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Sexual Hearing: The influence of sex hormones on acoustic communication in frogs

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Abstract

The majority of anuran amphibians (frogs and toads) use acoustic communication to mediate sexual behavior and reproduction. Generally, females find and select their mates using acoustic cues provided by males in the form of conspicuous advertisement calls. In these species, vocal signal production and reception are intimately tied to successful reproduction. Research with anurans has demonstrated that acoustic communication is modulated by reproductive hormones, including gonadal steroids and peptide neuromodulators. Most of these studies have focused on the ways in which hormonal systems influence vocal signal production; however, here we will concentrate on a growing body of literature that examines hormonal modulation of call reception. This literature suggests that reproductive hormones contribute to the coordination of reproductive behaviors between signaler and receiver by modulating sensitivity and spectral filtering of the anuran auditory system. It has become evident that the hormonal systems that influence reproductive behaviors are highly conserved among vertebrate taxa, thus studying the endocrine and neuromodulatory bases of acoustic communication in frogs and toads can lead to insights of broader applicability to hormonal modulation of vertebrate sensory physiology and behavior.

Keywords

anurans; arginine vasotocin; auditory system; gonadal steroids; sensory physiology

The most conspicuous and extensively studied behavior of anuran amphibians (frogs and toads) is their acoustic communication. In the vast majority of anurans, vocal signaling mediates sexual behavior: sexually active males produce courtship calls to attract females. In these species the tie between vocal communication and reproduction is unambiguous.

Reproduction in frogs occurs seasonally and, as is generally true for vertebrates, is regulated by steroid hormones and peptide neuromodulators (reviewed in Yamaguchi and Kelley, 2002). Given the role of acoustic signaling as the predominant mediator of sexual interaction

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in frogs, it is not surprising that their vocal communication systems are strongly influenced by hormonal systems. In anurans, seasonal changes in hormone levels correlate with seasonal changes in calling behavior (Varriale et al., 1986; Itoh et al., 1990; Itoh and Ishii, 1990; Gobbetti et al., 1991; Harvey et al., 1997; Polzonetti-Magni et al., 1998). In addition, endocrine state and/or peptide neuromodulator systems have been shown to impact the initiation of vocal signaling (Wada et al., 1976; Wada and Gorbman, 1977; Wetzel and Kelley, 1983; Penna et al., 1992; Boyd, 1994; Marler et al., 1995; Propper and Dixon, 1997; Solis and Penna, 1997; Semsar et al., 1998; Burmeister and Wilczynski, 2001), the characteristics of the calls that are produced (Marler et al., 1995; Chu et al., 1998; Klomberg and Marler, 2000; Kime et al., 2007), how vocal signals are received (Miranda and Wilczynski, this issue, Yovanof and Feng, 1983; Penna et al., 1992; Miranda, 2007), and which behaviors they induce (Schmidt, 1983; Boyd, 1994). Research in amphibians has also revealed that many of the hormones that modulate vocal communication are the same as those found in other vertebrate taxa, indicating that hormonal mechanisms influencing reproductive behaviors are evolutionarily conserved. Thus, studying the hormonal bases of acoustic communication in frogs presents a useful model for investigations of the action of these systems on vertebrate sensory physiology and behavior.

Frog acoustic communication

In the vast majority of vocal frog species, males are the sole callers. Under the classic paradigm, male frogs form lek-like aggregations during the reproductive season in which they produce a species-typical advertisement call that functions primarily to attract females for breeding, as well as to repel conspecific males. The production and reception of the advertisement call serve to coordinate the reproductive behaviors of the two sexes. Females use the acoustic cues provided by these calls to guide them toward sexually active males (i.e., engage in phonotaxis) and to choose among potential mates. Although this dimorphic call production pattern holds across thousands of species, there are some instances of female vocalization; these are most often “reciprocal” calls (Duellman and Trueb, 1986; Roy et al., 1995) produced in response to the advertisement calls of males (Given, 1987; Márquez and Verrell, 1991; Bush, 1997; Orlov, 1997; Schlaepfer and Figeroa-Sandi, 1998; Tobias et al., 1998). Despite these exceptions, the vast majority of study of the hormonal underpinnings of sexually specific behavior in frogs has focused on the male production of, and the female response to, advertisement signals.

