Androgen-induced alterations in vocalizations of female *Xenopus laevis*: modifiability and constraints

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**Summary.** We examined effects of exogenous androgen (testosterone and dihydrotestosterone) on vocalizations of ovariectomized, adult female South African clawed frogs, *Xenopus laevis*. When paired with sexually active males, all ovariectomized females exhibited ticking, the unreceptive or 'release' call. Ticking consists of low amplitude, regularly spaced clicks with a mean interclick interval of 154 ms. When androgen-treated and paired with sexually active males, these ovariectomized females also exhibited an aberrant call (atypical ticking) in which click multiples replaced the single clicks of ticking. MeanICI's for atypical ticking were 37 ms for click doubles and 22 ms for click quadruplets. Androgen treatment decreased the total time spent vocalizing (typical and atypical ticking) by ovariectomized females.

All androgen-treated females were then tested repeatedly with sexually receptive females in an attempt to elicit the male-typical vocalization, mate calling. Six of 17 females did not vocalize at all, even when gonadotropin injected. Eight females gave rapid (mean ICI, 36 ms) trains of clicks in an irregular temporal pattern (tick-like calls). Three females gave brief trills with alternating fast and slow components. Comparison of mate call-like vocalizations of androgen-treated females to mate calling of males reveals that calls in females are considerably shorter in duration (female: 0.32 min versus male: 45 min) and slower in tempo (ICI's; fast trill, female: 21 ms, male: 14 ms; slow trill, female: 36 ms, male: 28 ms). Incomplete masculinization of the vocal pattern of females by androgen treatment in adulthood may be due to developmental constraints on the modifiability of the neurons and muscles responsible for calling.

**Introduction**

*Xenopus laevis*, the South African clawed frog, exhibits sex-typical reproductive behaviors which are under the control of steroid hormones (Kelley and Pfaff 1976; Kelley 1982; Wetzel and Kelley 1983; Weintraub et al. 1985). During the breeding season in the wild (Kalk 1960), and at all times of year in the laboratory (Russell 1954) males emit a characteristic vocalization, the mate call. Mate calls are repetitive, amplitude-modulated trills which are believed to attract the female. Movement by the female results in the male's swimming over to her and grasping her with his forelegs. If the female is sexually receptive she will remain silent and flex her legs. If the female is sexually unreceptive she will terminate or avert a clasp attempt by extending her hind legs (Kelley 1982) and emitting a vocalization called ticking (Russell 1954). Ticking consists of slow trains of regularly emitted clicks which are not amplitude modulated. Thus the sexes of *Xenopus laevis* use acoustically distinct vocal patterns in their reproductive behaviors which convey different messages to conspecifics.

Male *Xenopus laevis* will tick when clasped by other males (Kelley and Long, unpublished). Ticking by a clasped male is heard less frequently than ticking by a female under the same stimulus conditions. Intact sexually receptive or unreceptive females have not yet been heard to give mate calls.

**Abbreviations:** C cholesterol; DHT dihydrotestosterone, HCG human chorionic gonadotropin; IBI interburst interval; ICI interclick interval; ovx ovariectomized; T testosterone

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Thus while ticking is a female-characteristic vocal pattern and mate calling is male-characteristic, the latter appears to be more sexually dimorphic than the former since it is not heard in females.

Male-typical reproductive behaviors depend upon circulating androgens (Kelley and Pfaff 1978; Wetzel and Kelley 1983). The ability of these hormones (testosterone and dihydrotestosterone) to induce claspers in adult females has also been examined (Kelley and Pfaff 1976). Gonadectomized females with androgen implants clasped other females in a manner indistinguishable from that of males. Though male-typical mate calling was not systematically studied, androgen-treated females did not call. This observation suggested that the male-typical behaviors, clasping and calling, may differ in the ease with which they can be evoked from adult females by hormone treatment. The present study represents a systematic attempt to evoke male-like mate calling from adult female frogs. The endocrine treatments used are those which resulted in restoration of mate calling to gonadectomized male frogs in a previous study (Wetzel and Kelley 1983) and which have been shown to control male reproductive vocalizations in many species (Kelley and Pfaff 1978). In order to study the behavioral specificity of androgen action, we also recorded the effects of these hormones on the female-typical vocal behavior, ticking.

