Springer Handbook of Auditory Research

Bruce A. Carlson
Joseph A. Sisneros
Arthur N. Popper
Richard R. Fay *Editors*

Electroreception: Fundamental Insights from Comparative Approaches

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Ted Bullock (left) and Carl Hopkins, 1984

This volume is dedicated to two of the true pioneers of electroreception research, Theodore Holmes "Ted" Bullock (1915–2005) and Carl D. Hopkins. The scientific ancestry of almost everyone in the world

today working on electroreception in fishes can, in some way, be traced back to Ted, one of the true giants of twentieth-century neuroscience.

Carl was one of Ted's postdocs. Carl, too, has an immensely strong history of scholarly contributions and training of students and postdocs in the areas of electroreception and behavior.

Ted and Carl were editors of the previous SHAR volume on Electroreception (Bullock, T. H., Hopkins, C. D., Popper, A. N., and Fay, R. R., 2005, Springer-Verlag, New York). Both were also champions of using comparative approaches to address fundamental questions about the neural control of behavior. We take great pleasure in dedicating this book to Ted and Carl — great friends and colleagues to all of the editors and mentors to two of the editors.

Acoustical Society of America

The purpose of the Acoustical Society of America (www.acousticalsociety.org) is to generate, disseminate, and promote the knowledge of acoustics. The Acoustical Society of America (ASA) is recognized as the world's premier international scientific society in acoustics, and counts among its more than 7000 members, professionals in the fields of bioacoustics, engineering, architecture, speech, music, oceanography, signal processing, sound and vibration, and noise control.

Since its first meeting in 1929, the ASA has enjoyed a healthy growth in membership and in stature. The present membership of approximately 7000 includes leaders in acoustics in the United States of America and around the world. The ASA has attracted members from various fields related to sound including engineering, physics, oceanography, life sciences, noise and noise control, architectural acoustics; psychological and physiological acoustics; applied acoustics; music and musical instruments; speech communication; ultrasonics, radiation, and scattering; mechanical vibrations and shock; underwater sound; aeroacoustics; macrosonics; acoustical signal processing; bioacoustics; and many more topics.

To assure adequate attention to these separate fields and to new ones that may develop, the Society establishes technical committees and technical groups charged with keeping abreast of developments and needs of the membership in their specialized fields. This diversity and the opportunity it provides for interchange of knowledge and points of view has become one of the strengths of the Society.

The ASA's publishing program has historically included The *Journal of the Acoustical Society of America, JASA-Express Letters, Proceedings of Meetings on Acoustics*, the magazine *Acoustics Today*, and various books authored by its members across the many topical areas of acoustics. In addition, ASA members are involved in the development of acoustical standards concerned with terminology, measurement procedures, and criteria for determining the effects of noise and vibration.

Series Preface

Springer Handbook of Auditory Research

The following preface is the one that we published in volume 1 of the Springer Handbook of Auditory Research back in 1992. As anyone reading the original preface, or the many users of the series, will note, we have far exceeded our original expectation of eight volumes. Indeed, with books published to date and those in the pipeline, we are now set for over 80 volumes in SHAR, and we are still open to new and exciting ideas for additional books.

We are very proud that there seems to be consensus, at least among our friends and colleagues, that SHAR has become an important and influential part of the auditory literature. While we have worked hard to develop and maintain the quality and value of SHAR, the real value of the books is very much because of the numerous authors who have given their time to write outstanding chapters and to our many co-editors who have provided the intellectual leadership to the individual volumes. We have worked with a remarkable and wonderful group of people, many of whom have become great personal friends of both of us. We also continue to work with a spectacular group of editors at Springer. Indeed, several of our past editors have moved on in the publishing world to become senior executives. To our delight, this includes the current president of Springer US, Dr. William Curtis.

But the truth is that the series would and could not be possible without the support of our families, and we want to take this opportunity to dedicate all of the SHAR books, past and future, to them. Our wives, Catherine Fay and Helen Popper, and our children, Michelle Popper Levit, Melissa Popper Levinsohn, Christian Fay, and Amanda Fay Sierra, have been immensely patient as we developed and worked on this series. We thank them and state, without doubt, that this series could not have happened without them. We also dedicate the future of SHAR to our next generation of (potential) auditory researchers – our grandchildren – Ethan and Sophie Levinsohn, Emma Levit, Nathaniel, Evan, and Stella Fay, and Sebastian Sierra.

Preface 1992

The Springer Handbook of Auditory Research presents a series of comprehensive and synthetic reviews of the fundamental topics in modern auditory research. The volumes are aimed at all individuals with interests in hearing research including advanced graduate students, post-doctoral researchers, and clinical investigators. The volumes are intended to introduce new investigators to important aspects of hearing science and to help established investigators to better understand the fundamental theories and data in fields of hearing that they may not normally follow closely.

Each volume presents a particular topic comprehensively, and each serves as a synthetic overview and guide to the literature. As such, the chapters present neither exhaustive data reviews nor original research that has not yet appeared in peer-reviewed journals. The volumes focus on topics that have developed a solid data and conceptual foundation rather than on those for which a literature is only beginning to develop. New research areas will be covered on a timely basis in the series as they begin to mature.

Each volume in the series consists of a few substantial chapters on a particular topic. In some cases, the topics will be ones of traditional interest for which there is a substantial body of data and theory, such as auditory neuroanatomy (Vol. 1) and neurophysiology (Vol. 2). Other volumes in the series deal with topics that have begun to mature more recently, such as development, plasticity, and computational models of neural processing. In many cases, the series editors are joined by a co-editor having special expertise in the topic of the volume.

Richard R. Fay, Chicago, IL, USA
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Volume Preface

A fundamental goal of neuroscience is to understand how the nervous system extracts biologically relevant information from the natural environment and how it uses that information to guide and coordinate behavior necessary for reproduction and survival. The electrosensory systems of weakly electric teleost fishes and those of nonteleost fishes are attractive systems for addressing basic questions about neuronal information processing and its relationship to natural behavior. Comparative approaches in these fishes have led to the identification of fundamental mechanisms that have shaped the adaptive evolution of sensory systems across animal taxa. Understanding how sensory systems encode and integrate information about the natural world has far reaching implications for advancing our knowledge in the basic biomedical sciences and in understanding how the nervous system has evolved to control behavior.

The primary goal of this book is to provide a comparative perspective on the topic of electroreception and review some of the fundamental insights gained from studies of electrosensory and electromotor systems. Although totally independent, this book follows from volume 21 in the Springer Handbook of Auditory Research series, *Electroreception* (Bullock, T. H., Hopkins, C. D., Popper, A. N., and Fay, R. R., 2005, Springer-Verlag, New York).

This volume begins with a brief history of electrogenesis and electroreception in fishes in Chap. 1 by Bruce Carlson and Joseph Sisneros. The chapter highlights how neuroethological studies of electric fish have contributed to our greater understanding of the neural mechanisms that are required to extract behaviorally relevant information. In Chap. 2, Clare Baker reviews the evolutionary and developmental origins of nonteleost lateral line electroreceptors with insights from comparative molecular approaches. Duncan Leitch and David Julius in Chap. 3 provide an exciting overview of electrosensory transduction, with comparisons across structure, afferent response, and cellular physiology that includes recent findings on the molecular mechanisms of electrosensory transduction.

Chapter 4 by Jason Gallant examines recent developments in our understanding of the evolutionary and embryological origins of electric organs. Michael Markham in Chap. 5 addresses the morphological and physiological basis for the generation

of electric organ discharges (EODs) and how specific features of the EOD waveform are generated. In Chap. 6, Ana Silva reviews how hormones affect the social behavior of South American weakly electric fishes through hormonal actions on electrosensory and electromotor systems. The subject of Chap. 7 by Rüdiger Krahe focuses on the ultimate evolutionary causes of EOD diversification in gymnotiforms and mormyrids, including ecological adaption, sexual selection, predation, and drift.

Chapter 8 by Sarah Stamper, Manu Madhav, Noah Cowan, and Eric Fortune highlights the use of control theory to reveal functional relationships between active sensing, task-related behaviors, sensing, and motor control, with a discussion of recently developed experimental systems that use artificially controlled feedback loops to perturb natural reafferent feedback in freely behaving animals. Then, Michael Metzen and Maurice Chacron (Chap. 9) review recent advances in the coding and processing of envelopes in the electrosensory system of gymnotiform fishes and how research on these fishes relates to fundamental insights into how envelopes are coded and processed in the mammalian auditory and other systems. This is followed by Chap. 10, in which Bruce Carlson discusses common themes and key differences in temporal coding across electrosensory and auditory systems and highlights how the comparative approach can uncover shared fundamental mechanisms and at the same time reveal the ultimate causes for differences between systems.

In Chap. 11, Krista Perks and Nathaniel Sawtell discuss how motor systems and behavior can influence the electrosensory processing of reafferent sensory input with implications for other sensory systems and brain structures, including the mammalian auditory system and the cerebellum. Then in Chap. 12, Sarah Nicola Jung and Jacob Engelmann provide an overview of the emerging research on spatial learning in weakly electric fish, with a discussion of the mechanisms by which active electrolocation can provide spatial information and its neural basis in the dorsal telencephalon.

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Chapter 1
A Brief History of Electrogenesis and Electroreception in Fishes

Bruce A. Carlson and Joseph A. Sisneros

Abstract The primary goal of this volume is to provide an updated perspective on the topics of electrogenesis and electroreception in fishes. Throughout, there is an emphasis on how comparative perspectives can inform general issues regarding the neural mechanisms of behavior, from detailed comparisons among related species having divergent phenotypes to broad comparisons across distantly related clades having similar phenotypes. The underlying theme throughout is that evolution provides a natural experiment that can be exploited to relate variation in behavior to variation in its neural substrates. This allows for the development and testing of hypotheses regarding the neural control of behavior and for distinguishing generally applicable principles from clade-specific differences. The chapters cover a range of topics including the evolution and development of electric organs and electroreceptors, electrosensory transduction, evolutionary drivers and biophysical bases of electric signal diversity, influences of hormones and motor systems on electrosensory processing, envelope and temporal coding, use of control theory to characterize active sensing, and the role of active electrolocation and spatial learning in behavior. In this introductory chapter, a brief history of research on electrogenesis and electroreception in fishes is presented, with a summary of some of the most important neuroethological studies in electric fish that have contributed greatly to our understanding of brain function and the neural basis of behavior. The field of electroreception research continues to provide fertile ground for using comparative frameworks to understand the neurobiology of animal communication, social behavior, orientation and navigation, and the evolution of information processing.

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Keywords Active electrolocation · Corollary discharge · Electric organ · Electric organ discharge · Electrocommunication · Electromotor · Electroreceptor · Electrosensory · Jamming avoidance response · Neuroethology · Passive electrolocation · Reafference

1.1 Introduction

It has now been over 14 years since the publication of *Electroreception* by Bullock et al. (2005). That volume provided a wide-ranging review of general topics in the field, such as electrosensory anatomy and physiology, plasticity in electrosensory systems, electrosensory-mediated behavior, electromotor control, evolution and diversity of electric fishes, and broad comparisons between the electrosensory system and other octavolateralis systems. Rather than updating the previous volume by providing a comprehensive general review, this new volume, *Electroreception: Fundamental Insights from Comparative Approaches*, narrows in on specific research questions that span more than one of these various subfields. Thus, this volume should be viewed as complementary to *Electroreception* (Bullock et al. 2005). Indeed, readers are encouraged to use the earlier volume as a general reference when diving into topics explored in the current volume, although all the chapters in the current volume have been written to stand on their own so that readers can choose how much they want to explore.

The research topics chosen have a long and distinguished history in the field, but they are also areas of active research in which new discoveries continue to accrue. The editors invited reviews from leading authorities to review some of the fundamental insights gained from studies of electrosensory and electromotor systems while paying particular attention to broadly relevant insights that have come about through a detailed focus on particular neural circuits, broad comparative approaches across species, or some combination of the two. A major goal of this approach is to provide a comparative and integrative perspective that illustrates how intensive research into specific topics in the field has informed important general questions in neuroscience. This chapter starts with a brief historical overview of the discovery of electrogenesis and electroreception in fishes (see Sects. 1.2 to 1.4). This is followed by highlighting major areas in which research on electric fishes has contributed to understanding the neural basis of animal behavior (see Sect. 1.5), culminating in an overview of the various chapters within this volume (see Sect. 1.6). This chapter closes by highlighting future directions and how comparative approaches to the investigation of sensory and motor systems may continue to reveal evolutionarily conserved solutions to fundamental problems in neuroscience (see Sect. 1.7).

1.2 Early Fascination with Electric Fishes

The earliest evidence of a human fascination with electric fishes dates back more than 5000 years ago to ancient Egypt (Moller 1995b; Finger and Piccolino 2011). Some of the earliest recorded paintings of electric fish can be found on Egyptian tombstones. On the tomb of Ti in Saqqara, Egypt, there is a limestone bas-relief painting known as *Ti Watching a Hippopotamus Hunt* that depicts the "electric catfish of the Nile" or *Malapterurus electricus* in a hunting scene with a number of other local fish species (Fig. 1.1). The electric catfish was often associated with the Egyptian gods Aker and Ra because these fish were common in dark, muddy waters, and it was believed they could navigate in the dark and thus assist the earth god Aker, protector and border guardian of earth's horizon, by helping guide the sun god Ra on his nightly journey into the dark netherworld. Of course, the painful, numbing sensations that resulted from handling these fish most likely contributed to their mythical status.

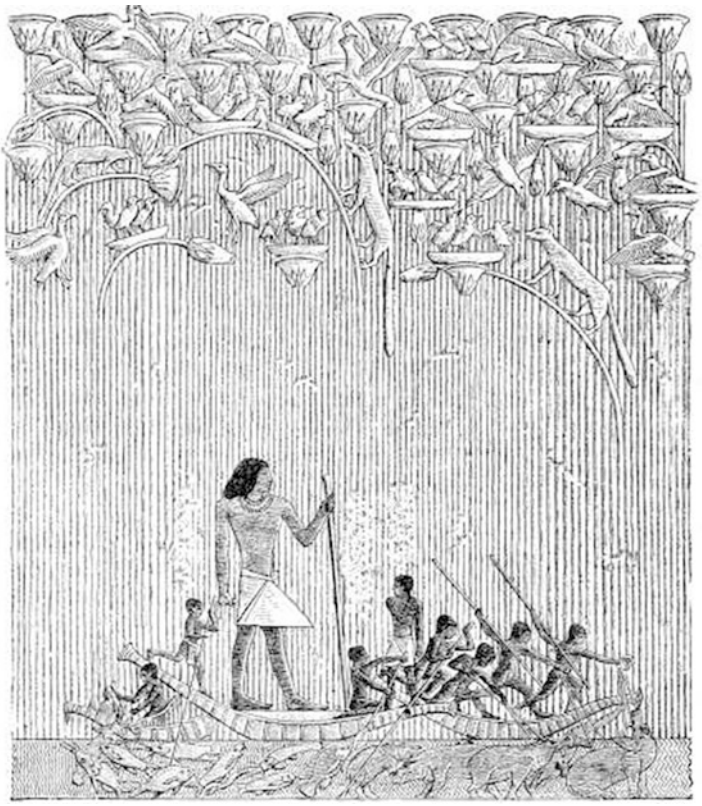

Fig. 1.1 A limestone bas-relief painting known as *Ti Watching a Hippopotamus Hunt* on the tomb of Ti in Saqqara, Egypt (c. 2400 BC). In this hippopotamus hunting scene in the marshes, the "electric catfish of the Nile" or *Malapterurus electricus* can be seen underneath the boat (*left*) with other local fish species

The Greeks and Romans were also familiar with electric fishes and the power of their strong electrical shocks, especially those of the electric torpedo ray (*Torpedo torpedo*). In the zoological treatise *Historia animalium*, Aristotle (374–322 BC; 1965) described how the electric torpedo ray captures prey by "…causing numbness in whatever small fishes it intends to overcome, catching them by the means which it possesses in its body, feeds on them; it hides itself in the sand and mud, and catches all the fish that swim towards it and become numbed as they are carried near." In another passage in *Historia animalium*, Aristotle mentions that torpedo rays can also cause numbness in humans. The Greek term for electric torpedo ray can be transliterated into "nárkē," whereas the Roman equivalent is "torporific." Both terms are based on the torpedo ray's ability to cause numbness. Several modern words have been derived from nárkē, including "narcotic," "narcotize," and "narcosis." The benumbing powers of the torpedo ray's electrical shocks were used in medicine during Greco-Roman times as a form of "electrotherapy" to treat pain and a variety of ailments, including gout and headaches (Finger and Piccolino 2011). During this time period, people could only speculate about the underlying source for the unusual power by which these fish produced such numbing shocks. The true physical basis of the fish's discharge would not be known until 2000 years later with the discovery of the force we now call electricity.

1.3 Discovery of Electrogenesis

By the early eighteenth century, the leading hypotheses as to the cause of the torpedo ray's powerful shocks were based on mechanical forces. The Italian scientist Stefano Lorenzini (1645–1725) first proposed that torpedo rays were capable of producing sudden contractions of specialized muscles known as *musculi falcati*. Lorenzini (1678) maintained that these violent contracting falciform muscles could produce a quick and explosive release of minute corpuscles that would then penetrate the receiver's nerves and block their function causing numbness. A similar hypothesis was put forth by the French scientist René-Antoine Ferchault de Réaumur (1683–1757), but he contended that the violent contractions of the *musculi falcati* alone could affect the nerves and muscles directly, causing numbness without the involvement of corpuscular emissions.

The case for animal electricity as the mechanism for the numbing effects produced by strongly electric fish was first put forth by the New Englander Edward Bancroft (1744–1820; Fig. 1.2), a physician, natural philosopher, and later fellow of the Royal Society of London. During the 1760s, Bancroft practiced medicine in Guiana where he had the opportunity to study *Electrophorus electricus* (known at that time as *Gymnotus electricus*), a fish feared by local natives. Bancroft referred to these fish as "torporific eels." Bancroft (1769) became convinced that "the shock of the Torporific Eel is not the immediate effect of muscular motion" but instead "is produced by an emission of torporific, or electric particles." Bancroft maintained that when a torporific eel is touched by a handheld rod while the other hand is joined

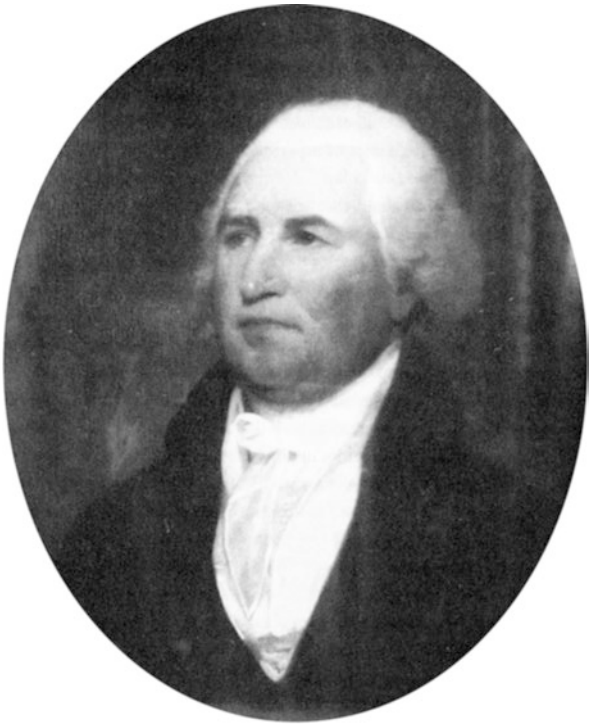

Fig. 1.2 Edward Nathaniel Bancroft (1744–1821) was an English physician, zoologist, botanist, and later a secret double agent during the American War of Independence. In 1763, Bancroft traveled to Dutch Guiana to practice medicine and would later write *An Essay on the Natural History of Guiana in South America* that includes details of his encounters with "torporific eels" (Bancroft 1769). After his return to London in 1771, Bancroft became a well-known authority on electric fishes

to another person, the eel can "communicate a shock perfectly resembling that of electricity, which is commonly so violent, that but a few are willing to suffer it a second time." In addition, Bancroft also observed when a person holds his finger in the water at a distance of two to three meters away from the eel and a discharge is elicited, the person at a distance can still receive a violent shock (Finger and Piccolino 2011). Thus, Bancroft's observations and experimental findings provided strong evidence as to the electrical nature of the torporific eel's shocks, and it is now typically referred to as the electric eel.

The first detailed analysis of the discharges from an electric fish was perhaps performed by John Walsh (1726–1795), a fellow of the Royal Society of London and member of the English Parliament. After his election into the Royal Society, Walsh was encouraged by Benjamin Franklin (1706–1790) to devote his scientific energies into studying torpedo rays and to specifically test the hypothesis that the torpedo ray's discharges were electrical in nature. Walsh traveled to La Rochelle and the Isle de Ré in France where he performed a number of experiments on torpedo rays. He focused on whether the shocks of torpedo rays could be transmitted from person to person in a long human chain similar to what could be elicited by a Leyden jar, an early form of electrical capacitor that consisted of a glass jar with metal foil layers on the inside and outside. Walsh discovered that the discharges of torpedo rays could be conveyed over distances up to 12 meters with metal wires (Finger and Piccolino 2011). He also noted that the shocks could not be conveyed by nonconductors such as glass or sealing wax. In addition, Walsh and his research

team failed to detect any muscle movements from the torpedo rays before or during shocks that Réaumur claimed were fundamental for the shocks to be felt.

Following his experiments, Walsh concluded that the torpedo ray's discharges had to be electrical by a natural force he called "torpedinal electricity." Based on his own dissections and those later described by the English surgeon and anatomist John Hunter (1728–1793) that detailed the electric organ anatomy of Walsh's French torpedo rays (Hunter 1773), Walsh became more convinced that the torpedo ray's "animal electricity" was associated with the "honeycomb"-like structures found under the skin on the torpedo ray's disk, which he began to refer to as "electric organs," a term still used today.

Perhaps the most convincing demonstration of the electrical nature of the shocks produced by strongly electric fish was Walsh's demonstration in 1773 that the electric eel could produce visible sparks under the right conditions (Finger and Piccolino 2011). During the nineteenth century, no other electric fish captured the public's imagination more than the electric eel. The allure of this fish was, in part, made famous by the German explorer and naturalist Alexander von Humboldt (1769–1859; Fig. 1.3), who detailed his encounters with electric eels in South America in

Fig. 1.3 Alexander von Humboldt (1769–1859) was a German geographer, explorer, and naturalist. As a celebrated explorer, he detailed his dangerous travels and scientific explorations in the New World from 1799 to 1804 in many illustrated volumes of his writings

one of the many illustrated volumes of his writings that vividly highlighted his dangerous travels and scientific explorations in the New World (see Finger and Piccolino 2011). One of von Humboldt's more famous and fantastic accounts describes how South American Chayma natives used horses to collect electric eels (Fig. 1.4). This unusual collection method resulted in an epic battle between eels and horses that left the electric eels exhausted and "electrically spent," which then allowed the Chayma natives to safely collect live specimens for von Humboldt to study. In his accounts, von Humboldt (1807) described a self-defensive behavior in which electric eels leaped out of the water and pressed their chins against the horses to directly electrify them.

A study performed over 200 years later provided support for this legendary account, revealing that eels naturally leap out of the water to attack perceived threats and that this acts to increase the electrical current delivered to the target and effectively activate the target's nociceptors (Catania 2016). Indeed, studies have revealed that the electromotor behavior of electric eels is far more sophisticated than previously appreciated, involving remote control and immobilization of potential prey (Catania 2014), concentrating electric fields on challenging prey items (Catania

Fig. 1.4 The epic battle between eels and horses was vividly described by Alexander von Humboldt (1807) . It shows the use of horses by local Chayma natives to collect electric eels. This unusual collection technique involved horsemen driving a herd of about 30 wild horses into a stagnant pool of electric eels that resulted in the terrifying deaths of two horses in the first few minutes as the electric eels vigorously defended themselves by repeatedly discharging their electric organs. The Chayma natives kept the horses from exiting the pool by waving branches and reeds to force them back into the water. Eventually, the remaining horses stumbled out of the pool with their manes erect and panting in anguish while the electric eels were equally exhausted and "electrically spent." After the battle, the Chayma natives safely collected five live specimens for von Humboldt to study

2015a), and using their strong electric fields to actively track the location of their target prey (Catania 2015b).

By the latter half of the nineteenth century, other researchers, including the Scottish surgeon James Stark (1811–1890), began to discover the presence of apparent electric organs in other fishes besides the strongly electric catfish, torpedo ray, and electric eel. Stark (1844) discovered that the flapper skate (*Raja batis*) possessed bilateral organs in the tail that were similar in structure to those of torpedo rays and electric eels. The presence of similar organs was soon found in a number of other fishes, including the unusual-looking African elephant fishes in the family Mormyridae and the South American knifefishes in the order Gymnotiformes. However, at the time, no researcher was able to successfully detect electric discharges produced by these organs. Thus, the mormyrids and gymnotiforms were thought to be "pseudoelectric" or "imperfectly electric," as referred to by the German physiologist Emil du Bois-Reymond (1818–1896). Hence, the term "pseudoelectric organ" became used to reference electric organs in fish that were incapable of producing perceptible discharges.

The presence of such pseudoelectric organs in mormyrids and gymnotiforms were thought to represent an incomplete stage of electric organ evolution. This posed a serious problem for Charles Darwin's theory of natural selection, one of several that he addressed in a chapter of his landmark *On the Origin of Species* entitled "Difficulties of the Theory" (1859, p. 150):

> "The electric organs of fishes offer another case of special difficulty; for it is impossible to conceive by what steps these wondrous organs have been produced. But this is not surprising, for we do not even know of what use they are. In the gymnotus and torpedo they no doubt serve as powerful means of defence, and perhaps for securing prey; yet in the ray… an analogous organ in the tail manifests but little electricity, even when the animal is greatly irritated; so little that it can hardly be of any use for the above purposes."

The problem for Darwin's theory was that strongly electric organs must have evolved from muscle by first passing through an intermediate stage of weakly electric organs, and these weakly electric organs must have performed some adaptive function to have evolved in the first place. The true nature of these so-called pseudoelectric organs and the solution to Darwin's conundrum would not be understood until the next century when electrical recording equipment became available and the first electric organ discharges (EODs) of electric fish were recorded and characterized.

1.4 Discovery of Electroreception

Research on weakly electric fishes can be traced to the mid-twentieth century due to both technological advancements in the amplification and visualization of electrical signals (reviewed in Moller 1995b) and a series of elegant studies by the British zoologist Hans Lissmann (1909–1995) at the University of Cambridge, UK. Lissmann (1951) first showed that the African knifefish *Gymnarchus niloticus* (monotypic sister taxon to the Mormyridae, which together make up the

Mormyroidea or mormyroids) produced continuous weak, wave-like EODs at frequencies of about 250–300 Hz that originated from the tail, where anatomists had previously described an electric organ structure. Lissmann also noted that when the animal's own recorded discharges were fed back into the water using electrodes, the fish was able to locate and attack the stimulating electrodes.

Lissmann (1958) would later go on to record pulse-type EODs from several mormyrid species. Based on "bursts of discharges" when pairs of mormyrids were in proximity, he suggested that "the electrical discharges may play a social role in the life of the Mormyridae," in-line with earlier observations by Möhres (1957) at the University of Tübingen, Germany. Both Lissman and Harry Grundfest (1903–1984) at Columbia University, New York, NY, also described weak EODs in several South American gymnotiform species other than the electric eel (Grundfest 1957; Lissman 1958). Finally, Lissmann and his research assistant Kenneth Machin (1924–1988) developed a model based on their detailed measurements and analysis of the bioelectric fields produced by *Gymnarchus niloticus* that could explain a function for the EODs. Based on this model and operant conditioning experiments, Lissmann and Machin (1958) concluded that the weakly electric knifefish could detect changes in the conductance of its own self-generated bioelectric field to locate objects in its environment and distinguish objects of varying conductivity and chemical composition through a mechanism now referred to as "active electrolocation." The results of their behavioral experiments also suggested that these fish must possess some specialized sensory receptor system capable of detecting weak, biologically relevant electric stimuli (see Baker, Chap. 2; Leitch and Julius, Chap. 3).

1.4.1 Detection of Electric Fields

The discovery of an electric sense in weakly electric fishes (Lissman 1958; Lissman and Machin 1958) prompted an immediate search for the electric sense organs that Lissmann would later initially identify as "electric pores" (reviewed by Fritzsch and Moller 1995). These electric pores were first described in detail by Lorenzini (1678) in torpedo rays (*Torpedo* sp.) where he observed pits in the ray's skin that corresponded to the "mouths" of the long canals or "canaliculi" that are characteristic of this class of electroreceptors known as ampullary electroreceptors. In elasmobranch fishes (sharks and rays), these ampullary electroreceptors bear the name of the discoverer and are known as the "ampullae of Lorenzini." In terms of their functional significance, the ampullae of Lorenzini were first thought to be pressure receptors based on behavioral responses of the dogfish (*Mustelus canis*) when pressure was applied to the receptor area (Parker 1909). The ampullae of Lorenzini were later found to be very sensitive to gross step changes in water temperature (Sand 1938) and even sensitive to mechanical stimulation (Murray 1957; Loewenstein 1960), but the applied stimuli used in these studies were not biologically relevant in the animal's natural environment (Bullock and Szabo 1986).

Fig. 1.5 Sven Dijkgraaf (1908–1995) was a Dutch comparative physiologist who was well-known for his work and insights on lateral line function and hearing in fishes, echolocation in bats, and animal sound production. Along with his student Adrianus Kalmijn, Dijkgraf also performed early recordings from primary afferents of ampullary electroreceptors in elasmobranchs. Dijkgraaf was professor and director of comparative physiology at the University of Utrecht, the Netherlands, where he worked for over 26 years

Later, it would be Richard Murray (1960) at the University of Birmingham, UK, who would provide the first experimental evidence that the afferents of the ampullae of Lorenzini were responsive and highly sensitive to weak electric stimuli. Soon after, Sven Dijkgraaf (1908–1995; Fig. 1.5) and his student Adrianus Kalmijn, both at Utrecht University, the Netherlands, characterized the response properties of ampullary afferents in more detail and showed that the afferents of ampullary electroreceptors responded to natural, electrical stimuli at frequencies of 0.1 to 30 Hz, with a sensitivity as low as a few microvolts per centimeter (Dijkgraaf and Kalmijn 1962, 1963).

Later, Kalmijn would be the first to demonstrate a functional role for the ampullae of Lorenzini in sharks and rays and their use in the detection of weak electric fields. In a series of landmark behavioral experiments, he showed that elasmobranch fishes use their ampullae of Lorenzini in passive electroreception to detect and locate buried prey (Kalmijn 1971) and use electric fields for orientation and navigation in their environment (Kalmijn 1978, 1982). Kalmijn (1982) was able to train round stingrays (*Urolophus halleri*) to orient in an electric field as weak as 5 nV/cm and then locate and bite a pair of stimulating dipole electrodes for a food reward. Based on the high electrosensitivity of the ampullae of Lorenzini, Kalmijn (1978) also suggested that elasmobranchs should be able to perceive the weak electric currents induced by the animal swimming through the magnetic field of the earth by a process known as geomagnetic induction, which could be used theoretically by elasmobranchs for compass orientation during migration and navigation. Consistent

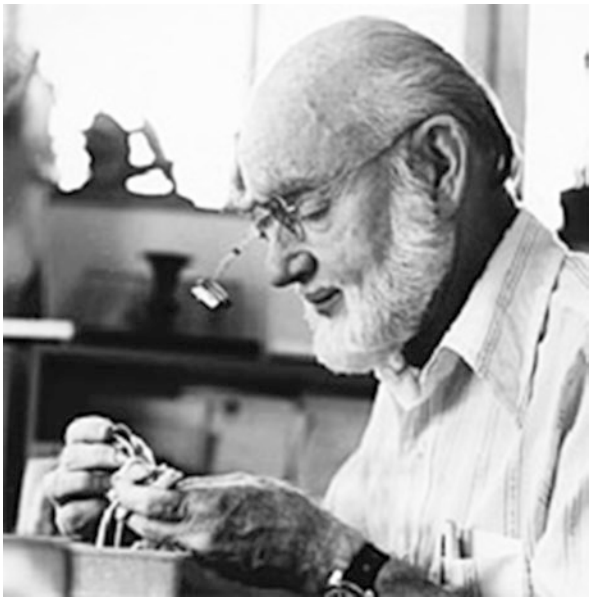

Fig. 1.6 Theodore "Ted" Holmes Bullock (1915–2005) was an American comparative neuroscientist who examined the physiology and evolution of the nervous system across many organizational levels and studied nearly all major groups including coelenterates, annelids, arthropods, echinoderms, mollusks, and chordates. Bullock was a pioneering and influential neuroscientist who championed the comparative approach and is considered to be one of the founding fathers of neuroethology. He spent most of his career at the Scripps Institution of Oceanography in La Jolla, CA, and was elected into the National Academy of Sciences in 1963. Photo from the Scripps Institution of Oceanography, with permission

with his hypothesis, Kalmijn (1982) demonstrated that round stingrays could be conditioned to orient within a magnetic field and thereby locate a specific place based on the magnetic field polarity for a food reward.

Around the same time that the ampullae of Lorenzini were discovered to be electrosensitive, two research groups, one led by Theodore Holmes Bullock (1915–2005; to whom this volume is dedicated; Fig. 1.6) at the University of California, Los Angeles, and the other group led by Alfred Fessard (1900–1982) and Thomas Szabo (1924–1993) at the National Center for Scientific Research, Paris, separately published their physiology studies that detailed the existence of a new class of electroreceptors known as tuberous receptors (Bullock et al. 1961; Fessard and Szabo 1961). The tuberous electroreceptors were identified in gymnotiforms and mormyroids, two groups of fish both capable of generating their own electric fields (Bullock 1982). These tuberous electroreceptors were named for their tuber-like anatomical arrangement in the skin and were found to respond to weak, high-frequency electrical stimuli greater than 50 Hz (Bullock et al. 1961; Fessard and Szabo 1961). The tuberous electroreceptors were later determined to be tuned at or near the frequency of the animal's own EODs and therefore play a critical role in active electroreception

and electrocommunication. Future studies would go on to describe in detail the morphology and physiological response properties of tuberous electrosensory systems in weakly electric fishes (see Metzen and Chacron, Chap. 9; Carlson, Chap. 10; Perks and Sawtell, Chap. 11). Much of our current understanding of information processing in the central electrosensory systems of weakly electric fishes owes its origins to two giants in the field, Curtis Bell at the Oregon Health and Sciences University, Portland, and Leonard Maler at the University of Ottawa, Canada (Bell and Maler 2005).

1.4.2 Generation of Weak Electric Organ Discharges

During this exciting time of research, the electric organs of mormyroids and gymnotiforms were becoming described in better detail. Grundfest and his Columbia University colleague Michael Bennett began to investigate in more detail the structure and function of electric organs in fishes (Bennett and Grundfest 1959, 1961). Bennett (1971) would later go on to propose a comprehensive and detailed model of the physiological and anatomical bases for EOD production by electric organs (see Gallant, Chap. 4; Markham, Chap. 5).

1.5 Electric Fishes and the Neuroethological Approach to Animal Behavior

Neuroethological studies of electric fish have contributed greatly to a basic understanding of brain function by integrating studies of cellular and systems neuroscience, behavior, and evolution (Zakon 2003; Rose 2004; Carlson 2006). This is due, in large part, to several unique experimental advantages. There is a direct 1:1 correspondence between EOD output and the central pattern-generating circuits that generate each EOD (Caputi et al. 2005). In an intact animal, this means that EOD timing provides a direct, noninvasive monitor of the output of the central electromotor system. In an in vivo electrophysiological preparation, paralysis is typically induced by pharmacologically blocking the neuromuscular junction, which also silences the electric organ. Nevertheless, a fictive EOD can easily be recorded from spinal electromotor neurons by placing an electrode near the tail, and this likewise provides a direct 1:1 readout of electromotor output. Many natural behaviors are generated in such a preparation, allowing researchers to monitor, stimulate, or interfere with the activity of individual neurons or specific brain regions during both stimulus presentation and the production of behavior (Hitschfeld et al. 2009).

Although EOD timing is controlled by central circuits, the EOD waveform is determined by the morphological and physiological properties of electrocytes in the electric organ. Here, too, this allows researchers to directly relate EOD waveform to

its underlying neural basis, and this has facilitated studies of species-, sex-, individual-, and dominance-related differences in EOD waveform as well as hormonal modulation of the EOD waveform (see Markham, Chap. 5; Silva, Chap. 6). With the advent of genomic and transcriptomic approaches, such studies have recently extended to the molecular level, linking the EOD waveform to ion channels and other proteins (see Gallant, Chap. 4). Comparative approaches ranging from the molecular to behavioral levels have addressed the roles of natural and sexual selection as well as drift in driving these evolutionary differences (see Krahe, Chap. 7).

On the sensory side, there is likewise a direct correspondence between individual EODs and receptor/primary afferent activation. This allows researchers to precisely manipulate the timing of presynaptic input to central sensory neurons in vivo by simply varying the timing of electrosensory stimuli. The same presynaptic inputs can be stimulated with the same timing using focal electrical stimulation in vitro. In both cases, the stimulation patterns have clear behavioral relevance because they represent patterns of electric signaling by the fish itself (in the case of active electrolocation) or by neighboring fish (in the case of electrocommunication). Thus, numerous studies have bridged in vivo studies of information processing with in vitro studies of synaptic and cellular physiology to gain insight into the processing of behaviorally relevant sensory input (see Metzen and Chacron, Chap. 9; Carlson, Chap. 10; Perks and Sawtell, Chap. 11). Recently, evolutionary developmental and electrophysiological studies have helped to elucidate the cellular and molecular basis of electrosensory transduction (see Baker, Chap. 2; Leitch and Julius, Chap. 3).

1.5.1 Active Electrolocation

The discovery of electroreception and its use in active electrolocation as first described by Lissmann and Machin (1958) provided an opportunity for a new generation of neuroethologists to examine this form of autocommunication in weakly electric fish. Autocommunication, in which the same individual is both sender and receiver, is also found in echolocating animals such as bats and dolphins (Griffin 1958). In this case, information about the surrounding environment is obtained by monitoring modulations (or in the case of echolocation, acoustic reflections) of their self-generated signals. During active electrolocation, the fish responds to changes in the local electrical impedance of its self-generated bioelectric field that enables it to "see" objects in the near field as changes in the intensity and waveform of electric signals across electroreceptors distributed throughout the body surface (von der Emde 1999). Objects with impedances that differ from the impedance of the surrounding water will cast electric "shadows" or "bright spots" on the electroreceptive surface, and the two-dimensional electric image of that object across the receptor array will depend on the object's electrical properties, shape, size, and distance from the fish. Although active electrolocation is effective for object detection and

discrimination, the effective range of this active sensing system is limited to about one to two body lengths from the fish (von der Emde 1999; Nelson 2005). Active electrolocation is also important to help fish maintain their body posture relative to the substrate and to control their distance to objects in the environment. In Chap. 8, Stamper, Madhav, Cowan, and Fortune use control theory to characterize active electrosensing behavior. In Chap. 12, Jung and Engelmann review the current research that focuses on the role of active electrolocation during spatial learning and how weakly electric fish may form spatial memories using their electric sense to aid in navigation in the natural environment.

1.5.2 Jamming Avoidance Response

In wave-type weakly electric fishes, the presence of a nearby fish with a similar EOD frequency can result in interference with their active electrolocation system. Both African and South American wave-type fishes have evolved a jamming avoidance response (JAR) to mitigate this interference (Bullock et al. 1975). The JAR was first discovered in the gymnotiform glass knifefish, *Eigenmannia* sp., by Akira Watanabe and Kimihisa Takeda (1963), both at the Tokyo Medical and Dental University, Japan. Later, Bullock and his colleagues (1972) at the Scripps Institution of Oceanography, La Jolla, CA, would describe the behavioral response in more detail and name the behavior the "jamming avoidance response."

The JAR and its underlying neural basis soon became the major research focus of Bullock's postdoc Walter Heiligenberg (1938–1994; Fig. 1.7). Over the course of his career at the Scripps Research Institute, La Jolla, CA, Heiligenberg and his colleagues helped establish the JAR as one of the most iconic neuroethological case studies (as described in Heiligenberg 1991). The neural circuity underlying the JAR has been studied in exquisite detail in the gymnotiform *Eigenmannia virescens*, and to date, it remains the only nonreflex vertebrate behavior for which the neural basis has been described in detail, all the way from sensory receptors that encode the relevant sensory information to motor effectors that drive the change in behavior. In Chap. 9, Metzen and Chacron review the JAR and expand on the more general roles of EOD modulations (i.e., envelopes) in electrosensory-mediated behavior. In Chap. 10, Carlson describes in detail how both African and South American wave-type fish detect the small phase modulations that are crucial for accurate performance of the JAR. Work led by Masashi Kawasaki at the University of Virginia, Charlottesville, has shown, remarkably, that the independently evolved JARs of African and South American electric fishes rely on the exact same computational algorithm but quite different neural circuitry to perform these computations (Kawasaki 1993, 2009).

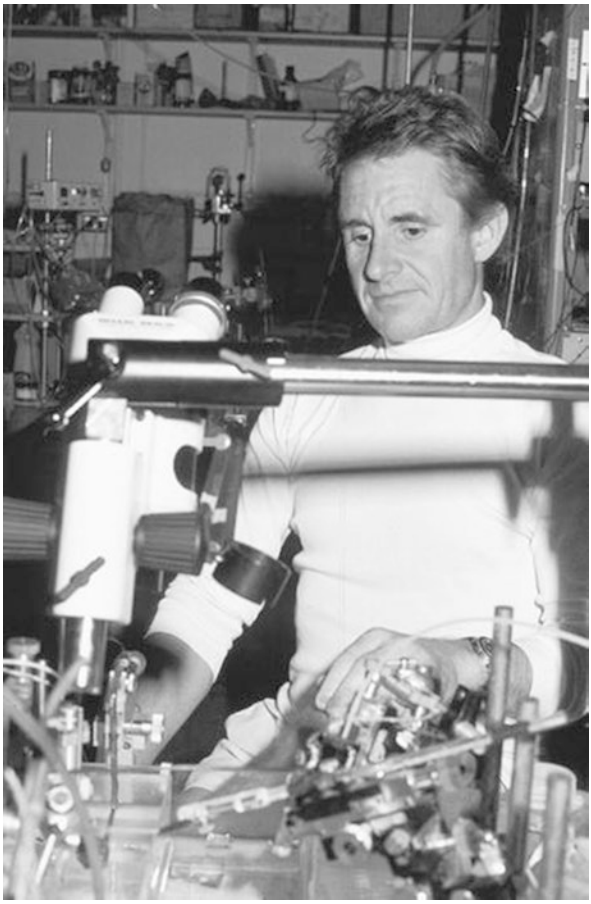

Fig. 1.7 Walter Heiligenberg (1938–1994) was a German comparative neuroscientist best known for his contributions to neuroethology based on detailed study of the jamming avoidance response (JAR) in the weakly electric glass knifefish *Eigenmannia virescens*. As a student of Konrad Lorenz, Heiligenberg studied the motivational behaviors of cichlid fish and crickets and conducted a successful quantitative demonstration of the law of heterogeneous summation. As a postdoc in Theodore Bullock's lab and later on the faculty at the Scripps Institution of Oceanography, Heiligenberg (1991) helped establish the JAR as the only nonreflex vertebrate behavior for which the neural basis has been described in detail, from sensory receptors that encode behaviorally relevant sensory information to motor effectors that drive the change in behavior

1.5.3 Electrocommunication

The study of the neural basis of electrocommunication in weakly electric fishes has been another rich area of investigation for neuroethologists. Möhres (1957) was the first to suggest that modulations of EOD rate in mormyrids had a communication function based on his observation that members of *Gnathonemus* sp. would often

interrupt or vary the frequency of their EODs during bouts of aggression and physical fighting. Lissmann (1958) also suggested that EODs may play a role in the social behavior of mormyrids. A subsequent study by Moller (1970) demonstrated clear changes in EOD frequency in *Gnathonemus* sp. in response to playback of electric stimuli, and this was followed by a detailed observational study that described electrical interactions between pairs of *Gnathonemus* sp. that varied with the distance between the fish (Moller and Bauer 1973). Since that time, numerous playback and observational studies in several species of weakly electric fish have removed any doubt that EODs play a central role in communication and social behavior in both mormyroid and gymnotiform fishes (reviewed in Moller 1995a; Kramer 1996).

In the 1970s and 1980s, species diversity of EOD waveforms in pulse-type fishes and EOD frequency in wave-type fishes were established from field and laboratory recordings, and this diversity was shown to be species-specific (reviewed in Kramer 1990; Moller 1995a). In 1972, Carl Hopkins (to whom this volume is dedicated), then at the University of Minnesota, Minneapolis, was the first to show sexually dimorphic differences in EOD frequency among individuals from a breeding population of wave-type *Sternopygus macrurus*. After moving to Cornell University, Ithaca, NY, Hopkins, along with his colleague Andrew Bass, discovered that sex differences in the EOD waveforms of pulse-type mormyrids were influenced by steroid hormones (Bass and Hopkins 1983; also see Silva, Chap. 6). In addition to species and sex differences in EODs, individual and dominance-related differences as well as developmental changes in EODs were later observed (reviewed in Moller 1995a). In Chap. 5, Markham describes in detail the current understanding of how EOD diversity relates to the biophysics of electrocytes, the electrically excitable cells that constitute the "battery" that makes up the electric organ. In Chap. 6, Silva addresses the hormonal regulation of social behavior in the South American gymnotiforms, from hormonal actions on electrocytes and the central nervous system that drive changes in EOD waveform and frequency, respectively, to the role of hormones in seasonality, circadian rhythmicity, and territorial aggression. As in the early studies of the communicative significance of EODs, playback experiments were essential in demonstrating the behavioral significance of species, sex, and individual differences.

1.5.4 Reafference and Exafference

Given the experimental accessibility of both electromotor and electrosensory systems, a fundamental question in neuroscience that has been studied extensively in electric fishes is how the central nervous system distinguishes between self-generated sensory input (reafference) and externally generated sensory input (exafference). Distinguishing among these sources of input and processing them separately are crucial to all three forms of electrosensing: passive electrolocation, active electrolocation, and electrocommunication. In the 1980s, Curtis Bell at the Oregon Health and Sciences University, Portland, was one of the first researchers to

investigate the role of sensory reafference in weakly electric fish and determine how animals perceive self-generated versus externally generated electric fields. Bell investigated the adaptive processing of electrosensory information that occurs in the cerebellum-like structures of the electrosensory lobes in weakly electric fish. In the context of electrolocation, Bell (1989) showed that associations between sensory inputs and corollary discharges within these cerebellum-like structures result in the generation of negative images of predictable features of sensory inflow that when added to the actual inflow of information removes the predictable features, thus allowing the unpredictable, externally generated sensory signals to be salient.

Similar noise suppression mechanisms were also observed in the elasmobranch electrosensory system by John Montgomery at the University of Auckland, New Zealand, and David Bodznick at Wesleyan University, Middletown, CT. In elasmobranch and teleost fishes, the animal's own ventilatory movements can create unwanted stimulation of the lateral line and electrosensory system that can potentially interfere with the detection of biologically relevant signals. Montgomery and Bodznick (1994, 1999) showed that there is an adaptive filter in the cerebellar-like circuits of medullary nuclei in the hindbrain for both senses (i.e., electrosensory dorsal nucleus and mechanosensory medial nucleus) that act to suppress self-stimulation through a common mode rejection mechanism. Montgomery and Bodznick (1994) also showed that fish can learn to cancel the effects of electrosensory and mechanosensory stimuli that are coupled to the fish's own movements. In Chap. 11, Perks and Sawtell describe in detail the underlying cells, circuits, and computations that underlie sensorimotor integration for processing exafferent and reafferent sensory input.

1.6 Fundamental Insights from Comparative Approaches

Comparative approaches to studying electrosensory systems have led to the identification of fundamental mechanisms for neuronal information processing and its relationship to natural behavior. A major goal of this volume is to provide a comparative perspective on the topics of electrogenesis and electroreception and to review some of the important insights gained from studies of electrosensory and electromotor systems.

In Chap. 2, Baker reviews the evolutionary and developmental origins of nonteleost lateral line electroreceptors with insights from comparative molecular approaches. Baker details how current gene expression results using "known candidate" gene and more recent unbiased transcriptomic (differential RNA sequencing) approaches suggest that the molecular mechanisms underlying electroreceptor development are highly conserved, with similar mechanisms underlying hair cell development. In addition, there exist a number of similar aspects in hair cell physiology of electroreceptor and lateral line systems, including transmission mechanisms at the level of the ribbon synapse. The high degree of similarity in the molecular development of the lateral line and electroreceptor systems suggests that

electroreceptors most likely evolved in the vertebrate ancestor via the diversification of lateral line hair cells rather than an independent evolution of electroreceptors and hair cells from a secondary ciliated cell.

In Chap. 3, Leitch and Julius provide an overview of the physiological mechanisms underlying electrosensory transduction. This exciting review of electrosensory transduction includes recent advances in genetic and patch-clamp electrophysiological techniques that have made possible comparisons of the molecular mechanisms underlying transduction in electrosensory systems and other hair cell-based sensory systems. Such comparisons have the potential to shed light on the mechanisms of stimulus transduction and filtering across diverse species and potentially reveal shared fundamental mechanisms for extracting biologically relevant information across octavolateralis systems.

Chapters 4 and 5 focus on electric organ development and the biophysical basis of electric signal diversity, respectively. In Chap. 4, Gallant provides a comprehensive review of electric organ development and discusses recent advances in the understanding of molecular mechanisms involved in electric organ development in light of a new comparative study of gene expression across multiple lineages of electric fishes. In addition, Gallant identifies areas of need for additional data on electric organ development along with the application of new molecular techniques that could lead to new insights into the evolution and development of electric organs.

In Chap. 5, Markham addresses the morphological and physiological basis for the generation of EODs and focuses on specializations in electrocyte morphology and physiology, including the diversity of ion-channel expression patterns in electrocytes that can have a strong influence on waveform diversity. As described by Markham, electrocyte morphology and innervation patterns are a major source of signal diversity in the African mormyrid fishes, whereas diversity of ion-channel expression patterns is known to be a major influence on waveform diversity in the South American gymnotiform fishes. Although convergent evolution of ion channels in these clades contributes to signal diversity, little is known about the ionic mechanisms of signal diversity in mormyroids, which highlights the need for broader comparative studies.

Chapters 6 and 7 focus on the influence of hormones on social behavior and on the ultimate evolutionary causes of EOD diversification in weakly electric fishes, respectively. In Chap. 6, Silva addresses how the social behavior of South American weakly electric fishes is influenced by neuroendocrine actions on electrosensory and electromotor systems. Silva highlights the contributions of four iconic gymnotiform species that help explain how hormones regulate social behavior. The chapter details how steroid hormones have long-term effects on the kinetic properties of ion channels in electrocytes that can produce sexually dimorphic differences in EOD frequencies, whereas neuropeptides can have short-term effects on amplitude modulations of the EOD waveform. These changes in EOD properties are shown to be adaptive to environmental and social demands.

In Chap. 7, Krahe focuses on the ultimate evolutionary causes of EOD diversification in gymnotiforms and mormyroids, including ecological adaption, sexual selection, predation, and drift. Krahe provides an extensive review of the role of

electric signaling in species diversification and how environmental and energetic constraints, ecological adaptations, predation, and sexual selection can be drivers of electric signal diversity.

Chapter 8 turns to active-sensing behavior. Stamper, Madhav, Cowan, and Fortune focus on the use of control theory to reveal functional relationships among active sensing, task-related behaviors, sensing, and motor control. Active sensing can be defined as the use of an animal's motor output to modulate the sensory information it receives. Stamper, Madhav, Cowan, and Fortune discuss a recently developed experimental system that uses artificially controlled feedback loops to perturb the natural reafferent feedback received by freely behaving animals to explore control strategies for active sensing in weakly electric fishes.

Then, in Chap. 9, Metzen and Chacron provide a comprehensive review on neural mechanisms utilized at different stages of sensory processing to extract behaviorally relevant information from stimulus envelopes and how stimulus envelope features can mediate behavior. The comparative focus of this chapter is on important parallels between the envelope-coding properties of the electrosensory system and other sensory systems, including how research on weakly electric fishes relates to fundamental insights into how envelopes are coded and processed by the mammalian auditory system. Metzen and Chacron also provide intriguing avenues for future research on envelope coding and processing.

In Chap.10, Carlson focuses on common themes and key differences in submillisecond temporal coding across electrosensory and auditory systems. The chapter highlights how comparative approaches can uncover shared fundamental mechanisms that have evolved convergently through natural selection to solve specific behavioral problems while at the same time revealing the ultimate causes for differences between systems. Carlson elaborates on how similar cellular and synaptic building blocks can be used to construct different circuit solutions to solve similar behavioral problems in different clades and how these differences may have arisen through some combination of chance, evolutionary history, and adaptation. As Carlson comments in Chap. 10, "these differences also make it clear that discoveries in one organism cannot be extrapolated to other organisms, highlighting the importance of comparative approaches in addressing general problems in neuroscience."

In Chap. 11, Perks and Sawtell provide a review of the substantial body of research that has elucidated the synaptic, cellular, and circuit mechanisms by which the electrosensory system of mormyrid fishes predicts and cancels self-generated and predictable sensory inputs. Additional functions of motor corollary discharge signals in weakly electric mormyrids fishes are explored and discussed. In the chapter, Perks and Sawtell address how motor systems and behavior can influence the electrosensory processing of reafferent sensory input, with implications for this research providing insight into other sensory systems and brain structures, including the mammalian auditory system and the cerebellum.

The final chapter provides an overview of the emerging research on spatial learning in weakly electric fish. In Chap. 12, Jung and Engelmann provide a summary of the mechanisms that can provide spatial information during active electrolocation and discuss how the complex dynamics of sensorimotor behaviors can enable

weakly electric fishes to actively generate sensory flow. Jung and Engelmann also provide a summary of spatial learning mechanisms in nonelectric and weakly electric teleost fishes. They also discuss the neural mechanisms by which active electrolocation can provide spatial information and its neural basis in the dorsal telencephalon. The authors conclude that comparative approaches using the sensory specialties of the active electrosensory system in weakly electric fishes may ultimately provide novel insights into the relationship between spatial cognition and forebrain networks in other animals, including mammals.

1.7 Future Directions and Concluding Comments

Historically, neuroscientists have used a variety of approaches and a large diversity of animal taxa to gain insight into brain function and the neural basis of behavior (Carlson 2012). Neuroethologists often selected eclectic research organisms because they were uniquely suited to studying the neural basis of specific behaviors, which often led to fundamental insights into general neural mechanisms for behavior across species. Following the molecular revolution, neuroscience research became increasingly focused on a handful of inbred, genetically tractable laboratory species. This work has undoubtedly led to numerous important insights, but the generalizability of many of the resulting discoveries remains unknown. This is a problem for both better understanding human brains and seeking general, fundamental theories of brain function. There is, however, reason to think that neuroscience may soon experience a renewed appreciation of the importance of species diversity (Brenowitz and Zakon 2015; Yartsev 2017). Experimental tools that can be applied across species are rapidly expanding, from transgenic manipulations to large-scale neural ensemble recordings in freely behaving animals. Applying these techniques in a diversity of species carefully chosen with regard to phylogenetic position, behavior, genomics resources, practicality, and accessibility offer the best chance of elucidating fundamental theories of brain function (Striedter et al. 2014). This volume was assembled in this spirit.

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Joseph A. Sisneros declares that he has no conflict of interest.

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Chapter 2
The Development and Evolution of Lateral Line Electroreceptors: Insights from Comparative Molecular Approaches

Clare V. H. Baker

Abstract In the jawless lampreys, most nonteleost jawed fishes, and aquatic-stage amphibians, the lateral line system has a mechanosensory division responding to local water movement ("distant touch") and an electrosensory division responding to low-frequency cathodal (exterior-negative) electric stimuli, such as the weak electric fields surrounding other animals. The electrosensory division was lost in the ancestors of teleost fishes and their closest relatives and in the ancestors of frogs and toads. However, anodally sensitive lateral line electroreception evolved independently at least twice within teleosts, most likely via modification of the mechanosensory division. This chapter briefly reviews this sensory system and describes our current understanding of the development of nonteleost lateral line electroreceptors, both in terms of their embryonic origin from lateral line placodes and at the molecular level. Gene expression analysis, using candidate genes and more recent unbiased transcriptomic (differential RNA sequencing) approaches, suggests a high degree of conservation between nonteleost electroreceptors and mechanosensory hair cells both in their development and in aspects of their physiology, including transmission mechanisms at the ribbon synapse. Taken together, these support the hypothesis that electroreceptors evolved in the vertebrate ancestor via the diversification of lateral line hair cells.

Keywords Ampullary organ · Electroreception · Electrosensory · Hair cell · Mechanosensory · Neuromast · Placode · Presynaptic ribbon · Ribbon synapse · RNA sequencing · Tuberous organ

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2.1 Introduction

Evidence from phylogenetic distribution, sensory receptor cell physiology, and innervation suggests that the ancestor of all living vertebrates had vestibular inner ears with mechanosensory "hair cells" detecting gravity and angular acceleration (see Fritzsch and Elliott 2017) plus a lateral line system comprising (1) a mechanosensory division, with sense organs containing hair cells that detect local water movement, and (2) an electrosensory division, with sense organs containing electroreceptor cells stimulated by low-frequency, cathodal (exterior-negative) electric fields (Bullock et al. 1983; Baker et al. 2013). The electroreceptors respond to minute direct-current standing electric fields around animals in water (arising from ions leaking across mucous membranes) that can be modulated by ventilation or limb movements, generating a low-frequency component (Bedore and Kajiura 2013).

The apical surface of lateral line hair cells, like that of vestibular hair cells, is characterized by a staircase array of actin-rich microvilli ("stereocilia" or "stereovilli") connected by tip links (the "hair bundle") and a primary cilium ("kinocilium") eccentrically positioned next to the tallest stereocilia (Fig. 2.1A; Jørgensen 2005). The apical surface of electroreceptor cells is more diverse, with a primary cilium and/or varying numbers of microvilli (Fig. 2.1B, C; see Sect. 2.1.2; Jørgensen 2005). Hair cells and electroreceptors all have basolateral presynaptic bodies ("ribbons") that tether many synaptic vesicles (Fig. 2.1; see Jørgensen 2005; Zanazzi and Matthews 2009). Depolarization of the hair cell or electroreceptor results in neurotransmitter release at these specialized "ribbon synapses" (see Zanazzi and Matthews 2009; Nicolson 2015) onto the terminals of afferent neurons whose cell bodies are collected in cranial ganglia. Innervation patterns in extant vertebrates (McCormick 1982; Bullock et al. 1983), including eptatretid hagfishes (Amemiya et al. 1985), suggest that in the vertebrate ancestor, the central targets of inner ear and lateral line afferent neurons were distinct nuclei in the octavolateral area in the rostral alar plate of the hindbrain. The octavolateral nuclei are (1) the ventral nucleus for inner ear afferents projecting via the eighth cranial nerve; (2) the medial nucleus for mechanosensory lateral line afferents projecting via the posterior lateral line nerve and the ventral root of the anterior lateral line nerve; and (3) the dorsal nucleus for electrosensory lateral line afferents projecting via the dorsal root of the anterior lateral line nerve (reviewed by Wullimann and Grothe 2014).

The inner ears and lateral line system are developmentally and evolutionarily independent (see Sect. 2.2.4.1). All vertebrates have inner ears, whereas the lateral line system was lost independently in the cyclostome lineage leading to myxinid hagfishes (Braun and Northcutt 1997) and with the transition to terrestrial life in the lobe-finned bony tetrapod lineage leading to amniotes (Fig. 2.2). (The lateral line system was also lost in a few direct-developing amphibian lineages without an aquatic larval stage; Schlosser 2002b.) The mechanosensory and electrosensory divisions are also independent. The mechanosensory division was lost in some

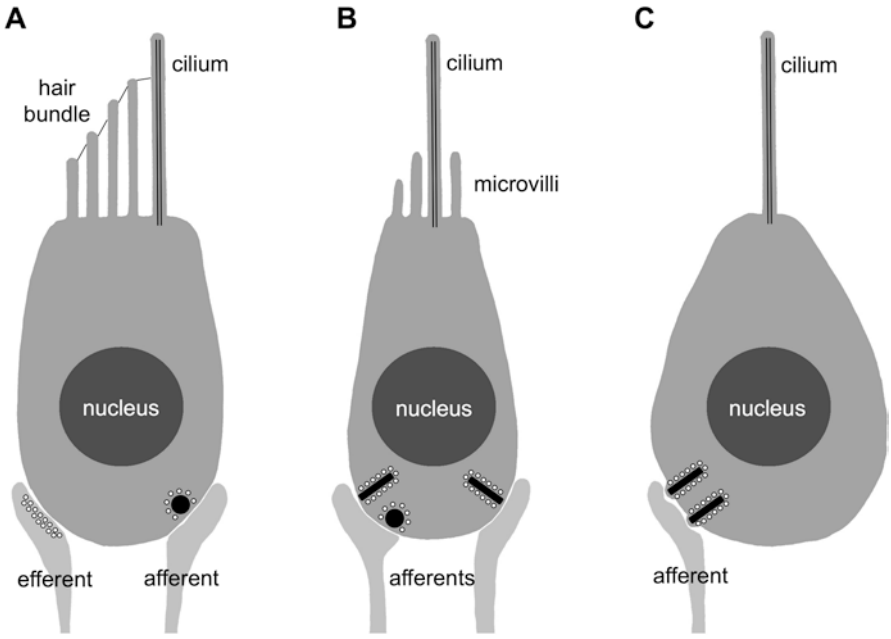

Fig. 2.1 Mechanosensory and electrosensory cells of the lateral line system. The apical surface varies, but they all have basolateral presynaptic bodies, surrounded by synaptic vesicles, opposite ribbon synapses with afferent lateral line nerve terminals. **A:** a hair cell characterized by a primary cilium (kinocilium) eccentrically positioned at the tallest edge of a staircase array of actin-rich microvilli (stereocilia) connected by tip links (hair bundle), with efferent as well as afferent innervation. **B:** an electroreceptor cell with a primary cilium and microvilli as found in, for example, ray-finned bony bichirs and lobe-finned bony lungfishes and amphibians. **C:** a pear-shaped electroreceptor cell with a primary cilium but without microvilli as found in, for example, cartilaginous fishes and ray-finned bony chondrostean fishes. Modified from Jørgensen (2011), with permission from Elsevier

aquatic caecilians that retain the electrosensory division (Schlosser 2002b). The electrosensory division was lost in the cyclostome lineage leading to eptatretid hagfishes (Braun and Northcutt 1997), in the lobe-finned bony tetrapod lineage leading to anuran amphibians (frogs and toads), and in the ray-finned bony fish lineage leading to neopterygian fishes (comprising gars, bowfin, and teleosts; Fig. 2.2; McCormick 1982; Bullock et al. 1983). A few teleost clades (see Sect. 2.1.2.3) possess an electrosensory division stimulated by anodal (exterior-positive) electric fields, with afferents projecting via both anterior and posterior lateral line nerves to distinct "electrosensory lateral line lobes" in the hindbrain (Bullock et al. 1983; Wullimann and Grothe 2014). The phylogenetic distribution suggests that teleost electroreception evolved independently at least twice (see Sect. 2.1.2.3.2; Bullock et al. 1983; Baker et al. 2013).

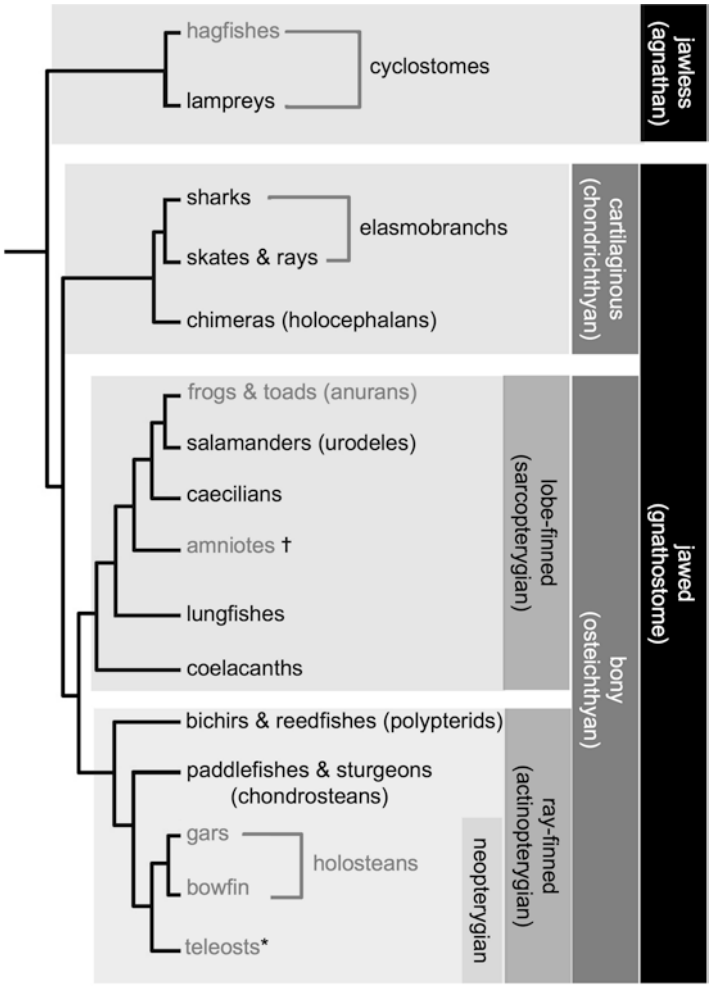

Fig. 2.2 Phylogenetic distribution of lateral line sensory divisions among living vertebrates. *Black text* indicates the presence of both the mechanosensory division, with afferents projecting to the medial octavolateral nucleus via the anterior and posterior lateral line nerves, and a low-frequency, cathodally sensitive electrosensory division, with afferents projecting to the dorsal octavolateral nucleus via the dorsal root of the anterior lateral line nerve. *Gray text* indicates the presence of the mechanosensory lateral line only, except for amniotes (†), which lost the entire lateral line system with the transition to life on land and ray-finned teleost fishes (∗), where a few clades possess anodally sensitive lateral line electroreception, with afferents projecting to electrosensory lateral line lobes. Adapted from Baker and Modrell (2018), with permission from Oxford University Press

2.1.1 The Mechanosensory Division of the Lateral Line System

Lateral line neuromasts (Fig. 2.3) are distributed in characteristic lines over the head and body, either superficially or in canals connected to the surface via pores (see Webb 2014). Each neuromast contains a central cluster of hair cells (Fig. 2.1A) that project into a gelatinous cupula in jawed vertebrates (Fig. 2.3). In addition to basolateral ribbon synapses with afferent lateral line nerve terminals (Figs. 2.1A and 2.3; see Nicolson 2015), neuromast hair cells receive efferent innervation from medullary octavolateral efferent nuclei (see Chagnaud and Coombs 2014; Wullimann and Grothe 2014). The hair cells are surrounded and underlain by supporting cells that send processes between the hair cells, while a layer of mantle cells forms the outer rim of the neuromast (Fig. 2.3; see Webb 2014).

Neuromast hair cells respond to local water movement, mediating a sense of "distant touch" important for behaviors including feeding, avoiding predators and obstacles, and intraspecific communication (Dijkgraaf 1963; Montgomery et al. 2014). Hair cells are directionally sensitive (Flock 1965; Hudspeth and Corey 1977). Hydrodynamic stimuli that displace the hair bundle in the direction of the tallest stereocilia and kinocilium open mechanically gated cation channels at the stereociliary tips, resulting in hair cell depolarization and, ultimately, glutamate release, increasing the firing rate of the afferent fiber, whereas displacement in the opposite direction hyperpolarizes the hair cell, decreasing the firing rate (see Chagnaud and Coombs 2014). Oppositely oriented hair cells are intermingled in

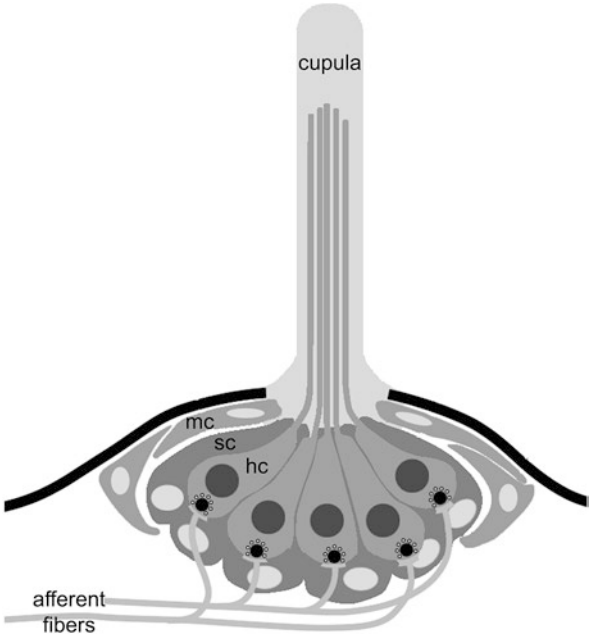

Fig. 2.3 A neuromast comprises a cluster of hair cells whose apical cilia project into a gelatinous cupula, surrounded and underlain by supporting cells, with an outer rim of mantle cells. Hair bundles and efferent fibers are not shown. hc, Hair cell; mc, mantle cell; sc, supporting cell. Modified from Ghysen and Dambly-Chaudière (2004), with permission from Elsevier

each neuromast (Flock 1965; López-Schier et al. 2004). During development and regeneration, hair cells of opposite orientation within the same neuromast become innervated by different afferent fibers (Nagiel et al. 2008; Dow et al. 2018).

2.1.2 The Electrosensory Division of the Lateral Line System

2.1.2.1 Electrosensory Organs in Jawless Fishes

Within the extant jawless fishes, lampreys have both electrosensory and mechanosensory lateral line divisions, although the neuromasts (all superficial) lack cupulae and efferent innervation (Braun 1996). Eptatretid hagfishes have a simple mechanosensory lateral line system only, whereas myxinid hagfishes lack even this (Bullock et al. 1983; Braun and Northcutt 1997). Molecular evidence overwhelmingly supports lampreys and hagfishes as a monophyletic clade, the cyclostomes (Fig. 2.2; see Shimeld and Donoghue 2012), suggesting that within hagfishes, the electrosensory division was secondarily lost in the eptatretid lineage, whereas the entire lateral line system was lost in the myxinid lineage, as previously suspected (Braun 1996).

Adult lampreys have both cranial and trunk epidermal "end bud" electroreceptor organs directly exposed at the surface, with supporting cells and electroreceptor cells lacking a primary cilium but with 80–90 short apical microvilli and basolateral spheroidal presynaptic bodies (Fig. 2.4; Jørgensen 2005). Both lateral line divisions are functional at ammocoete larval stages (Ronan 1988; Gelman et al. 2007). Ammocoetes lack electroreceptor organs, and their electroreceptor cells are thought to be lateral line-innervated epidermal "multivillous cells" with presynaptic bodies (Fig. 2.4; Jørgensen 2005).

2.1.2.2 Electrosensory Organs in Nonteleost Jawed Vertebrates

Within jawed fishes and amphibians, the "ancestral" electrosensory division (i.e., low-frequency, cathodally sensitive electroreceptors whose afferents project to the dorsal octavolateral nucleus via the dorsal root of the anterior lateral line nerve) is found in all lineages except the lobe-finned anuran amphibians (frogs and toads) and the ray-finned neopterygian fishes (teleosts and holosteans, i.e., gars and the bowfin), suggesting secondary loss of the electrosensory division within these lineages (Fig. 2.2; Bullock et al. 1983; Baker et al. 2013). Electroreceptor cells (see Leitch and Julius, Chap. 3) are found in "ampullary organs" (or "ampullae of Lorenzini"), named for their flask-like morphology. The sensory epithelium of electroreceptor cells and supporting cells is located at the base of a bulbous chamber from which a conductive jelly-filled duct (long in marine species; short in freshwater species) leads to a pore at the surface (Fig. 2.5; Jørgensen 2005). Each electroreceptor cell has an apical primary cilium, varying numbers of apical microvilli (from

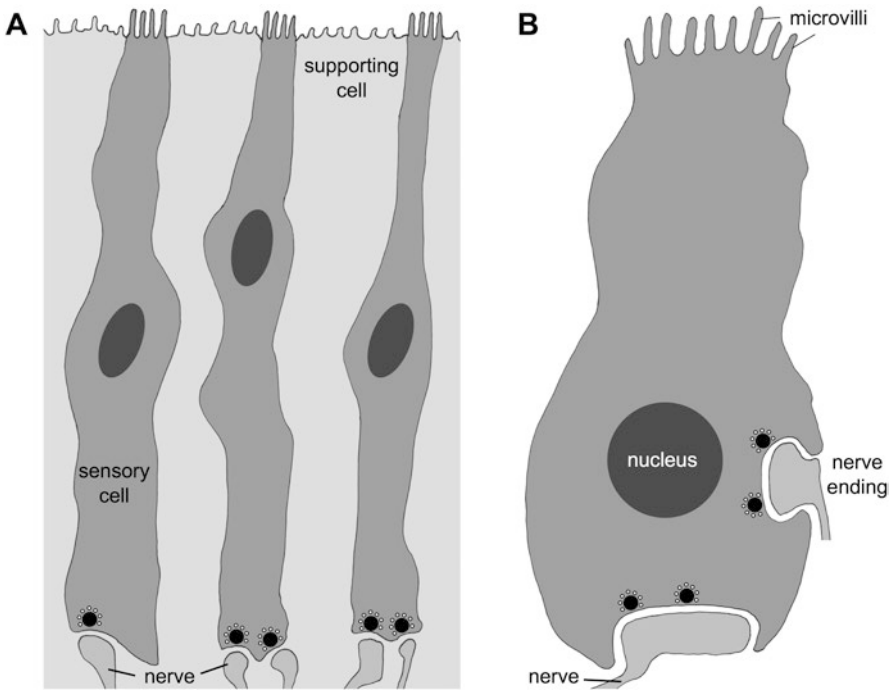

Fig. 2.4 Lamprey adult end bud electroreceptor cells (**A**) and ammocoete larval multivillous cells (not to scale; **B**). Individual supporting cells are not delineated. Redrawn after Jørgensen (2005), © Springer Science+Business Media, Inc., with permission

none to a couple of hundred), and basal presynaptic ribbons opposite afferent lateral line nerve terminals (Fig. 2.5; Jørgensen 2005).

2.1.2.3 Electrosensory Organs in Teleost Fishes: Independent Evolution

2.1.2.3.1 Overview of Teleost Electroreception

Within the teleosts, electroreception is found in two related clades within each of two distinct lineages (Bullock et al. 1983; Baker et al. 2013). In the osteoglossomorph lineage (Fig. 2.6), the electroreceptive species are the African notopterids (featherbacks or knifefishes) and their sister group, the mormyroids, which comprise the mormyrids (freshwater elephant fishes) plus the gymnarchid *Gymnarchus niloticus* (the aba). In the ostariophysan lineage (Fig. 2.6), the two related electroreceptive clades are the siluriforms (catfishes) and gymnotiforms (South American knifefishes).

Teleost electroreception differs significantly from nonteleost electroreception (see Leitch and Julius, Chap. 3). Teleost electroreceptors are stimulated by anodal stimuli and inhibited by cathodal stimuli, and the basal membrane is the voltage

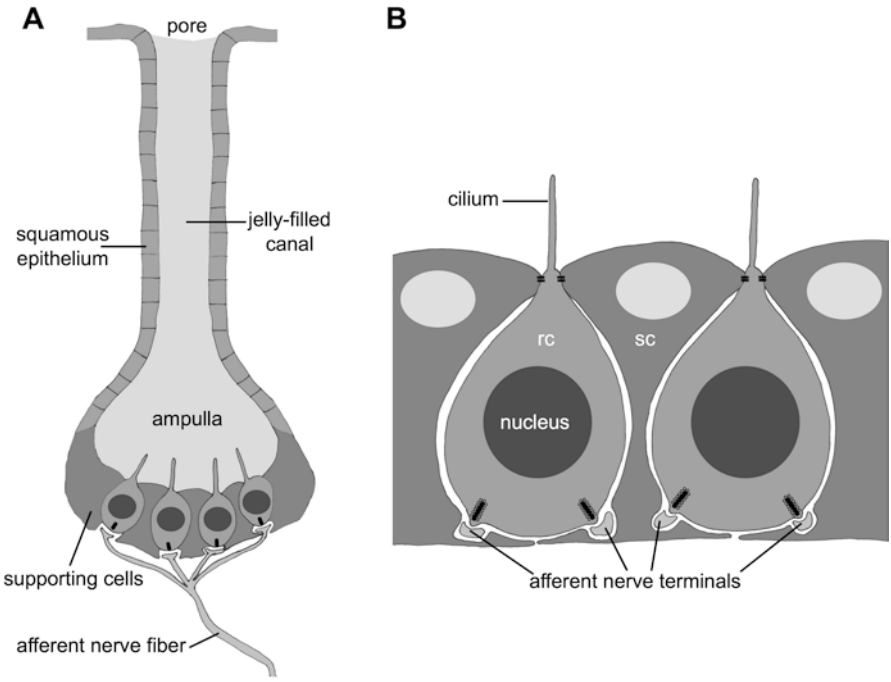

Fig. 2.5 **A:** a nonteleost jawed vertebrate ampullary organ. A surface pore opens to a conductive jelly-filled duct lined with squamous epithelium and ending in a bulbous chamber with a sensory epithelium at its base, comprising supporting cells (not delineated) and electroreceptor cells. Redrawn and modified from Sillar et al. (2016), with permission from John Wiley & Sons, Ltd. **B:** in the sensory epithelium, each electroreceptor cell has an apical primary cilium and variable numbers of microvilli (none in this example) with presynaptic ribbons opposite ribbon synapses with afferent lateral line nerve terminals. Apical tight junctions connect electroreceptor cells to neighboring supporting cells. rc, Receptor cell; sc, supporting cell. Redrawn and modified from Fields et al. (1993), with permission from Karger

sensor, whereas nonteleost electroreceptors respond to cathodal stimuli via the apical membrane (Bodznick and Montgomery 2005; see Leitch and Julius, Chap. 3). All electroreceptive teleosts have "ampullary" electroreceptors that respond to low-frequency environmental electric fields (passive electroreception). As in nonteleosts, the sensory epithelium containing ampullary electroreceptors is located at the base of a flask-like chamber, from which a mucus-filled duct leads to a surface pore (Fig. 2.7; Jørgensen 2005). Teleost ampullary electroreceptors have sparse microvilli, no primary cilium, and presynaptic ribbons (Fig. 2.7; Jørgensen 2005). The only teleost electroreceptors with a primary cilium are the ampullary electroreceptors of the osteoglossomorph African notopterid *Xenomystus nigri* (Jørgensen 2005). In contrast, all electroreceptors in nonteleost jawed vertebrates have a primary cilium (Jørgensen 2005).

