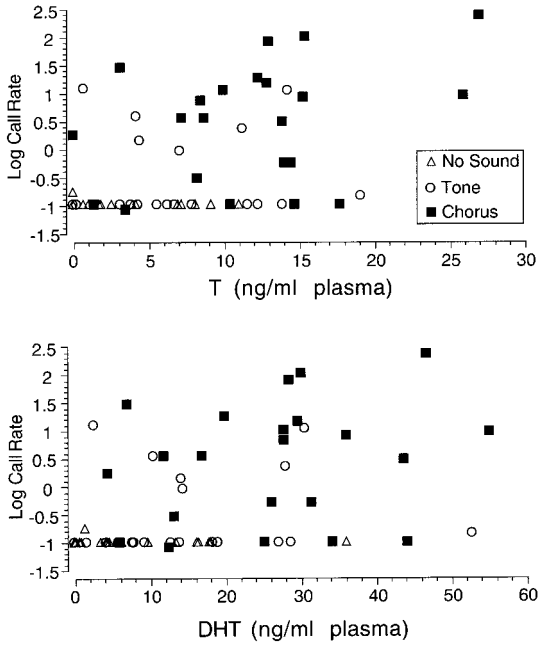

FIG. 5. Covariation of androgens and log-transformed call rate. In cases in which call rate was zero, animals were assigned a log-transformed score of −1.

relationship between androgens and calling (Fig. 5) suggests that changes in calling and androgens were simultaneous but independent. Because of the lack of variation in spontaneous calling, we did not examine the correlation between spontaneous calling and steroid levels.

To assess the independence of calling behavior and androgen levels, we analyzed the stimulus effect on posttreatment androgen levels for males with different calling rates (Fig. 6). Males who received the chorus and produced no calls had androgen levels that were higher than tone and no-sound males [MANOVA: $F(4, 78) = 4.21$, $P = 0.004$], supporting the interpretation that calling was not required to elevate androgen levels (Fig. 6A). In addition, chorus males who did not call were indistinguishable from males who had a low or high calling rate (Fig. 6B). Although there was no difference in androgens between callers and noncallers in the chorus group, we did find a difference between callers and noncallers in the tone group [MANOVA: $F(2, 21) = 4.17$, $P = 0.03$], suggesting that androgens influence calling in the absence of social cues.

DISCUSSION

Reception of the mating chorus had simultaneous effects on response calling and plasma androgens. Males receiving the chorus were far more likely to call, starting with the second day of exposure. In addition, reception of the mating chorus caused an increase in circulating androgens, in as early as 5 days. While long-term laboratory housing generally decreased androgen levels, reception of social signals increased circulating androgens to levels similar to, or higher than, those found at the time of arrival in the laboratory. The relative rapidity of the response and the fact that testes do not appear to be changing (as measured by GSI) suggest that the subjects were in a state of seasonal readiness, and social cues acted to facilitate androgen secretion. This physiological response is consistent with the life history of chorusing frogs. Although seasonal breeding can be predicted by seasonal cues such as day length and temperature (Paniagua, Fraile, and Sáez, 1990), appropriate timing of reproductive behavior is greatly influenced by the behavior of other males. It has been proposed that male frogs benefit from calling in aggregations due to reduced predation risk and increased attraction of females (Ryan, Tuttle, and Taft, 1981). For example, in the túngara frog, the number of females per male

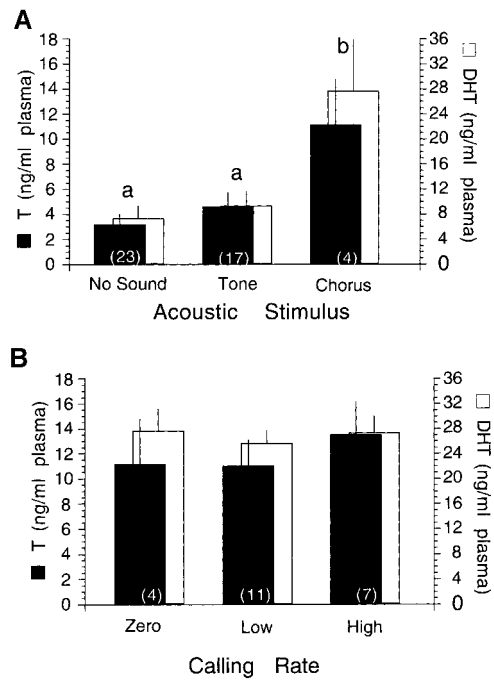

FIG. 6. Mean (SEM) posttreatment levels of T (■) and DHT (□) grouped by calling rate. (A) Effect of acoustic treatment for males that did not call. Groups that are statistically indistinguishable share a letter in common. (B) Effect of zero, low (0.1–9.9 calls / h), and high (10–240 calls / h) calling rate for males receiving the chorus. Subject number is indicated in parentheses.

increases with chorus size (Ryan *et al.*, 1981). A male frog, therefore, will benefit if he times his reproductive behavior with other males. The ability of social cues to stimulate androgen secretion in males exposed to short days may further reveal the relative strength of social cues in this species.