In parallel studies, we have described certain sexual dimorphisms in the vocal organ and in neurons of the efferent pathway for calling (Hannigan and Kelley 1981; Segil et al. 1983; Sassoon et al. 1983, 1986; Kelley and Fenstemaker 1983; Wetzel et al. 1985). We wish to compare the degree to which behaviors and their neuroeffector can and cannot be masculinized by androgen treatment in adulthood. Such a comparison should yield covarying constraints on functional and morphological modifiability; constraints which will help in identifying neuronal parameters essential for full masculine behavioral expression.

Materials and methods

*Animals and their maintenance.* Thirty-three laboratory bred female *Xenopus laevis* were purchased from Kelly Evans, Ann Arbor, Michigan. Upon arrival all frogs were toe-clipped for identification. Average pre-ovariectomy weight was 51.6 g (range: 36.2 to 93.7) and average post-ovariectomy weight was 45.9 g (range: 31.3 to 77.8 g). All animals were housed, three per tank, in 35 x 44 cm Nalgene containers. The tanks were filled with approximately 5 cm of standing 0.2% saline which was changed three times per week after feeding. The animals were maintained on a schedule of 14 h of light (00:00-14:00) and 10 h of darkness (14:00-00:00) at a temperature of approximately 18 °C.

*Surgery.* Frogs were randomly assigned to two groups. Twenty-three frogs were ovariectomized while the remaining ten frogs were sham operated (see Kelley et al. 1975; Kelley 1982 for operative procedures). All but one female survived the surgery and all females were sexually mature as indicated by the presence of large numbers of mature oocytes within the ovaries.

*Implant procedures.* Androgen and cholesterol pellets (diameter: 4 mm, height: 2 mm), 20 mg each, were prepared using a Parr Instrument Company pellet press. Testosterone (T; 4-androsten-17β-ol-3-one), dihydrotestosterone (DHT; 5α-androstan-17β-ol-3-one) and cholesterol (C; 5-cholesten-3β-ol) were obtained from Sigma Biochemical Co. Frogs were anesthetized by cold narcosis. A small incision (about 4 mm) was made on the dorsal surface above the lymph sac, pellets were inserted, pushed towards the caudal end of the lymph sac and the incision sutured closed. This procedure generally results in the formation of a vascularized fibrous capsule around the pellet and a gradual decrease in weight of the pellet. Nine ovariectomized females received T pellets; eight received DHT pellets. Ten sham operates and five ovariectomized females received C pellets. Pellets were numerically coded to permit observation of behavior without knowledge of the specific hormone treatment.

*General design.* This study consisted of two experiments. In Experiment I, measurements of female ticking in response to male mate calling and clasping were made before and after hormone implants. Two weeks after hormone implantation, females were tested for baseline ticking rate (pre-pellet condition). Immediately after testing, frogs received hormone implants. Four weeks after implantation animals were retested to determine the effect of androgen on ticking. The percentage of females vocalizing, the time spent vocalizing, the ICI and the peak fundamental frequencies of the individual clicks were measured. Past studies have shown that one month is sufficient time for T to elicit heavy nuptial pads and male-like clasping from ovariectomized female *Xenopus* and to restore clasping and mate calling to castrated males (Kelley and Pfaff 1976; Wetzel and Kelley 1983).

Ten sham operated and five ovariectomized females received C pellets. These groups were tested in order to control for non-specific changes in vocal behavior due to operative and pellet implantation procedures. Intact adult females in our colony are usually sexually unreceptive (Kelley 1982). Ticking of sexually unreceptive, intact females is not increased by ovariectomy (Kelley and Long, unpublished). Some intact (but no ovariectomized) females become spontaneously sexually receptive in early summer. The sham operated female control group thus also controlled for any seasonal decreases in ticking.

