Producing and Perceiving Frog Songs
Dissecting the Neural Bases for Vocal Behaviors in Xenopus laevis

Introduction

The vocalizations of frogs allow us to examine some important but unresolved issues in behavioral biology and neuroethology that include the bases for sexual attraction, categorization of social signals, and mechanisms of vocal communication. Our work has focused on the role of sex hormones in the development and adult function of vocal systems in South African clawed frogs (Xenopus laevis). We review here the mechanisms for song production, including the sexual differentiation of the vocal organ and the neural circuitry that drives it, and outline initial approaches to how attractive and repulsive vocalizations are perceived.

Vocal communication is used to establish territories, to repel rivals, and to attract potential mates. Species-specific advertisement calls of male frogs function in mate recognition by females, and the evolution of vocal differences is generally regarded as an important species isolation mechanism. The classic (and current, Nei and Zhang 1998) view of speciation is that of geographical isolation of populations, the accumulation of genetic differences between them, and ultimately reproductive isolation. In an alternative view, speciation can result from the introduction of a morphological, physiological or behavioral novelty that causes some individuals not to mate with one another, in spite of the fact that they could still do so, while permitting others to do so (Schwartz 1999). In using this approach to think about the introduction of novelty in frog songs, an immediate problem is the requirement for matching of production and perception (reviewed in Doherty and Hoy 1985). Does genetic variation have to coordinate changes in song with preference for the change (or vice versa; genetic coupling) or can production and perception evolve independently (genetic independence with selection leading to coevolution)? Divergence may arise first from changes in male vocalizations or from female preferences for specific vocal features. The latter forms the basis for the sensory exploitation hypothesis advanced by Ryan, Rand, and their colleagues (e.g., Ryan 1985, 1998; Ryan et al. 1990). X. laevis has an unusual vocal characteristic: females produce a fertility advertisement call, rapping, which stimulates male calling whereas their unreceptive call, ticking, suppresses male calling (Tobias et al. 1998). The X. laevis vocal system thus allows us to determine whether the same factors thought to be important in male advertisement also operate in females. The effects of female calls on male vocal and approach behaviors allow us to use both to probe the perceptual basis of attraction.
*Xenopus laevis* as an Experimental System

*X. laevis* is a totally aquatic pipid species that is native to southern Africa. Calls are made and heard underwater at night, in silt-filled ponds—circumstances that undoubtedly have contributed to its unpopularity for field studies. However, *X. laevis* has a rich intra- and intersexual communicatory repertoire, the ease with which it can be studied in the laboratory permits a number of mechanistic approaches that are more difficult in terrestrial frogs. At present we have a basic understanding of how calls are produced and how the sex of the caller determines its vocal behavior (see Kelley 1996 and Kelley and Tobias 1999 for detailed reviews of this topic).

The recent discovery of rapping, a female fertility advertisement that acts as an acoustic aphrodisiac for males (Tobias et al. 1998), opens a new avenue for the exploration of the perceptual features of vocal communication between the sexes.

Male Advertisement and Aggressive Calls

*X. laevis* relies on acoustic communication to convey location and reproductive status. The male advertisement call consists of a biphasic trill with fast and slow phases (Figure 12.1). Each trill lasts about 1 second and consists of a series of rapid clicks. Each click is a brief and noisy sound burst with most of its energy between 1.7 and 2.2 kHz. The duration of the fast phase of the trill is approximately 250 ms with a click rate of 70 Hz, whereas the slow phase lasts approximately 750 ms with a click rate of 35 Hz. The amplitude of successive clicks within the fast trill phase increases throughout the trill; we call this characteristic feature amplitude modulation. The advertisement call is prolonged; an individual male will call for many hours. Advertisement calls are given both by isolated males and by males in the presence of conspecifics. Laboratory studies, including recordings from the laryngeal nerve of vocalizing animals (Yamaguchi and Kelley 1998), reveal that only males produce the advertisement call (Picker 1983; Wetzel and Kelley 1983). The calls can act as positively phonotactic signals for a receptive female (Picker 1983; Tobias et al. 1998).