The osteoglossomorph mormyroids and the unrelated ostariophysan gymnotiforms (Fig. 2.6) are described as "weakly electric" teleosts. This is because they

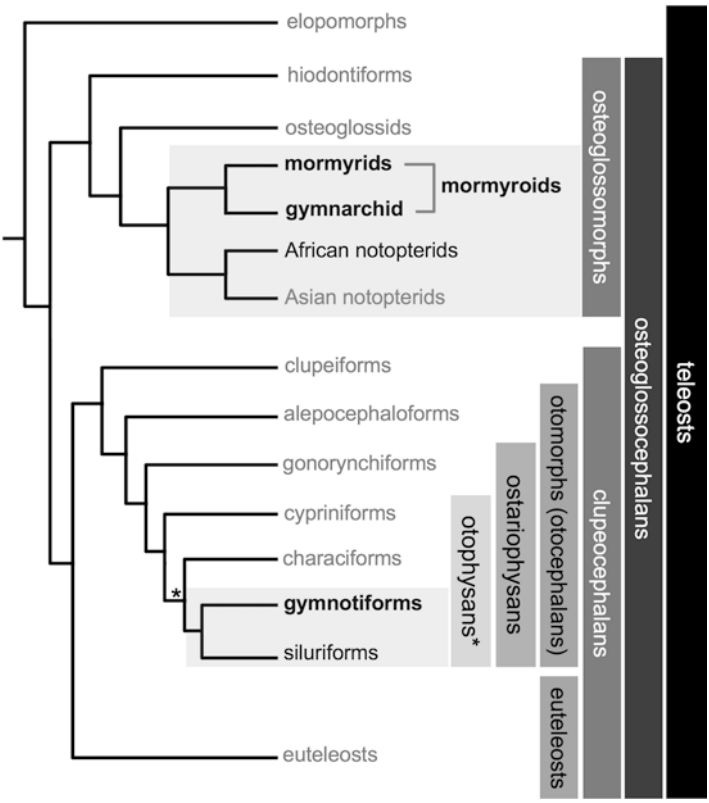

Fig. 2.6 Phylogenetic distribution of lateral line electroreception within teleosts. *Gray text* indicates the presence of the mechanosensory lateral line only. *Black text* indicates the presence of ampullary electroreceptors stimulated by low-frequency, anodal electric fields (passive electroreception) and electrosensory lateral line lobes in the hindbrain. *Bold black text* indicates the weakly electric fish clades, which also have electric organs and tuberous organs responding to high-frequency electric organ discharges (active electroreception). In otophysans (∗), there is a continuing debate over the sister-group relationships among characiforms, gymnotiforms, and siluriforms. The phylogeny follows that in Betancur-R et al. (2017)

possess not only ampullary electroreceptors but also electric organs (modified muscle or nerve cells) that generate high-frequency electric fields (see Gallant, Chap. 4), and "tuberous" electroreceptors that respond to these high-frequency electric organ discharges (see Leitch and Julius, Chap. 3). Tuberous organs are morphologically varied but united in lacking ducts and being plugged by loosely packed epidermal cells (Fig. 2.8; Jørgensen 2005). Tuberous electroreceptor cells, which are characterized by many microvilli apically and presynaptic ribbons basally, are located within an intraepidermal cavity (Fig. 2.8; Jørgensen 2005).

Teleost electroreceptor organs are found on both the trunk and head, innervated by posterior and anterior lateral line nerves, respectively, projecting to hindbrain electrosensory lateral line lobes (Fig. 2.9; see Bullock et al. 1983; Wullimann and

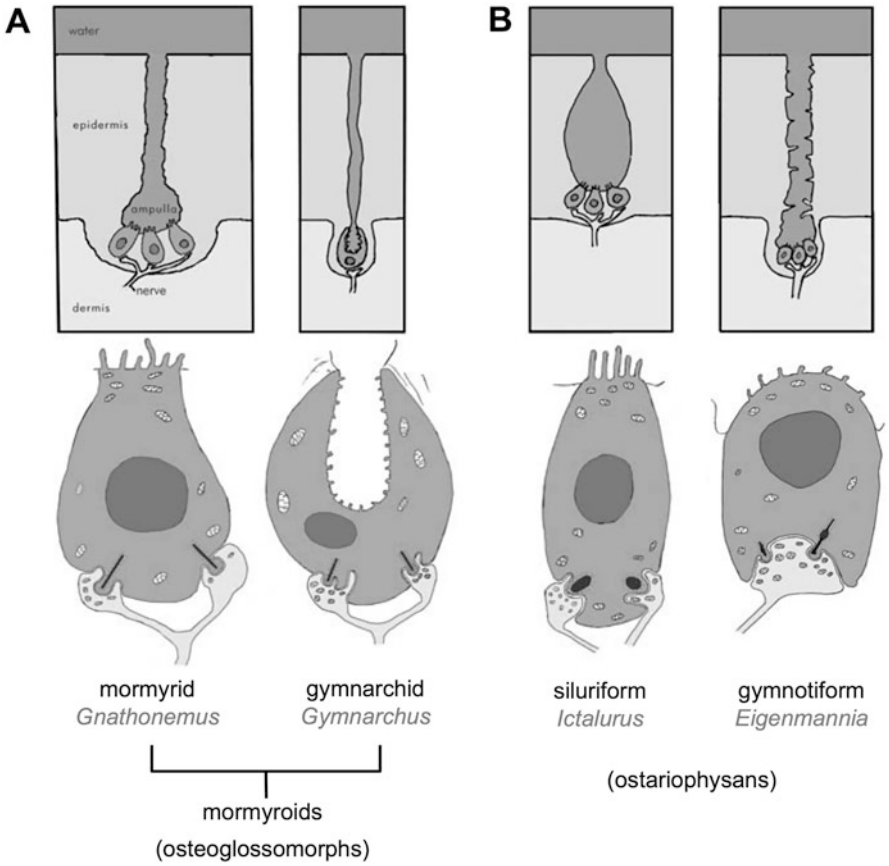

Fig. 2.7 Teleost ampullary organs (*top*) and electroreceptor cells (not to scale; *bottom*) from representative osteoglossomorph mormyroids (mormyrids plus the gymnarchid *Gymnarchus niloticus*; **A**) and ostariophysan siluriforms and gymnotiforms (**B**). Adapted from Jørgensen (2005), © Springer Science+Business Media, Inc., with permission

Grothe 2014). These share a cerebellum-like organization and circuitry with the medial octavolateral nuclei (the targets of mechanosensory lateral line afferents) and the dorsal octavolateral nuclei of nonteleosts (Fig. 2.9; (Bell et al. 1997; Bell and Maler 2005).

2.1.2.3.2 Electroreception Evolved Independently At Least Twice in Teleosts

Within the ray-finned bony fishes, "ancestral" electroreception, (i.e., stimulated by low-frequency, cathodal electric fields, with afferents projecting to the dorsal octavolateral nucleus via the dorsal root of the anterior lateral line nerve) is present in the basally branching lineages, namely, polypterids (bichirs and reedfishes) and

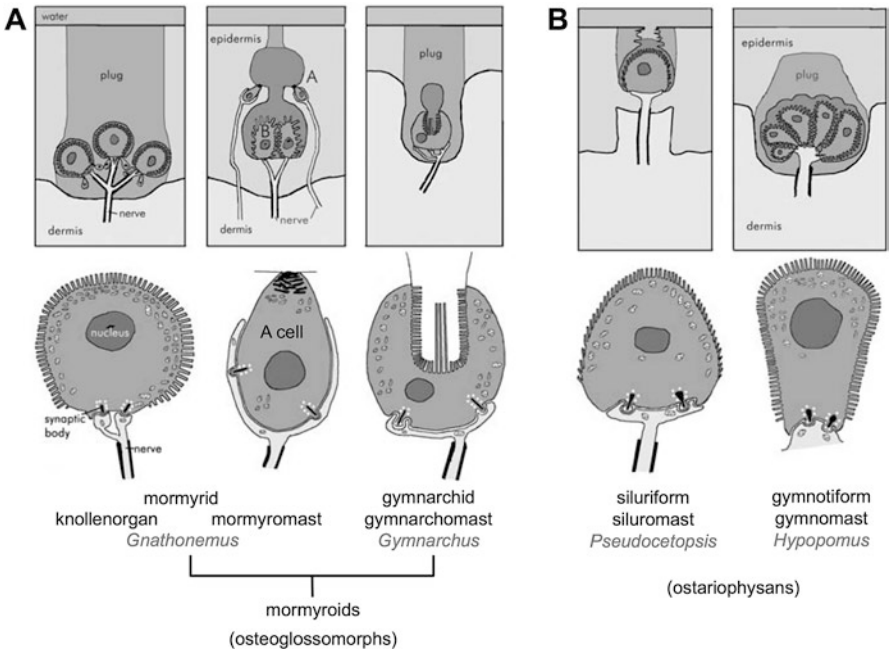

Fig. 2.8 Teleost tuberous organs (*top*) and electroreceptor cells (not to scale; *bottom*) from representative osteoglossomorph mormyroids (**A**) and from ostariophysan siluriforms and gymnotiforms (**B**). **A:** a mormyrid knollenorgan (*left*); a mormyrid mormyromast (*center*) containing both pear-shaped type A electroreceptor cells and knollenorgan-electroreceptor-like type B electroreceptor cells; and a gymnarchid gymnarchomast (*right*). Adapted from Jørgensen (2005), © Springer Science+Business Media, Inc., with permission

chondrosteans (paddlefishes and sturgeons), but is absent from neopterygians (Fig. 2.2). Given this phylogenetic distribution and the very different characteristics of teleost electroreception, as described in Sect. 2.1.2.3.1, the simplest hypothesis is that electroreception was lost in the ray-finned bony fish lineage leading to the neopterygian clade (Fig. 2.2) and evolved independently at least twice within teleosts (see Bullock et al. 1983; Baker et al. 2013). Less parsimoniously, ancestral electroreception could have been lost independently in each lineage.

In the osteoglossomorph lineage, the most parsimonious hypothesis is that anodally sensitive ampullary electroreception (with afferents projecting to a novel electrosensory lateral line lobe in the hindbrain) evolved along the stem leading to the common ancestor of mormyroids and notopterids (and was lost in the lineage leading to Asian notopterids) and that electric organs and tuberous electroreceptors subsequently evolved in the lineage leading to mormyroids (Fig. 2.6; see Lavoué et al. 2012; Baker et al. 2013). The less parsimonious hypothesis (assuming that novel trait evolution is less likely than trait loss) is that ampullary electroreception evolved independently in the lineage leading to African notopterids, and in the lineage leading to mormyroids (Fig. 2.6; see Lavoué et al. 2012; Baker et al. 2013).

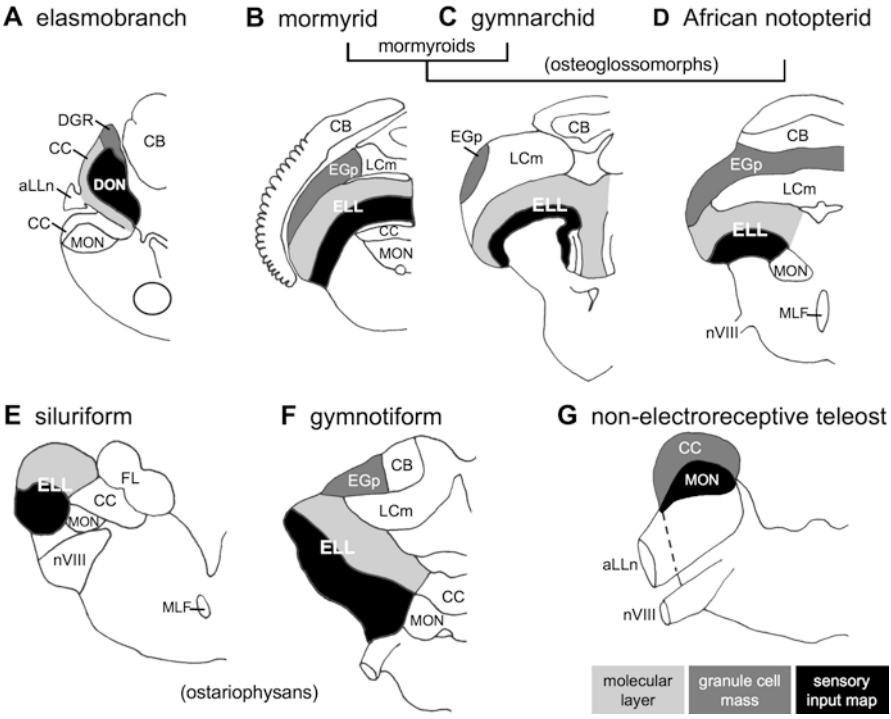

Fig. 2.9 First-order cerebellum-like electrosensory hindbrain structures in a nonteleost fish (an elasmobranch; **A**), osteoglossomorph teleost fishes (**B–D**), ostariophysan teleost fishes (**E** and **F**), and, for comparison, the cerebellum-like mechanosensory hindbrain structures from a nonelectroreceptive teleost (**G**). *Black areas* indicate where primary afferent fibers terminate (i.e., the sensory input map). *Dark gray areas* indicate the mass of granule cells whose parallel fibers form the molecular layer (*light gray areas*) of these structures. aLLn, anterior lateral line nerve; CB, cerebellum; CC, cerebellar crest; DGR, dorsal granular ridge; DON, dorsal octavolateral nucleus; EGp, eminentia granularis posterior; ELL, electrosensory lateral line lobe; LCm, molecular layer of the caudal lobe of the cerebellum; MLF, medial longitudinal fasciculus; MON, medial octavolateral nucleus; nVIII, eighth cranial nerve. Redrawn and modified from Bell et al. (1997), with permission from Karger and from Bell and Maler (2005), © Springer Science+Business Media, Inc., with permission

In the ostariophysan lineage, the picture is complicated by continued debate over the sister-group relationships among the siluriform, gymnotiform, and characiform clades within the otophysans (Fig. 2.6; Betancur-R et al. 2017; Dai et al. 2018). Under all scenarios, however, the most parsimonious hypothesis is that anodally sensitive ampullary electroreception (with afferents projecting to a novel electrosensory lateral line lobe in the hindbrain) evolved along the stem leading to the common ancestor of siluriforms and gymnotiforms. Subsequently, electric organs and tuberous electroreceptors evolved in the gymnotiform lineage, with ampullary electroreception being lost independently in any other lineages falling

within the clade containing siluriforms and gymnotiforms. The alternative would be that ampullary electroreception evolved independently in the lineage leading to siluriforms and in the lineage leading to gymnotiforms (Fig. 2.6; see Lavoué et al. 2012; Baker et al. 2013).

How might teleost electroreceptors have evolved? Like nonteleost electroreceptors, hair cells release neurotransmitter in response to sufficiently large anodal stimuli, which presumably directly depolarize the basal presynaptic membrane (e.g., Bodznick and Preston 1983; Münz et al. 1984). It has been suggested that greater sensitivity of the basal membrane to electrical stimuli, potentially achieved by increasing the density of basal voltage-gated calcium channels, could have been selected for in a subpopulation of hair cells (Bullock et al. 1983; Bodznick 1989). It seems plausible, therefore, that teleost ampullary electroreceptors evolved via the modification of neuromast hair cells such that their basal membranes responded to increasingly smaller electrical stimuli, and they lost the apical mechanosensory hair bundle (and cilium). The independent evolution of tuberous electroreceptors in the osteoglossomorph mormyroids and ostariophysan gymnotiforms could have involved the modification of either ampullary electroreceptors or neuromast hair cells. Furthermore, the evolutionary pathway could be different in the two lineages. Future comparative transcriptomic approaches, ideally at the single-cell level (e.g., Haque et al. 2017), would enable the transcriptomes of neuromast hair cells, ampullary and tuberous electroreceptors to be compared directly both within and across species. This could reveal the extent to which the evolution of different electroreceptor types in different teleost groups involved similar or wholly distinct molecular pathways and mechanisms.

2.1.3 Trigeminal Nerve-Mediated Electroreception in Monotremes and Dolphins

Monotreme mammals (the duck-billed platypus, *Ornithorhynchus anatinus*, and the echidnas, Tachyglossidae) and at least one fully aquatic eutherian mammal (the Guiana dolphin, *Sotalia guianensis*) independently evolved electroreception, mediated via naked afferent trigeminal nerve endings associated with accessory structures in the snout (Czech-Damal et al. 2013). In monotremes, the accessory structures are mucous or serous glands in the bill/snout (Fig. 2.10), whereas in the Guiana dolphin, they are whiskerless vibrissal crypts on the upper jaw (Czech-Damal et al. 2013). Given the independent evolution of trigeminal electroreception, it will not be considered further here. Future molecular work, should this prove feasible, may reveal whether there is any convergence with lateral line electroreception, for example, in the ion channels involved.

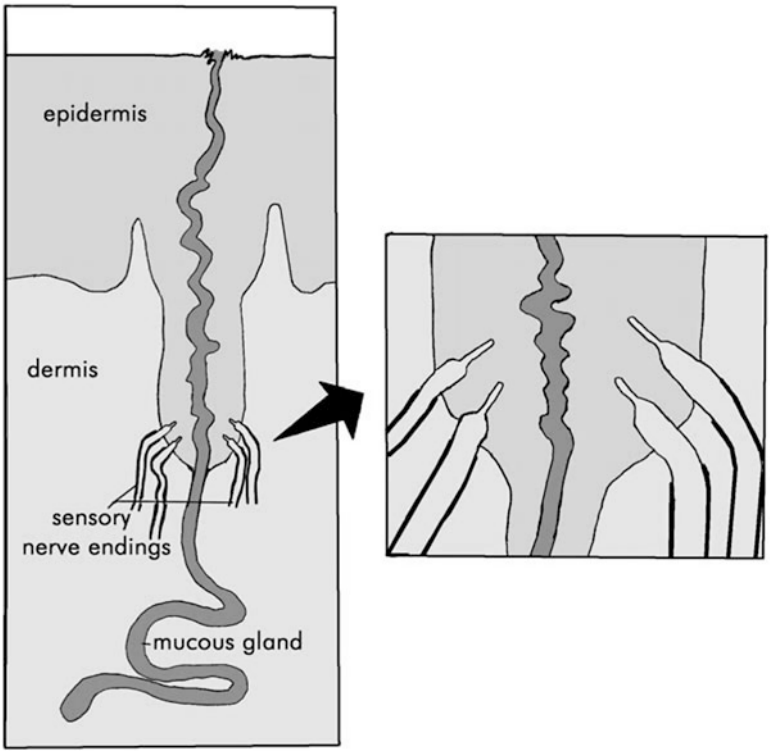

Fig. 2.10 A mucous gland from the bill of the duck-billed platypus (*Ornithorhynchus anatinus*) containing naked electroreceptive trigeminal nerve endings. Reproduced from Jørgensen (2005), © Springer Science+Business Media, Inc., with permission

2.2 Electroreceptor Development

2.2.1 An Introduction to Cranial Placodes

Following descriptions in the late nineteenth century of lateral line development in teleost (ray-finned bony) fish and urodele amphibian (lobe-finned bony tetrapod) embryos, a series of experimental grafting and ablation studies in both urodele and anuran amphibian embryos showed that lines of lateral line neuromasts, together with their afferent neurons in lateral line ganglia, originate from cranial lateral line placodes (LLPs; see Schlosser 2002a; Piotrowski and Baker 2014). The migrating posterior lateral line primordium of the zebrafish (*Danio rerio*, a cypriniform ostariophysan teleost; Fig. 2.6), which can be manipulated genetically to enable live imaging of migrating and differentiating cells and to study gene function, has become a key model for understanding the molecular mechanisms underlying collective cell migration, organ morphogenesis, and hair cell specification and regeneration (see Kniss et al. 2016; Dalle Nogare and Chitnis 2017). More recently,

ablation and fate-mapping studies revealed that individual LLPs in electroreceptive jawed vertebrates form ampullary organs as well as neuromasts and afferent neurons (Sect. 2.2.2; Baker et al. 2013).

LLPs are a subset of the cranial placodes. These simple patches of thickened columnar ectoderm form in characteristic positions on the embryonic vertebrate head and give rise (after, in some cases, extensive morphological changes) to a diverse array of different organs and cell types, which are critical not only for extero- and interoception but also for homeostasis and fertility (Schlosser 2010). All the hair cell-forming placodes develop as bilateral, paired structures from a "posterior placodal area" adjacent to the hindbrain (see Schlosser 2010). The otic placodes form the inner ears and their afferent neurons, which are located in the ganglia of cranial nerve VIII (Schlosser 2010). Phylogenetic analysis suggests that in the lineage leading to jawed vertebrates, there were three preotic LLPs and three postotic LLPs (Fig. 2.11; see Northcutt 2005a). The anterodorsal, anteroventral, and otic LLPs (the latter not to be confused with the inner ear-forming otic placode) are preotic, whereas the middle, supratemporal, and posterior LLPs (the latter forming the trunk lateral line) are postotic (Fig. 2.11; see Northcutt 2005a). The posterior placodal area also includes precursors of the epibranchial placodes, which form at

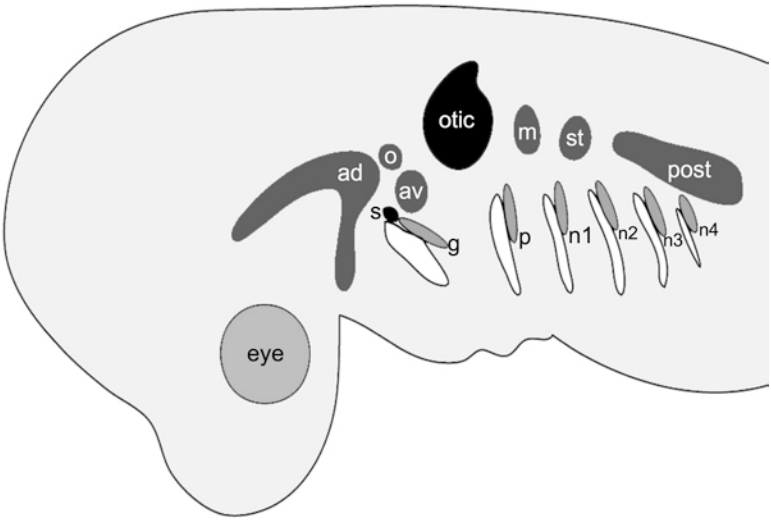

Fig. 2.11 An idealized cartilaginous fish embryo showing the relative positions of the placodes arising from the posterior placodal area. Dorsally, these are the placodes that form hair cells and their afferent neurons: the otic placode (which forms the inner ear, shown here at the otic vesicle stage), three preotic lateral line placodes (LLPs; anterodorsal, shown here as elongating to form the supraorbital and infraorbital sensory ridges, plus anteroventral and otic LLPs), three postotic LLPs (middle, supratemporal, and posterior), and the spiracular/paratympanic organ placode. The latter forms immediately dorsal to the geniculate placode, the first in the series of epibranchial placodes that develop dorsocaudal to each pharyngeal cleft (geniculate, petrosal, and nodose). ad, Anterodorsal; av, anteroventral; g, geniculate; m, middle; n, nodose; o, otic; p, petrosal; post, posterior; s, spiracular; st, supratemporal. Redrawn and modified from O'Neill et al. (2012)

the dorsolateral edge of each pharyngeal cleft (Fig. 2.11; see Schlosser 2010) and give rise to the gustatory and viscerosensory afferent neurons located in the distal ganglia of cranial nerves VII (geniculate), IX (petrosal), and X (nodose). Finally, in some extant jawed vertebrates, an additional "spiracular/paratympanic organ" placode develops immediately dorsal to the first epibranchial (geniculate) placode (Fig. 2.11); this is distinct from the LLP series and forms the hair cell-containing spiracular organ of nonteleost fishes and paratympanic organ of amniotes and associated afferent neurons (O'Neill et al. 2012).

Fate-mapping studies in chicken and African clawed frog (*Xenopus laevis*) embryos have shown that the cranial placodes originate from an "inverted U"/"horseshoe"-shaped ectodermal territory surrounding the rostral (anterior) neural plate, the "preplacodal ectoderm" (see Saint-Jeannet and Moody 2014; Schlosser 2014). This is defined by coexpression of members of the Six family of homeodomain transcription factors (encoded by vertebrate homologues of *Drosophila sine oculis*) and its transcriptional coactivator of the Eya family (encoded by vertebrate homologues of *Drosophila eyes absent*), whose expression is maintained in individual cranial placodes (Saint-Jeannet and Moody 2014; Schlosser 2014). The preplacodal ectoderm is likely to be a domain of competence to form placodes in response to local signals (see Schlosser 2010). Current models suggest that signals secreted from surrounding tissues (neural plate and endomesoderm) lead to the subdivision of the preplacodal ectoderm into three "multiplacodal" domains along the rostrocaudal axis, distinguished by the expression of different combinations of transcription factors (with some species-specific differences), within which individual cranial placodes are specified in response to more localized signaling from adjacent tissues (see Saint-Jeannet and Moody 2014; Schlosser 2014). The adenohypophysis, olfactory, and lens placodes develop from an anterior (rostral) Pax6/Otx2-positive domain; the profundal/trigeminal placodes develop from an intermediate Pax3/Otx2-positive domain, whereas the hair cell-forming placodes (otic, lateral line, and spiracular organ/paratympanic organ placodes, where present) and the epibranchial placodes develop from the Pax2/Sox2/Sox3/Gbx2-positive posterior placodal area (Saint-Jeannet and Moody 2014; Schlosser 2014).

2.2.2 Nonteleost Ampullary Organs Develop from Lateral Line Placodes that Elongate to Form Sensory Ridges

As noted in Sect. 2.2.1, extensive experimental evidence from grafting and ablation studies in urodele and anuran amphibian embryos plus recent genetic lineage-tracing work in zebrafish embryos has shown that neuromasts and their afferent neurons originate from LLPs (see Schlosser 2002a; Piotrowski and Baker 2014). Researchers only turned their attention to the developmental origin of ampullary organs in the last decade of the twentieth century. Histology and scanning electron microscopy in the axolotl (*Ambystoma mexicanum*, a urodele amphibian) suggested that all LLPs

except the posterior (which migrates onto the trunk) elongate to form "sensory ridges" (Northcutt et al. 1994). A row of neuromasts forms along the center of each ridge whereas ampullary organs differentiate later, on the flanks of the ridge (Fig. 2.12; Northcutt et al. 1994). LLP ablations and grafting experiments between pigmented and albino axolotl embryos subsequently confirmed that an individual-elongating LLP forms ampullary organs, neuromasts, and their afferent neurons (Northcutt et al. 1995). Focal labeling experiments using the fluorescent lipophilic dye DiI yielded the same results in a chondrostean ray-finned bony fish (the Mississippi paddlefish, *Polyodon spathula*; Modrell et al. 2011) and a cartilaginous fish (the little skate, *Leucoraja erinacea*; Gillis et al. 2012). Hence, ampullary organs originate from elongating LLPs in all major jawed vertebrate groups (Fig. 2.2).

Before LLPs begin elongating or migrating, neuroblasts delaminate from the pole nearest the otic vesicle and coalesce into individual ganglia that may fuse with other lateral line and/or other nearby ganglia (see Piotrowski and Baker 2014). The axons of these afferent neurons (and associated Schwann cells) track the primordium and innervate neuromast hair cells as they form (Fig. 2.12). First studied in amphibian embryos (see Piotrowski and Baker 2014), this has been investigated in depth for the migrating posterior LLP in zebrafish, where different transgenic lines and vital dye labeling can be combined to label hair cells, axons, and Schwann cells with different fluorescent reporters (e.g., Gilmour et al. 2004; Pujol-Martí et al. 2014). Efferents for neuromast hair cells, originating from hindbrain motor nuclei, reach their targets by following the sensory lateral line nerve (see Piotrowski and Baker 2014).

2.2.3 Experimental Evidence Is Lacking for the Embryonic Origin of Lamprey and Teleost Electroreceptors

In contrast to nonteleost jawed vertebrates, experimental evidence is lacking for the embryonic origin of both lamprey electroreceptors (see Sect. 2.1.2.1) and the various independently evolved teleost electroreceptors (see Sect. 2.1.2.3). There is strong support for the homology of lamprey and nonteleost jawed vertebrate electroreceptors from both innervation (projecting to the dorsal octavolateral nucleus via the dorsal root of the anterior lateral line nerve) and physiology, namely, stimulation by low-frequency cathodal electric fields (Bullock et al. 1983; Baker et al. 2013). Given this, it is likely that lamprey electroreceptors are also LLP derived, but this remains to be tested. The contribution of lamprey cranial placodes to neurons in cranial ganglia, including lateral line ganglia, has been fate mapped by vital dye labeling in the sea lamprey, *Petromyzon marinus* (Modrell et al. 2014). However, neuromasts have only been reported much later, at ammocoete larval stages (Gelman et al. 2007). Ammocoete larvae respond to weak cathodal electric fields (Ronan 1988), suggesting that electroreceptors, thought to be epidermal multivillous cells (see Sect. 2.1.2.1; Jørgensen 2005), are also present. It will be important to identify

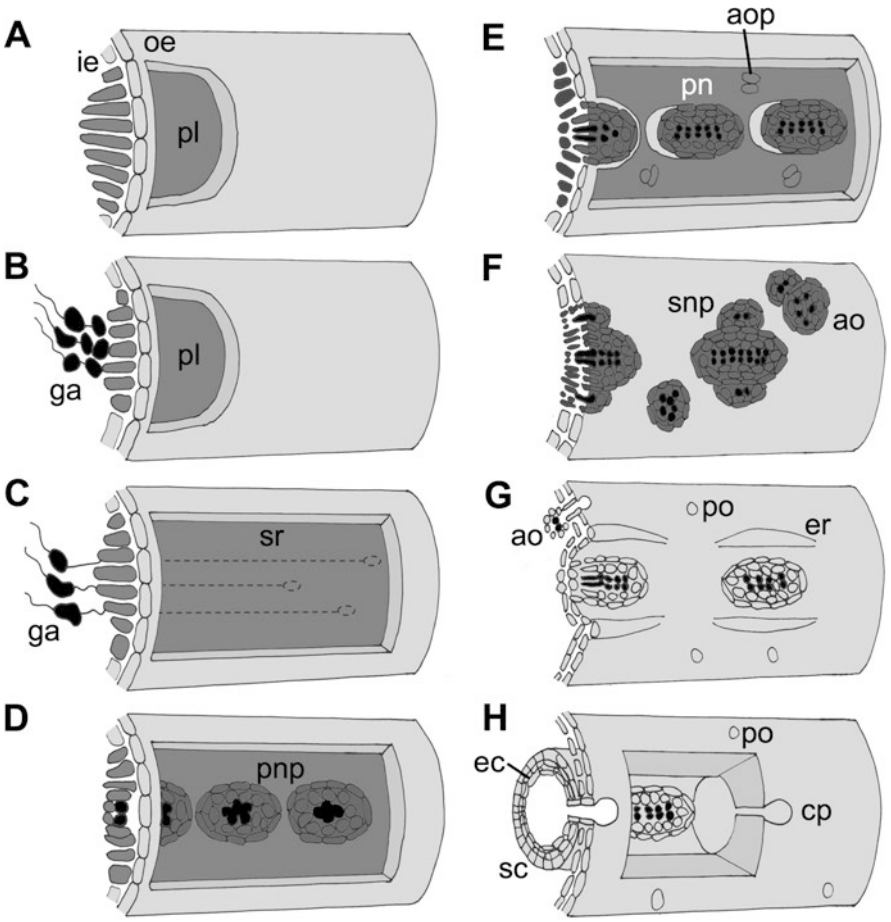

Fig. 2.12 Stages in the development of a LLP that forms ampullary organs as well as neuromasts. **A:** formation of the placode, i.e., a patch of columnar ectoderm within the inner ectodermal layer (in species with a bilayered ectoderm). **B:** neuroblasts delaminate from the placode and differentiate to form the afferent neurons of the lateral line ganglion. **C:** the placode elongates to form a sensory ridge, accompanied by the axons of lateral line afferent neurons. **D:** primary neuromast primordia form in a line along the center of the ridge. **E:** ampullary organ primordia form later, on the flanks of the ridge. **F:** first neuromasts and then ampullary organs erupt to the surface, following which secondary organs form by budding from the mantle zones of the primary organs. **G:** ectodermal ridges develop parallel to the neuromast lines, while ampullary organs invaginate. **H:** eventually, the neuromasts are enclosed within a primary ectodermal canal surrounded by a secondary connective tissue canal, with pores at the surface between adjacent neuromasts. ao, Ampullary organ; aop, ampullary organ primordium; cp, canal pore; ec, epithelial canal; er, ectodermal ridge; ga, ganglionic cells of lateral line nerve; ie, inner layer of ectoderm; oe, outer layer of ectoderm; pa, placode; pn, primary neuromast; pnp, primary neuromast primordium; po, ampullary pore; sc, secondary connective tissue canal; snp, secondary neuromast primordium; sr, sensory ridge. Redrawn and modified from Northcutt et al. (1994), ©1994 Wiley-Liss, Inc., with permission from John Wiley & Sons, Ltd

when lamprey neuromasts and electroreceptors form and to undertake longer term fate-mapping studies.

Similarly, fate-mapping experiments are needed to confirm the embryonic origin of teleost electroreceptors (see Sect. 2.1.2.3). Descriptive studies in mormyrid (osteoglossomorph) embryos suggested that all electroreceptors develop directly from basal epidermal cells (see Kirschbaum and Denizot 2011). However, apart from the large posterior (trunk) LLP, teleost LLPs are difficult to identify without molecular markers (see Northcutt 2005a). Lateral line nerves can always be identified before lateral line organs, leading some to suggest that nerves locally induce both cranial neuromasts and electroreceptors from surface ectoderm in ostariophysan (siluriform and gymnotiform) fishes (see Northcutt 2005a). Roth (2003) reported that unilateral ablation of the posterior lateral line nerve in siluriform (Wels catfish, *Silurus glanis*) embryos prevented electroreceptor development in trunk and tail skin without affecting neuromast development. In contrast, the only confirmed role for innervation in the mechanosensory lateral line is for postembryonic organ maintenance and for the "budding" of secondary neuromasts from primary neuromasts (see Piotrowski and Baker 2014). In the gymnotiform *Eigenmannia* (unidentified species), both ampullary and tuberous electroreceptors develop "adjacent to" the neuromast lines after they have formed, whereas in the channel catfish, *Ictalurus punctatus*, ampullary organs develop within the "lateral zones" of the sensory ridges formed by elongating LLPs on the head (see Northcutt 2005a). This was precisely what was observed using the same methods in axolotl embryos in which the LLP origin of ampullary organs was subsequently confirmed by ablation and grafting studies (see Northcutt 2005a). Fate-mapping studies of electroreceptive teleost LLPs are long overdue.

2.2.4 The Molecular Control of Lateral Line Placode Formation

2.2.4.1 Lateral Line and Otic Placodes Are Developmentally Independent

The Pax2/Sox2/Sox3/Gbx2-positive posterior placodal area adjacent to the hindbrain, within which the otic, lateral line, and epibranchial placodes develop (Fig. 2.11; see Sect. 2.2.1), is induced by fibroblast growth factor (FGF) signaling from endomesoderm and/or the hindbrain (see Saint-Jeannet and Moody 2014; Schlosser 2014). Within the posterior placodal area, Wnt signaling from the caudal hindbrain is required to specify an otic placode fate dorsally, whereas sustained FGF signaling from outpocketing pharyngeal pouch endoderm, which contacts the overlying ectoderm, specifies an epibranchial placode fate more ventrally (Fig. 2.11; Saint-Jeannet and Moody 2014; Schlosser 2014). In contrast, relatively little is known about the molecular control of LLP formation.

Transplantation studies in amphibian embryos showed that LLP induction is experimentally separable and temporally distinct from otic placode induction and

likely involves both the underlying mesoderm and the adjacent hindbrain (Schlosser 2002a). Recent experiments in zebrafish showed that a higher level of FGF signaling was needed for preotic LLP formation than for otic placode formation and that Wnt signaling (which, as noted earlier, specifies an otic placode fate) blocked the formation of both "anterior" (preotic) LLPs and the postotic posterior LLP (Nikaido et al. 2017). (Other postotic LLPs were not examined.) The specific FGF ligands required for preotic LLP formation remain unknown, as does their source, although possible candidates are FGF3 and FGF8, which are produced at the midbrain-hindbrain boundary and by the underlying mesendoderm at relevant stages (Nikaido et al. 2017).

The developmental independence of lateral line versus otic placodes plausibly underlies the evolutionary loss of LLPs in amniotes and some direct-developing frogs. The failure of LLPs to form in the common coqui (*Eleutherodactylus coqui*), for example, was shown (by reciprocal heterospecific grafting experiments between this direct-developing frog and the axolotl) to result not from the loss of LLP-inducing signals but from the loss of competence in head ectoderm to respond to such signals (Schlosser et al. 1999).

2.2.4.2 Different Lateral Line Placodes Have Different Molecular Requirements

Experiments in both zebrafish and axolotl have revealed significant heterogeneity in the molecular mechanisms underlying the formation of different LLPs. FGF signaling, although necessary for the formation of preotic LLPs in zebrafish (Sect. 2.2.4.1), inhibits the formation of the postotic posterior LLP (Nikaido et al. 2017). (Other postotic LLPs were not examined.) Retinoic acid treatment of late blastula-stage zebrafish embryos resulted in only one-third the normal number of LLP-derived neurons forming in the (preotic) anterior lateral line ganglion, but threefold more neurons in the posterior lateral line ganglion (Holder and Hill 1991). (Neuromasts were not examined.) This suggested that excess retinoic acid signaling reduced the preotic LLPs but expanded the posterior LLP. Similarly, experiments in which retinoic acid synthesis was blocked showed that retinoic acid is required in zebrafish at late gastrulation stages for the formation of the posterior LLP (Sarrazin et al. 2010; Nikaido et al. 2017) but not the preotic LLPs (Nikaido et al. 2017). (Other postotic LLPs were not examined.) In the axolotl, in which all LLPs except the posterior form ampullary organs as well as neuromasts and neurons (Northcutt et al. 1994), retinoic acid treatment at late gastrula/early neurula stages resulted in the loss of all ampullary organs and significantly fewer neuromasts but a larger posterior lateral line ganglion (Gibbs and Northcutt 2004b). This suggested that excess retinoic acid signaling reduced all ampullary organ-forming LLPs (i.e., all LLPs except the posterior) but expanded the posterior LLP. Taken together, these data suggest that the response to retinoic acid differs between the postotic posterior LLP (which migrates on the trunk) and all the other LLPs (which migrate/elongate on the head) rather

than differing between preotic and postotic LLPs. However, this hypothesis must be tested further.

Molecular differences in the induction of individual LLPs could explain the evolutionary loss of specific LLPs in different lineages. Within amphibians, the (preotic) otic LLP is missing in all anurans, some urodeles, and some caecilians (Schlosser 2002a; Northcutt 2005a). Within the teleosts, the postotic supratemporal LLP is missing in the channel catfish (Northcutt 2005a). Although neither otic nor supratemporal LLPs have been identified in the zebrafish (Andermann et al. 2002), they presumably exist because both the otic and supratemporal neuromast lines form (Raible and Kruse 2000). In axolotl embryos, the homeobox transcription factor gene *Hoxb3* is expressed specifically in the postotic middle LLP and the immediately adjacent hindbrain region (Metscher et al. 1997), although it is unknown whether Hoxb3 plays any role in LLP development.

Another question relates to the mechanisms underlying the formation of ampullary organs by different subsets of LLPs in different species. For example, ampullary organs are formed only by the preotic LLPs in the Mississippi paddlefish (Modrell et al. 2011), by the preotic LLPs plus the postotic supratemporal LLP in another chondrostean, the sturgeon *Scaphirhynchus platorynchus* (Gibbs and Northcutt 2004a), and by all LLPs except the posterior LLP in the axolotl (Northcutt et al. 1994).

2.2.5 Investigating the Molecular Basis of Nonteleost Electroreceptor Development

2.2.5.1 The Candidate Gene Approach

The candidate gene approach, based primarily on knowledge of the molecular basis of LLP development in nonelectroreceptive species, has identified some markers for developing ampullary organs and electroreceptors in nonteleost jawed vertebrates and signaling pathways likely to be important for ampullary organ development. The limited results obtained suggest significant parallels, but also some differences, across different vertebrate groups.

2.2.5.1.1 Transcriptional Regulators

In nonteleost ray-finned bony fishes, the first molecular marker identified for developing ampullary organs was the high mobility group (HMG) domain transcription factor gene *Sox3* in a chondrostean, the Mississippi paddlefish (see Baker et al. 2013). *Sox3* is expressed in the LLP-forming posterior placodal area (see Sects. 2.2.1 and 2.2.4.1) and throughout LLP development in the nonelectroreceptive African clawed frog (Schlosser and Ahrens 2004). *Sox3* is also expressed in the LLPs of two unrelated nonelectroreceptive teleosts, the medaka (*Oryzias latipes*, a

percomorph euteleost) and the zebrafish (a cypriniform ostariophysan; Fig. 2.6; Köster et al. 2000; Nikaido et al. 2007). In the Mississippi paddlefish, *Sox3* also proved to be expressed in the posterior placodal area and maintained throughout LLP development, including in developing ampullary organ fields and ampullary organs as well as in neuromasts (see Baker et al. 2013).

In an attempt to clone the "pan-placodal" marker *Eya1* (see Sect. 2.2.1) in a cartilaginous fish (the small-spotted catshark, *Scyliorhinus canicula*), the related family member *Eya4* was cloned; this was fortuitous because *Eya4* proved to be a specific marker for LLPs (and the otic placode) throughout their development, including in ampullary organs as well as in neuromasts (O'Neill et al. 2007). This expression pattern is conserved across all jawed vertebrates, i.e., in a ray-finned bony fish (the Mississippi paddlefish) and a lobe-finned bony tetrapod (the axolotl), as well as in another cartilaginous fish, the little skate (see Baker et al. 2013). Furthermore, immunostaining for the calcium-buffering protein "Pv3" (Heller et al. 2002), an oncomodulin-related β-parvalbumin (Pvalbβ1/Ocm; Modrell et al. 2017a) that is thought to be the major Ca^{2+} buffer in hair cells (Heller et al. 2002), revealed that *Eya4* expression in neuromasts and ampullary organs is restricted, respectively, to hair cells and electroreceptor cells in the Mississippi paddlefish, axolotl, and little skate (see Baker et al. 2013).

The pan-placodal homeodomain transcription factor gene *Six1* and its transcription coactivator gene *Eya1*, which are expressed from preplacodal stages and maintained in all cranial placodes and their derivatives except the lens in the African clawed frog (Schlosser and Ahrens 2004), are also expressed, as might be expected, in paddlefish LLPs, neuromasts, and ampullary organs, as well as in other cranial placodes (see Baker et al. 2013). Indeed, *Six1, Six2, Six4*, and all four *Eya* family members are expressed throughout the development of paddlefish LLPs, including in lateral line organs (see Baker et al. 2013).

Conserved expression across vertebrate groups is not seen for all genes, however. A study of homeobox gene expression in axolotl embryos, undertaken to test the hypothesis that a "*Hox* code" might pattern cranial placodes at different dorsoventral and rostrocaudal axial levels, identified *Msx2* and *Dlx3* expression throughout the development of all LLPs, including in neuromasts and ampullary organs, and *Hoxb3* expression specifically in the postotic middle LLP (Metscher et al. 1997). However, it was noted (although without showing any data) that *Msx2* and *Dlx3* are not expressed during lateral line organ development in the Mississippi paddlefish (Modrell and Baker 2012). Similarly, expression of the T-box transcription factor gene *Tbx3*, reported specifically in LLPs in the African clawed frog (Schlosser and Ahrens 2004), is restricted to LLP-derived neurons in the small-spotted catshark (O'Neill et al. 2007).

Overall, perhaps the most significant finding of the candidate gene approach was the conserved expression of *Eya4* throughout LLP (and otic placode) development specifically, and its maintenance in hair cells and electroreceptors, across the three major groups of jawed vertebrates (see Baker et al. 2013). *Eya4* encodes one of the four members of the Eya family of transcription coactivators, which also have phos-

phatase activity and cytoplasmic roles (see Rebay 2015). In the African clawed frog, high levels of the pan-placodal family member Eya1 and its transcription partner Six1 promote placode cell proliferation, whereas lower levels promote neuronal and sensory differentiation (Schlosser et al. 2008; Riddiford and Schlosser 2016, 2017). Mutations in human *EYA4* underlie nonsyndromic sensorineural hearing loss *DFNA10* (Schönberger et al. 2005). Together, these data suggest that Eya4 is likely to play both early and late roles in LLP development and in the differentiation of both electroreceptors and neuromast hair cells. Testing this hypothesis will require blocking Eya4 function in an experimentally tractable electroreceptive species, such as the axolotl. Genome editing using CRISPR/Cas9, which efficiently yields mutant phenotypes in axolotl and lamprey embryos injected at the one-cell stage (Flowers and Crews 2015; Square et al. 2015), is an exciting possibility. CRISPR/Cas9 could, in principle, also be used to mutate genes of interest in other electroreceptive species, provided that many fertilized eggs can be obtained during a sufficiently long spawning season to optimize the conditions for that species.

2.2.5.1.2 Signaling Pathways

As described in Sect. 2.2.4, Wnt signaling blocks the formation of both preotic LLPs and the postotic posterior (trunk) LLP in the zebrafish, whereas FGF signaling is required for the formation of preotic LLPs and blocks the formation of the posterior LLP (Nikaido et al. 2017). Nevertheless, both Wnt and FGF signaling are critical during later stages of posterior LLP development, for both neuromast formation and hair cell differentiation. Briefly, during the migration of the posterior lateral line primordium, Wnt activity in the leading domain results in the expression and secretion of FGF3 and FGF10, which activate FGF receptor 1 (FGFR1) in the trailing domain (for detailed reviews, see Thomas et al. 2015; Dalle Nogare and Chitnis 2017). Signaling through FGFR1 drives expression in the central cell of the proneural transcription factor gene *Atoh1* (required for hair cell formation in both the inner ear and lateral line; Millimaki et al. 2007; Costa et al. 2017) and the Notch ligand gene *DeltaA*. Atoh1 expression specifies the central cell as a hair cell progenitor and drives expression of a second Notch ligand gene, *DeltaD*, plus *Fgf10*. FGF10 secreted by the hair cell progenitor activates FGFR1 in its neighbors. This maintains the expression of Notch3, which, in turn, is activated by the Notch ligands expressed by the hair cell progenitor, resulting in the inhibition of *Atoh1* expression and thus of a hair cell fate ("lateral inhibition") in its neighbors. Furthermore, Notch and FGF signaling promote cell adhesion and apical constriction in the supporting cells, leading to the formation of "protoneuromasts," namely, epithelial rosettes of supporting cells around a central hair cell progenitor (see Thomas et al. 2015; Dalle Nogare and Chitnis 2017).

Only the migrating posterior LLP of the zebrafish has been studied in such detail. It is not known to what extent the roles played by these pathways are conserved even within the other LLPs of the zebrafish, let alone across the LLPs of

other teleost and nonteleost species. A recent study using small-molecule inhibitors took the first steps in this endeavor by investigating the roles of FGF and Notch signaling during the development of ampullary organs and neuromasts from elongating preotic lateral line primordia in the Mississippi paddlefish (Modrell et al. 2017b). During zebrafish posterior LLP development, inhibiting FGF signaling blocks *Atoh1* expression and hence blocks hair cell differentiation and also protoneuromast (epithelial rosette) formation (Thomas et al. 2015; Dalle Nogare and Chitnis 2017). In the Mississippi paddlefish, inhibiting Fgf signaling during placode elongation stages resulted in the formation of fewer neuromasts, but with more hair cells than usual, and accelerated (rather than blocked) the formation of ampullary organs, each of which had many more electroreceptor cells than during normal development (Modrell et al. 2017b). Although more work is needed to clarify the specific mechanisms involved, these results nevertheless suggest significant differences in the roles of FGF signaling in neuromast versus ampullary organ formation from elongating LLPs and also in neuromast formation from elongating versus migrating primordia.

In the migrating zebrafish posterior LLP, inhibiting Notch signaling expands the domain of *Atoh1* expression, which, in turn, causes a reduction in FGF signaling that blocks protoneuromast maturation (see Thomas et al. 2015; Dalle Nogare and Chitnis 2017). In the Mississippi paddlefish, blocking Notch signaling just before and during sense organ formation resulted in the formation of irregularly spaced neuromasts with supernumerary hair cells and in the clustering of ampullary organs (sometimes resulting in large domains of adjacent ampullary organs) with supernumerary electroreceptors (Modrell et al. 2017b). The supernumerary sensory receptor cell phenotype suggests that Notch signaling normally prevents supporting cells from adopting a sensory receptor cell fate in both ampullary organs and neuromasts, consistent with the data from zebrafish (see Thomas et al. 2015; Dalle Nogare and Chitnis 2017). However, the formation of neuromasts and ampullary organs with abnormal spacing after blocking Notch signaling in the Mississippi paddlefish differs from the failure of protoneuromast (epithelial rosette) maturation seen after blocking Notch signaling in zebrafish (Thomas et al. 2015; Dalle Nogare and Chitnis 2017).

Overall, the limited data gathered thus far from small-molecule inhibitor experiments in the Mississippi paddlefish (Modrell et al. 2017b) suggest that, apart from the importance of Notch signaling for preventing supporting cells from differentiating as sensory receptor cells, both FGF and Notch signaling may play different roles in the development of ampullary organs versus neuromasts from elongating LLPs and in neuromast development from elongating versus migrating LLPs. Before any conclusions can be drawn about conservation of mechanisms, it is essential to gather experimental evidence from more species as outgroups, both for the migrating posterior lateral line primordium (e.g., are the mechanisms identified in zebrafish conserved in the African clawed frog and/or the axolotl?) and for preotic LLPs, both from nonelectroreceptive species like the zebrafish and the African clawed frog as well as electroreceptive species like the Mississippi paddlefish and axolotl.

2.2.5.2 Insights from an Unbiased Transcriptomic (Differential RNA Sequencing) Approach

The candidate gene approach described in Sect. 2.2.5.1, i.e., studying in electroreceptive species the genes and signaling pathways identified in nonelectroreceptive species as being important for LLP and/or neuromast formation, can and has been fruitful. However, this approach is less likely to identify the molecular mechanisms required specifically for the development of ampullary organs/electroreceptors. For this, an unbiased transcriptomic approach holds more promise. Differential next-generation RNA sequencing (RNA-seq) analysis in late-larval stages of the Mississippi paddlefish generated a dataset of several hundred candidate genes that are putatively enriched in lateral line organs (Modrell et al. 2017a). Validation of a subset of these candidates in the Mississippi paddlefish revealed that critical components of the transcription factor network essential for hair cell development (see Costa et al. 2017), in particular, the basic helix-loop-helix (bHLH) transcription factor gene *Atoh1* and the POU-domain transcription factor gene *Pou4f3* (*Brn3c*), were expressed in developing ampullary organs as well as in neuromasts (Modrell et al. 2017a). Atoh1 is essential for the differentiation not just of hair cells but also, for example, of cerebellar granule neurons, Merkel cells and proprioceptive neurons, and intestinal secretory cells (Costa et al. 2017). Hence, the developmental context within which Atoh1 acts (for example, which other transcription factors are expressed) is critical for the phenotypic outcome; however, relatively little is known about how Atoh1 acts to promote hair cell development (Costa et al. 2017).

As a class II bHLH transcription factor, Atoh1 binds DNA as a heterodimer with a class I bHLH ("E-protein") binding partner for which it competes with other class II bHLH transcription factors (see Costa et al. 2017). Intriguingly, mouse embryonic stem cells develop as neurons when forced to express Atoh1 but form hair cell-like cells when forced to express Atoh1 plus Pou4f3 and the zinc-finger transcriptional repressor Gfi1 (see Costa et al. 2017). Pou4f3 and Gfi1 are each required for normal hair cell differentiation and survival (see Costa et al. 2017). Gfi1 is the vertebrate ortholog of *Drosophila* Senseless, which directly binds (via its zinc fingers) to proneural bHLH transcription factors, including the Atoh1 ortholog Atonal, modulating the transcriptional activity of both proteins (see Costa et al. 2017). The mouse embryonic stem cell data suggest that Gfi1 and Pou4f3 together somehow transform Atoh1 from a neuronal determinant to a hair cell determinant (see Costa et al. 2017). *Gfi1* is present in the lateral line organ-enriched dataset from the Mississippi paddlefish (Modrell et al. 2017a), although its expression has not yet been examined. The LIM homeodomain transcription factor Lhx3, which is expressed in all inner ear hair cells and regulated by Pou4f3 (Hertzano et al. 2007), was also expressed in developing ampullary organs as well as neuromasts in the Mississippi paddlefish (Modrell et al. 2017a).

Similarly, the HMG domain transcription factor Sox2, which interacts with Six1 (and/or Six4) and its transcriptional coactivator Eya1 in a physical complex that is sufficient to induce *Atoh1* in mouse cochlear explants (Ahmed et al. 2012; Zhang et al. 2017), was expressed in both developing ampullary organs and neuromasts in

the Mississippi paddlefish (Modrell et al. 2017a). In the mouse cochlea, Six1 activity is also required later to downregulate Sox2 expression (Zhang et al. 2017), enabling Atoh1 to drive hair cell differentiation (Dabdoub et al. 2008; Zhang et al. 2017). *Six1*, *Six4,* and *Eya1* (together with *Six2*, *Eya2*, *Eya3,* and *Eya4*) had previously been reported as being expressed throughout LLP development in the Mississippi paddlefish, including in developing ampullary organs as well as in neuromasts (see Baker et al. 2013). Furthermore, the *miR-183* family of microRNAs (*miR-183*, *miR-96,* and *miR-182*, processed from a single transcript), which are important for hair cell development and maintenance (Soukup 2009; Weston and Soukup 2009), are Atoh1 dependent in hair cells and may also be involved in downregulating *Sox2* expression (Weston et al. 2011, 2018) and fine-tuning the transcriptional response to Atoh1 in favor of hair cells (Ebeid et al. 2017). This family of microRNAs is expressed by axolotl electroreceptors as well as hair cells (Pierce et al. 2008).

Taken together, these data suggest that the molecular mechanisms underlying electroreceptor development are highly conserved with those underlying hair cell development, although functional experiments are needed to confirm this. The level of conservation also begs the question of how electroreceptors are specified as opposed to hair cells. The lateral line organ-enriched dataset from the Mississippi paddlefish provided one candidate: the proneural bHLH transcription factor gene *Neurod4* (*Ath3*, *NeuroM*), which was expressed in developing ampullary organs but not in neuromasts (as well as in sites expected from other species, including the brain, olfactory epithelium, eyes, and trigeminal ganglion; Modrell et al. 2017a). Neurod4 could specify an electroreceptor fate given its role in specifying other cell fates. In the retina, Neurod4 cooperates with the bHLH transcription factor Ascl1 (Ash1) and the homeodomain transcription factor Vsx2 (Chx10) to determine bipolar cell fate and is required together with the related bHLH transcription factor Neurod1 to specify amacrine cells (Hatakeyama and Kageyama 2004). Furthermore, different Neurod family members may be important for specifying different subtypes of hair cells. Neurod1 prevents otic neurons from expressing Atoh1 and adopting a hair cell fate and is required for the maturation of outer hair cells in the cochlea (Jahan et al. 2010), whereas Neurod6 is enriched in cochlear but not in vestibular hair cells (Elkon et al. 2015). Further studies are needed to determine the role(s) played by Neurod4 in electroreceptor development, the identity of its transcriptional partners, and whether or not this is conserved in developing ampullary organs outside chondrostean ray-finned fishes.

Overall, both the candidate gene (see Sect. 2.2.5.1.1) and unbiased transcriptomic (RNA-seq) approaches suggest that the molecular mechanisms underlying nonteleost electroreceptor development are likely to be highly conserved with those that underlie hair cell development. In particular, essentially all the transcription factor genes known to be important for hair cell development are also expressed in developing ampullary organs in the Mississippi paddlefish (Modrell et al. 2017a). This very close developmental relationship may also support a close evolutionary relationship between these cell types, as discussed in Sect. 2.3.

2.3 Electroreceptor Evolution

The homology of electroreceptors in lampreys and nonteleost jawed vertebrates is supported by both physiology and innervation: they are stimulated by weak, low-frequency cathodal (exterior-negative) electric fields (and inhibited by anodal fields) and innervated by lateral line afferents projecting to the dorsal octavolateral nucleus via the dorsal root of the anterior lateral line nerve (Bullock et al. 1983; Baker et al. 2013). As described in Sect. 2.2.2, fate-mapping experiments have shown that in representatives of the three major clades of jawed vertebrates (Fig. 2.2) individual LLPs give rise to ampullary organs as well as to neuromasts and lateral line neurons (see Baker et al. 2013). Furthermore, within these three clades, nonteleost ampullary electroreceptors and neuromast hair cells maintain expression of the transcriptional coactivator gene *Eya4* and express the calcium-buffering protein "Pv3" (see Baker et al. 2013), an oncomodulin-related β-parvalbumin (Pvalbβ1/Ocm; Modrell et al. 2017a). It will be important to extend the LLP fate-mapping and molecular studies to lampreys. Nevertheless, the shared physiology and innervation of lamprey and nonteleost jawed vertebrate electroreceptors support their being homologous, i.e., that the electrosensory division of the lateral line system evolved once, in the lineage leading to the common ancestor of all living vertebrates. The independent evolution of teleost electroreception is discussed in Sect. 2.1.2.3.2.

As proposed by Jørgensen (1982), electroreceptors could have evolved via the modification of hair cells. Alternatively, electroreceptors and hair cells could have evolved independently from a ciliated secondary sensory cell, which itself likely evolved via the diversification of an ancestral primary sensory neuron (Jørgensen 1982; also see Fritzsch and Elliott 2017). Sections 2.3.1 and 2.3.2 discuss the similarities between nonteleost electroreceptors and hair cells, then Sect. 2.3.3 brings these together to discuss hypotheses for electroreceptor evolution.

2.3.1 Morphological and Physiological Similarities Between Hair Cells and Nonteleost Electroreceptors

As noted in Sect. 2.1, hair cells and nonteleost ampullary electroreceptors are secondary sensory cells (i.e., lacking an axon), with basolateral presynaptic ribbons and a single apical primary cilium surrounded by varying numbers of actin-rich microvilli and basolateral ribbon synapses (Fig. 2.1; also see Sect. 2.3.2; Jørgensen 2005). Lamprey adult end bud electroreceptors and ammocoete-stage multivillous cells share all these characteristics except for the primary cilium (Fig. 2.4; Jørgensen 2005). However, the development of lamprey electroreceptors has not been characterized, so it is possible that an apical cilium forms but is subsequently lost, as occurs during mammalian cochlear hair cell development (Lu and Sipe 2016).

During hair cell maturation, the primary cilium (kinocilium) moves eccentrically and the apical microvilli (stereocilia) elongate in a graded fashion such that they

become organized into rows in a staircase array; they are connected at their distal tips by tip links and to the kinocilium by kinociliary links (Fig. 2.1A; see Lu and Sipe 2016). This stepped, linked array of stereocilia comprises the "hair bundle" that characterizes hair cells. Deflection of the hair bundle in the direction of the kinocilium (or of the tallest stereocilia in mammalian cochlear hair cells) increases tension on the tip links, which triggers the opening of cation-selective mechanoelectrical transducer channels (Nicolson 2017; Cunningham and Müller 2019). Cation entry depolarizes the hair cell, opening L-type voltage-gated Ca^{2+} ($Ca_v1.3$) channels clustered in the basolateral membrane at presynaptic ribbons (Safieddine et al. 2012; Nicolson 2015). Ca^{2+} entry via these $Ca_v1.3$ channels leads to synaptic vesicle exocytosis and neurotransmitter release (see Safieddine et al. 2012; Nicolson 2015).

Until 2017, the most detailed information about how nonteleost electroreceptors work had been gathered using ampullary organ preparations from various skate species (Bennett and Obara 1986; Bodznick and Montgomery 2005; also see Leitch and Julius, Chap. 3). Briefly, the electroreceptors are partially depolarized at rest by an inward "bias current," resulting in constant neurotransmitter release and tonic activity of the afferent fibers. Weak cathodal (exterior-negative) stimuli open apical voltage-gated Ca^{2+} channels, depolarizing the apical membrane and, in turn, depolarizing the basal membrane. This opens basal voltage-gated Ca^{2+} channels, leading to Ca^{2+} entry and neurotransmitter release, thus increasing spike frequency. Apical Ca^{2+} entry ultimately triggers a Ca^{2+}-activated outward K^+ current, repolarizing the apical membrane and terminating the depolarization of the basal membrane.

In both the little skate and the chain catshark (*Scyliorhinus retifer*), the $Ca_v1.3$ channel was identified as the apical low-threshold voltage-sensing Ca^{2+} channel (Bellono et al. 2017, 2018; also see Leitch and Julius, Chap. 3). In the little skate, as predicted from earlier work (Bennett and Obara 1986; Bodznick and Montgomery 2005), the Big Potassium (BK) channel is the large-conductance Ca^{2+}-activated K^+ channel working with the $Ca_v1.3$ channel to mediate electroreceptor membrane oscillations (Bellono et al. 2017). Although chain catshark electroreceptors express *Kcnma1*, which encodes BK, oscillations in this species are mediated by the voltage-gated K^+ channel $K_v1.3$, encoded by *Kcna3* (Bellono et al. 2018).

Specific channels involved in electroreceptor function have not been identified in bony fishes. However, analysis of the lateral line organ-enriched RNA-seq dataset from the Mississippi paddlefish showed that *Cacna1d*, encoding the $Ca_v1.3$ channel, is expressed in ampullary organs as well as in neuromasts (see also Sect. 2.3.2) and that *Kcna5*, encoding the $K_v1.5$ channel, and *Kcnab3*, encoding the β-subunit $K_vβ3$, are ampullary organ-specific (Modrell et al. 2017a). These expression data suggest the hypothesis, which remains to be tested, that the $Ca_v1.3$ and $K_v1.5$ channels mediate electroreceptor membrane oscillations in the Mississippi paddlefish, like the $Ca_v1.3$ and $K_v1.3$ channels do in the chain catshark (Bellono et al. 2018).

The BK channel has been localized to the primary cilium of both olfactory receptor neurons and principal cells in the rabbit nephron (Delgado et al. 2003; Carrisoza-Gaytán et al. 2017). Furthermore, the primary cilium of kidney cells is a specialized calcium-signaling organelle containing calcium-permeant channels at a high density, within which the Ca^{2+} concentration is effectively insulated from changes in

cytoplasmic Ca^{2+} (DeCaen et al. 2013; Delling et al. 2013). Other than in lampreys, all nonteleost electroreceptors bear a primary cilium (Jørgensen 2005). It seems plausible, therefore, that the $Ca_v1.3$ and BK channels may be localized to the primary cilium of electroreceptors, although this remains to be tested.

The importance of the $Ca_v1.3$ and BK channels for little skate electroreceptor function (Bellono et al. 2017) further emphasizes similarities between hair cells and nonteleost electroreceptors because basolateral $Ca_v1.3$ channel activity triggers neurotransmitter release at hair cell (but not photoreceptor) ribbon synapses (see Safieddine et al. 2012; Nicolson 2015; also see Sect. 2.3.2), whereas $Ca_v1.3$ and BK channel interaction in the basolateral hair cell membrane mediates electrical "tuning" (Fettiplace and Fuchs 1999). It may also be worth noting that in skate electroreceptors, weak anodal (lumen-positive) stimuli inhibit the resting discharge, but large anodal stimuli directly depolarize the basal membrane, resulting in neurotransmitter release (Bennett and Obara 1986). Similarly, neuromast hair cells release neurotransmitter in response to large anodal stimuli (e.g., Münz et al. 1984; Barry et al. 1988).

2.3.2 RNA Sequencing Data Suggest Nonteleost Electroreceptors Share Synaptic Transmission Mechanisms with Hair Cells

The specific mechanisms underlying transmission at the hair cell ribbon synapse are thought to be unique (see Zanazzi and Matthews 2009; Safieddine et al. 2012). As described in Sect. 2.1.2, all vertebrate electroreceptors have ribbon synapses (see Jørgensen 2005) as do vertebrate retinal photoreceptors, retinal bipolar cells, and pineal photoreceptors (see Zanazzi and Matthews 2009; Safieddine et al. 2012).

The main structural constituent of the presynaptic ribbon (and the only ribbon-specific protein known) is the protein Ribeye, which is generated via an alternative start site for the reduced nicotinamide adenine dinucleotide (NADH)-sensitive transcriptional corepressor gene *Ctbp2*, producing an N-terminal A-domain unique to Ribeye (see Zanazzi and Matthews 2009; Nicolson 2015). In mice lacking Ribeye, ribbons are abolished in the retina (Maxeiner et al. 2016) and cochlear hair cells (Becker et al. 2018; Jean et al. 2018) and synaptic transmission is impaired, confirming that the ribbon is important for rapid synaptic vesicle replenishment. In zebrafish neuromast hair cells, Ribeye protein depletion by morpholino injection and genetic mutation also showed its importance in clustering $Ca_v1.3$ channels at the presynaptic membrane (see Nicolson 2015; Lv et al. 2016).

There are significant differences in the mechanisms underlying synaptic transmission at the hair cell ribbon synapse versus other ribbon synapses (see Zanazzi and Matthews 2009; Safieddine et al. 2012). Hair cell synaptic vesicles are loaded with glutamate by the vesicular glutamate transporter Vglut3, whereas Vglut1 and Vglut2 are used at retinal photoreceptor and bipolar cell ribbon synapses and at central glutamatergic synapses (see Zanazzi and Matthews 2009). As noted in Sect.

2.3.2, synaptic vesicle exocytosis is triggered in hair cells by activation of the $Ca_v1.3$ channel, whose abundance and function is regulated by the auxiliary subunit $Ca_v\beta2$ (Neef et al. 2009), whereas retinal photoreceptors depend on the $Ca_v1.4$ channel (see Zanazzi and Matthews 2009; Nicolson 2015). Finally, synaptic vesicle exocytosis in hair cells is uniquely mediated by the multi-C2 domain transmembrane protein otoferlin, a calcium-sensitive type II ferlin (Hams et al. 2017; Michalski et al. 2017), rather than by neuronal soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs; see Safieddine et al. 2012).

Candidate gene analysis from the lateral line organ-enriched gene set generated by differential RNA-seq analysis in the Mississippi paddlefish (described in Sect. 2.2.5.2) revealed that in this species, late-larval ampullary organs as well as neuromasts express *Slc17a8* (encoding Vglut3), *Cacna1d* (encoding the pore-forming α-subunit of the $Ca_v1.3$ channel), *Cacnb2* (encoding $Ca_v\beta2$), *otoferlin,* and the Ribeye-specific A domain of *Ctbp2* (Modrell et al. 2017a). These expression data suggest that transmission mechanisms at the electroreceptor ribbon synapse are conserved with hair cells, although this remains to be tested.

2.3.3 Hypotheses for Electroreceptor Evolution: The Importance of Ribbons

Recent hypotheses about neurogenic placode evolution take as their starting point the variety of sensory and neurosecretory cell types found in the epidermis of invertebrate chordate filter feeders (i.e., the tunicates, the closest living invertebrate relatives of the vertebrates, and the cephalochordates, the outgroup to the tunicates and vertebrates, representing the most basally branching chordate lineage) and suggest how the evolution of ectodermal patterning in the vertebrate lineage may have enabled the concentration of such sensory and neurosecretory cells in patches on the head (see Patthey et al. 2014; Schlosser et al. 2014). This would be consistent with the cephalization and elaboration of placode-derived sense organs in the vertebrate ancestor, in the transition from filter feeding to predation, as originally proposed by Northcutt and Gans in their "New Head Hypothesis" (see Northcutt 2005b). Similarly, rather than focusing on the evolution of individual hair cell-forming placodes (otic before lateral line or lateral line before otic?), a "hair cell first" hypothesis has been proposed in relation to inner ear evolution, incorporating molecular evidence relating to the development of inner ear hair cells and their afferent neurons (see Fritzsch and Elliott 2017). As noted in Sect. 2.3.1, electroreceptors and hair cells could have evolved as separate, independent diversifications of a ciliated secondary sensory cell or hair cells could have evolved first, with electroreceptors subsequently evolving via the diversification of lateral line hair cells (Jørgensen 1982). (Electroreceptor evolution via lateral line hair-cell diversification most likely also occurred independently at least twice within teleosts; see Sect. 2.1.2.3.2.)

Intriguingly, in tunicates (the sister group of vertebrates), secondary sensory cells with microvilli, one or more apical cilia, afferent glutamatergic synapses, and

at least some gene expression patterns shared with hair cells have been described in the mechanosensory coronal organ of the ascidian oral siphon and in the appendicularian circumoral ring (see Burighel et al. 2011; Rigon et al. 2018). It is feasible that these tunicate secondary sensory cells and vertebrate hair cells evolved from the same mechanosensory cell type (whether a primary sensory neuron or a secondary mechanosensory cell) in the common ancestor of tunicates and vertebrates, i.e., that they are homologous.

A key difference between these tunicate secondary sensory cells and both hair cells and electroreceptors in vertebrates, however, is the absence of presynaptic ribbons (Burighel et al. 2011), which have not been reported in any cells in invertebrate chordates (Petralia et al. 2016). Because cephalochordates (amphioxus species) have homologues of vertebrate retinal and pineal photoreceptors, this suggests that ribbon synapses evolved independently in vertebrate retinal cells, pineal photoreceptors, and hair cells (see Baker and Modrell, 2018). As noted in Sect. 2.3.2, synaptic vesicle loading and exocytosis are mediated by different proteins at hair cell versus retinal ribbon synapses (Zanazzi and Matthews 2009; Safieddine et al. 2012). Electroreceptors are more closely related to hair cells in all these respects, at least as determined by gene expression in the Mississippi paddlefish (Modrell et al. 2017a), as well as in the molecular mechanisms underlying their development (see Sect. 2.2.5). If electroreceptors and hair cells evolved independently in the vertebrate ancestor via separate diversifications of a ciliated secondary sensory cell, then either this cell had already evolved ribbon synapses dependent on $Ca_v1.3/Ca_v\beta2$ channels, otoferlin, and Vglut3 and its development already involved all the molecular mechanisms that seem likely to be shared by hair cells and electroreceptors (see Sect. 2.2.5), or these features evolved independently in both hair cells and electroreceptors. It seems more parsimonious to suggest that electroreceptors evolved in the vertebrate ancestor via the diversification of lateral line hair cells to form a "sister cell type" (*sensu* Arendt et al. 2016). The selection pressure in early vertebrate evolution for the modification of a hair cell such that it depolarizes in response to low-frequency cathodal electric fields, perhaps involving the expression of $Ca_v1.3$ (and BK) channels at a high density in the primary cilium as well as in the basolateral membrane, could reflect the advantage of being able to detect not only local water movement but also nearby living prey items and/or predators.

2.4 Summary

Significant progress has been made in the understanding of electroreceptor development in nonteleost jawed vertebrates. This includes the experimental confirmation of the embryonic origin of ampullary organs (together with neuromasts and afferent neurons) from LLPs in representatives of all three major jawed vertebrate groups (lobe-finned bony fishes/tetrapods, ray-finned bony fishes, and cartilaginous fishes) and the identification of the transcriptional regulator *Eya4* and an oncomodulin-related β-parvalbumin as conserved markers of electroreceptors across all

nonteleost jawed vertebrates. The first inroads have been made into identifying signaling pathways involved in ampullary organ development and, more generally, into the molecular basis of LLP development, including heterogeneity of mechanism among different LLPs. The trickle of genes reported as expressed in developing ampullary organs in different species, based on candidate genes, has turned into a stream with the advent of differential RNA-seq analysis, enabling an unbiased approach. In the Mississippi paddlefish, at least, this has revealed very high levels of conservation of gene expression between developing ampullary organs and neuromasts, including essentially all the transcription factor genes known to be important for hair cell development plus genes required specifically for transmission at the hair cell ribbon synapse. This degree of conservation also suggests that electroreceptors most likely evolved in the vertebrate ancestor via the diversification of lateral line hair cells as opposed to the independent evolution of electroreceptors and hair cells from a secondary ciliated cell. The unbiased transcriptomic approach also identified the first-reported transcription factor gene expressed in developing ampullary organs but not in neuromasts, which could be involved in specifying electroreceptors rather than hair cells.

However, these advances are, for the most part, descriptive. Experimental evidence is still lacking for the embryonic origin of lamprey electroreceptors and the various independently evolved teleost electroreceptors. Nothing is yet known at the molecular level about these electroreceptors. More experimental evidence, from multiple species, is needed to understand the molecular mechanisms underlying the development of the elongating LLPs that form both ampullary organs and neuromasts, and how these differ from the mechanisms underlying the development of the migrating zebrafish posterior LLP, on which most LLP research is currently focused. Gene expression patterns are indicative, but experimental studies are needed to test hypotheses about gene function.

Technical advances in the second decade of the twenty-first century make the future of experimental research into electroreceptor development very bright. First, the reduced cost of next-generation transcriptome sequencing (RNA-seq), including from relatively small amounts of extracted RNA, plus software that assembles RNA-seq data without a genome sequence, together make unbiased transcriptomic approaches feasible in any species. Furthermore, single-cell RNA-seq should allow electroreceptor-specific transcriptomes to be generated (as opposed to tissue-level or electrosensory organ-level transcriptomes), enabling direct comparison to identify conserved and divergent features of different nonteleost electroreceptors and of ampullary versus tuberous electroreceptors in and between different electroreceptive teleosts. The results should also shed light on electroreceptor evolution, including in teleosts. Finally, genome-editing CRISPR/Cas9 technology (already used in the axolotl and lamprey) should enable gene function to be tested in both nonteleost and teleost electroreceptive species. Overall, these new technologies should enable spectacular future advances in our understanding of electroreceptor development and evolution.

Compliance with Ethics Requirements Clare Baker declares that she has no conflict of interest.

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Chapter 3
Electrosensory Transduction: Comparisons Across Structure, Afferent Response Properties, and Cellular Physiology

Duncan B. Leitch and David Julius

Abstract The first relays of the vertebrate electrosensory system arise from epidermal specializations with voltage-sensitive receptor cells that are tuned to the relevant frequencies of bioelectric fields. Despite diverse phylogenetic origins and adaptations to varying habitats, electroreceptor organs share a number of morphological and functional characteristics to facilitate the detection of low-intensity electric fields. Much of the current knowledge of physiological mechanisms underlying electrosensory transduction has been gleaned from in vivo electrophysiological recordings from primary electrosensory afferents or recordings of electrical impulses from the organs themselves. Recent advances in genetic and patch-clamp electrophysiological techniques have made detailed comparisons of the molecular mechanisms of transduction possible. These comparisons have the potential to shed light on convergent mechanisms of stimulus transduction and filtering among diverse species as well as broad themes of signal transduction relevant to other hair cell-based sensory systems.

Keywords Ampullary organ · Calcium channel · Elasmobranch · Electrophysiology · Electroreception · Electroreceptor · Ion channel · Potassium channel · Receptor cell · Sensory evolution · Sensory systems · Tuberous organ

3.1 Introduction

Natural, weak electric fields are universal phenomena throughout aquatic and marine environments and are generated by both electromagnetic properties of the planet (Keller 2004) and the movement or uneven distribution of ions across permeable surfaces in living organisms (Alberts et al. 2002). Throughout the vertebrate lineage, the ancient sensory modality of electroreception has been preserved in some clades

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while other vertebrate taxa have lost and subsequently independently reevolved the ability to detect these subtle electric fields (see Baker, Chap. 2).

In all sensory cells, specialized structural features and receptors are adapted to transduce salient external environmental signals into electric signals that are encoded by the nervous system, leading to detection of specific stimuli. In contrast to other, more ubiquitous sensory systems, investigations of electric field sensing have been challenging due to the diversity of electroreceptor organs, the signals they are adapted to transduce, and the technical limitations of direct measurement from electrosensory cells. Indeed, electroreception is unique in that the external voltage stimulus does not fully undergo a change into another form of energy to influence the nervous system, as is the case when, for example, light elicits electrical signals in the rod cells of the retina. However, by integrating results that span multiple levels of inquiry, from physiology to behavior, as well as investigations across a range of electroreceptor organ types, conserved principles of electrosensory transduction can be understood.

3.2 Electroreceptor Organs

Electroreception appears to have initially evolved in early chordates (Bullock et al. 1983) and has continued through all extant nonteleost fish taxa, except for hagfish, gars, and bowfins. Among amphibians, salamanders and caecilians appear to retain similar forms of electroreception, facilitated by homologous electroreceptor organs (Jorgensen 2005). Despite the apparent loss of electroreception in ancestors to most extant bony fishes, two distantly related teleost fish lineages that include the African Mormyroidea and South American Gymnotiformes have independently "reevolved" electroreception mediated by receptor organs that are distinct from ancestral homologues (New 1997; Alves-Gomes 2001). Fossil evidence of electroreceptive organs and their ubiquitous presence among phylogenetically diverse vertebrate taxa suggest that electroreception initially evolved in the vertebrate ancestral lineage in the early Paleozic (500–600 million years ago; Thomson 1977). Furthermore, anatomical observations from the distinct lobe-finned coelacanth *Latimeria* (Northcutt 1980; Webb and Northcutt 1997) have led to speculation that the earliest vertebrates had electroreceptive capabilities (Bullock et al. 1983). Although comparative topics of electroreceptor evolution and developmental morphology are given greater attention within this book (see Carlson and Sisneros, Chap. 1; Baker, Chap. 2), a basic review of the cellular structure of generalized groups of electroreceptors is helpful to understanding transduction properties through their specialized sensory cells.