In Experiment II the same androgen-treated or control females were tested with sexually active stimulus females to determine if androgen-treated females would mate call. Sexually active female stimulus partners were used because they are an effective stimulus for eliciting mate calling in males but they do not vocalize (Kelley and Long, unpublished), thus assuring that any recorded vocalizations were from the androgen-treated female. Animals were tested a total of four times over a four month period. Three tests were conducted with hormone alone and one with hormone and human chorionic gonadotropin (HCG). Treatment with T or DHT restores mate calling to castrated males; HCG injection increases the amount of time spent calling by androgen-treated castrated males (Wetzel and Kelley 1983). Experimental and control females were not given a pre-test for mate calling (i.e., recorded before hormone pellet...
implantation in a tank with sexually receptive females) because such females have never been heard to vocalize at all under these conditions (Kelley and Pfaff 1976; Kelley and Long, unpublished). Control females were reimplanted with cholesterol and injected with vehicle to control for nonspecific effects of pellet implantation or injection and possible seasonal effects.

**Testing procedure.** Four pairs of animals were tested in each session. Within a given tank, one frog was from the experimental or control group and one was a sexually active stimulus partner. To obtain sexually active stimulus partners, frogs were injected with 0.5 ml HCG (100 international units in distilled water, Sigma) at 09:00 on the day before the experimental recording. The stimulus partner was re-injected with 0.5 ml HCG at 09:00 the morning of the test-day and immediately placed in the recording tank for acclimation. At 14:45 that day, the experimental or control female was placed in the recording tank and at 15:00 the 1.5 h recording session began. Previous experiments indicated that a six hour interval between injection and recording yielded the greatest amount of calling from sexually active males (Wetzel and Kelley 1983). This interval was therefore chosen for our studies of females. The taping session began with the onset of the dark portion of the light/dark cycle. The animals remained in the tank overnight and were returned to their home tanks the next morning.

**Recording apparatus.** Four ten-gallon aquaria were fitted with plexiglass tops. Tanks were lined with closed cell foam (Aires, 4.4 cm thick) to reduce noise and echoes. The final volume of the tanks was 42 × 17.8 × 25.4 cm. Wilcoxon hydrophones (model HS05) were used to transduce sound and were suspended from the plexiglass top to a height of approximately 7.5 cm from the tank bottom, in the center of the tank. All vocalizations emitted during taping were recorded using Akai model GX-253 tape recorders on Scotch 207 magnetic tape. During recording sessions, the tanks were lined with polyethylene bags (Mobil) filled with standing tap water. An uncontrollable variable in this paradigm was the position of the frog in the tank and thus its distance from the hydrophone. Absolute call amplitude could not, therefore, be determined.

**Data analysis and statistics.** The percentage of animals vocalizing and the time spent vocalizing in each condition were recorded. Frequency characteristics of individual clicks, the basic unit of mate calling and ticking, were analyzed using a Princeton Applied Research FFT real time spectrum analyzer (model 4512) interfaced to a Hewlett-Packard X-Y plotter (Model 7035B). To avoid including frequencies due to resonance properties of the recording tanks, only those peaks below 4 kHz were measured (see Wetzel and Kelley 1983, for a more detailed discussion of acoustical conditions). Calls were randomly selected for analysis for each animal in the various experimental conditions. The time between successive clicks, the interclick interval (ICI), was measured in milliseconds directly from sonograms of vocalizations (Kay Sonograph model 6061B). Data on time spent calling, interclick intervals and frequency maxima of spectra are described as means. The experimental design permits comparison of data from individuals across treatment conditions. Changes in behavior were evaluated using non-parametric statistical analysis (Sign test, Wilcoxon matched-pairs signed-rank test; Siegel 1956).