We conducted a series of field observations on vocal behaviors of *X. laevis* during their extended breeding season in 1995 and again in 1997. The observation sites were natural ponds in the vicinity of Cape Town, South Africa. The ponds contained very high population densities and were heavily siled with decomposing vegetation that formed the pond substrate. We could record vocal behaviors at night using a hydrophone but could not observe the frogs. In 1995 we sampled several ponds across the breeding season (July to December; the end of winter and the beginning of spring).

![Figure 12.1. Sound spectrograms of male vocal behavior in Xenopus laevis. Advertisement calling is given by isolated male; growing functions as a male–male release call; chirping is given during male–male encounters. In these situations chirping and growing can alternate.](image)

In 1997 we made repeated recordings at a single pond at the beginning of the breeding season (June to August).

Each pond contained many sexually receptive males (as judged by nuptial pad development, reviewed in Kelley 1996). Every male with well-developed nuptial pads called when removed from the pond. In the ponds themselves, however, only a subset of males called on a given night; on some nights, no males called. That relatively few males call in a pond where most males are capable of song suggests that calling is suppressed in many males. What is the source of this suppression? One possibility is the establishment of a calling dominance hierarchy due to male–male aggressive
interactions. To explore this idea we have categorized the vocalizations given by pairs of males in a laboratory setting and have determined whether these vocal behaviors can be recorded in the Cape Town ponds, particularly at the beginning of the breeding season.

Two distinct vocal behaviors, growling and chirping, accompany male-male interactions (Figure 12.1). Growling is a rapid trill made up of low-frequency (< 1.0 kHz) clicks. Our observations and those of Picker (1980) suggest that growling functions as a release call when one male attempts to clasp another. Growling is thus probably functionally analogous to female ticking (see below) and some common acoustic features—such as the low (< 1.0 kHz) frequencies comprising individual clicks—could play a role in release from amplexus.

Chirping (Figure 12.1) is a brief trill made up of higher frequency clicks. Growling and chirping are produced together (Figure 12.1) by male-male pairs both during attempted amplexus and without clasp attempts. Both growling and chirping have been recorded from the natural ponds; the relationship of these male-male interactions to the determination of which male produces advertisement calls is a topic of current study in the laboratory.

Female Attractive and Repulsive Calls

Female X. laevis also use vocalizations to convey their state of sexual receptivity. A female about to oviposit produces a rapid (~ 12.5 Hz) series of clicks, rapping (Figure 12.2), in response to a calling male (Tobias et al. 1998). Rapping stimu-
lates both the male's approach (phonotaxis) and his vocalizations. Males answer rapping by increasing the length and amplitude modulation of the fast trill phase of the advertisement call as well as decreasing the duration of the slow trill phase of the call; this alteration results in a distinct and intense answer call (Figure 12.2). As long as the female raps, the male answers. If rapping slows down, the male reverts to advertisement calling. Thus sexually receptive pairs duet before amplexus; if the sexes are prevented from initiating amplexus, duetting is prolonged.

Sexually unreceptive females produce a different call, ticking (Figure 12.2). Ticking inhibits male calling (Tobias et al. 1998). Ticking is a slow (~6 Hz) series of clicks, part of a suite of behaviors produced by sexually unreceptive females in response to attempted amplexus by males. Males initially respond to tapes of ticking with the answer call but then, in sharp contrast to the response to rapping, stop calling as long as ticking continues (Figure 12.2). Both ticking and rapping have been recorded from the natural ponds. Ticking and rapping are click trains; sound frequencies in female clicks are distinct from those of male clicks, permitting identification during duets.

Mechanisms of Vocal Production: The Larynx

In X. laevis all calls are made and heard underwater and one would thus expect to find some differences in sound production and perception relative to terrestrial anurans. The larynx, or vocal organ, has been modified to produce sounds using a "click" mechanism that does not require respiration. The larynx (Figure 12.3) is a box-like structure of muscle and cartilage that communicates with the buccal cavity, anteriorly, and with the lungs, posteriorly. The "skeleton" of the larynx is made up of several kinds of cartilage: the predominant form is hyaline cartilage, but the larynx also contains calcified cartilaginous rods, the thyrohyals, and sound-producing disks of arytenoid cartilage. As first described by Yager for X. boreas (Yager 1992), adult male X. laevis larynges also contain elastic cartilage, a type absent from the mature female (Fischer et al. 1995). The size and complexity of the hyaline cartilages differ markedly in the sexes and results in a more elaborate internal lumen in males than in females.