Electroreceptor organs (i.e., peripheral adaptations of the body surface that facilitate the detection of external electric fields) are part of the octavolateralis system, which also includes specialized vestibular, auditory, and mechanically sensitive neuromast organs derived from the lateral line system (homologous to the mammalian auditory nerve; McCormick 1982). Both the mechanosensory neuromasts and the electrosensory ampullary organs are innervated together by multiple distinct branches of the lateral line system, as shown in anatomical preparations

from several gnathostome (jawed vertebrate) groups (Northcutt 1997; Baker et al. 2013b). They also share a common embryonic lineage from specific placodes (Modrell et al. 2011; Gillis et al. 2012) as well as unique sensory hair cell morphology (Baker et al. 2013b).

Electroreceptor organs can be broadly classified into two functionally and morphologically distinct groups: (1) *ampullary*-type and (2) *tuberous*-type organs. Ampullary organs are present in a large range of taxa, including most nonteleost fish and Siluriformes, Gymnotiformes, and Mormyroidea among a handful of teleost fish (Bullock et al. 1983) as well as in some amphibian species (Fritzsch and Wahnschaffe 1983; Rose 2004). Tuberous organs are found only in two distantly related freshwater weakly electric teleost groups (Gymnotiformes and Mormyroidea; Bullock 1982) and in blind catfish (*Pseudocetopsis*; Andres et al. 1988); however, their sensory function in blind catfish is unknown.

3.2.1 Ampullary Receptors

The widespread appearance of ampullary receptors among diversely related extant vertebrates and its subsequent reevolution in weakly electric fishes underscore the biological importance of these sense organs. In both teleosts and nonteleosts, ampullary organs detect very weak, low-frequency stimuli, with the greatest sensitivity between about 0.1 and 20 Hz, depending on taxa (Tricas and Carlson 2012). Ampullary organs are typically associated with roles in passive detection of low-frequency electric fields generated by other living aquatic organisms. In behavioral experiments, they have been shown to be involved in the detection of the electric fields generated by prey in elasmobranchs (sharks, skates, and rays; Fig. 3.1A;

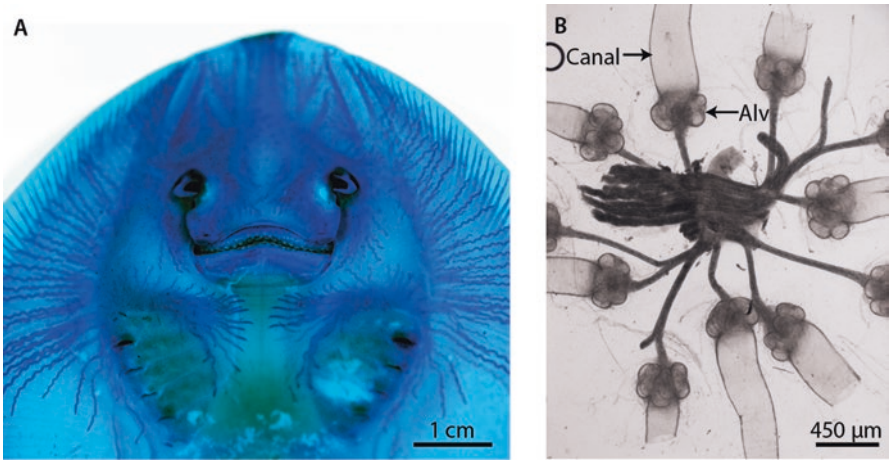

Fig. 3.1 Ampullae of Lorenzini. **A:** Alcian blue stains of long gel-filled canals radiating from CN VIII in a juvenile little skate *Leucoraja erinacea*. **B:** light microscopy of receptor cell-filled alveoli (Alv) and associated nerves

Kalmijn 1971, 1974, 1982; Tricas 1982) and amphibians (Himstedt et al. 1982) along with the detection of electrical signatures associated with potential predators (Kempster et al. 2013a, b). Beyond their contribution to predation, ampullary organs have been implicated in the detection of conspecifics, including passively generated electric fields (Tricas et al. 1995) and the unusually low-frequency signals produced by weakly electric skate species (Sisneros et al. 1998).

3.2.1.1 Ampullae of Lorenzini Structural Properties

Perhaps the most widely appreciated ampullary organs are the elaborate ampullae of Lorenzini from elasmobranch fish and chimeras (Fields et al. 1993) because they have been noted for both for their distinctive pit/pore appearance and their exquisite sensitivity (less than a nanovolt per centimeter; Kalmijn 1982; Kajiura and Holland 2002). Like the ampullary organs of teleost fish, the ampullae of Lorenzini share a common structural motif of an epidermal invagination, forming a pore with a gel-filled canal leading to a layer of electrosensory receptor cells housed in an enlarged reservoir or "ampulla" vessel (Fig. 3.1B; Zakon 1986; Jorgensen 2005). The high-impedance canal wall, formed by two layers of flattened epithelial cells, terminates on a basement membrane containing voltage-sensitive sensory cells. The low-impedance jelly-like matrix filling the interior of the canal allows the interior lumen to be isopotential to the water adjacent to the pore, contributing to detection of potential differences between the interior potentials of the animal at the base of the ampullary structure and the external environment.

One morphological distinction between the electroreceptive periphery of batoids (such as skates) and sharks is the relative distribution of ampullae into distinct clusters (Fig. 3.2; Raschi 1986; Tricas 2001). Skate ampullae are organized into superficial ophthalmic, buccal, hyoid, and mandibular clusters, with a clear variation in canal length, whereas the buccal cluster may be absent in benthic rays (Camilieri-Asch et al. 2013; Gauthier et al. 2018). Ampullae originating from the hyoid cluster are typically the longest and extend over the lateral regions of the pectoral disc; however, those from the mandibular cluster are typically the shortest and project to the margins of the lower jaw (Tricas 2001). The clear distribution and relative lengths of the ampullary organs suggest the electrosense could facilitate the detection of conspecifics (those producing both active electric organ discharges or weak standing bioelectric fields) as well as the bioelectric fields encountered during benthic foraging (Bratton and Ayers 1987; Sisneros and Tricas 2002). In contrast, sharks with their cylindrically shaped heads could sample bioelectric fields in a three-dimensional space. They lack the hyoid cluster found in skates; however, the superficial ophthalmic cluster is elaborated into prominent dorsal and ventral groups that project to the tip of the snout and between the snout and the eye, respectively (Tricas 2001; Tricas and Sisneros 2004). This physical alignment of electrosensory input with the visual fields could provide higher resolution spatial information during predation, including moments prior to attack when the eyes are rolled back into the head.

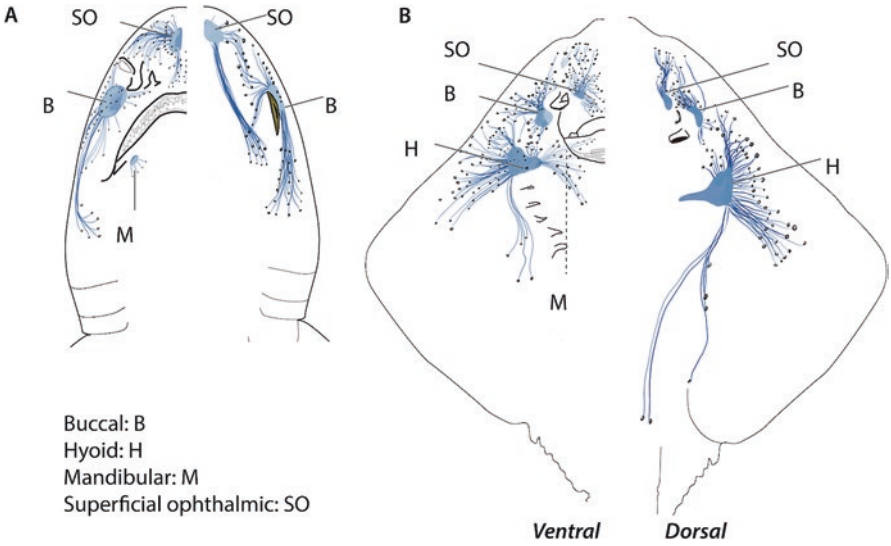

Fig. 3.2 Schematic representations of ampullary organ distribution in two elasmobranchs. The ampullary organ pores (*black dots*) and canal structure (*blue lines*) lead to discrete clusters of ampullary receptor cells, as drawn from Alcian blue-stained specimens. **A:** in the chain cat shark (*Scyliorhinus retifer*), the ampullary organs originate from supraophthalmic (SO), buccal (B), and mandibular (M) clusters and are concentrated near the mouth and nares. **B:** little skates (*Leucoraja erinacea*) share a similar arrangement to cat sharks but have a prominent hyoid (H) cluster. Their ampullary organs are also distributed across much of the pectoral disc, with some individual organs having notably lengthy canals as well as particular concentrations of ampullae near the mouth

Beyond the spatial arrangement of the ampullary organs, the skin itself demonstrates morphological adaptations to facilitate bioelectric sensing. The exterior skin resistance of fish ranges from a few hundred ohms per square centimeter in freshwater species to about 10 thousand ohms per square centimeter in marine elasmobranchs (Bennett 1965). Other estimates place the average resistance across marine elasmobranch skin and body as two orders of magnitude greater than an equivalent volume of seawater (Kalmijn 1987). With the fish's body acting as an insulator to external electrical fields, approximately half of the voltage is attenuated by the skin itself and the remainder by the internal body tissues between the external surface and the position of the receptor cell. With canals occasionally approaching lengths of one-third of the total body length, as in marine rays (Szabo et al. 1972), long canals could facilitate more appreciable comparisons between the lumen isopotential to the environment and the internal reference potential within the fish's body. In more general terms, longer ampullary canals appear to contribute to heightened electrical sensitivity. In interspecific comparisons, the length of canals from the ampullae of Lorenzini appear to correspond to habitat in that marine elasmobranchs have much longer canals compared with estuarine or freshwater species, as has been noted in the freshwater ray *Potamotrygon* (Szabo et al. 1972). These "microampullae" are similar in length to the ampullary organs of freshwater teleost species, terminating

Table 3.1 Comparisons of ampullary and tuberous receptor morphological and functional properties contributing to stimulus transduction

Electroreceptor	Taxon	Structural motif	Canal composition	Number of receptors per organ	Skin
Ampullary organ					
Ampullae of Lorenzini	Elasmobranchs	Long canal to sensory cell, shorter canal in freshwater species	Jelly-like matrix	Hundreds	High resistance (marine); relatively low resistance (freshwater)
Ampullary (microampullae)	Teleost	Short canal to sensory cell	Jelly-like matrix	1–20 (including amphibians)	High resistance
Tuberous organ					
Tuberous	Gymnotiform (Teleost)	Short canal to sensory capsule	Loosely packed epithelial cell plug	Usually 20–30	Relatively low resistance
Knollenorgan	Mormyriform (Teleost)	Short canal to sensory capsule	Loosely packed epithelial cell plug	>10	Relatively low resistance
Mormyromast	Mormyriform (Teleost)	Short canal to sensory capsule	Loosely packed epithelial cell plug	3–5	Relatively low resistance

just below the epidermis, presumably as an adaption to the low conductivity of freshwater (see Table 3.1).

Although the majority of the canal is composed of high-resistance accessory support cells, the composition and electrical properties of the jelly-like substance that fills the ampullae of Lorenzini continues to provoke discussion (Waltman 1965; Brown 2010). This mucopolysaccharide-filled jelly (Doyle 1967) appears to be secreted from epithelial cells lining the inner wall of the canal (Raschi 1986), and its continual release has been proposed as providing both a stable electrical resistance within the canal and a protective, buffering mechanism for the sensory epithelia relative to rapid fluctuations of the ionic environmental composition (Loewenstein and Ishiko 1962; Whitehead et al. 2015). Potassium appears to be concentrated, particularly near the base of the ampullae compared with the pore opening, suggesting efflux of potassium by the sensory cells (Murray and Potts 1961). Although strong potassium inward currents have been demonstrated in other octavolateralis receptor cells (Russell and Sellick 1976), inward currents across the apical face of the electrosensory cell (in contact with the lumen and jelly) are carried by calcium (Clusin and Bennett 1979a, b).

A variety of speculative functions have been ascribed to the jelly matrix, including specialized thermoelectric capabilities that contribute to thermosensation via temperature-dependent changes to conductivity (Brown 2003, 2010). Gel expressed from the organs has been proposed to be operating as an ion channel-free thermoelectric

semiconductor, creating voltage changes between two silver wires when heated or cooled. However, other measurements using salt bridge electrodes (which preclude electrochemical effects that occur when using metal electrodes) have found the conductive properties of the ampullary gel to be similar to those of seawater (Fields et al. 2007). Intriguingly, measurements using proton-conducting devices immersed in room temperature jelly have shown an unusually high proton conductivity (2 mS/cm), the highest recorded from any biological material and rivaling state of the art synthetic proton-conducting polymers (Josberger et al. 2016). It has been suggested that polyglycan keratan sulfates and their acid groups donate protons to the jelly to facilitate the unusually high conduction. More recently, proteomic analyses have confirmed the presence of keratin sulfate as well as calreticulin and parvalbumin α-like protein that have been implicated in calcium- and potassium-channel regulation in muscle cells (Zhang et al. 2018).

At the base of the jelly-filled canal, the typical elasmobranch ampullary organ ends in a bulbous swelling of pear-shaped electrosensory cells that make tight junctions with adjacent supporting cells in the alveolar surface (Fig. 3.3; Waltman 1965). These tight junctions and desmosomes, visible through transmission electron

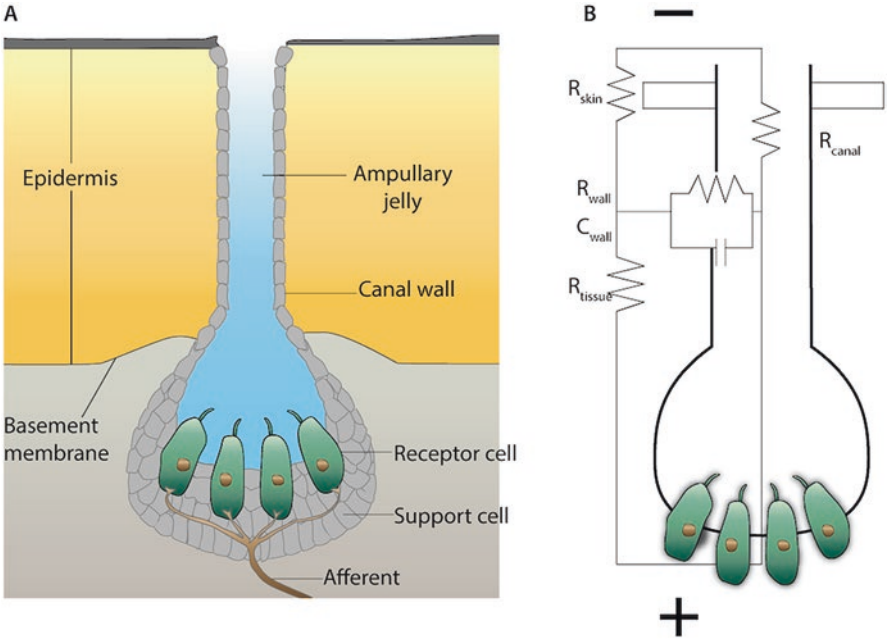

Fig. 3.3 Schematic representation of an ampullary organ (elasmobranch ampulla of Lorenzini) and the equivalent circuit. **A:** ampullary organ consists of a long gel-filled canal that connects the external marine environment to the receptor cells found in the base of the epithelium. Depolarization of the receptor cell results in synaptic release on the afferent. **B:** in the presence of outside negative stimuli, current is shunted through the relatively low-resistance (R) canal to the receptor cell. The high-capacitance (C) wall contributes to the loss of some high-frequency stimuli. Modified from Bennett (1967) and Keller (2004), with permission

microscopy, are thought to minimize attenuation of the stimulus currents to the receptor cell surface by providing a nonconductive epithelial barrier. Roughly 1% of the apical face of the receptor cells is in contact with the lumen, and the cells demonstrate a distinct polarity, with a single kinocilium that projects toward the canal. The presence of a single kinocilium in ampullary receptor cells is found in all elasmobranchs, sturgeons, and paddlefish (Jorgensen 2005), whereas lungfish (Jorgensen 1984) and caecilian amphibians also have microvilli that may contribute to varying the apical surface area of the receptor cell facing the lumen, reflecting species-specific adaptations to the conductivity of the habitat. Salamanders have ampullary receptor cells that appear to only have microvilli (Fritzsch and Wahnschaffe 1983). The configuration of polarized apical and basal surfaces, separated by tight junctions and contacting environments of varying composition, has been noted in other hair cells, including those in the cochlea (reviewed in Hudspeth 2005; Wang et al. 2015).

The basal membrane of the ampullary receptor cell makes synaptic contact with afferent fibers of the electrosensory system. There are 20–30 invaginations within the presynaptic surface that feature an elaborate synaptic ribbon (Fig. 3.4). These structures have been identified in other octavolateral receptors as well as in

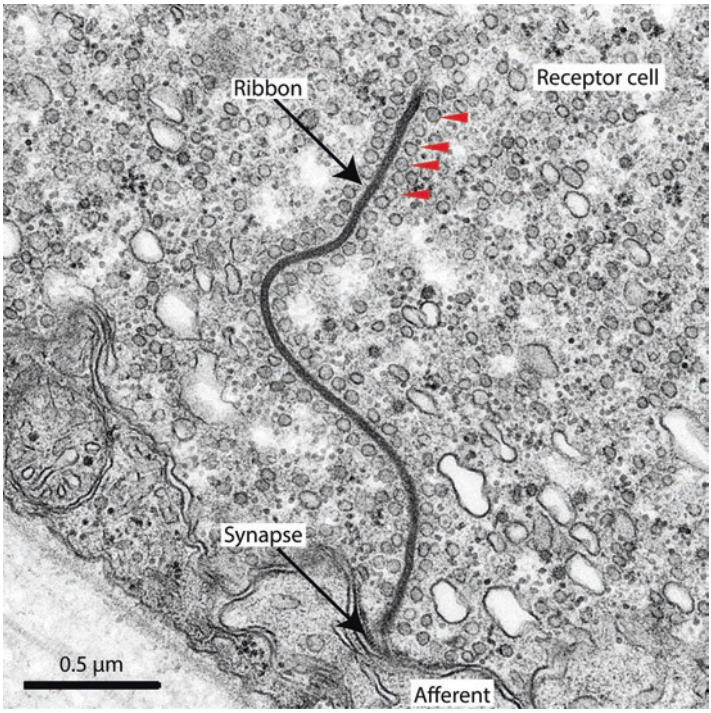

Fig. 3.4 Electron micrograph of ribbon structure at the basal membrane of little skate *Leucoraja erinacea* electrosensory receptor cell. The long ribbon structure facilitates continuous vesicle (*red arrowheads*) release at the synapse on the postsynaptic afferent nerve

retinal photoreceptors where they modulate the rate of release in response to stimulus intensity and allow rapid, continuous excitation (Matthews and Fuchs 2010). Although it is unclear whether the ribbons provide a tethering structure to accumulate vesicles of neurotransmitter or actively shuttle vesicles toward the synapse, the ribbons found in electroreceptor cells appear particularly long in comparison to those found in mammalian hair cells. Moreover, the ribbon invagination on the presynaptic membrane appears to flatten out with decreasing electrical sensitivity in the ampullary receptor cells of rays (Fields and Ellisman 1985; Fields et al. 1987). These morphological measurements suggest a plastic relationship between synaptic architecture and stimulus sensitivity.

3.2.2 *Tuberous Organ Structural Properties*

The tuberous organs of weakly electric teleost fish (Fig. 3.5) facilitate electrolocation via distortions of the fish's own self-generated electric organ discharge (EOD), and communication via detection of conspecific EODs. Although there is a great degree of morphological variation (Szabo 1965; Jorgensen 2005), most tuberous organs follow a basic structural motif of a short epidermal canal that enlarges into an inner chamber containing 20–30 receptor cells (Table 3.1; Szabo 1965, 1974).

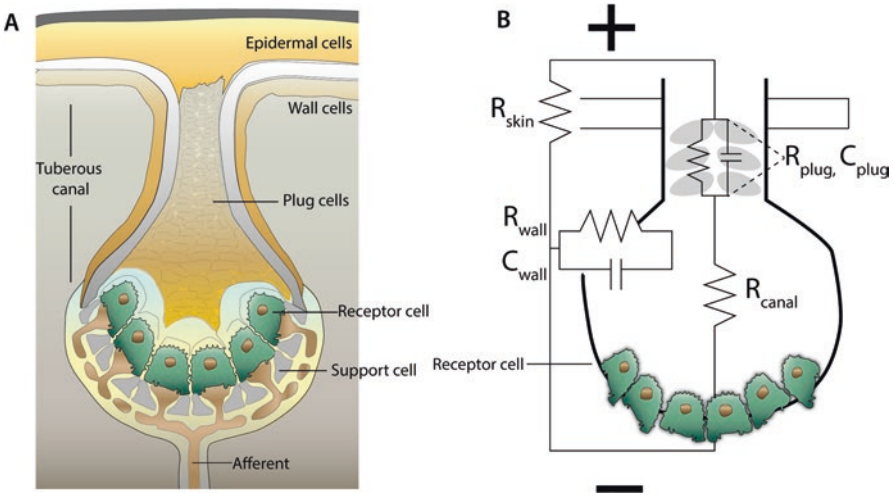

Fig. 3.5 Schematic representations of a tuberous organ (gymnotiform) and the equivalent circuit. **A:** tuberous organs have an epithelial cell-filled plug that functions as a series capacitance and a separate capsule that isolates the receptor cells from the external environment. High-frequency signal loss is minimized because the wall of the organ has a relatively low capacitance. **B:** in the presence of outside positive stimuli of appropriately high frequency, the tuberous cells are depolarized and initiate synaptic release on the afferent. Modified from Bennett (1967) and Keller (2004), with permission

In comparison to the jelly-filled matrix of ampullary organs, tuberous receptors are plugged by a loose cap of epithelial cells, with the extracellular space between these support cells in contact with the water external to the body of the fish (Wachtel and Szamier 1966). Also different from ampullary organs, the base of the tuberous organ has a covering membrane present between the loose epithelial plug and the sensory epithelium. Tight junctions between these covering cells and the support cells of the tuberous canal may attenuate changes to ionic composition near the receptor cells in the reservoir at the base of the organ as might occur during chemical fluctuations of the fish's environment (i.e., changes in salinity; Zakon 1986). Along with the stacked layers of epithelial cells that form the "cell plug," multiple layers of epithelial cells also comprise the walls of the tuberous canal, imparting a low capacitance. This anatomical configuration presumably attenuates the shunting of high-frequency stimulation to which the receptor cells are preferentially tuned (Bennett 1971). The epithelial plug also acts as a high-pass filter tuned specifically for the EOD of each species, and its thickness has been shown to correlate with the frequency of the EOD; weakly electric fish with higher frequency EODs have more layers of epithelial cells within the tuberous plug (Zakon 1986).

In comparison to the distinctive kinocilium of marine ampullary receptor cells, tuberous receptor cells are densely covered in elaborate patterns of microvilli. These are found in greater numbers than in the freshwater ampullary cells of amphibians and serve to expand the surface area of the apical membrane, with a configuration thought to both increase capacitance and decrease input resistance (Bennett 1967). Indeed, the receptor cells themselves reside primarily within the lumen of the capsule at the base of the tuberous organ canal, with ~95% of the receptor surface area exposed to the conductive extracellular space compared with the small (~1%) apical surface that faces the lumen in ampullary receptors. The remaining 5% of the surface of the tuberous receptor cell is held at the basal surface, with tight junctions to adjacent supporting epithelial cells, creating two polarized surfaces. Transduction of the tuberous electrosensory cell is initiated on this comparatively small basal surface that is the location of synaptic contact with afferents and voltage-sensitive ion channels (Kawasaki 2005).

3.2.3 Mammalian Trigeminal Electroreceptor Structural Properties

The passive electric sense of mammals (as observed in the semiaquatic platypus, terrestrial echidnas, and Guiana dolphin) pose a number of striking differences from the more widely appreciated fish electric senses (Czech-Damal et al. 2013). Although the platypus has about 40,000 electroreceptor organs distributed in distinct stripes across the entire bill (Manger and Pettigrew 1996), echidna species have 2,000 or less organs found only along the most distal portion of the snout (Pettigrew 1999). Their electroreceptive organs appear to have evolved independently from the fish lineages and are an elaboration of the trigeminal system that typically provides

somatosensory innervation to facial areas in other vertebrates, in contrast to the octavolateral origins among fish and amphibians (Asahara et al. 2016). Indeed, monotreme electroreceptor organ structures are markedly different in that their presumptive electrosensory cells are bare nerve endings within specialized dermal mucus gland organs (Gregory et al. 1989; Andres et al. 1991). Unlike other electrosensory peripheries, there is no secondary innervation afferent in monotreme receptor cells. The sensory cell itself arises from the trigeminal ganglion.

Anatomical and behavioral studies have also described passive electroreception in the Guiana dolphin (Czech-Damal et al. 2011). Because Guiana dolphins appear to preferentially feed on bottom-dwelling fish, electroreception could facilitate successful predation when foraging through mud (de Gurjao et al. 2003). Externally, dolphin electroreceptor organs are visible as four to seven pores that are distributed on each side of the animal's slender rostrum. These pores form hairless vibrissal crypts similar to the follicle sinus complexes associated with mechanosensation and are densely innervated by the infraorbital branch of the trigeminal ganglion. The inner lumen of the ampullary-shaped crypts is filled with a matrix of keratinous fibers and a glycoprotein-composed biogel. It has been hypothesized that this gel functions in the conduction of electrical stimuli to intraepithelial nerve fibers at the base of the organ (Czech-Damal et al. 2011, 2013).

3.3 Physiological Properties of Electrosensory Organs and Innervating Afferents

Electrosensory transduction begins with the transformation of the primary stimulus of an external voltage change to a chemical signal to afferents of the electrosensory system. Although the first steps of this process are initiated by the specialized electrosensory cells at the base of each electroreceptive organ, the majority of ionic physiological properties of electroreception have been observed from preparations that have recorded the extracellular activity of afferent nerves innervating entire intact electroreceptor organs and the intracellular recordings of receptor cells. These have provided initial approximations of the ionic currents that are modulated during electric stimulus presentation. For a more comprehensive review of these topics beyond the scope of this chapter, see Bennett (1971) and Bennett and Obara (1986) who have elucidated many of the common motifs of electroreceptor organ activity modulation.

3.3.1 Basic Themes of Electroreceptor Organ Transduction and Response Properties

Although electroreceptor organs are commonly recognized in relation to their peripheral morphology, functional designations have also been used to describe the response properties when presented with electrical stimuli. *Tonic* receptors

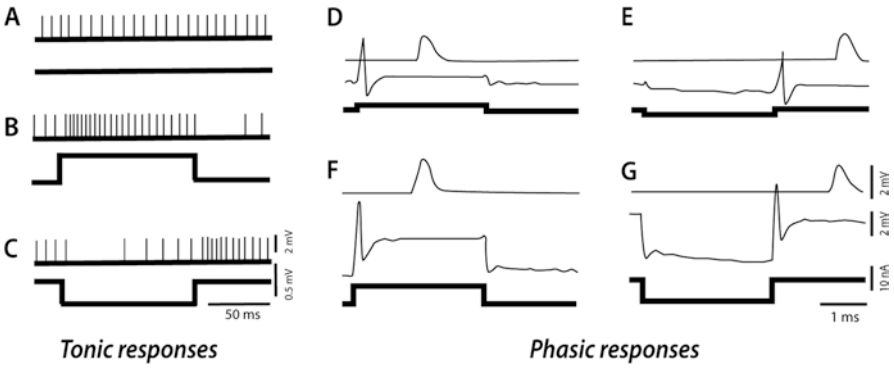

Fig. 3.6 Electroreceptor organ stimulus sensitivity. **A-C:** schematized responses of an ampullary (tonic) receptor from gymnotid fish. *Top:* voltage measurements from afferent nerve. *Bottom:* stimulating potential presented to the receptor pore. **A** represents the tonic, resting discharge. **B:** excitatory response to an anodal (outside-positive) stimulus showing an increase in the impulse frequency in the afferent nerve recording, followed by a silent period at the end of stimulation. **C:** inhibitory response to a cathodal (outside-negative) stimulus, with reduced impulse frequency in the afferent nerve. **D-G:** schematized responses of mormyrid *Gnathonemus* knollenorgan (phasic) receptors to transient steps in an external electric field. *Top:* voltage measurements from an afferent nerve. *Center:* voltage measurements from a receptor opening. *Bottom:* stimulating potential presented external to the receptor opening. Brief all-or-none spike response from the knollenorgan and the subsequent afferent impulse to the onset of an anodal stimulus (**D**) and offset of a cathodal stimulus (**E**). The stimulus intensity was 0.3 mV. Larger amplitude stimuli (2 mV) also show the same responses, with a transient spike produced by the organ at the onset of an anodal stimulus (**F**) or offset of a cathodal stimulus (**G**), both producing impulses in afferent nerve fibers. Adapted from Bennett and Obara (1986), with permission

(ampullary) maintain a tonic baseline of afferent discharge that is modulated in the presence of external electric stimuli (Fig. 3.6A).

In the ampullary organs of freshwater teleosts, the tonic afferent discharge frequency is increased in the presence of an anodal (outside-positive) stimulus. The pore and lumen are positive relative to the basal face of the receptor. Anodal stimuli depolarize the basal surface of the receptor cell, increasing calcium conductance and transmitter release. Current flows inward into the basal face of the receptor cell. When the stimulus is cathodal (outside negative), the tonic afferent discharge frequency is reduced as current moves out of the hyperpolarized cell (Table 3.2; Fig. 3.6A, B).

In contrast to the ampullary organs of freshwater fish, the ampullae of Lorenzini of elasmobranchs, chondrosteans (paddlefish and sturgeon), sarcopterygians (lungfish and coelacanth), and amphibians are excited by cathodal stimuli and inhibited by anodal stimuli (Bodznick and Montgomery 2005). In this case, the apical surface of the receptor cell, which contains voltage-gated calcium channels, becomes depolarized while the basal surface is hyperpolarized. Current flows inward through the apical face of the receptor cell, mediating calcium influx and transmitter release, which consequently increase the tonic firing rate of the afferent. In contrast, anodal stimuli reduce calcium conductance and transmitter release and inhibit the tonic firing rate (Fig. 3.6C).

Table 3.2 Comparisons of electric stimuli providing maximal excitation of electroreceptor organ afferents

Electroreceptor afferent	Taxa	Base response	Best stimulus frequency	Maximal stimulus orientation
Ampullary				
Ampullae of Lorenzini	Elasmobranchs	Tonic	Low-frequency (1–20 Hz) extrinsic field	Cathodal (positive internal)
Ampullary (microampullae)	Teleost	Tonic	Low-frequency (1–20 Hz) extrinsic field	Anodal (positive external)
Tuberous				
Gymnotiform tuberous	Gymnotiform (Teleost)	Phasic	Higher frequency (100 Hz to 2 kHz, varied tuning) to EOD	Anodal (positive external)
Knollenorgan	Mormyrid (Teleost)	Phasic	Higher frequency (100 Hz to 20 kHz broadly tuned) to EOD	Anodal (positive external)
Mormyromast	Mormyrid (Teleost)	Phasic	Higher frequency (10 kHz broadly tuned) to EOD	Anodal (positive external)

EOD, electric organ discharge.

Tuberous organs, such as those found in the South American and African weakly electric species and some catfish, are *phasic* receptors (Fig. 3.6D-G). The two subtypes of these receptors encode different high-frequency qualities of the modulation of the actively generated electric field (e.g., EOD). One class encodes the timing of the EOD and the other encodes the EOD amplitude. Related afferent fibers from these receptors are typically inactive (in contrast to the tonically active ampullary organs) and respond quickly to stepwise changes in voltage to produce submillivolt-to-millivolt amplitude all-or-none action potentials in electrosensory afferents or the receptor cells themselves (in mormyrids) that can be recorded external to the tuberous organ. Tuberous receptor cells are depolarized by outside positive stimuli that direct an inward current through the basal surface of the receptor. Transduction in both the amplitude-coding mormyromast receptors and gymnotiform receptors appear to be mediated by potassium and calcium conductances as demonstrated in pharmacological manipulations in electrophysiological recordings (Bennett and Obara 1986).

3.3.2 Response Properties of Teleost Ampullary Organs

Most biophysical insight into freshwater "microampullary" organs and their afferents has been drawn from recording preparations with *Ictalarus* and *Krypotopterus* catfish species. In these fish, high-magnesium and low-calcium solutions in contact with the basal (but not apical) membrane of the receptor cells block postsynaptic activity, suggesting a role for calcium channels on the basal face in the initiation of voltage transduction (Teeter and Bennett 1981; Andrianov et al. 1997). In contrast, the apical face does not appear to be electrically excitable.

Despite progress in cell-attached patch-clamp electrophysiological recording preparations in *Kryptopterus*, no transduction channels have been identified yet in freshwater ampullary species. However, these limited datasets of the voltage dependence in current-voltage curves have suggested that ion channels are indeed present in the receptor cells, and these are apparently found in very low densities (Struik 2001; Peters and Denizot 2004).

Beyond investigations of the microampullary organs of freshwater fish, the relatively long macroampullary organs of marine teleosts have also been examined in a variety of biophysical preparations and in greatest detail in the marine catfish *Plotosus* (Sugawara and Obara 1984a,b). In vivo preparations have shown that the apical face appears to be electrically unresponsive. Voltage- and current-clamp measurements have indicated that electrogenic Na^+/K^+ pumps are found in the basal membrane, and these appear to mediate a characteristic direct current (DC) potential to supply an outward current from the receptor cells (Sugawara 1989a). This steady outward "bias" contributes to a sustained L-type calcium current (Sugawara 1989b) that is enhanced by anodal stimulation that also initiates an outward calcium-dependent potassium current (Bennett 1971; Bennett and Obara 1986). The presence of a calcium-activated potassium current has been further substantiated by voltage-clamp manipulations that showed that this inward current is blocked by potassium-channel blockers as well as calcium-channel blockers (Sugawara and Obara 1984b; Bennett and Obara 1986).

The presence of a second calcium current was characterized in patch preparations on the basal membrane of the receptor cell (Sugawara 1993). When blocking the potassium channels, two calcium currents were identified, with one having a long-lasting conductance and the other being more transient. It has been suggested that the long-lasting voltage-dependent channel with a low closing probability may contribute to the characteristic steady outward calcium current/bias current.

In sum, physiological recordings from *Plotosus* and other teleosts have established several common characteristics of ampullary organs and their activation. First, a calcium-dependent bias current holds the ampullary organ depolarized, providing continual chemical stimulation to the innervating afferents of the receptor cell (Teeter et al. 1980; Teeter and Bennett 1981). Second, stimulation via anodal electrical sources acts to increase the calcium current in a linear fashion, whereas cathodal stimuli decrease this current and modulate neurotransmitter release (most likely through glutamate). Finally, a transient calcium-dependent potassium current is present on the basolateral membrane of the receptor, which contributes to repolarization of the ampullary organ.

3.3.3 Response Properties of Elasmobranch Ampullary Organs

The morphologically distinctive ampullae of Lorenzini have provided perhaps the greatest wealth of biophysical information regarding tonic-receptor electrosensory transduction due to their significantly lower threshold of excitation compared with

freshwater teleost ampullary organs. However, the elasmobranch ampullae of Lorenzini are stimulated by cathodal (negative-external, positive-internal) fields in contrast to the anodal stimuli that excite teleost ampullary organs. At the level of afferent recordings, the long canals of the ampullae of Lorenzini and the alveoli of electroreceptor cells are suitable for dissection and voltage-clamp preparations. The basal membrane of the receptor cell can be submerged in one medium and electrically isolated from the lumen of the canal via an air bridge. Although ex vivo ampullary preparations have recorded thresholds (2 μV) about 10 times greater than suggested by behavioral experiments (Clusin and Bennett 1979b), this reduced voltage sensitivity may be a reflection of damage to the organ structure during experimental preparation. Indeed, more recent in situ afferent recordings have demonstrated entrained neural responses to sinusoidal electric field gradients of 20 nV/cm or less (Tricas and New 1997).

In keeping with observations of the tonic outward current noted in teleost ampullary organs, single afferents from elasmobranch organs are tonically active, with low-voltage oscillations (Clusin and Bennett 1979b; Bennett and Obara 1986) as a consequence of a "bias current" keeping receptor cells depolarized and steadily releasing transmitter (Clusin and Bennett 1977). In preparations that separate the apical and basal membranes of the ampulla, these oscillations appear to be generated by near-threshold stimulation that produces an inward current on the apical face (Clusin and Bennett 1979a). This configuration stands in contrast to teleost ampullary receptor cells that are excitable only on the basolateral membrane, proximal to the chemical synapse to the afferent. Through pharmacological manipulation with calcium-channel or potassium-channel blockers applied to the basal face, it appears that these oscillations are initiated by calcium currents. Bennett proposed a sequence initiated by calcium activation of the apical face causing a large depolarization of the membrane that, in turn, contributes to depolarization of the basal membrane, initiating transmitter release. Next, potassium activation (through either voltage or calcium influx) on the basal face of the receptor causes repolarization of both faces, leading to deactivation of calcium on both faces and deactivation of potassium on the basal membrane once again. The cycle begins again with depolarization of the apical membrane, and the oscillatory activity is continued (Bennett 1971; Bennett and Obara 1986). More recent experiments have investigated the identity of calcium currents of the apical face with pharmacological manipulations, implicating L-type calcium channels and noting the absence of N-type calcium or potassium channels (Lu and Fishman 1994a, b).

Based on ampullary organ afferent recordings, an intriguing correlation has been drawn between receptor sensitivity and habitat. Marine species (among both the teleost and elasmobranch fishes) appear significantly more sensitive in comparison to freshwater species, as inferred from voltage-clamp experiments. Although skates (Murray 1962, 1965) and the marine catfish *Plotosus* (Obara 1976) respond to stimuli in the single microvolt range or less, freshwater species such as *Kryptopterus* (Teeter and Bennett 1981) and sturgeon (Teeter et al. 1980) show thresholds in the range of tens to hundreds of millivolts. Behavioral experiments in euryhaline Atlantic stingrays (*Daysatis sabina*), which occupy habitats ranging

from freshwater to brackish lagoons, have corroborated these current-response properties. Freshwater-habituated specimens require 200–300 times stronger stimuli to elicit electrosensory-driven feeding responses compared with brackish or saltwater specimens (McGowan and Kajiura 2009), underscoring the significance of habitat adaptation over shared phylogeny in ampullary organ sensitivity. A similar reduction in sensitivity has also been noted in behavioral responses of obligate freshwater stingrays (Harris et al. 2015). However, thresholds of electrical sensitivity of freshwater elasmobranch ampullary afferents have yet to be assessed electrophysiologically except in the freshwater ray *Potamotrygon* that respond to millivolt-amplitude stimuli (Szamier and Bennett 1980). It also is probable that thresholds in sensitivity are significantly influenced by the impedance and conductivity properties of freshwater or saltwater.

3.3.4 Response Properties of Tuberous Organs

Phasic or tuberous receptors, recognizable as the short-length (tuber-like), epithelial cell-filled organs distributed in mormyrid and gymnotiform lineages, are specialized for the detection of high-frequency EODs for the purposes of electrolocation and social communication. Intracellular recordings from individual tuberous receptors have not yet been obtained, so much of their physiology and function has been gleaned from primary afferent recordings or from receptor potentials recorded external to the organs themselves (Kawasaki 2005; Tricas and Carlson 2012). Anatomically, tuberous organs are dispersed across and within the epidermis (Szabo 1965, 1974), and individual organs house one to tens of receptors cells compared with the stereotyped innervation patterns of ampullary organs that each contain hundreds of receptor cells, further complicating experimental access and manipulation (Table 3.1). However, in light of the dearth of experimental data suggesting transduction mechanisms, a few clear patterns in tuberous organ electrosensory transduction can be observed and are discussed in Sects. 3.3.4.1 and 3.3.4.2, with particular respect to comparisons to ampullary electroreceptors.

3.3.4.1 Response Properties of Mormyrid Phasic Receptors

In African mormyrids, there are two distinct forms of tuberous receptors: the large time-coding knollenorgans (Fig. 3.6D-G) and the medium-sized amplitude-coding mormyromasts. In general terms, “time-coding” tuberous receptor cells encode the timing of EODs, producing a phase-locked action potential or spike in response to each EOD (Hopkins and Bass 1981; Zakon 1986). Individual knollenorgans respond to the onset of positive-voltage steps and the offset of negative-voltage steps. Because they are distributed over both sides of the body, when presented with an electrical potential, knollenorgans on one side of the body receive a positive inward-voltage

current, whereas organs on the opposite side receive an outward-voltage current. In the presence of an EOD signal of a complex waveform, different individual knollenorgans would respond to different stimulus components of the signal based on their distribution on the body surface relative to the position of the electric field. Furthermore, individual knollenorgans are capable of following stimulation rates up to 500 Hz, permitting the detection of an interspike interval in response to repeated EODs (Bell and Grant 1989; Baker et al. 2013a). Knollenorgans typically contain 1–10 large (40- to 50-μm-diameter) receptor cells, with each individual receptor positioned within its own capsule in the larger receptor capsule (Szabo 1974; Bennett et al. 1989). Recordings from afferent nerves to the knollenorgans have shown very brief, all-or-none action potentials in response to the onset of anodal stimuli or the offset of cathodal stimuli (Bennett and Obara 1986).

Unique to knollenorgans, the receptor cells appear to generate action potentials rather than graded receptor potentials, and the synapses from the receptors to afferents appear electrical rather than chemical in nature. (Szabo 1967; Bennett and Obara 1986). The spikes themselves appear to involve at least two conductances based on the latency of the rising and falling phases of the action potentials. Blocking sodium currents does not affect the spikes produced by the receptor cells but prevents the afferent from becoming electrically excitable, along with immobilizing muscle contraction including the EOD (Zipser and Bennett 1973). Furthermore, experimental manipulation of the media bathing the organs themselves has highlighted a contribution of external ionic concentrations to the characteristic "ringing" or oscillations of the receptor (Peters and Dénizot 2004). Applications of a high-magnesium solution to the basal face of intact organs appears to produce little effect on electrically evoked responses in knollenorgan afferents (Steinbach and Bennett 1971).

In contrast to the temporally coding knollenorgans and their pear-shaped end bulb with receptor cells, "amplitude-coding" mormyromasts have a unique morphology, with two distinct groups of receptor cells physically segregated into upper and lower chambers. The afferents of the cells in the upper chamber (A-type, as described by Szabo and Wersäll 1970) and in the lower chamber (B-type) project to distinct layers of the electrosensory lateral line lobe (ELL) in the cerebellum (Bell et al. 1989). The central terminals of these synapses form a combination of gap junction and chemical synapses on the granule cells of the ELL (Bell 1990). Electrophysiological recordings from the afferents and the central terminals have described lower stimulus thresholds for fibers arising from A-type sensory cells compared with those from B-type sensory cells. Comparing observations across various mormyrid species, mormyromast afferents appear to respond to suprathreshold stimuli by generating bursts of spikes that vary in latency and number of spikes per burst as a consequence of stimulus amplitude (Zakon 1986; Heiligenberg 1991), suggesting that latency of the first spike is integral to encoding the stimulus intensity (Bell 1990). Synaptic function appears to be inhibited by high magnesium, and distinct calcium and potassium conductances also appear necessary for intrinsic receptor cell activity (Zipser and Bennett 1976).

3.3.4.2 Response Properties of Gymnotiform Phasic Receptors

Similar to mormyrids, South American gymnotiforms (knife fish) have physiologically and morphologically distinct time-coding and amplitude-coding tuberous electroreceptors, and these are adapted to function with respect to nature of the fish's EOD. Like the amplitude-coding momyromasts of the Mormyridae, gymnotiform tuberous electroreceptors appear to rely on at least two conductances, including one mediating spontaneous oscillating activity and another contributing to setting the threshold for receptor cell excitability. These afferent responses are graded in amplitude at both the onset and offset of anodal stimuli, and oscillations can be readily recorded as the skin resistance increases, as in the case of the skin drying (Zipser and Bennett 1973). Intriguingly, the oscillations of these phasic receptors are insensitive to sodium blockers, as is the case in the mormyrids, suggesting the influence of calcium and potassium conductances. Further substantiating the contribution of these conductances, the application of potassium and calcium blockers to preparations of the gymnotiform *Sternopygus* abolished or inhibited oscillatory receptor potentials (Zakon 1984, 1986).

3.4 Cellular Basis of Transduction

Despite considerable progress in identifying morphological and receptor-organ level specializations contributing to electrosensory transduction, mechanistic explanations for exceptional low-threshold sensitivity have remained challenging. Proposed overlapping mechanisms have included potential specializations of voltage-sensitive channels in receptor cells, ionic conductances that perpetuate a bias current within receptor cells, and probable specializations in receptor or afferent synaptic structure and innervation. These themes are explored in the following discussion of the cellular physiology of elasmobranch ampullary receptor cells (see Sect. 3.4.1).

3.4.1 Understanding Electrosensory Transduction: Examples with Molecular and Physiological Characterization of Elasmobranch Ampullary Receptor Cells

Recent advances in understanding molecular specializations of electrosensory cells have been facilitated by experimental approaches that integrate direct receptor cell measurements combined with genetic and behavioral techniques. Moreover, comparative approaches between elasmobranch taxa with unique electrosensory and behavioral repertoires have offered the chance to elucidate different cellular mechanisms underlying specializations of the electrosensory system. Whereas sharks appear to preferentially rely on their electrosensory systems for predation and geomagnetic navigation cues (Kalmijn 1982; Tricas 2001), electrogenic skates rely

on electroreception for the detection of bioelectrical fields associated with potential prey and predator avoidance as well as detection of the EODs of their conspecifics (Bratton and Ayers 1987; Sisneros and Tricas 2002). However, because all elasmobranchs rely on ampullary receptor systems for electroreception, they provide a unique framework for examining adaptations in cellular transduction mechanisms and potential contributions to sensory behavior.

Unbiased next-generation transcriptional profiling techniques (i.e., RNA sequencing) have recently been used to examine conserved genetic profiles among electric organ tissues (Gallant et al. 2014; see Gallant, Chap. 4) and in lateral line sensory organs, including electroreceptors (Modrell et al. 2017; see Baker, Chap. 2). Through comparisons of gene expression in a variety of body tissues from both *Leucoraja erinacea* and the chain catshark (*Scyliorhinus retifer*), orthologs of the *a*-subunit of the voltage-gated calcium channel 1.3 ($Ca_V1.3$; *cacna1d*) were shown to be differentially enriched in the ampullary organs of both species (Bellono et al. 2017, 2018). The $Ca_V1.3$ is an L-type calcium channel, in-line with observations of a low-threshold calcium current in previous electrophysiological measurements with intact ampullary organ preparations (Lu and Fishman 1995a,b). Patch-clamp electrophysiological measurements from electroreceptor cells dissociated from the ampullary organs of skates and cat sharks demonstrate the presence of a low-threshold L-type calcium current.

Interestingly, sequence alignment comparisons of the $Ca_V1.3$ from skate and cat shark compared with *cacna1d* homologues from nonelectroreceptive model organisms revealed a unique structural motif. Ampullary-specific isoforms of *cacna1d* from skate and cat shark both have an insertion of four positively charged residues in an intracellular loop domain (Fig. 3.7A). Both cat shark and skate $Ca_V1.3$ alone (i.e., patch-clamp preparations using heterologous cell systems) demonstrate voltage activation profiles that are lower in threshold compared with mammalian homologues, recapitulating current measurements from dissociated electroreceptor cells themselves. Moreover, the charged residue motif present in skate and cat shark homologues appears to confer the lower threshold of voltage responses (Fig. 3.7B). By experimentally neutralizing the charge in skate $Ca_V1.3$ or adding the charged motif to rat $Ca_V1.3$, it is possible to manipulate the voltage sensitivity in the $Ca_V1.3$. More specifically, adding the charged motif to rat $Ca_V1.3$ confers elasmobranch-like voltage sensitivity to the rat homologue and, conversely, neutralizing the charged motif in skate $Ca_V1.3$ increases the voltage sensitivity threshold. These effects are presumably mediated by an electrostatic interaction involving the voltage sensor domain to prime or partially activate the channel, demonstrating structural adaptions in voltage-sensitive ion channels involved in the earliest stages of electrosensory transduction (Bellono et al. 2017).

Beyond initiation of electrosensory transduction via the calcium current, electrophysiological recordings from intact ampullary organs have implicated potassium currents as the modulators of electrosensory cell activity. Electrophysiological measurements, gene expression profiling, and behavioral experiments have identified the contribution of the calcium-activated large-conductance big potassium (BK) channel to the modulation of voltage-activated responses in skate electrosensory

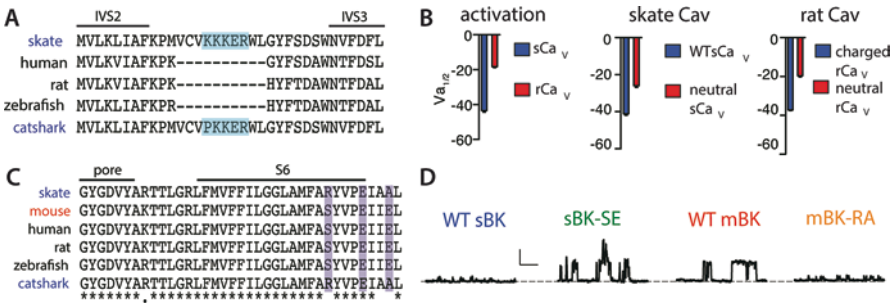

Fig. 3.7 Electroreceptor-specific ion channels. **A:** voltage-gated calcium channel (Ca_V) 1.3 ($Ca_V1.3$) amino acid sequence alignment reveals a positively charged insertion motif in domain IV in both skates and cat shark electroreceptors. **B:** half-maximal activation voltage is reduced in skate Ca_V (sCa_V) compared with that in rat Ca_V (rCa_V; *left*). Charge-neutral sCa_V activation voltage is similar to that in rCa_V (*center*). Conversely, the charged rCa_V activation voltage resembles wild-type (WT) sCa_V (*right*). **C:** calcium-activated large-conductance big potassium channel (BK) alignment reveals amino acid alterations near the pore in skate and cat shark electroreceptor cells. **D:** WT skate BK (sBK) channel shows a reduced current amplitude compared with the WT mouse BK (mBK) channel. Mutation of arginine (R) and alanine (A) in the sBK (sBK-SE) channel to mouse cognate residues produces a single-channel conductance nearly identical to the mBK channel, and mutation of mBK to sBK (mBK-RA) residues produces a conductance similar to the sBK channel. Scale bars: 20 pA (*vertical*); 20 ms (*horizontal*). Adapted from Bellono et al. (2017)

cells (Bellono et al. 2017). Similar to the molecular adaptations found in skate and cat shark $Ca_V1.3$, the *a*-subunit of the skate BK channel has discrete alterations within an intracellular domain near the pore, a location that has been previously implicated in altering channel conductance (Fig. 3.7C; Fodor and Aldrich 2009; King et al. 2016). Strikingly, experimentally mutating this small motif to the residues found in the skate homologue of the BK channel was sufficient to produce skate-like current responses (i.e., single-channel conductance and open-dwell time) in the mouse BK channel, and, conversely, introduction of the mouse cognate residues conferred mouse-like current properties to the skate BK channel (Fig. 3.7D).

Although both skate and cat shark electrosensory cells rely on the $Ca_V1.3$ to initiate the major depolarizing current in response to low-threshold voltage stimuli, the contributions of modulating potassium currents demonstrate marked differences between the two species. Although skate cells rely on calcium coupling through the BK channels, electrophysiological recordings from cat shark cells suggest reduced functional interaction between calcium and potassium currents. Instead, potassium currents in cat shark electrosensory cells are mediated by voltage-gated potassium channels. The voltage-gated potassium channel (K_V) 1.3 ($K_V1.3$) is the predominant potassium channel expressed in cat shark ampullary organs, although the $K_V1.3$ does not appear to be present in skate ampullary organs. Cloned cat shark $K_V1.3$ has a voltage threshold shifted to more depolarized values compared with the human homologue and produces a conductance that can be repetitively stimulated with undiminished amplitude responses, whereas human $K_V1.3$ rapidly inactivates with repetitive stimulation. It is thought that cat shark $K_V1.3$ demonstrates decreased open state stability, thereby requiring less negative voltage to bring the channels

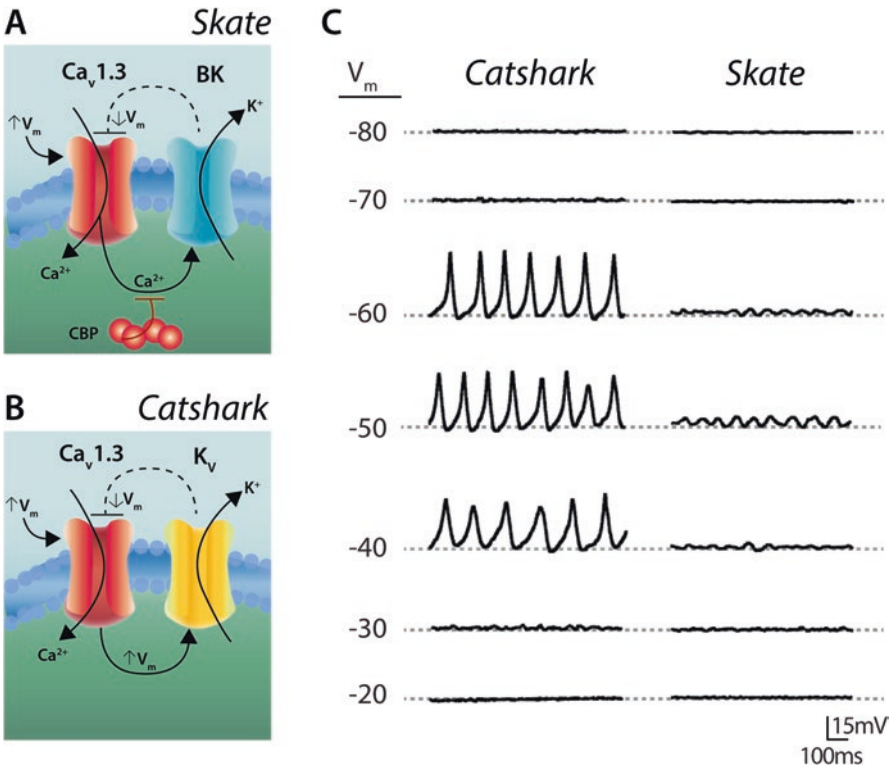

Fig. 3.8 Two models of electrosensory transduction tuning in elasmobranch fishes. **A:** little skate receptor cells rely on a low-voltage threshold $Ca_V1.3$ and the influx of calcium to activate the BK channel and thereby modulate oscillations of the membrane voltage (V_m). CBP, calcium-binding proteins. **B:** cat shark receptor cells also rely on a low-voltage threshold $Ca_V1.3$. However, $Ca_V1.3$ is coupled via a voltage to a high-threshold voltage-gated potassium channel ($K_V1.3$), which shapes V_m response properties. **C:** electrosensory cell V_m responses to current injection. Cat shark cells respond with robust, repetitive spiking that is relatively invariant in amplitude and frequency across a range of stimulus amplitudes and held membrane potential. In contrast, skate cells exhibit a tunable membrane oscillation that changes in amplitude and frequency in response to variation in the stimulus amplitude and membrane potential

back to a resting state and prompting fast channel closure compared with human $K_V1.3$ (Bellono et al. 2018).

Two different models emerge for the initiation of electrosensory transduction via modulation of tonic activity in elasmobranch receptor cells (Fig. 3.8A). Low-frequency membrane voltage oscillations or ringing is a ubiquitous property of receptor cells (see Metzen and Chacron, Chap. 9; Carlson, Chap. 10) and other receptors of the octavolateral system. Results from afferent recordings have suggested that calcium and potassium conductances contribute to the characteristic rhythmic depolarization and hyperpolarization cycle (Zakon 1986). Skate electrosensory cells rely on the $Ca_V1.3$, functionally coupled via calcium influx, to activate an outward potassium current and consequently shape the resting membrane voltage

oscillations (Fig. 3.8B). Injecting current into skate receptor cells modulates the frequency and amplitude of these oscillations. This nonsaturating cellular response is also supported by observations of skate electroreceptor cell morphology, with skate cells possessing large "refilling" pools of vesicles that could facilitate graded responses to increasing stimulus voltage. In stark contrast, cat shark electroreceptor cells respond with robust, repetitive membrane voltage spiking with relatively little amplitude variation when the current is injected. The low-voltage threshold and inactivation properties of cat shark $Ca_V1.3$, electrically coupled to the $K_V1.3$ with its high-voltage threshold, rapid deactivation, and weak inactivation, appear sufficient to shape the membrane spiking responses of cat shark electrosensory cells (Bellono et al. 2018). Extrapolating from these membrane responses, the initial stages of cat shark electrosensory transduction at the level of the sensory cell appear to respond with less variation across a range of voltage stimuli of differing frequency and amplitude, whereas skate electrosensory transduction appears to be more "tunable," with graded responses changing in membrane oscillation amplitude and frequency at ethologically relevant frequencies of electrical stimuli (i.e., low frequencies similar to conspecific EODs and biogenic electric fields of respiring prey).

In reflection of the shared developmental ancestry and evolution of the electrosensory system, homologues of the $Ca_V1.3$, BK channel, and K_V are enriched in both the lateral line mechanoreceptive organs and hair cells of the cochlea. Although it remains to be seen how widely conserved similar structural changes affecting ion-channel activity have been employed among the homologous and independently evolved electrosensory systems and the specific electric stimuli to which they are adapted, comparative experimental considerations that take genetic, physiological, and behavioral approaches into account seem best poised to further clarify electrosensory transduction on a mechanistic level.

3.5 Summary

Despite the seemingly straightforward transmission of an extracellular electrical stimulus to an internal electrical signal capable of interpretation by the central nervous system, the electrosensory system is a highly tuned modality, mediating fundamental communication, navigation, and predator avoidance/prey localization processes. Although the independent evolution of electroreception in various vertebrate lineages could have resulted in significant morphological and functional variation among diverse extant electrosensory systems, their similarities are striking. Electroreceptors occur in two basic forms. Ampullary organs are pore-formed, long-tubed, epithelial structures that are tuned to low-frequency signals, mediate the passive detection of bioelectric fields, and are found among all electroreceptive species. Tuberous organs are cell-covered, short-tubed structures that are tuned to relatively high-frequency signals, corresponding to the electric organ discharges of conspecific weakly electric fish. In both basic types of electroreceptor organs, morphological adaptations facilitate the detection and comparison of external electrical stimuli by receptor cells at the base of the structure.

Beyond a shared general electroreceptor organ form, the receptor cells themselves share several specialized traits that promote electric transduction. These include tight junctions around the sensory cells with neighboring high-resistance epithelial cells, creating asymmetrical apical and basal surfaces through which ions flow through channels and pores to mediate voltage detection. More specifically, the apical surface of the receptor, which is covered by an array of microvilli and/or a kinocilium, may initiate electrosensory transduction through inward calcium currents (at least in elasmobranch ampullary receptor cells), with voltage modulation provided by the basal membrane that possesses voltage-sensitive ion channels, contributing to stimulus-driven neurotransmitter release. Calcium and potassium conductances in response to weak voltage stimuli have been suggested from electrophysiological recording preparations both from electrosensory afferents innervating individual organs and from recordings of voltage potential within the organs themselves. Preparations such as these have also demonstrated endogenous oscillations of the membrane potentials, generated by the receptor cells, and these are amenable to modulation by electrical stimuli, suggesting possible mechanisms contributing to enhanced sensitivity and specificity in tuning for certain frequencies. More recently, patch-clamp experiments from receptor cells have confirmed the presence of voltage-gated calcium channels and potassium channels with unique structural motifs compared with their nonelectrosensing homologues, and these appear to facilitate the low-voltage threshold and receptor cell membrane voltage oscillations when coupled together.

New mechanistic insight into electrosensory transduction will almost certainly arise through the adoption of a comparative perspective, as suggested by this book. With a diverse range of independently adapted species relying on electrosensory systems for distinct behaviors, the meaningful activation range of voltage-dependent conductances is likely to be equally diverse. Furthermore, even among individual species, the electrosensory system has been shown to exhibit remarkable plasticity associated with both age and seasonality (see Tricas and Sisneros 2004) similar to other fundamental hair cell-mediated sensory systems (Bass 2016). To date, comparisons among species varying in habitats of different conductivity and shared phylogeny have posed intriguing questions regarding adaptation of sensory transduction mechanisms. The integration of burgeoning cellular- and molecular-level analyses with classical physiological and behavioral methods seems to create a fertile area for identifying the convergent evolution of the transduction mechanisms of electroreception. Beyond electroreception, this approach could also address broader neurobiological questions of signal transduction as well as physiological and behavioral adaptation of fundamental sensory input.

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Chapter 4
The Evolution and Development of Electric Organs

Jason R. Gallant

Abstract There have been six independent origins of electric organs within extant vertebrates. In each lineage, the electric organs are derived from either skeletal muscle precursors or from fully differentiated skeletal muscles. Remarkably little is known about the mechanisms underlying this process. With recently acquired genomics datasets from a diverse array of electric fishes, however, this is beginning to change. These new data provide an opportune time for a comprehensive review of electric organ development. This chapter provides a brief introduction on the prospects, progress, and major obstacles to understanding electric organ development, followed by a brief overview of skeletal muscle development. This is followed by a consideration of data accumulated over the past 150 years on electric organ development, ranging from early histological observations to the characterization of novel microRNAs that regulate electric organ development to the first attempts at examining mechanisms of development in comparative genomics framework. The purposes of this chapter are to (1) synthesize a broad literature on electric organ development; (2) introduce the reader to more recent advances in understanding the molecular mechanisms of electric organ development that have occurred in the past 20-30 years; (3) consider these historical and more contemporary references in light of a new comparative study of gene expression across multiple lineages of electric fishes; (4) summarize the current broader themes in electric organ development; and (5) identify the needs for new research programs to answer lingering questions.

Keywords Convergent evolution · Development · Electric organs · Genomics · Histology · Muscle · Myogenic regulatory factors · RNA sequencing · Stem cells

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4.1 Introduction

Vertebrates have evolved a multitude of adaptive traits to exploit resources and habitats in the air, on the land, and in the water. Several studies have begun to elucidate the genetic and developmental processes underlying major vertebrate traits such as fins (Davis et al. 2007), limbs (Schneider et al. 2011), feathers (Harris et al. 2002), and teeth (McCollum and Sharpe 2001). Few of these structures have evolved repeatedly, particularly in extant lineages where molecular and developmental studies are possible. This prevents the analysis of molecular and developmental processes underlying novel traits in a comparative framework, limiting insights into the degree of constraint and repeatability of the evolutionary processes underlying novel vertebrate traits.

Of the few traits that have evolved multiple times in vertebrates, one of the most distinctive is the electric organ. These have evolved to produce electric fields for the purposes of communication, navigation, and, in extreme cases, predation and defense. In contrast with most other vertebrate traits, there have been six independent origins of electrogenesis (Fig. 4.1) within extant vertebrate lineages. The taxonomic diversity of electrogenic fishes is so broad that Darwin (1859) considered the multiple origins of electric organs difficult to reconcile with his theory of natural selection. Although it has been more than 150 years since the publication of *The Origin of Species*, remarkably little is known about the "steps by which these organs have been produced" despite their clear benefit as a model for understanding general principles of how complex vertebrate tissues may have evolved repeatedly.

Because this chapter is aimed at the newcomer to electric fish, it is prudent to begin with a consideration of why electric organ development is of broad interest. First, the study of electric organs should appeal to students of evolution and development because systems that produce novel structures are not often biologically replicated in evolution. Electric organs have evolved multiple times (Fig. 4.1) and could therefore be tremendously informative in understanding the constraints that operate on the evolution of gene regulatory networks. Second, of interest to vertebrate biologists more generally is the role of gene duplication in the evolution of novel vertebrate structures. Electric organs have evolved in two lineages that predate and three lineages that follow the hypothesized teleost-specific whole genome duplication (see Fig. 4.1). In this sense, specific hypotheses about how whole genome duplication contributes to the evolution of novelty may be directly addressed by electric organ biology. As an example, Thompson et al. (2014, 2016), using electric fish as a model, have created a compelling hypothesis for how genes become "neofunctionalized" through the combined effects of dosage compensation and genetic drift. Finally, electric organs undergo a relatively "rare" developmental process, essentially transforming from one fully differentiated cell type to another (discussed in Sect. 4.2). Together with the well-known abilities of some electric fishes to completely regenerate their electric organs, characterizing electric organ development may one day have broad impacts in fields such as developmental biology, regenerative medicine, and even biological engineering. The ability to produce

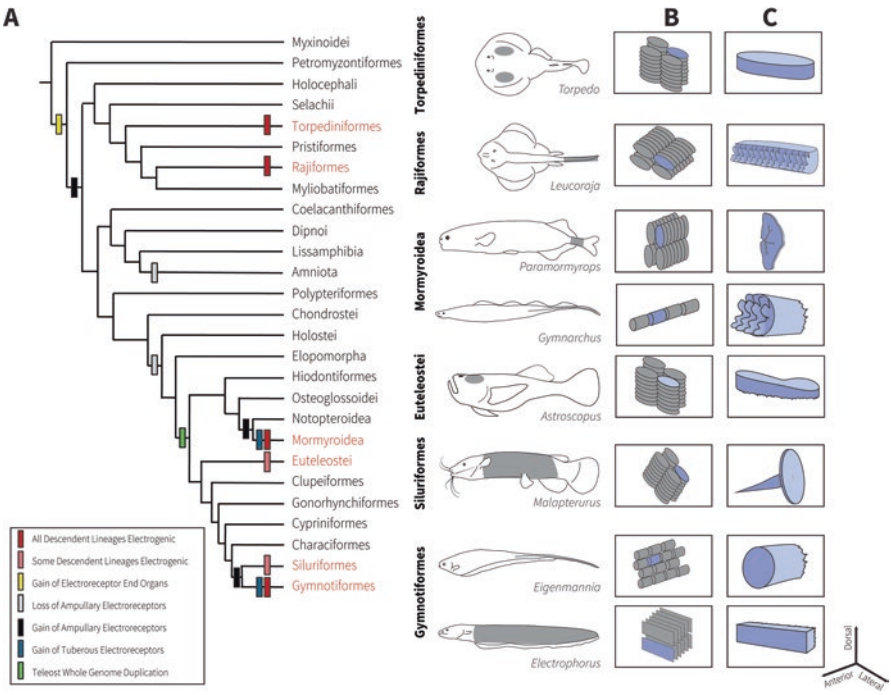

Fig. 4.1 **A:** phylogenetic distribution of electrogenic lineages with the major events in the evolution of electroreception (see Baker, Chap. 2) and electrogenesis highlighted. *Red,* taxa that have independently evolved electric organs. *Three right columns:* representative sketches of species in each of the major electrogenic taxa, approximate location, and size of electric organs (*gray*). The electric organ (**B**) is composed of electrocyte cells (**C**)**.** For each species, a schematic of the three-dimensional configuration of the electrocytes in the electric organ (**B**) and of the three-dimensional anatomy of an electrocyte (**C**) is shown. Note that these schematics are not to scale and mainly serve to orient the reader to the text in Sect. 4.3.

"biological batteries" from stem cells may one day inspire new classes of artificial biological devices with their own power supplies (Ozbolat and Hospodiuk 2016).

Although there have been several comprehensive reviews on the anatomy and physiology of electric organs (e.g., Bennett 1971; Bass 1986; Markham 2013), there has to date been no comparative reviews of electric organ development across the six lineages of electrogenic fishes. Therefore, the first purpose of this chapter is to provide a synthesis of the broad literature on electric organ development, which has been actively studied for over a period of about 150 years (see Sect. 4.3). The second purpose of this chapter is to introduce the reader to more recent advances in understanding the molecular mechanisms of electric organ development that have occurred in the past 20-30 years (see Sect. 4.4). The third purpose of this chapter is to consider these historical and more contemporary references in light of a new comparative study of gene expression across multiple lineages of electric fishes (see Sect. 4.4). The fourth purpose is to summarize the

broader themes achieved thus far in the field (see Sect. 4.5). The final purpose of this chapter is to provide some sense of where the field is heading and identify important questions that should be addressed when considering new research on this exciting topic (see Sect. 4.5).

The study of electric organ development is a difficult task. Perhaps the most obvious and fundamental problem with understanding electric organ development is the paucity of embryological materials available for developmental work; electric fish species are not easily cultured in the laboratory. The majority of early developmental studies were based on the serendipitous availability of embryos and small specimens obtained from the field. Because of this problem, information about the most critical phase of development, the point at which electric organ tissue is specified, is often missed. Because of incomplete embryological series, key time points in the development of electric organs may also be missed. This can lead to misinterpretations about the development of electric organs, as was the case in the electric eel *Electrophorus electricus* (see Sect. 4.3). It was therefore a major breakthrough when Kirschbaum (1975), discovered the environmental factors necessary to promote gonadal maturation in some electric fish species under laboratory conditions, making it possible to breed two lineages of weakly electric fish, the Gymnotiformes and Mormyroidea.

Another means of circumventing this problem has been to consider the postembryonic development and regeneration (see Sect. 4.3). This form of development has been used by several researchers as a proxy for understanding the development of electric organs. This strategy was used to study the gymnotiformes *Sternopygus* (Patterson and Zakon 1997) and *Eigenmannia* (Baillet-Derbin 1978). Despite the success of these studies, it motivates essential questions about the similarity between embryonic and postembryonic developmental mechanisms (Schwassmann et al. 2014; see Sect. 4.3.4).

Another limitation of understanding electric organ development is the inconsistency in techniques applied to various electric fish species. Although the majority of species have been studied using light microscopy, some species have been investigated using electron microscopy, which grants considerable insights into the biochemical and structural properties of electric organs and their precursor cells. An even smaller number of electric fish species (see Sect. 4.4) have been studied using modern molecular biological techniques (e.g., in situ hybridization, immunohistochemistry, next-generation sequencing) that also serve to greatly enhance the conclusions about the developmental origins of these materials.

A final problem with the study of electric organ development is the relatively descriptive nature of the work, which is problematic for identifying general comparative themes in electric organ development. Although researchers clearly read each other's work and communicate about their findings, studies of electric organ development lack clear hypothesis testing, particularly across lineages. This is further complicated by the incredible taxonomic diversity of electric fishes, which derive their electric organs from a variety of muscles and muscle precursors, even within the same taxonomic groups. One of the purposes of this review is to highlight

the common "themes" in electric organ development studies, which will hopefully motivate clear hypotheses to test with newly available genomic data (see Sect. 4.4).

4.2 Electric Organ and Skeletal Muscle Development: A Primer

A more practical issue in approaching the literature on electric organ development is that of terminology. Because of the relatively wide time span over which the studies were performed, the breadth of researchers and disciplines involved, the varied techniques utilized, there are a large number of synonymous terms and potentially terms that are only used by one researcher. To remedy this issue, Sect. 4.2.1 begins with a brief overview of the organization, major structures, and development of teleost skeletal muscle, the tissue most closely related to electric organ tissue in every taxon that has evolved electric organs. This allows for a common conceptual framework in which to approach electric organ development as well as a standardized set of terms by which one can consider the development of electric organs. Wherever possible, attempts are made to use terminology common to muscle development to describe the major ontogenetic events in electric organs.

In Sect. 4.2.2, major structures and features common to all electric organs are considered. The references within these sections will provide a much larger degree of detail than can be provided. For a more in-depth review of the form and function of electric organs, please see Bennett (1971), Bass (1986), and Markham (2013) as well as Markham (Chap. 5).

4.2.1 Skeletal Muscle: Anatomy and Physiology

The lateral musculature in fishes is divided into segmentally arranged myotomes. In gnathostomes, myotomes have characteristic "W"-like shapes, whereas in more basal vertebrate lineages, the shape is simpler (Katz 2002). The myotomes form multiple nested "cones" that enable the force transmission necessary for the wave-like motions of the body used for swimming (Katz 2002). The myotomes themselves are made up of individual muscle cells (muscle fibers), and individual myotomes are separated by a collagenous sheet of tissue called a myoseptum.

Another widely recognized characteristic of fish muscle is the nearly complete separation of muscle fiber types at the anatomical level. Oxidative (slow-twitch) muscle fibers, deeply red in color and used in long duration, low-intensity activity, are located deep and close to the midline, whereas the remaining volume of muscle is glycolytic (fast-twitch) muscle fibers used in high-intensity movements (Bone 1978; Ochi and Westerfield 2007). The relative proportions of the two muscle types vary dramatically, as any sushi aficionado may appreciate.

Unlike many other cells, muscle cells are highly enriched in mitochondria and are multinucleated, partially as a consequence of their unique development. Muscle cells consist of multiple bundles of myofibril proteins surrounded by a specialized membrane called the sarcolemma. Bundles of myofibril proteins inside muscle cells are arranged in a highly regular fashion, which consists of repeating sections of sarcomeres appearing as alternating light and dark striations, giving muscle its characteristic appearance. Sarcomeres consist of many long filamentous proteins; chief among these are myosin, actin, troponin, and tropomyosin.

Innervation of muscle cells occurs in specific locations, named the neuromuscular junction, which consist of a highly folded sarcolemma enriched for acetylcholine receptors. On stimulation with acetylcholine, these receptors open, allowing for the rapid influx of sodium. Like neurons, the sarcolemma propagates action potentials using voltage-gated sodium channels (typically using $Na_V1.4$; see Zakon et al. 2006; Arnegard et al. 2010). Unlike neurons, however, the sarcolemma propagates action potentials through an elaborate network of transverse tubules (T-tubules), allowing action potentials to propagate not only along the cell but deeply into the cell. The action potentials propagate toward intracellular calcium stores in the sarcoplasmic reticulum. Specialized extensions of the sarcoplasmic reticulum called terminal cisternae meet the T-tubule network such that they are closely apposed in an arrangement known as a triad.

As action potentials propagate via the T-tubule network, this leads to stimulation of L-type Ca^{2+} dihydropyridine receptors (DHPRs) in the T-tubules, which, in turn, physically interact with ryanodine receptors located in the terminal cisternae (Franzini-Armstrong and Protasi 1997). As ryanodine receptors open, Ca^{2+} is released into the intracellular space. Ca^{2+} binds to troponin, unmasking myosin binding sites on the actin molecule. In the absence of ATP, actin and myosin remain bound (the source of rigor mortis on an animal's death), whereas in the presence of ATP, myosin undergoes a conformational change that causes both the myosin head to move and then detach from the actin molecule. Due to the conformational change of the myosin head, the result is a ratcheting motion of myosin along the actin molecule, causing the two filaments to slide past one another and the physical contraction of the cell (Rome 2001).

4.2.2 Skeletal Muscle Development

In all vertebrates, muscle cells originate from paraxial mesoderm, tissue immediately adjacent to the developing neural tube of vertebrates. In fishes, unlike other amniotes, the paraxial mesoderm is specified by the combinatorial actions of fibroblast growth factor (FGF) signals and two T-box domain-containing proteins, spadetail and floating head (Watabe 2001; Bentzinger et al. 2012). These combined signals activate the expression of the early myogenic regulatory factors (MRFs) *Myod* and *Myf5*, the earliest recognizable markers of commitment to the myogenic fate. In contrast with other vertebrates, fish myogenic precursor cells express *Myod*

much earlier in development, after gastrulation but before the formation of somites and segmentation (Ochi and Westerfield 2007). Initially appearing as two triangular fields flanking the developing notochord, this population of cells extends into a single layer of cuboidal cells, called adaxial cells, immediately adjacent to the notochord (Currie and Ingham 2001; Ochi and Westerfield 2007). Adaxial cells are morphologically distinct from the surrounding paraxial mesoderm cells, are molecularly distinct, and are characterized by the expression of *engrailed 1* and *2* (Ochi and Westerfield 2007).

A subset of adaxial cells migrate to the lateral edge of the developing somite, forming the superficial "slow-twitch" muscle cells (i.e., express "slow" myosin heavy chain isoforms), whereas another subset of nonmigratory adaxial cells, termed the "muscle pioneer" cells, remain medial (Devoto et al. 1996; Ochi and Westerfield 2007). Muscle pioneer cells are among the first to elongate and differentiate into striated, multinucleated myotubes. Because of their early differentiation, they are thought to serve a role as intermediate targets for early motor neuron growth cones and to facilitate the formation of myosepta between adjacent myotomes, thus instructive in the formation of myotomes. The remaining cells between the lateral edge of the somite and the pioneer cells ultimately become "fast-twitch" muscle fibers (i.e., express "fast" myosin heavy chain isoforms). The decision between fast and slow fiber types is mediated partly by the positional gradients of hedgehog, FGF8, and retinoic acid signals (Ochi and Westerfield 2007).

Cells committed to differentiating into mature muscle cells express the early MRFs *Myod* and *Myf5* and are referred to as myoblasts. Myoblasts, in contrast with their mature progeny, are spherical, consist of a single nucleus, and are proliferative. After receiving the appropriate developmental signal, myoblasts cease proliferation and begin to modify their extracellular matrices and cell-adhesive properties to facilitate alignment into long chains. Next, the cells fuse into large, multinucleated cells, termed myotubes, through the expression of a family of proteins called meltrins that physically act to fuse cell membranes (Gilbert and Barresi 2016). It is at this point that the MRFs *myogenin and mef2* become active, upregulating sarcomeric and other muscle-specific proteins. As muscle-specific protein expression continues, the myotubes "mature" into muscle fibers, recruiting additional myoblasts to fuse with the growing myotube, eventually increasing the size of the mature myofiber (Gilbert and Barresi 2016).