**Differentiating the source of vocalizations.** Data analysis required distinguishing which frog of an experimental pair was vocalizing. Since there are no obvious throat movements which accompany the underwater vocalizations of *X. laevis*, it is impossible to visually identify the sound source. However, observation of the animals in Experiment I using a red light during the taping session indicated that mate calling was heard simultaneously with the approach of the male. Immediately after the male clasped the female, the female stretched out rigidly and ticking was heard; mate calling continued during the initial stages of the clasps attempt. (See Kelley 1982, for a more extensive description of female unreceptive behaviors). *Xenopus laevis* have only one laryngeal sound source (Yager 1982; Tobias and Kelley 1985); it is most unlikely that a single frog ticked and mate called simultaneously. Thus, in Experiment I, the male was mate calling and the female was ticking. In Experiment II, the source of recorded vocalizations was more easily identified. Since the sexually active female stimulus partner is mated by HCG injection (Kelley and Long, unpublished), any recorded vocalizations were due to the androgen-treated experimental male.

**Results**

**Experiment I: Ticking**

The female vocalization, ticking, was first described by Russell (1954) who likened the tick to the sound 'made by winding a wrist watch'. The call, consisting of repetitive single clicks, is emitted by an unreceptive female in response to the approach of a mate calling male or to a male's clasping attempt (Russell 1954; Kelley 1982). Together with leg extension and movement, ticking results in unclasping by the male and is thus analogous to function to the release call of other anura (Kelley 1982; Weintraub et al. 1985). A sonogram of ticking is illustrated in Fig. 1A. The call consists of regularly emitted clicks with a mean ICI of 154 ms (Table 1). Analyses of individual clicks (Fig. 1D) reveal amplitude maxima at approximately 0.7 and 1.3 kHz (Fig. 1E, see Table 1).

All ovariectomized females ticked in response to clasps assaults by males. After testosterone, four of nine females did not vocalize. All females vocalized after DHT treatment. After one month of androgen treatment, some recorded vocalizations of females displayed altered temporal characteristics (Fig. 1B and C). Experimental females began to produce double, triple and quadruple click bursts in addition to 'typical' single clicks. The mean ICI for doublets (Fig. 1B) was approximately 37 ms and for quadruplets (Fig. 1C) was 22 ms (Table 1). Despite the induction of click multiples, the interval from the first click of one burst to the first click of the next burst (IB1) was approximately 155 ms (Fig. 1B). This interval closely resembles that characteristic of ticking; we have therefore termed the androgen altered pattern of click bursts described above 'atypical ticking'.