Chemical control systems

There are two hormonal systems that are widely recognized to influence reproductive behaviors, including acoustic communication, in anurans. The first is a neuromodulator system comprised of arginine vasotocin (AVT), a peptide hormone produced by neurons in the telencephalon and diencephalon. The second is an endocrine system consisting of the suite of steroid hormones (e.g., testosterone, dihydrotestosterone, estrogen) produced by the gonads and regulated by a signaling cascade beginning with another neuropeptide produced in the basal forebrain, gonadotropin releasing hormone (GnRH). Below, we will give a brief summary of the primary features of these two systems.

Arginine vasotocin

The neuropeptide AVT is the homologue of the mammalian arginine vasopressin (AVP), and functions primarily as a systemic regulator of osmolarity and blood pressure. The peptide is released into general circulation by neurosecretory cells in the preoptic area (POA) or, in mammals, in the anterior hypothalamic nuclei. Additional extrahypothalamic, telencephic AVT-containing neurons have been found in the anuran brain that do not appear to be neurosecretory cells, and are likely to instead play a neuromodulatory role within the CNS (for review, see Wilczynski and Chu, 2001). These neuromodulatory cell populations provide a mechanism by which AVT can affect behavior by acting directly within the brain, in addition to its canonical systemic effects. Via neuromodulatory activity, AVT and AVP have been shown to affect a wide range of social behaviors among vertebrate groups, including aggression, territoriality, courtship behaviors, and mating (reviewed in Goodson and Bass, 2001; Yamaguchi and Kelley, 2002; Marler et al., 2003; Wilczynski et al., 2005). In frogs, there is substantial evidence that AVT administration impacts production of advertisement and territorial calls by males (Marler et al., 1995; Propper and Dixon, 1997; Chu et al., 1998; Semsar et al., 1998), and some indication that it enhances sexual receptivity in females (Boyd, 1994). Local concentrations of the peptide and its m-RNA have been located in regions of the frog brain involved with acoustic signal production; thus, it is probable that the peptide is implicated in the modulation of acoustic communication behavior (reviewed in Emerson and Boyd, 1999; Moore and Rose, 2002).

Gonadal steroids

In accordance with the widely accepted vertebrate model, steroid hormones in anurans play critical roles in the organization and differentiation of sexually dimorphic body structures during development. In addition, gonadal steroids are key regulators of the expression of sex-specific mature reproductive behaviors. For example, in frogs, advertisement calling is androgen dependent (Wada et al., 1976; Wada and Gorbman, 1977; Wetzel and Kelley, 1983; Burmeister and Wilczynski, 2001) and the neural pathway subserving vocal production concentrates sex steroids (Kelley et al., 1975; Morrell et al., 1975; Kelley, 1981). Regulation of these steroids occurs through the hypothalamic-pituitary-gonadal, or reproductive (Vilain and McCabe, 1998), axis (HPGA). At the apex of the HPGA are the GnRH-releasing neurons, which in amphibians, including frogs, are distributed along the midline of the basal forebrain (Rastogi et al., 1998; Burmeister and Wilczynski, 2005). The secretion of GnRH stimulates the release of pituitary gonadotropins [e.g., luteinizing hormone (LH)], which in turn regulate the synthesis and release of steroid hormones from the gonads. Thus, plasma levels of gonadal steroids are ultimately regulated by the GnRH neuronal population, making these cells a primary site for regulation of reproductive physiology and behavior (Gore, 2002).