It is important to note that a subset of paraxial mesoderm cells, although somewhat committed to the myogenic fate by the early expression of *myod*, remain relatively undifferentiated through development. These cells support one of the most important features of muscle, its regenerative properties, well-known by even the most modest athlete. These cells express a combination of *pax3* and *pax7* and microRNAs (miRNAs) that are thought to inhibit muscle differentiation (Bentzinger et al. 2012). These cells can divide asynchronously to produce satellite cells and stem cells that can replenish the pool of satellite cells (Fauconneau and Paboeuf 2001). Satellite cells can proliferate and differentiate in response to stress and injury and either can be incorporated into existing muscle fibers (muscle hypertrophy) or form new muscle fibers (Fauconneau and Paboeuf 2001; Bentzinger et al. 2012).

4.3 Electric Organ Development

4.3.1 Electric Organs: General Features and Themes

With these general principles of skeletal muscle development in mind, the stage is set for considering electric organ development. Darwin (1859) observed that electrogenic fishes are "remote in their affinities." Indeed, the independent origins of electrogenesis appear to span vertebrates, with two lineages of elasmobranch fishes (members of the order Torpediniformes and the family Rajidae) and four lineages of teleost fishes (the superorder Mormyroidea, the order Gymnotiformes, the family Malapturidae, and the genus *Astroscopus*). In all cases, development has been at least superficially studied using light microscopy; in many cases, using electron microscopy; and in some systems, using modern molecular biology approaches.

In all cases, myogenic electric organs are composed of individual cells, termed electrocytes (synonymous with electroplax). Following conventions established in muscle development as well as in some electric organ development literature, this chapter refers to fully differentiated electric organ cells as electrocytes and their precursor cells as electroblasts. In general, electrocytes *tend* to (1) be much larger than skeletal muscle cells; (2) have fewer and/or poorly organized myofibril proteins; (3) have disrupted coupling between excitation of the cell membrane and contraction of any remaining myofibril proteins; (4) exhibit strong cellular polarity, with a single innervated face and an uninnervated face characterized by elaborate folds (canniculi); and (5) have organized connective tissue septa to "'direct" the flow of current through the organ. Evidence for each of these features for each taxon of electric fish is considered in Sect. 4.3.

The mechanisms underlying the development of the electric organs seem to differ considerably between taxa, both in terms of the embryological origin of the tissue and in whether the nascent organ is induced by the presence of neuronal tissue or autonomous of it. Depending on the taxon under consideration, fully differentiated electrocytes resemble the structure of skeletal muscle more or less closely. This is in large part due to the developmental history of the precursor cells. As exemplified by *Electrophorus electricus* (see Sect. 4.3.4), electric organ cells can derive directly from mesodermal precursor cells, but in most lineages, electrocytes develop from fully differentiated skeletal muscle fibers. This mode of development, called transdifferentation (Patterson and Zakon 1997), is a relatively rare mode of development in vertebrates but occurs most often in myogenically derived cells (Patapoutian et al. 1995). In this mode of development, electrocytes can develop from extraocular muscles (i.e., *Astroscopus*) or from axial muscles in other lineages (e.g., Mormyroidea). In addition, electric organ development may be conditional on the presence of innervation (e.g., *Sternopygus*; see Sect. 4.3.4) or may, as in many lineages, be autonomous (e.g. Mormyroidea and *Torpedo*; see Sects. 4.3.2 and 4.3.3), where electrocytes are well differentiated before innervation is even present.

Finally, electric organs change throughout postembryonic growth. In at least two lineages, the Mormyriformes of Africa and the Gymnotiformes of South

America, the fully differentiated adult electric organ is preceded by the development of a distinct larval electric organ (see Sects. 4.3.3 and 4.3.4). The electrocytes of the larval organ tend to resemble adult electrocytes but generally lack the anatomical specializations of the adult organ. Electrocytes and the electric organs they comprise, like muscles, must also grow with the organism and be repaired when damaged. Although not extensively characterized in all electrogenic lineages, it appears that, like muscle cells, satellite cells are involved in this process (see Sect. 4.3.4).

The ensuing subsections consider each of the major taxa of electrogenic fishes in turn, first describing the anatomy of the electric organ and then considering its development, bearing in mind the themes enumerated in this section. For the convenience of the reader, Fig. 4.1 illustrates both the three-dimensional organization of electrocytes inside the electric organ and the overall shape and major features of individual electrocytes in each lineage.

4.3.2 Rajiformes

Skates in the family *Rajidae* are a group of approximately 200 species distributed across approximately 20 genera (Eschmeyer and Fong 2018). These saltwater skates are not well-known for their electrogenic abilities, possibly due to the fact that the fishes make weak discharges somewhat infrequently (Bennett 1971); however, all are characterized by a weak electric organ located in the tail.

Skate electrocytes are located medially in the tail between longitudinally running muscle fibers (Bennett 1971). The organs are spindle shaped (Ewart 1889a) and run most of the length of the tail. The individual electrocytes comprising the organ are oriented anterior-posterior (Fig. 4.1), innervated on the anterior face, and bounded by connective tissue septa (Ewart 1889a; Bennett 1971). In large skates, there can be more than 10,000 electrocytes per organ, and each electrocyte can have a surface area of 2 square millimeters (Ewart 1892). The anterior face is directly innervated by the electromotor nerves.

There are two alternative morphological configurations of electrocytes present in skates: cup-shaped and disc-shaped cells. Cup-shaped cells are moderately convex and relatively smooth on both faces, with a slightly greater number of canniculi on the posterior (uninnervated) face (Bennett 1971). Disc-shaped cells, in contrast, have more elaborate canniculi (Ewart 1889a) but lack the convex bending. Both classes of cells contain a layer of striated, filamentous material between the two faces (Ewart 1889a, b).

Ewart (1889a, b, 1892) published a series of studies on the development of electric organs from three species of *Raja* using observations from light microscopy. Based on a preliminary survey of eight species, Ewart (1889a) concluded that the disc-shaped electrocytes of Rajidae are the derived condition and the cup-like electrocytes are likely ancestral.

In young embryos, the tail is composed of fully differentiated muscle fibers. From these muscle fibers, electrocytes begin to form in embryos as small as 7 centimeters. In these specimens, muscle fibers closest to the notochord develop a "club"-like morphology and are surrounded by diffuse connective tissue. As these electroblasts age, the "head" of the club enlarges, flattens, and forms a shallow cup, which Ewart (1889a) posits is facilitated by the activity of myofilaments still present in the anterior part of the cell. The posterior face simultaneously develops canniculi that increase in their size and fuse with each other. Ewart (1889a) notes the presence of a "prong-like" backward extension of the original muscle fiber that is retained through development, much longer than the developing electroblast. This long process, a clear remnant of the muscular origin of these cells, eventually atrophies as the skate ages beyond 60 centimeters (Ewart 1889a).

4.3.3 Torpediniformes

The electric rays in the order Torpediniformes are a group of approximately 70 species distributed across approximately 4 families (Nelson 1994). All species are well-known for their ability to produce strong electric discharges from two large organs located in the head.

The electric organs of *Torpedo* and *Narcine* are kidney shaped, dorsoventrally flattened, and bilaterally located lateral to the eyes. Each organ is made up of 500-1,000 closely packed columns, each consisting of approximately 1,000 dorsoventrally flattened electrocytes as large as 5-7 millimeters in diameter and stacked like coins (Bennett 1971). The ventral surface is innervated, with the nerve fibers entering the space between electrocytes, and the dorsal face is uninnervated, consisting of many canniculi (Fig. 4.1).

The electric organ forms in the segments containing the first four branchial arches (Mellinger et al. 1978) from sheets of cells organized in columns of dorsal and ventral plates. The cells are observed initially to contain single nuclei containing myofibrils and then fuse into multinucleated myotubes with recognizable sarcomeric structures, including both thick and thin filaments. These cells are comparatively similar to surrounding muscle, aside from their distinct anatomical arrangement (Fox and Richardson 1978).

When the larvae are approximately 40 millimeters in length, the muscle cells that comprise the future electric organ appear to rotate approximately 90° with respect to the body axis. This is caused by a profound change in cell shape from a myotube to a rounded myotube and then eventually to horizontally flattened electrocytes (Fox and Richardson 1978). During this change in shape, nuclei become repositioned on the equatorial plane of the rounding cell. As development proceeds, the adjacent electroblasts interdigitate as they expand horizontally, stacking to form columns. The myofibrils contained within each electrocyte become contorted and disorganized, breaking into isolated components. Finally, disassembly of the myofibrils begins, with a longitudinal splitting followed by loss of A-bands, which

results in the isolation of Z-bands with thin filaments attached, beginning at the ventral pole and then spreading to the dorsal pole, completing the morphological transformation into an electrocyte.

The dorsal and ventral poles of each cell begin to diverge in their appearance. The ventral, innervated surface is characterized by smooth secretory vesicles that secrete an amorphous, unknown substance, which is apparently missing from the dorsal surface (Fox and Richardson 1979). In contrast, the dorsal surface begins to contain more cellular organelles, including a high concentration of heterogeneously sized vacuoles. These vacuoles fuse with the dorsal surface, creating pseudopodia that "loop" back to the membrane, creating the canniculi that characterize the dorsal surface (Fox and Richardson 1979).

Satellite cells are observed to be concentrated on the dorsal (uninnervated) surface, diagnosed by their round shape and mononucleated appearance. As the electric organ grows, these mononucleated cells are "enveloped" by the dorsal canniculi, and, eventually, the membranes fuse, increasing the number of nuclei present in the growing electrocyte (Fox and Richardson 1979).

The parallel efforts of Fox and Richardson (1978, 1979) and Mellinger (1978) are largely in agreement, with the exception of the role of innervation. Fox and Richardson (1979) report, based on both light microscopy and electron microscopy, that although the nerve is present through most of the developmental stages of the electric organ, no synaptic contacts between the tissues are present; only after the organ has formed do neurites penetrate the interelectrocyte space and establish synaptic contact. In contrast, Mellinger et al. (1978) suggest an inductive role for the nerve, although the observations that support this are restricted to light microscopy. Gautron (1974) reported that surgical denervation of adult electric organs leads to slow degeneration of electrocytes, including the reappearance of myofibril bundles in the cytoplasm. This finding suggests that although innervation may not play a role in the early development, it may serve a role in the terminal differentiation of electrocytes and perhaps maintenance of their phenotype.

4.3.4 Mormyroidea

The monotypic Gymnarchidae and the more than 200 species of Mormyridae all have weakly electric, myogenic electric organs (Sullivan et al. 2000). After hatching, mormyrids develop a distinct larval electric organ that completely degenerates by the time the larvae have become approximately 25 millimeters long. The organ is "replaced" by an anatomically and biochemically distinct adult electric organ, which develops after hatching but is not active until the larvae have become approximately 15 millimeters long. *Gymnarchus niloticus* maintains a single electric organ from hatching to adulthood. This electric organ closely resembles the structure of the larval organ in mormyrids. This, together with the phylogenetic relationship between the Gymnarchidae and Mormyridae (Sullivan et al. 2000), supports the hypothesis that these structures are homologous and suggests that the adult mormyrid organ is a derived structure among the Mormyridae (Hopkins 1999).

4.3.4.1 The Larval Mormyrid Organ/*Gymnarchus* Organ

The larval electrocytes are characterized by extensive membrane invaginations on both faces. The cytoplasm contains few organelles but many vesicles, which open into the invaginations of the plasma membrane, as well as irregularly shaped vacuoles containing an unknown "coating material" (Denizot et al. 1978). The faces are characterized by the presence of many nuclei and mitochondria as well as other cytoplasmic organelles. Another striking feature of the larval organs is the presence of abundant myofibrils. Rather than the parallel arrangement of muscle fibers found in muscles, myofibrils are arranged orthogonally such that muscle fiber bundles run in different directions, although sarcomeric structures (Z-lines and H-zones) are visible (Denizot et al. 1978). Myofibrils apparently do not extend into the stalk. The larval organ extends from the edge of the skull to the end of the dorsal fin and consists of four tubes of electrocytes, two dorsal and two ventral, all of which are located medially within the lateral muscle. The electrocytes are distinct from muscle fibers in that they are barrel shaped and oriented approximately 45° to the anterior-posterior axis (Denizot et al. 1978).

In *Gymnarchus,* the electrocytes are arranged in eight long tubes (referred to in earlier literature as "spindles"), four on each side of the body, located medially within the lateral muscle but extending to the tip of the tail (Dahlgren 1914; Srivastava and Szabo 1972). As in the larval mormyrid organ, the electrocytes are barrel shaped and innervated on the posterior face, although there is no stalk present (Fig. 4.1). Although both faces are "moderately convoluted" (Srivastava and Szabo 1972), the anterior (uninnervated) face reportedly has many small canniculi absent on the relatively smooth innervated face.

The electrocytes that comprise the larval electric organ in mormyrids are arranged in parallel on myotomes, each myotome contributing both muscle cells and electrocytes (Denizot et al. 1978). The outer portion of the myotome is devoted chiefly to the muscle cells and the inner portion is devoted to the larval electric organ, although the boundary between the muscle cells and electrocytes within a myotome is ambiguous and "intermediate" stages can be observed (Denizot et al. 1978). Very little else is known about the development of the larval organs in mormyrids. Detailed studies have been performed on *Gymnarchus niloticus* (Dahlgren 1914; Srivastava and Szabo 1972). Development of the *Gymnarchus* electric organ occurs between the 9th and 15th day of embryonic life (Dahlgren 1914). Dahlgren (1914) reports that the columns of electrocytes develop at different rates, a fact leveraged to observe different points of development within the same juvenile individuals. A subsequent study (Srivastava and Szabo 1972) examined a series of embryological material.

Electrocytes derive from two differentiated muscle fibers on the inner edge of myotome, which detach from the main myotome to form an electroblast (Dahlgren 1914; Srivastava and Szabo 1972). Later in development, muscle cells intermediately situated between the myotome and electroblast degenerate (Srivastava and Szabo 1972). The electroblast initially appears elongate, syncytial, and multinucle-

ated with a visible pair of two distinct bundles of myofibrils. As the primordium increases in length and width, the myofibril bundles begin to fuse, still showing transverse striations. As the electroblasts continue to grow, the myofibrils begin to occupy a central position. Throughout the process, the number of nuclei increase and additional myofibril bundles appear in addition to the central bundle, which join the central bundle and add to its thickness; transverse striations are still visible (Srivastava and Szabo 1972).

As these electroblasts continue to grow, the anterior ends remain pointed and the posterior ends become thick and round as the middle portion of the primordium becomes wider and more barrel shaped, likely due to the appearance of vesicles secreting an "amorphous substance" (Srivastava and Szabo 1972). As these vacuoles increase in number and size, the myofibril bundle disintegrates into filaments and transverse striations disappear.

As the transverse striations disappear, the nerve endings make contact with the posterior face of the electrocyte (Srivastava and Szabo 1972). A second vacuole type, unassociated with the nucleus, begins to appear on the posterior face. As these vacuoles increase in number, they fuse with the membrane, leaving canniculi on the posterior face.

As the primordium grows, the electromotor nerve approaches the posterior end, increasing the number of vacuoles in the central portion of the electrocyte as the myofibril bundle continues to disintegrate and striations disappear; only after this does synaptogenesis begin (Srivastava and Szabo 1972).

4.3.4.2 The Mormyrid Adult Organ

4.3.4.2.1 Anatomy

The adult electric organ consists of 4 columns of electrocytes, 2 on each side of the body surrounding the spinal cord, each composed of approximately 100 electrocytes (Bennett 1971; Bass 1986). Each electrocyte is flattened in the anterior-posterior dimension, consisting of anterior and posterior faces approximately 0.5 millimeter in diameter (Bass 1986). Typically, the posterior face is characterized by finger-like invaginations (Bass 1986) that fuse to form a stalk structure that is innervated by electromotor neurons. This innervation occurs away from the electrocyte on either the anterior or posterior side. In some cases, where innervation occurs on the anterior face, the electrocyte face is penetrated by the stalk system, which has consequences for the electric signal it produces (see Fig. 4.1; Bennett 1971; Gallant et al. 2011; also see Markham, Chap. 5). Penetrations are apparently unique to mormyrid electrocytes. Each electrocyte is bounded by a connective tissue septum, and the entire organ is surrounded by a connective tissue sheath. The anterior and posterior faces are characterized by numerous canniculi (Bass et al. 1986). Unlike many other electric fish species, myofibril bundles, complete with sarcomeric structures, are retained in the center of the electrocyte parallel to the two faces (Bass et al. 1986).

4.3.4.2.2 Development

Early studies of juvenile *Mormyrus rume* obtained from the field (Szabo 1960) indicate that the anterior aspect of the organ develops earlier than the posterior aspect. Szabo (1960) describes the most anterior cells as still attached to the myoseptum, suggesting that electrocytes originate from already differentiated muscle fibers. A subsequent study on *Pollimyrus* drew on observations from a developmental series of individuals bred under laboratory conditions and utilized light microscopy and electron microcopy observations (Denizot et al. 1982). This study does not describe these earliest stages of development, commenting only that electrocytes initially derive from 7 to 10 myotomes from tissue "that resembles myoblast tissue" (Denizot et al. 1982). More studies of the early development of adult electric organs are needed to clarify if adult electric organs, like their larval counterparts, arise from differentiated myotubes or from presomitic mesodermal precursor cells.

The first recognizable, differentiating electroblasts are found in 10- to 12-millimeters specimens (Denizot et al. 1982). These cells initially retain a myotome-like arrangement at approximately 45° angles to the anterior-posterior axis and are bounded by loose connective tissue. At this early stage, electroblasts already possess stalks, but there is no indication of penetrations (Denizot et al. 1982). Electrocytes also possess many myofilaments, similar to those of the larval electrocytes, which are retained into adulthood (Denizot et al. 1982). By time the larvae have reached 13 millimeters in length, the electrocytes have begun to lose their myotome-like arrangement and by 15.5 millimeters in larval length, the electrocytes are regularly arranged. The amount of muscle lateral to the developing electric organ is substantially decreased as the electrocytes increase in size. By 16 millimeters in larval length, the electrocytes begin to flatten and widen and nuclei of the stalk system become less heavily stained. Satellite cells are observed on the posterior face surrounding stalks and electrocytes, which appear to "facilitate" the formation of penetrations (Denizot et al. 1982). Penetrations begin to appear when the larvae reach 19 millimeters in length and increase in number as the fish grows beyond 33 millimeters.

No synapses are observed when the larvae are 10-12 millimeters long, and the adult electric organ is incapable of discharge at this time. Electric organ discharges are first observed in larvae that are 15.5 millimeters in length (Denizot et al. 1982). Denervation of the electric organ by Szabo and Kirschbaum (1983) revealed that disrupted innervation does not appear to affect differentiation of the electrocytes.

4.3.5 Gymnotiformes

Gymnotiformes are a diverse group of about 200 species (Albert and Crampton 2006), all of which are considered to have myogenic electric organs (with the exception of *Apteronotus;* but see Kirschbaum 1983). The diversity of electric organs among Gymnotiformes is considerable. *Electrophorus* is well-known for its ability

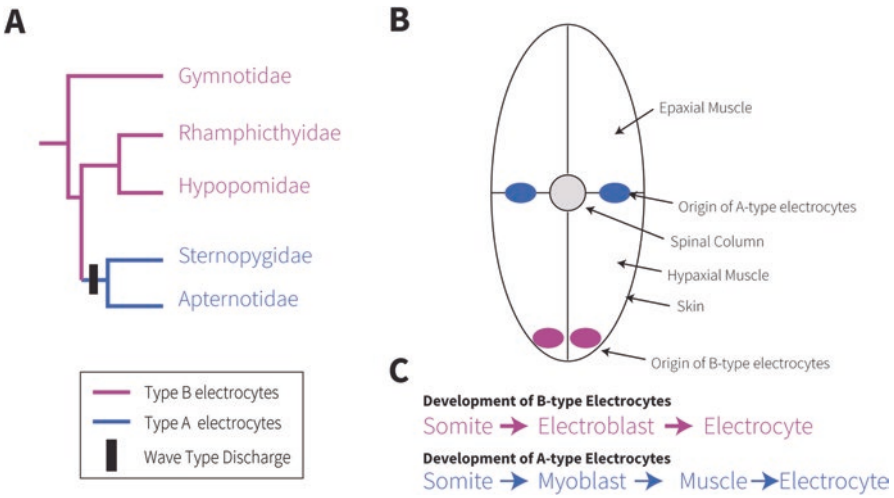

Fig. 4.2 **A:** condensed phylogeny of the major families of Gymnotiformes (after Crampton and Albert 2006). According to this phylogeny, pulse-type fishes are ancestral and wave-type discharges are derived. Type A and type B electrocytes (following the classification of Kirschbaum and Schwassmann 2008) show that type B electrocytes are ancestral and typical of pulse-type fish, whereas type A electrocytes are derived and characteristic of wave-type species. **B:** organization of musculature in a schematic cross section of a gymnotiform shows the location of epaxial and hypaxial musculature, highlighting the distinct origins of type A and type B electrocytes. **C:** distinct developmental mechanisms appear to underlie the difference between type A and type B electrocytes. See Fig. 4.3 for more details.

to produce strong and weak electric discharges using three electric organs: the main organ, the organ of Sachs (Sachs 1877), and Hunter's organ (Hunter 1775). Although *Electrophorus* is singular in this group for its strong discharge abilities, several other species are known to have multiple "accessory organs" (Bennett 1971; Stoddard 2002). A large group of species can continuously discharge their electric organ, leading to a quasi-sinusoidal electric organ discharge, the so-called wave-type discharging fishes, whereas another large group produces intermittent electric organ discharges, the so-called pulse-type discharging fishes (see Fig. 4.2). In addition, many wave-discharging species develop distinct larval and adult organs, but all pulse-type fishes (families Gymnotidae, Hypopomidae, and Ramphichthydiae) retain their "larval" organ through adulthood (Franchina 1997; Albert and Crampton 2006; Pereira et al. 2007; Schwassmann et al. 2014). Some species (e.g., *Sternopygus*) have well-characterized abilities to regenerate large portions of their electric organs after loss due to predation (Dunlap et al. 2016).

Despite the considerable diversity of Gymnotiformes, there is a relative paucity of developmental material available for analysis. The majority of developmental materials are obtained from field-captured specimens, although there have been successes in breeding Gymnotiformes in captivity (Kirschbaum 1975; Franchina 1997). This problem has been circumvented by some researchers by drawing on the

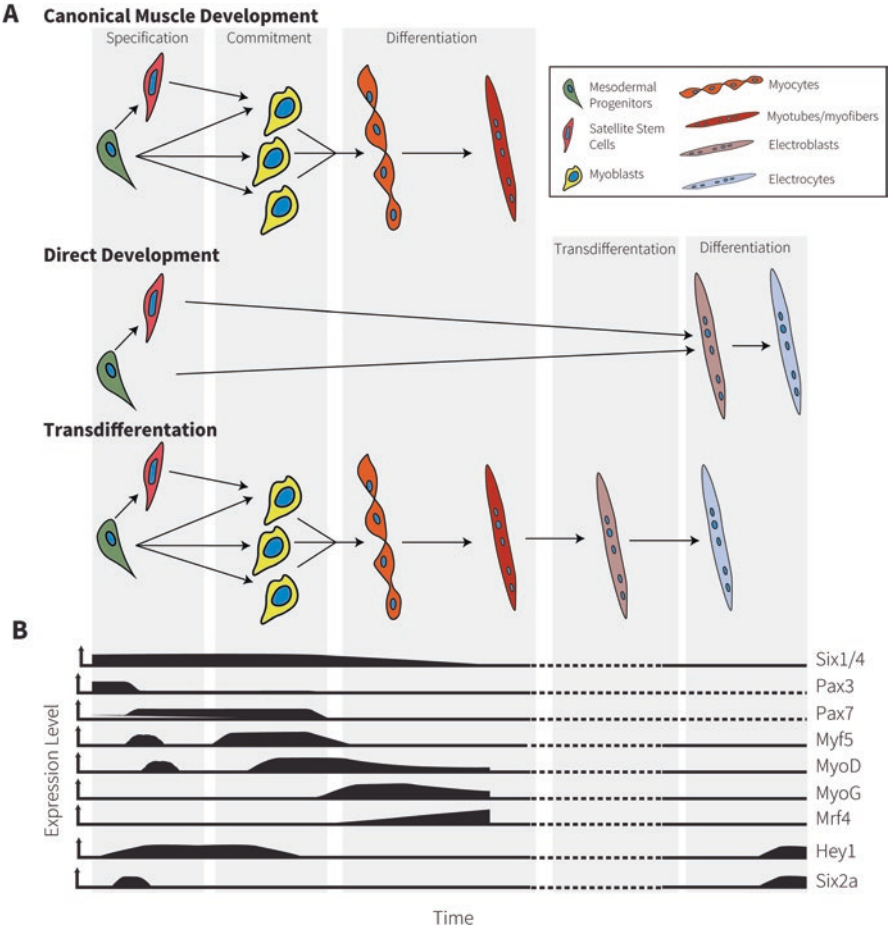

Fig. 4.3 **A:** comparison of canonical muscle development (*top*) to two distinct modes of electrocyte development. The first, exemplified by *Electrophorus*, where electrocytes differentiate directly from presomitic precursor cells (direct development, *center*). The second, exemplified by most other species of electric fish, whereby cells develop like normal muscle and then transdifferentiate into electrocytes (*bottom*). **B:** major transcription factors and myogenic regulatory factor (MRF) profiles at the various stages of muscle and electric organ development (Bentzinger et al. 2012; Gallant et al. 2014). Note that there are no data for gene expression in electroblasts at present (*dotted lines*). The major stages in development are highlighted (*gray boxes*).

regenerative capacity of Gymnotiformes. Unlike other taxa of electric fish, some Gymnotiformes are capable of regenerating the posterior (i.e., tail) portion of their bodies, possibly in response to intense predation (Dunlap et al. 2016). Thus, in both *Sternopygus* and *Eigenmannia*, surgical removal of the electric organ prompts regeneration of the electric organ, enabling an entire body of studies on the developmental mechanisms in these genera without the need for embryonic materials (Baillet-Derbin 1978; Zakon and Unguez 1999).

The Gymnotiformes vary widely in terms of the organization of their electric organs. In pulse-type Gymnotiformes, electrocytes are cylindrical in shape, about 300 microns in diameter, and about 200 microns thick (Bennett 1971; Bass 1986). Stalklike processes extend from the electrocyte, which receives the innervation and may be on the anterior or posterior face (Bennett 1971; Bass 1986). *Electrophorus* has posteriorly located, short stalks where innervation terminates (Bass 1986). The uninnervated anterior face is characterized by the presence of canniculi (Bennett 1971; Bass 1986). The electrocytes of *Gymnotus* are exceptional among pulse-type fishes, because the electrocyte faces are smooth and are innervated directly on the posterior side without stalklike process (Fig. 4.1). *Gymnotus* lack formal accessory organs but have groups of cells that discharge asynchronously, serving an analogous physiological function (Castello et al. 2009; Rodriguez-Cattaneo and Caputi 2009; Crampton et al. 2013; Rodriguez-Cattaneo et al. 2013).

Wave-type discharging fish (i.e., *Eigenmannia* and *Sternopygus*) have more "cigar-shaped" electrocytes (about 1-2 millimeters long and 200 microns in diameter; Bennett 1971; Bass 1986) and are loosely arranged in multiple columns of electrocytes that parallel the body axis. Innervation is typically on the posterior face, and extensive canniculi characterize the uninnervated face (Bennett 1971; Bass 1986). Stalks are not typically found in wave-type species (Fig. 4.1).

The developmental origins of electrocytes in Gymnotiformes have been a subject of debate in the literature; this is partially motivated by the sheer diversity of species considered together with a paucity of developmental materials for study. Early studies of *Electrophorus* suggested that electrocytes originated from skeletal muscle precursors (e.g., Fritsch 1883), whereas others claimed that electrocytes originated from undifferentiated presomitic mesodermal precursor cells in a defined germative zone (e.g., Keynes 1961).

Evidence from numerous developmental studies (Wachtel 1964; Szabo 1966; Esquibel et al. 1971) seem to support the former hypothesis, and Szabo (1966) attempted to reconcile the two by accepting the position of the germative zone postulated by Keynes (1961) but claiming that the early electroblasts passed through an intermediate skeletal muscle phase, in-line with histological evidence that he obtained. Schwassmann et al. (2014), after obtaining a much larger sample of field-captured embryological materials, demonstrated unambiguously that electrocytes originated from a group of metamerically organized, undifferentiated embryonic trunk mesoderm cells, supporting the original hypothesis of Keynes (1961).

These most recent results are in apparent contrast to findings in *Sternopygus* by Patterson and Zakon (1996, 1997) and in *Eigenmannia* by Baillet-Derbin (1978) who both studied the regeneration of the electric organ as a proxy for understanding the development of the electric organ. Baillet-Derbin (1978) and Patterson and Zakon (1997) demonstrated that electrocytes derived from striated muscle cells, complete with sarcomeric structures. Using sophisticated cell labeling and electron microcopy and light-microscopy observations, Patterson and Zakon (1993) was able to unambiguously show that the source of these muscle fibers were satellite cells near the wound, which first differentiated into muscle and then into mature electrocytes.

The apparent contradiction of these results could be resolved by a comparative synthesis by Kirschbaum and Schwassmann (2008). By examining embryonic materials from eight species representing each of the major families of Gymnotiformes, it becomes evident that there are two electrocyte types present in Gymnotiformes. Type A electrocytes, characteristic of wave-type Gymnotiformes (i.e., *Eigenmannia, Sternopygus, and Apteronotus*), originate inside both hypaxial and epaxial myomeres. Type B electrocytes, characteristic of the pulse-type species (i.e., *Electrophorus, Gymnotus, Rhamphythis, and Brachyhypopomus*), originate from a distinctive germative zone below hypaxial muscle. Intriguingly, Gymnotiformes with type B electrocytes lack distinct larval and adult electric organs (Albert and Crampton 2006), whereas Gymnotiformes with type A electrocytes develop distinct larval and adult organs (see Fig. 4.2).

The apparent contradiction of results on the origins of electrocytes in Gymnotiformes may therefore be partly explained by two distinct developmental mechanisms: that type A electrocytes originate between muscle fibers and retain muscle fiber-like morphology for several weeks and that type B electrocytes differentiate directly from a mesodermal precursor cell into electrocytes with no intermediate stage resembling skeletal muscle. This is a satisfying explanation for a standing enigma in Gymnotiformes, but additional studies are necessary to explore this concept further. One key question without an answer was raised by Schwassmann et al. (2014): how closely related are the developmental mechanisms regulating postembryonic/regenerative properties to embryonic mechanisms? A second question is the apparent discordance between the observations of several authors' study of *Electrophorus* development that seem to support development from skeletal muscle rather than from undifferentiated presomitic mesoderm. One possible explanation is the considerable postembryonic development, noted by Szabo (1960), that likely occurs as the animal grows. It is conceivable that mechanisms of "growth" of the electric organ and embryonic development may be difficult to differentiate in juvenile specimens.

4.3.5.1 Type A Electrocytes

No studies have described the embryonic development of myogenic electric organs in wave-type discharging electric fish. Instead, Baillet-Derbin (1978) and Patterson and Zakon (1997) leveraged the regenerative properties of Gymnotiformes (see Sect. 4.3.5) to examine the development of the electric organ. In both cases, (1) a blastema forms at the wound site following surgical amputation of the tail; (2) blastemal cells cluster to form fully differentiated, multinucleated muscle cells (i.e., expressing normal sarcomeric proteins); (3) the earliest recognizable electroblasts contain myofilaments and even sarcomeric structures but are much larger in cross-sectional area than muscle; and (4) these myofilaments quickly disassemble, followed by the invagination of the posterior (innervated) face to form canniculi that coincides with the appearance of innervation in *Eigenmannia* (Baillet-Derbin 1978; Patterson and Zakon 1997).

Drawing on multiple lines of evidence, Patterson and Zakon (1993) concluded that the cells that form the blastema after amputation are satellite cells, including the fact that the cells express *Pax7*, which is characteristic of stem cells (Weber et al. 2012; see Sect. 4.2.2). Unguez and Zakon (1998a) delineated fast and slow muscle isoforms and discovered that following differentiation, centrally located fast muscle fibers fuse to form electrocytes, whereas more peripherally located slow muscle fibers do not.

In a follow-up experimental study, Unguez and Zakon (1998b) examined the changes in protein expression and electrocyte morphology in *Sternopygus*. Most surprisingly, they found that although muscle cells did not change their biochemical profile, electrocytes began to express sarcomeric proteins, myosin heavy chain, and tropomyosin within weeks of spinal transection. Striking electron micrographs reveal the formation of new sarcomeric structures in denervated electrocytes, suggesting an "inhibitory" role of innervation on the maintenance of the electrocyte phenotype.

4.3.5.2 Type B Electrocytes

Light-microscopy observations with immunohistochemistry revealed that vertically aligned electrocytes in *Brachyhypopomus gauderio* form near the ventral boundary of the ventral mass of hypaxial musculature, which appear in specimens about 6 days old (Franchina 1997). Electrocytes take on a cylindrical shape with tapered ends and lack stalks. In later stages, the electric organ extends rostrally and caudally as the electrocytes begin to flatten, separate into rows, and develop stalks on the posterior face.

In *Electrophorus electricus*, electrocytes originate from a "germinal zone" in the ventral tip of the myotome (Szabo 1960; Keynes 1961; Schwassmann et al. 2014) and appear to differentiate from the anterior portion of the animal such that the posteriormost electrocytes are the most developed (Szabo 1960; Schwassmann et al. 2014). Sach's organ is the first to develop, followed by the main organ, and eventually Hunter's organ (Szabo 1960). These cells lose their cell membrane and are described as "mere nuclei" (Schwassmann et al. 2014) before rapidly producing electroplasm, aligning, and forming a new syncytial membrane. Myofilaments appear near several of the electroblast nuclei, although they are relatively sparsely distributed without sarcomeric arrangement. As development continues, electrocyte nuclei become more numerous at the posterior (innervated face) of the electrocytes, which are characterized by newly forming canniculi. At this phase, the electromotor neurons are making synaptic contacts with the posterior face (Schwassmann et al. 2014).

4.3.6 Siluriformes

The family Malapteruridae consists of 2 genera and approximately 20 species, all of which are electrogenic. Best known among these is *Malapterurus electricus,* well-known for its strong electric discharges. The first histological analyses concluded

that the electric organ originated from the glandular portions of the epidermis, an idea attributed by Johnels (1956) to Fritsch (1883). A single study on the development of *Malapterurus* was performed by Johnels (1956).

The electric organ of *Malapterurus* lies in the skin and surrounds the body over most of its length. This electric organ is unusual among electric fish in that the millions of constituent "lily pad"-like electrocytes are irregularly organized, superficially located, and surround the entire body near the skin much like a jacket. The electric organ is innervated by two nerves originating from the first spinal segment and branching to innervate the electrocytes. The cells are disc shaped, about 1 millimeter in diameter, and 20-40 microns thick. A conical region in the center of the caudal face produces a stalk that protrudes and is innervated by a motorneuron (Fig. 4.1). The electrocytes are flanked on both sides by a layer of connective tissue isolating the electric organ from the skin and the body.

Analysis of the electric organ in 11.4- and 12.7-millimeters specimens revealed small but adultlike electrocytes in the vast majority of the body. Despite this, a distinct germinal zone was located in the rostral portion of the electric organ, dorsal to the pectoral fin near the shoulder girdle. Here, the interior and exterior connective tissue layers surrounding the electric organ meet and attach to the shoulder girdle. A few researchers noted a small "deficiency" in the muscle wall in this region of adult *Malapterurus* that is more pronounced in the juvenile fishes at the point at which the electric nerve enters the organ. Here, electrocytes can be observed forming, although there are few histological details about these cells to permit further interpretation.

4.3.7 Euteleostei

A single genus of marine perciformes, *Astroscopus*, is known to be electric. These unusual and enigmatic fishes have been poorly studied compared with many of the other species considered in this chapter.

The electric organ in *Astroscopus* is located just behind the eye (Bennett 1971) and consists of two irregular vertical columns that surround slender extraocular muscles (White 1918; Bennett 1971). Each column is composed of approximately 200 parallel plates, separated by connective tissues and consisting of approximately 20 electrocytes laying side by side (White 1918; Dahlgren 1927; Bennett 1971). The electrocytes are flattened horizontally and are densely innervated on the dorsal surface. The ventral surface has many short papillae and canniculi that increase the surface area (Fig. 4.1). Only the innervated face is active during discharges, created by postsynaptic potentials in the dorsal surface (Dahlgren 1927; Bennett 1971).

There is only one study on the development of *Astroscopus*, performed by White (1918), that found that the electric organ derives from muscle cells comprising four of the six extraocular muscles of each eye. The earliest stages of electric organ development were observed in embryos of 4-14 millimeters in length. Future electrocyte cells absorb stain more darkly and are smaller than other muscle cells that

comprise the four extraocular muscles. Electric organs derive from the lateral edge of the four muscles farthest from the eye (White 1918).

By the time the larvae have become about 14 millimeters in length, these cells have increased to about 6× times the diameter of normal surrounding muscle cells and their nuclei have moved close to the cell membrane. At this stage, the cytoplasm contains numerous vacuoles that form the papillae of the ventral face. By the time the larvae have become about 33-35 millimeters, the 4 electric organs are well developed and separate from the eye muscles, and the electrocytes begin to assume their flattened shape, broadening laterally without deepening in the dorsal-ventral aspect. The dorsal face changes in structure to a flat smooth structure, whereas the ventral surface retains it numerous papillae (White 1918; Dahlgren 1927). Similar to other electric fishes, the *Astroscopus* electrocyte is polarized (White 1918; Bennett 1971) along the dorsal-ventral body axis.

4.4 Comparative Genomics

The earliest studies concerning the molecular biology of electric organs employed a variety of candidate gene approaches, examining the expression patterns of mRNAs and proteins identified to be important in developmental processes, homologous to those found in canonical model systems. These studies were followed by next-generation sequencing approaches in the past decade, which has led to a proliferation of genomics and RNA sequencing and proteomic datasets for electric organs, motivating "unbiased" surveys of both gene identity and gene expression. For a summary of these techniques, see Pitchers et al. (2016).

From a molecular biology perspective, the most comprehensively surveyed group of electric fish are the Gymnotiformes, chiefly the species *Sternopygus macrurus* (Kim et al. 2009; Güeth et al. 2013; Pinch et al. 2016) and *Electrophorus electricus,* which were the first species of electric fish to have a completed genome, along with full somatic mRNA and miRNA transcriptomes and an annotated proteome (Gallant et al. 2014; Traeger et al. 2015, 2017). Several studies have focused on the proteomics and transcriptomics of the neuromuscular junction in *Narcine* (Nazarian et al. 2007, 2011; Mate et al. 2011) and may provide datasets that have relevance to development in future analyses. Relative latecomers to molecular techniques are the mormyrid electric fish (Gallant et al. 2012; Lamanna et al. 2014, 2015), which now also have a completely sequenced genome and somatic transcriptome (Gallant et al. 2017).

Although comparative biology is in the "DNA" of the electric fish research community, efforts to compare molecular mechanisms between lineages have only just begun in the past few years. The first of these studies assembled the genome and somatic transcriptomes of *Electrophorus* and the electric organ and skeletal muscle transcriptomes of several other species (the gymnotiforms *Eigenmannia and Sternopygus,* the siluriform *Malapterurus,* and the mormyroid *Brienomyrus*), representing three independent origins of electroreception (Gallant et al. 2014).

This study identified orthologous genes between each of these lineages and compared expression in adult electric organs across these taxa, with a particular focus on genes with well-annotated functions in vertebrates. The overall result of this study was the discovery of several groups of genes, with known biological functions, that shared highly similar patterns of gene expression across each of the independently evolved electric organs.

A key limitation to the study by Gallant et al. (2014) and to nearly every other dataset on the molecular biology of electric organs is that they are based on adult tissue samples. As such, they give only a snapshot of one point in time, namely, after the electric organ has already formed, and therefore miss the period of embryonic electric organ development. This thereby limits the ability to interpret the role of particular genes in the evolution and development of electric organs; however, there is still great value in these studies. First, they give a biochemical "inventory" of electrocytes across many species. Second, they describe patterns of gene expression that are the *consequence* of embryonic developmental processes and reflect signatures of ongoing postembryonic development/growth. Much in the way that examining cosmic background radiation may give insights to how the "big bang" may have unfolded, so too might these studies provide insights into the mechanisms of development of electric organs.

In efforts to integrate these comparative results with previous molecular studies, this section has been organized by the themes discussed in Sect. 4.3.1. Section 4.5 considers where new data are needed, which may identify opportunities for future studies and the application of new techniques.

4.4.1 What Are the Common Features of Electric Organs Across Lineages?

Analyses of the transcriptome of the electric organ of *Electrophorus* (Gallant et al. 2014; Traeger et al. 2015) revealed strong upregulation of many genes associated with ion transport, including voltage-gated ion channels and transporters, acetylcholine receptor activity, and Ca^{2+} binding, although these were not explicitly examined in other species. One notable example is the Na^+/K^+-ATPase α-subunit, of which there are several paralogues in fishes, where it appears that species have evolved the use of different subunits for membrane repolarization.

Expression analysis of the *scn4aa* gene (Zakon et al. 2006; Arnegard et al. 2010) reveals that weakly electric fish have convergently neofunctionalized the voltage-gated sodium channel $Na_V1.4a$, normally expressed in muscle, to generate action potentials in electrocytes. A more detailed review of these studies is provided by Markham (Chap. 5). This robust result has been confirmed in many studies (e.g., Gallant et al. 2014; Lamanna et al. 2014).

The abundance of sarcomeric proteins appears to be lower than in skeletal muscle for all electric organs studied thus far, which has been a robust finding in both ultrastructural studies (discussed in Sect. 4.3) and numerous molecular studies

(Mate et al. 2011; Gallant et al. 2012; Lamanna et al. 2015). Intriguingly, the relative amount of sarcomeric proteins between electric fish lineages is more variable; mormyrids have a much higher amount of sarcomeric proteins (Gallant et al. 2012; Lamanna et al. 2014, 2015) than Gymnotiformes (Cuellar et al. 2006) and Torpediniformes (Mate et al. 2011), which has been noted by ultrastructural studies (see Sect. 4.3). Although most electric fish species seem to achieve a low level of sarcomeric proteins by repressing mRNA expression, the Gymnotiformes *Sternopygus* seems to be exceptional in this regard. Several studies have indicated that *Sternopygus* electrocytes express a full complement of sarcomeric mRNAs, essentially at the same levels found in skeletal muscle (Gallant et al. 2014; Pinch et al. 2016) but lack their proteins (Cuellar et al. 2006), which has implicated a presently unknown mechanism of posttranscriptional repression. This issue is revisited in Sect. 4.4.2. Despite the heterogeneity in the amount of sarcomeric protein and the mechanism by which this paucity arises, the genes *smyd1a, smyd1b*, and *hsb11*, all implicated in the assembly and maintenance of sarcomeric integrity, are highly downregulated in all electric fish lineages examined (Gallant et al. 2014).

As reviewed in Sect. 4.2, muscle cells translate action potentials into the release of Ca^{2+} from the sarcoplasmic reticulum, which causes the sarcomeres to contract. A sudden change in the shape of the electrocytes would have deleterious effects on the strength of electric signals, thereby affecting the efficiency of electric signaling. Because no electrocytes are known to be contractile, it would appear that the excitability of electrocytes and the ability to contract has been decoupled. Gallant et al. (2014) noted that a consistent feature of electric organs appears to be the downregulation of the DHPR *cacna1sa.* In skeletal muscle, this would have the result of preventing the release of Ca^{2+} from the sarcoplasmic reticulum on depolarizing the plasma membrane, regardless of how much sarcomeric protein is present.

To maximize the strength of electric fields, current dissipation must be minimized and the current must be conducted unidirectionally through the electric organ. This is partially achieved through the uniform orientation of individual electrocytes (Bennett 1971). An additional property that may facilitate this is a key structural similarity of all electric organs outlined above: connective tissue septa forming the boundaries of individual electrocytes and surrounding the electric organ, which may further prevent the dissipation of current throughout the body. Although the biochemistry of these septa has not been explicitly examined, Gallant et al. (2014) noted the expression of two collagen genes, *col6a6* and *col141a1*, a glycosyltransferase (*gyltl1b*), and dystrophin (mutations of which cause muscular dystrophy). These proteins may act in concert to form the collagenous sheaths that facilitate current flow through the electric organ.

4.4.1.1 Cell Size

Another convergent feature of electrocytes, as described in Sect. 4.3, is that electrocytes are much larger than muscle fibers. The mechanisms by which electrocytes achieve this remarkable cell size is presently unknown, although it may involve a

combination of both embryonic and postembryonic mechanisms. Gallant et al. (2014) note the upregulation of several members of the insulin-like growth factor-signaling pathway genes, including ligands (e.g., *gill*), effectors (e.g., *pik3r3b*), and proregulatory factors (e.g., *net-37*) within this pathway, as well as profound down-regulation of negative inhibitors (e.g., *fbxo40*). Because insulin-like growth factor-signaling pathways have been implicated in organism size polymorphisms as well as in changes in individual tissue size, these may be good candidate genes for the regulation of cell size in electric organs as well (Gallant et al. 2014).

4.4.2 What Is the Role of Postembryonic Growth in the Development of the Electric Organ?

Denervation studies of *Sternopygus* and *Torpedo* electrocytes (Gautron 1974; Unguez and Zakon 1998b) have led to the hypothesis that the electromotor neurons may have an inductive role in the development of electrocytes. The results obtained in *Sternopygus* in turn led to the hypothesis that electromotor neurons may post-transcriptionally regulate protein expression (Zakon and Unguez 1999). Strong evidence for this hypothesis was provided by Cuellar et al. (2006), demonstrating that mRNAs for sarcomeric proteins in electric organs are expressed at comparable levels in muscle but sarcomeric proteins are not. The potential mechanisms that underlie the regulation of proteins are currently unidentified, but the potential mechanisms in the light of current evidence are reviewed by Güeth et al. (2013). To contrast with these results, denervation studies in mormyrids (Szabo and Kirschbaum 1983) demonstrate that the electric organ persists without neuronal input. In many of the electric fish lineages described in Sect. 4.3, differentiation of the electrocyte is well underway before synaptogenesis has begun. The role of innervation in the development and maintenance of electrocytes is an area that needs more careful and thorough study.

4.4.3 Do Electrocytes Arise from Fully Differentiated Muscles or Mesodermal Precursors?

Histological studies on electric organ development considered in Sect. 4.3 have motivated at least two distinct pathways by which electric fish appear to achieve fully differentiated electrocytes (Fig. 4.3). Efforts to characterize the molecular basis of the developmental mechanisms regulating the progression of electrocytes through these pathways have been largely fruitless. One potentially useful piece of data is the varying degrees of sarcomeric protein expression as well as the timing of their expression during development. These data support at least two distinct pathways to an electrocyte: one that relies on fully differentiated muscles transdifferentiating into electrocytes and another direct pathway from mesodermal precursors (Fig. 4.3A).

A potentially important source of information in understanding the mechanisms underlying these various developmental mechanisms are the transcription factors (Fig. 4.3B). Transcription factors have been a favorite subject of examination in the molecular biology of electric organs, particularly the myogenic regulatory factors (MRFs). To date, MRFs have been identified in Torpediniformes, Mormyriformes, and Gymnotiformes through a variety of approaches. As described in Sect. 4.2, MRFs act in a hierarchical fashion to specify, commit, and eventually cause differentiation of skeletal muscle cells by working to activate muscle-specific genes. MRFs are activated by transcription factors that pattern the early presomitic mesoderm.

Three transcription factors, *six2a, hey1a,* and *hey1b,* are normally expressed at low levels in differentiated skeletal muscle (Fig. 4.3B). However, in all lineages of electric fish examined to date, they are highly abundant (Gallant et al. 2014; Lamanna et al. 2015). The downstream targets of these genes are the MRFs *myod*, *myogenin,* and *six4b*. The expression of *myod* has been observed in both *Sternopygus* (Kim et al. 2004, 2009; Pinch et al. 2016) and *Torpedo* (Neville and Schmidt 1992; Asher et al. 1994) to be at comparable levels to those of skeletal muscle. In contrast, *myogenin* and *six4b* are expressed at very low levels in nearly every electric fish lineage, with the exception of *Sternopygus* (Kim et al. 2004, 2009; Gallant et al. 2014; Pinch et al. 2016). This is consistent with the relatively high levels of sarcomeric mRNAs characteristic of this lineage.

Mormyrid electric organs all highly express *erh, mef2aa,* and *mef2b,* all transcription factors downstream of or parallel to *myogenin* (Gallant et al. 2014, 2017; Lamanna et al. 2015). A recent study demonstrated that *mef2aa* is among the 50 most abundant genes expressed in the electric organ (Gallant et al. 2017). This is in stark contrast to the electric organ of *Sternopygus*, which expresses a variety of MGFs, including *mrf4* and *mef2,* at comparable or slightly higher levels in electrocytes than in skeletal muscle (Kim et al. 2004, 2009; Pinch et al. 2016).

4.5 Summary

The independent origins of electrogenesis span vertebrates (Fig. 4.1), represented by two lineages of elasmobranch fishes and four lineages of teleost fishes. Despite the considerable diversity of taxa represented by the term "electric fish," electric organs share many aspects of their form and physiological function. These similarities are likely the result of two major modes of development (see Fig. 4.3) originating from the same developmental precursor, namely, presomitic mesoderm, which also ultimately forms muscle. Although the majority of electric organs are the result of transdifferentation of skeletal muscle, it would appear that pulse-type Gymnotiformes in particular (e.g., *Electrophorus*; see Sect. 4.3.4.2) may derive their organs directly from undifferentiated myoblasts or somatic mesoderm. Throughout this process, the electromotor nerves may or may not play an inductive role. In mormyrids, *Malapterurus*, Rajidae, and *Astroscopus*, electrocytes seem to

form without synaptic contact, whereas in some Gymnotiformes as well as in *Torpedo*, denervation studies have revealed a role in the maintenance of the organ.

Synthesizing information from what is known about skeletal muscle development (see Sect. 4.2) with observations of electric organ development (see Sect. 4.3) and available molecular data (see Sect. 4.4), we are left with glimmers of insight as to key molecular mechanisms that may regulate the development of electric organs. First, the MRF *myogenin* is almost universally repressed in electric fish, potentially by "upregulated" the transcription factors *six2a*, *hey1a*, and *hey1b*. Second, mRNA for sarcomeric proteins seems to be tightly coupled to myogenin expression. In *Sternopygus*, *myogenin* and sarcomeric transcripts are comparable to muscle, and in all other electric fish lineages, both *myogenin* and sarcomeric mRNAs are low in abundance among electrocytes compared with skeletal muscle. Mormyrids, despite relatively low levels of myogenin expression, seem to have relatively high levels of sarcomeric proteins compared with electrocytes in other electric fish lineages. This may correspond to relatively high levels of transcription factors downstream of *myogenin* (i.e., *mef2a*).

A striking outcome of this comparative treatment of electric organ development is the uniqueness of the *Sternopygus* electric organ compared with nearly every other electric fish species examined thus far. *Sternopygus* has been observed to contain both type A and type B electrocytes within the same individual, suggesting that *Sternopygus* may represent a "more primitive or earlier, evolutionary pathway" (Schwassmann et al. 2014). This pathway may well be the pathway that is recapitulated by regeneration in the wave-type electric fishes *Eigenmannia* and *Sternopygus*. Alternatively, *Sternopygus* could represent a distinct developmental mechanism from those in all other electric fishes. Until detailed studies of the embryonic development of *Sternopygus* are performed, this will remain a mystery. Regardless, this highlights the importance of comparative approaches in attempting to understand the "general principles" in development. Given the volume of studies on *Sternopygus*, one must be careful not to assume that the mechanisms at play in *Sternopygus* are necessarily representative of other electric fish.

4.5.1 Need for New Data

There are several obvious places for additional data to understand electric organ development. First, more developmental studies need to be performed on a series of well-fixed embryological materials for a variety of species, particularly *Astroscopus*, Rajidae, and Malapteruridae.

Second, given the proliferation of genomics data, new developmental studies should be considered in the context where transcriptomics and genomics can be maximally utilized. A comparative, developmental series of gene expression will likely provide the greatest insight into understanding the mechanisms of gene expression. These studies should also consider the spatial patterns (i.e., in situ hybridization) in addition to the temporal patterns of gene expression. In species

where developmental series are difficult or impossible to obtain, any transcriptomic and genomic data would be of great importance. Along these lines, additional data on the development and developmental genetics of larval organs would also be of great importance.

Third is the need for a hypothesis testing framework in studies of electric organ development. This is particularly important concerning many of the genes identified by next-generation sequencing screens and will require the construction of new tools (e.g., CRISPR/Cas9 gene editing, antisense RNA interference, viral-mediated gene transfer) to evaluate the hypothetical roles of particular genes in developmental contexts. Thankfully, these techniques and tools are readily applied in "noncanonical" model systems, and the prospects for applying these techniques in electric fish look bright (Pitchers et al. 2016).

Other hypotheses about electric organ development may be tested without the application of sophisticated gene manipulation techniques. Although a handful of studies have examined the role of denervation of the electric organ, this has not been systematically evaluated. Establishing the role that electromotor neurons play in both inducing electric organ development and maintaining its phenotype should be straightforward experiments, but they have not yet been universally applied to representative species for all independent origins of electric organs.

Fourth is the need for a cleaner separation between embryonic development and postembryonic development/growth. Vertebrate embryos are typically born with a set number of muscles. Throughout life,. the number and size of individual fibers may change, largely through exercise and potentially injury. The developmental mechanisms that underlie the embryonic development of muscle and these postembryonic mechanisms are distinguishable. Electric organs face similar problems of damage and the need to increase in size as the animal grows. It is likely that histologists, in their reliance on juvenile animals, may have conflated these two processes, particularly given the conflicting accounts of *Electrophorus* development described in Sect. 4.3 and the studies of regeneration in *Sternopygus* and *Eigenmannia.* How do electric organs grow and repair to meet the demands of living fish? Are these mechanisms like those of muscle or distinct in different lineages? Are there distinct populations of electrocyte stem cells?

Finally, an area of great importance in electric organ form and function is the development of highly polarized cells from symmetrical precursors. Despite the nearly universal description of vacuoles creating canniculi in the uninnervated face of electrocytes, there are hardly any insights into the mechanisms underlying this process. Although the innervated face is characterized in some species by the proliferation of folds, the answers to the developmental mechanisms underlying this likely lie in the profound literature on the development of the neuromuscular junction. In contrast, the canniculi of the various taxa seem to occur around the time that innervation develops but on the side opposite the innervation. Schwartz et al. (1975) hypothesized, based on ultrastructural analyses in several species, that these canniculi may be homologous to the T-tubules in skeletal muscle, but this hypothesis remains essentially untested.

4.5.2 New Techniques and Approaches

New insights into the evolution and development of electric organs will also be motivated by the application of new techniques. One particularly exciting area is that of "regulomics": how the protein profile of a cell is modified by networks of gene regulation but also by phosphorylation and small molecules such as miRNAs. There have been a few of these studies, mainly in *Electrophorus,* that have identified the mechanisms of regulation that have not widely been considered in other electric fish lineages. For instance, Traeger et al. (2015) discovered 18 novel miRNAs in *Electrophorus electricus* and characterized 294 miRNAs that are conserved in other species. Of these, 18 were differentially expressed between adult muscle and the electric organ (Traeger et al. 2015). Several of these miRNAs play inhibitory roles (i.e., miR-193, -218, and -365) in muscle development. One very highly expressed miRNA, referred to as mir-11054, was found to be exclusively expressed in the electric organ and is apparently novel to *Electrophorus*. This mRNA is expressed 30-fold higher in electric organs versus that in smooth muscle, is not expressed in other somatic tissues, and derives from the intron of the inward-rectifier K^+-channel gene *kcnj12b*, which is highly abundant in electrocytes. The functional role of this "electromiR" is presently unknown.

A second area of regulation is phosphorylation, an important posttranslational cell regulatory mechanism, which has been poorly studied in electric fish and may have very important implications for development. Recently, Traeger et al. (2017) constructed an improved genomic assembly and annotation of *Electrophorus* and performed a comprehensive analysis of the proteome using cutting-edge isotope-assisted quantitative mass spectrometry. This analysis revealed numerous known and previously uncharacterized phosphorylation sites in a variety of transcription factors and membrane-bound proteins and revealed specific differences in the abundance of phosphoproteins between each of the three organs of *Electrophorus*. The differences in the abundance of phosphoproteins and cellular regulators of phosphorylation will undoubtedly be an important area of research in trying to understand how electric organs develop and function.

4.5.3 Concluding Remarks

Efforts to understand the evolution of electric organ development has been an ongoing enterprise in biology as old as *The Origin of Species*. Histological techniques, applied in every major lineage of electrogenic fishes, have set the stage for a new generation of genomics studies. Although there are still many unanswered questions, the answers have far-ranging implications for essential questions in evolutionary biology, physiology, and developmental biology. The arrival of new datasets and a growing set of new tools make the prospect of identifying the "steps by which these wondrous organs have been produced" (Darwin 1859) ever brighter.

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Chapter 5
Biophysical Basis of Electric Signal Diversity

Michael R. Markham

Abstract The electric sensory and communication signals of electric fish show remarkable diversity in their waveforms, and this diversity is driven by selective pressures related to reproduction, sensory ecology, predation avoidance, and the metabolic costs of signaling. These electric signals are generated by electrocytes, electrically excitable cells that comprise the electric organ. Although the signaling rate is controlled by a brainstem pacemaker or command nucleus that coordinates the discharge of electrocytes within the electric organ, waveform diversity arises primarily from the underlying biophysics of electrocytes, including their passive electrical properties, morphology, voltage-gated ion channels, and regulatory pathways that modify electrocyte function. Electrocyte morphology and innervation patterns are a major source of signal diversity in the African mormyrid electric fishes, whereas diversity of ion-channel expression patterns has a strong influence on waveform diversity in the South American gymnotiforms. Convergent evolution of ion channels in both clades further contributes to signal diversity. Little is known about the ionic mechanisms of signal diversity in mormyrids. Additionally, asynchronous activation of distinct electric organ regions with different electrocyte properties enhances waveform complexity in some gymnotiforms. Signal diversity associated with development and sexual dimorphism arises from the effects of steroid hormones on electrocyte ion channel kinetics, and the rapid changes in signal waveform are mediated by the effects of peptide hormones on electrocyte action potentials and ion channel function. These processes have been investigated primarily in a small number of gymnotiforms, highlighting a great need for broader comparative studies across gymnotiform species and between mormyrids and gymnotiforms.

Keywords Action potential · Electric organ · Electric organ discharge · Electrocyte · Ion channels · Melanocortin hormones · Sexual dimorphism · Steroid hormones

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5.1 Introduction: The Dimensions of Electric Signal Diversity

The signals generated by electric fish are extremely diverse in some ways that are obvious to any casual observer and also in many ways that are not as readily apparent. Electric signal diversity is most apparent in the hundreds of freshwater electric fish species, where signals vary across multiple dimensions and timescales. These fishes are distributed broadly across African (Roberts 1975) and South American (Albert and Crampton 2005) waters. The independent origin of electrogenesis in the African mormyrids and the South American gymnotiforms provides a rare opportunity for comparative analyses of signal diversity mechanisms across two independent evolutionary replicates on two different continents (Lavoué et al. 2012). Understanding the mechanisms of this signal diversity requires integrative and comparative studies across levels of biological organization from genes to molecules, cells, organisms, social networks, and ecology. The end result of such comparative approaches to understanding the biophysical mechanisms of electric signal diversity is the promise of discovering both the general principles of signal production as well as the mechanisms of convergence and divergence in signal biophysics.

5.1.1 Strong Versus Weak Electric Signals; Fast Versus Slow Electric Signals

Fish generate electric signals known as electric organ discharges (EODs). One obvious difference among these electric signals is in the amplitude of the EOD, which would occur to anyone brave enough to put their arm first into an aquarium housing a weakly electric gymnotiform or mormyrid fish and then into an aquarium housing an electric catfish or eel. In the first case, the electric signal of the gymnotid or mormyrid is so weak as to be imperceptible, whereas in the latter instance, the electric signal of the catfish or eel is strong enough to cause extreme pain (Catania 2017).

Another rather obvious difference among electric signals is the EOD rate that can be easily appreciated by listening to EODs transduced to sound with an inexpensive audio amplifier. Some electric fish species generate EODs at very low rates with long, irregular intervals between EODs, sounding like a Geiger counter or a stuttering gas lawn mower. Other species produce regular, high-frequency EODs (about 100–2000 Hz) that sound like pure tones from the middle musical octaves. This difference is the basis for one of the most fundamental distinctions in electric signal diversity, the difference between pulse fish and wave fish.

5.1.2 Diversity of Electric Signal Waveforms

The true breadth of signal diversity begins to appear when comparing the time-voltage waveforms of individual EODs. It is this dimension of signal diversity that is the focus of this chapter. The EOD is typically recorded "head to tail" with two differential electrodes located in the longitudinal axis more than a body length from the fish. EODs recorded in this manner reveal vast differences in the waveforms between species (Fig. 5.1). These far-field EODs are species specific, showing variation in signal duration, the number of positive and negative phases, inflection points within a phase, and the order of positive and negative phases. In some species, EOD waveforms are also sexually dimorphic and can even be individually specific in some cases (McGregor and Westby 1992). The far-field EODs likely are not useful for electrosensing but are potentially effective as communication signals at this distance (Aguilera et al. 2001).

When the EOD is recorded from gymnotiforms using electrodes at various locations within a body length of the fish, these near-field waveforms show remarkable spatiotemporal variation (Assad et al. 1999; Caputi 1999). The near-field waveforms often bear little to no resemblance to the far-field signal, but the near-field signals likely are crucial for electrolocation and communication. The significance and mechanisms of near-field signal diversity are detailed in an excellent earlier review (Caputi 1999) and in a recent study (Waddell et al. 2016).

5.1.3 Diversity in the Spectral Content of Electric Signals

An important feature of electric signals is the power spectrum of the signal that represents the relative power in the signal across a range of frequencies ranging from 0 Hz DC to 10 kHz or higher. Although the power spectrum is determined exclusively by the time-voltage waveform of the EOD, differences in the power spectra are often not readily apparent by examining the differences in time-voltage waveforms (Fig. 5.2). Accordingly, electric signals that seem quite similar when presented as time-voltage recordings can have very different power spectra, with important consequences for both the communication and sensory functions of the signal. Low-frequency components of electric signals (approximately 0–50 Hz) activate ampullary electroreceptors and have important communication functions, whereas higher frequency components of the signal (approximately 100 Hz to 10 kHz) are detected by tuberous electroreceptors and serve both sensory and communication functions (see also Baker, Chap. 2; Leitch and Julius, Chap. 3).

For pulse fish, monophasic signals with a baseline at or near 0 V carry the majority of their energy in the low-frequency range, whereas the addition of one or more additional phases that make the signal symmetrical about 0 V greatly suppresses

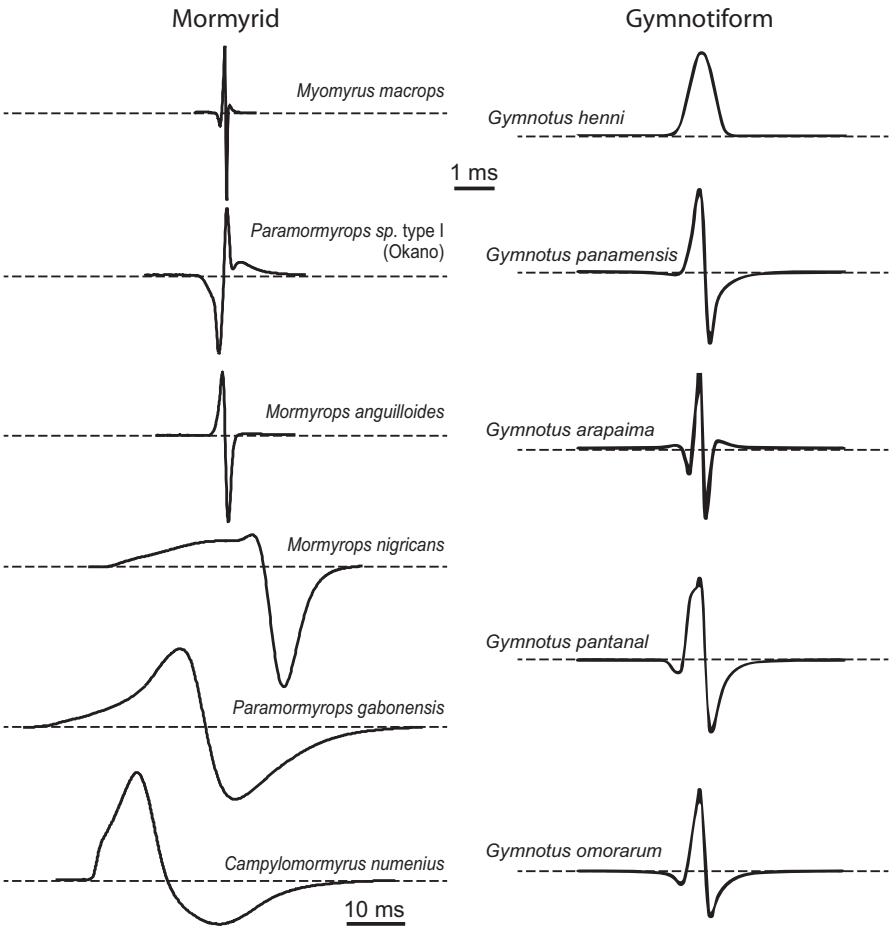

Fig. 5.1 Diversity of electric signal waveform in pulse-type mormyrid (*left*) and gymnotiform (*right*) fish. *Solid lines*, electric organ discharges (EODs) with head-positive potentials plotted upward; *dashed lines*, 0 V. Amplitudes are scaled to similar peak-to-peak amplitudes. Scale bar, 1 ms for all recordings except for the mormyrid *Campylomormyrus numenius* (*bottom left*; scale bar, 10 ms). The EODs from mormyrid species span families within the mormyrid clade, whereas the gymnotiform waveforms show the immense diversity within the genus *Gymnotus*. Signal diversity in *Gymnotus* is representative of the diversity seen across the other pulse-type gymnotiform families. Mormyrid EODs adapted from Arnegard et al. (2010b); gymnotiform EODs adapted from Crampton et al. (2013)

low-frequency energy while maintaining the high-frequency components. In wave fish, the power spectrum includes peaks at the fish's EOD frequency as well as at higher harmonics of the fundamental frequency. If the baseline of the signal is near 0 V, then the signal also carries significant energy in the low-frequency range (with a peak at 0 Hz). However, most wave fish offset the EOD baseline below 0 V or their discrete EODs are symmetrical about 0 V, thereby suppressing the low-frequency energy in the signal (Fig. 5.2).

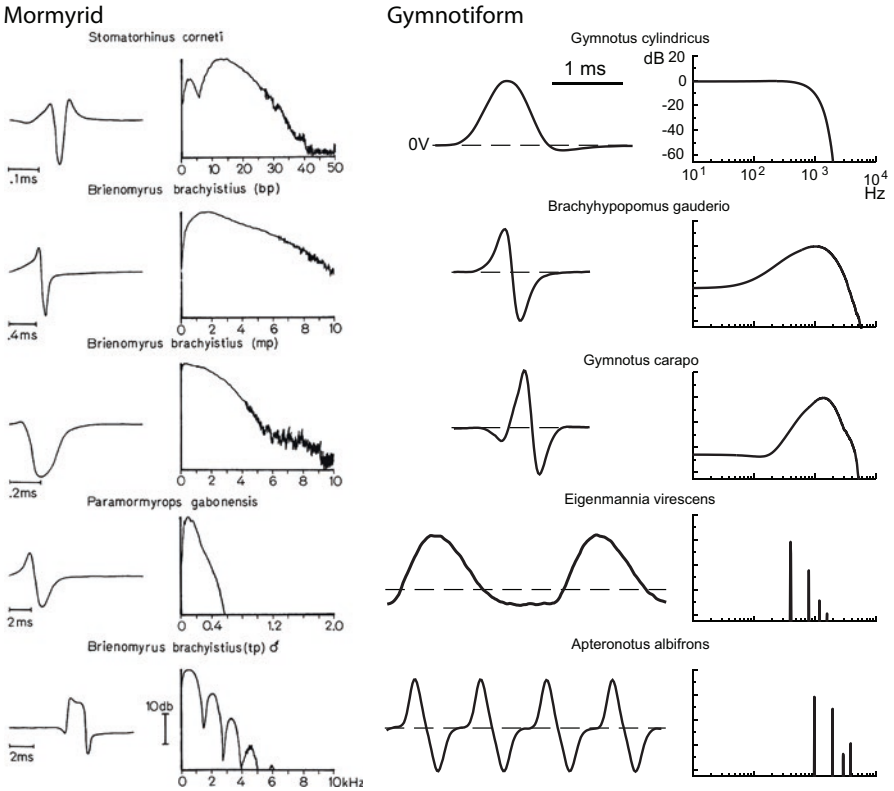

Fig. 5.2 Power spectra of diverse signal waveforms. EOD waveforms for mormyrid (*left*) and gymnotiform (*right*) fish are shown next to their power spectra. Note that the mormyrid species designated as *Brienomyrus brachyistius* have since been revised. Signals for which the time-voltage waveform is symmetrical around zero show spectral suppression of energy in the low-frequency range of ampullary electroreceptors. Continuous waveforms from wave-type fish have narrow spectra consisting primarily of the EOD frequency and its harmonics. Pulse fish have a much broader spectral content. Mormyrid waveforms and spectra adapted from Hopkins (1980); gymnotiform waveforms and spectra adapted from Stoddard and Markham (2008)

5.1.4 Plasticity of Electric Signal Properties

Finally, the diversity of EOD waveforms in some species also extends to waveform variations that occur over timescales ranging from minutes to months, including developmental changes during maturation, seasonal variation, circadian changes in the waveform, and rapid waveform changes in response to stress and social encounters (Fig. 5.3). These waveform modulations also produce corresponding changes in the power spectrum of the signal. Most commonly, electric signal plasticity involves changes to the amplitude and/or the duration of the signal, under the control of multiple hormonal axes.

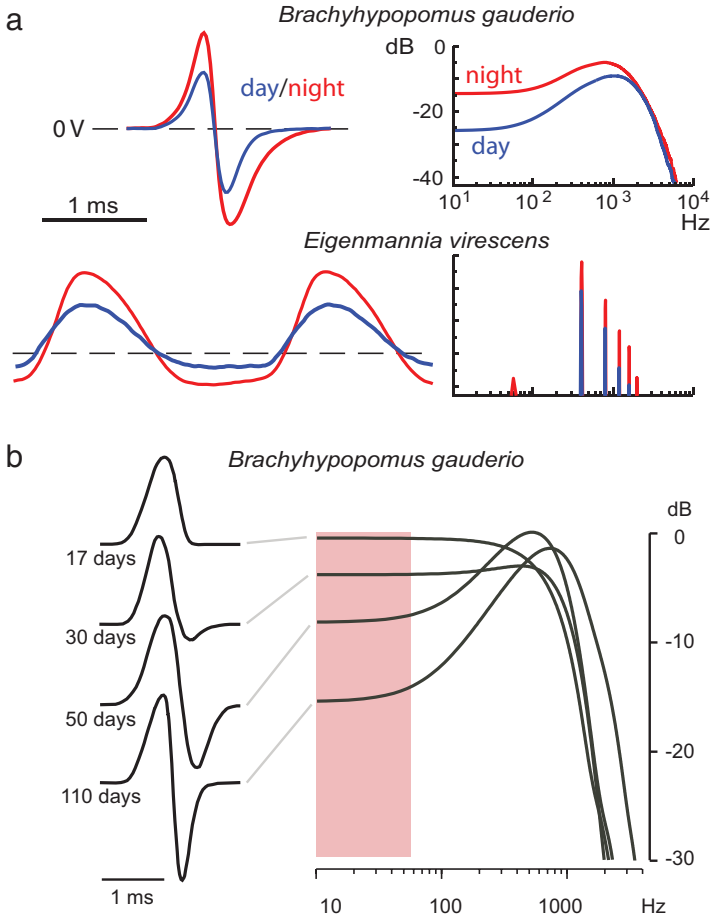

Fig. 5.3 Circadian and developmental changes in signal waveforms and power spectra. **a:** Day-to-night changes in EOD waveform for the pulse gymnotiform *Brachyhypopomus gauderio* (*top*) and the wave gymnotiform *Eigenmannia virescens* (*bottom*). *Brachyhypopomus gauderio* waveforms show enhanced amplitude and extension of the second phase at night, whereas *Eigenmannia virescens* waveforms show only increased signal amplitude. Power spectra (*right*) show that increasing the head-negative second phase of the EOD (P2) duration in *Brachyhypopomus gauderio* produces a marked increase in low-frequency content of the signal (*left*), whereas signal amplitude enhancement in *Eigenmannia virescens* changes the spectral power but not the frequency content. **b:** Development of EOD waveform in *Brachyhypopomus gauderio*. A series of EODs were recorded at progressive ages (days postfertilization). Waveforms have been rescaled to standardize the amplitude of the first phase. Young juveniles produce a monophasic signal (17 days) with maximal power in the spectral range of the ampullary electroreceptors (*red box*). Over the course of ~3 months, the signal becomes increasingly biphasic with the addition and subsequent enhancement of the negative second phase of the signal. At 110 days, the signal is nearly symmetric around 0 V, thereby significantly suppressing energy in the low-frequency range of the ampullary electroreceptors. Adapted from Stoddard and Markham (2008)

5.2 Why Are Electric Signals Diverse?

Given this broad diversity in electric signal characteristics, a fundamental question is why these signals are so diverse. Ultimately, electric signal waveforms are determined by evolutionary forces, as is true for any animal signal. Electric signals, however, are distinctive among animal signals because they are used both for communication and for the basis of an active electrosensory process that yields electric "images" of the animal's environment (see Jung and Engelmann, Chap. 12). As a result, these electric signals are shaped by a combination of evolutionary forces that act on communication signals and selective pressures that act on active sensory signals. In some cases, these selective forces can exert opposite pressures on a signal. For example, sexual selection might favor high-amplitude signals while predator avoidance instead favors low-amplitude signals. These competing forces often lead to tradeoffs in signal characteristics or to behavioral or life-history adaptations that balance signal costs and benefits. Most importantly, the sometimes peculiar biophysical mechanisms of electric signal production are made far more understandable with an appreciation of the sometimes conflicting evolutionary forces that act on them. A comprehensive review of the evolutionary influences on signal diversity is provided by Krahe (Chap. 7), but it will be useful here to survey a subset of these selective factors to provide context for appreciating the many proximal mechanisms of signal diversity.

5.2.1 Predation Risk

Most animal signals are detectable by one or more sympatric predators, exposing the signaler to potential injury or death. Such predation costs would generally be expected to favor signal characteristics that reduce the salience of the signal to predators. In the case of electric signals in fish, the primary predation risk arises from electroreceptive predators that possess ampullary electroreceptors. This situation would likely favor electric signals of lower amplitude as well as signals where the power spectrum has reduced energy in the lower frequency range of ampullary electroreceptors (0–50 Hz). This is not a universal solution, however, because some large piscivorous weakly electric fish species can use their tuberous electroreceptors to detect and consume their smaller weakly electric cousins based on high-frequency signal components.

5.2.2 Metabolic Cost

In addition to predation costs, electric signals also have metabolic costs. Any animal signal incurs some degree of metabolic cost, and energy devoted to signaling is not available for other essential physiological functions such as locomotion, body maintenance, or immune function. Accordingly, with all other factors being equal, sig-

nals that require less metabolic investment would be preferable, thereby favoring low-amplitude signals, adaptations that increase the efficiency of signal production, and adaptations that maximize power transfer from the fish to the surrounding water. Despite these pressures, recent findings suggest that the metabolic cost of electric signal production can be quite high, consuming from 10 to 30% or more of the animal's daily energy budget (Salazar and Stoddard 2008; Salazar et al. 2013).

5.2.3 Sexual Selection

Sexual selection can exert a strong evolutionary influence on animal signals when female sensory biases result in the exaggeration of certain signal characteristics in males. This process generally favors male signals that are more conspicuous, and for electric fish, this translates into higher amplitude signals with enhanced low-frequency spectral content, a situation that increases both the predation risk and the metabolic cost of the signal. Sexual selection is also likely to be the driving force behind the sexual dimorphism in electric signals observed in many mormyrid and gymnotiform species, and evidence suggests that sexual selection for signal diversification has driven speciation in some instances (Arnegard et al. 2010a).

5.2.4 Reproductive Isolation

In many locations in both Africa and South America, multispecies assemblages of closely related species are sympatric. These conditions increase the risk of costly reproductive interference through mismating between heterospecifics when species recognition signals are not sufficiently different to distinguish between species. Another cause of reproductive interference is masking interference in which the communication signals of two species are sufficiently similar in their spectral characteristics to disrupt communication within each species. In such situations, selective forces tend to promote and maintain diverse signal characteristics, a phenomenon known as reproductive character displacement that has been clearly documented in gymnotiforms (Crampton et al. 2011) and may contribute to signal diversity in some mormyrid clades (Arnegard et al. 2010a).