Androgen reduced the percentage of females
Fig. 1. A-G. Vocalizations of female *Xenopus laevis*: ticking and atypical ticking. The basic unit of a vocalization is a click, a brief burst of sound. A Sonogram of ticking from an ovariectomized female. Frequency of recorded vocalizations is shown on the ordinate and time on the abscissa. Each recording represents 2.5 s of calling and was prepared using a Kay Sound spectrograph. Ticking is a female-typical vocalization consisting of regularly emitted clicks with interclick intervals of approximately 155 ms. Ticking is not amplitude modulated. B Sonogram of atypical ticking. This vocal pattern is given by androgen-treated females in response to a clasp attempt by a male. While the interval between the initial click of a click burst (IBI) remains at approximately 155 ms, doublet clicks with ICI's of approximately 36.6 ms are given instead of the single clicks of ticking. C Sonogram of atypical ticking given by a gonadectomized, androgen-treated female. Triplet and quadruplet click bursts are illustrated. Individual females giving atypical ticks continued to emit typical ticks although less frequently. D Individual click from ticking of an ovariectomized female. E Fast Fourier transform spectral analysis of the click (from ticking) illustrated in D. Peak frequencies are approximately 0.7 and 1.3 kHz. F Individual click from atypical ticking. G Spectral analysis of the click in F. Note the introduction of higher frequency components (>2 kHz) than are found in typical ticking. Peak frequencies are 0.6, 1.8 and 2.0 kHz.
Fig. 2A–D. Vocalizations of androgen-treated females.
A Sonogram of a tick-like call given by a long-term dihydrotestosterone-treated female tested with a sexually receptive female stimulus partner. Conventions as in Fig. 1.
B Individual click of a tick-like call.
C Spectral analysis of the click in B. The component frequencies are tick-like (i.e., contain no frequencies greater than 2 kHz).
D Sonogram of a ‘sawing’ vocalization from a one month androgen-treated female. Mean ICI is 20 ms.
Fig. 3A–G. Mate calling by a male (A, D, E) and mate call-like vocalization (B, C, F and G) of a long term testosterone-treated ovariectomized female tested with a sexually receptive stimulus female partner. A Sonogram of male mate calling. The fast and slow trill phases which comprise one mate call (Wetzel and Kelley 1983) are illustrated. B Sonogram of male-like mate calls given by a gonadectomized adult female treated for 4 months with testosterone. Fast and slow components resembling those of a male mate call can be identified although the ICI in the androgen-treated female is longer in both the fast and the slow part of the mate call. Note that androgen-treated females can emit vocalizations with very short ICI’s (Fig. 2D) but do not use these short ICI’s in their male mate call-like vocalizations. C Another characteristic of mate call-like vocalizations of androgen-treated females is the tendency to pause after the fast portion of the trill. This female could call smoothly (see B) but also gave many examples of interrupted trilling. Pauses between fast and slow portions of the trill ranged from 150 to 220 ms. D Individual click from male mate calling. E Spectral analysis of the click in D. Peak frequencies are approximately 1.8 and 3 kHz. F Individual click from female mate call-like vocalization. G Spectral analysis of the click in F. Peak frequencies are at 0.5, 1.8, 2.2 and 2.6 kHz. Note the retention of low frequency components (less than 1 kHz) found in the female click.
giving typical ticks (pre-pellet: 100%; post-pellet: 33%; T; 25% DHT). All of the DHT treated and 55% of the T-treated females produced atypical ticking. In addition to atypical ticking, we recorded bursts of multiple, rapid clicking from one T-treated and one DHT-treated female (Fig. 2D). Such calls overlapped with mate calls from males in our studies and were thus most probably made by females. Sound spectrographs of this vocalization are similar to a vocalization recorded from pairs of androgen-treated females, termed 'sawing' (Kelley and Long, unpublished). In males, 'sawing' is elicited by clasping (Russell 1954) and may function as an agonistic signal (Rabb 1973). Sawing was also recorded from two T-treated females in Experiment II.

The amount of time spent ticking by individual females was also reduced by androgen treatment. Before pellet treatment the mean time spent ticking was 18.3 min (T), 17.1 min (DHT), 20.4 min (sham C) and 19 min (ovx C). After pellet insertion the mean time spent ticking was 0.2 min (T), 0.6 min (DHT), 14 min (sham C) and 20 min (ovx C). Testosterone and DHT significantly reduced time spent ticking (sign test; \( P < 0.005 \)); C did not reduce ticking (sign test; \( P > 0.5 \)). No female exhibited atypical ticking before hormone treatment. After treatment, mean time spent atypical ticking was 5.7 min (T), 5.0 min (DHT) and 0 min (C). Taken together, these results indicate that T and DHT reduce the total time spent vocalizing (ticking plus atypical ticking). There were no significant differences between the effects of T and DHT in any of the behavioral parameters measured.

The suppression of vocal behaviors by androgen could reflect an androgen-induced increase in female sexual receptivity; sexually receptive females tick less than when unreceptive (Kelley 1982; Weintraub et al. 1985). Observation of androgen-treated ovariectomized females, however, indicates that they remain sexually unreceptive as measured by display of leg extension. It is also possible that androgen-treated females vocalize less because they inspire less sexual activity (mate calling, clasp attempts) in males. Ovariectomy itself decreases female attractiveness (Kelley 1982). While it is possible that androgen treatment makes these females even less attractive, a measure of male sexual activity (amount of time spent mate calling) was not different between androgen- and cholesterol-treated females (mean time spent mate calling: T, 18 min; DHT, 15 min; C, 20 min). We thus suggest that androgen suppresses female ticking directly rather than acting via changes in female receptivity or attractiveness to males.

