OFFPRINTS FROM THE EVOLUTION OF THE AMBHIBIAN AUDITORY SYSTEM
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Chapter One

THE AMPHIBIAN AUDITORY SYSTEM AS A MODEL FOR NEUROBIOLOGY, BEHAVIOR, AND EVOLUTION

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I. INTRODUCTION

As anyone who has taken a high school or college biology class knows, amphibians have long been used as models to illustrate basic principles in the biological sciences. William Harvey's observations of frogs nearly 400 years ago led to his radical theory that blood actually circulated through the body. He described his results in *Anatomical Dissertation Concerning the Motion of the Heart and Blood in Animals* in 1628, and presumably undergraduates have been forced to find the aortic arches in dissected frogs ever since. Harvey was not alone in his choice of frogs as model systems. Many of the pioneers in the fields of neurobiology and sensory physiology—people such as Galvani, Volta, Mueller, von Helmholtz, Sechenov, Yerkes, and Hodgkin—used amphibians at some time as model systems for extracting fundamental principles of neural and behavioral processes. This preference for amphibian models continues. Amphibians are used as experimental models in such diverse areas as the development and plasticity of the nervous system (Constantine-Paton and Capranica, 1975; Zakon, 1983), the cellular basis of sensory transduction (Hudspeth, 1985), the hormonal control of behavior (Kelley, 1986), the interaction of natural and sexual selection (Ryan, 1985), and the mechanisms of speciation and reproductive isolation (Blair, 1964; Littlejohn, 1981).

Each of those modern examples mentioned above has something in common: at some time researchers have used the amphibian auditory system to explore basic questions in these areas. Each of these topics, and several more, are covered by the contributors to this book, whose chapters provide discussions ranging from the anatomy, physiology, and development of the auditory system, through the behavioral context in which audition is used and its integration with systems for producing acoustic signals, to the evolutionary principles integrating these areas.

II. AMPHIBIANS AS MODELS

A. Emphasis on Anurans

The bulk of research on the amphibian auditory system has centered on the anurans (frogs and toads) for an obvious reason: they are the only amphibian group that engages in acoustic communication. For this reason, anuran auditory research encompasses not only anatomy and physiology, but acoustic communication and its attendant mechanisms and behaviors. Furthermore, because acoustic communication is used during reproductive behavior, the structure and function of the signals, and the manner in which the auditory system processes them, have important evolutionary implications in such areas as sexual selection, speciation, and reproductive isolation. This does not diminish the importance of research on the auditory system of the two other amphibian orders, the urodeles (newts and salamanders) and gymnophions (apodans). If anything, too little work on these other orders has been done. Nevertheless, anurans are especially attractive as research subjects because their auditory system allows integrative studies of neural, behavioral, and evolutionary biology in a way offered by very few other systems.

B. Advantages of Anurans

Anurans are not unique in their use of the auditory system during social behavior. Many insects, birds, fish, and mammals do the same. However, anurans offer many practical advantages that recommend them for integrative studies of their auditory system.

One nontrivial advantage is that, as Harvey discovered centuries ago, frogs and toads are good laboratory animals for anatomical and physiological experiments. They are small, hearty, and tolerate anesthesia and surgery well. It takes very little experience with animals to realize that it is easier (and safer) to work with treefrogs than with Howler monkeys. Furthermore, it is actually possible to place laboratory studies of anuran audition in an ethologically relevant context. Anuran communication signals are species-specific and highly stereotyped, and each species' repertoire is small. The fact that frog communication signals can vary, and that this variability can have communicative significance (Wells, this volume), is often unappreciated. Still, even with this variability, frogs surpass most other vertebrates in the constancy and simplicity of the signals they emit and respond to socially. The presence of such a small, stereotyped repertoire necessarily limits the possible parameters that frogs use to guide their behavior. Once identified, the resultant small set of stereotyped acoustic parameters can be compared with the coding properties of the nervous system (Walkowiak, this volume; Zakon and Wilczynski, this volume).