5.3 Physiological Mechanisms of Electric Signal Production

In all electric fish, the electric signal originates from postsynaptic potentials and/or action potentials (APs) generated by electrocytes in the electric organ. The timing and pattern of electrocyte activation is regulated by a brainstem pacemaker nucleus in gymnotiforms and the mormyroid wave species *Gymnarchus niloticus* ("aba knife"). In mormyrids, electrocyte activity is controlled by a medullary command

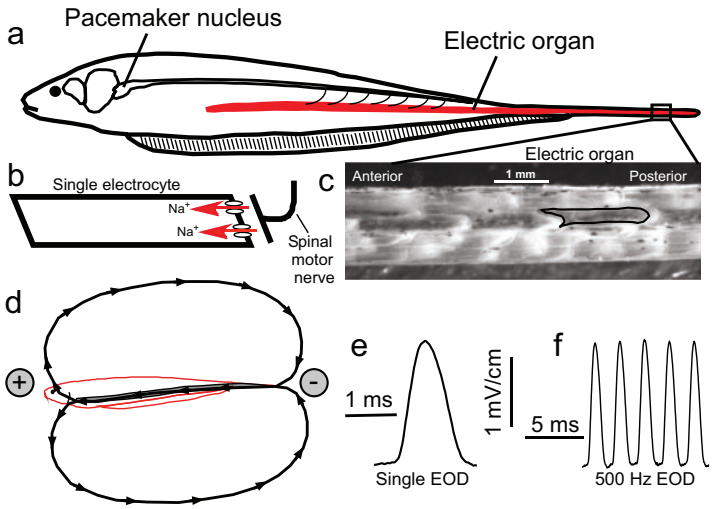

Fig. 5.4 General schematic of EOD production. **a:** The electric signal, an EOD, is produced by the near-simultaneous action potentials (APs) of electrocytes in the electric organ (EO). A medullary pacemaker nucleus initiates the electrocyte APs via spinal electromotor neurons that innervate the electrocytes, forming a broad cholinergic synapse. **b:** Simplified electrocyte schematic. Electrocytes are large cells, often greater than 1 millimeter in length, innervated on the posterior end of the cell. Activation of the cholinergic synapse initiates the AP when sodium enters the cell via voltage-gated sodium channels. The electrocyte geometry and localization of Na^+ channels to the posterior region causes the Na^+ current to move along the rostral-caudal body axis. **c:** A section of the EO from the tail of *Eigenmannia virescens*, with skin removed to expose the electrocytes that are densely packed within the EO. One electrocyte is outlined in *black*. **d:** The near-simultaneous APs of all electrocytes in the EO sum to generate current that moves forward toward the head, then follows a return path (*black line*) through the water to the tail. By convention, the current moving toward the head is measured as positive (upward). **e:** A single EOD is a monophasic pulse. **f:** EOD waveform from a fish with an EOD frequency of ≈500 Hz. Adapted from Ban et al. (2015)

nucleus. The resulting simultaneous or near-simultaneous potentials generated by hundreds to thousands of electrocytes sum to produce the EOD (Fig. 5.4). Variations in EOD rate and timing carry important social information, and the central circuits controlling EOD timing have been well characterized in both gymnotiforms and mormyrids (Caputi et al. 2005).

Beyond the differences in signal amplitude and rate or timing, the biophysical mechanisms that shape electric signal diversity center primarily on the biophysical properties of electrocytes, with contributions also from their innervation patterns and their arrangement within the electric organ. The electrocytes are a central link in the electrosensory and electrocommunication systems of electric fish. They are the target of central control by the pacemaker/command nucleus, their function is regulated by multiple hormonal axes, and they are the cellular source of the electric signal that is the primary information carrier in the environment and the input to the electrosensory system. Accordingly, the focus for the remainder of this chapter will be on the cellular biophysics of electrocyte function that give rise to the broad diversity of electric signal waveforms in mormyrid and gymnotiform fishes.

5.3.1 General Biophysics of Excitable Cells

Electrocytes are electrically excitable cells, and, as such, they share certain biophysical features in common with all electrically excitable cells including neurons and myocytes. The key features of excitable cells are their passive membrane resistance and capacitance, the maintenance of ionic gradients across the cell membrane, and the presence of synaptic receptors and voltage-gated ion channels that regulate ionic currents across the membrane. These properties together give rise to the postsynaptic potentials and APs generated by excitable cells.

The most fundamental biophysical properties of any excitable cell are its membrane resistance and membrane capacitance. The membrane resistance is determined by the number of ion channels in a conductive state at any given moment, with resistance increasing as the number of conducting channels decreases and vice versa. The membrane resistance of the cell determines the magnitude of membrane voltage changes in accordance with Ohm's law (voltage = current × resistance). For a given input current (say a postsynaptic current), the magnitude of the resulting membrane voltage response is a linear function of the membrane resistance. Higher resistances will produce larger voltage responses and vice versa.

The cell membrane also acts as a capacitor, which is to say that the membrane is able to both store and release the electrical charge on its surface. Membrane capacitance is a linear function of the total membrane area of the cell, and it is important because any current delivered to the cell will first charge the membrane capacitance before any ionic current begins to cross the membrane to change the membrane voltage. Once the input current terminates, the charge stored on the membrane will be released. Cells with greater surface area (and therefore greater capacitance) can store and release more charge. The rate at which the membrane stores and releases charge is affected by the membrane resistance. At higher resistances, the charging and discharging of the membrane capacitance are slower, as is the rate of any changes in membrane voltage. The opposite is also true: at low resistances, charge movement on and off of the membrane is faster and the corresponding changes in membrane voltage are also faster.

All excitable cells also maintain concentration gradients of several key ion species across the cell membrane. These gradients are maintained by active transport mechanisms, typically transmembrane enzymes known as transporters or pumps, that require energy to transport ions against their concentration gradients. These include transporters for calcium (Ca^{2+}), chloride (Cl^{-}), sodium (Na^{+}), and potassium (K^{+}). The most important transporter for the present discussion is Na^{+}/K^{+}-ATPase, also known as the sodium-potassium pump. For every catalytic cycle, this transmembrane protein hydrolyzes one ATP in order to transport three sodium ions to the extracellular space and two potassium ions to the intracellular space. As a result, most excitable cells maintain Na^{+} gradients with much higher concentrations outside the cell than inside and K^{+} gradients with much higher concentrations inside the cell than outside.

These concentration gradients give rise to equilibrium potentials for each ion species such that when the membrane becomes more permeable to a particular ion, the membrane potential will move toward the equilibrium potential of that ion. In the case of most excitable cells, the equilibrium potential for sodium (E_{Na}) is approximately 60 mV, whereas the equilibrium potential for potassium (E_K) is approximately −90 mV for neurons and −100 mV for skeletal muscle. These equilibrium potentials set the limits on membrane voltage during an AP, with the minimum being the E_K and the maximum being the E_{Na}. As a result, the maximum voltage excursion during the AP is approximately 150 mV.

The activation of ionotropic receptors and voltage-gated ion channels allows ionic current to flow across the membrane and is thereby responsible for the membrane voltage changes characteristic of electrically excitable cells. The synaptic activation of ionotropic receptors or the activation of voltage-gated ion channels can have depolarizing or hyperpolarizing effects on the cell depending on the ion selectivity of the channel. The canonical AP begins from the resting potential that is near the E_K due to the resting membrane being predominantly permeable to K^+. The AP is initiated by synaptic stimulation of excitatory receptors permeable primarily to Na^+ that depolarize the membrane. The ensuing rapid activation of voltage-gated Na^+ channels produces an inward current of Na^+ that depolarizes the membrane toward the E_{Na}. Subsequently, Na^+-channel inactivation terminates the inward Na^+ current, and activation of voltage-gated K^+ channels produces an outward K^+ current that repolarizes the membrane toward the E_K. Following each AP, the Na^+/K^+-ATPases restore the proper ionic gradients.

5.3.2 The Special Case of Electrocytes

Electrocytes operate by the same functional principles as any excitable cell but are unique excitable cells in many ways. The sheer size of an electrocyte is one of the most notable characteristics. They are multinucleated cells that can be up to two millimeters in length and/or width. With such a large membrane surface area, electrocytes have extremely large membrane capacitances, on the order of tens to hundreds of nanofarads, orders of magnitude larger than observed in neurons where membrane capacitances are on the order of picofarads. Electrocytes also are notable for very low membrane resistances, usually well below 1 MΩ and as low as 10 kΩ in most cases (Bennett 1961; Markham and Stoddard 2013), compared with values for neurons and myocytes that fall in the range of tens to hundreds of megaohms. The high membrane capacitance and low resistance have important consequences for the electrical properties of the electrocyte. By virtue of the large capacitance, the electrocyte membrane can store and release a great deal of electrical charge. Furthermore, the low resting membrane resistance allows this charge to be stored and released very quickly but also means that the cell requires very large input currents to change the membrane voltage.

Beyond the sheer size of electrocytes, their morphologies are fascinating. In all but one clade of weakly electric fish, adult electrocytes are derived from skeletal muscle. The South American genus *Apteronotus* is the sole exception because the adult electric organ is of neural origin (Kirschbaum 1983). The electrocytes in apteronotids themselves are enlarged terminals of the spinal motor neurons. Myogenic electrocytes are generally cylindrical cells with flattened areas (faces) of electrically excitable membrane oriented such that the membrane currents across these areas are directed along the rostral-caudal body axis (Figs. 5.5 and 5.6). Electrocytes can be elongated cigar-shaped cells such as for the wave-type gymnotiform *Eigenmannia virescens* ("glass knifefish"; Fig. 5.6b) or the wave-type mormyroid *Gymnarchus niloticus.* For pulse-type gymnotiforms and mormyrids, electrocytes are flattened and disc-like, with widths being very narrow relative to the diameter (Fig. 5.6a). The disc-like electrocytes sometimes also feature stalks that protrude from the flattened membrane surfaces (Figs. 5.5, *bottom*, and 5.6a).

Fig 5.5 (continued) corresponding EOD waveforms are below the outlines. In the schematic representations of electrocyte function, active synaptic inputs are represented by *black triangles* and inactive synaptic inputs are represented by *gray triangles*. *Solid dashed lines*, activated excitable membrane; *arrows*. direction of membrane current flow. *Top***:** monophasic EODs in both gymnotiform and mormyroid fish are produced by electrocytes innervated on the posterior membrane where only the innervated membrane is active. These generate a single AP on the posterior membrane following synaptic activation, which produces headward current flow and a monophasic head-positive EOD pulse. *Center*: biphasic EODs in gymnotiforms and mormyrids are typically produced by disk-shaped electrocytes where both the anterior and posterior membranes are electrically active. Synaptic activation elicits an AP on the posterior membrane that creates headward current flow and the head-positive EOD phase (P1). A subsequent AP on the noninnervated anterior membrane produces the P2. *Bottom*: multiphasic EOD waveforms are produced by different mechanisms in the gymnotiform *Gymnotus carapo* and the mormyrid *Brevimyrus niger*. In *Gymnotus carapo*, the EOD is a multiphasic waveform with two initial head-negative components (a and b), followed by a head-positive phase (c), and then a final head-negative phase (d). This waveform is produced by the asynchronous activation of three distinct electric organ regions populated by three types of electrocytes (*blue*, *green*, and *red*). Two populations are innervated on both faces, and the third population is innervated only on the posterior face (Macadar et al. 1989a). In one type of doubly innervated electrocytes, synaptic activation elicits an AP on both the anterior and posterior membranes (*blue area*). In the second type of doubly innervated electrocyte, activation of the anterior synapse produces only a postsynaptic potential and activation of the posterior synapse produces an AP (*green area*). Electrocytes innervated only on the posterior membrane (*red area*) produce an AP on the posterior membrane followed by an AP on the anterior membrane. The spatiotemporal activation pattern of these three electrocyte populations produces the complex multiphasic EOD (Caputi 1999). In the mormyrid *Brevimyrus niger*, the EOD is a multiphasic waveform that begins with a head-negative component (**a**), followed by a head-positive phase (**b**), and then a final head-negative phase (**c**). The *Brevimyrus* EOD is generated by the near-simultaneous activation of a single population of electrocytes. The electrocytes are innervated from the anterior side on a stalk that then penetrates through the electrocyte to join the posterior membrane. Synaptic activation initiates an AP in the stalk, and the propagation of this AP along the stalk through the electrocyte penetration produces the initial head-negative phase (**a**). The subsequent initiation of an AP on the posterior membrane face produces the head-positive second phase (**b**), and the resulting AP on the anterior membrane face produces the final head-negative phase (**c**). Adapted from Markham (2013)

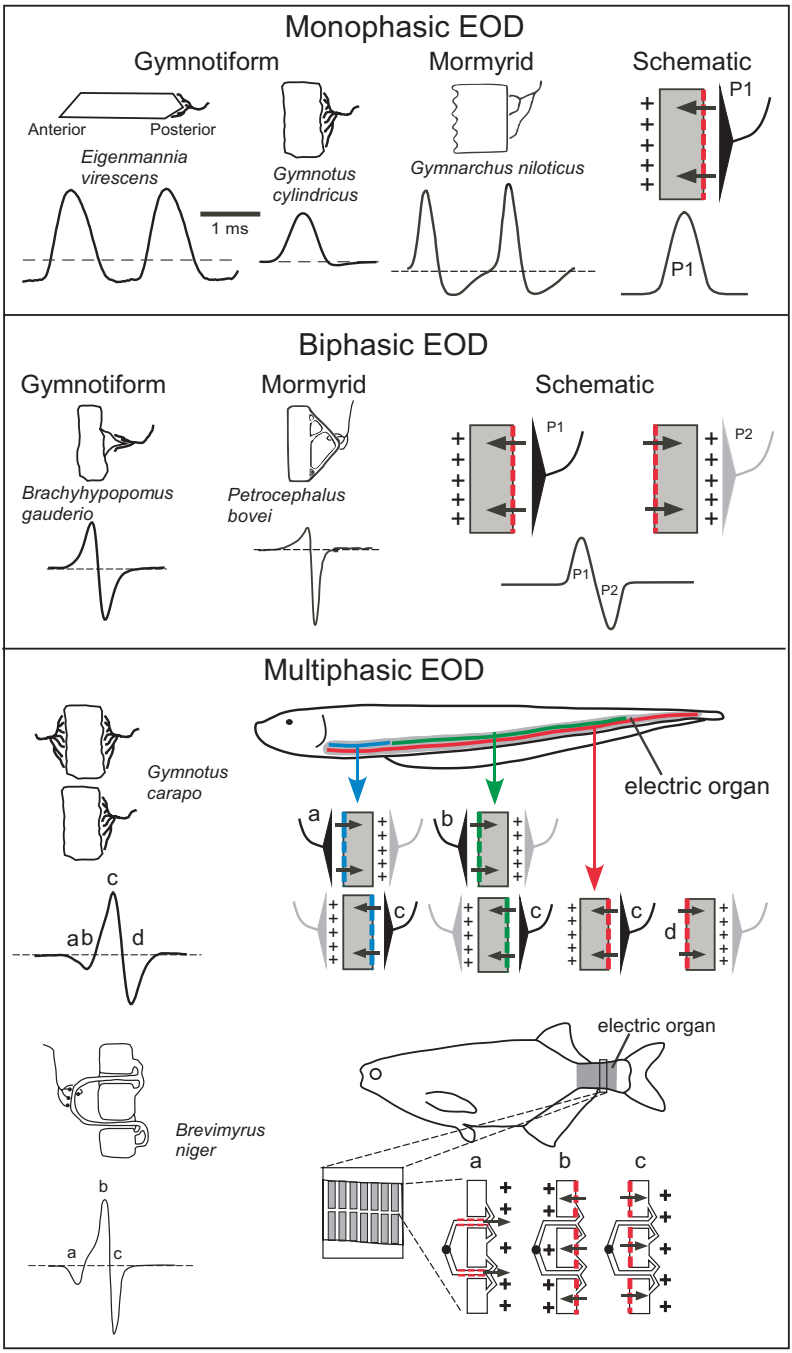

Fig. 5.5 Mechanisms of EOD generation and EOD waveform diversity in gymnotiform and mormyrid fish. Line drawings are cross sections of electrocytes from representative species and the

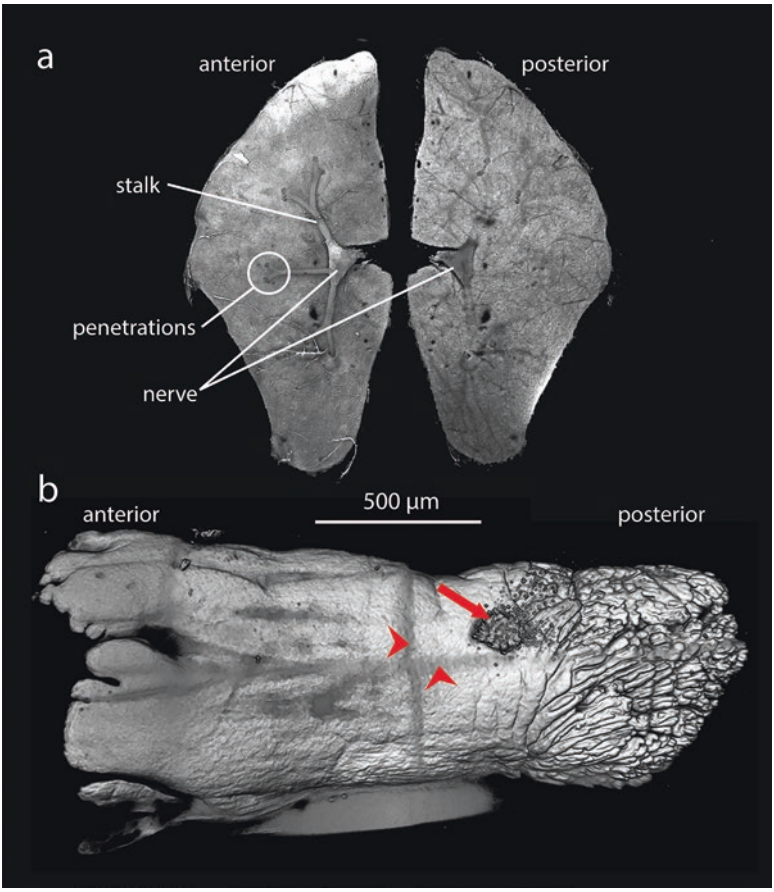

Fig. 5.6 Electrocyte morphology. **a:** Confocal 3-D projections of the anterior and posterior face of a single electrocyte from *Paramormyrops kingsleyae* showing the innervating nerve, stalks, and sites of stalk penetrations through the electrocyte. Electrocyte thickness is 60 µm. Image courtesy of Jason Gallant; annotations adapted from Gallant et al. (2011). **b:** 3-D reconstruction from serial confocal scanning through a live *Eigenmannia virescens* electrocyte injected with rhodamine B dextran (10,000 MW). *Arrow*, site of dextran injection; *arrowheads*, horizontal and vertical lines that are artifacts caused by the image tile overlap. The posterior face contains deep invaginations that dramatically increase the surface area of the cell, whereas the anterior face features large lobes penetrated by capillaries. Image adapted from Ban et al. (2015)

Finally, electrocytes are also unique in the magnitude of the ionic currents that generate their postsynaptic potentials and APs. Although the whole cell ionic currents in neurons and myocytes are at most a few nanoamperes, ionic currents in electrocytes typically are on the order of 1 µA, and in some cases, they exceed 10 µA in magnitude (Sierra et al. 2007; Markham et al. 2013). An important consequence of such large ionic currents is the metabolic demand incurred by the Na^+/K^+-ATPases that restore the ionic gradients after each AP at a cost of one ATP for every three Na^+

ions that enter the cell during the AP. The resulting metabolic costs associated with electric signal production are significant and can consume up to 30% of the animals' daily energy budget (Salazar et al. 2013; Lewis et al. 2014).

5.4 Physiological Mechanisms of Signal Diversity

The simplest EOD waveform in both gymnotiforms and mormyrids is a monophasic pulse produced by electrocytes where only one face is electrically excitable while the other is electrically passive. Synaptic input at large cholinergic synapses innervated by the spinal motor neurons initiates an AP on the excitable face where Na^+ current enters the cell via voltage-gated Na^+ channels, creating an axial current within the electric organ. If, for example, the active face is the posterior electrocyte membrane, the net positive current is directed headward. Subsequent repolarization by voltage-gated K^+ channels terminates the AP. The result is a monophasic head-positive pulse shaped by the depolarization and repolarization of the innervated posterior membrane (Fig. 5.5, *top*).

Species with monophasic EODs include both pulse and wave fish. In the case of wave fish, the interval between EODs is approximately the same as the EOD duration, creating a sinusoidal signal, whereas in monophasic pulse fish, the EODs are separated by long intervals. Across monophasic species, EOD duration varies over a wide range, from a few hundred microseconds to tens of milliseconds or more. And within wave species, frequency differences among conspecifics are associated with different EOD durations, with higher frequency individuals having shorter duration EODs and vice versa.

In species with more complex biphasic EOD waveforms, at least some of the electrocytes in the electric organ have two electrically excitable faces. An AP is initiated first on the posterior innervated face (AP1), followed closely by an AP on the opposite face (AP2; Fig. 5.5, *center*). The two successive APs with their ionic currents directed in opposite directions create a biphasic electrocyte discharge that is shaped both by the the waveforms of the two APs and the delay between the APs (Bennett 1961; Markham and Zakon 2014). For gymnotiform species with complex multiphasic EOD waveforms, the mechanisms of signal complexity are best understood in *Gymnotus carapo* ("banded knifefish"). Waveform complexity in *Gymnotus carapo* arises from the asynchronous recruitment of several electrocyte populations with different discharge characteristics (Lorenzo et al. 1988; Caputi 1999). Some electrocytes in *Gymnotus carapo* are innervated on both the anterior and posterior faces, and both faces produce APs as they are activated asynchronously. Another population of doubly innervated electrocytes produce a synaptic potential just on the anterior face, whereas activation of the posterior face elicits an AP. A third population of electrocytes is innervated only on the posterior face, and synaptic activation elicits an AP on the posterior face followed by an AP on the anterior face. The spatiotemporal pattern of activation across these electrocyte populations produces the multiphasic EOD waveform measured head to tail (Fig. 5.5, *bottom*) as well as producing a large spatial variation in EOD waveforms measured at different locations near the body (Caputi 1999).

Many mormyrid species produce multiphasic EODs with complexity comparable to *Gymnotus carapo*, but the underlying waveform complexity mechanisms are quite different. Mormyrid electric organs consist of a single, relatively homogeneous population of electroctyes. It is the particular innervation pattern and morphological complexity of these electrocytes that produces the complex multiphasic EODs. In mormyrids with complex EOD waveforms, electrically excitable stalks penetrate the electrocyte (Fig. 5.5, *bottom*) that contribute to waveform complexity when the APs are propagated along the stalks toward the electrocyte body (Fig. 5.5, *bottom*; Bennett and Grundfest 1961). The morphological complexity in mormyrid electrocytes yields diversity and complexity of EOD waveforms that rival or exceed EOD diversity and complexity in gymnotiforms (cf. Crampton and Albert 2006; Arnegard et al. 2010a).

5.5 Cellular Biophysics of Signal Diversity

5.5.1 Expression Patterns of Diverse Ion Channels

Electrocyte morphology is clearly important for shaping the EOD waveform in both gymnotiform and mormyrid fishes, but morphology alone cannot account for the vast differences in signal durations and waveforms. The particular complement of ion channels expressed by the electroctye and their kinetics and voltage dependence as well as their localization on the membrane plays a central role in determining the EOD waveform.

Patch- and voltage-clamp recordings from electrocytes across several genera and species have revealed that electrocytes in different species express a remarkably diverse complement of ionic currents. Patch-clamp studies of *Electrophorus electricus* (electric eel) electrocytes (Shenkel and Sigworth 1991) showed that the dominant ionic currents of the electrocyte were voltage-gated Na^+ currents and inward rectifier K^+ currents. In the monophasic wave-type gymnotiform *Sternopygus macrurus* ("longtail knifefish"), electrocytes express voltage-gated Na^+ currents, inward rectifier K^+ currents, and delayed rectifier K^+ currents (Ferrari and Zakon 1993). For both *Electrophorus* and *Sternopygus*, the EOD and electrocyte APs are monophasic and relatively long duration, generally several milliseconds or more. In *Eigenmannia virescens*, a high-frequency wave fish with brief EODs approximately 1 ms long, electrocytes express an inward rectifier K^+ current and a transient Na^+ current and, surprisingly, the predominant repolarizing ion current is not a voltage-gated K^+ current but is instead a Na^+-activated K^+ current (Markham et al. 2013; Ban et al. 2015).

Electrocytes that produce brief, biphasic discharges apparently recruit a much broader complement of ionic conductances. The biphasic electrocytes of *Steatogenys elegans* ("barred knifefish") express an inward rectifier K^+ current, two distinct transient Na^+ currents, a delayed rectifier K^+ current, and an inactivating A-type K^+ current (Markham and Zakon 2014). *Gymnotus carapo* electrocytes express two

functionally distinct inward rectifiers, a transient Na^+ current, a delayed rectifier K^+ current, an inactivating A-type K^+ current, and a persistent Na^+ plateau current (Sierra et al. 2005, 2007).

Data for electrocyte ionic currents are available for just this small subset from the 200+ gymnotiform species but shows a striking diversity of ion-channel mechanisms that contribute to signal diversity. This suggests that recruitment of different ion-channel mechanisms is a major driver of signal diversity in this clade. Unfortunately, very little electrophysiological data on the ionic currents expressed in mormyrid electrocytes are currently available. The complex, multiphasic EOD waveforms in many mormyrid species are likely attributable to electrocyte morphology and multiple patterns of stalk penetration (Alves-Gomes and Hopkins 1997; Gallant et al. 2011). This suggests the interpretation that selective pressures for signal diversity resulted in the recruitment of diverse ion-channel combinations in gymnotiform electrocytes, whereas in mormyrids, signal diversity arose by increased variability in electrocyte morphologies and membrane properties (Bass et al. 1986). However, the extreme diversity of EOD durations in mormyrids, ranging from hundreds of microseconds to more than 10 ms (Hopkins 1999), is also likely a function of the particular ion channels expressed in mormyrid electrocytes and their particular kinetics. This highlights a clear need for both molecular and electrophysiogical data regarding the ionic currents expressed in mormyrid electroctyes.

5.5.2 Ionic Mechanisms of Signal Diversity

Signal diversity between species, as outlined in Sects. 5.4 and 5.5.1, arises from the morphology and ionic conductances of electrocytes together with their innervation pattern. Given the apparently broad diversity of ion channels expressed by the electrocytes of different clades, how does the particular complement of ion channels expressed by electrocytes ultimately determine signal waveform?

5.5.2.1 Signal Duration in Monophasic Signals

The underlying mechanisms regulating EOD duration are differences in the electrocyte AP duration, which are associated with differences in the kinetics of the underlying voltage-gated Na^+ and K^+ channels. Slower channel kinetics are associated with longer duration electrocyte APs and vice versa (Fig. 5.7). The relationship between ion-channel kinetics and signal duration has been most thoroughly investigated in the wave fish *Sternopygus macrurus*, where EOD frequencies range from ~70 to 150 Hz and EOD durations vary over a fourfold range between individuals (from ~3 to ~12 ms). In this case, the kinetics of both the voltage-gated Na^+ channels and the voltage-gated K^+ channels are tightly coregulated across electrocytes from fish with different EOD durations (McAnelly and Zakon 2000). Increased

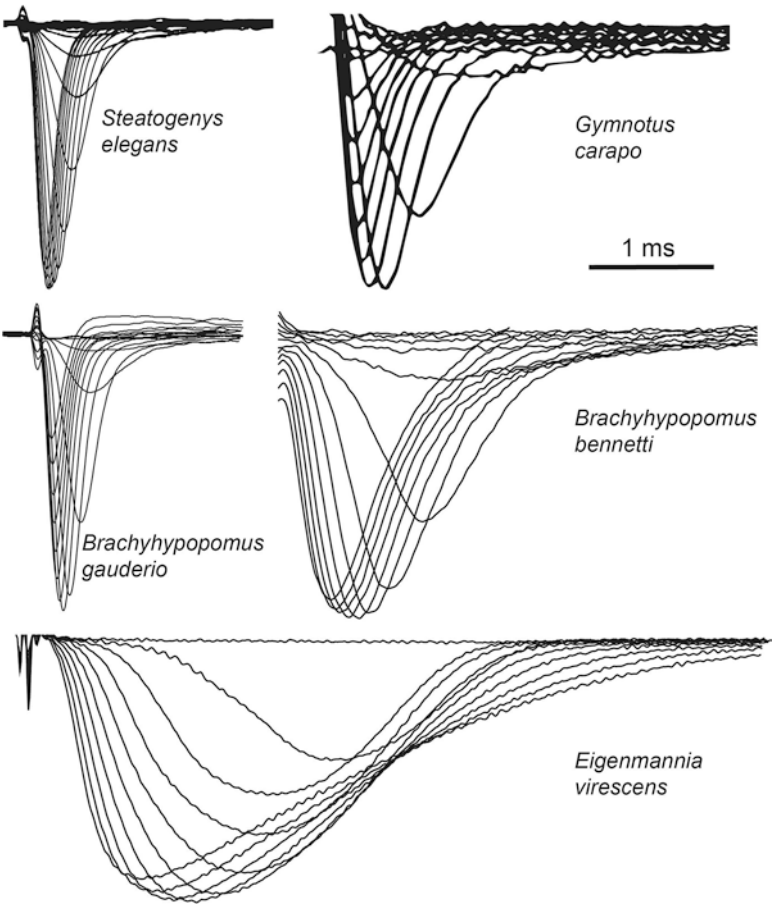

Fig. 5.7 Variation in Na^+-current kinetics across gymnotiform species for which data are available. Shown are families of Na^+ currents elicited by increasingly depolarizing voltage steps under two-electrode voltage clamp (*Gymnotus carapo*, *Brachyhypopomus bennetti*, *Eigenmannia virescens*) or single-electrode patch clamp (*Steatogenys elegans*, *Brachyhypopomus gauderio*). In all species, Na^+ currents are the product of $Na_V1.4$ voltage-gated Na^+ channels, yet the activation and inactivation kinetics are vastly different across species, with current durations ranging from ~0.5 to more than 3 ms. Data for *Steatogenys elegans* from Markham and Zakon (2014), for *Gymnotus carapo* adapted from Sierra et al. (2005), for *Brachyhypopomus gauderio* adapted from Markham (2013), for *Brachyhypopomus bennetti* from David Saenz (with permission), and for *Eigenmannia virescens* from Markham et al. (2013)

EOD duration (lower EOD frequency) is associated with the slower kinetics of both currents, and the Na^+- and K^+-current kinetics are tightly correlated (Fig. 5.8).

It would be possible to shape AP duration by regulating the kinetics of either the depolarizing Na^+ current or the repolarizing K^+ current in isolation, raising the question of why both currents are regulated in tandem. One likely explanation is that coregulating Na^+- and K^+-channel kinetics minimizes the energetically wasteful

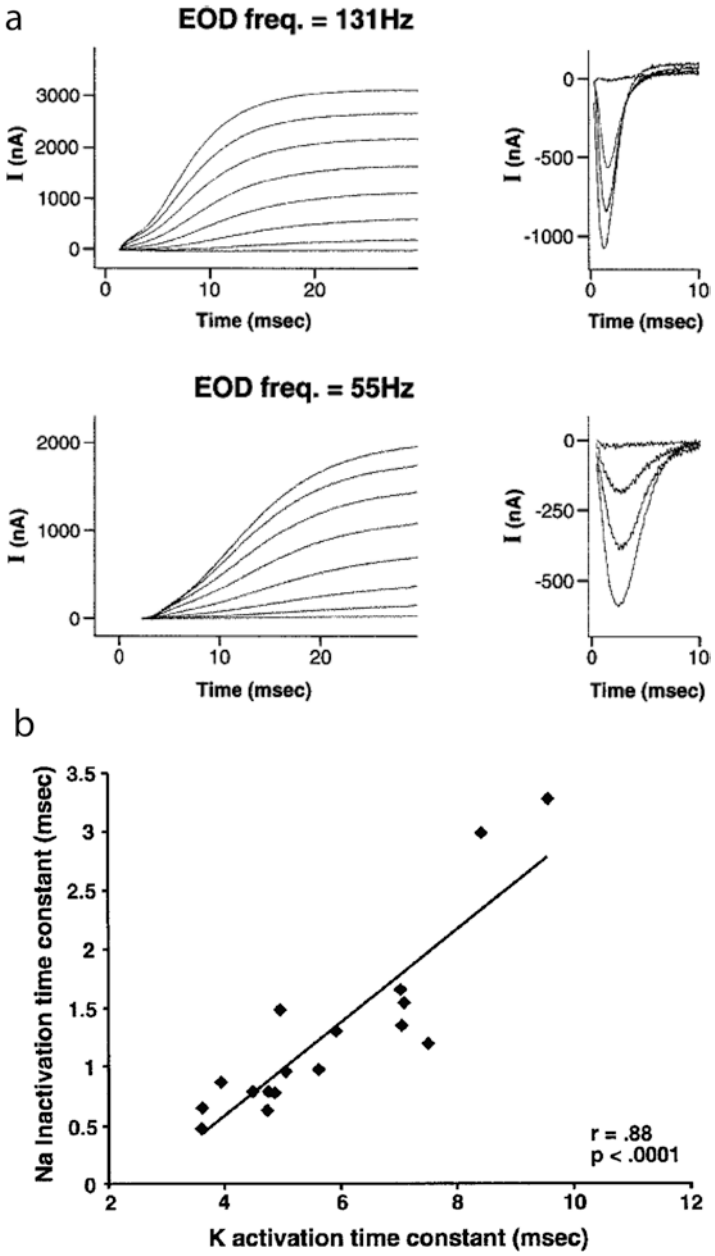

Fig. 5.8 Coregulation of Na^+- and K^+-current kinetics in *Sternopygus macrurus*. **a:** Current families for voltage-dependent K^+ currents (*left*) and voltage-dependent Na^+ currents (*right*) in an electrocyte from a high-frequency (freq.) fish (131 Hz) and an electrocyte from a low-frequency fish (55 Hz). Activation of the K^+ current is much faster for the high-frequency fish and activation/inactivation of the Na^+ current is also faster in the high-frequency fish. **b:** K^+-current activation time constants and Na^+-current inactivation time constants are tightly correlated across electrocytes from fish with different EOD frequencies. Adapted from McAnelly and Zakon (2000)

overlap of Na^+ and K^+ currents during the AP (Alle et al. 2009; Carter and Bean 2009). That is, when both the Na^+ and K^+ conductances are simultaneously active, the inward Na^+ current is offset by the outward K^+ current and does not contribute to changes in membrane potential, yet the Na^+ must still be returned to the extracellular space with the associated metabolic costs incurred by Na^+/K^+-ATPase. Accordingly, the coordinated regulation of kinetics in two molecularly distinct ion channels is likely driven by pressures to reduce the metabolic costs of EOD production.

In the high-frequency wave fish *Eigenmannia virescens*, EOD frequencies range from about 200–600 Hz (Scheich 1977), with EOD durations of about 1–2 ms. Sustaining such high firing rates presents two related challenges for electrocytes. The first is the generation of very brief APs, and the second is minimizing the refractory period following each AP. In *Eigenmannia virescens* electrocytes, the voltage-gated Na^+ currents show extremely fast activation/inactivation kinetics and a time constant for recovery from inactivation of about 300 μs, with complete recovery from inactivation in less than 1 ms (Markham et al. 2013). Thus, Na^+-channel kinetics in *Eigenmannia virescens* electrocytes are well suited to both brief and high-frequency APs.

Electrocytes of *Eigenmannia virescens* appear to be unique in that they repolarize the AP with Na^+-activated K^+ (K_{Na}) channels (Markham et al. 2013) rather than voltage-gated K^+ channels in other species where data on electrocyte ionic currents are available (Ferrari and Zakon 1993; Markham 2013). As electrophysiological data become available for a broader range of species, the expression of K_{Na} channels in electrocytes may turn out to be more widespread than expected. However, given the transition from voltage-gated K^+ channels in *Sternopygus macrurus* electrocytes to the molecularly distinct class of K_{Na} channels in *Eigenmannia virescens* electrocytes, the question arises as to what functional adaptation the K_{Na} channels might serve in *Eigenmannia virescens*. Computational simulations suggest that repolarizing the electrocyte AP with K_{Na} channels might serve to further minimize the wasteful overlap of Na^+ and K^+ currents in electrocytes with brief APs (Markham et al. 2013), thereby improving the energy efficiency of EOD production for high-frequency wave-type fish. Subsequent findings, however, suggest that K_{Na} channels serve a different purpose in *Eigenmannia virescens* because the K_{Na} channels in these electrocytes are found on the opposite end of the electrocyte, more than 1 mm from the voltage-gated Na^+ channels (Fig. 5.9; Ban et al. 2015). This arrangement is especially puzzling because, in other systems, micrometer-scale colocalization of Na^+ and K_{Na} channels is necessary for K_{Na}-channel activation (Budelli et al. 2009; Hage and Salkoff 2012).

5.5.2.2 Signal Waveform in Multiphasic Signals

Electrocytes that produce biphasic discharges do so by the sequential generation of APs on two distinct regions of excitable membrane as described in Sect. 5.4 (Fig. 5.5, *center*). The AP1-AP2 delay in this type of electrocyte must be very tightly

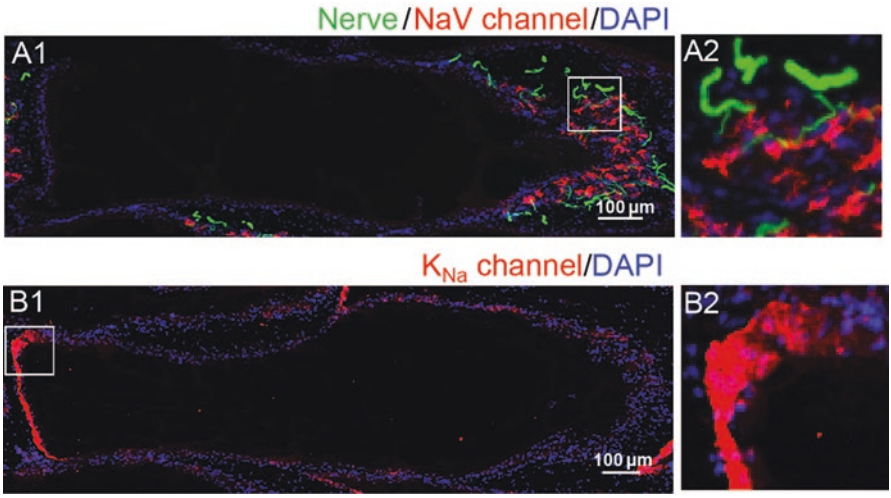

Fig. 5.9 Expression patterns of voltage-gated Na^+ channels (Na_V) and sodium-activated K^+ channels (K_{Na}) in an electrocyte from *Eigenmannia virescens*. Cells were immunolabeled with antibodies for nerve (3A10), Na_V channels, and K_{Na} channels. 4′,6-Diamidine-2′-phenylindole dihydrochloride (DAPI) labels nuclei that reside just under the membrane in these multinucleated electrocytes, thereby providing a general outline of the cell morphology. **A1**: only the posterior region (*right*) is innervated, with axons of innervating spinal motor neurons labeled with 3A10 (*green*). **B1**: K_{Na} channels are expressed only on the anterior face (*left*). **A2** and **B2:** enlarged areas from white-line boxes. Adapted from Ban et al. (2015)

regulated to maintain the electrocyte discharge waveform. Where precise data are available, the AP1-AP2 delay ranges from approximately 30–100 ms (Markham and Stoddard 2005; Markham and Zakon 2014), and very small differences in this delay, even a few microseconds, can significantly distort the resulting biphasic electrocyte discharge (μEOD; Markham and Zakon 2014).

Several mechanisms are possible for maintaining the AP1-AP2 delay in biphasic electrocytes. In some cases, both electrocyte faces are innervated so the timing of the respective APs is controlled by the spinal motor neurons (Macadar et al. 1989b; Caputi et al. 1994). The maintenance of a precise AP1-AP2 delay in biphasic electrocytes where only the posterior membrane is innervated is less easily understood. An intuitive assumption is that the initiation of the AP1 on the posterior membrane depolarizes the anterior membrane and initiates the AP2 with some propagation delay. This mechanism, however, would be insufficient to maintain an extremely precise delay. The earliest hypothesis for the reliable AP1-AP2 delay in electrocytes was that differences in passive properties between the two excitable membranes controlled the order and timing of the two APs (Bennett 1961). For example, a higher resistance or lower capacitance on the innervated membrane would result in a larger and/or faster depolarization of that membrane and earlier initiation of the AP1.

In the one case where the mechanisms of the AP1-AP2 delay have been investigated, it is active ionic mechanisms that regulate the AP1-AP2 delay. The biphasic electrocytes from *Steatogenys elegans* maintain a delay of ~30 μs between the two

APs. The innervated posterior face generates the AP1 followed by the AP2 on the noninnervated anterior face, and this delay is rigidly maintained within ±3 μs across a variety of stimulus conditions that would be expected to disrupt the timing of the 2 APs and the resulting μEOD waveform. Patch-clamp recordings of voltage-gated Na^+ currents on each electrocyte face revealed that the activation voltage of the Na^+ channels on the posterior face was approximately 10 mV lower than for the Na^+ channels on the anterior face. Computational simulations support the conclusion that this difference alone is sufficient to account for the precise AP timing observed in *Steatogenys elegans* electrocytes (Markham and Zakon 2014). This, of course, does not rule out different mechanisms for regulating AP timing in other species but simply provides evidence of potentially varied biophysical mechanisms for achieving a particular signal characteristic.

The two APs generated by biphasic electrocytes have different durations, with the AP2 typically being longer than the AP1 (Bennett 1970, 1961). In the two multiphasic gymnotiforms where the electrocyte ionic currents have been characterized (*Gymnotus carapo* and *Steatogenys elegans*), electrocytes express inactivating A-type K^+ channels (K_A) in addition to the classical delayed rectifier K^+ channels found in electrocytes of monophasic wave fish (Sierra et al. 2007; Markham and Zakon 2014). The activation kinetics of these K_A channels are extremely rapid, and in *Steatogenys elegans* they are the dominant repolarizing current for both AP1 and AP2, with computational simulations suggesting that a higher density of K_A channels on the innervated posterior membrane is responsible for shortening the AP1 duration relative to the AP2 duration (Markham and Zakon 2014), and the same mechanism seems likely in *Gymnotus carapo* electrocytes. An important and unanswered comparative question is what biophysical mechanisms regulate the AP duration in biphasic electrocytes of mormyrids in which 1 or more phases of the complex signal waveform can exceed 20 ms (Hopkins 1999), far longer than any multiphasic discharge observed in gymnotiforms, which are at most a few milliseconds in duration (Crampton and Albert 2006).

5.5.2.3 Biophysical Mechanisms of Signal Cloaking

Almost all weakly electric fish have developed mechanisms for centering the EOD energy on 0 V DC, thereby "cloaking" the signal from electroreceptive predators sensitive to low frequencies (Stoddard and Markham 2008). In wave-type fish with monophasic head-positive EODs, the noninnervated anterior faces on the electrocytes generate a head-negative DC current that sums with the head-positive APs (Bennett 1961) to center the EOD energy around 0 V. This occurs in *Gymnarchus niloticus* through the passive discharge of the sizable capacitance of the anterior membrane. In wave-type gymnotiforms with myogenic electric organs, such as *Eigenmannia* and *Sternopygus*, the cellular mechanism underlying this head-negative DC component remains unknown. A persistent active process seems likely because this DC potential decays over the course of 10–15 ms after electrocyte discharges are silenced (Bennett 1961). Solving this puzzle would provide an

intriguing comparative perspective concerning the parallel or convergent evolution of similar mechanisms for reducing predation risk.

The remaining gymnotiform and mormyrid species also reduce low-frequency spectral energy in the EOD but accomplish this with a different mechanism at the level of individual electrocytes. By generating biphasic or multiphasic signals with roughly equal head-positive and head-negative phases, the signal has approximately as much energy above 0 V DC as below, which nulls the DC component of the signal and attenuates the low-frequency energy. This enhancement of signal complexity likely serves to make the signal less conspicuous to electroreceptive predators (Stoddard 1999; see Krahe Chap. 7).

5.5.3 *Molecular Evolution of Ion Channels Contributes to Signal Diversity*

At the root of the ionic mechanisms of signal diversity is the molecular evolution of electrocyte ion channels. The whole genome duplication that preceded the radiation of teleosts (Hurley et al. 2007) provided these fishes with two paralogs of every gene. In both gymnotiforms and mormyrids, the presence of a second gene for each ion channel allowed the exclusive expression of one paralog in electrocytes where functionally significant modifications that might otherwise be crippling or fatal might instead produce adaptive diversity. The best example is the $Na_V1.4$ sodium-channel gene that is expressed in vertebrate skeletal muscle. In both gymnotiforms and mormyrids, one $Na_V1.4$ paralog ($Na_V1.4a$) is expressed only in electrocytes, whereas its paralog $Na_V1.4b$ is expressed in both muscle and electrocytes (Zakon et al. 2006; Arnegard et al. 2010b).

The $Na_V1.4a$ channels expressed only in gymnotiform and mormyrid electrocytes have rapidly accumulated mutations at locations in the channel gene known to affect channel kinetics (Zakon et al. 2006; Arnegard et al. 2010b), likely because this gene was released from purifying selection pressures in skeletal muscle and subject to positive selection on EOD waveform divergence. Interestingly, some of the mutations that presumably drive signal diversity in the electric signals are associated with disease states when they occur in human sodium channels (Zakon et al. 2006). The rapid evolution of sodium-channel genes in electrocytes has likely been accompanied by a similarly rapid evolution of other key ion channels that accompanied the broad divergence of EOD waveforms and waveform regulation mechanisms.

More recent results have shown that the molecular evolution of electrocyte voltage-gated K^+ channels also plays an important role in shaping EOD waveforms (Swapna et al. 2018). The wave-type mormyroid *Gymnarchus niloticus* generates electrocyte APs and EODs that are more than 1 ms in duration, whereas the pulse-type mormyrid *Brienomyrus brachyistius* (“baby whale”) produces much shorter electrocyte APs and EODs (approximately 200 ms long). Transcriptomic analyses showed that the same voltage-gated K^+ channel, $K_V1.7a$, is expressed at high levels in the electrocytes of both species and that this K^+ channel had undergone rapid

molecular evolution in mormyrids compared with the more basal *Gymnarchus*. Electrophysiological analysis of these $K_V1.7a$ channels expressed in *Xenopus* oocytes showed that *Brienomyrus* $K_V1.7a$ activates at more hyperpolarized membrane potentials than *Gymnarchus* $K_V1.7a$ because of the insertion of a patch of negative amino acids near the voltage-sensing element of *Brienomyrus* $K_V1.7a$. By activating at lower membrane potentials, the *Brienomyrus* channel activates much sooner after the initiation of the AP, thereby terminating the AP more rapidly than the *Gymnarchus* channel that would activate much later after AP onset. This change appears to be sufficient to account for the different durations of *Gymnarchus* and *Brienomyrus* EODs, demonstrating that relatively small molecular changes can have profound impacts on electric communication signals.

Taken together, these findings from just two electrocyte ion channels emphasize that further identification and characterization of additional signaling mechanisms subject to rapid evolution in electrocytes is a key area for future investigation. Given the apparently broad range of ion channels responsible for shaping the electrocyte AP across species, it seems highly likely that molecular tuning of ion-channel function across multiple ion-channel families is a major contributing factor underlying electric signal diversity.

5.6 Mechanisms of Signal Development and Plasticity

5.6.1 Developmental Changes

The ontogenic development of electric organs is an area of intense investigation, now aided by recent genomic advances (see Gallant, Chap. 4). Larval electric organs are replaced during development by adult electric organs in many mormyrid and gymnotiform species (Franchina 1997; Kirschbaum and Schwassmann 2008). The neurogenic organs of apteronotids also arise after the development and loss of a myogenic larval organ (Kirschbaum 1983). In many mormyrids where adult electrocytes show complex patterns of innervation and stalk morphology, the adult electric organ is preceded by a larval organ in which the electrocytes resemble (and may be homologous to) the structurally simpler electrocytes of *Gymnarchus niloticus* (Westby and Kirschbaum 1977, 1978). In some gymnotiform species, the larval electric organ is not replaced by a distinct adult organ, but instead, electrocytes are transformed during development into adult electrocytes (Franchina 1997; Kirschbaum and Schwassmann 2008). *Brachyhypopomus pinnicaudatus* (“feathertail knifefish”; now *Brachyhypopomus gauderio*) is one species where larval electrocytes are transformed into adult electrocytes over the course of approximately 3 mo. The larval electrocytes are elongated cylindrical cells resembling the electrocytes of monophasic wave fish such as *Eigenmannia virescens* and they produce a head-positive monophasic signal. During development, these larval electrocytes gradually compress in length, becoming increasingly box-like, and these changes are accompanied by the gradual addition of a second head-negative phase (Fig. 5.3; Franchina 1997). A fascinating yet

unanswered question is what changes in the ion-channel expression and localization of the electrocyte accompany the developmental transformation from a monophasic electrocyte to a biphasic electrocyte.

5.6.2 *Steroid Hormones and Sexual Dimorphism of Electric Signals*

A comprehensive review covering the central and peripheral hormonal regulation of electric signaling behavior is offered by Silva (Chap. 6). The focus here is on the biophysical mechanisms by which these hormones regulate and modulate electric signal production. Sexual dimorphism in electric signals was first described in the gymnotiform *Sternopygus macrurus* (Hopkins 1972), and subsequently, sexually dimorphic EOD waveforms were reported in many gymnotiform (Hagedorn and Carr 1985; Hopkins et al. 1990) and mormyrid (Hopkins 1980, 1981) species. These sex differences are regulated by steroid hormones. Experimental administration of exogenous androgens increases EOD duration and enhances low-frequency spectral content ("masculinizes" the EOD) of juvenile and female fish by altering the AP waveforms of the electrocytes in both mormyrid (Bass and Hopkins 1985; Bass and Volman 1987) and gymnotiform (Hagedorn and Carr 1985; Mills and Zakon 1991) species. In mormyrids, estrogens also increase EOD duration (Bass and Hopkins 1985), whereas estrogens have the opposite effect of reducing EOD duration in gymnotiforms (Dunlap et al. 1997). The ionic mechanisms by which steroid hormones modify electrocyte APs have been investigated in several gymnotiform species, but no comparable data are yet available for mormyrids.

5.6.2.1 Steroid Hormone Regulation of Voltage-Gated Ion-Channel Kinetics

In the monophasic wave fish *Sternopygus macrurus*, exogenous androgens increase electrocyte AP duration by slowing the kinetics of the voltage-gated Na^+ current of the electrocyte (Ferrari et al. 1995) and estrogen treatment shortens the electrocyte AP waveform by speeding up the inactivation kinetics of the voltage-gated Na^+ current of the electrocyte (Dunlap et al. 1997). A similar pattern was found for the kinetics of the voltage-gated K^+ currents of the electrocytes (McAnelly and Zakon 2007). The molecular mechanisms behind these steroid-induced effects are complex but fascinating.

Sodium channels consist of a single α-subunit that by itself can form a functional channel. The addition of accessory β-subunits often alters the functional properties of these channels. *Sternopygus* electrocytes express two different α-subunits, $Na_V1.4a$ and $Na_V1.4b$, and the *$Na_V1.4b$* gene is expressed as both long ($Na_V1.4bL$) and short ($Na_V1.4bS$) splice variants. $Na_V1.4b$ is found in both

skeletal muscle and in electrocytes, whereas $Na_V1.4a$ is expressed only in electrocytes (Zakon et al. 2006). Expression levels of *$Na_V1.4a$* in electrocytes is constant regardless of EOD duration, but expression levels of $Na_V1.4bL$ are correlated with shorter EOD durations. Androgen treatment suppresses $Na_V1.4bL$ expression levels in electrocytes, which is the likely mechanism behind longer duration male EODs. Expression levels of a sodium-channel β1-subunit expressed in electrocytes ares also correlated with shorter EODs and are also suppressed by androgen treatment (Liu et al. 2007). The role of both $Na_V1.4bL$ and the β1-subunits in accelerating Na^+-channel inactivation (thereby shortening electrocyte APs) has been confirmed in heterologous expression systems, providing strong evidence that regulation of their expression levels in electrocytes is the mechanism through which androgens control EOD duration at the level of electrocytes (Liu et al. 2007, 2008).

A similar picture emerges for the voltage-gated K^+ channels in *Sternopygus* electroctyes, which express three different K^+-channel genes from the K_V1 family. One of these genes, *$K_V1.2b$*, shows no difference in expression levels across individuals, whereas expression levels of *$K_V1.1a$* and *$K_V1.2a$* are correlated with shorter EOD durations. Treatment with steroid hormones that change EOD duration produce corresponding changes in the expression levels of these genes (Few and Zakon 2007). Voltage-gated K^+ channels differ from voltage-gated Na^+ channels because they are formed as tetramers of channel subunits, either homotetramers of a single subunit variety or heterotetramers of different subunits from the same family. Heterotetrameric channels typically exhibit functional properties intermediate between the properties of the various subunits. In *Sternopygus*, changing the relative representation of *$K_V1.1a$* and *$K_V1.2a$* subunits in the voltage-gated K^+ channels of the electrocyte is likely the mechanism underlying the effects of steroid hormones on K^+-channel kinetics and electrocyte AP duration.

5.6.2.2 Regulation of Multiphasic Signal Waveforms

Sexual dimorphism of biphasic electric signals is observed in a subset of both mormyrid and gymnotiform species, with the predominant sex difference being that males exaggerate the duration of one or more of the phases of the signal (Hopkins et al. 1990; Hopkins 1999). Regulation of this sexual dimorphism is mediated by steroid hormones, and where experimental evidence is available, the signal regulation occurs at the level of the electrocytes in both mormyrids and gymnotiforms (Hagedorn and Carr 1985; Bass and Volman 1987). In the biphasic gymnotiform *Brachyhypopomus occidentalis*, males show a prolonged extension of the negative second phase of the signal. Hagedorn and Carr (1985) found that this results from the selective broadening of the electrocyte AP2 in males, whereas the width of AP1 remains relatively constant. A similar mechanism is at work in the related *Brachyhypopomus gauderio* (Markham and Stoddard 2013).

5.6.3 Temperature-Dependent Signal Changes

In some temperate-zone gymnotiforms, the signal waveform shows temperature-sensitive changes. In both *Brachyhypopomus pinnicaudatus* and *Gymnotus carapo*, fish that are not in the reproductive condition decrease the amplitude of the EOD head-negative second phase when the water temperature increases from ~20 °C to above 28 °C (Caputi et al. 1998; Ardanaz et al. 2001). One would suspect that this is a function of temperature-induced accelerations in biophysical kinetics, but these changes occur much more slowly than the change in water temperature and the signal modulations are often transient, indicating that these temperature effects are actively initiated and not simply a matter of temperature-dependent kinetics. Additionally, this is a steroid-dependent effect because sexually mature fish in the breeding condition and nondifferentiated fish given testosterone implants do not exhibit temperature-related changes in signal waveform (Silva et al. 1999; Quintana et al. 2004). Analyzing the underlying cellular mechanisms of this temperature sensitivity and the role of androgen regulation is an important area for future experimental work, especially given the potential implications for reproduction given the imminent thermal disruptions from climate change.

5.6.4 Metabolic Stress and Signal Plasticity

In at least some species, signal characteristics are modulated in response to metabolic stress induced by hypoxia or food restriction. The wave-type gymnotiforms *Eigenmannia virescens* and *Apteronotus leptorhynchus* ("brown ghost knifefish") reduce the signal amplitude within minutes of exposure to hypoxic conditions while the signal frequency remains constant (Reardon et al. 2011). This response to metabolic stress likely serves to reduce the metabolic costs of EOD production, which are known to be extremely high for *Eigenmannia* (Lewis et al. 2014), whereas no data for signal costs are yet available for *Apteronotus*. These hypoxia-induced reductions in signal amplitude might result from an absolute energy shortfall in the electric organ or might instead be a proactive physiological mechanism for conserving energy in hypoxic conditions.

Under metabolic stress caused by one or more days of food restriction, *Eigenmannia virescens* reduces the signal amplitude but not the signal frequency as it does under hypoxia. However, these reductions in amplitude occur over the course of hours to days, much more slowly than hypoxia-induced changes. Reduced signal amplitude during food restriction does not reflect an absolute energetic limitation in the electric organ because full signal amplitude rapidly recovers during social encounters. Instead, the reduction in signal amplitude is a proactive response mediated by the levels of the peptide hormone leptin (Sinnett and Markham 2015). Leptin could be acting via a central endocrine pathway to regulate signal amplitude, it could be acting directly on electrocytes, or both mechanisms could be present.

Interestingly, the pulse-type gymnotiform *Brachyhypopomus gauderio* does not reduce the signal amplitude during food deprivation. Instead, males increase their signaling effort in social contexts, perhaps a terminal investment in reproduction (Gavassa and Stoddard 2012). Different signaling strategies under food deprivation between *Eigenmannia virescens* and *Brachyhypopomus gauderio* could be a function of their different reproductive life-histories. *Brachyhypopomus gauderio* are semelparous single-season breeders that rarely survive to a second reproductive season. In contrast, *Eigenmannia virescens* are iteroparous breeders that live for many years.

5.6.5 Biophysical Mechanisms of Rapid Signal Plasticity

In addition to developmental changes in signal waveforms and the sexual differentiation of signal waveforms over the course of weeks to months, signal waveforms in some gymnotiform species also vary on shorter timescales of minutes to hours in response to environmental conditions and social encounters. These rapid signal modulations occur in both monophasic wave fish where waveform modulations are primarily in signal amplitude (Markham et al. 2009b; Sinnett and Markham 2015) and biphasic pulse fish where changes are found in the amplitude of both phases and the duration of the second phase (Fig. 5.3; Franchina and Stoddard 1998; Franchina et al. 2001). These observations suggest that these rapid signal modulations are produced by moment-to-moment modulations in the underlying biophysics of the electrocytes.

Research on the rapid neuroendocrine regulation of the EOD waveform in the monophasic wave fish *Sternopygus macrurus* (Markham et al. 2009b) and in the biphasic pulse fish *Brachyhypopomus gauderio* (Stoddard et al. 2003; Markham et al. 2009a) led to identification of melanocortin peptide hormones as factors that act directly on electrocytes to produce rapid changes in the signal waveform. These melanocortin hormones, such as adrenocorticotropic hormone or α-melanocyte-stimulating hormone, bind to G-protein-coupled receptors in the electrocyte membrane and activate an intracellular cAMP/protein kinase A (PKA) pathway that then modulates the electrocyte biophysics.

In *Sternopygus macrurus*, rapid signal modulations include rapid increases in signal amplitude within minutes of social encounters (Fig. 5.10a) and circadian increases in signal amplitude at night when the fish are active (Fig. 5.10b). These modulations of signal amplitude in *Sternopygus* are mediated by circulating melanocortin peptides that activate the cAMP/PKA pathway where PKA upregulates the trafficking of preformed voltage-gated Na^+ channels and inward rectifier K^+ channels into the electrocyte membrane, increasing signal amplitude by up to 40% within a matter of minutes (Fig. 5.10c; Markham et al. 2009b). This process is remarkable for its speed and raises the question of why such a large pool of ion channels would be available but not inserted in the membrane

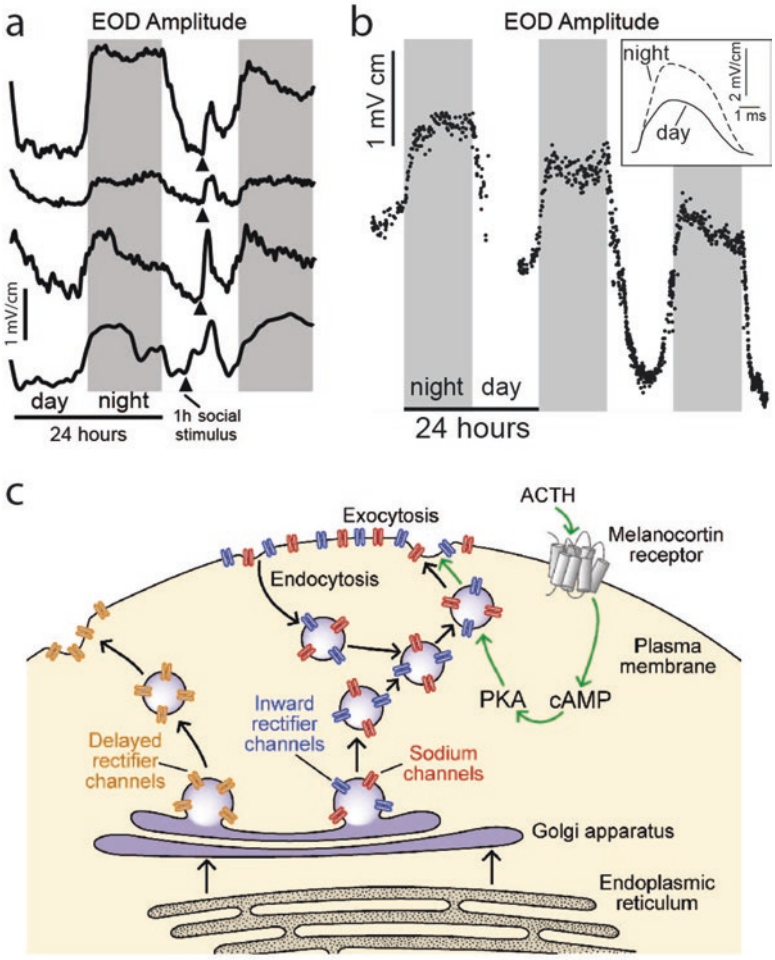

Fig. 5.10 Rapid signal amplitude modulations in *Sternopygus macrurus* are caused by hormonally regulated trafficking of voltage-gated ion channels into the electrocyte membrane. **a:** EOD amplitudes of 4 representative fish (*black lines*) recorded approximately every 60 s over 2 days. A second fish added to the recording tank for 1 hour on the second day (*arrowheads*) caused rapid and transient increases in EOD amplitudes of all fish. **b:** EOD amplitudes of a representative fish (*black dots*) recorded approximately every 60 s over 3 days. The signal amplitude increases at night during darkness (*gray areas*) and decreases during the day with the lights on (*white areas*). *Inset:* superimposed EOD waveforms from the same fish taken at nighttime maximum and daytime minimum. **c:** Schematic diagram of the cellular mechanisms underlying rapid signal amplitude changes in *Sternopygus macrurus* electrocytes. Ion channels are synthesized in the endoplasmic reticulum, then processed and packaged into vesicles in the Golgi apparatus. Delayed rectifier potassium channels undergo exocytosis to the cell surface and are stable there. Inward rectifier K^+ channels and voltage-gated Na^+ channels are constitutively cycled into and out of the membrane. This process is modulated when the melanocortin peptide hormone ACTH activates a G-protein-coupled melanocortin receptor that elevates cAMP and activates protein kinase A (PKA). PKA accelerates only the exocytosis of channels into the membrane, thereby increasing the density of Na^+ and inward rectifier K^+ channels in the electrocyte membrane, producing a higher magnitude of both conductances. Adapted from Markham et al. (2009a)

at all times. A likely explanation is that the ability to reduce signal amplitude during periods of rest or low social interaction confers significant metabolic savings by reducing the Na^+ influx during the electrocyte AP. With a ready pool of preformed ion channels at the ready, signal amplitude can be returned to maximum on demand without the significant delays associated with protein synthesis and processing.

In the biphasic gymnotiform *Brachyhypopomus gauderio*, rapid waveform modulations include changes in overall signal amplitude as well as changes in the duration of the P2 of the signal. These signal modulations increase the active range of the signal as well as significantly increase the low-frequency content of the signal (Fig. 5.3). As with *Sternopygus*, these modulations occur on a circadian rhythm where amplitude and P2 duration increase at night when fish are active and then decrease to a minimum during daytime hours (Stoddard et al. 2007). Rapid increases in signal amplitude and P2 duration also accompany social challenges. These signal modulations are regulated by melanocortin hormones that activate an intracellular cAMP/PKA pathway in *Brachyhypopomus* electrocytes as is the case in *Sternopygus*. The cellular mechanisms, however, are quite different.

The biphasic electrocyte discharge (μEOD) from *Brachyhypopomus gauderio* electrocytes is produced by a sequence of two APs; AP1 initiates first on the innervated posterior membrane followed approximately 75 μs later by initiation of AP2 on the noninnervated posterior membrane, with AP2 being a broader spike than AP1 (Fig. 5.11a). Application of the melanocortin peptide ACTH changes the μEOD waveform in the same manner as the electric signal in vivo: the amplitude of the head-positive EOD phase (P1) and P2 are increased as well as the duration of P2 (Fig. 5.11b, c). The increased P2 amplitude and duration are both a function of the selective broadening of AP2, whereas AP1 width is unchanged. Increases in P1 amplitude, interestingly, do not arise from changes in AP1 or AP2 amplitude because both remain constant. Instead, the AP1-AP2 delay increases by ~35 μs, allowing an increased influence of AP1 on P1 amplitude (Markham and Stoddard 2005).

A number of studies have now shown that steroid and peptide hormones have interactive effects on electrocyte discharge waveform and the resulting signal waveform. In addition to regulating sex differences in baseline EOD characteristics, steroid hormones also regulate the extent and nature of signal waveform changes in response to social interactions and injections of melanocortin hormones where androgens enhance the responsiveness of the signal to melanocortin hormones (Allee et al. 2009; Goldina et al. 2011). These findings provide compelling evidence that electrocytes are the cell-autonomous point of convergence where long-term effects of steroid hormones shape the nature of short-term signal modulation by peptide hormones. The mechanisms by which steroid and peptide hormones interact to coregulate the electrocyte discharge waveform, however, remain unknown and a fertile area for further investigation.

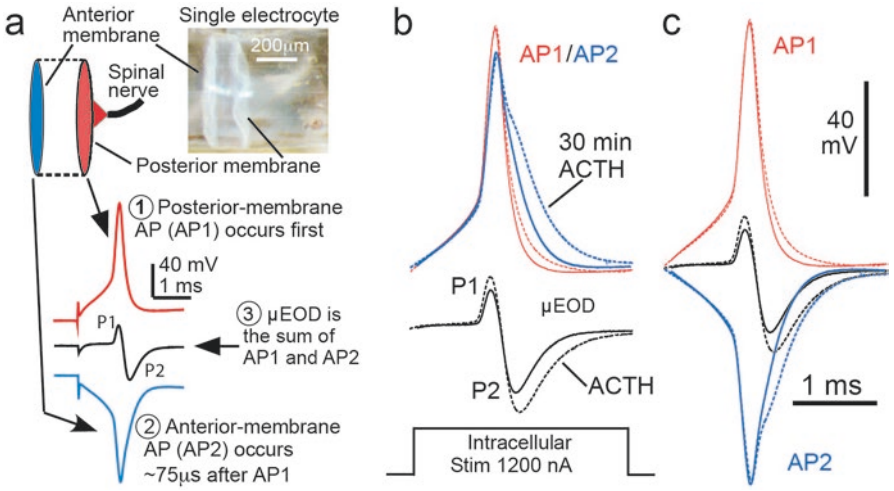

Fig. 5.11 Mechanisms of rapid signal waveform modulation in *Brachyhypopomus gauderio.* **a:** A biphasic electrocyte discharge (µEOD) is produced by two action potentials (APs) generated in close succession. The innervated posterior membrane fires first (AP1; *red*) followed approximately 75 microseconds later by an AP on the noninnervated anterior membrane (AP2; *blue*). AP2 is inverted in this figure to reflect the fact that the ionic currents producing AP2 are directed in the direction opposite of the ionic currents that produce AP1. These two APs sum to produce the biphasic µEOD. Adapted from Markham and Stoddard (2013). **b:** Rapid µEOD waveform changes are initiated by the melanocortin peptide adrenocorticotropic hormone (ACTH). *Solid lines*, baseline recordings; *dashed lines*, recordings after 30 min of exposure to ACTH in vitro. ACTH causes increased P1 and P2 amplitude in the µEOD as well as a pronounced increase in the duration of P2. The amplitudes of AP1 (*red*) and AP2 (*blue*) do not change, and AP2 is broadened while AP1 duration is constant. **c:** Increased amplitude and duration of µEOD P2 is produced by the broadening of AP2 (*blue*), whereas the increased amplitude of µEOD P1 results from an increase of ~35 µs in the delay between AP1 and AP2 (*red*). Because AP1 and AP2 partially overlap, increasing the AP1-AP2 delay increases the µEOD P1 amplitude by "unmasking" the effects of AP1. Data from Markham and Stoddard (2005)

5.7 Summary: Current State of Knowledge, Critical Gaps, and Prospects for the Future

From the early work by Bennett (1961) on the cellular basis of electric signal diversity and continuing through the most recent advances in understanding electrocyte biophysics, the picture that emerges is one of a system that is ripe for comparative analyses that promise important discoveries about the evolutionary shaping of the physiology and biophysics of signal production. The present survey of the known mechanisms of signal diversity shows a remarkable variety of morphological, biophysical, and endocrine mechanisms that shape signal diversity across timescales ranging from evolutionary time to the microsecond-scale timing of bioelectrical events. The mechanisms of diversity across species are themselves remarkable, made all the more interesting by the fairly recent discoveries concerning the mechanisms

by which excitable cells such as electrocytes modify their molecular-level functional properties with surprising speed.

The current state of knowledge, however, must be recognized as far from complete. Perhaps the most glaring gap at the moment is the lack of data regarding the ionic mechanisms of signal diversity in mormyrids. Related to this, knowledge about the ionic mechanisms of signal diversity in gymnotiforms is limited to just a handful of species, with no data available for the entire apteronotid family. Heterologous expression of electrocyte ion channels based on genomic data from apteronotids (Thompson et al. 2018) and mormyrids (Nagel et al. 2017; Swapna et al. 2018) will help to fill these gaps, but the need remains for data on electrocyte ionic currents in situ. A broader comparative dataset across continents and across species on each continent, coupled with ecological and life-history data would enable a better understanding of how and why evolutionary- and life-history forces have shaped the particular biophysical mechanisms that shape signal waveforms.

Even for the species where data are available for the ionic mechanisms of signal production, key questions remain about the mechanisms that regulate the signal waveform. For example, in all known cases where EOD waveforms are rapidly modulated by stress, social encounters, or circadian cues, activation of PKA is the key intracellular factor mediating changes in electrocyte excitability (McAnelly et al. 2003; Markham and Stoddard 2005). The exact phosphorylation events that regulate electrocyte biophysics, however, are unknown. Possibilities include phosphorylation of ion channels, vesicular trafficking components, or other regulators of ion-channel function.

It is also important to note that both mormyrid and gymnotiform fishes show sexual dimorphism in their EOD waveforms, regulated by steroid hormones. However, only a subset of gymnotiform species exhibit rapid circadian and socially induced EOD modulations, and no mormyrid species observed to date exhibit rapid EOD waveform modulation. Broad comparative assays for rapid signal modulation across species could determine if this pattern is reliable and begin to address the question of what conditions supported the emergence (and possible loss) of social and circadian EOD modulations in gymnotiforms and why rapid EOD waveform plasticity is not found in mormyrids.

Given that electrocyte morphology plays such a large role in signal diversity both across species and developmentally within species, investigating the mechanisms that determine electrocyte morphology will be essential for a full understanding of signal diversity. The ongoing revolution in genomics and genetic manipulation techniques should enable progress on this front at a rate that would have been considered impossible just a few years ago. This, coupled with ongoing progress in understanding the genetic mechanisms guiding the developmental origin of electrocytes (Gallant et al. 2014; Pinch et al. 2016), will be essential for a full understanding of the cellular mechanisms of signal diversity. Alongside efforts to better understand morphology, continued investigation of the molecular evolution in molecules key to electrical excitability are necessary for a complete understanding of what exactly makes the electrocytes of one species produce a signal so different from even closely related sympatric species. To answer this question is ultimately to understand the biophysical basis of signal diversity in electric fish.

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Chapter 6
Hormonal Influences on Social Behavior in South American Weakly Electric Fishes

Ana C. Silva

Abstract This chapter highlights the contributions of four iconic Gymnotiform model species to the understanding of the neuroendocrine control of social behavior. In weakly electric fish, social behavior includes electric signaling in addition to locomotor displays. The central circuitry commanding the electric organ discharge (EOD) is well-known, and thus electrocommunication displays can be easily linked to the structures responsible for their modulation. Sexually dimorphic frequency (central) and waveform (peripheral) modulations are reviewed. In *Sternopygus macrurus,* androgens decrease the EOD frequency and broaden the pulse duration, whereas estrogens induce opposite effects. Long-term steroid hormone effects, acting directly on the ion-channel kinetic properties of electrocytes, combine with short-term peptide EOD waveform modulations to adapt electric signaling to environmental and social demands. Closely related species of the family Apteronotidae exhibit diverse sexual dimorphisms in EOD frequency, indicating that the actions of steroids may change their valence and sensitivity across species. The electric signal plasticity of *Brachyhypopomus gauderio* in response to seasonal, daily, and social changes of the environment is outstanding. The interplay of steroids and peptidergic hormones explain long- and short-term modulation of EOD amplitude, duration, and rate. In *Gymnotus omarorum*, gonadal-independent hormonal mechanisms are involved in the regulation of territorial aggression and in the emergence of the dominant subordinate status.

Keywords *Apteronotus* · *Brachyhypopomus* · *Gymnotus* · Melanocortins · Sexual dimorphism · Signal plasticity · *Sternopygus* · Steroid hormone actions · Territorial aggression · Vasotocin

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6.1 Introduction

Social behavior consists of a set of interactions among individuals of the same species, including communication, allogrooming, aggression, mating behavior, and parental behavior. Social behavior varies according to context and thus must be finely regulated to ensure that individuals respond appropriately to others in a range of circumstances. The neuroendocrine control of social behavior emerges from the adaptation of hormonal mechanisms embodied in rather conserved neural circuits across species.

It is currently accepted that social behavior arises as an emergent property of a social behavior network that includes the medial preoptic area, lateral septum, anterior hypothalamus, ventromedial hypothalamus, periaqueductal gray, medial amygdala, and bed nucleus of the stria terminalis (Newman 1999). Initially described in mammals, these circuits appear to be highly conserved across vertebrates (O'Connell and Hofmann 2011). Several signaling molecules, including hormones (steroid hormones, hypothalamic neuropeptides), neurotransmitters, and neuromodulators, shape activity of the social behavior network. From this perspective, the diversity in social behavior would be achieved by plasticity in the weighting of activity across the network (Newman 1999). Thus, each social behavior arises from a distinct spatiotemporal pattern of activation of the social behavior network, which is the result of the plastic orchestration of neuroanatomical pathways and neuroendocrine messengers within species. In addition, evolutionary pressures induce adaptive changes in the actions of these molecules that account for species differences.

Hormones are the context-dependent coordinators of social behavior (Adkins-Regan 2005). They coordinate behavioral and physiological sequences over time (in the short term and across the life span), establish the duration of events and sequences by regulating onset and offset, and modify the nervous system appropriately.

Steroid hormones play a major role in the control of social behavior. They are small molecules derived from cholesterol through a biosynthesis pathway that renders sex steroids (androgens, estrogens, and progesterone) and glucocorticoids. Bioactive steroid hormones are identical across species, and they are synthesized in peripheral endocrine glands (gonads and adrenals) as well as in the brain (Adkins-Regan 2005). The biosynthesis and liberation of steroid hormones depends on a three-tiered system termed the hypothalamus-hypophyseal-gonadal axis for sex steroids and hypothalamus-hypophyseal-adrenal axis for the control of the classic stress response. The gonadotrophin-releasing hormone (GnRH) is the hypothalamic-releasing peptide that increases the levels of the hypophyseal polypeptide hormones follicle-stimulating hormone (FSH) and luteinizing hormone (LH), the gonadotrophic hormones that stimulate increases in gonadal sex steroids. The corticotropin-releasing hormone (CRH) is the hypothalamic-releasing peptide that increases the levels of the hypophyseal polypeptide hormone adrenocorticotropic hormone (ACTH; and other hormones) that, in turn, stimulates glucocorticoids from the adrenal cortex.

Neurohypophyseal hormones of the vasopressin-oxytocin family (and their homologs for nonmammalian species) are also key modulators of social behavior. In addition to their peripheral actions, these nonapeptides are liberated in precise brain regions where they promote species-specific, sexually dimorphic, and/or phenotype-dependent actions in several vertebrate taxa (Goodson and Bass 2001; Johnson and Young 2017). Extensive evidence supports the idea that social behavior diversity across vertebrates has evolved in close association to the evolution of the vasopressin-oxytocin family of hypothalamic nonapeptides.

Active electroreception is the main system of communication in electric fish. Their electric organ (EO) discharge (EOD) carries information about species, sex, individual identity, maturity, season, time of the day, and motivational state (Caputi et al. 2005). All these context-dependent features are hormonally regulated to adapt their behavior to changes in the physical and social environment (Dunlap et al. 2017). Although information about species is stable, sexual maturity changes seasonally. Information about environmental changes (e.g., the beginning of the dark night phase) must be processed within minutes, and motivational states during social interactions must be modulated on even shorter timescales.

Electric fish have proven to be advantageous model systems for studying how the brain coordinates this broad range of modulations for several reasons: (1) they are continuously broadcasting electric information; (2) their electric signaling behavior depends on the firing of a simple and well-known neural circuit (Fig. 6.1); (3) they are amenable to both field and laboratory studies and thus to examining the natural relevance as well as the mechanisms of their behavior; and (4) they belong to a basal branch of vertebrate phylogeny (teleosts), and hence the discoveries made in them have the potential of being universal across vertebrates.

Two groups of freshwater weakly electric fishes have evolved independently: the family Mormyridae in Africa and the order Gymnotiformes in South America (Carlson and Sisneros, Chap. 1). All gymnotiform species emit regular EODs that are generated by a relatively simple and well-described electromotor pathway (Bennett 1971; Caputi et al. 2005).

A spontaneously firing hindbrain structure, the pacemaker nucleus (PN), commands the timing of the EOD (Fig. 6.1A). The PN contains two neuronal types: actual pacemaker neurons and bulbospinal projection neurons (relay cells), which activate electromotor neurons in the spinal cord. Although the PN itself produces and maintains the regular discharge of the EOD, it receives multiple superior inputs that modulate its firing rate. Specifically, inputs from the diencephalic prepacemaker nucleus (PPn) modulate PN firing to produce gradual rises and brief transient accelerations termed chirps, whereas inputs from sublemniscal prepacemaker nucleus (sPPn) drive frequency decreases or interruptions.

The stereotyped species-specific EOD waveform depends on the spatial organization and innervation pattern of the peripheral EO as described by Markham (Chap. 5) and shown in Fig. 6.1B. In all gymnotiform species, the EO extends through the entire ventral portion of the fish body, and except for the family Apteronotidae in which it is organized by an extension of the axons of the electromotor neurons, the EO is composed of myogenic cells called electrocytes.