Gonadotropins exert their effects by binding to a transmembrane receptor, for example, the luteinizing hormone receptor (LHR). LHR was traditionally thought to be expressed only in the gonads, in accord with the role of gonadotropins in stimulating the release of gonadal steroids. However, LHR has since been found in the neural tissues of birds, mammals and frogs (Lei et al., 1993; You et al., 2000; Rao et al., 2004; Yang et al., 2007). These data suggest that gonadotropins may act directly in the brain to regulate reproductive behaviors, in addition to inducing steroid hormone production (Lei and Rao, 2001). Indeed, recent research suggests that gonadotropin works within the CNS of male *Xenopus laevis* (the South African clawed frog) to induce advertisement calling (Yang et al., 2007). This suggests a neuromodulatory role in the control of acoustic communication in this species (Yang et al., 2007).

Hormonal modulation of acoustic signal reception

As in any communication system, acoustic communication consists of two primary units, a signaler and a receiver. The principal elements involved in communication differ between these two units: for the signaler, it is the sound-production apparatus, and for the receiver, the auditory apparatus. On the receiving end, there is evidence from numerous vertebrate species, including humans, that acoustic perception is influenced by circulating hormones (Hinde and Steele, 1964; Komisaruk et al., 1972; Wright and Crow, 1973; Elkind-Hirsch et al., 1992; Penna et al., 1992; Sisneros et al., 2004; Walpurger et al., 2004; Maney et al., 2006; Lynch and Wilczynski, 2008). The modification of acoustic signal processing by hormones may facilitate communication during periods of reproductive behavior. Studying endocrine-based modulation of acoustic perception in animals such as frogs, however, is quite difficult, particularly when using a behavioral approach. Signal perception is only apparent when the focal individual expresses an overt response to acoustic stimuli. If no behavioral response is induced by an applied stimulus, it is impossible to determine if the signal was perceived. Perhaps as a result of these challenges, the bulk of research on the role of hormones in the modulation of frog acoustic communication has focused on the signaler. Herein, however, we will focus on the smaller, but rapidly growing, body of literature that examines the way in which hormones modulate the reception of vocalizations by the frog auditory system.

Female phonotaxis

One of the most evident behaviors observed during anuran acoustic communication is female phonotaxis towards the source of the conspecific advertisement call. Thus, phonotaxis provides an excellent behavioral assay for explorations of the impact of hormones on acoustic signal reception and sexual motivation in frogs. For example, studies of female túngara frogs (*Physalaemus pustulosus*) have demonstrated that maximal receptivity (i.e., positive phonotactic response to any conspecific male signal) and permissiveness (i.e., phonotactic selectivity assessed by a response to a synthesized call known to be less attractive than the conspecific call) correspond to peaks in circulating levels of the steroid hormones estrogen and progesterone (Lynch and Wilczynski, 2005). In addition, injection with human chorionic gonadotropin (hCG), which is an agonist at LHRs, thus inducing release of gonadal hormones, also increases receptivity and permissiveness in this species (Lynch et al., 2006). A subsequent study demonstrated that female túngara frogs injected with estradiol show a similar likelihood of phonotaxis, and similar call preferences, as non-manipulated females in breeding condition. These results suggest that estradiol fluctuations are primarily responsible for the changes in female sexual behavior seen over the breeding cycle (Chakraborty and Burmeister, 2008). Taken together, these studies indicate that positive phonotaxis and the ability to discriminate calls covary with circulating gonadal hormone levels in female túngara frogs, and suggest that estradiol plays a principal role in this hormonal effect. In addition to the studies on *P. pustulosus*, work with other frog species have shown that female receptivity and phonotactic efficiency is enhanced by injections of both hCG and AVT (Diakow, 1978; Diakow and Nemiroff, 1981; Raimondi and Diakow, 1981; Picker, 1983; Schmidt, 1983; 1985; Boyd, 1992; 1994).