The cartilaginous skeleton is flanked by bipennate laryngeal dilator muscles that insert, at one end, onto a tendon attached to the arytenoid disks. In X. boreas, where the larynx is sufficiently transparent that disk movement can be observed, clicks are produced when the arytenoid disks open (Yager 1992); we assume that a similar mechanism operates in X. laevis. The force required for disk movement is supplied by contraction of the bipennate muscles in response to activity in the laryngeal nerve; provided that muscle activity generates suprathreshold force, each cycle of muscle contraction and relaxation is associated with the production of a single click.

This relatively simple mechanism of vocal production has been useful in analyzing the physiological properties of male and female larynges that are associated with their
distinctive calls. The larynx can be removed and stimulated in vitro via the laryngeal nerves; muscle contraction and resultant tension on the tendon can be recorded as can the actual sounds produced (Tobias and Kelley 1987). Because the isolated larynx produces sounds that closely resemble actual vocalizations when stimulated via the nerves, respiration is clearly not required for sound production. However, the glottal nerve runs with the axons of laryngeal motor neurons, and trains of stimuli to the nerve result in glottal opening. It is possible that in the animal, access to the buccal cavity (or the lungs) is coordinated with vocalization and that glottal opening, for example, contributes to acoustic features of the call. The production of vocal patterns is widely believed to have arisen from the intrinsic rhythms of respiration. The aquatic habitat is a secondary adaptation (i.e., the pipid taxonomy is believed to have originated from terrestrial frogs) and it would be surprising if no remnant of respiratory control could be found in vocal output pathways.

One question is whether sexually differentiated features of the distinctive male and female calls can be tied to characteristics of the vocal organ. There are four basic differences between male and female vocal behaviors: the dominant frequency of each click, the rate at which clicks are given, the temporal pattern of the click trains, and the amplitude modulation of click trains. We do not know how the dominant frequency of each click is determined. Fast Fourier transform analyses reveal that clicks produced by isolated larynges contain more high-frequency components (> 4 kHz) than are found in actual clicks; some of the acoustic features of clicks may be shaped by resonance properties of extralaryngeal structures. In general the dominant frequency of clicks in male vocalizations is higher (~ 1.7 kHz) than that in female vocalizations (~ 1.2 kHz). The lumen of the hyaline cartilages is considerably more complex in males than in females and could contribute to differences in click frequencies. However the shape of the larynx does not impose an invariant constraint on click frequency. The clicks in growling (< 1 kHz) for example, produced only by males, are lower in frequency than the clicks in advertisement calls (~ 1.7 kHz).

Male and Female Laryngeal Muscle

The best understood laryngeal correspondence is between click rate and twitch characteristics of laryngeal muscle fibers. The fast trill portion of the advertisement call is a short (250 ms), rapid (70 Hz) click train and is produced by bipennate muscles that can contract and relax at rates up to 100 Hz. Female muscle tetanizes at rates as slow as 35 Hz and cannot contract and relax completely at rates faster than 25 Hz (Tobias and Kelley 1987). Since, as long as enough fibers are involved, each contraction of the bipennate muscle is accompanied by a click, the contractile properties of the muscle fibers themselves set limitations on the rapidity with which calls can be produced. Males can produce both fast and slow trills, whereas females can only produce slow trills.

What accounts for sex differences in the contraction rate of laryngeal muscle fibers? The entirely fast twitch muscle of males is the result of a masculine developmental program that relies on gonadal secretion of steroid hormones, particularly the androgens (reviewed in Sassoon et al. 1987; Kelley 1996). At the end of metamorphosis males and females have equivalent numbers of laryngeal muscle fibers and most are slow twitch. Males then proceed to add fibers at an average rate of approximately 150 a day until the adult complement is reached 6 months later (Marin et al. 1990). From this point on, muscle fibers start to convert from slow to fast twitch and it is not until the last slow twitch fibers change over, generally 10 to 12 months after metamorphosis is complete, that males can produce the fast trills of advertisement calls (Tobias et al. 1991a). Castration at metamorphosis or 6 months later halts, but does not reverse, conversion of slow to fast twitch fibers (Tobias et al. 1991b). The larynges of juvenile frogs, either males or females, can be converted to entirely fast twitch by the provision of a testis transplant or exogenous androgen that will also reverse the effects of castration at an early age (Tobias et al. 1991b; Watson et al. 1993).