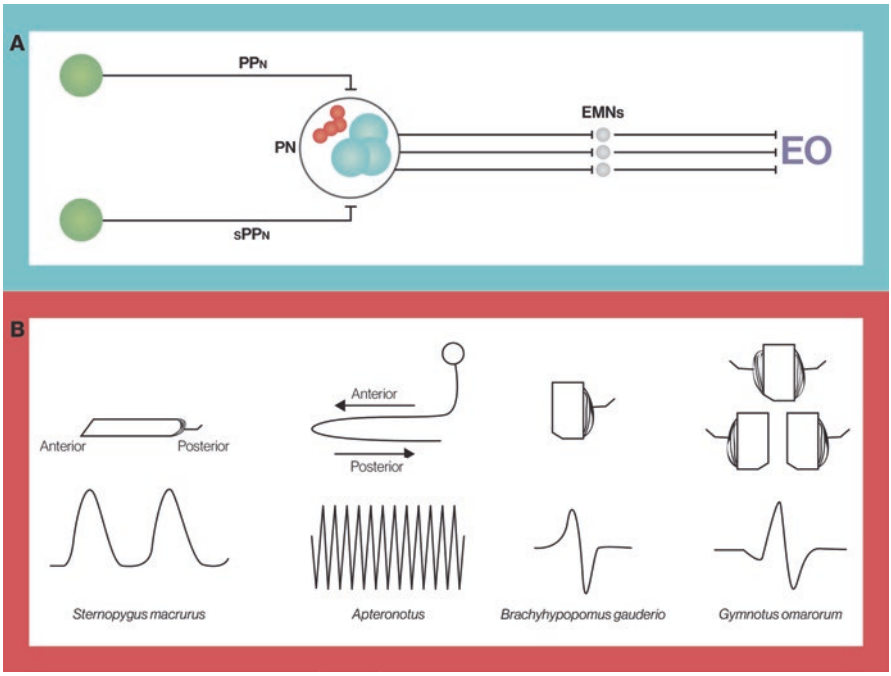

Fig. 6.1 **A:** schematic illustration of the electrogenic system of a gymnotiform fish. The medullary pacemaker nucleus (PN) contains both pacemaker neurons (*red*) and bulbospinal relay neurons (*blue*) that synapse on the electromotor neurons (EMNs) in the spinal cord that, in turn, innervate the electrocytes in the electric organ (EO). The PN receives 2 main central inputs: one from the diencephalic prepacemaker nucleus (PPn) and a second from the sublemniscal pacemaker nucleus (sPPn). **B:** schematic illustration of the representative innervation pattern of the EO (*top*) and EO discharge (EOD; *bottom*) waveforms of *Apteronotus*, *Sternopygus macrurus*, *Brachyhypopomus gauderio*, and *Gymnotus omarorum*

The shape of the EOD waveform, in terms of the number of phases and their polarities, depends on the geometry of the electrocytes, the site(s) of innervation, and the number of electrically excitable faces on the electrocytes. Two groups of gymnotiform species can be identified according to the relationship between the duration of the EOD and its timing. In wave-type fish, the discharge interval is regular and similar to the duration of the EOD, producing an overall EOD resembling a sinusoidal wave. In pulse-type fish, the interval between discharges is longer than the duration of each EOD. Consequently, wave-type species display higher EOD frequencies than pulse-type species, whereas pulse-type species tend to exhibit more complex EOD waveforms and relatively larger amplitudes than wave-type species. The output of the electrogenic system modifies its firing rate and waveform to environmental demands along a wide range of timescales. Thus, it is clear how electrocommunication displays can be linked to the structures responsible of their modulation. Waveform and amplitude behavioral modulations can be traced directly to the peripheral EO, whereas modulations of the rhythm of EOD emission are

determined by neurons of the hindbrain PN, which, in turn, can be influenced by superior prepacemaker inputs.

This chapter highlights the contributions of four iconic model species in the order Gymnotiformes to the understanding of neuroendocrine control of communication and social behavior in vertebrates. Because signaling is crucial for the information exchange among conspecifics inherent to social behavior, Sect. 6.2 focuses on the contributions of three model systems to the understanding of the hormonal modulation of communication systems. The most striking contribution of *Sternopygus* as a model system for behavioral endocrinology is the demonstration of the molecular mechanisms by which long-term effects of steroid hormones combine with short-term peptide EOD waveform modulations to adapt ongoing electric signaling to real-time demands.

Second, fish in the family Apteronotidae display a huge diversity of sexually dimorphic (and monomorphic) EOD frequency modulations as well as a diversity of sensitivity of the underlying neural pathways to hormones. Such diversity makes the genus *Apteronotus* an excellent model system for studying the evolution of sexually dimorphic behavior across vertebrates.

Finally, the genus *Brachyhypopomus* is outstanding for its signal plasticity and has become a valuable model system in which the mechanisms underlying seasonal, daily, and sexually dimorphic modulations of electric behavior have been unraveled successfully.

Section 6.3 describes a model system in which the neuroendocrine control of social behavior has been directly approached. The agonistic behavior of *Gymnotus omarorum* is the best understood example among teleosts of pure territorial aggression unrelated to reproduction and has contributed a new comprehensive evaluation of the role of steroid hormones and hypothalamic neuropeptides in the regulation of aggression.

6.2 Hormonal Modulation of Electric Signaling

6.2.1 *Multilevel Sexual Dimorphism in the Electrocommunication System of* Sternopygus macrurus

Among Gymnotiformes, sexual dimorphism in electric signaling and its hormonal bases are probably best understood in the longtail knifefish *Sternopygus macrurus*. Its sexual dimorphism in EOD frequency and duration is a classic example of long-term actions of steroid hormones with opposing effects of androgens and estrogens (Mills and Zakon 1987; Dunlap et al. 1997). In addition, *Sternopygus macrurus* has become a relevant model system for understanding the mechanisms of action of steroid hormones at the molecular level in vertebrates.

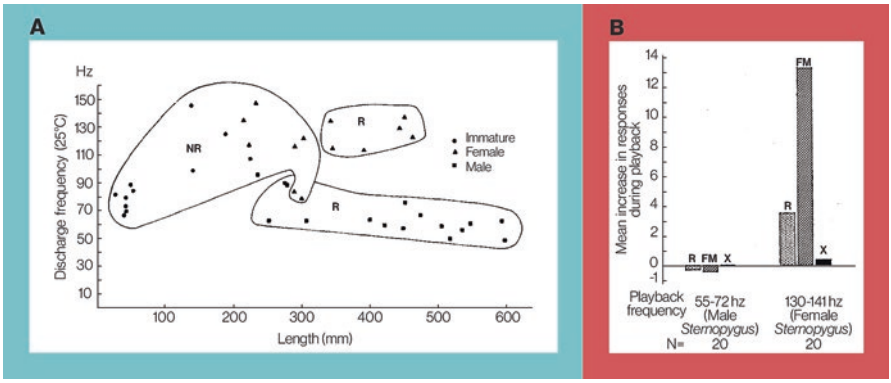

Fig. 6.2 Sexual dimorphism of EODs in *Sternopygus macrurus*. **A:** discharge frequencies of immature (*circles*), female (*triangles*), and male (*squares*) *Sternopygus macrurus* from a natural population plotted against the length from tip of the snout to end of the caudal filament. R, fish in reproductive condition; NR, fish in nonreproductive condition. From Hopkins (1974). **B:** mean increases over controls of responses by *Sternopygus macrurus* males to playback experiments. R, number of rises; FM, number of times when the discharge frequency reached a maximum; X, interruptions. The experiments compare responses to playback of pure sine waves with frequencies corresponding to the electric discharge of male and female *Sternopygus*. From Hopkins (1972), with permission from AAAS

Sternopygus macrurus was the first known example of a fish with sexual differences in its electric discharges. Mature males discharge at a rate of 50–60 Hz, with an EOD duration of around 20 ms, whereas mature females discharge at higher frequencies (up to 200 Hz) and lower EOD durations (around 5 ms). The sex differences in EOD frequency and duration of *Sternopygus macrurus* meet all the requirements of a sexually dimorphic trait. First, pioneer studies demonstrated that males and females from a natural population in Guyana, South America, differ in the steady-state frequency of their discharges (Fig. 6.2A; Hopkins 1974). Second, systemic androgen treatment broadens EOD duration while simultaneously lowering EOD frequency (Fig. 6.3A; Mills and Zakon 1987). Third, male EOD traits are honest indicators of maleness because androgen levels in males correlate inversely with EOD frequency (Zakon et al. 1990, 1991). Fourth, playback experiments demonstrate that sex differences in electric discharges have communicative significance and are used during courtship. For example, males are more responsive to artificial sine waves within the range of female frequencies than to those mimicking male discharges (Fig. 6.2B; Hopkins 1972).

The electric sexual dimorphism of *Sternopygus macrurus* is a good example of activational mechanisms of steroid hormones with coordinated but opposite effects of androgens and estrogens on EOD traits. Androgens decrease EOD frequency, which is set by the medullary PN, and broaden pulse duration, which is determined by the electrophysiological properties of the electrocytes (Fig. 6.3A; Mills and Zakon 1987). Conversely, estrogens increase EOD frequency and decrease pulse duration (Fig. 6.3B; Dunlap et al. 1997). Because of these coordinated effects, the

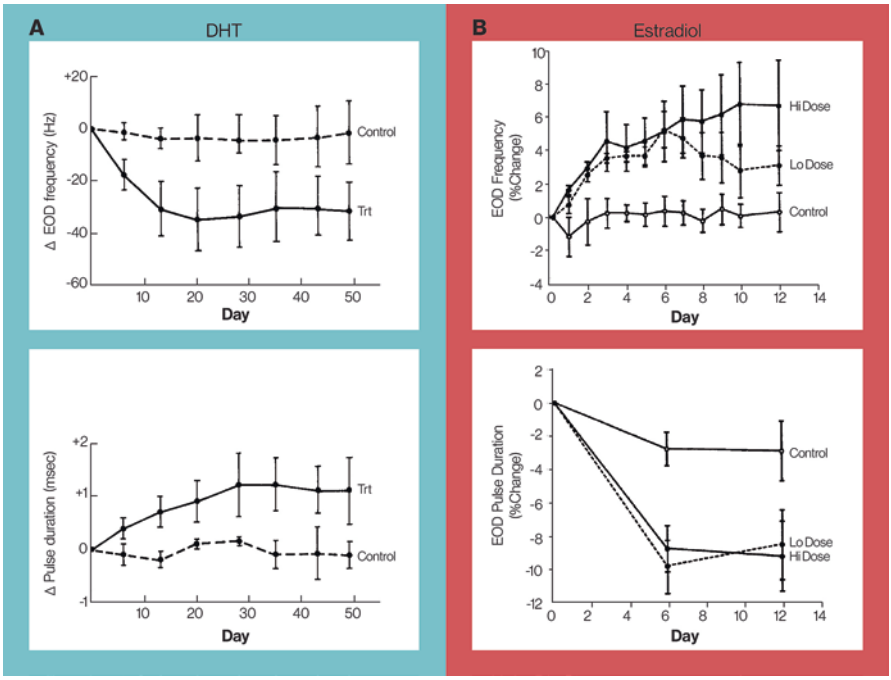

Fig. 6.3 Modulation of *Sternopygus macrurus* EODs by steroid hormone administration **A:** effects of dihydrotestosterone (DHT) treatment on EOD frequency (*top*) and duration (*bottom*). Each point represents the average change (±SD) from the pretreatment (day 0) values of DHT fish (Trt) in comparison to control fish. From Mills and Zakon (1987), with permission from Springer Nature. **B:** effects of estradiol treatment on EOD frequency (*top*) and duration (*bottom*). Each point represents the average change (±SE) from the pretreatment (day 0) values of gonadectomized fish implanted with either low doses (Lo dose) or high doses (Hi dose) of estradiol in comparison to fish implanted with an empty capsule (Control). Adapted from Dunlap et al. (1997)

quasi-sinusoidal shape of *Sternopygus macrurus* EOD is maintained despite the two- to threefold changes in frequency induced by steroid hormone actions. The possibility that hormone actions on pulse duration could be a collateral effect of their effects on EOD frequency was ruled out by demonstrating that local androgen implants in the EO increase pulse duration without affecting EOD frequency (Few and Zakon 2001). Thus, the sexual dimorphism of *Sternopygus macrurus* relies on coordinated, multilevel steroid hormone actions impacting directly on central neuronal networks as well as on peripheral effectors, in which androgen and estrogen receptors have been identified (Zakon 2000).

Two principal observations suggested that the ultimate target of steroid hormone actions in the EO were the ion channels themselves. First, in contrast to other weakly electric fish species, hormone actions occur with no changes in electrocyte morphology, ruling out the possibility that spatial changes in the organization of these cellular units are responsible for the hormone-induced changes in the EOD (Mills et al. 1992). Second, the responses of individual electrocyte action potentials (APs)

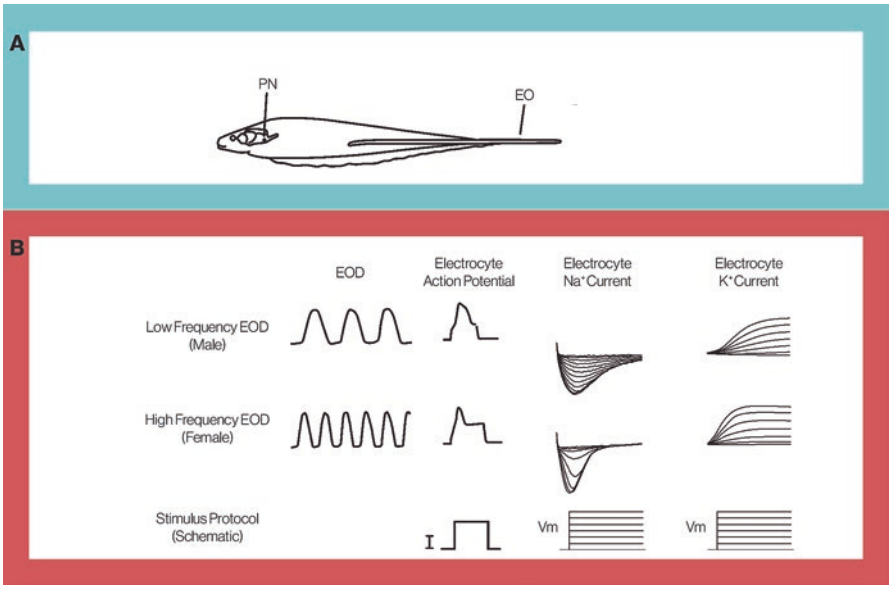

Fig. 6.4 Sexual dimorphism in EOD frequency and duration of *Sternopygus macrurus*. **A:** schematic illustration of the electrogenic system. The PN nucleus sets the EOD frequency, whereas the EOD duration is shaped at the peripheral level (EO). **B:** males show low-frequency EODs and long-duration EOD pulses, whereas females show high-frequency EODs and short-duration pulses. The EOD pulse represents a compound action potential (AP) produced by the simultaneous firing of the electrocytes. Electrocyte APs from males are long and those from females are short. The electrocyte AP is shaped by an inward Na^+ current and an outward delayed rectifier K^+ current. The kinetics of these two currents covary with EOD frequency and thus directly determine AP duration that, in turn, shapes the EOD pulse duration. I, stimulus currect internsity; Vm, Membrane potential. Adapted from McAnelly and Zakon (2007)

parallel the effects of steroid hormones on the whole EOD. That is, males and androgen-treated individuals have longer APs than females and estrogen-treated individuals (Fig. 6.4; Mills and Zakon 1991).

The AP of *Sternopygus macrurus* electrocytes is shaped by the kinetics of an inward Na^+ current and a delayed rectifier K^+ current. Electrocytes that generate short APs have Na^+ currents that inactivate and K^+ currents that activate rapidly, whereas electrocytes of fish that generate long-duration APs have Na^+ currents that inactivate and K^+ currents that activate slowly (Fig. 6.4; McAnelly and Zakon 2007). Chronic androgen treatment slows the inactivation of the electrocyte Na^+ current (Ferrari et al. 1995) and the activation of the delayed rectifier K^+ current (McAnelly and Zakon 2007). These changes in both Na^+ and K^+ channels lengthen electrocyte AP duration and hence EOD pulse duration, causing the masculinization of the EOD at the peripheral EO. Conversely, estradiol speeds up the inactivation of the Na^+ current, thus shortening EOD pulse duration (Dunlap et al. 1997).

Steroid hormones exert their actions directly on Na^+ and K^+ channels via genomic pathways. Both Na^+- and K^+-channel genes have been cloned in electric fish. Two

Na^+ channels (*$smNa_V1.4a$* and *$smNa_V1.4bL$*), a sodium-channel auxiliary subunit (*b1*), and several K^+ channels of the Kv1 family are expressed in the EO (Zakon et al. 2006; Few and Zakon 2007). The following model has been proposed to explain how steroids shape EOD pulse duration by acting differentially on the expression of these three Na^+-channel genes (Dunlap et al. 2017). The Na^+ channel *$smNa_V1.4a$* is expressed equally in all individuals and is hypothesized to inactivate slowly. The Na^+ channel *$Na_V1.4bL$* (with fast inactivation) and *b1* (which acts on Na^+ channels to further speed inactivation) are expressed at higher levels in females (with short electrocyte AP duration) than in males (with long electrocyte AP duration; Liu et al. 2007, 2008). Furthermore, androgens suppress expression of *$smNa_V1.4bL$* and *b1* (Liu et al. 2007, 2008). Thus, as a consequence of the action of steroids on the EO, males and females express a different proportion of Na^+-channel genes; males have low levels of *$smNa_V1.4bL$* and *b1* but relatively higher levels of *$smNa_V1.4a$*, which would generate a slowly inactivating current and thereby lengthen the electrocyte AP.

The long-term effects of steroids on the EO interact with the short-term peptidergic modulation of electrocyte excitability and EOD waveform. Peptide hypophyseal hormones such as ACTH or α-melanocyte-stimulating hormone (α-MSH) are responsible for rapid circadian and socially controlled changes in EOD amplitude in *Sternopygus macrurus* as well as in other gymnotiform species (Sect. 6.2.3; Markham 2013). Under the control of these peptide hormones and intracellular second messengers (e.g., phosphokinase A), *Sternopygus macrurus* rapidly enhances EOD amplitude (by 40%) at night and during social encounters through selective trafficking of Na^+ channels into electrocyte membranes (Markham et al. 2009a). Thus, this hormonally controlled mechanism of ion-channel trafficking allows *Sternopygus macrurus* to adaptively boost its EOD amplitude in response to environmental demands and to save the extra energy cost of maintaining a high-amplitude signal during its resting periods.

6.2.2 Frequency Modulations in Apteronotidae

Interspecific variation in communication systems has evolved to aid in reproductive isolation, especially in sympatric species. The same highly conserved hormones act on similarly conserved central nervous pathways across vertebrates in a highly precise, species-specific way to render this diversity. To understand the evolution of neuroendocrine mechanisms, it has been useful to find closely related species whose social neural networks respond differently to the same hormonal stimulus. South American weakly electric fish of the family Apteronotidae have contributed outstanding evidence in this respect.

Apteronotidae is the most speciose family of wave-type weakly electric fish of the order Gymnotiformes and the only one in which the electric organ is not myogenic (see Markham, Chap. 5). Instead, their electric organ is neurogenic, consisting of an array of electromotor neuron axons. This peculiarity of apteronotid species

allows them to reach the highest EOD frequencies among Gymnotiformes (up to 2000 Hz) and enables the use of frequency modulations (rather than waveform modulations) as the main communication signals (Turner et al. 2007). Therefore, the control of sexual and other context-dependent changes of electric behavior is necessarily attained by hormones and neuromodulators acting exclusively on central neural pathways.

Among the Apteronotidae, only one reported species (*Apteronotus albifrons*, the black ghost knifefish) shows the sexual dimorphism in EOD frequency observed typically in other Gymnotiformes, with males having lower discharges rates than females (Dunlap et al. 2017). Several species show no sex differences in EOD frequency (*Apteronotus magdalensis, Apteronotus bonapartii, Adontosternachus devenanzii,* and *Parapteronotus hasemani*). Finally, some species (*Apteronotus leptorynchus*, the brown ghost knifefish; *Apteronotus rostratus*; and *Sternarchogiton natteri*) show the opposite sexual dimorphism, with males discharging at higher frequencies than females (Smith 2013; Dunlap et al. 2017).

This intriguing example of reversed sexual dimorphism among closely related species has been examined in detailed comparisons of *Apteronotus leptorhynchus* and *Apteronotus albifrons* (Zakon and Dunlap 1999). As shown in Fig. 6.5A, the sexual dimorphism in EOD frequency of *Apteronotus leptorhynchus*, in which males discharge at higher frequency (850–1100 Hz) than females (600–800 Hz), is more clear and can be used reliably for sex identification. In *Apteronotus albifrons*, males discharge at a lower frequency (850–1100 Hz) than females (1000–1200 Hz), but there is a lot of overlap between the sexes. As predicted from the direction of the sex difference in EOD frequency, the administration of the nonaromatizable androgen 11-ketotestosterone (11-KT) masculinizes EOD frequency in both species, inducing an increase in EOD frequency in *Apteronotus leptorhynchus* (Meyer et al. 1987) and a decrease in EOD frequency in *Apteronotus albifrons* (Dunlap et al. 1998). Interestingly, *Apteronotus albifrons* exhibits population differences in sexual dimorphism associated with a differential sensitivity to androgen treatment. EOD frequency in a Colombian population is more sexually dimorphic and more androgen sensitive than the EOD frequency of a Brazilian population (Ho et al. 2013). Thus, the direction and magnitude of sex differences in EOD frequency across species and populations are not only a matter of changing the valence of hormonal actions on EOD frequency but also of the sensitivity by which neural circuits respond to hormones.

In addition to the sexual dimorphism of their regular wave-type EOD frequency, every apteronotid species that has been studied so far also produces transient rhythm modulations of their EOD during social interactions (Zakon et al. 2002). EOD modulations fall into two broad categories: chirps, which are acute and short (10- to 200-ms) increases in EOD frequency, often accompanied by decreases in EOD amplitude, and rises, which are smaller and longer (1- to 10-s) gradual increases in EOD frequency (Smith 2013; Dunlap et al. 2017). Chirps are highly variable across apteronotid species in many aspects of chirp structure (e.g., EOD frequency shift, duration, EOD amplitude modulation, and post-EOD frequency undershoot), suggesting they can act as a species-identifying signal (Turner et al. 2007).

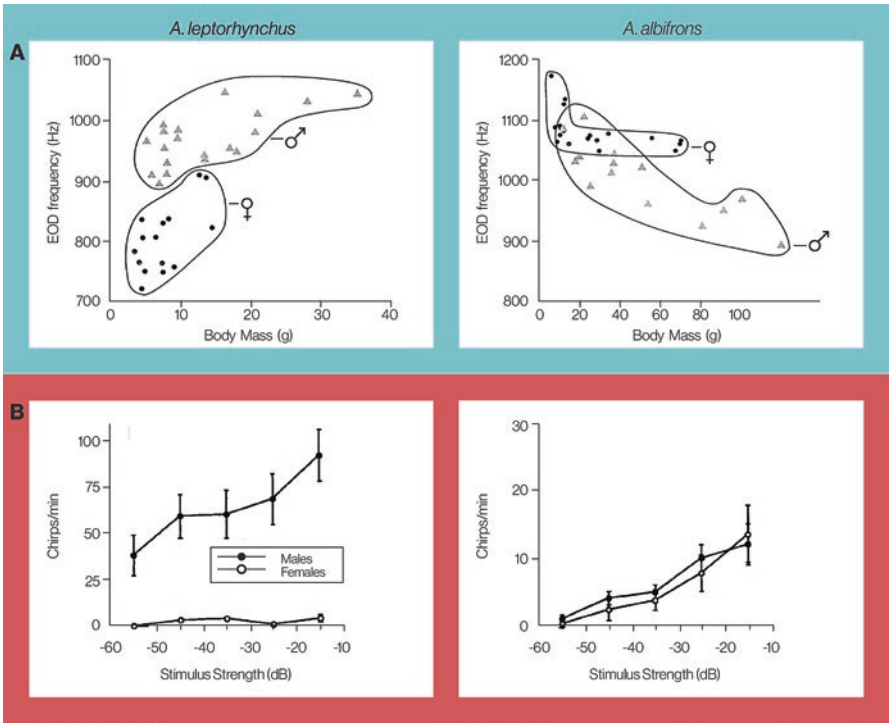

Fig. 6.5 Differences in the sexual dimorphism of electric signaling between the brown ghost *Apteronotus leptorhynchus* and the black ghost *Apteronotus albifrons*. **A:** brown ghost females have lower EOD frequencies than males, whereas black ghost females have higher EOD frequencies than males. From Zakon and Dunlap (1999). **B:** brown ghosts show a large sex difference in chirp rate, whereas black ghosts show no sex difference in this parameter. Error bars are ±SE. From Dunlap et al. (1998), with permission from Springer Nature

Apteronotus leptorhynchus and *Apteronotus albifrons* show distinct sexually dimorphic chirping behavior (Zakon and Dunlap 1999; Smith 2013). *Apteronotus leptorhynchus* show a large sex difference in chirp rate as well as in the percent of individuals that chirp, whereas *Apteronotus albifrons* show no sex differences in these parameters (Fig. 6.5B; Dunlap et al. 1998). Chirp structure is sexually dimorphic in both species (Engler et al. 2000; Smith 2013), but these sex differences vary between species. High-frequency chirps are exclusively emitted by males in *Apteronotus leptorhynchus*, whereas males of *Apteronotus albifrons* emit longer high-frequency chirps than females. Low-frequency chirps involve a larger shift in EOD frequency in males than in females of *Apteronotus leptorhynchus*, whereas males of *Apteronotus albifrons* emit longer low-frequency chirps than females.

As demonstrated by a study in *Apteronotus leptorhynchus* in the 1980s, chirps can be readily evoked under laboratory conditions by stimulating fish with a sinusoidal signal at an amplitude and frequency that mimic the discharge of conspecifics (Dye 1987). Playback experiments strongly suggest that EOD frequency signals sex

in apteronotid species because fish chirp differently to male versus female playbacks in species that exhibit sexual dimorphism in EOD frequency (Smith 2013).

Strong evidence indicates that sex differences in chirp rate and structure in *Apteronotus leptorhynchus* are regulated in part by the activational effects of androgens. Gonadectomy in adult male *Apteronotus leptorhynchus* eliminates sexual dimorphism in chirp rate (Dunlap et al. 1998). Treatment of female *Apteronotus leptorhynchus* with androgens partially masculinizes the chirp response to playbacks by increasing the chirp rate, shifting the EOD frequency, and causing EOD amplitude distortion (Dulka and Maler 1994). In male *Apteronotus leptorhynchus* interacting electrically with a conspecific fish, the chirp rates correlate with plasma levels of 11-KT (Dunlap 2002). Interestingly, species differences in androgen sensitivity parallel the degree of sexual dimorphism across species. In other words, *Apteronotus leptorhynchus,* which is dimorphic in chirp rate, increases chirping in response to androgens, whereas the monomorphic chirp rate of *Apteronotus albifrons* is insensitive to androgen treatment (Dunlap et al. 1998).

6.2.3 *Context and Hormonal Dependent Signaling of* Brachyhypopomus gauderio

Brachyhypopomus gauderio is a pulse-type weakly electric fish occurring at the southern boundary of the gymnotiform continental distribution in South America. The EOD of *Brachyhypopomus gauderio* changes in response to the physical and social environment on two timescales and with two levels of action. Two hormone classes, melanocortins and androgens, act on the peripheral EO to mediate short-term and long-term modulations of signal amplitude and duration, respectively, observed during social interaction. At least three hormone classes, amines, neuropeptides and androgens, act on the central command nucleus, PN, to mediate the short-term and long-term modulations in EOD rate. Another remarkable feature of *Brachyhypopomus* is how laboratory and field studies have been effectively combined to render a comprehensive view of the relevance of signal plasticity for coping with real-life demands.

6.2.3.1 Hormonal Control of Electric Organ Discharge Waveform Modulations

In its natural habitat, *Brachyhypopomus gauderio* breeds during the austral summer (Silva et al. 2002). Males are larger than females, and during the breeding season, other sexually dimorphic traits appear (Hopkins et al. 1990; Caputi et al. 1998). Males show elongated and flattened tails that remain short and cylindrical in females and display sexually dimorphic biphasic EODs. As shown in Fig. 6.6A (in recordings in which EOD amplitudes have been normalized), males generate EODs with

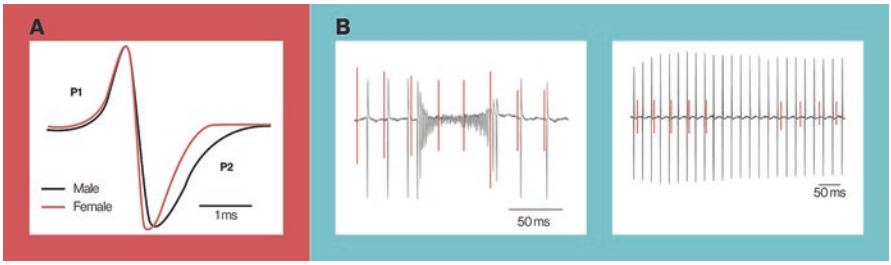

Fig. 6.6 Sexual dimorphism in the electric signaling of *Brachyhypopomus gauderio*. **A:** male and female biphasic EOD pulses with the head-positive initial phase (P1) and head-negative late phase (P2). The male EOD (*black*) is longer in duration than the female EOD (*red*). Modified from Caputi et al. (1998). **B:** transient EOD rate modulation during courtship in male-female dyads. Only the male (*black*) emits chirps, while the female (*red*) maintains its regular discharge (*left*). Only the female (*red*) interrupts its EOD, whereas the male (*black*) maintains its regular discharge (*right*). Modified from Perrone et al. (2009)

a longer duration of the second head-negative phase (Caputi et al. 1998). Because males are larger than females, the amplitude of the EOD is also larger in males than in females (Franchina and Stoddard 1998). These sexually dimorphic changes in EOD waveform are driven in the EO by the classical signaling pathway, which involves androgens crossing the plasma membrane, entering the cytoplasm and binding to the androgen receptor, and translocating the complex to the nucleus where it modulates gene transcription and subsequently protein synthesis (Silva et al. 2002; Allee et al. 2009). Androgen implants (testosterone and 11-KT) masculinize the EOD waveform by increasing the duration of the second phase of the EOD in sexually immature males and females, whereas only 11-KT induces an increase in EOD amplitude (Silva et al. 1999; Goldina et al. 2011). In addition, in the field during the breeding season, the EOD amplitude correlates with fish body size while the duration of the second phase of the EOD correlates with testosterone and 11-KT levels in males and females and with gonadal size in males (Gavassa et al. 2011). Thus, by sensing a conspecific EOD waveform, *Brachyhypopomus gauderio* can gather useful information about the size and physiological state of the signaler that can be used for either courtship or agonistic encounters (Curtis and Stoddard 2003; Zubizarreta et al. 2012). Interestingly, field and laboratory studies have shown that the reliability of the information encoded in the EOD waveform increases with fish density (Gavassa et al. 2012). The EOD amplitude and tightness of its correlation with fish body length increase in highly populated environments, thus reinforcing signal honesty under the most socially competitive scenario.

Brachyhypopomus gauderio occurs within the temperate zone of South America (20–35°S), where seasonal changes in water temperature are probably the most important environmental cue to first trigger sexual gonadal maturation in the spring and to subsequently achieve breeding in summer (Silva et al. 2002). A long-term field study, in which a natural population of *Brachyhypopomus gauderio* was followed across seasons, demonstrated that morphological and electrophysiological indicators of sexual maturity are associated with high water temperatures

(Silva et al. 2003). Moreover, in the laboratory, gonadal maturation and EOD sex differences can be induced by mimicking the seasonal increase in water temperature (Quintana et al. 2004).

Most electric fishes live in the tropics where there are few seasonal changes in water temperature. Instead, tropical gymnotiform species rely on seasonal changes in water conductivity associated with the alternation of rainy and dry seasons as environmental cues for the onset of breeding (Kirshbaum 1995). In contrast, in the southernmost populations of gymnotiforms, seasonal increases in water temperature influence the hypothalamus-hypophyseal axis to induce gonadal maturation and the subsequent increase in gonadal steroid levels, which, in turn, induce secondary indicators of sexual maturity such as morphological and EOD sex differences. During the nonbreeding season, the EOD waveform of *Brachyhypopomus gauderio* is sensitive to acute increases in water temperature and undergoes a striking change from a biphasic to almost a monophasic signal (Silva et al. 2002). Nevertheless, during the breeding season, when water temperature can reach daily peaks of 27 °C, circulating androgens decrease this peripheral effect, protecting the waveform and allowing it to remain a reliable sign of male reproductive state regardless of temperature conditions (Silva et al. 1999).

Male *Brachyhypopomus gauderio* adjust the degree of their sexual dimorphism in EOD waveform to rapid environmental and social changes (Fig. 6.7A; Franchina et al. 2001). EOD duration and amplitude increase minutes after the beginning of the dark phase and also by social interaction. In a similar way as in *Sternopygus macrurus* (Sect. 6.2.1), these rapid waveform changes are controlled by the melanocortin peptide hormones ACTH and α-MSH (Fig. 6.7A; Markham et al. 2009b). The injection of cyclic-α-MSH, which acts as a silent antagonist of α-MSH activity in vitro, attenuates EOD enhancements that normally follow lights-out or interaction with conspecifics (Markham et al. 2009b). As reviewed in Sect. 6.2.1 for *Sternopygus macrurus*, melanocortin receptors in the EO of *Brachyhypopomus gauderio* activate the cAMP/protein kinase A (PKA) pathway, which increases EOD amplitude and duration, in part by differentially modulating the timing of electrocyte APs (Markham and Stoddard 2005) and by promoting ion-channel trafficking to the excitable membranes of the electrocyte (Markham et al. 2009b). Also, as in *Sternopygus macrurus* (Sect. 6.2.1), although androgens affect the EOD waveform on a long-term timescale of days to months, the melanocortin peptide hormones modify the EOD waveform to environmental changes within minutes. In addition, androgens and melanocortins interact in the modulation of the EOD waveform because androgens enhance the responsiveness of EOD waveform to melanocortin injections (Allee et al. 2009; Goldina et al. 2011).

6.2.3.2 Hormonal Control of Electric Organ Discharge Rate Modulations

All nocturnal animals need to enhance their temporal resolution during nighttime, and many gymnotiform fish do this by increasing the rate of their EOD emission. Arousal in *Brachyhypopomus gauderio* is thus characterized by a nocturnal increase

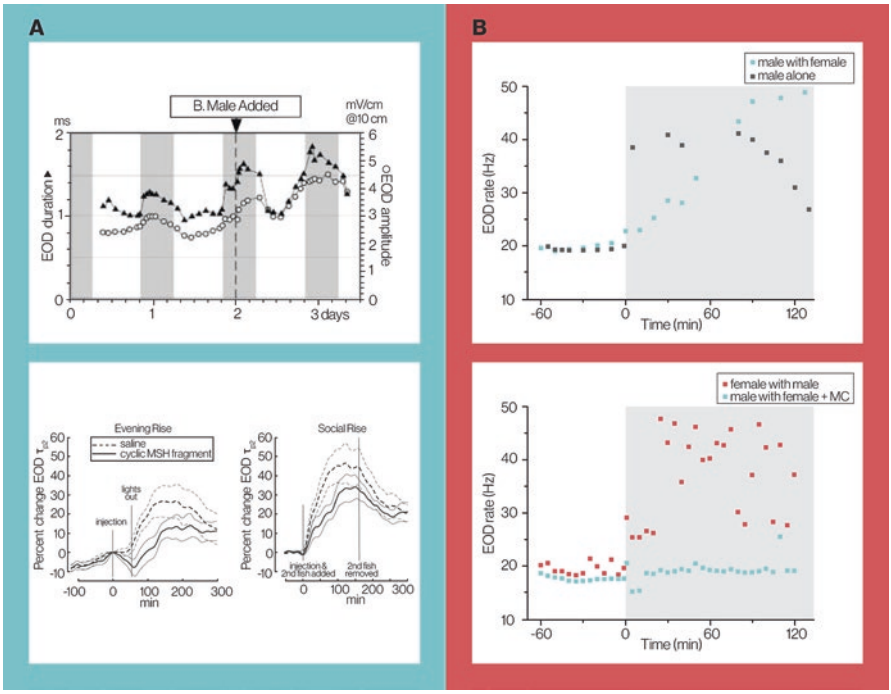

Fig. 6.7 Social modulation of daily changes in the EOD of *Brachyhypopomus gauderio*. **A:** nocturnal increase of EOD waveform and amplitude. *Top*: a male that has been isolated for one week is recorded first in isolation; EOD amplitude (*open circles*) and duration (*solid triangles*) peaked during night (*gray boxes*). When a second stimulus male was added to the tank, EOD amplitude and duration increased a few minutes later and reached higher values than previous maxima. The following night, EOD amplitude and duration increased further. From Franchina et al. (2001), with permission from Springer Nature. *Bottom*: injections of cyclic α-melanocyte-stimulating hormone (α-MSH) in vivo attenuate circadian and socially induced EOD waveform enhancements. *Black lines*, means; *gray lines*, 95% confidence intervals. *Left:* injection of cyclic α-MSH 60 min before lights out delays and attenuates the circadian rise in EOD P2 time (τ_{P2}). *Right*: cyclic α-MSH injections just before addition of a conspecific delay and attenuate the socially induced increases in EOD τ_{P2}. All records are rescaled to the magnitude at the time of injection. Reprinted from Markham et al. (2009b), with permission from Elsevier. **B:** both breeding males and females show a long-lasting increase in their EOD rate when recorded in social interaction. *Top*: nocturnal increase in EOD rate only occurs during the first dark hour in isolated males. Modified from Silva et al. (2007). *Bottom*: nocturnal increase in EOD rate is abolished in social males when the arginine vasotocin (AVT) antagonist Manning compound (MC) is administered 15 min before sunset. *Gray boxes*, occurrence of night. Modified from Perrone et al. (2010)

in the EOD rate that relies on daily changes in the firing rate of the medullary PN. The nocturnal increase in the EOD rate is part of a circadian activity-rest rhythm that is melatonin dependent and persists in free running conditions of darkness in the laboratory and in the wild (Stoddard et al. 2007; Migliaro and Silva 2016). This nocturnal increase in the EOD rate in *Brachyhypopomus gauderio* changes seasonally, and during breeding, it is also socially modulated (Fig. 6.7B; Silva et al. 2007).

When a breeding male is recorded during sexual interaction with a female, a steady increase in the EOD rate is observed immediately after sunset (Fig. 6.7B, *top, blue circles*). However, when the same male is recorded in isolation one night later, the nocturnal increase in the EOD rate is lower and returns to diurnal values around 150 min after sunset (Fig. 6.7B, *top, black circles*).

Although the basic nocturnal increase in the EOD rate is modulated by the circadian system via melatonin, the social component of this increase is under neuropeptidergic modulation (Fig. 6.7B; Perrone et al. 2010). As mentioned in Sect. 6.1, hypothalamic nonapeptides of the vasopressin-oxytocin family modulate social behavior, with prominent differential actions between species, sexes, and social contexts across vertebrates (Goodson and Bass 2001; Johnson and Young 2017). The administration of arginine vasotocin (AVT; the teleost equivalent of mammalian vasopressin) induces steady increases in the EOD rate in male and female *Brachyhypopomus gauderio*, recorded in vivo either in isolation or in pairs, as well as increases in the firing rate of the PN when administered to in vitro brain slices (Perrone et al. 2010). Although the competitive AVT antagonist, Manning compound, does not abolish the nocturnal increase in the EOD rate observed in isolated individuals (Migliaro and Silva 2016), it prevents AVT actions on the EOD rate, including the occurrence of the extrasocially dependent component of nocturnal increase observed in breeding males during social interaction (Fig. 6.7B, *bottom, blue circles*; Perrone et al. 2010). In addition, AVT influences the nocturnal increase in the EOD rate of breeding males differently during courtship than during agonistic male-male encounters (Perrone et al. 2010), demonstrating that AVT has also subtler context-dependent actions on electric signaling in this species.

Only during breeding, *Brachyhypopomus gauderio* produce transient sexually dimorphic social electric signals in addition to the regular emission of their biphasic EOD: males produce chirps and accelerations, whereas females interrupt their EOD emission (Fig. 6.6B; Silva et al. 2008). Male chirps are short (25- to 260-ms) increases in the EOD rate (up to 500 Hz) with EOD amplitude dips, whereas accelerations are gradual and smaller increases in the EOD rate with almost no distortion of the EOD amplitude. These social electric signals have been thoroughly characterized in realistic social reproductive and agonistic contexts in the wild as well as in laboratory settings (Perrone et al. 2009).

Social electric signals, such as chirps and interruptions, arise from descending inputs to the PN in gymnotiform fish. The neural mechanisms underlying the generation of EOD chirps have been unraveled by early studies in the genus *Brachyhypopomus*. Inputs from two premotor nuclei called the PPn and the sPPn cause transient changes in the output of the PN (Fig. 6.1).

Chirps are produced via glutamatergic input from the PPn acting on AMPA-kainate receptors in the dendritic arbor of relay cells in the PN, whereas interruptions are generated via glutamatergic input from the sPPn acting on NMDA receptors of the soma of the same relay cells (Kawasaki and Heiligenberg 1990; Kennedy and Heiligenberg 1994). In both cases, glutamatergic input depolarizes relay cells and uncouples them from the regular input of pacemaker neurons. For chirps,

AMPA-mediated depolarization induces an acceleration of relay cell firing rates; for interruptions, NMDA-mediated depolarization on relay cells is strong enough to inactivate their Na^+ channels and cause them to cease firing (Kawasaki and Heiligenberg 1989; Spiro 1997).

During the emission of social electric signals, the orderly functional activation of the *Brachyhypopomus* electrogenic system is transiently disrupted, and the whole EOD rate follows the firing of momentarily uncoupled relay cells. However, the PN is not a homogeneous nucleus passively responding to central influences to produce EOD rate modulations. Rather, the PN has a complex cytoarchitectural organization with topographic differences that probably support its plastic functionality (Silva et al. 2008).

Seasonal and sex specificity of social electric signals in *Brachyhypopomus gauderio* rely on the remarkable functional, but not the morphological, plasticity of the PN (Fig. 6.8; Quintana et al. 2011a, b). The PN commands EOD interruptions in both breeding and nonbreeding adults, but its ability to generate chirps is only exhibited by males during the breeding season. The maps presented in Fig. 6.8 clearly illustrate two aspects of the distinctive responses of the PN to localized injections of glutamate in in vivo experiments. The first conclusion is that there are topographic differences in glutamate responses across seasons in all experimental groups of fish. In addition, although the PN of nonbreeding adults and breeding females behave similarly to glutamate injections, a very restricted area of relay cells acquires the capability to respond with chirps in breeding males. In vitro preparations containing the PN confirmed these observations because the injection of AMPA to the PN extracted from breeding males, but not from females, generated chirp-like bursting activity in vitro (Quintana et al. 2014).

The seasonal and sexual changes of the PN in *Brachyhypopomus gauderio* are probably modulated by the direct actions of androgens. The expression of androgen receptors in relay and pacemaker neurons is enhanced in breeding males, and this upregulation can be controlled by circulating androgen levels rising naturally at the beginning of the breeding season (Pouso et al. 2010).

6.3 Hormonal Modulation of the Agonistic Behavior of *Gymnotus*

Gymnotus omarorum, the banded knifefish, is the most abundant species of weakly electric fish at the southern boundary of gymnotiform continental distribution in South America. Among teleosts, *Gymnotus omarorum* displays the best understood example of pure territorial aggression (Quintana et al. 2016). During the nonbreeding season, when gonads are regressed and no reproductive motivation drives competition, males and females of this sexually monomorphic species fiercely defend territories in intrasexual and intersexual encounters. *Gymnotus omarorum* is thus a relevant model system to understand gonadal-independent mechanisms regulating

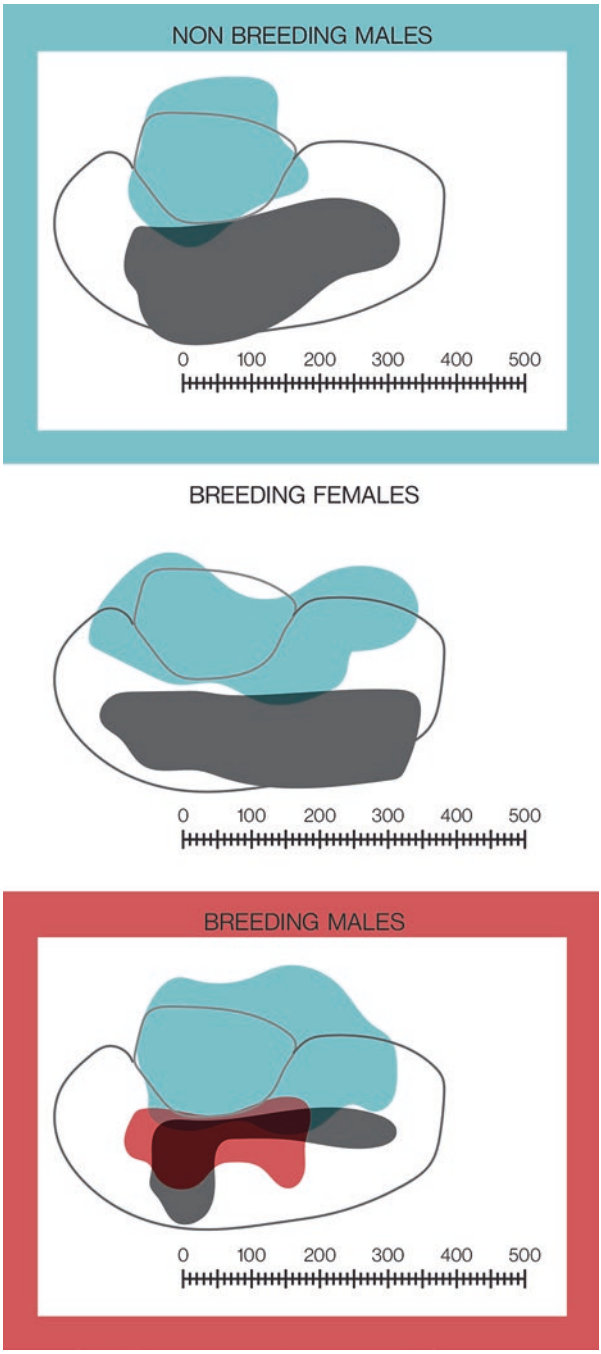

Fig. 6.8 Effects of glutamate injections in the PN of *Brachyhypopomus gauderio*. Comparative maps show glutamate actions according to the location of injection sites within the PN based on a 50-μm medial section (the dorsal area [*top*] represents the pacemaker neurons and the ventral

aggression. In freely moving fish, it is possible to analyze the neuroendocrine mechanisms underlying both the priority access to territories in the wild and the emergence of the dominant-subordinate status in laboratory settings.

6.3.1 Aggression in the Context of Territoriality

Agonistic behavior is the social behavior by which conspecifics resolve conflict situations through the establishment of dominant-subordinate status (King 1973). When space is the resource animals compete for, territory is the area from which intruders are excluded by some combination of advertisement, threat, and/or attack (Brown 1975). As a form of social dominance, territoriality is thus mediated by agonistic encounters between conspecifics. Reproductive males and male-female dyads usually defend territories during the breeding season, and this type of territorial aggression has been traditionally linked to the action of gonadal androgens (Adkins-Regan 2005; Wingfield 2005). Less frequently, territorial defense can also be observed year-round. For example, in wood rats (Caldwell et al. 1984) and song sparrows (Wingfield 1994), both males and females display territorial behaviors in the absence of reproductive drive and hence independently of circulating androgens. Pioneering studies in *Gymnotus carapo* (Black-Cleworth 1970) described the social hierarchical organization of this species and the persistence of a nonreproductive territoriality, which is probably associated with its feeding habits. More recent field studies demonstrated that *Gymnotus omarorum* holds equal-size territories between males and females across seasons in the wild. During the day in the nonbreeding season, adult individuals rest, on average, 1.4 m apart from each other regardless of sex, and body size is the only predictor of territory size. During breeding, the body size of adult *Gymnotus omarorum* is larger than in the nonbreeding season, and thus the individual territory size is also larger (mean interindividual distance = 2.3 m). In contrast to the nonbreeding season, circulating steroid hormone levels correlate with territory size during breeding. Interestingly, although territoriality in *Gymnotus omarorum* occurs year-round, its motivation and, therefore, its underlying mechanisms change seasonally. In the nonbreeding season, when foraging is the only drive, territories are established independently of circulating steroid hormone levels. During breeding, reproduction imposes a more complex territorial competition in which individual quality depends on gonadal hormone levels as traditionally observed in territorial species across vertebrates.

Fig. 6.8 (continued) area [*bottom*] represents the relay neurons). Glutamate produces EOD rate increases (*blue*) when administered to the pacemaker neuron area and EOD interruptions when administered to the relay neuron area (*black*) in all the experimental groups. Only in breeding males, glutamate injections induce chirp-like activity (*red*) when administered to a restricted area of the PN. Scale bar, μm. Modified from Quintana et al. (2011b)

Territoriality is mediated by agonistic encounters that can be tested in laboratory settings. During the nonbreeding season, a clear dominant-subordinate status emerges within minutes in dyadic encounters of *Gymnotus omarorum* in which the initial interindividual distance resembles the one observed in the wild (Perrone et al. 2019). The larger contender always wins the fight (with no outcome reversion in the following 36 h), holds the territory after contest resolution, and excludes the subordinate from its conquered resource. Taken together, the territorial behavior of *Gymnotus omarorum* during the nonbreeding season is the clearest example among teleosts of sexually monomorphic territory defense attained by agonistic encounters independent of gonadal steroid levels.

6.3.2 Steroid Modulation of Nongonadal Steroid-Dependent Behavior

The nonbreeding territoriality of *Gymnotus omarorum* offers the unusual opportunity of evaluating alternative nongonadal hormone-dependent mechanisms. To ensure that space is the only resource that animals fight for, nonbreeding adults (males or females) with the same previous experience and residence time are placed in equal-size compartments separated by a removable glass gate (Batista et al. 2012). When the gate is lifted, all fish engage in rapid agonistic encounters in which the dominant-subordinate status is set in less than 3 min. As in any other agonistic behavior, subordinate *Gymnotus omarorum* end the struggle when they decide to stop attacking and retreat. In addition to retreating, subordinates take advantage of the electric channel of communication to further signal their surrender. (1) They first interrupt their EOD to hide from the dominant. (2) They then emit transient high-rate electric signals termed chirps. (3) Finally, they adopt a lower postresolution EOD basal rate than dominants (Perrone and Silva 2018).

As expected from the nonbreeding spacing of *Gymnotus omarorum* in the wild, body-size asymmetry is the only predictor of contest outcome in nonbreeding agonistic contests (Batista et al. 2012). Furthermore, in this sexually monomorphic species, with no body-size differences between males and females, intra- and intersexual nonbreeding agonistic contests are indistinguishable and individual sex has no significant influence on the contest outcome (Quintana et al. 2016). Indeed, gonadal hormones do not play a role in the modulation of the nonbreeding agonistic behavior of *Gymnotus omarorum.* Plasma 11-KT levels of dominant males are similar to those of isolated males with no agonistic experience, and plasma estrogen levels in dominants females do not differ from those of isolated females (Quintana et al. 2016). The clearest evidence of the gonadal independence of the nonbreeding agonistic behavior of *Gymnotus omarorum* was provided by the fact that castration does not influence the agonistic encounters at all (Jalabert et al. 2015). Neither contest outcome, timing, aggression levels, nor submissive displays differ between dyadic encounters with castrated males and with sham-operated males (Jalabert et al. 2015).

Steroids are not only synthesized in peripheral endocrine glands (e.g., gonads and adrenals) but also within the nervous system. Steroid levels in the blood do not always reflect those of neurosteroids in specific brain regions, and gonadectomy obviously does not eliminate sex steroids from the brain. Therefore, in *Gymnotus omarorum,* the independence of nonbreeding territorial aggression from gonadal steroids does not rule out the possibility that it is under the control of sex steroids from other sources. In some species of rodents and songbirds in which aggression persists across seasons, it has been demonstrated that the gonadal steroidal regulation of the breeding aggression switches to a neurosteroidal regulation during the nonbreeding season (Heimovics et al. 2015). In particular, the neurosynthesis of estradiol by the conversion of testosterone mediated by aromatase enzyme activity has rapid effects on the nonbreeding aggression of song sparrows and *Peromyscus* mice (Heimovics et al. 2015). In an analogous way, the nonbreeding agonistic behavior of *Gymnotus omarorum* depends on normal aromatase activity (Jalabert et al. 2015). When the aromatase inhibitor fadrozole is administered to the potential dominant (larger fish of the dyad) before the encounter, the agonistic behavior is totally distorted and dominance can no longer be predicted by body-size asymmetry (Jalabert et al. 2015). Aromatase inhibition also produces a fast (within 30 min) inhibition of the aggression levels, suggesting that the underlying mechanisms involve rapid estrogenic nongenomic signaling mechanisms as has been reported in the nonbreeding aggressive behavior of mammals and birds (Heimovics et al. 2015).

6.3.3 Status-Dependent Vasotocin Modulation

As described in Sect. 6.3.2, the asymmetry in the behaviors of dominants and subordinates during the nonbreeding agonistic encounters of *Gymnotus omarorum* is outstanding. Dominants are highly aggressive, whereas subordinates signal submission in a precise sequence of locomotor and electric traits: retreating, decreasing their EOD rate, and emitting transient electric signals (Quintana et al. 2016). Interestingly, this asymmetrical behavior arises within a few minutes from an initial symmetrical state among contenders placed in equal-size compartments with the same previous experience. Therefore, the clear status-dependent asymmetry in the behavior of contenders observed after resolution necessarily relies on rapid and distinctive neuroendocrine mechanisms that control the emergence of either dominance or subordination. Given their well-known context-dependent actions (mentioned in Sects. 6.1 and 6.2.3.2), hypothalamic neuropeptides of the vasopressin-oxytocin family are good candidates to mediate the emergence and consolidation of dominant-subordinate status. Indeed, multiple lines of evidence link AVT with the control of dominance and aggression. In an oversimplified view, AVT is conceived as a universal modulator of aggression, with demonstrated actions on contest outcome, contest dynamics, and aggression levels across vertebrates (Insel and Young 2000; Johnson and Young 2017).

Following the same cytoarchitectural pattern observed in other teleost species, three populations of AVT neurons (parvo cells, magno cells, and giganto cells) occur exclusively in the preoptic area of the *Gymnotus omarorum* brain (Pouso et al. 2017). AVT projections widely spread across the brain, including brain areas related to the control of both social and electromotor behaviors such as prepacemaker areas and the medullary PN (Pouso et al. 2017). In several teleost species, when long-term dominance is maintained without reversion, changes in the number and/or size of AVT neurons between dominants and subordinates have been reported (reviewed in Silva and Pandolfi 2018). It has not been easy to identify commonalities among teleost examples of AVT neuron changes related to either dominance or subordination. However, Greenwood et al. (2008) postulated that two subsystems of AVT neurons alternatively activate and control distinct aspects of social behavior. The magno and giganto AVT populations modulate circuits that stimulate courtship and/or aggressive behaviors, whereas AVT parvo cells most likely act on circuits that induce behaviors related to social subordination. According to this model, the behavioral displays of dominants and subordinates depend on the relative activation of these two AVT subsystems in particular social contexts.

Less is known about distinctive actions of AVT in establishing dominant-subordinate status in teleosts. Pharmacological experiments offer indirect evidence for the status-dependent actions of AVT, but only a few studies in teleosts have explored these actions by comparing the same treatments on both dominants and subordinates. For example, in the bluehead wrasse, AVT inhibits aggression by territorial dominant males but enhances aggression of nonterritorial subordinate ones (Semsar et al. 2001). By pharmacological manipulations of the AVT system, the nonbreeding agonistic behavior of *Gymnotus omarorum* provides the clearest example of nonoverlapping status-dependent effects of AVT among teleosts (Fig. 6.9; Perrone and Silva 2018). When AVT is administered to potential subordinates (smaller fish of the dyad) before the encounter, it induces an increase in the electric signaling of submission that is partially reversed by its competitive antagonist Manning compound. AVT inhibits postresolution EOD rate, emphasizing electric submission (Fig. 6.9A), and it also enhances the emission of submissive social electric signals (offs and chirps) as well (Perrone and Silva 2018). In contrast, AVT acts as a promoter of aggression in dominants but has no effects on the aggression levels of subordinates (Fig. 6.9). It seems clear that AVT is normally secreted by dominants (but not by subordinates) during the agonistic contest because the administration of Manning compound induces a decrease in the aggression levels of dominants (but not of subordinates) compared with saline controls (Fig. 6.9).

Although status-dependent actions of AVT have been previously described in vertebrates, the pharmacological modulation of the AVT system in the agonistic behavior of *Gymnotus omarorum* contributes two novel aspects that reinforce the value and complexity of neuropeptidergic modulation. First, in contrast to previous reports in mammals and fish (Ferris 1992; Huffman et al. 2015), the AVTergic system does not regulate the contest outcome in *Gymnotus omarorum*. Rather, the AVTergic system likely adopts two distinctive configurations once the decision of winning or losing the contest is already made. Second, in contrast to other species in which opposite actions among contenders were reported on the same trait (e.g., aggression levels;

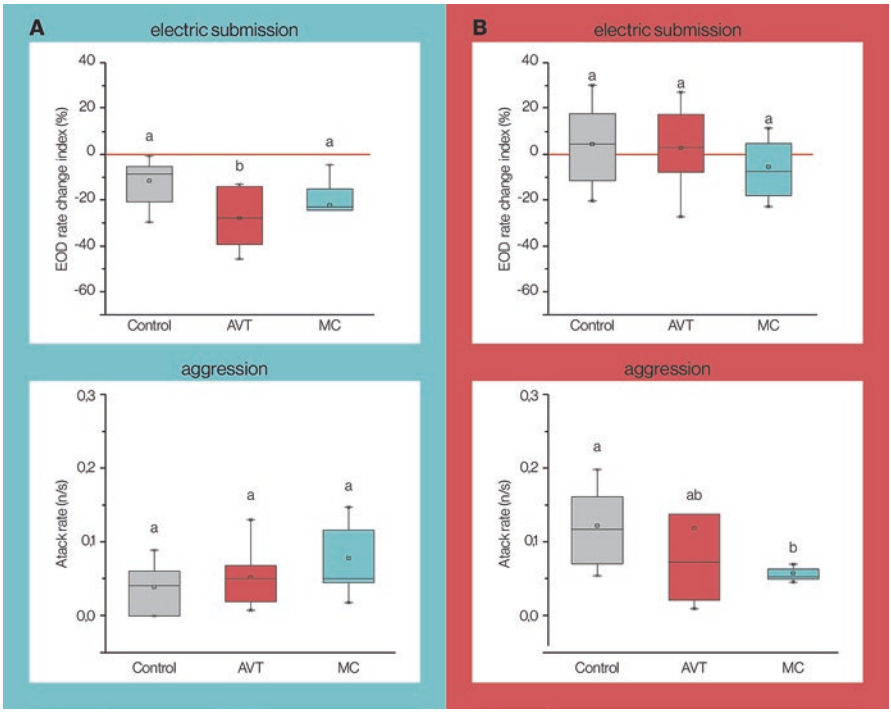

Fig. 6.9 Status-dependent AVT actions in the agonistic behavior of *Gymnotus omarorum*. **A:** subordinates. The EOD rate change index of subordinates after AVT treatment is more pronounced with respect to both saline subordinate controls and MC-treated subordinates. Neither AVT nor MC treatment affects aggression levels in subordinates. **B:** dominants. Neither AVT nor MC treatment affects the EOD rate change index in dominants. *Horizontal red lines*, zero value of the index meaning no change in EOD rate; *boxes*, means; *line in boxes*, medians; top and bottom lines, 25–75% interquartile range; error bars, minimum (*bottom*) and maximum (*top*) values. a and b, Significant difference: same letters mean nonsignificant difference; different letters mean significant difference. Aggression levels in dominants decrease after AVT blockade with MC with respect to saline dominant controls. Modified from Perrone and Silva (2018)

Semsar et al. 2001), in *Gymnotus omarorum*, AVT affects independent displays in dominants and subordinates. This reinforces the idea of a dual status-dependent configuration of the AVTergic system that alternatively promotes the activation of distinctive hormonal targets and behavioral effectors.

6.4 Summary

This chapter does not attempt to give an exhaustive revision of all background studies and previous reports on hormonal modulation in weakly electric fish, which have been recently reviewed elsewhere (Dunlap et al. 2017). Rather, the purpose of this chapter is to focus on selected exemplary studies by which electric fish have contributed to the understanding of the neuroendocrine modulation of social behavior in vertebrates.

From an evolutionary perspective, hormones shape sociality by bridging the gap between environmental demands and internal responses. Therefore, social behavioral performance is always the result of the dynamic relationship between the different levels in which hormones act in response to the pressures of a physically and socially changing environment. In other words, hormones act in a context-dependent manner, constantly adjusting their actions to changes in the environment. All the examples presented in Sects. 6.2 and 6.3 contribute to the understanding of the neuroendocrine mechanisms by which hormones can handle different contexts and exert distinctive responses either between closely related species or between males and females or dominants and subordinates of the same species.

Signaling is crucial for communication among conspecifics during social behavior. Weakly electric fish have the advantage of possessing a sophisticated communication system that depends on a rather simple and well-known neural pathway. Section 6.2 highlights the mechanisms by which gonadal steroid hormones control the emission of sexually dimorphic electric signals acting at both central and peripheral targets. More importantly, Sect. 6.2 describes how the interaction between gonadal steroids and hypothalamus-hypophyseal peptidergic hormones modulate seasonal, circadian, and social electric signal plasticity.

Section 6.3 reviews studies that face the challenge of evaluating the role of hormonal modulation in actual complex behaviors of naturally behaving fish. *Gymnotus omarorum* is the only species among teleosts known to defend territories independent of gonadal steroids and to present the clearest status-dependent example of AVT modulation of agonistic displays.

Overall, gymnotiform weakly electric fish emerge as superlative model systems for the understanding of the neuroendocrinological bases of social behavior. This chapter reviews evidence of their contribution in the comprehension of (1) the mechanisms of steroid hormone actions at the molecular level; (2) the mechanisms and evolution of sexually dimorphic behaviors; (3) the interplay of short- and long-term hormonal mechanisms underlying signal plasticity acting on both central and peripheral targets; and (4) the gonadal-independent mechanisms of the regulation of territorial aggression and of the emergence of dominant-subordinate status.

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Chapter 7
Evolutionary Drivers of Electric Signal Diversity

Rüdiger Krahe

Abstract The electric signals of weakly electric fishes have seen impressive diversification since the independent origin of electric organs in the ancestors of Gymnotiformes and Mormyroidea approximately 100 million years ago. Whether the primary selective advantage of electric organs lay in their use for communication or for active sampling of the environment is unclear and may be difficult to determine. Several evolutionary innovations in both signal generation and sensory processing appear to have widened the available signal space and thus promoted dramatic radiations. Sensory drive mechanisms are unlikely to have played a major role in the diversification of signals, except for a potential role of flow regimes. Life in faster flow appears to promote faster sensory sampling and thus higher electric organ discharge rates. It seems likely that signal diversification has been driven more strongly by biotic factors. Sexual selection on signal properties and reproductive character displacement appear to have had a strong influence on signal waveform and the associated spectral properties and also on discharge frequency. Diverse evidence suggests that predation by eavesdropping electroreceptive predators has favored the reduction of low-frequency power in the signals. The observation of male signals with strong low-frequency power in sexually dimorphic species is consistent with handicap signals in that they might increase the risk of predation and also the energetic cost of signal generation. Low-frequency male signals may also have been favored by sensory bias of the receiving animals because these signals might also activate the passive, ampullary electrosensory system.

Keywords Energetic constraints · Eavesdropping · Genetic drift · Gymnotiform · Mormyrid · Neural innovation · Predation · Reproductive character displacement · Sexual selection · Weakly electric fish

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7.1 Introduction

The ability to produce electric signals has evolved at least six times independently among fishes (Gallant et al. 2014; see Carlson and Sisneros, Chap. 1; Gallant, Chap. 4). Two of these six groups are the focus of this chapter. Both of them are teleosts and rich in species: the Central and South American Gymnotiformes (called knifefishes) and the African Mormyroidea (called elephant fishes). The defining feature of electric fishes is the ability to produce weak electric fields and sense perturbations of this self-generated field. Accordingly, these fishes are usually referred to as weakly electric fishes, although it should be noted that the strongly electric eel is a member of the Gymnotiformes and uses, in addition to weak electric fields, strong discharges for prey capture and defense (Catania 2016). In Mormyroidea and Gymnotiformes, the electric organ discharge (EOD) acts as a dual-use signal by supporting an active electric sense and by serving a communication function (see Stamper, Madhav, Cowan, and Fortune, Chap. 8; Jung and Engelmann, Chap. 12). Because of the dual function of the EOD, the diversity of evolutionary drivers that have shaped electric signal diversity may be quite complex. In contrast, the songs of birds and orthopteran insects, the calls of frogs, and the waving of the large claw of fiddler crabs are examples of pure communication signals and are expected to have been shaped largely by selective forces related to the communication function (Bradbury and Vehrencamp 2011).

How diverse are the electric signals of weakly electric fishes? One basic categorization of electric signals (and the fish producing them) is in pulse type and wave type (Fig. 7.1). Pulse-type fish generate discrete EOD pulses separated by pauses that are longer than the pulses and are usually of variable duration. Wave-type fish, on the other hand, produce highly periodic, quasi-sinusoidal signals. Both wave-type and pulse-type fishes are found in Africa as well as in South and Central America, although there is only a single species of wave-type fish in Africa, in the family Gymnarchidae. All other African species belong to the Mormyridae and produce pulse-type EODs. In contrast, there are similar numbers of wave- and pulse-type species among Gymnotiformes (Moller 1995). The diversity of wave-type electric signals resides, to a large degree, in species-specific and often sex-specific frequency ranges of the discharge rate of the electric organ. EOD frequencies can be as low as 25 Hz in *Sternopygus branco* and as high as 2,200 Hz in *Sternarchella schotti* (Crampton and Albert 2006). An additional component of diversity exists in the details of the EOD waveform, which can be largely sinusoidal in a female or immature *Eigenmannia* (glass knifefish), or it can contain several phases due to strong harmonic structure of the power spectrum, for example, in species of the genus *Sternarchorhynchus* (Fig. 7.1). In the context of aggressive encounters and courtship, wave-type species can vary their EOD frequency in a transient manner in species-typical ways (e.g., Turner et al. 2007; Smith et al. 2016), adding yet another dimension of diversity to the electric signals of wave-type fishes.

The diversity of pulse-type signals lies largely in the diversity of the EOD waveforms (Fig. 7.1), which differ in duration, number, amplitude, and polarity of phases as well as in spectral properties. The mechanistic underpinnings of waveform diversity

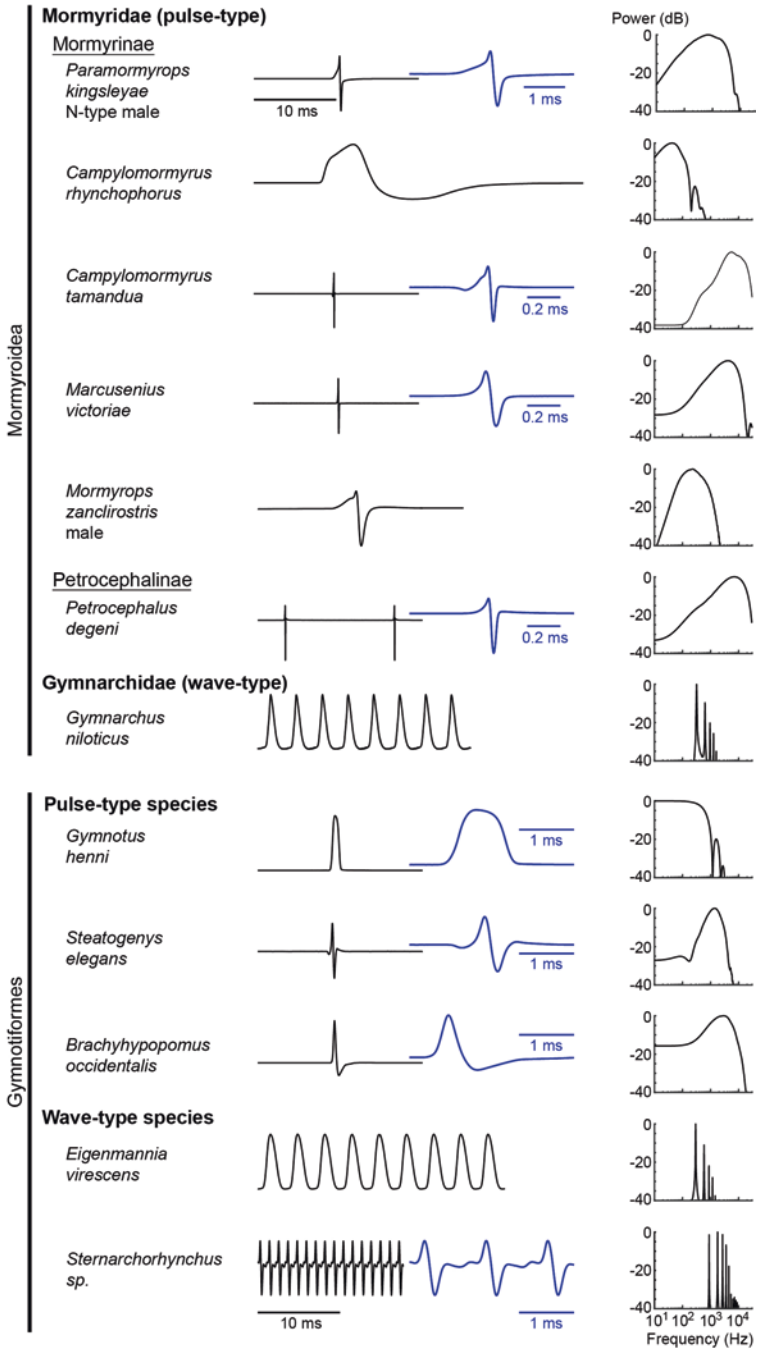

Fig. 7.1 Electric organ discharge (EOD) diversity of mormyroid and gymnotiform weakly electric fishes. *Left column, black lines:* EODs are all displayed on the same timescale. *Center column, blue lines:* for comparison of waveform details, brief EOD pulses are shown at the indicated expanded time scale. *Right column:* power spectra of the waveforms for the respective species. The EODs of the following species were kindly provided by J. R. Gallant (*Paramormyrops kingsleyae*, *Mormyrops zanclirostris*, and *Gymnarchus niloticus*); S. Mucha and F. Kirschbaum (*Campylomormyrus rhynchophorus* and *Campylomormyrus tamandua*); S. Mucha (*Marcusenius victoriae* and *Petrocephalus degeni*); K. L. Ackerly (*Steatogenys elegans*); and S. Picq (*Brachyhypopomus occidentalis*)

are discussed by Markham in Chap. 5. The range of discharge rates is much narrower than that of wave-type fish (maximal rates of around 150 Hz). Nevertheless, for a group of sympatric species of *Brachyhypopomus*, a kind of EOD rate range fractionation has been reported that could, in principal, support species recognition (Waddell et al. 2016). Similar to frequency modulations for communication in wave-type fish, pulse-type fish also show various modulations of their EOD rate in aggressive and courtship contexts (e.g., Lorenzo et al. 2006; Scheffel and Kramer 2006).

A further feature of the electric signals of weakly electric fish that adds diversity is the spatial geometry of the time-varying electric field generated by the EOD. Systematic variation in the EOD waveform along the body has been described for a number of gymnotiform species. Because this spatiotemporal variation usually disappears at distances of approximately one body length from the fish, it has been discussed mostly in connection with the electrolocation of objects that only acts at a short range (e.g., Rodríguez-Cattáneo et al. 2013). At larger distances, the EOD field resembles that of a dipole, which is usually considered to be the relevant signal for communication purposes. Nevertheless, it is conceivable that body region-specific EOD waveforms could play an underappreciated role in communication interactions at close distances (Waddell et al. 2016). In the discussion of the evolutionary drivers of electric signal diversity, the present chapter focuses mostly on the EOD waveform as measured at some distance from the fish and on the frequency of the discharge because these are the EOD characteristics for which most information is available. EOD frequency modulations and the spatiotemporal structure of the EOD field will, however, be touched on briefly in the appropriate sections.

In trying to understand the mechanisms leading to the diversification of signals, the interest is usually in determining which biotic and abiotic selective forces have had a directional, stabilizing, or disruptive effect on signal properties. Before addressing a set of potential drivers of diversification, this chapter starts with a discussion of random processes in signal divergence among populations and species.

7.2 Evolutionary Drivers of Electric Signal Diversity

7.2.1 Genetic Drift Versus Selection

The term genetic drift refers to changes in the allele frequencies in a population that are caused by random differences in survival and reproduction among individuals. One might think that genetic drift should be a “nondriver” of signal divergence due to its randomness. Nevertheless, genetic drift has been shown to have potent effects on signal divergence in systems such as the calls of Amazonian *Allobates* frogs and of greenish warblers (Irwin et al. 2008; Amézquita et al. 2009). Therefore, when considering the evolution of signal properties in any system, the obvious null hypothesis should be that differences in signal characteristics between populations and species can be explained by the random effects of genetic drift. If genetic drift

is responsible for differences in signals between populations or species, strong correlations are expected between signal distance (some quantification of how different the signals are) and some measure of neutral genetic distance (Wilkins et al. 2013). Strong evidence for genetic drift playing a role in shaping signal diversity among allopatric populations comes from gymnotiform pulse fish, *Brachyhypopomus occidentalis*. The potential for gene flow between allopatric populations of this Panamanian species is small because these populations live in separate drainages flowing independently into the Caribbean or the Pacific Ocean (Bermingham and Martin 1998; Picq et al. 2014). The significant positive correlation between signal distances (based on waveform cross-correlation) and genetic distances (based on differences between DNA sequences generally accepted as evolving neutrally) strongly suggests a sizable role of drift in the evolutionary divergence of EODs in this gymnotiform species (Fig. 7.2; Picq et al. 2016). The mormyrid fish, *Paramormyrops kingsleyae*, shows clinal variation in several EOD waveform parameters across populations in Gabon (Gallant et al. 2011). Signal distances have, however, not been correlated with neutral genetic distances between populations yet, leaving open whether genetic drift has played an important role in signal divergence in this species.

The example of *Brachyhypopomus occidentalis* provides good evidence that genetic drift needs to be taken into account when discussing the evolution of electric signals (or any other signals). It is conceivable that drift has played a larger role in Gymnotiformes than in Mormyroidea because most sympatric assemblies in Central and South America appear to be polyphyletic in origin, suggesting speciation in allopatry (with little opportunity for gene flow) and subsequent assembly of local communities (Albert and Crampton 2005). Among mormyrids, on the other hand, there are several examples of species flocks that have apparently undergone radiation in sympatry (Arnegard et al. 2005; Lamanna et al. 2016), suggesting a larger role for speciation in the presence of gene flow, which tends to counteract genetic drift. Nevertheless, given that drift explains approximately 27% of the variance in the example of *Brachyhypopomus occidentalis*, other factors have likely contributed to signal divergence as well. The remainder of this chapter discusses the potential contributions of habitat and biotic factors to EOD evolution.

7.2.2 Habitat Factors

There is a long history of research into habitat properties influencing the evolution of communication signals, in particular, acoustic and visual communication (Bradbury and Vehrencamp 2011). Constraints imposed by the transmission channel differ between modalities. Electric signals attenuate much faster with distance than sounds, but they are not degraded by absorption, echoes, and scattering as are acoustic signals (Brenowitz 1986). In contrast to sound signals, the electrostatic fields of weakly electric fish do not experience frequency-dependent attenuation, which means that their temporal structure is not affected by distance from the

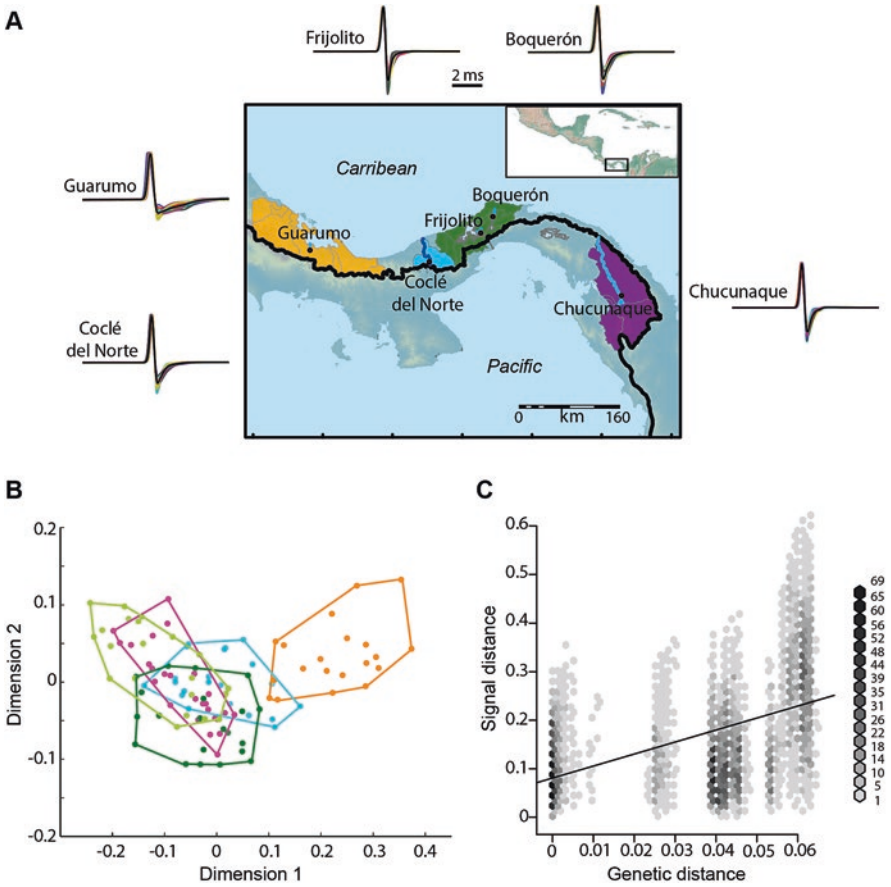

Fig. 7.2 EOD waveform variation among populations of *Brachyhypopomus occidentalis* from Panama is consistent with a major effect of genetic drift. **A:** map indicating the five recording and sampling sites for *Brachyhypopomus occidentalis* in Panama. All populations were from mutually isolated drainages except Frijolito and Boquerón. Around map are EOD waveforms from each population (mean waveform in *black*; $n \geq 20$ individuals in each case). **B:** minimum polygons enclosing EOD waveforms from each recording locality in a two-dimensional representation of signal space (obtained through multidimensional scaling of signal cross-correlations). Each point represents the EOD of a recorded male ($n = 109$). **C:** pairwise signal distances (Euclidian distances between points from **B**) are linearly correlated with genetic distance, even after controlling for geographic distance ($r = 0.517$; $P = 0.001$). *Numbers at right:* binning of counts of data points into hexagonal cells. Modified from Picq et al. (2016)

emitter (Hopkins 1986). However, electric fields are compressed by nonconducting boundaries, such as rocks or the water surface, and they are affected by the electrical conductivity of the water (Knudsen 1975; Fotowat et al. 2013). Sections 7.2.2.1 through 7.2.2.8 address various habitat properties and whether and how they might be evolutionary drivers of electric signal diversity. Because more is known about the

habitat features of gymnotiforms than of mormyroids, these sections may appear biased toward knifefishes.