Phonotactic behavior is a motor response driven by the reception of acoustic signals, thus the coincident modulation of phonotaxis and hormone levels suggests that hormones may act directly on acoustic processing in female frogs. Only a few studies have tested this possibility. Yovanov and Feng (1983) found that auditory evoked potentials recorded from the midbrain (i.e., torus semicircularis) of female leopard frogs (*Lithobates pipiens*, formerly *Rana pipiens)* in response to tones representing frequencies contained in the conspecific advertisement call increased in amplitude after injection with the estrogen estradiol. The behavioral implications of this result, however, are unclear because estrogens have not been shown to promote female receptiveness in this species (Diakow et al., 1978). In female green treefrogs (*Hyla cinerea*), implantation with testosterone significantly increases midbrain auditory thresholds for frequencies corresponding to the male advertisement call, but not for frequencies outside these spectral bands (Miranda and Wilczynski, this issue, Miranda, 2007). Thus, testosterone apparently decreases the female treefrogs’ sensitivity to the conspecific call. This result may be explained by examining the pattern of natural testosterone fluctuation in *P. pustulosus*. Female túngara frogs have the highest circulating testosterone concentrations prior to the expression of maximal receptivity and permissiveness (Lynch et al., 2005; Lynch and Wilczynski, 2005). If this pattern holds in female *H. cinerea*, the reduction of sensitivity to the spectral range of the advertisement call during periods of high circulating testosterone may help inhibit premature mating behavior that precedes peak reproductive readiness (Miranda, 2007). Altogether, these electrophysiological studies, along with others showing seasonal fluctuations in neural activity in the auditory midbrain of both sexes of fire-bellied toads (*Bombina bombina*), grass frogs (*Rana temporaria*) and the gray tree frog (*Hyla chrysoscleis*) (Walkowiak, 1980; Hillery, 1984) provide intriguing evidence of the complex influence of sex hormones on sensitivity and spectral filtering of the anuran auditory system.

In a recent study, Lynch and Wilczynski (2008) took a different approach to investigating hormone-induced modulation of auditory signal processing. Instead of employing electrophysiological techniques, the investigators used *in-situ* hybridization to measure levels of expression of the activity-dependent immediate early gene *egr-1* (early growth response 1) in the auditory midbrain of female túngara frogs injected with hCG and/or exposed to recordings of the conspecific mating chorus. Immediate early genes, such as *egr-1*, are commonly used in communication studies (Hoke et al., 2004; Mello et al., 2004; Hoke et al., 2005; Burmeister et al., 2008) because the location and abundance of *egr-1* mRNA in the auditory midbrain serves as an indicator of patterns of neuronal activity induced by the processing of auditory signals. Lynch and Wilczynski (2008) found that injection of hCG significantly increased *egr-1* induction in one of the nuclei (the laminar nucleus, see below) of the auditory midbrain in response to chorus exposure (figure 1). These data suggest that elevated gonadotropin levels enhance the stimulatory effect of the conspecific calls. It remains to be determined whether this enhancement is due to the downstream effects of hCG application (i.e., the induced release of gonadal hormones which influence midbrain activity via steroid receptors) or the direct action of the gonadotropin through LHRs on forebrain neurons which, in turn, modulate midbrain activity (Lynch and Wilczynski, 2008). The results of this study indicate that in sexually receptive females, higher concentrations of circulating hormones may increase the responsiveness of particular central auditory nuclei to conspecific signals. Future experiments employing this technique, which can provide a brain-wide view of neuronal activity modulation by hormones and sound, will provide an additional source of information about the mechanisms by which hormones work within the CNS to modulate acoustic processing.

Modulation of auditory signal processing

How do hormones influence the reception and processing of acoustic signals? This is a complex question, but some progress has been made in understanding hormonal modulation of activity in the frog central auditory system. The midbrain torus semicircularis (TS) is an auditory nucleus that is considered to be the amphibian homolog of the mammalian inferior colliculus (Wilczynski and Endepols, 2007). The TS is the primary auditory processing center in the frog CNS, so it is an obvious starting point for investigating the influence of sex hormones on auditory processing. This nucleus integrates ascending auditory pathways, receives both ascending and descending auditory inputs, and serves as a sensorimotor integrator (Wilczynski, 1988; Wilczynski and Endepols, 2007). The TS is comprised of several sub-nuclei of which the two most widely studied are the principal nucleus and the laminar nucleus (LN). The LN has extensive connections to premotor and motor areas, and is therefore likely to bear significant responsibility for audiomotor integration (Endepols and Walkowiak, 2000). In addition, this nucleus has been found to contain cells that concentrate both androgens and estrogens (Kelley et al., 1975; Morrell et al., 1975; Kelley et al., 1978; Kelley, 1980; di Meglio et al., 1987). Thus, the LN potentially represents a key access site through which gonadal hormones can influence the processing of communication signals, including the modulation of motor correlates of signal reception, such as phonotaxis. To date, however, relatively little is known about the direct impact of hormone action on the processing of communication signals in the TS.