In addition, it is a tractable problem to relate the call parameters and the concomitant nervous system processing to the natural behavior of these animals. The social behavior that acoustic signals elicits in frogs also consists of a small, highly stereotyped repertoire. The two most often studied are phonotaxis (usually by females) and antiphonal calling (by males) in response to the male's advertisement call. One thus often has a relatively reliable bioassay for each sex with which to assess the function of various call parameters. Moreover, frogs respond well to synthetic versions of their species' call that mimic only a few key parameters of the natural call, and they often appear unconcerned about other sensory cues: a loudspeaker is an adequate substitute for a conspecific. The simplicity of both signal and behavior and the reliability with which one elicits the other allows for elegant studies of communication behavior (Gerhardt, this volume; Wells, this volume) and of sensorimotor integration (Schneider, this volume).

Furthermore, the consequences of acoustically mediated behavior—mating success—are relatively easy to study. Most frogs gather into reproductive assemblies that can be readily and closely observed, and in which members are remarkably refractory to the moderate disturbances caused by the researcher. In most species, amplexus between males and females lasts for a long time, fertilization is external, and the result, a clutch of eggs, can be observed and measured. Because reproductive success can be determined, the evolutionary consequences of the investigated behavior can be assessed (Littlejohn, this volume; Ryan, this volume).

Finally, anurans exhibit more interspecific diversity than is often realized (Rand, this volume). Some species produce spectrally complex calls, some produce extremely simple calls. Some incorporate temporal cues, some do not. They occupy a wide variety of habitats and employ a wide variety of reproductive strategies. This diversity provides the raw material for comparative anatomical, physiological, and behavioral studies that address basic evolutionary questions.

III. THE NEUROETHOLOGY OF ANURAN COMMUNICATION

A. General Questions in Neuroethology

A relatively new discipline that seeks to integrate mechanistic and evolutionary questions is neuroethology, the study of neural and hormonal control of natural behavior. Animal communication is a major target of neuroethological research, and anuran acoustic communication is an important model system for this investigation. Because of its integrative nature, neuroethology necessarily requires a rigorous assessment and understanding of both natural behavior, including the characteristics of communication signals, and neural processing, including anatomical pathways, neurophysiology, and, where applicable, hormonal interactions. In addition, an awareness of the evolutionary implications of the findings is essential. For all the reasons discussed above, the anuran auditory system offers a good model system for undertaking neuroethological studies.

B. Acoustic Communication in Bullfrogs

Capranica established the frog as a model system for the neuroethology of acoustic communication with a series of papers published even before the word "neuroethology" became part of the scientific lexicon (summarized in Capranica and Moffat, 1983). These studies became models themselves for how one undertakes neuroethological research, and the specific model he proposed for communication based on results in the bullfrog (*Rana catesbeiana*) quickly became the framework against which he and others assessed the neural variability coincident with the diversity of anuran communication signals and strategies.

Capranica attempted to relate the spectral parameters of the bullfrog's advertisement call to the coding properties of its peripheral auditory system in order to explain behavioral responses of these frogs to a conspecific call. The bullfrog call is a broadband signal with two distinct spectral peaks, one at about 200 Hz and one at about 1500 Hz. Energy is greatly reduced between these peaks. A signal with energy at both peaks is necessary to elicit antiphonal calling in a male. Adding energy to the midfrequency trough inhibits the behavior.

The characteristics of the peripheral auditory system bear an interesting relationship to the signal and behavior. Bullfrogs (and all other frogs) have two auditory papillae in each ear. The two organs have different frequency sensitivities. In bullfrogs, the basilar papilla (BP) tuning matches the high-frequency peak in the advertisement call while the amphibian papilla (AP) contains hair cells activated by low frequencies, including the 200-Hz peak in the call, and mid-frequencies corresponding to the trough between the two peaks. Furthermore, excitation of the midfrequency portion of the AP inhibits the low-frequency population (the nonlinear phenomenon of two-tone suppression).

Capranica's research led to the following model of call recognition: the bullfrog's advertisement call is designed to activate both papillae simultaneously, and such coactivation is necessary for the behavior to be expressed. Adding midfrequency noise to the call suppresses the neural activity generated by the low-frequency peak, thereby tricking the nervous system into thinking that only the BP was excited and so shutting off the behavior. By this simple peripheral mechanism the bullfrog can recognize its species-specific communication signal from, for example, broadband noise or the calls of other species.

Capranica's coactivation model also generated a prediction about central processing of the signal: if the coactivation of the peripheral papillae is necessary for eliciting behavior, then inputs from the two organs should converge onto neural feature detectors at some point. Physiological studies have shown that indeed such a convergence occurs at higher auditory centers (Fuzessery, this volume). In fact, in ranid frogs much of the higher auditory processing appears skewed toward combining input from the papillae in various ways.