The results described above suggest that androgen secretion from the gonads is required for the masculinization of muscle fiber type in the X. laevis larynx. Since it is clearly not adaptive to convert all nonvocal muscles to fast twitch we have asked what mechanisms confine the effects of gonadal androgen to the larynx. One possibility is that androgen sensitivity is limited to laryngeal muscle. Sensitivity to androgen is conferred by the expression of an intracellular protein, the androgen receptor, which binds the hormone; the receptor/hormone complex accumulates in the cell nucleus and influences function by regulating transcription of target genes. We have cloned the X. laevis androgen receptors and have analyzed their pattern of expression in larynx during development (Fischer et al. 1995). Laryngeal muscle and cartilage, particularly in juveniles, express extremely high levels of receptor, among the very highest levels of any vertebrate tissue. However, all muscle has some androgen receptor and thus could be influenced by circulating hormones.

Different myosin heavy chains (produced by separate genes) have different adenose triphosphatase (ATPase) activities that contribute substantially to differences in speed of muscle contraction and relaxation. Thus another possibility is that laryngeal muscle expresses unique, androgen-
sensitive contractile proteins. We examined this possibility by screening a laryngeal cDNA library for myosin heavy chain (MHC) genes expressed predominately in adult males (Catz et al. 1993). The screen revealed an abundant MHC that we have called laryngeal myosin heavy chain or LM. Using a probe containing untranslated 3’ sequence (important because this region is poorly conserved among myosin heavy chains), we can detect LM expression in all laryngeal muscle fibers of adult males, but in only some of females. LM expression is regulated by gonadal androgens; castration at the end of metamorphosis prevents the male-typical increase in LM expression, and LM expression is increased by exogenous androgen secretion in both sexes (Catz et al. 1995).

In vertebrates the MHC genes expressed by embryos are different from those expressed in newborns and in adults. In amino acid sequence, the portion of the LM gene that we have examined is more like embryonic fast myosins than those of neonates or adults. It thus seems likely that this tissue-specific, androgen-regulated gene arose from the duplication of—and then divergence from—an ancestral embryonic, fast MHC. That divergence probably included changes that permit regulation of the gene by several hormone systems. Recent studies by Chris Edwards in the laboratory (Edwards et al. 1999) suggest that the secretion of the pituitary hormone prolactin is required to establish the androgen sensitivity of LM expression. Male muscle fibers cannot become fast twitch in response to androgen without the normal developmental sequence of exposure to prolactin at the end of metamorphosis.

Temporal Pattern of Calling

The twitch characteristics of laryngeal muscle fibers set limits on the rate at which clicks can be produced. The temporal pattern of calling (including click rate) is generated by the central nervous system. This feature was first demonstrated by the vox in vitro experiments in which isolated larynges were driven to produce rapid trills (males: advertisement calling) or slow trills (females: ticking) by stimulating the laryngeal nerves at the behaviorally appropriate rates (35-70 Hz vs. 7 Hz); the rate of click production mirrored the rate of stimulation (Tobias and Kelley 1987).

What was not clear from these experiments was the actual temporal pattern produced by the central nervous system during vocal behavior. For example, the central nervous system might generate a single pattern conveying a sexually receptive state and an alternative pattern for the unreceptive state; the pattern for advertisement calling might be identical to that for rapping and that for growing be identical to ticking. The call produced would be determined by how faithfully the larynx could follow the brain; the twitch properties of laryngeal muscle fibers could, for example, filter the growing input into a ticking output or an advertisement calling input into rapping. To examine this question, Ayako Yamaguchi in the laboratory has recorded en passant from the laryngeal nerves of vocalizing male and female frogs (Yamaguchi and Kelley 2000). These studies reveal a unique pattern of nerve activity that corresponds 1:1 with the vocalizations produced by the animal. Thus sexually differentiated laryngeal muscle fiber characteristics do not shape a state-dependent neural output (receptive vs. unresponsive) into a sexually appropriate vocal behavior, but rather subserve the distinct demands of a varied and sexually dimorphic vocal repertoire.