**Experiment II: Mate calling**

Male mate calling is a metallic sounding amplitude-modulated trill (Fig. 3A; Wetzel and Kelley 1983). The basic unit of each call, the click (Fig. 3D) has a peak fundamental frequency of 1.8 kHz with an additional peak at 2.0 kHz (Fig. 3E; Table 1). Clicks are produced at two rates, fast and slow, having mean ICIs of 14 ms and 28 ms, respectively. One male call consists of one fast trill and one slow trill sequence (see Wetzel and Kelley 1983).

Females were tested for mate calling four times over the course of four months; three times with androgen alone and once with androgen and HCG. Experimental and control females were tested six and 11 weeks after hormone implant, given a hormone pellet (if the first implant could not be palpated at the time, 10 females), and then retested after two weeks. One week later, females were injected with HCG and tested for the final time.

**Results.** Six of 17 experimental females did not vocalize in the 12 total hours of testing. The remaining 11 of the adult, ovariectomized females treated with androgen vocalized, while the control females never vocalized. The vocalizations fell into two categories: tick-like (Fig. 2A) and mate call-like (Fig. 3B).

**Tick-like vocalizations.** Most vocalizations of androgen-treated females resembled fast ticks rather than male-like mate calls. Tick-like vocalizations, given by eight of the 11 females that vocalized, were characterized by variable and long ICI's, lack of identifiable fast and slow trill components and lack of amplitude modulation. A sonogram of a tick-like call is illustrated in Fig. 2A. The peak frequencies of individual clicks from these vocalizations (Fig. 2C) were more similar to those of normal ticks (Fig. 1E) than to those of the clicks comprising female mate call-like vocalizations (Fig. 3E, see also Table 1). A single click from a tick-like call is illustrated in Fig. 2B and can be compared to clicks of ticking (Fig. 1D), male mate calling (Fig. 3D) and female mate call-like vocalizations (Fig. 3F).

**Mate call-like vocalizations.** The vocalizations in the second category, heard from three of the eleven females that vocalized, were trills resembling abnormally slow mate calls. Two of these females had been treated with T, one with DHT. Sonograms of calls from one of these females are shown...
Table 1. Temporal and frequency characteristics of calls and their component clicks emitted by intact females and males, and androgen-treated, gonadectomized females

<table>
<thead>
<tr>
<th>Vocalization type</th>
<th>Sex and hormone treatment of experimental frog</th>
<th>Sex and hormone treatment of stimulus partner</th>
<th>Mean ICT ms $\pm$ SEM (of clicks, of frogs)</th>
<th>Frequency maxima of individual clicks $\pm$ SEM in kHz (of spectra, of frogs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ticking</td>
<td>Female ovx</td>
<td>Male intact</td>
<td>$154 \pm 2$ (151.12)</td>
<td>$0.68 \pm 0.06$ $1.34 \pm 0.03$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ (5.5)</td>
</tr>
</tbody>
</table>
|                  | Female intact                               | Male intact                                   | $161 \pm 3$ (36.3)                        | $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-$ $-

All vocalizations were recorded and sonograms prepared. Measurement of interclick intervals was made from these sonograms; the number of clicks and the number of individual animals used are indicated as is the standard error of the mean (SEM) for each determination. Acoustic properties of individual clicks from each vocalization type were determined from individual click spectrograms (intensity versus frequency). Frequency maxima were defined as discrete peaks whose amplitude was greater than or equal to 20% of the maximum amplitude on that spectrogram.

* fast trill portion  
* slow trill portion  
* data from Wetzel and Kelley (1983)

in Fig. 3B and C. These vocalizations had fast and slow trill components with ICT’s of 21 ms and 35 ms, respectively (Table 1). Female mate call-like trills often contained 150 to 200 ms pauses between the fast and slow portions of the call (Fig. 3 C).