Capranica's research quickly became the framework—the model—for neuroethological research on animal communication. The specific model he proposed for bullfrog call recognition was eventually shown to hold true for other ranids and some hylids. But as Capranica and others in the field investigated more and more species, it became clear that a papilla coactivation model could not account for call recognition in all anurans (Zakon and Wilczynski, this volume). For example, many anurans have calls whose energy excites only one papilla; some like the spring peeper (*Hyla crucifer*) have calls that are virtually pure tones. Many toad species have calls with identical spectral composition that are separable only by temporal features such as amplitude modulation, or trill, rate. Capranica and coworkers have also begun to investigate frogs with frequency modulated calls in which the frequency sweep activates the papillae sequentially rather than simultaneously.

Far from being detrimental, this diversity represents the strength of anuran acoustic communication as a neuroethological model system. It has also confirmed the importance of Capranica's work. By using Capranica's approach to investigate call processing in different species, and by using his explanation of ranid call processing as a reference against which variations in call characteristics can be compared, one can potentially discern how the nervous system can constrain call variability or be modified with it. In addition to generating a deeper understanding of audition in general, such a comparison can illuminate basic evolutionary processes and principles.

IV. DIVERSITY AS A TOOL

A. Call Diversity and Neural Coding

Communication is interesting to evolutionary biologists because it requires the congruence of a sender's signal and the receiver's neural mechanisms for detecting and recognizing it. The congruence likely results from coevolution. Thus one assumes that some features of the receiver's auditory system are adaptations for processing the stereotyped, species-specific communication signal. Indeed, one can often see such neuroethological correlates in the properties of the central auditory system (Fuzessery, this volume; Walkowiak, this volume).

However, exploring the diverse amphibian auditory systems has shown that identifying whether a neural character is an adaptation that has evolved specifically for communication is more complicated than it may seem. Consider the two-tone suppression important in bullfrog call recognition. Is this an adaptation specifically for this type of communication? Without the call diversity manifested by anurans this question could not be answered. But by testing different species with different call types one can show that, in fact, two-tone suppression like that present in the bullfrog is present in all anurans whether or not they use a low-frequency peak in their call. It is even present in the spring peeper, which does not use the AP for communication. Therefore, two-tone suppression is a conservative feature of the anuran auditory periphery. Anurans possess this trait because they have inherited a particular type of auditory system, not because they evolved it as an adaptation for communication, although, as Capranica demonstrated, it is used for communication purposes in bullfrogs.

Considering other parts of the amphibian auditory system gradually leads to a sophisticated appreciation of biobehavioral evolution. The basilar papilla, for example, is a peripheral auditory organ whose tuning is species-specific in anurans and nearly always close to a major spectral peak in the communication signal. Yet it is also present in most urodeles and all gymnophions, who, as far as we know, use no acoustic communication signals. The *presence* of the BP is thus a general feature of amphibians, but its *properties* appear to adapt to serve acoustic communication where it is present.

Similar consideration can be given to characteristics of central auditory processing. Rose, Brenowitz, and Capranica have studied the coding of amplitude modulation (AM) in the frog midbrain, where they have found neurons tuned to different modulation rates (reviewed in Capranica and Rose, 1983). The distribution of temporally tuned units varies among species coincident with variations in AM rates in the advertisement call, and their properties shift with temperature just as the call and the behavioral responses to it shift with temperature. Are temporally tuned neurons adaptations for communicating with an amplitude modulated call? Probably not. Rose (1986) has argued that the type of tuning present in frogs is a basic feature of vertebrate auditory systems. Thus, once again, amphibians provide a model for investigating a fundamental feature of vertebrate auditory systems and an example of how evolutionary processes shift the properties of the system within that basic framework. Fuzessery (this volume) raises a similar question concerning the nonlinear summation of AP and BP inputs in the thalamus. This summation suggests specializations for call detection. But is it common to all anurans, and if so do the properties shift in predictable ways? Fuzessery suggests using the natural diversity of frogs to explore this problem.