Upstream from the TS, forebrain limbic areas have also been implicated as possible CNS centers for hormonal modulation of acoustic processing. The ventral hypothalamus (VH) and the POA are the two forebrain nuclei known to be important in the control of GnRH secretion

(Ball, 1981) and, like the LN, have been shown to concentrate steroid hormones (Endepols and Walkowiak, 1999). Projections from these forebrain areas into the TS could be another mechanism by which TS activity is modulated by hormonal secretion. Interestingly, robust ascending auditory connections from the TS to the VH and POA via thalamic relay nuclei have been characterized in anurans (Neary, 1988; Wilczynski et al., 1993). This anatomical pathway provides a means by which acoustic stimuli could influence endocrine state by altering the secretion of GnRH, and thus influencing the activity of the HPGA axis. Indeed, POA and other hypothalamic neurons have been found to respond to auditory stimulation (Wilczynski and Allison, 1989; Allison, 1992; Hoke et al., 2005), and it is known that the reception of communication signals induces steroid hormone production in male (Burmeister and Wilczynski, 2000; 2001; Chu and Wilczynski, 2001; Burmeister and Wilczynski, 2005) and female frogs (Lynch and Wilczynski, 2006).

In a recent study Kime et al., (2007) found that systemic AVT administration caused male túngara frogs to alter their calling activity in a manner that mimicked changes induced by exposure to a conspecific chorus; this correlation led the authors to hypothesize that AVT release may be stimulated by the receipt of conspecific vocalizations, and play a role in mediating social regulation of the frogs' acoustic communication behavior (Kime et al., 2007). The mechanisms by which AVT may act within the frog brain to modulate communication behavior are as yet uncharacterized.

Taken together, this group of studies suggests that for both gonadal steroids and AVT, important incurrent pathways exist in which reception of conspecific vocal signals impact the release of hormones which, in turn, modulate the activity of the auditory system to affect appropriate sexual behavior during the breeding season. We must await further study before being able to describe the subtle interplay between external stimuli and hormonally modulated central integration that leads to acoustically triggered physiological and behavioral response.

Conclusion

Hormones play a vital role in regulating the sexual behavior of vertebrates, from frogs to humans, and hormonal systems are highly conserved across vertebrate taxa. Acoustic communication is the primary mediator of sexual behavior in the majority of anuran species, thus it provides an excellent system within which to explore the diversity of ways in which hormones work to ensure appropriate and successful reproductive activity. To coordinate breeding, both signalers and receivers are under selection to produce appropriate and temporally coordinated behaviors during the reproductive season. Although the majority of research in anurans has focused on hormonal influences on vocal signal production, intriguing evidence is emerging for a potent role of hormones in regulating signal reception and processing. By exploring the mechanisms by which hormonal activity within the auditory system of frogs connects the external social environment with internal physiological state, we have the potential to learn a great deal about the most basic processes that underlie vertebrate social behavior.