7.2.2.1 Flow Regime

Weakly electric fishes occur in flow regimes from stagnant swamps and floating meadows to deep river channels and rapids (Moller 1995; Crampton and Albert 2006). Living in a lentic habitat (e.g., a swamp) entails slower relative velocities of objects such as prey in the environment than when living in a lotic habitat (e.g., rapids). Therefore, one would expect rates of sensory sampling of the environment to be higher in faster flowing habitats, whereas low rates of sensory sampling may be entirely sufficient in low-flow environments. This idea was originally proposed by Hopkins and Heiligenberg (1978) as part of a scheme for the evolution of EOD waveforms and frequencies from an ancestral low-frequency pulse-type signal to more regular pulsatile signals with higher rates and to high-frequency wave-type EODs. To some degree, this idea appears to be borne out by the EOD rates of gymnotiform fishes (Lissmann 1961; Crampton 1998b). In deep river channels of the Amazon, high-frequency wave-type species of the family Apteronotidae (ghost knifefishes) are found in medium- to fast-flowing regions, whereas lower frequency wave-type fishes are seen in parts with slack current (Crampton and Albert 2006). Examples of the latter are the Sternopygidae and the tamandua knifefish *Orthosternarchus tamandua*, the lowest frequency species among the ghost knifefishes, with an EOD frequency in the range of 420–470 Hz. Few pulse-type species occur in faster flowing habitats. The ones that do (*Steatogenys*, *Rhamphichthys* and, *Gymnorhamphichthys*) are characterized by relatively high EOD rates (50–100 Hz) and more regular discharges than species in streams and floodplains (Crampton and Albert 2006; Crampton 2011). A positive relationship between flow velocity and sensory sampling rate has recently also found support in a study of the genus *Brachyhypopomus*, with species with low pulse rates occurring in nonflowing floodplain systems and species with higher rates being more prevalent in flowing streams (Waddell et al. 2016). The large and overlapping EOD frequency ranges seen in different flow regimes suggest, however, that factors other than just temporal acuity of sensory sampling must be playing a role.

Whether the fast-flowing water of Amazonian deep river channels can be seen as the drivers behind the extremely high EOD frequencies seen in some apteronotid species (up to 2200 Hz in *Sternarchella schotti*) is unclear. The frequency range of EOD amplitude modulations caused by prey is limited to below 25 Hz in fish swimming up to 10 cm/s (Nelson and MacIver 1999). At this swim speed, an EOD frequency of ca. 1,000 Hz, as in *Apteronotus albifrons*, appears like oversampling. The bandwidth of amplitude modulations caused by prey should increase approximately in proportion to the relative velocity of the EOD emitter and the prey object. What the relevant velocities are in different habitats (taking into account sustained swimming and burst swimming) has not been explored. Laboratory experiments to determine critical swim speed and burst swim speed for different species should be instructive.

Unfortunately, no systematic information is available on links between flow regime and discharge rate in the African weakly electric fishes.

7.2.2.2 Habitat Complexity

Mormyrids as well as gymnotiform wave- and pulse-type species can discriminate objects that differ in resistance and capacitance in ranges corresponding to the resistive and capacitive properties of plant and animal materials (von der Emde 1999). In principle, EODs with a broad power spectrum might be better suited for discriminating capacitive object properties in structurally complex habitats. The brief EOD pulses with broad power spectra produced by certain pulse-type species should offer advantages over EOD pulses with narrower spectral properties or the spectrally quite narrow EODs of wave-type fish. The best support for this notion comes from the finding that open-water habitats, which are structurally simple, are dominated by wave-type gymnotiforms, whereas structurally more cluttered habitats are dominated by pulse-type species (Crampton 2006). However, there is little other support for this idea because most habitats are shared by species with different spectral EOD properties. In addition, many pulse-type species show strong sexual dimorphism in the EOD waveform and thus spectral properties, although both sexes share the same habitat (Hopkins 1999a). A comparative study of 11 species of the genus *Gymnotus* also failed to find any correlation between the EOD waveform and microhabitat properties (Crampton et al. 2013). Because there is only one wave-type species (*Gymnarchus niloticus*) in Africa and the pulse-type mormyrids are found in all habitats from highly complex swamps to the open waters of rivers and lakes (Moller 1995), no correlation between EOD type and habitat structure can be established for African weakly electric fishes.

In summary, the EOD waveform has so far not been found to be related to any structural habitat properties (e.g., Crampton et al. 2013). However, it is conceivable that, to some degree, this lack of evidence is due to the geometry of EOD measurements. So far, comparisons of EOD properties and habitat complexity have been based on EOD recordings with one electrode in front of the head and one behind the tip of the tail, the so called head-tail configuration. More relevant for electrolocation performance than the head-tail EOD may be, at least in pulse-type gymnotiforms, the local EOD as measured, in particular, in the rostral region of the fish. The rostral region shows the highest density of electroreceptor organs on the body and may act as an electrosensory fovea (Castelló et al. 2000). Local EOD waveforms in this area can deviate considerably from the waveform recorded in the head-tail configuration and often contain much more power at low frequencies than the latter (Fig. 7.3; Rodríguez-Cattáneo et al. 2013; Waddell et al. 2016). The rostral local EODs may even have sufficient low-frequency power to activate ampullary electroreceptors, thus recruiting the "passive electrosense" to active, EOD-based sensing. Whether properties of the rostral EOD are correlated with habitat properties, such as the capacitive properties of dominant prey species, is, however, not known.

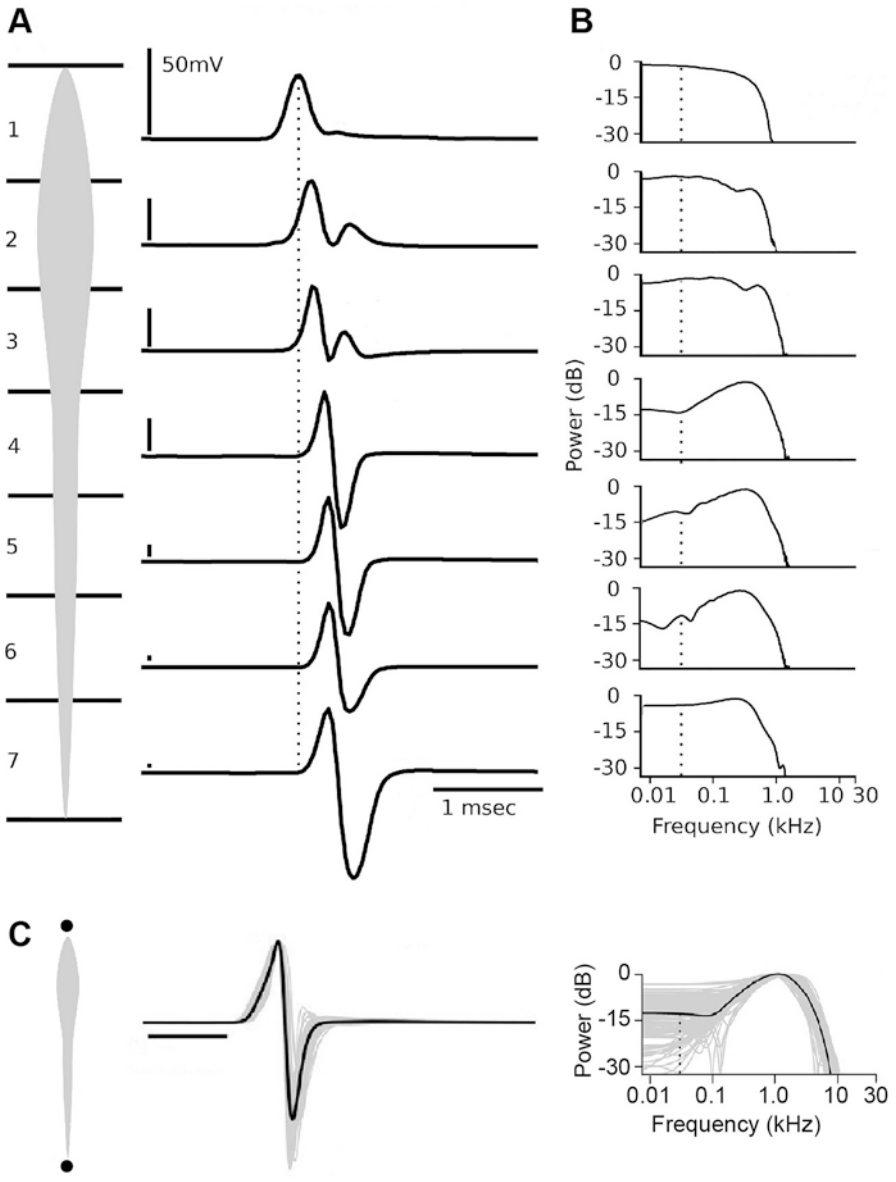

Fig. 7.3 In the rostral part of the body of *Brachyhypopomus beebei*, the locally measured EOD contains strong power at low frequencies, which is attenuated with distance and also when measured between head and tail. **A:** electromotive force (emf) EOD patterns (*right*) recorded with the multiple air-gap procedure from a single immature animal (148 mm total length) at various positions along the fish (*left*). **B:** power spectral density plots for the corresponding emf-EOD waveforms. *Dotted lines*, power at 30 Hz, which is well in the sensitivity range of ampullary electroreceptors. **C:** EOD waveforms (*center*) and associated power spectral densities (*right*) of 125 specimens of *Brachyhypopomus beebei* recorded between electrodes at the head and at the tail (*left, black dots*). Modified from Waddell et al. (2016)

7.2.2.3 Electrical Conductivity and Impedance Matching

An environmental parameter of obvious relevance for electric fields is the electrical conductivity of the water. It is determined by the concentration of dissolved ions and is generally low in most electric fish habitats (Moller 1995; Crampton 2006). Because conductivity affects the amplitude of the EOD and the sensitivity of the tuberous electroreceptors, which are tuned to the EOD (Knudsen 1974, 1975), it is not surprising that a relationship can be seen between water conductivity and the organization of the electric organ. *Brachyhypopomus* species from habitats with extremely low-conductivity water (10 μS/cm) were found to have long and thin electric organs (i.e., long and thin tails) with only three columns of electrocytes, whereas species from higher conductivity habitats have shorter electric organs with four columns of electrocytes and species from still higher conductivity areas have the shortest and thickest electric organs with five columns (Hopkins 1999a). Thus, to maintain prey detection distances and communication distances, these fish appear to match the impedance of the water by increasing the voltage output in low-conductivity settings and the current output in high-conductivity habitats. Apart from the described effects of electrical conductivity changes on EOD output and a correlation between conductivity and electric organ structure, there is no evidence for conductivity to act as an evolutionary driver of electric signal diversity.

7.2.2.4 Temperature

Physiological processes in general are strongly temperature dependent and so is the generation of EODs as well as their sensory processing (Enger and Szabo 1968; Hopkins 1976). It is not obvious, however, in what way temperature might be shaping the evolution of electric signals in particular directions. Interestingly, though, the vast majority of wave-type gymnotiform fishes are found in relatively temperature-stable habitats, such as river channels. In other habitats, they appear to prefer the most temperature-stable microhabitats (Crampton 2006). Pulse-type species, on the other hand, are often found in quite temperature-variable habitats, such as floating meadows, which can show a daily variation of 5–10 °C (Crampton 2006), and temperate lakes with daily fluctuations as large as 18 °C at sunrise to 33 °C late in the day (Silva et al. 1999). Based on this differential distribution of pulse- and wave-type gymnotiforms (which knows many exceptions), the thermal-trap hypothesis proposes that wave-type fish are constrained to relatively temperature-stable environments because their EOD frequency and the spectral tuning of their tuberous electroreceptors might diverge with the changing temperature, which would lead to a loss in sensitivity of their electrosensory system (Stoddard 2002). The proposed divergence between EOD frequency and receptor tuning is based on different temperature coefficients (Q_{10}) reported for EOD frequency and the best frequency of tuberous afferent fibers. Given that the Q_{10} determined for receptor afferents of *Eigenmannia* was based on a limited sample size ($n = 2$) and an admittedly crude measurement of local temperature near the receptor pore (Hopkins 1976), more

research, in particular on the temperature dependence of the electrosensory system, is required before it can be concluded that a thermal-trap mechanism is constraining wave-type fish to temperature-stable habitats.

7.2.2.5 pH

Weakly electric fishes in Africa and South and Central America are found in waters with a wide range of pH values, from 3.5 to well over 7 (Moller 1995; Crampton 1998b). No systematic relationships have been described between pH level and electric signal properties.

7.2.2.6 Dissolved Oxygen Concentration

The concentration of dissolved oxygen (DO) in the water has a clear effect on the distribution of fishes, including weakly electric fishes (Crampton 1998a; Diaz and Breitburg 2009). It is less clear whether DO concentration can be viewed as an evolutionary driver of electric signal properties. Crampton (1998a) observed that, for most of their lives, almost all wave-type gymnotiforms are restricted to highly oxygenated water bodies. He originally hypothesized that the high-frequency firing of the electric organ incurs a high-energetic cost that cannot be satisfied in regions of low DO. Signal costs have since been excluded as the decisive difference between pulse- and wave-type species (see Sect. 7.2.3.5). Rather, wave-type fish perform scan swimming, a back-and-forth swimming motion that likely serves the electrical scrutinizing of nearby objects and that may be energetically too costly in oxygen-deprived habitats (Lannoo and Lannoo 1993; Julian et al. 2003). In addition, wave-type gymnotiforms are unable to breathe air, which limits their oxygen acquisition strategies in hypoxic water compared with most pulse-type species (Crampton 1998a). Thus, the fact that pulse-type signals are usually associated with slow-flowing or stagnant water bodies in South America may not be due to a particular adaptation of these signals to low DO conditions but rather reflect the inability of wave-type fish to gulp air. As discussed in Sect. 7.2.3.4, pulse-type fish may have more options than wave-type fish to save metabolic energy when challenged with hypoxia by reducing both the amplitude and rate of discharge of their electric organ. EOD rate reduction in response to exposure to hypoxia has also been observed in mormyrids to happen on the timescale of minutes to hours (Sukhum et al. 2016; Ackerly et al. 2018). It is not known, however, whether the average EOD rates of pulse-type fishes living in hypoxic habitats are reduced compared with species living in better oxygenated water bodies. Thus, although the generation of EODs and the associated processing likely carries a sizable metabolic cost and should therefore be under considerable selection pressure to be energetically efficient (see Sect. 7.2.3.4), there is so far no obvious association between DO concentration and EOD properties.

7.2.2.7 Lightning

Thunderstorms and lightning are particularly prevalent in the tropical and subtropical areas in which weakly electric fish are found, with mean flash rates of up to 70 flashes per square kilometer per year in parts of the Democratic Republic of Congo (Cecil et al. 2015). With multiple thunderstorms happening simultaneously in the tropics at any given time and electromagnetic waves propagating over long distances, Hopkins (1973) suggested that lightning is a major source of electrical noise for weakly electric fish. It is conceivable that lightning can interfere with electrolocation and electrocommunication due to the strong overlap of the power spectra of pulse-type fish EODs and those of lightning. Intriguingly, fish may even use this overlap and produce irregular, "lightning-like" discharge patterns to be less conspicuous to electroreceptive prey or predators (Hopkins 1973). Thus, matching of the power spectrum to that of lightning and producing EOD pulses at highly variable rates, mimicking the temporal patterns of lightning strikes, may have been favored by natural selection. The highly regularized interpulse intervals observed during communication interactions fit this idea because they should maximally stand out from the irregular background pattern. For lightning to be a factor in signal divergence, one would expect to see regional differences in lightning density as measured in the water and correlated differences in interpulse interval patterns and in how regularized communication patterns of different species are. No such correlations have been reported.

7.2.2.8 Conclusions on Habitat Properties as Evolutionary Drivers of Electric Signal Diversity

In visual and acoustic communication systems, there is now strong evidence for signal characteristics and sensory systems to be shaped by transmission properties of the habitat, a process termed sensory drive (Endler 1992; Wilkins et al. 2013). Signals that stand out more in a given habitat and lead to stronger activation of the sensory system of the receiver will be favored. When habitats differ in some property that affects signal transmission, it can lead to signal divergence and even support reproductive isolation as shown for stickleback fish from British Columbia and African cichlids from Lake Victoria (Boughman 2001; Seehausen et al. 2008). So far, the evidence for a sensory drive mechanism that supported divergence of the electric signals is weak. The clearest relationship between habitat and EOD properties may be between flow regime and EOD frequency, as originally envisaged by Lissmann (1961). A faster sensory world (i.e., flow velocity) may require more frequent and more regular sensory sampling. The correlation between flow velocity and EOD frequency is, however, not strong, with higher frequency wave-type fish spending at least part of their lives in low-flow environments and some pulse-type fish living in relatively high-flow habitats.

7.2.3 Biotic Factors

Section 7.2.3 summarizes the potential biotic effects on electric signal evolution. These can result, for example, from interactions with other species in the form of eavesdropping by predators on communication signals or reproductive interference. The former has long been recognized as a factor in signal evolution, such as in the red coloration of guppies (Endler 1980). Examples of reproductive interference are reduced hybrid fertility or masking of communication signals by those of heterospecifics. A likely consequence is the divergence in characters related to the interference, called reproductive character displacement (Pfennig and Pfennig 2009). These characters can be, but do not have to be, signal properties. A major driver of signal divergence in many groups is thought to be sexual selection, which can promote speciation in allopatry by shifting signal properties in different directions in different populations. It is, however, also thought to play an important role in sympatric speciation, often likely through interplay with ecological selection (Maan and Seehausen 2011). Competition for access to mating partners and mate preferences can both impose considerable costs on senders, either by exposing senders to risk of predation or by forcing them to spend a disproportionate amount of metabolic resources on signal generation. Therefore, Sect. 7.2.3.4 is devoted to the energetic costs of electric signal production. Last, but not least, innovative traits can open up new opportunities, be it previously inaccessible ecological niches or new signal space.

7.2.3.1 Jamming by the Signals of Others

A signal that is used for active sensing of the environment is potentially sensitive to interference from the signals of conspecifics or heterospecifics (Heiligenberg 1991; Corcoran and Moss 2017). Weakly electric fish are exposed to such jamming and have evolved different strategies to reduce its effects on their electrolocation performance (Heiligenberg 1991; Kawasaki 2009). Many wave-type species shift their EOD frequency away from that of a nearby neighbor if the neighbor's frequency is close to theirs. For pulse-type fish, jamming has been shown to be most disruptive if own and foreign pulses coincide (Heiligenberg 1976; Heiligenberg et al. 1978). To reduce such coincidences, they transiently change their rate of discharge. Apart from these transient behavioral responses, there is evidence that jamming played a role in the evolution of EOD duration in pulse-type fishes. As the probability of coincidence of own and foreign EOD is proportional to the product of EOD rate and duration, one would expect a negative correlation between the average EOD rate and the duration of the EOD pulses. Such a negative correlation has indeed been observed among gymnotiform pulse fish in Surinam (Hopkins and Heiligenberg 1978). Similarly, mormyrid species leading a more gregarious life and, consequently, being exposed to more intense jamming, tend to have shorter EOD pulses than species with larger interindividual distances (Hopkins 1980).

7.2.3.2 Reproductive Isolation

The discovery of several species flocks among African mormyrids, including likely cases of incipient speciation, suggests that sympatric speciation may have been a common mechanism of diversification in this group of fishes (Arnegard et al. 2005; Lamanna et al. 2016). In contrast, gymnotiform species assemblages appear to be polyphyletic, with speciation having happened in allopatry (Crampton 2011). In both cases, there is strong evidence for EOD diversification playing an important role in prezygotic reproductive isolation, as originally proposed by Hopkins and Heiligenberg (1978).

EOD signal space tends to be well partitioned in sympatric multispecies assemblages in gymnotiform (e.g., Hopkins and Heiligenberg 1978; Kramer et al. 1981) as well as in mormyrid fishes (Arnegard et al. 2005; Lamanna et al. 2016). Among mormyrids, two recently described rapid radiations show interesting differences with respect to the role of ecological selection. One of them is a sympatric species group of the genus *Campylomormyrus* that is found in the rapids of the lower Congo River and shows remarkable diversity in EOD waveform and similarly remarkable diversity in snout morphology (Fig. 7.4; Feulner et al. 2007; Lamanna et al. 2016). The prominent tube snouts of the *Campylomormyrus* species aid in the grasp-suction feeding of insect larvae from highly structured river bottoms (Marrero and Winemiller 1993). The strong divergence in feeding morphology in this species group suggests rapid ecological specialization, although confirmation of differences in feeding behavior, for example, through stomach content analysis, is still missing. Genetically closely related species of *Campylomormyrus* show large differences in EOD waveform, whereas the EOD of one of them, *Campylomormyrus compressirostris*, is much more similar to that of another sympatric but more distantly related species, *Campylomormyrus tamandua* (Feulner et al. 2007; Lamanna et al. 2016). This pattern is consistent with an allopatric origin of *Campylomormyrus tamandua* and rapid sexual selection acting on EOD properties in the closely related group of species. At this point, it remains unresolved whether ecological adaptation of morphological features or sexual selection on EOD properties triggered the radiation of *Campylomormyrus*, but it seems clear that they are now acting in the same direction.

The second rapid radiation of mormyrids is that of the genus *Paramormyrops* in the Ivindo River of Gabon (Fig. 7.5; Sullivan et al. 2002; Arnegard et al. 2010). The morphological differentiation in this radiation is much less pronounced than in *Campylomormyrus*, in particular, when compared with their strong divergence in EOD waveforms. An analysis of trait divergence rates yielded a much faster divergence in EOD signal space than in ecological traits, such as body shape or size, or in a measure of trophic ecology (Fig. 7.6; Arnegard et al. 2010). Indeed, very little divergence was seen in body size or trophic ecology in the entire sample of species, including *Paramormyrops*, but also in other, more distantly related mormyrids from the same site. Body shape showed a gradual divergence with increasing genetic distance but has clearly been outraced by signal divergence for even the most closely related species (Fig. 7.6, *black lines*).

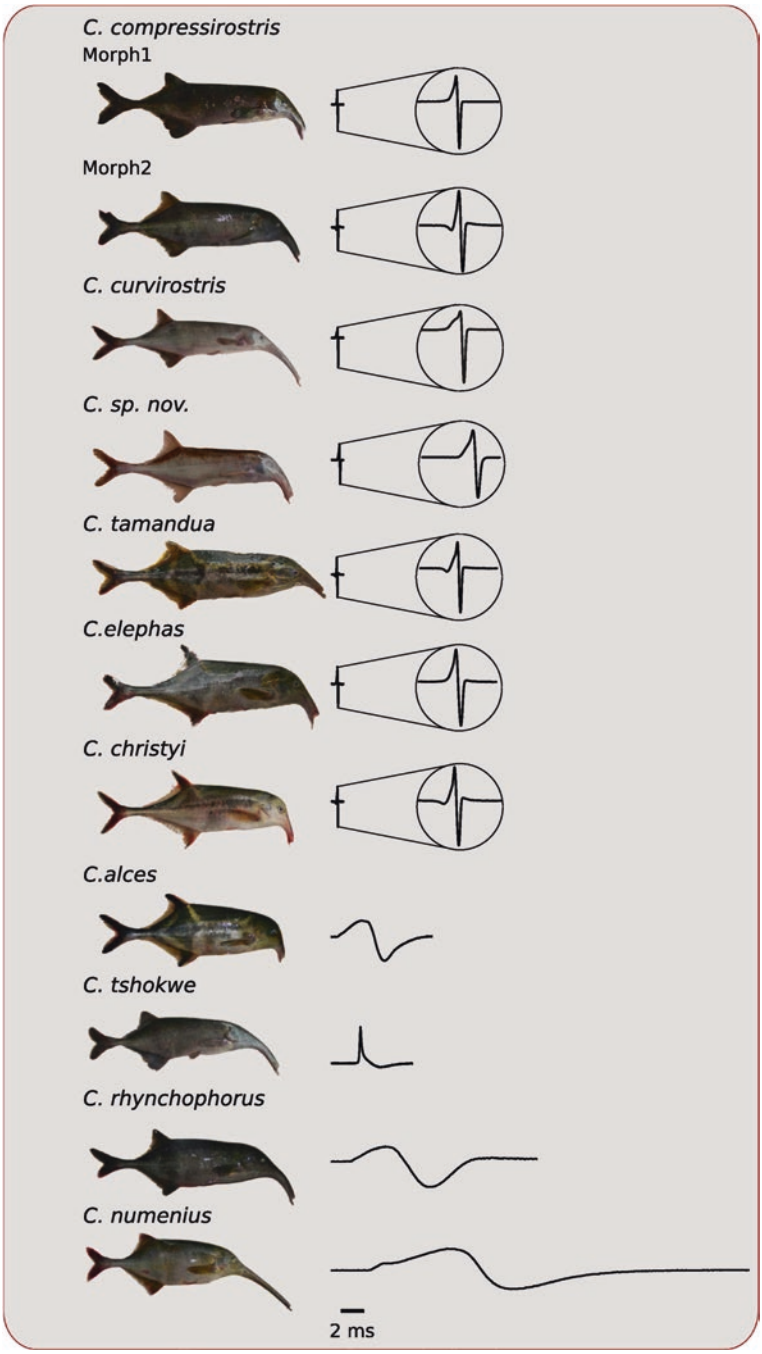

Fig. 7.4 Sympatric species of the mormyrid genus *Campylomormyrus* captured in the Congo rapids near Kinshasa/Brazzaville differ in morphology, in particular, snout morphology (*left*), and EOD waveform (*right*). All EOD waveforms are shown on the same timescale for comparison. The seven topmost signals are also shown with 10× time expansion (*circles*). From Lamanna et al. (2016)

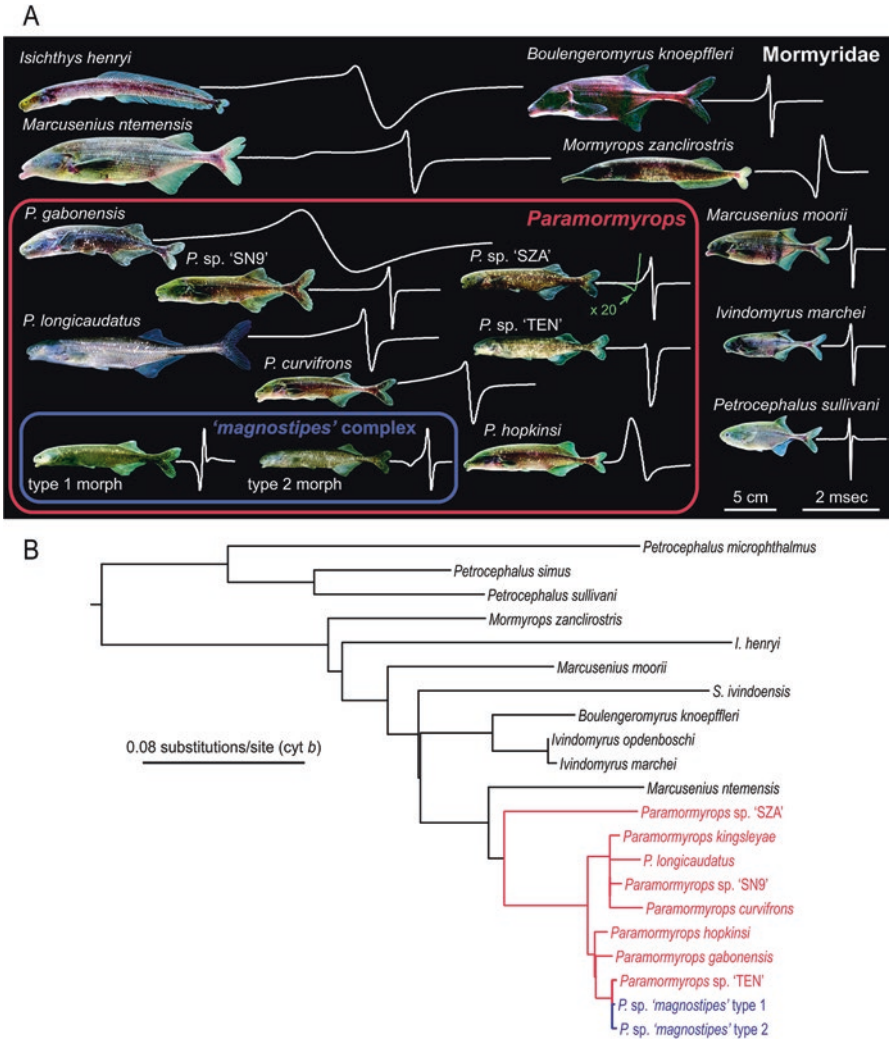

Fig. 7.5 Sympatric mormyrid species of the Ivindo River of Gabon and their phylogenetic relationships. **A:** morphology and EOD waveforms of representative taxa from Loa Loa Rapids at three nested levels of phylogenetic relatedness (*black, red,* and *blue lines*). EODs are plotted with head-positive voltage up. **B:** phylogenetic tree of Ivindo River mormyrids. The maximum likelihood branch lengths were calculated from cytochrome *b* sequences. *Gymnarchus niloticus* was used as the outgroup. From Arnegard et al. (2010)

Interestingly, both radiations included distinct EOD morphs that showed neither genetic nor morphological differentiation. It is conceivable that these EOD differences are signatures of incipient speciation (Arnegard et al. 2005; Lamanna et al. 2016). Mate-recognition experiments with the *Campylomormyrus* species support that EOD morphs may play a role in assortative mating (Feulner et al. 2009a). Thus,

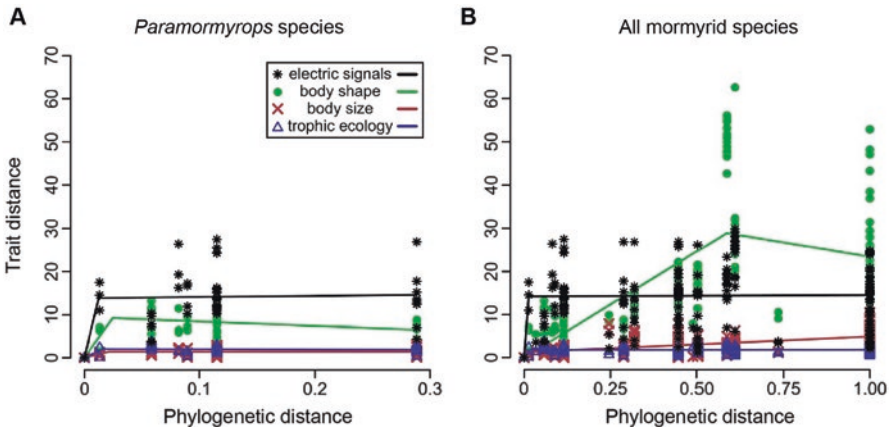

Fig. 7.6 EOD waveforms (*black lines*) of Ivindo River mormyrids have diverged much faster than other traits related to morphology and trophic ecology. Rates of trait divergence for electric signals, body shape, body size, and a measure of trophic ecology were calculated for the Loa Loa Rapids community of the Ivindo River of Gabon. **A:** trait distance versus phylogenetic distance for the *Paramormyrops* species flock. **B:** trait distance versus phylogenetic distance for all mormyrids at this site including *Paramormyrops*. Color-coded lines in each plot are based on a breakpoint regression. In each case, the slope of the initial pre-breakpoint segment describes the initial divergence rate during species radiation. From Arnegard et al. (2010)

incipient speciation in this group might indeed be triggered by sexual selection of EOD properties and not by ecological specialization.

Sympatric speciation has so far been deemed unlikely for gymnotiform species assemblages. This does not exclude, however, the potential for reproductive interference because of the similarity of EOD properties among distantly related sympatric species, a situation that could lead to reproductive character displacement (RCD). RCD is the process of divergence between sympatric species in mating traits as a consequence of selection to reduce reproductive interactions (Pfennig and Pfennig 2009). It is commonly considered to be an important factor in the diversification of communication signals (Hoskin and Higgie 2010). Demonstrations of RCD usually rely on a comparison of signal properties of two species or populations in an allopatric setting with their signal properties in sympatry. Larger differences in sympatry than allopatry point to RCD. In an elegant twist of this approach, Crampton et al. (2011) compared the developmental trajectories of EOD properties in the genus *Gymnotus*, positing that RCD should be at full display in mature animals ready to reproduce, but less so in juveniles. Weakly electric fish are particularly amenable to such a comparison because these fish, in contrast to the vocalizations of birds or insects, produce EODs throughout their lives. Indeed, both mature males and mature females of different, syntopic species showed little overlap of their EODs in multivariate signal space compared with postlarval animals, large juveniles, and immature adults (Fig. 7.7). Direct overlap was only observed in species from different habitats, consistent with the prediction for RCD. Thus, RCD appears to have contributed considerably to electric signal divergence, both in settings of sympatric speciation and on secondary contact.

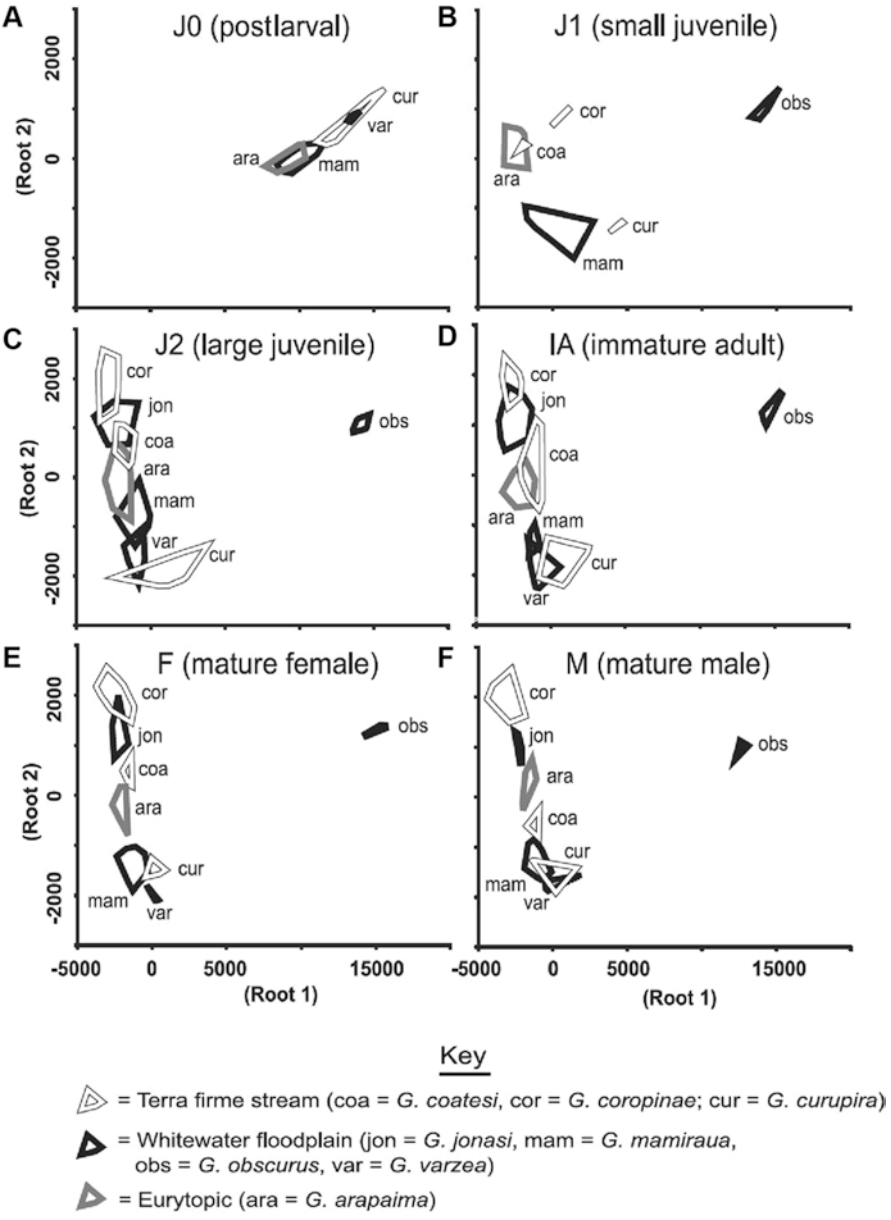

Fig. 7.7 The EOD waveforms of syntopic species of the gymnotiform genus *Gymnotus* diverge during maturation and achieve less interspecific overlap in adults than in immature animals. **A-F:** ordination of the EODs of eight sympatric species of *Gymnotus* in multivariate signal space by a linear discriminant analysis. Polygons represent the boundaries in signal space for each species at six ontogenetic stages. As the animals mature, the EODs of species from the same habitat become more and more dissimilar. The three habitats occupied by the eight species are indicated by the pattern of the boundary frame. From Crampton et al. (2011)

7.2.3.3 Predation by Electroreceptive Predators

In many communication systems, effective signaling puts the sender at risk of eavesdropping by predators able to sense the signals (e.g., Endler 1980; Falk et al. 2015). Gymnotiform weakly electric fishes are known to suffer predation from electric eels, other large gymnotiforms, and piscivorous catfishes (e.g., Westby 1988; Merron 1993). With their ampullary electroreceptor organs, catfish (Siluriformes) are able to sense the low-frequency electric fields emanating from muscle activity of hidden prey organisms (Kalmijn 1974). Catfish should therefore also be able to sense EODs if there is sufficient power at low frequencies. This is the case for monophasic EOD waveforms that produce a DC offset and for bi- or multiphasic EODs if they are of long duration and asymmetrical (Bennett 1971; Hopkins 1988). EODs with a sizable DC component are produced, for example, by several pulse-type gymnotiforms (Fig. 7.1). Such signals should activate not only the ampullary receptors of catfish and electric fish but also the tuberous receptors of electric fish species that themselves produce EODs dominated by low-frequency power, such as the electric eel.

In 1999, Stoddard proposed that predation by electroreceptive predators has had a shaping influence on the complexity of EOD signals of gymnotiforms (Stoddard 1999, 2002). Assuming that the basal EOD waveform was a monophasic, head-positive pulse that is dominated by low-frequency power (as shown by *Electrophorus electricus* and some members of the closely related family Gymnotidae), Stoddard (1999) suggested that predation pressure has favored the evolution of biphasic and multiphasic EODs as well as of wave-type EODs. The addition of a second, head-negative phase to a monophasic pulse leads to the attenuation of low-frequency power (Fig. 7.1, *right column*). The sine wave-like EOD of wave-type fish (Sternopygidae and Apteronotidae), which does not contain a DC component, is then a derived character that provides full escape from detection by ampullary electrosensory systems. Central to the original hypothesis for the evolution of electric signal complexity was the assumption that a monophasic pulse as produced by *Electrophorus* constitutes the plesiomorphic EOD type, which has, over the course of gymnotiform evolution, been replaced by bi- or multiphasic or by wave-type EODs in most lineages. In the light of recent work, the original hypothesis requires some revision, but the idea of eavesdropping by predators as an evolutionary driver of EOD properties certainly maintains its merit.

The evidence in favor of a role of predation in shaping EOD properties is fivefold.

(1) In a playback experiment, an electric eel was more sensitive to monophasic than biphasic pulses, even though the latter had twice the peak-to-peak amplitude of the former (Stoddard 1999).
(2) Weakly electric fish have been found to make up a large proportion of the fish ingested by certain large catfish species, both in Africa and in South America (Reid 1983; Merron 1993). It should be noted, though, that some catfish species living in Amazonian deep river channels prey heavily on apteronotids and other

gymnotiforms with very little low-frequency power in their EODs (Barbarino-Duque and Winemiller 2003) and thus do not appear to rely on their ampullary electrosense to detect EODs. In other cases, the use of the catfish ampullary sense seems more likely.

(3) The low-frequency power of the short female EOD pulses of the bulldog (*Marcusenius macrolepidotus*), an African mormyrid fish, is quite small, whereas in males, it increases considerably with increasing EOD duration (Kramer 1997). In playback experiments, sympatrically living catfish, *Clarias gariepinus*, were much more sensitive to the long-duration EODs of the males than to the much shorter EODs of the females (Hanika and Kramer 1999, 2000). In fact, no response could be elicited from catfish with female EODs of natural signal strength, whereas the maximum detection distance for the signal of a large male *Marcusenius macrolepidotus* was calculated to be as long as 1.5 m.

(4) At least two gymnotiforms with monophasic EODs, and thus maximal low-frequency power in their signal, occur in areas devoid of the main electroreceptive predators: electric eels, large pimelodid catfish, and river stingrays. One of the two, *Gymnotus cylindricus*, used to be considered a close relative of *Electrophorus electricus* and its EOD therefore to be a plesiomorphic character. A more recent phylogenetic study of the genus *Gymnotus* has, however, found solid evidence for the ancestral state of the EOD in this genus to be multiphasic and for loss of phases to have occurred multiple times (Lovejoy et al. 2010). *Gymnotus cylindricus* is now considered to be derived from ancestors with a multiphasic EOD. The same holds for *Gymnotus henni*, the other monophasic *Gymnotus*, which also occurs in Central America, outside the range of the main predators. The question remains what factors might have promoted the loss of phases and the gain in low-frequency power in a situation of release from predation. Conceivably, the answer lies in the recruitment of the fish's own ampullary receptors by the monophasic EODs. The monophasic EOD might thus aid in active sensing. Another, nonexclusive possibility is that low-frequency power is attractive to females because of a sensory bias.

(5) Another species with monophasic EOD, *Brachyhypopomus bennetti*, has been proposed to be a Batesian mimic of the electric eel's discharge (Stoddard 1999). *Brachyhypopomus bennetti* has a remarkably large electric organ (Sullivan et al. 2013) and a larger amplitude EOD than other *Brachyhypopomus* species. Its EOD waveform closely resembles that of the electric eel as does its average EOD rate and interpulse variability (Crampton 1998b; Stoddard 1999). Although these features do suggest Batesian mimicry, their protective function is unclear because field collections show similar proportions of specimens with predator-induced tail damage of *Brachyhypopomus bennetti* and a congener with a lower amplitude and biphasic EOD (Sullivan et al. 2013). In addition, any protective function via mimicry would suffer if the model (*Electrophorus electricus*) is much rarer than the mimicking species, which appears to be the case in the habitat of *Brachyhypopomus bennetti* (Crampton 2006).

In summary, biogeographic data, stomach content analyses of predators, and playback experiments with catfish and the electric eel support the hypothesis that eavesdropping by electroreceptive predators is a relevant evolutionary driver of electric signal diversity. Reduction of low-frequency power through generation of multiple EOD phases, short pulses, or wave-type signals may aid signal cloaking (Stoddard and Markham 2008). For the various mechanisms that lead to a reduction in low-frequency power, see Chap. 5 by Markham.

7.2.3.4 Energetic Constraints

The generation of signals comes at some metabolic cost for the signaling organism. Given that energy budgets are limited, form and frequency of signaling may be constrained by energy availability. This has been demonstrated impressively in sage grouse, where display effort comes at a sizable energetic cost and is negatively correlated with body condition (Vehrencamp et al. 1989). In comparison to sage grouse and most other communication systems, the situation in electric fish is complicated by the fact that the EOD serves the dual purpose of electrolocation and communication. The signal is being produced not just during the reproductive season but throughout the year, day and night, although the cost of electric signaling may increase considerably during the mating season, at least for males (see below in this section and Sect. 7.2.3.5).

In most communication systems, energetic costs to the sender are due in large part to muscle activity, such as in abdominal drumming of wolf spiders or vocal calling in hylid tree frogs (Stoddard and Salazar 2011). Electric signals are different in that they only require the generation of action potentials in nerve cells of the circuit controlling EOD generation and in the electrocytes of the electric organ (see Markham, Chap. 5). Costs therefore arise predominantly from the activity of the Na^+/K^+-ATPase that maintains the ionic gradients across the cell membrane of electrocytes and neurons. The energetic cost of EOD production can thus be assumed to correlate with the amount of sodium current flowing during each electrocyte action potential and with their rate of firing. The amount of sodium ions flowing across the membrane depends on ion-channel density and the kinetics of the channels involved. In particular, temporal overlap between the sodium and potassium currents entails so-called waste current. Minimization of the waste current depends critically on the optimal timing of inactivation of voltage-gated sodium channels relative to the activation of potassium channels (e.g., Alle et al. 2009).

Larger currents across the electrocyte membrane provide for a stronger EOD, which translates into larger ranges of electrolocation and electrocommunication. Given that electrostatic fields drop off with the cube of distance, a doubling of communication distance would require an eightfold increase in signal amplitude (Heiligenberg 1975; Knudsen 1975). Therefore, it is conceivable that the EOD range is limited by energy availability. As for the EOD rate, the energetic expense for EOD generation is expected to go up linearly with the rate in the case of pulse-type fish because the sodium-current flow of successive action potentials can be

assumed to be independent of each other. In this respect, energetic considerations for pulse-type EODs are identical to those for cortical neurons with variable firing rates (Attwell and Laughlin 2001). Wave-type electric fish are special in that no other systems are known in which neurons fire at comparably high and sustained frequencies as the neurons and electrocytes of the electromotor system (note that parts of the electrosensory system of wave-type fish fire at the same high frequencies). Therefore, it seems likely that these animals have evolved mechanisms to maintain high firing rates at some minimum of metabolic cost per action potential.

What do we know about the energetic cost of EOD production? Estimates have varied widely. Based on earlier voltage and current measurements from the electric organ and assumptions on the metabolic rate of mormyrids, Hopkins (1999a) estimated the energetic cost of EOD production to be approximately 1% of standard metabolic rate. The standard metabolic rate assumes that the animal is resting and immobile. Considering that a fish's metabolic rate increases drastically with active swimming (e.g., Schurmann and Steffensen 1997) while the EOD rate is not expected to increase as much, the energetic cost of the EOD could be viewed as rather low, if not negligible. In-line with a low estimate, a study of 23 species of gymnotiforms covering all 5 families found that their routine metabolic rates (routine metabolic rate measurements permit minimal but unquantified amounts of movement) were no higher than expected for other tropical fish species that are non-electric (Julian et al. 2003). This suggested that the EOD is either energetically relatively inexpensive or that electric fish trade off their metabolic investments in other functions against their investment in electrogeneration.

More recent estimates based on routine metabolic rate measurements are, however, higher. In an elegant experimental dissection of the costs of EOD production of a pulse-type gymnotiform fish, Salazar and Stoddard (2008) pharmacologically isolated the proportion of the animal's total energy budget spent on the EOD. Female *Brachyhypopomus gauderio* were estimated to use approximately 3% of their daily energy budget on EOD production compared with 11–22% for males (see also Sect. 7.2.3.5). For the wave-type gymnotiform fish, *Eigenmannia virescens*, the cost of EOD generation per pulse was estimated to be lower than in *Brachyhypopomus gauderio*, but this lower per-pulse cost is compensated by the higher discharge frequency of the wave-type species (Salazar et al. 2013). Overall, the cost of EOD generation in *Brachyhypopomus gauderio* and *Eigenmannia virescens* appears to be similar, which suggests a trade-off between energy allocation to a higher EOD amplitude in pulse-type fish versus a higher discharge rate in wave-type fish. Including estimates of ATP consumption for the electromotor circuitry and EOD-associated electrosensory processing, Salazar et al. (2013) estimated close to 30% of the routine metabolic rate to be allocated to the active electric sense.

These recent estimates suggest that electric signaling comes at a sizable cost, in particular because the signals are produced continuously instead of seasonally. Several lines of evidence support that these fish are indeed signaling under tight energetic constraints. First and foremost, many species of pulse-type weakly electric fish lower their rate of discharge during the day and only raise it as they become active in the evening, which was observed as early as 1965 in the Brazilian Amazon

by Lissmann and Schwassmann (1965). That discharge rate reduction may be an energy-saving measure for pulse-type fishes is supported by experiments in which fish were exposed to decreasing levels of DO. The animals lowered their EOD rate drastically as DO reached hypoxic levels (Crampton 1998a; Sukhum et al. 2016). With a lower EOD rate, the fish appear to be sacrificing temporal resolution of sensory input in order to maintain essential organismal functions.

In wave-type gymnotiforms, the kinetics of voltage-gated sodium and potassium channels have been shown to be tightly coregulated according to the individual-specific EOD frequency (McAnelly and Zakon 2000, 2007), thus minimizing the waste current for that frequency (see Markham, Chap. 5). In principle, lowering the EOD frequency should still save ATP in proportion to the rate reduction in wave-type fish. Nevertheless, these fish do not show daytime reductions in frequency and they reduce EOD frequency only minimally when challenged by hypoxia (Reardon et al. 2011). EOD frequency changes are observed on a developmental timescale and under hormonal control over the course of days (Dunlap et al. 2017) as well as transiently on a timescale of milliseconds to seconds in communication interactions (Zakon et al. 2002). However, on intermediate timescales, wave-type fish appear to be locked in at their individual-specific EOD frequency.

Whether wave-type fish with higher EOD frequencies incur higher energetic costs compared with fish with lower frequencies is still an open question. For an individual fish, to increase the EOD frequency from its baseline has been shown to be expensive if the EOD amplitude is to be maintained (Lewis et al. 2014). In fact, increases in frequency during chirps are correlated with amplitude decreases in *Apteronotus leptorhynchus* such that larger frequency excursions come with larger loss in amplitude (Engler et al. 2000; Turner et al. 2007). The question is whether longer term changes in the baseline that may be under hormonal control necessarily have a strong effect on the energy budget. Because fish appear to adjust the kinetics of their voltage-gated sodium and potassium channels according to their baseline EOD frequency (McAnelly and Zakon 2000, 2007), increasing the baseline frequency may be much less expensive than transient increases. Fine tuning of ion-channel kinetics to minimize the waste current could involve various mechanisms, including expression of different channel genes, splice variants, and phosphorylation of channel proteins. Whether some of these mechanisms, which might reduce energetic penalties of high-frequency EODs, are at work in electrocytes remains to be seen.

Another option to reduce the energetic load of signal production is to boost amplitude only when needed and keep it at lower values at other times, just sufficient to maintain basic functionality. Circadian variation in EOD amplitude has so far been demonstrated for several gymnotiform pulse-type fishes (but not for mormyrids) and, among wave-type fishes, for some sternopygids (Stoddard et al. 2007; Markham et al. 2009). These fish boost their EOD amplitude at night by 25–40% compared with minimum daytime levels. As shown for *Sternopygus macrurus*, this change in amplitude is mediated by ion-channel trafficking controlled by pituitary hormones (Markham et al. 2009). The selective addition of voltage-gated sodium channels to the excitable membranes of electrocytes boosts the amount of sodium

current flowing during electrocyte action potentials and thus the amplitude of the externally measured EOD but only at night, fitting their nocturnal life style.

That energy availability is a limiting factor for EOD amplitude is also supported by drastic drops in amplitude in fish exposed to hypoxia (Reardon et al. 2011). Interestingly, in apteronotids for which no circadian variation in EOD amplitude has been reported, a dramatic amplitude reduction sets in just when DO levels drop below the fish's critical oxygen tension, the level at which the animal's metabolic rate shifts from being independent of to being dependent on oxygen concentration. In *Eigenmannia*, on the other hand, the amplitude drop is more gradual and sets in at DO levels far above the critical oxygen tension of this species (Reardon et al. 2011). This suggests that *Eigenmannia* are able to adjust the EOD amplitude as a means to manage their energy budget under metabolic stress. Future work should address whether *Eigenmannia* show indeed a controlled drop in EOD amplitude to manage their energy budget and whether they achieve this through ion-channel trafficking as described for the circadian amplitude control of *Sternopygus* (Markham et al. 2009).

The daytime reductions in EOD rate in pulse-type fish and the amplitude reductions observed in some gymnotiform pulse- and wave-type species should have the dual effect of lowering the energetic demand of signal production during times of low activity and at the same time reducing conspicuousness to electroreceptive predators.

7.2.3.5 Sexual Selection

There is considerable evidence for sexual selection having shaped the EODs of weakly electric fish, which is not surprising because one of its functions is in communication (Andersson 1994). Examples of sexual dimorphism in EOD properties abound, such as differences in EOD frequency in wave-type gymnotiforms and waveform differences in pulse-type gymnotiforms and mormyrids. In principle, sexual dimorphism in EOD properties could also be due to sex-specific ecological selection because of differences in resource use between males and females. However, no such sex differences in resource use have been reported for weakly electric fish. In addition, there is no evidence for EOD waveform adaptation to specific habitats or foraging requirements (see Sect. 7.2.2.2). Thus, it seems fair to assume that sexual dimorphisms in EOD waveform or frequency are a consequence of sexual selection. As a side note, it even is conceivable, if not likely, that the generation of EODs per se evolved in the context of sexual communication as a mechanism to stimulate the sexual partner's ampullary electrosense (Stoddard 2002) and that it did not evolve primarily as a mechanism of active sensing as originally proposed by Lissmann (1958), but this hypothesis is difficult to test in gymnotiforms and mormyroids. Synodontid catfish may offer a clearer case because some of them appear to have evolved the ability to produce EODs by modifying a sonic muscle used to generate acoustic communication signals (Boyle et al. 2014). More detailed phylogenetic and behavioral studies are needed to verify if EOD evolution in this

group represents an example of exploitation of hidden sensory biases in the catfish ampullary electric sense (Ryan and Keddy-Hector 1992).

Sexual dimorphism in the EOD waveform has been described for many pulse-type fishes (Fig. 7.8). A general feature in all these cases is that male signals are of longer duration than the corresponding female EODs and that they contain more low-frequency energy (e.g., Hopkins 1999a; Stoddard et al. 2006). Two additional features, described in some gymnotiform pulse-type species, are that reproductive males boost the amplitude of their EODs more at night than females and discharge their electric organ at a higher rate (e.g., Silva et al. 2007; Stoddard et al. 2007). All three features, longer pulse duration, larger amplitude, and higher EOD rate, have been shown to be energetically costly (Salazar and Stoddard 2008; Markham et al. 2009). Apart from the extra metabolic expense to be paid by males, all three factors are expected to increase conspicuousness to electroreceptive predators, such as catfish (see Sect. 7.2.3.3). These points are consistent with male competition for access to reproductive females, imposing metabolic costs and also risks on males. In fact, in *Brachyhypopomus gauderio*, EOD generation is relatively inexpensive for

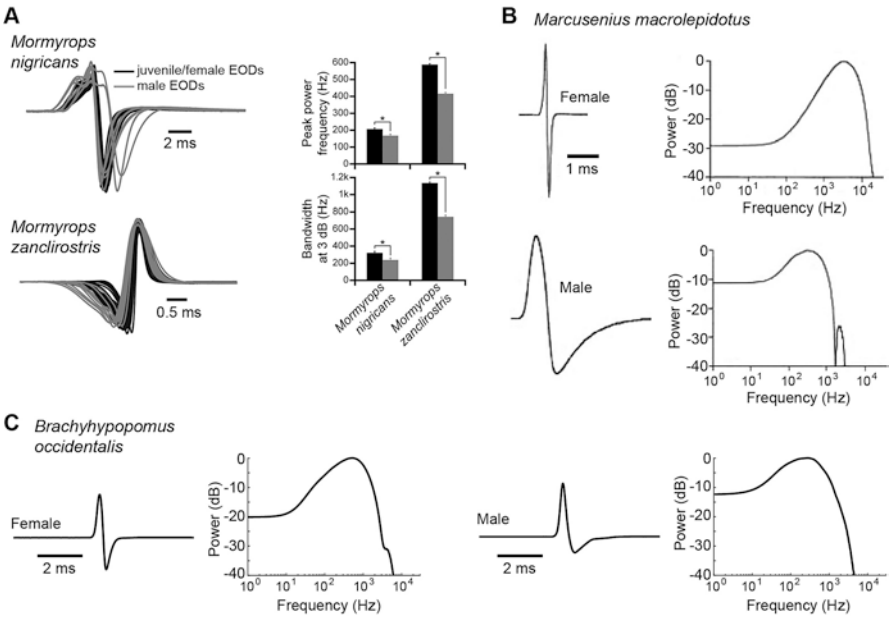

Fig. 7.8 A sample of representative sexually dimorphic EOD waveforms of gymnotiform and mormyrid species. In each species, the male signal is longer in duration and contains more low-frequency power. **A:** *Mormyrops nigricans* and *Mormyrops zanclirostris* (Mormyridae). EOD waveforms of multiple immature and female specimens (*black*) and of mature males (*gray*) are overlaid. Male signals have lower frequencies of peak power and narrower bandwidth. *Asterisks*, significant difference ($P < 0.01$). **B** and **C:** EOD waveforms (*left*) and associated power spectral density plots (*right*) for female and male *Marcusenius macrolepidotus* (Mormyridae; **B**) and *Brachyhypopomus occidentalis* (Gymnotiformes; **C**). **A** modified from Carlson and Arnegard (2011); **B** modified from Hanika and Kramer (2000); recordings for **C** kindly provided by S. Picq

females, with an estimated 3% of the daily energy budget compared with up to 22% for males (Salazar and Stoddard 2008; see also Sect. 7.2.3.4).

That male EODs carry a larger risk than female EODs of being detected by electroreceptive predators is supported by several findings. First, indirect support comes from the field observation that a much larger percentage of male than female *Brachyhypopomus pinnicaudatus* was found to have damaged tails, although it could not be verified if the tail damage resulted from predator attacks or aggressive encounters with other males of the same species (Hopkins et al. 1990). Second, an electric eel was twice as likely to approach an electrode playing a monophasic EOD (i.e., with strong low-frequency energy content) than an electrode playing a biphasic EOD (i.e., with much reduced low-frequency energy content), even though the biphasic signal was of twice the peak-to-peak amplitude of the monophasic signal (Stoddard 1999). Third, the African predatory catfish, *Clarias gariepinus*, was much more sensitive to playback of male than of female EODs of *Marcusenius macrolepidotus*. The EOD of male *Marcusenius lepidotus* can be ten times longer in duration than the EOD of females and contains much more energy in the low-frequency range of the ampullary electrosensory system of catfish (Hanika and Kramer 1999, 2000). That the greater sensitivity of catfish to the EODs of reproductive male *Marcusenius macrolepidotus* translates to an actual greater risk of predation is supported by stomach content analysis of field-captured specimens of *Clarias gariepinus* (Merron 1993).

Sexual dimorphism in EOD properties is also common among wave-type gymnotiforms. In several members of the family Sternopygidae, mature males discharge their electric organ at lower frequencies than females (Hopkins 1972, 1974). Even though these fishes avoid low-frequency energy in their signals by producing highly periodic discharges and generating a head-negative DC offset on which the head-positive monophasic EOD pulses ride (Bennett 1971), the EOD frequencies of males of certain *Sternopygus* species drop sufficiently low to be in the range of ampullary electroreceptors. The frequency of adult male *Sternopygus branco* can be as low as 25 Hz (Crampton and Albert 2006). Sex differences in EOD frequency are also observed in many of the high-frequency wave-type species (Apteronotidae), although their presence and which sex has the higher frequency signals appears to be evolutionarily quite labile (Smith 2013). In any case, the high frequencies and the lack of DC offset of apteronotid EODs should make them immune to ampullary electroreceptive predators, such as catfish. Whether there is any sex- and EOD-related risk of being captured by large apteronotid predators is unknown (see Sect. 7.2.3.3).

A positive correlation between EOD frequency and body size in male *Apteronotus leptorhynchus* suggested a role for sexual selection in the evolution of high EOD frequency, but this correlation is not found consistently in this species (e.g., Dunlap 2002; Dunlap et al. 2002). A strong correlation between body size and EOD frequency was found, however, in a field study of the apteronotid *Sternarchorhynchus* sp. (Fugère et al. 2011). Competition and playback experiments of these highly territorial animals demonstrated that they use EOD frequency as an indicator of domi-

nance. Whether EOD frequency also plays a role in sexual behavior could, however, not be established.

Rapid modulations of EOD rate, such as chirps, have been described in the aggressive and courtship behavior of both wave-type and pulse-type weakly electric fishes (Carlson and Hopkins 2004; Lorenzo et al. 2006) and may be instrumental in synchronizing spawning of male and female fish (Silva et al. 2008; Henninger et al. 2018). In many species, these transient signals are DC unbalanced and thus contain considerable low-frequency power, which would expose, at least, the signaler to extra risk of predation because it would activate ampullary electroreceptors (Stoddard 2002). The evolution of such communication signals with strong low-frequency power on a DC-balanced baseline EOD may then be driven by a combination of factors: (1) the risk assumed by the signaler, which may indicate its high quality, consistent with the handicap principle (see below in this section); (2) transiently boosting EOD frequency could drive up the energetic cost of EOD production, and the ability to do so could also serve as an indicator of the quality of the signaler. This metabolic cost factor has not been estimated so far; and (3) the third factor favoring low-frequency power in communication signals could be the added stimulation of the ampullary electrosensory system of the intended receiver. Assuming that the usual use of the ampullary electrosensory system is in foraging, its stimulation by communication signals might constitute exploitation of a sensory bias in the receiver (Endler 1992).

The group whose chirping behavior has been studied most intensely are the apteronotids. Chirp parameters, such as frequency excursion, duration, and rise and fall times, appear to be evolutionarily highly labile and could very well support species recognition (Turner et al. 2007; Smith et al. 2016). Interestingly, many apteronotids appear to have evolved mechanisms to avoid a DC component in their chirps, which should protect them from low-frequency-sensitive predators (Bennett 1971). At least one apteronotid species, the tamandua knifefish, however, has recently been shown to produce EOD interruptions that introduce a considerable DC offset (Smith et al. 2016). This type of communication signal is discussed as being plesiomorphic among apteronotids. It is thus conceivable that predation pressure has driven the evolution of communication signals that avoid low-frequency power in this group.

If sexual dimorphism is based on intersexual selection, one predicts to find female preferences for the more extreme male signal properties. The clearest evidence for female preference for long-duration male EODs comes from playback experiments with the African fish *Marcusenius pongolensis* in which male EOD duration is positively correlated with body size (Machnik and Kramer 2008). Females preferring males with longer EODs would thus be choosing larger mates, a choice pattern that is common among fishes (Ryan and Keddy-Hector 1992). Obviously, signal differences can also arise from intrasexual competition, and there is evidence for that from the same species of African electric fish: male *Marcusenius pongolensis* attacked playbacks of longer EOD pulses more vigorously than playback of shorter ones (Hanika and Kramer 2005).

Female preference for male signals that are costly in terms of metabolism and/or predation risk is consistent with the handicap principle proposed many years ago by Zahavi (1975). It posits that a male's superior quality is demonstrated by its ability to afford a "handicap," which could be energetically wasteful signaling or producing signals that increase the predation risk of the signaler (Bradbury and Vehrencamp 2011). Both of these aspects appear to be met by the EODs and their modulations in many weakly electric fishes. That these costs are biologically relevant is supported by the short duration of extreme male signal phenotypes. Duration and/or amplitude of the signals are under control of hormonal systems that limit the extra costs and risks for males to the nighttime and/or the breeding season (Dunlap et al. 2017; see also Silva, Chap. 6).

In summary, sexual selection has likely been a major evolutionary driver of electric signals in weakly electric fishes. This point has also been suggested strongly in a phylogenetic comparison of EOD properties and a suite of ecologically relevant traits in the mormyrid genus *Paramormyrops* (Arnegard et al. 2010). EOD waveform properties showed much faster divergence in this genus than ecological traits (Fig. 7.6; see Sect. 7.2.3.2). Given the dual function of the EOD in electrolocation and communication, it is tempting to think of the EOD as a potential magic trait. Magic traits have been defined as traits that are, at the same time, subject to divergent ecological selection and contribute to non-random mating (Servedio et al. 2011). They can thus drive rapid speciation. The evidence for sexual selection (non-random mating) acting on EOD properties is strong, as discussed above. If sexual and ecological selection acted in conjunction on the EOD, this could indeed lead to rapid speciation as proposed for a group of mormyrids (Feulner et al. 2009b). So far, however, the evidence for divergent ecological selection on the EOD, due, for example, to specific features of different microhabitats, is lacking.

7.2.3.6 Neural Innovations

Evolutionary novelties can have dramatic effects on species diversification (Erwin 2015). Famous examples are the evolution of eyes in the Cambrian and of feathers in the Cretaceous. The presence of a new trait is, in itself, certainly not sufficient to drive a radiation. Other factors promoting divergence are required, such as sexual selection or some form of ecological selection. The success and radiations of weakly electric fishes are likely tightly linked to the origins of electric organs and the use of EODs in electrolocation and communication. The evidence for a decisive role of neural innovations in diversification is particularly strong in African weakly electric fishes. All but one species (*Gymnarchus niloticus*) belong to the Mormyridae, whose electrocytes are innervated by spinal motor neurons on stalks protruding from the electrocyte membrane (see Fig. 7.9A). The electrocytes in one subgroup, the Petrocephalinae, have stalks protruding from their posterior membrane. The electrocyte stalks of their sister group, the Mormyrinae, can protrude from the posterior or anterior face, and the stalks can penetrate the electrocyte once or twice or they

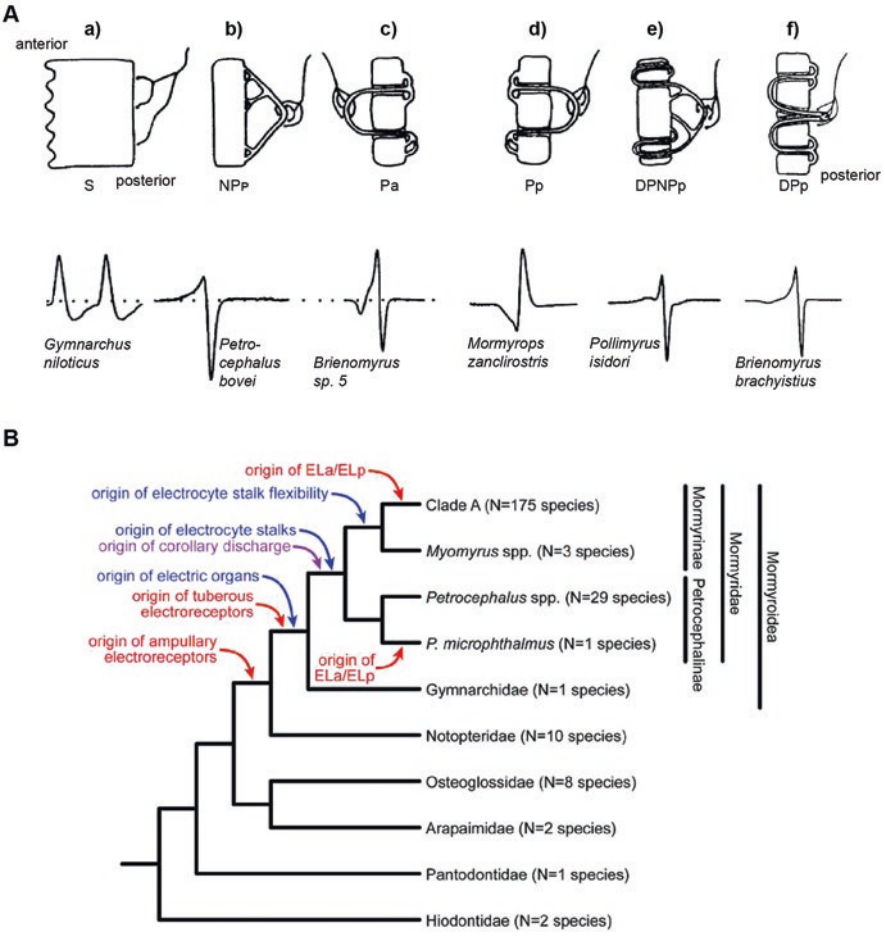

Fig. 7.9 Evolutionary innovations increase signal space. **A:** evolution of flexibility in the morphology of electrocyte stalks in Mormyrinae increased the possible number of EOD phases, which can vary in amplitude and direction of voltage change (**c-f**). The plesiomorphic state is represented by the stalkless (S) electrocyte of *Gymnarchus niloticus* (**a**). Electrocyte stalks appear in Petrocephalinae and are of the non-penetrating type with posterior innervation (NPp; **b**). Pa, penetrating stalk with anterior innervation; Pp, penetrating stalk with posterior innervation; DPNPp, doubly penetrating and non-penetrating stalk with posterior innervation; DPp, doubly penetrating stalk with posterior innervation. Modified from Hopkins (1999b). **B:** cladogram of osteoglossomorph fishes shows the origins of sensory innovations (*red*) and of motor innovations (*blue*). The estimated number of extant species in each lineage is given in parentheses. An enlarged exterolateral nucleus subdivided into anterior (Ela) and posterior (ELp) parts evolved twice, once in the genus *Petrocephalus* and once in the species-rich clade A. Modified from Carlson and Arnegard (2011)

can be non-penetrating. The species-specific pattern of electrocyte stalk morphology is directly linked to properties of the EOD, such as the number and amplitude of waveform phases (Fig. 7.9A; Hopkins 1999b; see also Markham, Chap. 5). It seems likely that the appearance of flexibility in electrocyte stalks in the Mormyrinae has been instrumental in their diversification by opening up the space of possible EOD variation. The availability of a larger signal space may have offered the opportunity for sexual selection to take EODs in different directions and promote speciation, which is supported by a much more pronounced sexual dimorphism in EOD waveforms in Mormyrinae compared with Petrocephalinae. Indeed, the Mormyrinae contain approximately 180 recognized species compared with around 30 recognized species in the Petrocephalinae (Fig. 7.9B). A closer look clarifies that most of the Mormyrinae are members of the so-called clade A, one of whose defining features is another evolutionary novelty, an enlarged and subdivided nucleus in the midbrain, the exterolateral nucleus (Carlson et al. 2011). The neural circuitry of this nucleus compares the timing of input from knollenorgan electroreceptors from different parts of the body surface and thus is critical for detecting variation in EOD waveform of signals produced by other fish (Vélez et al. 2017; see also Carlson, Chap. 10). The picture that has emerged is that enhanced neural power of processing temporal information has permitted the diversification of EODs and species seen in clade A.

In the context of this chapter, it is important to note that neural innovation is not itself an evolutionary driver of signal diversity but that it allowed enhanced utilization of the EOD signal space for selection to act on. Based on the much faster speed of signal divergence compared with divergence in morphology, size, and trophic ecology shown for a species-rich genus of Mormyrinae (Fig. 7.6; Arnegard et al. 2010), sexual selection may be the most likely driver of the massive divergence seen in this group.

7.2.3.7 Conclusions on Biotic Factors as Drivers of Electric Signal Diversity

Biotic factors have been strong drivers of electric signal diversity. In particular, sexual selection, reproductive character displacement, and selection on signal properties by electroreceptive predators have likely played important roles in shaping EOD diversity. Evolutionary innovations, such as larger size and subdivision of the exterolateral nucleus in the midbrain of clade A mormyrids, have opened up new dimensions of signal space for selection to act on. The energetic costs of signal generation and the associated sensory processing can be seen as a constraint but not an evolutionary driver of diversity. Daytime reductions in EOD rate and amplitude provide savings in energy and thus support higher rates and amplitudes during the animals' active phase at night. Tight correlation between the kinetics of sodium and potassium currents appear to moderate the metabolic cost of sustained high-frequency firing in wave-type fish, enabling evolutionary drivers to push EODs to higher frequencies.

7.3 Summary

Sexual and ecological selection along with genetic drift have been shaping the diversity in electric signals seen today. Weakly electric fish provide an interesting example of how innovation in structures (e.g., electrocyte stalks), neural circuitry (e.g., the exterolateral nucleus in clade A mormyrids), and ion-channel expression (see Gallant, Chap. 4; Markham, Chap. 5) can open up the available signal space, which has the potential to promote species radiation. The main evolutionary drivers acting on this signal space since electric organs evolved independently in Gymnotiformes and Mormyroidea approximately 100 million years ago (Lavoué et al. 2012) have likely been related to the communication function of the EOD, although other factors, such as an effect of flow regime on sensory sampling rate, may have played a significant role as well.

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Chapter 8
Using Control Theory to Characterize Active Sensing in Weakly Electric Fishes

Sarah A. Stamper, Manu S. Madhav, Noah J. Cowan, and Eric S. Fortune

Abstract Animals routinely use their own motor outputs to modulate the sensory information they perceive, a process termed "active sensing." This chapter highlights the use of control theoretic approaches to reveal the functional relationships between active sensing, task-related behaviors, sensing, and motor control. Specifically, recently developed experimental systems use artificially controlled feedback loops to perturb natural reafferent feedback in freely behaving animals. Such perturbations allow quantitative and systematic descriptions of control strategies for active sensing.

Keywords Closed loop · *Eigenmannia* · Electroreception · Feedback control · Gymnotiformes · Image stabilization · Jamming avoidance response · Open loop · Ribbon fin · Station keeping

8.1 Introduction to Active Sensing

Animals use behavior to control the acquisition of sensory information through a variety of processes collectively referred to as "active sensing." The simplest definition of active sensing is the use of motor outputs for the purpose of acquiring or

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modulating sensory information. Active sensing is found across animal taxa and sensory modalities. A handful of species are active sensing specialists; these species have adaptations for the generation of sensing signals. The best known of these species have evolved specialized organ systems for electroreception, echolocation, whisking, and hydrodynamic imaging (Nelson and MacIver 2006). Each of these species has complementary motor and sensory adaptations that work together to gather information from their environment. For example, weakly electric fishes have specialized neuromotor systems known as electric organs that generate an electric field (Heiligenberg 1991a) that can extend to over a meter around the fish (Tan et al. 2005; see also Gallant, Chap. 4). The corresponding sensory adaptations in these fishes are modified electroreceptive cells that detect the electric fields produced by these organs (Meyer and Zakon 1982). Together, the electric organ and specialized electroreceptors can detect nearby objects and conspecifics and are used in behaviors that range from prey capture to social communication (Heiligenberg and Bastian 1984; Caputi 2017).

The most common form of active sensing, however, does not involve specialized organ systems but is mediated through movement. An animal's movements often dramatically alter and/or regulate the information that its sensory receptors receive from the environment (Hofmann et al. 2014). For example, many animals move their pinnae in relation to attention; such movements have been described in a variety of species including echolocating bats (Ghose and Moss 2006) but also in animals such as foxes (Koop and Velimirov 1982) and cats (Populin and Yin 1998). Indeed, movement often determines what information is available to a sensorium.

Active sensing can also be profoundly affected by social context. When animals are near each other, they can perceive, and sometimes even exploit, the sensing signals used by nearby animals. This occurs, for example, when animals move in a herd or fish swim in a school. The competing signals from conspecifics often interact with the animal's own sensing signals (Griffin et al. 1963; Heiligenberg 1991a). Active-sensing signals are also public and therefore subject to "eavesdropping" where other animals can intercept, and potentially exploit, information carried in these signals. Examples of eavesdropping are found in both invertebrate (Stowe et al. 1995; Lichtenberg et al. 2011) and vertebrate (Fenton and Ratcliffe 2004; Götz et al. 2006) animals.

Social context can introduce a new category of sensorimotor challenges for animals vis-à-vis their use of active sensing (Partan and Marler, 1999). Specifically, there is a categorical difference in the sensory environment of animals when they are alone compared with when they are in groups (Tan et al. 2005; Stamper et al. 2010). Adding to the complexity is the fact that animals frequently use signals that serve dual purposes: sensing and social signaling (Metzner 1999; Dawson 1991). Animals routinely use their own movement to modulate both the effects of nearby interference produced by conspecifics and the social signals between individuals.

Weakly electric fishes rely on each of these forms of active sensing. These animals generate a weak electric field produced and detected by specialized organs that is used in the control of many locomotor and social behaviors (Heiligenberg 1991a). The fish also use movement for active sensing, both in the context of locomotor control and in social behaviors. Both the electric field and movement are also used in social behaviors in these fishes.

One of the advantages of weakly electric fish is that the spatial distribution of the active sensing signal, the electric field, is directly related the position of the fish (Madhav et al. 2018). As a result, the sensory consequences of movement can be computed by monitoring the position of fish relative to objects and conspecifics over time (Nelson and MacIver 1999).

8.2 Properties of Active Sensing

8.2.1 Active Sensing via Movement

The most common form of active sensing is movement, which is often tuned to the sensory demands of the task. For example, if a task is to determine the texture of an object, people tend to move their hand back and forth in a lateral rubbing movement (Lederman and Klatzky 1987; Hollins and Risner 2000). This movement activates mechanoreceptors in the hand (e.g., Merkel disks) that respond to indentation of the skin and have restricted receptive fields that allow for the very fine spatial resolution required for tactile discrimination. However, if instead the task is to determine the weight of an object, people tend to make "hefting" movements where they move the hand holding the object up and down (Gibson 1962). This type of movement primarily activates muscle stretch receptors that can detect the load on a given limb but do little in terms of discriminating textures.