Click Amplitude

A characteristic feature of the male’s advertisement call is variation in the amplitude of individual clicks in the fast trill portion; clicks are initially soft and become progressively louder (amplitude modulation). An individual male can produce advertisement calls with both amplitude modulated and nonmodulated fast trills. The answer call (a modified advertisement call elicited by rapping), however, is always markedly amplitude modulated. How is the progressive increase in click loudness produced?

Recordings of electromyographic (EMG) activity from the vox in vitro preparation reveal that the loudness of individual clicks is directly proportional to EMG amplitude: the more muscle fibers that are active, the louder each click (Tobias and Kelley 1987). An increase in the number of contracting laryngeal muscle fibers reflects both a progressive increase in neurotransmitter release from individual synapses (facilitation, Tobias et al. 1993; Ruel et al. 1998) and a progressive increase in the activity of laryngeal motor neurons (recruitment, Yamaguchi and Kelley 2000). Thus the extent to which motor units (laryngeal motor neurons and the muscle fibers they innervate) are recruited and facilitated determines the progressive loudness of individual clicks in the fast portion of the advertisement call. Amplitude modulation, like the temporal pattern of vocal behaviors, is a function of the central nervous system, both recruitment of laryngeal motor neurons in the hindbrain and facilitation of the vocal neuromuscular synapse in the larynx.

A striking characteristic of the male vocal synapse is that a single action potential invading the presynaptic terminal releases only a small amount of neurotransmitter, an amount insufficient to depolarize the muscle fiber to the threshold level required to produce an action potential and a muscle contraction (Tobias and Kelley 1988). Action potentials in
muscle require repetitive activity in the laryngeal nerve; the weak male synapse strengthens with use (facilitation). With weak vocal synapses, synaptic failures occur even during repetitive nerve activity; neuromuscular transmission is unreliable (Tobias and Kelley 1988). At most vertebrate neuromuscular synapses, each action potential in a motor axon releases sufficient (or more than sufficient) neurotransmitter to evoke a muscle fiber action potential. Even the laryngeal synapse of adult female *X. laevis* is stronger than that of males and synaptic failures are infrequent (Tobias and Kelley 1987, 1988; Tobias et al. 1998). Does improved vocal courtship compensate for the synaptic failures that characterize the weak vocal synapses of male *X. laevis*?

At the level of individual synapses, subthreshold neurotransmitter release permits facilitation, one of the cellular mechanisms responsible for the progressive amplitude increase of fast trills. If this feature—increasing click loudness—is important for attracting females (and/or discouraging male competitors) then it may reflect the process of sexual selection believed to shape characteristics of anuran advertisement calls (Wells 1977). Progressive increases in click amplitude accompany some advertisement calls. Preliminary experiments in the laboratory suggest that these calls can be more attractive to females than advertisement calls with constant amplitude fast trills (Tobias et al. 1991c). The male answer call is also highly amplitude modulated. We do not know whether answer calls are more attractive to females than advertisement calls. One possible scenario is that the answer call evoked from a male by a rapping female also attracts other gravid females, increasing the probability that a male will encounter an ovipositing female with which to mate.

The Localizability of Sounds

The first requirement of a mating system is locating a sexual partner; advertisement calls have to provide accurate location information. A parsimonious explanation for specific acoustic features of *Xenopus* calls (and those of the túngara frog; see Ryan and Rand, this volume) is that some are more easily localized than others. The localizability of natural sounds has been examined in a number of vertebrate species and several features of easily located sounds have been identified; louder sounds are easier to locate than softer ones, longer sounds are found more readily than shorter ones. Sounds such as clicks with abrupt onsets are more easily localized than sounds that gradually wax or wane in intensity. Repeated click trains with distinctive signatures (e.g., progressive increases in amplitude) may be more easily localized than single clicks or more monotonous trains (see Marler 1955; Erulkar 1972). The broad range of frequencies present in *X. laevis* clicks, their rapid onsets, the frequent repetition of clicks, the amplitude of advertisement and rapping, and the amplitude modulation of advertisement and answer calls all suggest an adaptation for easily localized sounds. How *X. laevis* actually localizes sounds, however, is not well understood (Elepfandt et al. 1995; Elepfandt 1996).