Measurement of the peak fundamental frequencies of individual maxima of clicks from mate call-like vocalizations of females reveal peaks at approximately 0.5, 1.8, 2.2 and 2.6 kHz (Fig. 3 G; see Table 1). A click from mate calling and its component frequencies are illustrated in Fig. 3 (D and E) for comparison. Compared with the frequency spectra for typical ticking or tick-like vocalizations (Fig. 1 E, Fig. 2 C) these mate call-like clicks have more high frequency components (see Table 1).

HCG. In the final behavioral test, androgen-treated and control females were injected with human chorionic gonadotropin (HCG) as this glycoprotein hormone has been shown to increase the amount of time spent calling by gonadectomized, androgen-treated males (Wetzel and Kelley 1983). Two frogs vocalized only after HCG injection, two (that had vocalized with T alone) did not vocalize at all following HCG injection, and four vocalized both with and without HCG injection. None of the control frogs vocalized either with or without HCG injection. For those frogs that did vocalize, HCG did not increase the amount of time spent calling. (Mean values: pre-HCG: $T = 0.36$ min, $DHT = 0.07$ min; post-HCG: $T = 0.32$ min,
DHT = 0.6 min. P > 0.10, Wilcoxon matched-pairs signed-rank test on individual comparisons). All three females that gave male-like mate calls, did so both before and after HCG injection. No apparent difference in the quality of calls was noted. There were no significant differences between the effects of T and DHT during any of the four mate calling tests in any of the parameters measured.

Discussion

Vocal behavior - modifiability and constraints. Some characteristics of calls made by adult females can be changed by androgen and some cannot. For example, androgen suppresses ticking, induces high frequency components in clicks and induces atypical tick bursts. However, androgen does not alter the fundamental temporal characteristics of ticking. The interval between the initial clicks of atypical tick bursts remains tick-like (approximately 155 ms).

For mate calling, androgen did induce some (3 of 17) ovariecutomized females to call in a male-like fashion. Control females never mate called. All gonadectomized adult males mate call after androgen replacement; amount of time spent vocalizing is further increased by gonadotropin (Wetzel and Kelley 1983). In the present study, only 63% of gonadectomized females vocalized at all when treated with androgen; gonadotropin had no effect on the amount or quality of calls. Of those androgen-treated females that did call, most gave tick-like vocalizations rather than the mate call appropriate for the stimulus situation (pairing with a sexually receptive female partner). The temporal and acoustic properties of calls from the three mate calling females differ from similar calls made by males. The female calls are slower (ICI's 70-80% greater than male values) and more raggedly modulated than calls given by males. Female calls are interrupted by 150-200 ms pauses between fast and slow trill components, pauses not heard in males. Gonadotropin-treated males mate call for an average of 45 min during a 1/2 h recording session with a stimulus female (Wetzel and Kelley 1983). The maximum time spent calling by any androgen-treated female (mate call and tick-like vocalizing) was 4.5 min for a 1 1/2 h session. Finally, individual clicks within calls retain the low frequency components characteristic of females even as higher frequencies are induced by androgen treatment. The behavior of androgen-treated females reveals limitations in ability to generate a sustained mate call vocal pattern or a mistaken perception of the stimulus situation.

Calling neuroeffectors - modifiability and constraints. We know something of how calls are produced in Xenopus laevis and where, within the "circuitry" for calling, androgen acts. Each click is produced by movement of the arytenoid discs of the larynx, the vocal organ, effected by contraction of paired bipinnate muscles (Ridewood 1898; Yager 1982; Tobias and Kelley 1985). These muscles are innervated by motor neurons of cranial nerve IX-X of the caudal medulla (Kelley 1980; Simpson et al. 1986). Afferents to laryngeal motor neurons have been described anatomically (Wetzel and Kelley 1983; Wetzel et al. 1985) and electrophysiologically (Schmidt 1974). All CNS nuclei and muscles identified as participants in vocal production contain androgen receptors (Kelley et al. 1975; Kelley 1981; Segil et al. 1983). In fact, the vocal pathway appears to be the predominant androgen-target system in the X. laevis CNS. Androgen treatment can increase the excitability of motor neurons in X. laevis (Fulk et al. 1981).