Anuran auditory diversity might eventually also provide examples of the adaptive significance of anatomical variation in the central nervous system. Neary (this volume) describes an unexpectedly heavy interconnection between the auditory system and the hypothalamus in bullfrogs. Is this an anuran adaptation for the use of acoustic communication in reproductive behavior? A comparison of these interconnections among select anurans and between anurans and the mute urodeles and gymnophions could very well yield something that has rarely been accomplished: an ethological interpretation of neuroanatomical variation.

The idea that characters shift within a preset framework during evolution is an interesting one because it implies that while characters can change, they are constrained as well. The idea of constraints on evolutionary change is currently receiving intense scrutiny. Anuran audition has provided a model to investigate this problem, in terms of both neural changes coinciding with call evolution and the more basic problem of call evolution itself (Ryan, this volume).

B. Mechanisms Controlling Call Diversity

A more fundamental question than how differences in neural systems reflect differences in call structure is why the differences exist at all. That is, what are the evolutionary mechanisms responsible for call diversity within a group of animals? Using the considerable diversity one finds among anuran calls as raw material for study, and taking advantage of the ability to perform rigorous behavioral studies on these vertebrates, one can potentially elicit the basic evolutionary principles underlying the evolution of, and the constraints on, character diversity.

1. *Factors Enhancing Call Diversity*

One factor responsible for differences among species-specific calls may be habitat selection. That is, have environmental characteristics selected for call parameters that maximize transmission or fidelity? The adaptation of a communication signal to the habitat in which it is used is often assumed, but rarely tested. The stereotypic nature of each anuran species' call, and the ability to discern the call parameters necessary for recognition eases the formulation and interpretation of environmental acoustics experiments to test this conjecture.

Similarly, the ready response of females to natural and synthetic calls and the relative ease of measuring reproductive success in anurans allows rigorous tests of sexual selection on the species-specific call. These same considerations make possible an assessment of a third, highly controversial, proposed mechanism for generating diversity: character displacement. This concept posits that differences between species in characters used for mate recognition (the advertisement call in this case) become exaggerated when the species can interbreed but the resulting hybrids are at a selective disadvantage. To date, the only rigorous tests of this phenomenon have used anurans as model systems (Littlejohn, 1981; Nevo and Capranica, 1985).

2. *Constraints on Diversity*

While several evolutionary mechanisms can lead to diversity among communication signals, it is becoming apparent that many factors can also constrain the evolution of a communication system. For example, the characteristics of the peripheral receptor system may constrain the possible acoustic cues used during communication (Zakon and Wilczynski, this volume). Energetic or mechanical constraints can limit features of call production and thus indirectly impact on auditory evolution. The influence of these and other factors are discussed at length by Ryan (this volume).

Ryan adds the observation that, at a higher level of analysis, the properties of the auditory system may actually constrain speciation among anurans. It is startling to contemplate that, all things considered, the auditory system, through its use in reproductive behavior, could be both a significant driving force and a major constraint on the evolution of this group of vertebrates.

C. Urodeles and Gymnophions

These two orders offer an interesting and important comparison with anurans precisely because they do not use their auditory system for acoustic communication. They are vertebrates that share a recent common ancestry with anurans (Duellman, this volume) but that have auditory systems uninfluenced by the natural and sexual selective pressures associated with using audition for communication and reproductive behavior. Thorough comparisons could provide insights into the way such selective pressures manipulate the neural substrates of perception and behavior.

Although the type of anatomical and physiological comparisons among the amphibian orders necessary for such insights have not been done, morphological studies of the periphery and part of the central nervous system have been undertaken to determine the evolution of amphibian audition. These studies have been important in generating an understanding of the general patterns of vertebrate auditory system evolution and the principles behind this evolution (Lombard and Bolt, this volume; Will and Fritzsch, this volume; Fritzsch, this volume; McCormick, this volume).

V. DISCUSSION

Given the characteristics of the amphibian auditory system documented in this book, one must be careful how the system is used as a model. In some ways, it can be used as a strict model, or substitute, for other vertebrate auditory systems. Indeed, it has been used that way in explorations of the basic processes of auditory transduction (Lewis and Lombard, this volume) and development (Zakon, this volume). However, one should not lose sight of the fact that the amphibian auditory system is unique in many ways. Its peripheral apparatus may have evolved independently from that of other vertebrates (Lombard and Bolt, this volume) and it must contend with the phenomena of metamorphosis and growth (Heatherington, this volume; Fritzsch, et al., this volume; Shofner, this volume). It may be very different from other vertebrates in some physiological (Eggermont, this volume) and anatomical (Neary, this volume; Wilczynski, this volume) features. Moreover, the diversity one sees anatomically (Jaslow et al., this volume; Will, this volume; Will and Fritzsch, this volume) and behaviorally (Rand, this volume; Wells, this volume) may often make it difficult to use one amphibian species as a model for another.