Social cues are often the product of interactions between individuals, and in such cases it can be difficult to differentiate the effect of the reception of cues from the production of responses. In our data, males receiving the chorus showed increases in calling that were, as far as we measured, simultaneous with increases in plasma androgens. However, the absence of a significant correlation between calling and androgens argues against the first two models in Fig. 1, whereby social effects on androgens (Fig. 1A) or calling (Fig. 1B) were mediated *only indirectly* through the other. The fact that chorus males who produced no calls at all had higher androgens than tone and no-sound males demonstrates that calling was not required for social effects on androgens, effectively ruling out model A as the main influence of social signals on androgens (Fig. 1A). Further, the fact that chorus males with different levels of calling had the same androgen levels suggests that call production does not influence androgens in a "dose-dependent" manner. However, it is possible that call production may influence androgen levels if calling rate were to approach levels observed in the field. For example, calling males may have higher (Marler and Ryan, 1996) or lower (Mendonça, Licht, Ryan, and Barnes, 1985) androgen levels than noncallers in the field. Such findings raise the possibility that call production may have an effect on androgen levels in field-breeding males.

Although there is no correlation between androgens and calling in any group, our data do not rule out all effects of androgens on calling (Fig. 1B). A threshold level of androgens is likely required for the expression of calling behavior; indeed, the dependence of sexual behavior on androgens has been well documented in anurans (Wada, Wingfield, and Gorbman, 1976; Wada and Gorbman, 1977; Wetzel and Kelley, 1983; Solís and Penna, 1997), as well as other vertebrates. The fact that calling males in the tone group had higher androgen levels than noncallers supports the notion that a threshold level of androgens is required for calling in the absence of social cues. Understanding the relationship between calling, androgens, and social cues, however, requires simultaneous manipulation of social cues and androgen levels.

Males receiving the chorus stimulus also showed elevated levels of corticosterone. Unlike androgens, CORT did not change over the course of laboratory housing, and generally levels of CORT were low. The exception was the chorus group that increased CORT in response to the chorus stimulus. One possible explanation is that elevated CORT is a physiological preparation for the challenges of reproductive behavior, such as the energetic challenges of calling (Prestwich, Brugger, and Topping, 1989) or increases in intrasexual competition (Mendonça *et al.*, 1985; Orchinik, Licht, and Crews, 1988). However, a lack of correlation between calling and CORT suggests that elevated CORT is not in preparation for, or in response to, the physiological demand of calling. In addition, we have found low levels of CORT in field-bled males [mean (SEM) = 2.4 (1.0) ng/ml plasma, $n = 10$] who were calling far more vigorously than our laboratory-housed males (Burmeister and Wilczynski, unpublished data).

A second possibility is that chorus males are stressed by the experience of hearing a chorus under artificial circumstances. We suspect that males receiving the chorus are demonstrating a stress response that is caused by an inability to dynamically regulate their physical relationship with their neighbors. Advertisement calls are used both to attract females and to repel neighboring males. In many chorusing species of frogs, males regulate their distance from other males by regulating the amplitude of their neighbors' calls (Wilczynski and Brenowitz, 1988; Brenowitz, 1989). They do so through agonistic encounters, as well as by simply moving toward or away from neighbors. In our experimental chambers, subject males are unable to regulate the amplitude of their neighbors' calls by either of these mechanisms. As a result, we postulate that the presence of loud, unresponsive neighbors stressed our subject males. Social interactions, especially competitive ones, are often stressful (Fox, White, Kao, and Fernald, 1997) and may be especially so in cases where the individual is unable to exert control.

Our data are consistent with independent influences of social signals on calling and androgens (Fig. 1C). The appreciation that social and environmental signals can influence physiology in vertebrates is well established and growing, but how these influences reach the basal forebrain areas that ultimately control hormone secretion remains largely unknown (Ball, 1983). Previous work has provided evidence for the neuroanatomical circuit by which social signals may influence the endocrine system in anurans. The preoptic area (POA) and ventral hypothalamus (VH) are major targets of the ascending auditory system of

anurans (Neary, 1988; Wilczynski, Allison, and Marler, 1993). Furthermore, mating call-responsive neurons have been demonstrated in both these nuclei (Allison and Wilczynski, 1991; Allison, 1992). As in other vertebrates, POA and VH are known to be important in the control of GnRH secretion (Ball, 1981); thus, the auditory system may have direct access to cells that influence the production or secretion of GnRH, as has been demonstrated in the ring dove (Cheng, Peng, and Johnson, 1998). In addition, the POA is important in the production of mating calls and is believed to be the trigger for call production by the pretrigeminal nucleus (Wada and Gorbman, 1981; Schmidt, 1984; Wetzel, Haerter, and Kelley, 1985). Interestingly, the pretrigeminal nucleus has also been shown to possess acoustically responsive neurons (Aitken and Capranica, 1984). This interaction between the auditory system and vocal production pathway may be related to acoustically evoked mate calling. Not surprisingly, many of these same brain regions that are involved in reception and production of social signals are also responsive to circulating sex steroids (Kelley, Morrell, and Pfaff, 1975; Morrell, Kelley, and Pfaff, 1975; Kelley, 1981). The neuroanatomy of these interrelated systems of hormonal regulation, reception of cues, and production of responses points to an increasingly apparent principle of vertebrate reproductive behavior: we cannot simply regard one (hormones) as controlling the other (behavior). In contrast, the emerging view is one of a dynamic, interactive system, in which each element influences every other.

ACKNOWLEDGMENTS

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