The auditory periphery of *X. laevis* includes the tympanic disk, amphibian and basilar papillae, and their associated nerve fibers (Figure 12.4; Weyer 1983; Elepfandt 1996). The tympanic discs are oblongs of thin cartilage into which one end of the columnella inserts, the other end being inserted into the oval window. The middle ear cavity is air filled and communicates with the contralateral cavity via a midline eustachian tube. The auditory sensitivity of the peripheral apparatus is well matched to the dominant frequencies of the clicks. Measurements of inner ear potential changes (reflecting activity of the papillae; Weyer 1983) reveal maximum sensitivities at 1200 to 2500 Hz (Figure 12.4), a range that includes the dominant frequencies of clicks from most vocal behaviors. Behavioral tests (Figure 12.4; Elepfandt 1996) reveal maximum frequency discriminations in the range 1400 to 2500 Hz (2% limen), values that are in good agreement with the electrophysiological recordings of Weyer. Biophysical studies reveal that the amplitude and phase of vibration of the tympanic disks varies with sound direction and frequency (reviewed in Elepfandt 1996). How these differences translate into the ability to localize sound sources is not yet clear, but could be augmented by interaural comparisons carried out within auditory regions of the central nervous system. Brain regions that participate specifically in sound localization are present in other vertebrates (the nucleus laminar is of birds for example; reviewed in Knudsen and Brainard 1993), but have not yet been identified in *X. laevis*.

The Anuran Auditory System: Sex Differences

How are anuran communication signals perceived? Answers to this question might shed some light on the pre-existing preferences thought, in the sensory exploitation hypothesis (see Ryan and Rand, this volume), to shape vocal behaviors. For the most part, these issues have been examined in terms of the biases in the female's nervous system (assayed by phonotaxis to male advertisement calls). It is not clear, however, whether a specific auditory preference must represent a sexually dimorphic character; the auditory preference could be characteristic of both sexes in a particular species and exploited by both sexes, or it could be sex-specific and shape distinct features of male and female vocal behavior. In *X. laevis* we have the opportunity to examine the female's response to the male advertisement and answer
calls as well as the male's response to both repulsive and attractive female calls (ticking and rapping). Pacific treefrogs (see Brenowitz et al., this volume) present an opportunity to look at neural coding of salient auditory stimuli used in male-male competitions (which should also, provided that they influence reproductive opportunities, be subject to sexual selection).

Sound Frequency

The best evidence for sexually differentiated auditory processing comes from the work of Narins and Capranica (1976, 1980) on the Puerto Rican tree frog, Eleutherodactylus coqui. Males have a two-note call, Co and Qui; the first note is used in male-male territorial encounters whereas the second note is attractive to females. The Co note is a constant frequency (1.1–1.2 kHz) whereas the Qui note is an upwards frequency sweep (1.8–2.2 kHz). The anuran inner ear contains two papillae (see Figure 12.4), amphibian and basilar, tuned to different frequency ranges: low to mid (amphibian) and high (basilar). The papillae are innervated by axons of neurons that form the eighth nerve, and relative frequency sensitivity can be determined by an axon's best excitatory frequency (BEF)—the frequency most effective, at the lowest amplitude, in increasing the firing rate of eighth nerve axons.

The major result of these studies is the higher proportion (relative to the opposite sex) of neurons with BEFs in the Co range in males and in the Qui range in females. Further the sharpness of tuning of nerve axons with high frequency sensitivity (innervating the basilar papillae) was greater in males than in females; for low and midrange frequencies (amphibian papillae), results were the same in the sexes. Does this finding mean that females are deaf to Co and males to Qui? No. As a female approaches a male and the loudness of his call increases, her auditory system should respond to Co notes; only at relatively greater distances (and lower sound amplitudes) should the sex difference be apparent. A similar argument applies to a male approaching another male; responses to Qui should be robust at shorter distances.