The real value of the amphibian auditory system's role as a model comes from the fact that it is above all an information processing system. It is in this sense that its use was described above. Surveying patterns of diversity one might ask what changes occur when an information processing system adopts a communication function. Once communication becomes a premier function, how do evolutionary mechanisms manipulate the system and the signal it receives (Ryan, this volume)? How does it encode complex signals (Zakon and Wilczynski, this volume; Fuzessery, this volume; Walkowiak, this volume)? How does it reliably separate them from environmental noise (Narins and Zelick, this volume) and code their location (Eggermont, this volume; Rheinlaender and Klump, this volume)? How is the system interfaced with neural networks for the behaviors it must direct (Neary, this volume; Schneider, this volume)?

These are questions whose answers span many areas of biology and impact on fundamental questions in each. This is the true role of a good model system. It gives us more than a substitute for another, less convenient, preparation. It gives us a lens through which to observe questions basic to many systems and allows us to probe for answers at many different levels. The anuran auditory system is such a model system. Its use spans neural, behavioral, and evolutionary biology, and it promises to contribute greatly to the understanding of them all.

VI. SUMMARY

The diversity in habitat, call structure, and reproductive strategy among amphibian species makes the amphibian auditory system especially good for integrative tests of neural, behavioral, and evolutionary questions, while the advantages of amphibians as experimental animals make these integrative studies tractable. Acoustic communication in anurans provides a model system for looking at the way in which the auditory system changes to match the diversity of calls and the way in which both the auditory and vocal control systems might constrain the evolutionary mechanisms that ultimately generate call diversity. Comparisons of anurans and the other amphibian orders could provide information on the evolution of terrestrial hearing and how basic neural patterns can change in response to selective pressures associated with communication. The amphibian auditory system is therefore an important model system for uncovering basic information about vertebrate hearing and for exploring theoretical problems in many areas of biological science.

REFERENCES

Blair, W. F. (1964) Isolating mechanisms and interspecies interactions in anuran amphibians. *Quart. Rev. Biol.* **39,** 334–344.

Capranica, R. R., and Moffat, A. J. M. (1983) Neurobehavioral correlates of sound communication in anurans. In *Advances in Vertebrate Neuroethology* (Ewert, J.-P., Capranica, R. R., and Ingle, D., eds.), pp. 701–730, Plenum Press, New York.

Capranica, R. R., and Rose, G. (1983) Frequency and temporal processing in the auditory systems of anurans. In *Neuroethology and Behavioral Physiology* (Huber, F., and Markl, H., eds.), pp. 136–152, Springer-Verlag, Berlin, Heidleberg, and New York.

Constantine-Paton, M., and Capranica, R. R. (1975) Central projection of optic tract from translocated eyes in the leopard frog (*Rana pipiens*). *Science* **189,** 480–482.

Hudspeth, A. J. (1985) The cellular basis of hearing. *Science* **230,** 745–752.

Kelley, D. B. (1986) Neuroeffectors for vocalization in *Xenopus laevis:* Hormonal regulation of sexual dimorphism. *J. Neurobiol.* **17,** 231–248.

Littlejohn, M. J. (1981) Reproductive isolation: a critical review. In *Evolution and Speciation, Essays in Honor of M. J. D. White* (Atchley, W. R., and Woodruff, D. S., eds.), pp. 298–334, Cambridge University Press, Cambridge.

Nevo, E., and Capranica, R. R. (1985) Evolutionary origin of ethological isolation in cricket frogs, *Acris. Evol. Biol.* **19,** 147–214.

Rose, G. (1986) A temporal processing mechanism for all species? *Brain Behav. Evol.,* **28,** 134–144.

Ryan, M. R. (1985) *The Tungara Frog, A Study in Sexual Selection and Communication.* University of Chicago Press, Chicago.

Zakon, H. H. (1983) Reorganization of connectivity in amphibian central auditory system following VIIIth nerve regeneration: Time course. *J. Neurophysiol.* **49,** 1410–1427.