It seems most likely that androgen-induced changes in the calls of these frogs are the result of action on the vocal organ and its nervous innervation. Androgen induces growth of laryngeal cartilages and hypertrophy of existing muscle fibers (Segil et al. 1983; Sassoon et al. 1986). Androgen treatment can also alter the electrophysiological characteristics of laryngeal muscle (Tobias and Kelley 1985), its histochemical staining pattern (Gray et al. 1985) and the somal size of laryngeal motor neurons in females (Hannigan and Kelley 1983). These androgen-induced changes in the vocal organ and its motor neurons most probably contribute to the androgen-induced changes in vocal behaviors described here.

However, the male and female vocal pathways differ in several ways. The male larynx is larger and contains more muscle fibers (Sassoon et al. 1983, 1986). Male motor neurons are more numerous and have longer dendrites than those of females (Hannigan and Kelley 1981; Kelley and Penstemaker 1983). Connections between certain brain nuclei of the calling circuit are more robust in males (Wetzel et al. 1985). Many of these cellular parameters in the female cannot be masculinized by androgen treatment in adulthood. For instance, the number of muscle fibers in the adult female cannot be increased by androgen administration (Sassoon et al. 1986). The number of motor neurons and their dendrite length is not increased by testosterone treatment (Hannigan and Kelley 1983; Kelley and Shih, unpublished). These limitations on the neuroeffectors for vocalization in female Xenopus laevis most probably contribute to
constraints on androgen-induced calling described here.

Ticking versus mate calling. The larynx produces both mate calling (in males) and ticking. Differences in which vocal pattern is produced are likely to arise from differences in firing of the laryngeal motor neurons, the `final common path' for both ticking and mate calling. Do hormones play a role in switching the vocal behaviors from one pattern to another? Evidence from the present study on females suggests that androgen inhibits ticking elicited by a claspng male and facilitates mate-like mate calling given in response to a sexually receptive female. In groups of sexually active males (with high circulating androgen levels) observed under laboratory conditions, daisy chain arrays of claspng males are frequently found. We have wondered why male ticking (an effective stimulus for clasp release) is inhibited under these circumstances. One possibility is that in males, as in females, androgen suppresses the ticking vocal pattern. Androgen might act to switch the vocal pattern from ticking to mate calling by facilitating the action of one set of different inputs to laryngeal motor neurons.

Is mate calling a sexually differentiated behavior? Reproductive behaviors vary in their dependence on hormones and in the ease with which behaviors typical of one sex may be elicited in the other (Goy and McEwen 1980). Some behaviors require only the presence of the appropriate hormone in adulthood and can be evoked with ease in either sex. Claspng in X. laevis is a behavior of this sort (Kelley and Pfaff 1976). Others can rarely be elicited in the opposite sex without altering the developmental history of exposure to hormones (e.g., song in zebra finches; Gurney and Konishi 1982; Gurney 1982). We suspect that mate calling, like song in zebra finches, is a sexually differentiated behavior and that constraints on the ability of adult females to mate call stem from their developmental history of hormone exposure. Different components of calling (e.g., click acoustics, interclick intervals, temporal patterning) may be modulated by hormonal action on discrete vocal components (e.g., larynx, motor neurons, CNS vocal nuclei) and may be affected by endocrine action during different developmental periods. The goal of our coordinated behavioral and anatomical studies is to understand how steroid hormones `harness' the cellular machinery of neuron and muscle differentiation and produce the sexually dimorphic behaviors characteristic of the adult.

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References


Kelley DB, Pfaff DW (1976) Hormone effects on male sex behavior in adult South African clawed frogs, Xenopus laevis. Horm Behav 7:159–162