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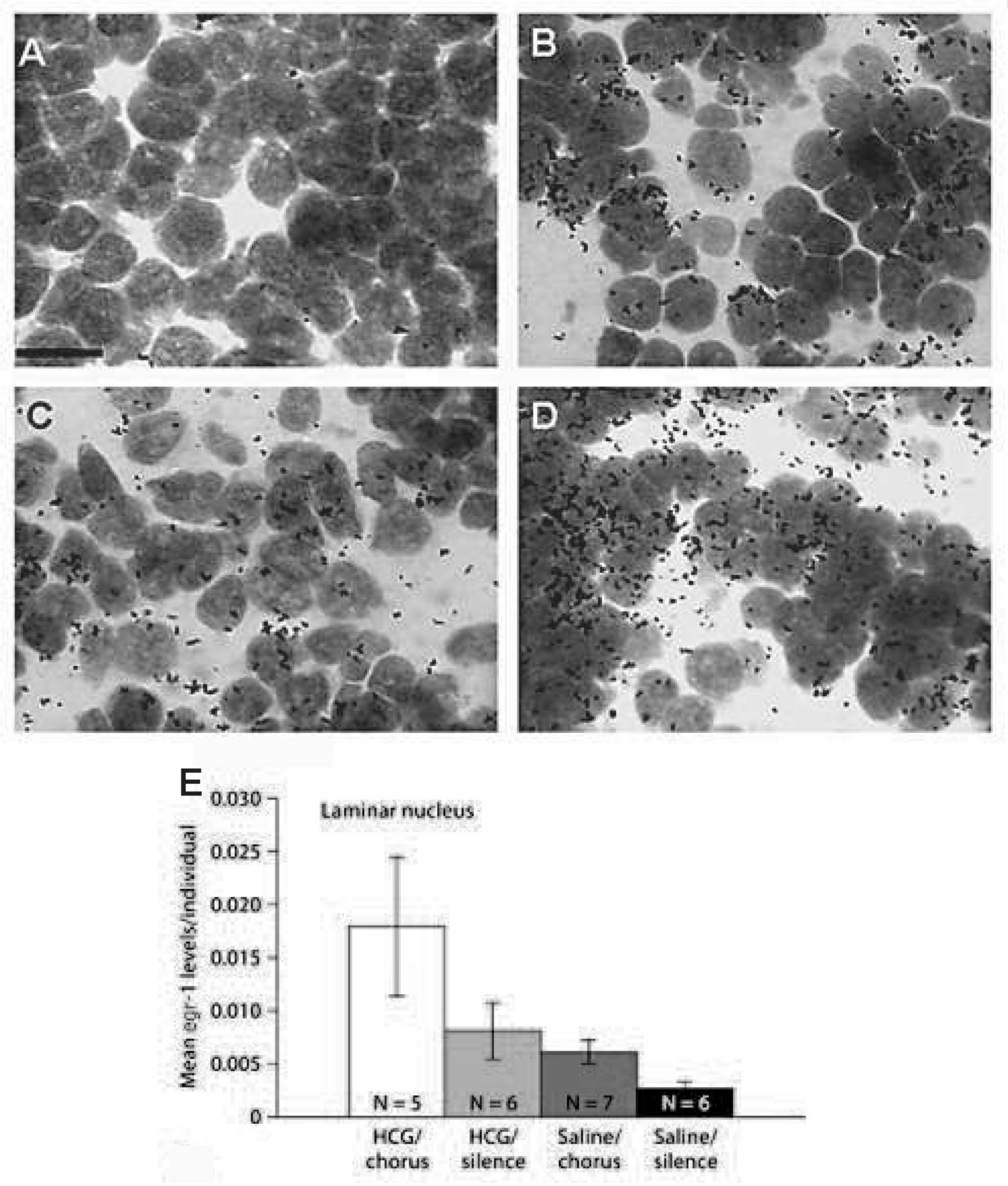

Figure 1.

A–D Four photomicrographs representing the quantitative difference in *egr-1* mRNA expression in the laminar nucleus (LN) of *Physalaemus pustulosus.* **A** *Egr-1* mRNA in saline/silence condition. **B** *Egr-1* expression in the saline/chorus condition. **C** *Egr-1* expression in the human chorionic gonadotropin (hCG)/silence condition. **D** *Egr-1* expression in the hCG/chorus condition. The area within the field of view covered by black spots (silver grains) was counted to determine the amount of *egr-1* expression. Scale bar = 1 µm. **E** Mean *egr-1* mRNA levels in the LN of females treated with hCG or saline then exposed to natural mate choruses or silence (modified and reprinted with permission from S. Karger AG, Basel).