Temporal Aspects of Sounds, Inter- and Intrasexual Communication

Ticking and rapping have very different effects on male vocal behaviors in X. laevis (inhibition and excitation, respectively) and this differential response has enabled us to design a series of experiments aimed at determining how these female vocalizations are distinguished. These vocalizations differ primarily in rate; the individual clicks in ticking and rapping are not distinguished, but the former is a
slow (~4 Hz) whereas the latter is a more rapid (~12 Hz) trill. To determine how males label click trains we have constructed artificial trills at intervals intermediate between ticking and rapping and are examining the amount of time males spend calling in response to these artificial calls.

Of particular interest is whether the calls are labeled categorically (see Wytenbach et al. 1996 for a discussion of this issue). For example, up to some boundary rate, males may respond to artificial calls as though to ticking and past this boundary, as though to rapping. Alternatively there may be a simple monotonic relationship between the rate of click production by the female and the male's vocal response. These outcomes have different implications for how female songs are decoded by the male's nervous system. In the first case, the nervous system initially sorts the click trains into one of two categories: vocalizations are stimulated if acoustic stimuli are categorized as tick-like and suppressed if categorized as tick-like. In the second, there is no initial categorization and vocalization is simply a direct function of the rate of stimulation. Our preliminary data suggest that the first scenario is correct and that the criteria for labeling are stringent.

When males hear rapping they can switch from advertisement to answer calling within a few hundred milliseconds. The differential response to the two vocal behaviors (ticking: suppression; rapping: stimulation) is slower, on the order of seconds, and could require processing in higher brain regions involving neurons that are tuned to the rate of stimulation by clicks. Studies by Rose and colleagues (Alder and Rose 1998 and reviewed by Brenowitz et al., this volume) reveal the presence of rate-selective neurons in the torus semicircularis (Figure 12.5), a midbrain region that processes temporal aspects of sound in many vertebrate species.

Male Pacific treefrogs defend calling territories and switch from a trill with a faster repetition rate (advertisement call) to one with a slower rate (encounter call) in response to a vocalizing intruder. Males discriminate between call-appropriate trill rates in playback experiments. Alder and Rose (1998) have recently described a population of rate-sensitive neurons in the torus of these frogs whose properties match call features. Neurons responsive to fast trill rates only start firing after the eighth click (multiple clicks are needed to determine rate) and the response continues after termination of the stimulus. These toral neurons have features that could be used as part of an encounter call detection system. Their presence in the anuran torus suggests that it is in this region that we may find portions of the neural machinery used in discriminating ticking from rapping in X. laevis. The torus of X. laevis (Figure 12.5) contains several candidate nuclei for participation in rate tuning including one, the laminar nucleus, that is a target for gonadal steroids (Kelley 1980). This system should provide a rich arena in which to investigate the neural processing of courtship signaling and whether it differs in the sexes.

Conclusions

Xenopus laevis live in dark, silt-filled ponds and call at night. Under these conditions, sound plays a preeminent role in social communication; X. laevis has a rich and complex repertoire of vocal signals. The two female-specific calls, rapping and ticking, have powerful but opposite effects on male calling. Rapping stimulates whereas ticking suppresses male songs. Both sexes can advertise their sexual readiness, females via rapping and ticking and males via their advertisement calls. Males also communicate vocally and some male–male duets appear to function in the establishment of vocal dominance. We are beginning to understand the cellular and molecular mechanisms that produce sexually differentiated vocal behaviors. Male- and female-specific developmental programs require secretion of sex-specific
eroidal steroids that act on both the vocal organ and on the neural pathways for vocal production in the central nervous system. In the larynx, androgen controls muscle fiber addition and muscle fiber type. In the central nervous system, androgen prevents the death of developing vocal motor neurons. Estrogen controls synaptic strength. How vocal behaviors are perceived is less well understood, although the major brain pathways and some sex differences in auditory processing have been described in other anurans. Of particular interest is how the temporal characteristics of calls are coded for in the central nervous system. Understanding, for example, how the rapid trills of rapping and the slower trills of creaking, respectively, excite or suppress a calling male is a fascinating problem.

References