Indeed, there are many ways in which animals move specifically for the purpose of gathering sensory information (Fig. 8.1). Animals can move to

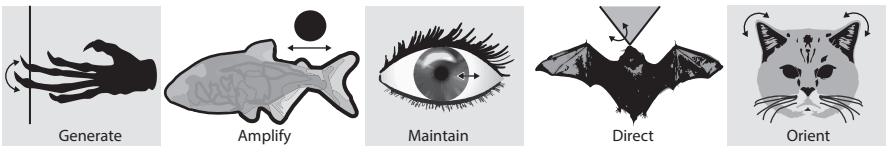

Fig. 8.1 Examples of categories of active-sensing movements. *Left to right:* aye-ayes generate both tactile and auditory feedback by tapping their fingers; blind cave fish use hydrodynamic imaging to amplify feedback; involuntary fixational eye movements reduce perceptual fading and possibly statistically whiten afferent signals (Mostofi et al. 2016); bats move their heads to direct echolocation calls to prey items and other targets while flying; and cats orient their pinnae in relation to sound sources to improve auditory perception

(1) Generate a sensory signal. Consider the "aye-aye" lemur *Daubentonia madagasceriensis*, which is nocturnal and forages for insects that live in the subsurface of tree cavities. To detect an insect, the aye-aye makes a rapid tapping motion (termed "percussive foraging" or "tap scanning") on the surface of the wood and listens for the returning echoes (Erickson 1994; Erickson et al. 1998). This behavior is not specific to aye-ayes; it has also been observed in woodpeckers and some monkeys (Phillips et al. 2004).

(2) Amplify a sensory signal. The blind cave fish *Astyanax jordani* uses its mechanosensory lateral line for hydrodynamic imaging (Windsor et al. 2010). To investigate novel objects, these fish rapidly accelerate and glide past the object (Von Campenhausen et al. 1981; Hassan 1989). It appears that this rapid acceleration produces a flow field around the fish's body that is modified by the presence of stationary objects (Hassan 1985; Windsor et al. 2008). The fish controls its swimming speed and pattern (acceleration glide) in order to optimize the activation of their mechanoreceptors (neuromasts; Teyke 1988).

(3) Maintain a sensory percept. Sensory receptors commonly have high-pass filtering properties and therefore reject stationary or very low frequency signals. This filtering is often known as "adaptation," which can have profound effects on sensing. If an image is stabilized perfectly on the retina, then there is no relative movement and the photoreceptors would adapt over a period of a few seconds. The perceptual consequence is that the visual pattern would disappear, which is known as "perceptual fading" (Ditchburn and Ginsborg 1952). One-way animals overcome adaptation, which leads to perceptual fading, is by maintaining high-frequency movements, such as in the form of temporally punctate saccades (Ahissar and Arieli 2012) or continuous, broadband motions (Stamper et al. 2012).

(4) Direct a sensory signal. In echolocating bats, the sonar beam is highly directional and narrow (a 60–90° cone from the midline; Surlykke et al. 2009a), which is beneficial for detecting targets within the range directly in front of the bat but less so for detecting objects located off-axis. To solve this problem, bats use movement to direct the beam across a wider swath of the environment. Specifically, they move their head back and forth in a scanning motion to increase the sensory volume for the detection of prey and other objects in their environment (Ghose and Moss 2006; Surlykke et al. 2009b). The sonar beams of dolphins are also highly directional and narrow (Au and Moore 1986).

(5) Orient their receivers or receptor arrays. Bats (Pye and Roberts 1970; Ghose and Moss 2006), foxes (Koop and Velimirov 1982), and cats (Populin and Yin 1998) each use ear movements to help localize the direction of a sound source. For example, surgically immobilizing the bat's ears before an obstacle-avoidance task leads to decreased performance, especially for targets that require elevation processing (Mogdans et al. 1988). In short, movement is used to modulate the spatial relationships between the signal source and the sensorium to improve sensory perception.

8.2.2 *Relationships Between Task-Directed and Sensing Movements*

A common feature of active sensing via movement is that the movements for sensing are distinct from the movements the animal uses to complete a behavioral task. In some behaviors, the movements for sensory acquisition and task completion are performed during separate temporal epochs. For example, if the task is to report features of a stationary image (e.g., time of day, number people in the image), the eye movements for active sensing are nonoverlapping with the motor task of verbally reporting the answer (Fig. 8.2; Yarbus 1967).

Such behaviors are amenable to information-theoretic approaches based on information maximization (Yang et al. 2016). However, when the active sensing and task-dependent movements occur contemporaneously, such theoretical approaches are more challenging to apply. Consider, for example, the change in a person's reaching pattern as they flip a light switch. In the light, the person would first use eye movements to find the light switch and then plan arm movements to execute the task of flipping the switch (Yang et al. 2016). However, in the dark, the person would scan a hand along the wall while trying to move toward the switch and flip it. In the latter case, sensing movements are not temporally independent from task-directed movements.

Another feature of active sensing is that such movements depend on the availability of information across modalities. In the above example, individuals compensate for the loss of *visual* information by using manual scanning to generate

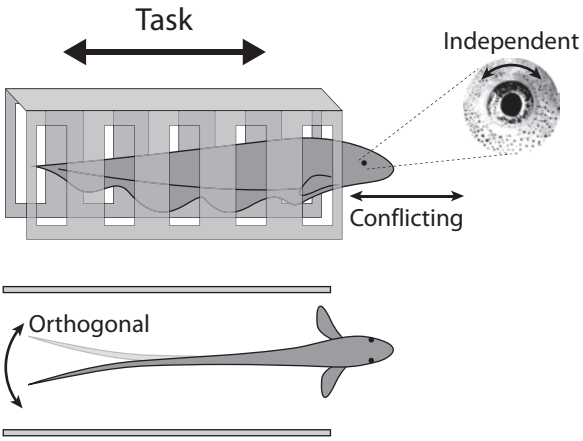

Fig. 8.2 In refuge tracking in weakly electric fish, fish swim forward and backward to remain within the refuge: the Task. Active movements for sensing can be independent of such task-related swimming movements, such as moving the eyes. Active-sensing movements can also be orthogonal to the task-related movements, such as swaying the tail back and forth within the refuge, or be conflicting, in the same dimensions as the task-related movements, as is the case for va-et-vient active-sensing movements

somatosensory feedback. In both the light and dark, the task goal, flipping the switch, is identical. But in the dark, the person adds active-sensing movements that are not mechanically related to performing the task goal but are nevertheless necessary to obtain sufficient sensory information to achieve the task.

Animals simultaneously engage in both categories of movements: those related to the task mechanics and those related to active sensing. These two categories of movement may be related in the following ways:

(1) *Independent.* The sensory apparatus is on a different part of the body from that performing the mechanical task, such as ocular movements in support of manual tasks.
(2) *Orthogonal.* The sensory apparatus is mechanically coupled to the task effector, but the movements are largely orthogonal, such as tapping perpendicular to a wall while reaching along the wall to flip a light switch or tail bending of electric fish during refuge-tracking behavior (Stamper et al. 2012).
(3) *Overlapping/Conflicting.* The sensory apparatus is mechanically coupled to the task effector, and the movements for active sensing overlap with the physical degrees of freedom required to achieve the task. In this way, an active-sensing movement may be in the opposite direction as that required to achieve the task goal and therefore in direct conflict. The back-and-forth active-sensing movements that electric fish use during refuge-tracking behavior is an example of this sort of conflict (Stamper et al. 2012).

During the execution of any mechanical task, animals can exhibit different active-sensing behaviors contemporaneously, each having a potentially different relationship (independent, orthogonal, overlapping) with the task goal. For example, tail-bending and fore-aft movements are simultaneously used in electric fish during refuge tracking (Stamper et al. 2012). Just as movements for active sensing depend on task-oriented movements, progress toward achieving task goals can modulate active-sensing movements.

Given the dynamic relations between active-sensing and task-oriented movements that evolve during behavior, how can a biologist identify the neural mechanisms for the control of these two categories of movement? This is particularly challenging because both categories routinely stimulate the same receptor systems.

8.2.3 Correlations in Sensing and Motor Systems

Movement, irrespective of whether it is generated with respect to achieving a task or for active sensing, results in strong correlations in activity between the motor and sensory systems in the brain. Indeed, it is increasingly appreciated that animals rely on the correlations between an animal's behavior and sensing to enhance task-dependent sensory perception. The relationship between motor activity and sensory

information arises through "reafferent" feedback (sensing produces movement, and movement, in turn, creates sensory signals; Pearson 2008; Hofmann et al. 2014). Reafferent information is often used in the control of movement (Gritsenko et al. 2009; Knill et al. 2011). Sensory–motor correlations also can arise internally, through descending and other pathways within the brain, for example, sensory predictions and corollary discharge (Alviña and Sawtell 2014; see Perks and Sawtell, Chap. 11).

These sensory–motor correlations, however, present a challenge for scientists because they can obscure the role of brain activity in sensory perception versus motor control. In other words, because strong temporal correlations between motor output and sensory inputs occur at all levels of biological organization, from the mechanics of the behavior to the activity of neurons in the brain, these sorts of correlations cannot be naively used to disentangle the respective roles of sensory and motor control systems. For example, neurons in a song control nucleus in the brains of songbirds respond to playback of the bird's own song, an exclusively sensory signal, with a pattern of activity that is almost identical to the activity seen when the bird is singing (Dave and Margoliash 2000). This result might be surprising: the auditory activity during playback is mediated by spiking activity that originates from the cochlea and reaches these neurons via ascending auditory pathways, whereas motor activity affects downstream targets to control the contractions of muscles in the syrinx to produce sound. It seems surprising that neurophysiological codes used to represent ascending sensory information would be identical to the descending motor output used to control the production of forces in the syrinx given the distinct physics between sound production and sound transduction. However, the fact that output from the syrinx always stimulates the cochlea suggests that there will be strong correlations in sensory (auditory) and motor (singing) activity in the brains of these birds.

The relationships between the motor and sensory systems are dynamic because they serve multiple behaviors with differing task-dependent goals. Because the spatiotemporal structure of behaviors differs, so must the spatiotemporal structure of correlations between the motor and sensory systems. In other words, the computational structure of neural substrates for control are dynamically tuned in relation to the behavioral tasks (Chacron et al. 2003). These dynamic changes in correlations pose an additional challenge for neuroscientists because neurons within brain circuits retune in relation to behavioral context.

The tight coupling between the sensory and motor systems pushes neuroscientists toward conducting experiments in awake, behaving animals rather than in anesthetized animals. One consequence of using anesthesia for experiments is the immobilization of the animal, which opens feedback loops. This experimental opening of feedback loops eliminates correlations between sensory and motor activity in the brain, which can both alter patterns of neural activity and lead to fundamental misinterpretations of the roles of sensory responses in the control of behavior (Szwed et al. 2003; Mosconi et al. 2010).

8.3 Using Control Theory to Study Closed-Loop Sensorimotor Systems

The challenge of understanding active-sensing mechanisms is that the sensory and motor circuits operate together to produce behavior. Motor circuits generate movement (i.e., the behavioral output) that results in sensory feedback that is processed by the nervous system to modulate ongoing motor output. These "closed-loop systems" offer dramatic improvements in the regulation of behavior, including increasing the speed of responses and stabilizing motor performance (Cowan et al. 2014).

8.3.1 Control-Theoretic Approaches

Understanding the dynamics of these types of systems are the focus of control systems theory. The application of control-theoretic approaches allows scientists to quantitatively and independently probe control subsystems to understand their roles in the performance of the intact closed-loop system (Roth et al. 2014). Control-theoretic approaches to the study of closed-loop dynamical systems take advantage of the linkages between the inputs and outputs of a system (Cowan et al. 2014). These analyses often involve the application of small perturbations in feedback information. This approach has proven to be extremely successful in predicting the performance of designed systems, where the feedback topology and the system dynamics are specified by humans.

This approach differs from those commonly used in behavioral neuroscience. Although neuroscientists are keenly aware of feedback loops at multiple levels of organization, from within neurons to the brain circuits to the entire organism and beyond, many experiments focus on a unidirectional flow of information from sensory systems through to motor systems. Experiments that do explicitly engage the roles of feedback most commonly use techniques that completely eliminate the flow of information through feedback pathways. Examples include the use of lesions or injections of anesthetics into brain areas or the application of masking signals such as noise. Of course, many of the approaches and strategies that are used by neuroscientists to analyze how animals control behavior are limited by technical and practical issues.

Although the application of control-theoretic approaches to the analysis of feedback control in animals may be useful in decoding neural mechanisms, these approaches involve additional challenges. First, unlike in artificial systems, the feedback topology and system dynamics of animal behavioral systems are rarely known. Biological feedback loops that can impact the control of behavior occur at levels of organization from molecules to ecosystems; it is fair to say that the full topology for feedback control has not been revealed for any organism. Second, experimental perturbations of feedback may be difficult or impossible to achieve during behavior or within neural circuits due to lack of experimental access to feedback pathways. Third, perturbations of feedback can lead to categorical shifts

in behavior. Experimental perturbations can drive the animal to switch tasks or adopt different strategies for achieving the task goal. This is particularly challenging in relation to the study of active sensing when the animal is typically behaving with two simultaneous goals (achieving a task goal and controlling active sensing). Perturbations are likely to interact with both goals in ways that drive nonlinear changes in behavior.

8.3.2 Closing the Loop on Active-Sensing Systems

Systems that rely on closed-loop modulation of reafferent feedback are being developed to help understand control strategies across a wide range of behaviors in animals from flies (Roth et al. 2012; Reiser and Dickinson 2008) to fish (Ahrens et al. 2012; Madhav et al. 2013) to rodents (Chen et al. 2013; Ravassard et al. 2013; Aronov and Tank 2014; Sofroniew et al. 2014; Aghajan et al. 2015). The benefit of these systems is the tight control over the sensory experience of the animal, often during neural recordings (Maimon et al. 2010; Ahrens et al. 2012; Sofroniew et al. 2014).

These newly developed closed-loop systems typically constrain the animal (e.g., glued to a stick, mounted in agar, or head fixed to a microscope) to reduce the routes for sensory feedback and behavioral state of the animal. Once within the system, the motor output of the animal is monitored in real time. Information from the behavior of the animal is translated into sensory signals delivered to the animal as a form of feedback (Kim et al. 2018). The behaving animal then responds to this modified feedback that it perceives as being generated through natural sources of reafference.

Creative modulation of these feedback systems can give the experimenter almost complete control over an animal's behavior. Perhaps the most compelling application of this approach is the ability to induce the animal to repeatedly produce nearly identical behaviors (Madhav et al. 2013). This is important for the analysis of brain mechanisms because neurophysiological activity is inherently noisy. Neuroscientists rely on repeated measures and averaging to identify and characterize neural encoding strategies, which can be difficult to achieve in awake freely behaving animals. Using real-time manipulation of the animal's feedback, it is possible to either elicit *repeated motor signals* for analysis of activity in areas of the brain that contribute to motor control and/or deliver *repeated sensory stimuli* to awake, behaving animals to analyze activity in areas of the brain used in sensory perception.

8.3.3 Reconciling Terminology of Control Theory and Biology

The mathematical analysis of transformations between input and output fall naturally within the purview of control theory. Control theory was originally developed to control a known machine (the "plant," as in a manufacturing plant), and it allows

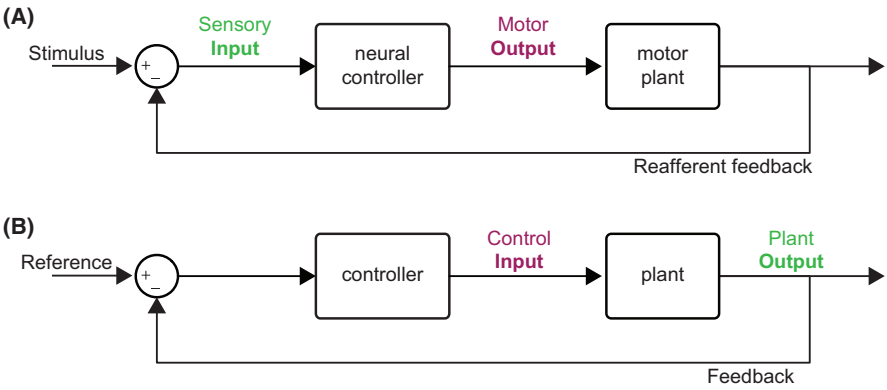

Fig. 8.3 Simplified block diagram representations of a biological system (**A**) and an engineering feedback control system (**B**) with the same topology. Biology and engineering differ in their nomenclature of inputs and outputs. In biology, input typically refers to the sensory signals and output refers to the motor commands from the neural controller. In engineering, input refers to the signal generated by the controller that drives the plant and output refers to the signals generated by plant dynamics

the user to design a "controller" that drives states (relevant parameters) of the plant to desired values. The same techniques of design can be inverted to analyze neural control. In this case, the plant is the animal, more precisely, its motor system and its physical interaction with environment.

Importantly, neuroscientists and engineers use semantically opposite terminology to refer to the same network (Fig. 8.3). In engineering, *inputs* refer to the signals sent by the control system to the plant, whereas *outputs* refer to signals measured by sensors. In neuroscience, *inputs* refer to signals measured by the nervous system, whereas *outputs* refer to motor activity.

8.4 Electric Fishes as a Model System for the Study of Feedback Control

The challenges of studying the closed-loop dynamics that dominate active sensing can be, in part, mitigated through the choice of animal model systems and behavioral tasks. Specifically, behavioral systems that facilitate the manipulation of feedback signals are particularly useful for the application of control-theoretic approaches.

Weakly electric fishes are unusually well-suited animals for these sorts of approaches due to at least three features. The first and most important is the self-generated electric field that facilitates the manipulation of sensory feedback. Second is a ribbon fin for locomotion that permits these fish to swim omnidirectionally (Blake 1983; Snyder et al. 2007). This high degree of maneuverability affords nearly

symmetrical forward and backward swimming performance that allows (Lannoo and Lannoo 1993; MacIver et al. 2001) the animal to accurately track a longitudinally moving refuge. Finally, these fish use a combination of movement and sensory feedback mediated by the electric field in multiple behaviors. Such behaviors include refuge tracking, social interactions, and prey capture, each of which features categorically different task parameters and goals.

8.4.1 Electric Field

The primary feature that makes weakly electric fish well suited for the exploration of the relationships between the motor and sensory systems via manipulation of feedback signals is the electric field. The electric field is generated by an electric organ in the tail and along the sides of the animal. The electroreceptors that detect the electric field are embedded in the skin across the body. Electrosensory systems share properties with both the visual and auditory systems. Like the visual system, the signal propagates at the speed of light and there a topographic representation of the external spatial world across the receptor array. Objects that are closer to the receptor array cast sharper gradients along their edges than objects that are further away (Babineau et al. 2006).

Like auditory systems, electrosensory information is encoded in relation to the frequency and amplitude of signals. Indeed, for social signals in which the electric fields of two or more individuals interact, the interaction can be described with regard to the frequency of modulations of both amplitude and phase and/or timing (see Metzen and Chacron, Chap. 9). Similarly, reafferent feedback caused by changes in the animal's electric organ discharge (EOD) and/or by the movement of the animal also affect the amplitude and phase perceived by the fish.

These spatiotemporal properties of electrosensory signals are often linked to task-dependent categories of electrosensory feedback. For example, the interaction between a swimming weakly electric fish and small prey items leads to relatively slow (approx. <10 Hz), localized activation of electroreceptors (Nelson and MacIver 1999), whereas social signals can lead to faster (approx. >10 Hz), global (entire receptor sheet) activation of the electroreceptors (Chacron et al. 2003; Cowan and Fortune 2007). The spatiotemporal properties of feedback from different tasks can overlap, leading to degradation of performance.

Electrosensory feedback is pervasive across tasks in weakly electric fishes. Because the spatiotemporal parameters of the electric field are closely tied to the movement of the fish, almost all behaviors performed by these fish result in modulations of electrosensory feedback. This includes social communication in which the relative motion of two fish can be perceived by both individuals.

Critically, electrosensory feedback from both movement and social communication, are amenable to the types of perturbations used in control-theoretic approaches to the study of the motor and sensory systems. This is due, in part, to analog and digital technologies for the generation and control of electrical signals. Specifically,

electrosensory feedback can be detected and characterized through direct measurement of the electric field and via video monitoring of the position of the fish. This feedback can, in turn, be modulated in real time via artificial electric signals generated in the tank or via manipulations of the movements of nearby objects.

8.4.2 Ribbon Fin

Weakly electric gymnotiform fishes and one group of mormyroid fishes (*Gymnarchus*) use longitudinal undulating fins to generate primary locomotor forces. These fins use traveling deformations along the length of the fin to produce force in either a rostral or caudal direction (Blake 1983). In many species of gymnotiform fishes, fish produce counterpropogating waves that can increase manueverability in the fore-aft direction (Sefati et al. 2013). Indeed, some species, such as *Eigenmannia*, are like aquatic hummingbirds, using the counterpropagating waves to hover in position and make small, precise fore-aft movements.

Ribbon fin locomotion offers a distinct advantage in the study of sensory feedback because locomotion can occur without bending or movement of the body axis. In this way, propulsive movements, which can be restricted to the ribbon fin, are decoupled from task-oriented and active sensing-oriented movements. In *Eigenmannia*, this specialization enables the fish to use tail-bending movements for active sensing (Stamper et al. 2012) because they do not need to rely on them for locomotion as in so many other species (Colgate and Lynch 2004). In weakly electric fishes that use ribbon fin locomotion, measurements of body position and pose are strongly correlated to task-oriented and active sensing-oriented goals rather than to locomotor mechanism. Indeed, ribbon-finned propulsion may have evolved, in part, as a mechanism to stabilize electrosensory information by decoupling locomotor-based contamination.

8.5 Experimental Control of Feedback in Weakly Electric Fishes

As described in Sect. 8.3.2, the manipulation of feedback signals is an important component of control-theoretic approaches to the study of animal systems. Unlike stimulus/response paradigms where a predetermined stimulus is played to the animal and the response is measured, control-theoretic approaches use stimuli that are generated (at least in part) by measuring behavior in real time and manipulating feedback to the animal. The result is that the stimulus reflects the consequences of the ongoing behavior of the animal (Kim et al. 2018), as is the case in naturally occurring behaviors. There is a rich history of such manipulation in electric fish, particularly with the study of the jamming avoidance response, and more recently, this approach has been used in the analysis of refuge tracking.

8.5.1 Closing the Loop on Social Behavior

When two or more weakly electric fish are in close proximity, within about 1 meter of each other, the electric fields sum, often producing emergent modulations of the amplitudes and phases/timing of electrosensory signals (Tan et al. 2005). For wave-type fish, the amplitude and phase modulations occur at the difference frequency between a fish's EOD and that of a conspecific (Heiligenberg 1991a). These modulations can be detected by electroreceptors in the skin of these fishes and such modulations used in social communication (see Metzen and Chacron, Chap. 9). Unlike the amplitude and phase modulations that are produced by small prey items, social signals are broad and diffuse, often affecting the entire electrosensory array of the animal (Chacron et al. 2003).

The diffuse, spatially distributed electrosensory interference caused by conspecific signals is a key feature that facilitates the study of the neural mechanisms of social signaling. Because social signals are spatially diffuse, artificial social signals can be delivered using simple pairs of electrodes around the fish, with little sensitivity to the specific geometry. Indeed, many studies rely on stimulus geometries that are experimentally convenient but not biologically plausible (e.g., across the body of the fish).

The social behavior that has been studied most intensively is the jamming avoidance response (JAR) in *Eigenmannia*. The JAR remains one of the best understood behaviors, from the level of the entire organism to the contributions of single neurons within computational networks in the central nervous system. The analysis of the JAR was facilitated not only by the geometry of social signals but also critically by the ability to manipulate feedback signals. This was possible, in part, due to two "quirks" of the organization of the control system for the JAR. First, *Eigenmannia* do not have internal feedback signals (efference copy or corollary discharge) within the brain of the fish (see Perks and Sawtell, Chap. 11). As a result, the sensory feedback used in the control of the JAR is mediated solely through external sensory cues that are experimentally accessible. Second, the JAR behavior does not require movement of the animal; it can be elicited reliably in immobilized fish.

But perhaps the most important tool used in the decoding of the JAR is the modulation and even replacement of the natural electrosensory (reafferent) feedback loop. This can be achieved using a combination of pharmacological blockade of the electric organ and replacement of the autogenous electric field with an artificial mimic (fictive EOD). In this way, the natural electrosensory feedback loop is eliminated (see Fig. 8.4). This manipulation enables the replacement of natural feedback with experimentally defined signals. Critically, although the production of the electric field is blocked, the neural signal that controls the electric organ remains intact and can be monitored using electrodes placed adjacent to the animal. As a result, the responses of the fish to manipulations and modulations of electrosensory feedback can be directly measured and used in real time for the generation and modulation of the experimentally defined signals presented to the animal.

The classic experiments of Heiligenberg (1991a,b) relied on manipulation of feedback loops for the control of the JAR. Electrosocial signals were presented in two different external feedback topologies. In a closed-loop topology, artificial signals were tied to the output of the neural control system of the animal by triggering the production of the simulation of the fish's own electric signal based on the timing of spinal activity for the control of the electric organ. In an alternate, open-loop topology, the signals were generated independent of the activity of the neural control system (see Fig. 8.4).

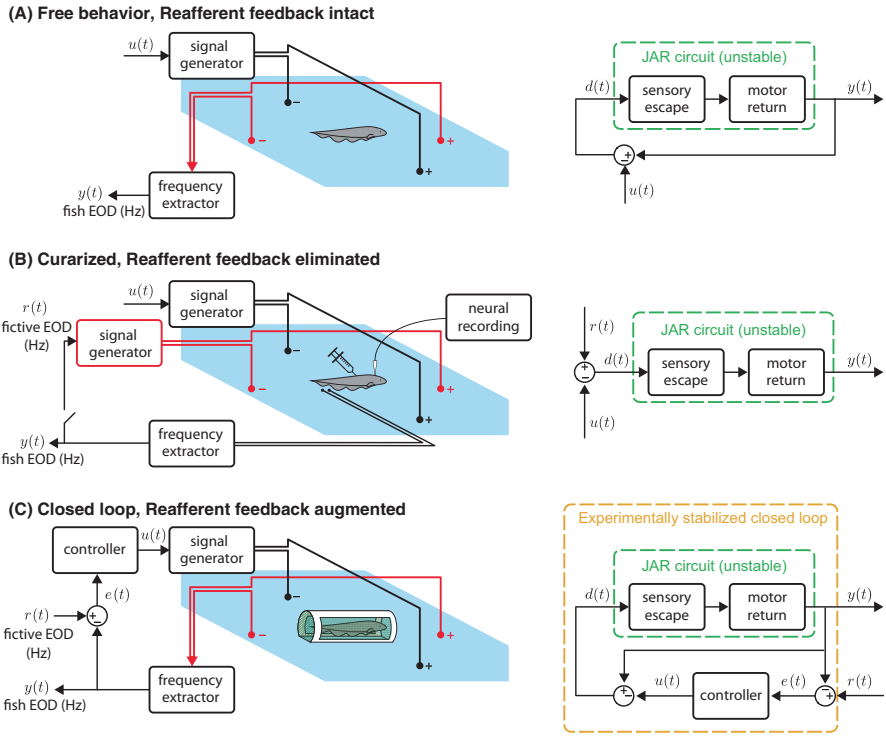

Fig. 8.4 Three different experimental topologies for investigating the jamming avoidance response (JAR) circuit. *Left:* experimental setup; *right:* control topology. **A:** an intact animal responds to the interaction between its own signal [$y(t)$] and artificially generated conspecific electrosocial signals [$u(t)$] that sum to produce $d(t)$. EOD, electric organ discharge. B: pharmacological blockade (syringe) of the electric organ eliminates natural feedback from the fish's own signal [$y(t)$]. Electrodes placed very close to the electric organ can detect the residual EOD $y(t)$ to control the generation of an artificial mimic [$r(t)$] of the fish's own EOD (fictive EOD). Alternatively, $r(t)$ can be independent of the fish's EOD, thereby creating an open-loop experimental condition. **C:** addition of an augmented feedback control system that controls the artificial signal [$r(t)$] in relation to the fish's intact EOD [$y(t)$] allows moment-to-moment manipulation of reafferent electrosensory feedback [$e(t)$]

Precise manipulations of feedback were the key to unlocking the computational organization of the JAR. For example, the spatial organization of feedback was modified to show how the relative phase of the autogenous and heterogeneous signals are computed from the spatial distribution of phase differences across the electroreceptor array (Heiligenberg 1991b). Similarly, the sensitivity to beat rate (at the difference frequency) and not the individual frequencies of the autogenous and heterogeneous electric fields was demonstrated by generating the same temporal pattern of beats using equally spaced pairs of frequencies in open-loop experiments.

There are very few vertebrate behaviors (the JAR among them) for which the complete computation and neural control system has been elucidated from sensory afferents, through central nervous system circuits, and to motor outputs. The classical description of the JAR was made without a formal quantitative model of the behavior. In contrast, the physiological description of the vestibulo-ocular reflex (VOR) relied on detailed engineering control systems modeling (Robinson 1976). Such modeling has been vital in the analysis of a wide range of other sensorimotor systems (Cowan et al. 2014; Roth et al. 2014) and served an essential role in interpreting neurophysiological activity underlying the VOR (Robinson 1977). Analogous modeling of the dynamics of the JAR (e.g., in the form of differential equations and feedback control theory) was not completed until recently (Madhav et al. 2013), decades after the pioneering work describing the underlying circuitry.

Unlike the VOR, which is a *stable* sensorimotor control system, the JAR is an "escape behavior" and is *unstable* in the sense that trajectories are dynamically driven away from the equilibrium condition that would occur if the heterogeneous signal were precisely matched in frequency to the fish's own EOD (i.e., identical frequencies, 0-Hz beat frequency; Madhav et al. 2013). Standard stimulus–response techniques rely on small perturbations from an equilibrium; these perturbations would inevitably drive an unstable system away from this unstable equilibrium. Thus, modeling the dynamics of the unstable JAR is challenging.

To overcome this, a novel experimental topology was developed that utilized a new layer of feedback (Madhav et al. 2013). The concept involves stabilizing the naturally unstable biological system by applying artificial, low-latency closed-loop feedback signals during an experiment. This allows the application of system identification techniques to the artificially stabilized system. The model thus computed is of the combined system, the animal along with the artificial feedback. However, because the feedback is a deterministic quantity that is computed via an algebraic relationship to the biological variables, the model for the underlying, unstable biological system can be "reverse engineered" post hoc.

In this particular work, the JAR was stabilized around the unstable equilibrium of being "exactly jammed" by using a closed-loop artificial feedback system (Fig. 8.4). Specifically, the system measures the frequency of the EOD of the fish and generates an artificial sine wave at that frequency, which is presented to the fish. To stabilize the EOD frequency of the fish, when its frequency drifts lower, for example, the frequency of the artificial signal can be moved slightly lower yet, eliciting a JAR that drives the fish's frequency back to the original frequency. Through this constant

measurement of the frequency of the EOD while eliciting the JAR behavior to drive the fish's EOD frequency up and down, the EOD frequency can be artificially stabilized. Once stabilized, the JAR dynamics can be elucidated using standard engineering system identification analysis (Cowan et al. 2014), resulting in a new, parsimonious representation of this classical system.

This parsimonious representation affords two advantages. First, the response of the system to any input stimulus can be simulated. Second, the structure of the model constrains the possible neural mechanisms. For example, the model for the JAR is composed of two abstract mathematical components. The "escape," which causes the fish's frequency to diverge from that of the interfering conspecific, and the "return," which is a spring-like component that causes the fish's frequency to converge back to a preferred frequency in absence of interference, a return that can be altered with long-duration jamming signals (Oestreich and Zakon 2005).

It was discovered that the escape function is different for each individual and is also asymmetrical in the direction of frequency shifts. The return, on the other hand, is symmetrical and highly stereotyped across individuals. This high-level model thus indicates developmental or social factors that might shape the plastic neural mechanics of an individual's escape function while keeping the circuitry responsible for the return essentially unchanged.

8.5.2 Closing the Loop on Refuge Tracking

Another example in which natural feedback loops can be experimentally modulated is in refuge-tracking behavior (Rose and Canfield 1993; Cowan and Fortune 2007). In this behavior, which occurs in several species of weakly electric fishes, individuals swim forward and backward to maintain their position within a refuge (Roth et al. 2011; Stamper et al. 2012). The neural goal of the refuge-tracking task is similar to the VOR: to stabilize a sensory image on receptor arrays. When either the refuge or fish moves, the sensory image of the refuge is translated proportionally along the receptor surface. The fish detects this "sensory slip" and swims to stabilize the position of the image on receptor arrays (Cowan and Fortune, 2007; Roth et al. 2011).

Contemporaneously, fish commonly produce transient "va-et-vient" back-and-forth movements (Toerring and Møller 1984) that are used for active sensing. These movements are often in direct conflict with the task-oriented goal of following the position of the refuge (Fig. 8.2). Indeed, these back-and-forth movements significantly increase the swimming effort used in tracking the movement of the refuge (Stamper et al. 2012).

These back-and-forth movements were shown to be a form of active sensing because they are modulated by the sensory information available to the fish (Stamper et al. 2012). The fish produce larger back-and-forth movements in the dark than in the light. Similarly, the magnitudes of these active-sensing movements increase as the conductivity of the water is increased (Stamper et al. 2012). Increasing conductivity decreases electrosensory salience by decreasing sensory volume (Snyder et al. 2007),

thereby affecting the contrast of nearby electrosensory images such as the refuge wall (Babineau et al. 2007). These results are interpreted to indicate that the active-swimming movements are specifically produced to alter the spatiotemporal patterns of feedback through the electrosensory system (Stamper et al. 2012).

Both the task-directed movement, swimming to maintain position with the refuge, and the movements for active sensing determine the relative velocity of the object and the fish and therefore determine the temporal patterns of spiking in electroreceptors. Because the reafferent stimulation for both task-oriented and active-sensing movements is mediated by the same sensorimotor systems in the same linear dimension (rostrocaudal axis of the fish), it is difficult to determine whether any specific movement is related to the task goal or active sensing.

To carefully examine the roles of reafferent feedback in refuge tracking, the feedback needs to be controlled deterministically and repeatably. This was done (Biswas et al. 2018) by detecting the position of the fish and altering the trajectory of the refuge in real time to produce an experimentally controlled error signal. The feedback was thus altered to produce an "augmented reafferent feedback" system (Fig. 8.5). Normally, if a fish were to swim 1 cm forward within a normal refuge, the fish would experience a 1-cm head-to-tail relative slip of the sensory image from the refuge. Using this closed-loop experimental setup, as the fish moves, the sensory slip can be reduced (by moving the refuge in the same direction) or amplified (by moving the refuge in the opposite direction).

Experimental changes in reafferent gain likely have different impacts on task-dependent swimming and active sensing. On the one hand, because the goal of refuge tracking is to maintain position within the refuge, changes in reafferent gain are expected to result in commensurate changes in swimming: the fish may cancel the experimental gain through increases or decreases in its own swimming. On the other hand, the impacts of experimental changes in reafferent gain on the production of back-and-forth active-swimming movements depends not on the tracking task but on the sensory goal. How might sensory feedback be used to manage these two simultaneous behavioral goals, tracking and active sensing?

To answer this sort of question, it is useful to "separate" the roles of movement toward achieving the task and in active sensing as much as is possible. For example, in an effort to examine the control of active sensing in refuge tracking, one can reduce the task-level behavior (remaining in the refuge) by not moving the refuge. That is, the fish could achieve the task by simply remaining in place. Any movements of the fish were therefore a result of either active sensing, tracking error, or other unrelated movements.

Fish produced active-sensing movements under these conditions that were, as expected, modulated by lighting conditions. When the fish were in the light and could use visual cues, their active movements were small. In the dark, however, fish rely more almost exclusively on electrosensory cues and produced large active movements. By changing the reafferent feedback gains while measuring active movements, it was shown that fish maintain the sensory slip between the fish and the refuge. Fish use a strategy that minimizes energy expenditure: the fish performed longer drifts with fewer reversals of direction (Biswas et al. 2018).

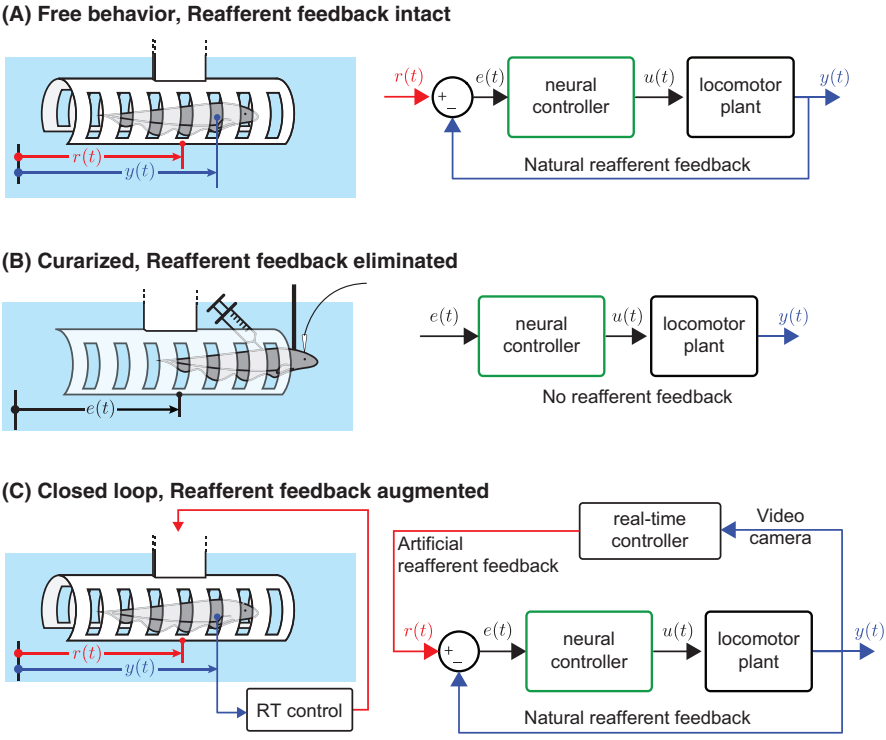

Fig. 8.5 Three different experimental topologies for investigating the refuge-tracking behavior. *Left:* experimental setup; *right:* control topology. A: an intact animal moves freely [$y(t)$] back and forth in a refuge [$r(t)$] at position $y(t)$. The fish receives natural reafferent feedback in the form of sensory slip: $e(t) = r(t) - y(t)$. **B:** reafferent feedback can be eliminated by immobilizing fish via pharmacological blockade of neuromuscular junctions. Neural recordings can be made while moving the refuge with previously recorded $e(t)$ or other trajectories. **C:** addition of a real-time controller for the refuge position [$r(t)$] based on feedback of the animal's position [$y(t)$] allows moment-to-moment manipulation of the $e(t)$ signal in the intact, freely swimming fish

These sorts of augmented reality systems can also be used as a form of behavioral clamp. For example, reafferent feedback arising from the fish's own swimming can be eliminated by moving the refuge to precisely match the fish's own movements. Under this "reafferent clamp" condition, the refuge can be used to impose any arbitrary stimulus by superimposing desired signals on top of the behavioral clamp. In this way, for example, previously recorded reafferent signals can be replayed to the freely swimming fish.

The ability to play back an arbitrary reafferent signal to a freely moving animal is generally applicable and could be a powerful tool in neurophysiological studies. Neuronal spiking activity is inherently noisy, and therefore, any signal stimulus–response pair will not fully represent the underlying relationships between them. To address this issue, neuroscientists typically present multiple repeats of a stimulus

while recording the activity of neurons, permitting the characterization of the firing statistics, including the structure of the noise and the response.

This approach has proven challenging in awake, behaving animals: the timing and production of behavior is both subject to its own variation and under the control of the animal. This has been addressed by observing long bouts of free behavior and "binning" the resulting behaviors into similar epochs, effectively relying on behavioral serendipity to probe the stimulus space.

A potential advantage of behavioral clamps in virtual or augmented reality systems, in contrast, is that they allow the presentation of test signals designed to address specific questions about neural coding in freely behaving animals. It is sensible (and standard) to replay exafferent signals that mimic the sensory experience of a stationary animal to an immobilized animal. In contrast, feedback from active-sensing movements only makes sense in the context of freely moving animals, and augmented reality systems provide a novel means by which to replay stimuli in a behavioral relevant context.

8.6 Summary

Sensing is not a static process but rather is dynamically tuned by the animal depending on the task it is performing and its social and sensory context. Active sensing can be used in at least five ways, including to generate, amplify, maintain, or direct sensory signals or orient receptor arrays. Animals can use a variety of strategies for active sensing that often include the generation of reafferent feedback via movement. These movements for active sensing are produced contemporaneously with movements for achieving behavioral tasks and can be independent of, orthogonal to, or in conflict with the goals of the task. The interplay between task-oriented and active-sensing behavior requires specialized strategies to disentangle the relationships between sensory and motor signals. Control theory, the study of the behavior of dynamical systems and feedback regulation, provides the tools and approaches for decoding the structure of these systems. Specifically, control theory highlights the critical role of reafferent feedback in behavioral control and enables the application of experimental modulation of feedback topology as an approach to understanding the organization of biological control systems.

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Noah J. Cowan declares that he has no conflict of interest.

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Chapter 9
Envelope Coding and Processing: Implications for Perception and Behavior

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Abstract How envelopes are processed in the electrosensory system and how this gives rise to behavioral responses has been the focus of extensive research. This chapter provides a comprehensive review on the mechanisms the brain exploits at different stages of sensory processing to extract meaningful information about these stimulus attributes and how this mediates behavioral responses. After a brief review of the relevant anatomy and circuitry, the natural statistics of envelopes in the electrosensory system are discussed in detail. This is followed by a review of the current state of knowledge as to the cellular and network mechanisms that give rise to envelope responses in the electrosensory system. In particular, it is highlighted how electrosensory neurons can optimally encode envelopes by matching their tuning properties to natural statistics. There is an emphasis throughout the chapter on the important parallels with the mammalian auditory and other systems, along with interesting future avenues of research.

Keywords Envelope · Information theory · Neural coding · Neuromodulation · Parallel processing · Second-order statistics · Sensory processing · Wave type · Weakly electric fish

9.1 Introduction

The electrosensory system of weakly electric fish has long served as a model for understanding how neural circuits extract the relevant features of incoming sensory input to give rise to behavior. This is exemplified by the classic work of Heiligenberg (1991) on the neural circuits that give rise to the jamming avoidance response (JAR) described in detail in Sect. 9.3.2. Continuing in this tradition, research in the

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electrosensory system has focused on how other stimulus features are processed by the brain to give rise to behavior.

This chapter provides a comprehensive review of how envelope stimulus features are processed by the electrosensory system. Natural sensory stimuli frequently consist of a fast time-varying waveform (i.e., first-order attribute) whose amplitude (i.e., second-order attribute or envelope) varies independently on a longer timescale (Lewicki 2002; Theunissen and Elie 2014). The temporal envelope thus can be described as the line connecting successive peaks in the stimulus waveform. Envelopes are found ubiquitously in natural sensory input and have been found to carry information that is critical for perception in many sensory systems. This includes speech recognition in the auditory system (Smith et al. 2002; Zeng et al. 2005), contrast discrimination in the visual system (Mante et al. 2005), the amplitude of whisking in the somatosensory system (Fee et al. 1997), or the determination of distance and orientation of a conspecific in the electrosensory system (Yu et al. 2012). Understanding how envelopes are processed in the brain has, however, proven a challenging problem, which is, in part, because nonlinear operations are necessary. Here we review how envelopes are processed by the electrosensory system of weakly electric fish, thereby giving rise to behavioral responses. Throughout, this chapter emphasizes parallels with other sensory systems. The chapter is organized as follows: after a brief introduction to weakly electric fish (Sect. 9.2), behaviorally relevant envelope stimuli are first explained before covering the pathways and mechanisms that are involved in their processing in the electrosensory system.

9.2 Envelopes in Weakly Electric Fishes

9.2.1 The Electrosensory System

The electrosensory and auditory systems are both components of the octavolateralis system and therefore presumably represent the products of evolutionary processes acting to modify common ancestral mechanosensory systems. Indeed, both auditory and electrosensory peripheral receptors are derived from sensory hair cells (Duncan and Fritzsch 2012; Modrell et al. 2017). However, in the electrosensory system, the principal receptors are stimulated by electric current, whereas the driving stimulus in the auditory system is due to mechanical movement of the signal-transducing structures (Crawford 1992; Hudspeth 2014). Furthermore, it has been suggested that similar physiological and morphological adaptations could improve temporal coding in both sensory systems (Carr 2004; see also Carlson, Chap. 10).

Gymnotiform weakly electric fish possess a specialized electric organ whose discharges generate an oscillating electric field (i.e., the electric organ discharge [EOD]) around the animal's body. Perturbations of this field due to objects or the EODs of conspecifics in the vicinity are sensed by peripheral P-type tuberous electroreceptors. These receptors give rise to electrosensory afferent (EA) fibers whose

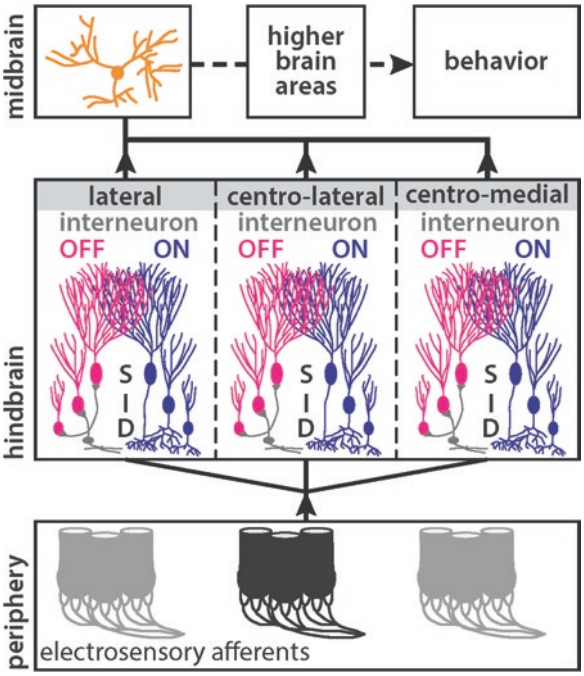

Fig. 9.1 Electrosensory pathway. Electric organ discharge (EOD) perturbations are sensed by an array of peripheral electroreceptors (*bottom*) in the skin of the fish that synapse onto deep (D), intermediate (I), and superficial (S) ON- and OFF-type pyramidal cells within three maps (lateral segment [LS]; centrolateral segment [CLS]; centromedial segment [CMS]) the hindbrain electrosensory lateral line lobe (ELL; *center*). ON-type pyramidal cells receive direct electrosensory afferent (EA) input, whereas OFF-type pyramidal cells receive disynaptic input via an inhibitory interneuron. Pyramidal cells synapse onto neurons within the midbrain torus semicircularis (TS; *top left*). From there, information is relayed through higher brain areas to finally induce behavior

probability of firing increases with increasing EOD amplitude. Each EA trifurcates and projects in a topographical fashion to three maps within the electrosensory lateral line lobe (ELL) of the brain (Fig. 9.1, *bottom*), the structural organizations of which are identical: the centromedial (CMS), centrolateral (CLS), and lateral (LS) segments (Fig. 9.1, *center*; Krahe and Maler 2014). It should be noted that the medial segment (MS) of the ELL receives input from ampullary afferents responsible for detecting exogenous electric fields (i.e., passive electrolocation) that are not considered here.

Within each ELL map, EAs terminate ventrally in the deep neuropil layer consisting of GABAergic interneurons and the basilar dendrites of pyramidal cells. Pyramidal cell apical dendrites reach into the molecular layer and receive feedback signals from higher order brain areas. Pyramidal cells can be divided into multiple classes; the most basic distinction is between ON- and OFF-type cells (Clarke et al. 2015). ON-type cells (Fig. 9.1, *center, blue*) have a basilar dendrite receiving direct

input from EAs and respond with increased spiking activity to increases in EOD amplitude. In contrast, OFF-type cells (Fig. 9.1, *center, red*) lack a basilar dendrite and instead receive disynaptic inhibitory input from EAs via local interneurons (Fig. 9.1, *center, grey*). As such, OFF-type cells respond with decreased spiking activity to increases in EOD amplitude. Based on physiological, morphological, and molecular criteria, both groups can be further subdivided into deep (D), intermediate (I) and superficial (S) cell types (Fig. 9.1, *center*; Maler 2009). Pyramidal cells are the sole output of the ELL and project to the midbrain torus semicircularis (TS; Fig. 9.1, *top left*). TS neurons project to higher brain areas such as the nucleus electrosensorius, which projects to the prepacemaker nucleus. The prepacemaker nucleus projects to the pacemaker nucleus, which then sends command signals to the electric organ, thereby completing the sensorimotor loop. This circuitry is described elsewhere (Heiligenberg 1991; Metzner 1993).

9.2.2 *Envelopes in the Electrosensory System*

In the electrosensory system of wave-type weakly electric fish, envelopes are ubiquitous features of the natural environment (for reviews, see Stamper et al. 2013; Clarke et al. 2015). It is important to note that envelopes in the electrosensory system occur exclusively during interactions with either con- or heterospecific weakly electric fish. Figure 9.2 shows a situation when two fish are located close (<1 m) to one another. Because of interactions between their EODs, each conspecific experiences a sinusoidal amplitude modulation (i.e., beat; Fig. 9.2, *bottom right*) with a frequency that is equal to the difference between the two individual EOD frequencies. This beat arises because of alternating regions of constructive and destructive interference when the instantaneous EOD frequencies do not vary in time (Fig. 9.2, *right*). It is important to realize that the EOD is a carrier and that the relevant stimuli consist of both

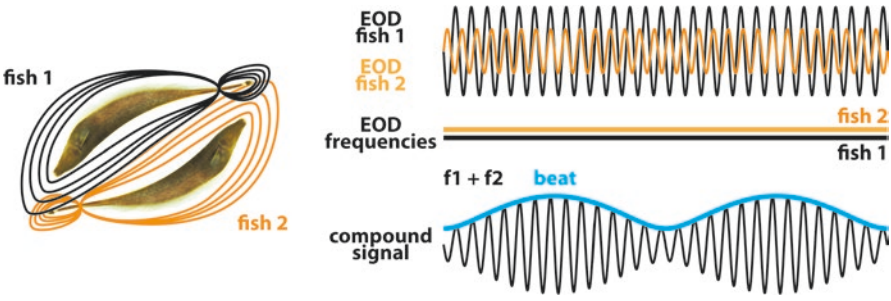

Fig. 9.2 *Left:* two weakly electric fish with their electric fields generated by their individual EODs. The EOD waveforms of both fish (*top right*) show alternating regions of constructive and destructive interference when the instantaneous EOD frequencies do not vary in time (*center right*). Interference between the two EODs leads to a sinusoidal amplitude modulation (i.e., a beat) of the summed signal (*bottom right*)

amplitude and phase modulations, of which the latter ones are not considered here in detail (see Carlson, Chap. 10 for details on detecting phase modulations). The envelope can thus be thought of as the amplitude modulation of the beat. It is worthwhile to note important differences between envelopes in the electrosensory system, which is an active sense, and those occurring in passive senses (e.g., visual, auditory) for which the envelope is instead the amplitude modulation of the sensory signal (e.g., luminance or sound pressure). Apart from this, envelopes in the electrosensory system share many similarities with envelopes found in other systems.

9.2.2.1 Electrosensory Envelopes During Behavioral Contexts

In the electrosensory system, envelopes arise from different interactions between animals, leading to a distinction between movement and social envelopes. For fish that are stationary, the movement envelope is constant, whereas in moving fish, it varies with the relative positions of both fish (Fig. 9.3a). Specifically, the envelope

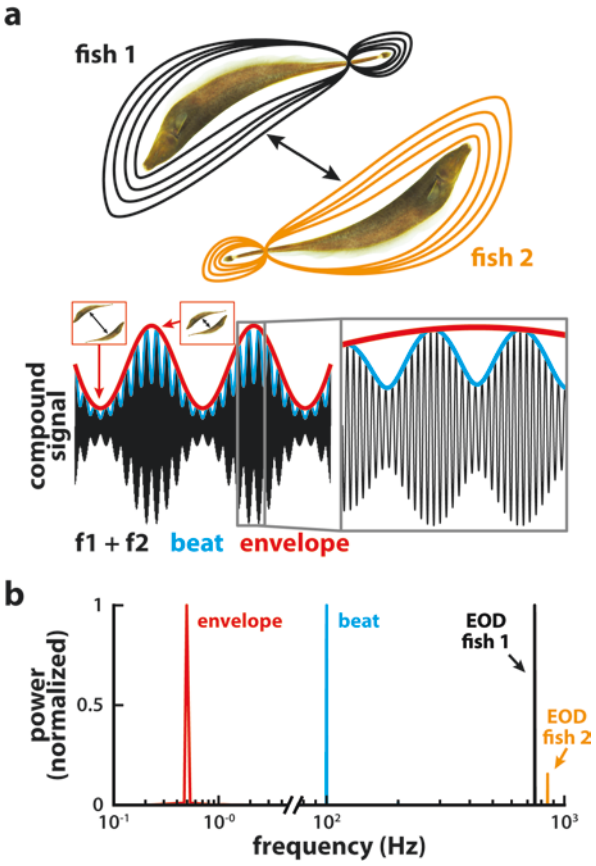

Fig. 9.3 **a:** *Top:* electrosensory movement envelopes are generated by the relative locomotion between two fish. *Bottom:* EOD waveform from *Apteronotus leptorhynchus* (*black*) with beat and envelope waveforms. Note that the envelope corresponds to the depth of modulation of the beat. *Inset, bottom right:* snippet of all waveforms on a shorter timescale. **b:** Power spectra show the power for each of the signals: the individual EOD signals, the beat, and the envelope

increases when the distance between the two fish decreases and vice versa (Fig. 9.3a, *bottom*). In this way, movement creates an envelope by altering the depth of modulation of the beat, which occurs in relation to changes in both the distance and orientation between the two fish (Yu et al. 2012; Fotowat et al. 2013). In general, envelopes caused by movement tend to contain lower temporal frequencies (<1 Hz) than the beats (Fig. 9.3b). The information extracted from the time-varying envelope is correlated with the relative position between the two fish (Yu et al. 2012; Metzen and Chacron 2014).

On the other hand, social envelopes occur when three or more fish are close together and are stationary (Fig. 9.4a). In this case, the EODs of the fish sum and the compound signal then consist of two prominent beat frequencies (Fig. 9.4b, *blue*; Stamper et al. 2012). If the two beats consist of different frequencies, the interaction between these two frequencies create an envelope that oscillates at the difference between the two beat frequencies as they cycle in and out of phase with respect to one another (Fig. 9.4a, b, *bottom*). Importantly, such social envelopes do not require

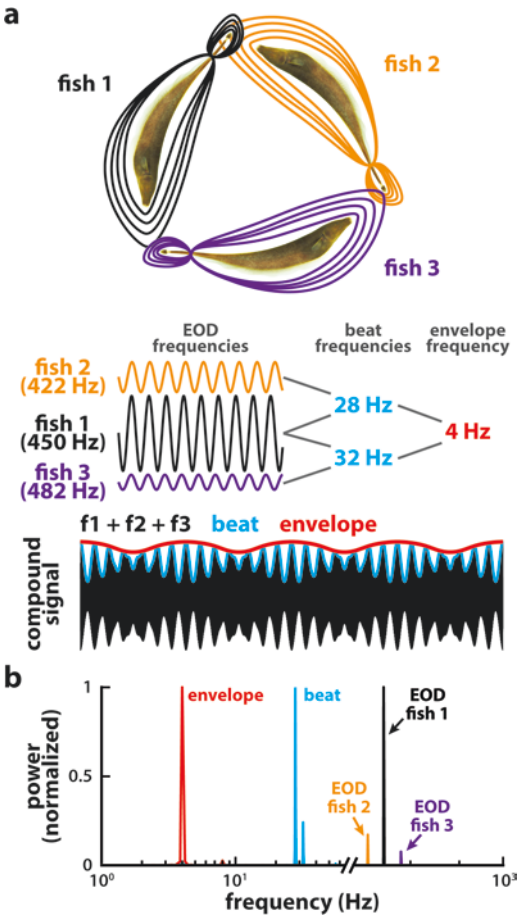

Fig. 9.4 **a:** *Top:* social envelopes are generated by the interaction of the EODs of three or more weakly electric fish. *Center:* three EOD signals with their individual frequencies and relative intensities. Due to the differences in the individual EOD frequencies, two prominent beat frequencies arise that, in turn, create an envelope. *Bottom:* compound signal is the sum of the EOD signals of the three fish that has a beat and an envelope. **b:** Power spectra show the power for each of the signals: the three individual EOD signals, the two beat signals, and the envelope

movement. For example, if there are three stationary fish with EOD frequencies of 450 Hz (fish 1), 422 Hz (fish 2), and 482 Hz (fish 3), then fish 1 would experience two beats with frequencies of 28 Hz and 32 Hz (Fig. 9.4a, *center* and *bottom*). It is the interference between both beats that then creates an envelope with a frequency of 4 Hz (Fig. 9.4a, *center* and *bottom*). In general, social envelopes tend to contain higher temporal frequencies (>1 Hz) than movement related envelopes (Stamper et al. 2010; Fotowat et al. 2013). Importantly, in the natural situation, movement and social envelopes will often occur in conjunction with one another. This is especially the case for gregarious species of weakly electric fish (e.g., *Eigenmannia*). A third class of envelope occurs during natural communication calls and has been reviewed elsewhere (Marsat et al. 2012).

9.2.3 Statistical Properties of Envelopes Found in the Natural Electrosensory Environment

This section reviews some of the known statistical properties of envelopes in the electrosensory system. Electrosensory envelopes that are generated through locomotion have been characterized as a function of the relative movement between two freely swimming fish (Fig. 9.5a; Yu et al. 2012; Fotowat et al. 2013). During periods when two moving fish come close together, the envelope increases, whereas the envelope is much lower during periods when the fish are farther apart (Fig. 9.5b, c; Yu et al. 2012; Metzen and Chacron 2014). Furthermore, the spectral power of movement envelopes mainly consists of low frequencies that decay with increasing frequency, and this can be described as a power-law relationship with negative exponent (Fig. 9.5d; Fotowat et al. 2013; Metzen and Chacron 2014). The fact that spectral power decays as a power law as a function of increasing temporal frequency is a signature of scale invariance (Simoncelli and Olshausen 2001), which has also been found for natural sounds (Lewicki 2002). Growing evidence suggests that the coding strategies of the brain have adapted to the statistics of natural sensory input, thereby allowing optimal information transmission and perception (Wark et al. 2007). As detailed in Sect. 9.4.2.2, there is evidence that the electrosensory system optimally processes envelopes based on their statistical structure, which has strong consequences on the behavioral responses. Before reviewing such evidence, however, behavioral responses to envelopes are reviewed first (see Sect. 9.3).

9.3 Behavioral Responses to Envelopes

The electrosensory system of weakly electric fish is particularly well suited for studying neural coding of envelopes because these animals display clear behavioral responses to envelopes. As seen in Sect. 9.3.1, these are innate (i.e., do not require training) and can be easily elicited in a laboratory setting (Metzen and Chacron

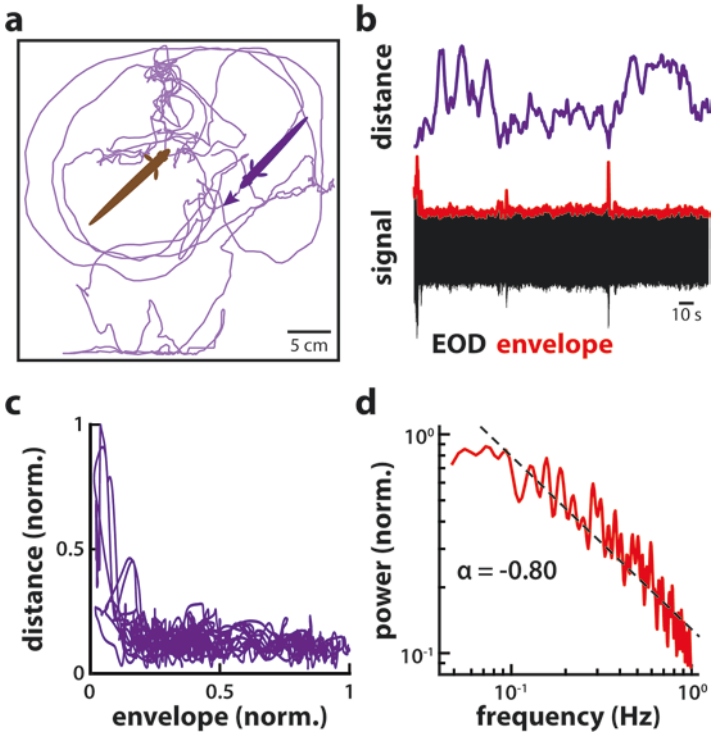

Fig. 9.5 **a:** Movement trajectories of two individual fish where fish 1 (*brown*) is stationary and fish 2 (*purple*) is swimming. **b:** The distance between individuals varies over time as a function of the movement of fish 2, thus creating an envelope. **c:** The amplitude of the envelope depends on the relative distance between two moving fish. **d:** The power spectrum of movement envelopes decays as a function of frequency and can be described by a power-law fit

2014). Importantly, such behavioral responses are also observed in immobilized animals (i.e., do not require actual movement), which greatly facilitates understanding their neural basis because neural recordings can be obtained while the animal behaves.

9.3.1 Movement Envelope Tracking Response

Wave-type gymnotiform weakly electric fish of the genera *Apteronotus* and *Sternopygus* display behavioral responses to movement envelopes (Metzen and Chacron 2014; Martinez et al. 2016). Indeed, when stimulated with either a sinusoidal beat or noise whose envelope varies sinusoidally (Fig. 9.6a, *top*), the animals' EOD frequency actively follows the detailed time course of the envelope around a mean value that is positively offset with respect to the baseline EOD frequency (Fig. 9.6a; *Apteronotus leptorhynchus* [brown ghost knifefish], *orange*; *Apteronotus*

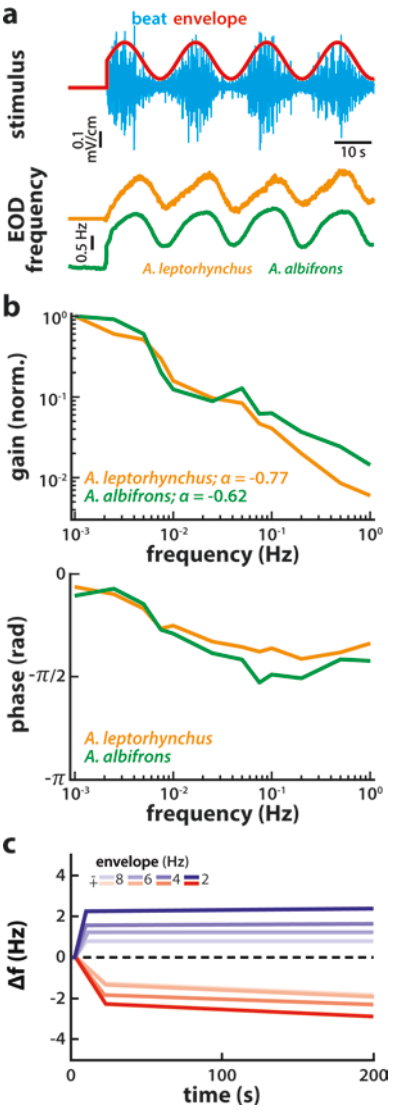

Fig. 9.6 **a:** Example envelope stimulus (*top, red*) and EOD frequency response for two species of the genus *Apteronotus* (*Apteronotus leptorhynchus* and *Apteronotus albifrons*; *bottom*). **b:** Gain (*top*) and phase (*bottom*) as a function of envelope frequency for the two *Apteronotus* species tested. **c:** Stimulus protocol to demonstrate the social envelope avoidance response in *Eigenmannia*. The fish respond to the sums of two sinusoids that contain a low-frequency envelope. Two trial types are shown, where $beat_1 = -50$ Hz and $beat_2 = +52$ Hz (+2-Hz envelope; *blue lines*) and where $beat_1 = -52$ Hz and $beat_2 = +50$ Hz (−2-Hz envelope; *red lines*). The fish shift their frequency down for positive envelopes (*blue trials*) and up for negative envelopes (*red trials*) (Stamper et al. 2012)

albifrons [black ghost knifefish], *green*). This "tracking behavior" demonstrates that detailed information about the low-frequency components of movement envelopes is retained in the brain. Such behavioral responses have been characterized using linear systems identification techniques (i.e., transfer functions). In particular, behavioral sensitivity (i.e., the gain, or the number by which the input must be multiplied to get the output) decreases as a power law with increasing frequency (Fig. 9.6b, *top*). Interestingly, the power law exponent observed for both species is similar to that observed for natural envelopes over more than three orders of magnitude (Fig. 9.6b, *top*), suggesting that this behavior is matched to natural statistics.

In contrast, the phase (i.e., the relative time by which one must shift the input to match the output) indicates that the behavior lags the envelope stimulus and that this lag increases as a function of increasing frequency (Fig. 9.6b, *bottom*). These behavioral responses are plastic as they habituate to repeated stimulus presentations; such changes are thought to be mediated by top-down signals. It is worthwhile to note that envelope-tracking responses consist of relatively small changes in EOD frequency and were thus likely missed in previous studies. Finally, although the functional role of such envelope-tracking behavior remains unclear, studying these behavioral responses and their neural basis has proven invaluable for understanding how envelopes are processed in the electrosensory system.

9.3.2 Social Envelope Avoidance Response

Weakly electric fish also display behaviors in response to social envelopes. Before reviewing these, this section briefly reviews the JAR. Consider the situation described in Fig. 9.3 where interference between the EODs of two fish gives rise to a beat. The beat frequency is then determined by the difference between both EODs and, when low (<10 Hz), can significantly hamper the animal's ability to electrolocate (i.e., to detect prey or other objects in the environment). Some species of weakly electric fishes will then alter their EOD frequencies, thereby increasing the beat frequency and consequently minimizing its deleterious effects. The ethology and neural circuits responsible for the JAR behavior have been fully elucidated and are reviewed elsewhere (Heiligenberg 1991).

Eigenmannia virescens performs a behavior conceptually similar to the above-described JAR but in response to social envelopes (Stamper et al. 2012). It is thus important to note an important distinction between movement and social envelopes. Although movement envelopes are independent of the carrier and contain behaviorally relevant information (e.g., distance), social envelopes are instead completely determined by interference between the EODs of three or more fish and can constitute a nuisance for detecting other more relevant signals (e.g., those caused by prey). Indeed, experiments by Stamper et al. (2012) show that *Eigenmannia* will either increase or decrease its EOD frequency in response to these social envelope stimuli. This behavior was termed the envelope avoidance response (EAR) in relation to the JAR (Watanabe and Takeda 1963; Bullock et al. 1972). Specifically, when an individual fish is presented with two high-frequency (e.g., ~50-Hz) beats whose interferences gives rise to a low-frequency envelope, the animal shifts its EOD frequency (Fig. 9.6c). The amount by which the animal changes its EOD frequency decreases with increasing envelope frequency (Fig. 9.6c). As a result, the change in EOD frequency increases the envelope frequency to be between 5 and 15 Hz (Stamper et al. 2012). It is important to realize that presenting each high-frequency beat by itself will not give rise to such a change in the fish's own EOD frequency. As such, the EAR behavior is different from the JAR. The algorithms and neural pathways that mediate the EAR behavior have been reviewed elsewhere and share some similarities with those used for the JAR behavior (Stamper et al. 2013).

9.3.3 Implications of Behavioral Responses to Envelopes on Their Encoding in the Brain

This section highlights the important point that the envelope-tracking and envelope-avoidance behaviors have been investigated in different species. Importantly, it is not known whether the frequency offset observed during envelope-tracking behavior in *Apteronotus* is related to the EAR in *Eigenmannia*. Although desirable, it is not fully known to what extent a given species can distinguish movement from social envelopes. However, a study by Thomas et al. (2018) on *Apteronotus albifrons* shows that this species is capable of distinguishing between envelopes originating from movement and social interactions. This was demonstrated because the response lag in behavioral tracking was greater for social envelopes with low beat frequencies compared with movement-related envelopes (Thomas et al. 2018). Further studies are, however, needed to understand how these behavioral responses are generated in the brain. Nevertheless, behavior demonstrates that envelopes are relevant in the electrosensory system and thus must be processed in the brain. In particular, the envelope-tracking behavior indicates that information pertaining to the detailed time course of the envelope must be retained in the brain. With these important points in mind, Sect. 9.4 focuses on understanding how envelopes are processed in the electrosensory system.

9.4 Envelope Coding

This section reviews how electrosensory neurons respond to envelopes. It is important to understand that to extract the envelope (i.e., demodulate) of a signal, a nonlinear operation is necessary. This is because the frequency content of the envelope can be quite different from that of the underlying carrier.

9.4.1 Electrosensory Periphery

First, the emphasis is at the level of the electrosensory periphery with EAs. EAs are characterized by high-baseline (i.e., in the presence of the animal's unmodulated EOD) firing rates (200–600 Hz) and increase their firing rates linearly with increasing EOD amplitude over a certain range (Chacron et al. 2005; Gussin et al. 2007). However, EAs display the fundamental property of excitability and can fire, at most, one action potential per EOD cycle (Scheich et al. 1973). As such, large (typically >40% of baseline) changes in EOD amplitude will elicit nonlinear responses that consist of either rectification (i.e., cessation of action potential firing) or saturation (i.e., the firing rate is maximum and less or equal to the EOD frequency).

9.4.1.1 Envelope Coding by Single Electroreceptor Afferents

As mentioned in Sect. 9.4.1, nonlinear operations are necessary for a neuron to respond to envelopes. Mathematical models have suggested that electroreceptors could respond to envelopes provided that the envelope signals exceed a certain threshold amplitude value (Longtin et al. 2008; Yu et al. 2012). These have been confirmed by experimental studies. Indeed, EAs tend to respond to low-amplitude beats with modulations in firing rate that are linearly related to the beat and with nonlinear phase locking (i.e., firing during only a portion of the beat cycle) when the amplitude is high (Metzen et al. 2016; Metzen and Chacron 2017). This has important consequences for determining whether a single EA responds to the envelope or not. If the envelope amplitude is low enough, then the increase in firing rate during one half of the beat cycle will be compensated for by the decrease in firing rate during the other half of the beat cycle. As such, the average firing rate during each beat cycle is then the same. However, the situation changes when the envelope amplitude is large enough to elicit static nonlinearities such as rectification and/or saturation because changes in envelope will then elicit changes in the overall firing rate. The exact threshold for which EAs respond to envelopes depends on multiple factors such as the baseline firing rate as well as the beat frequency content (Savard et al. 2011; Stamper et al. 2013).

The tuning properties of EAs to movement envelopes have been investigated in detail. EAs form two classes based on whether their responses are in or out of phase with the envelope. While one afferent class increases its spiking activity in response to increases in the envelope (Fig. 9.7a, *left, orange*), the other class instead responds with decreased spiking activity (Fig. 9.7a, *left, green*) and thus have phase preferences with respect to the envelope that are shifted by 180° (Fig. 9.7a, *right*). Despite this opposing response profile to envelope stimuli, the sensitivities (i.e., the gain) of both classes to the frequencies contained in movement envelopes are not significantly different from one another (Fig. 9.7a, *center*). The gain of the response to the sinusoidal envelope is largely independent of beat frequency. This is because the changes in firing rate elicited by envelopes can be accurately predicted from the linear filtering properties to first-order stimuli and static nonlinearities (Metzen and Chacron 2015). Consider two cases illustrated in Fig. 9.7b and assume that the envelope varies slowly with respect to the beat (i.e., that the envelope does not vary much during one cycle of the beat and thus is effectively constant). An EA with a low-baseline firing rate responds (Fig. 9.7b, *bottom left*, *solid orange curve*) in a linear fashion to the sinusoidal stimulus (Fig. 9.7b, *top left*) except for stimulus phases for which the firing rate is equal to zero (i.e. rectification). For comparison, the hypothetical firing rate response in the absence of rectification is also shown (Fig. 9.7b, *left*, *dashed orange curve*). The mean firing rate response (Fig. 9.7b, *left*, *solid orange line*) is then positively offset with respect to its value in the absence of rectification (Fig. 9.7b, *left*, *dashed black line*). It is then easy to see that sinusoidal stimuli of larger amplitude will give rise to more rectification (i.e., the firing rate will be zero during a greater portion of the stimulus cycle), leading to a greater posi-

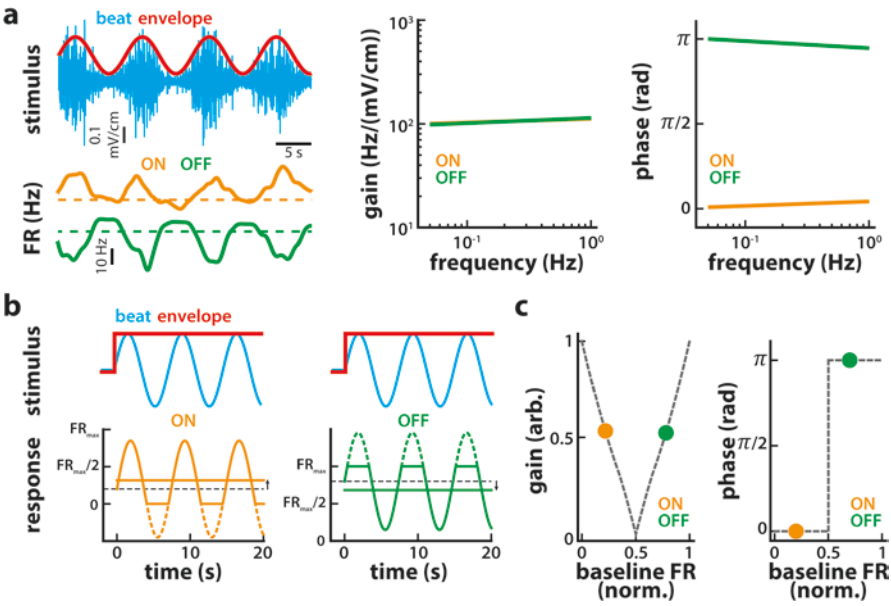

Fig. 9.7 **a:** Typical responses of ON- and OFF-type EAs (*left bottom*) to a sinusoidal envelope (*left top*). While ON-type EAs respond to an increase in envelope with an increase in firing rate, OFF-type EAs respond to an increase in envelope with a decrease in firing rate. Gain (*center*) and phase (*right*) to the envelope as a function of envelope frequency for ON- and OFF-type EAs. **b:** Schematic showing why EAs with low-baseline firing rates (*left*) and high-baseline firing rates (*right*) give ON- and OFF-type responses, respectively, to the envelope of a sinusoidal stimulus. While ON-type EAs display rectification at zero, causing an increase in mean firing rate (*bottom left, horizontal orange line*) during stimulation compared with what it would be if no rectification occurred (*bottom left, dashed horizontal black line*), OFF-type EAs display saturation, causing a decrease in mean firing rate decreases (*bottom right, green horizontal line*) during stimulation compared with what it would be if no saturation occurred (*bottom right, dashed black horizontal line*). *Dashed orange* and *green lines*, responses without the static nonlinearity. **c:** *left:* gain to the envelope as a function of normalized baseline firing rate (i.e., the baseline firing rate divided by the maximum firing rate obtained during stimulation; *solid circles*) displays a minimum when the normalized firing rate is equal to 0.5. *Right:* phase of the response as a function of normalized baseline firing rate. Responses were ON-type when the normalized baseline firing rate was less than 0.5 and OFF-type otherwise (*solid circles*)

tive offset of the mean and thus the response of low firing rate EAs will be in phase with the envelope.

In contrast, the right panel of Fig. 9.7b shows the response of an EA with a high-baseline firing rate just below the maximum firing rate. This neuron displays saturation in response to the sinusoidal stimulus (Fig. 9.7b, *right*, *solid green curve*). Thus, the mean firing rate is then negatively offset (Fig. 9.7b, *right*, *solid green line*) with respect to its value in the absence of saturation (Fig. 9.7b, *right*, *dashed black line*). Stimuli of larger amplitudes will elicit more saturation and thus a greater negative offset. The response of EAs with high-baseline firing rates will then be out of phase with the envelope. In this context, the gains of the envelope response for

EAs whose baseline firing rate is in the middle of the dynamic range are predicted to be smaller than for EAs displaying either low- or high-baseline firing rates (Fig. 9.7c, *left*) Moreover, responses of EAs with normalized baseline firing rates less than 0.5 are in phase with the envelope stimulus, whereas those of EA-normalized baseline firing rates greater than 0.5 are out of phase (Fig. 9.7c, *right*; Metzen and Chacron 2015).

EAs can also respond to mimics of social envelopes, although their responses have not been as systematically characterized as for movement envelopes. Studies have shown that EAs can respond to such envelopes provided that the underlying beat amplitude is large enough (Savard et al. 2011; Stamper et al. 2013). Surprisingly, single EAs can display phase locking to beats with frequencies >400 Hz, thereby responding to envelopes. Similar to what has been shown for movement envelopes, EAs with lower baseline firing rates tend to respond more robustly to envelopes (Savard et al. 2011).

Overall, the mechanisms by which single peripheral EAs can respond to envelopes are similar to what has been observed in other systems. For example, in the auditory system, single peripheral auditory fibers respond to envelopes by phase locking to the carrier (Heil 2003). However, because auditory fibers display much lower baseline firing rates than EAs, their responses tend to be largely in phase with the envelope.

9.4.1.2 Envelope Coding by Correlated Activity in Electroreceptor Afferent Populations: Implications for Vestibular and Auditory Processing

EAs also encode envelopes at the population level. This is particularly important for low amplitudes because single EAs then do not encode for the envelope. Interestingly, a strong covariation between the time course of the pairwise neuronal correlation coefficient among EAs and the stimulus envelope has been demonstrated (Metzen et al. 2015). The reason for this is that the spiking activity of pairs of EAs is more similar during periods when the envelope is high (Fig. 9.8a, *top left*) than when the envelope is low (Fig. 9.8a, *top right*). This ratio of synchrony (i.e., the time-varying correlation coefficient) of paired EA spiking activity (Fig. 9.8b, *orange*) over the time course of the envelope is reflected by a strong relationship between the correlation coefficient and the envelope (Fig. 9.8c, *left*). This can be further demonstrated by computing the variance accounted for (VAF), which ranges between 0 (no predictability) and 1 (optimal predictability). The VAF can be used to quantify the degree to which the envelope could be predicted from the correlation coefficient. High VAF values for paired EA spiking activity (Fig. 9.8c, *right*) indicate that the correlation coefficient is a reliable predictor of the envelope (Metzen et al. 2015).

By contrast, the instantaneous firing rate from single EAs (Fig. 9.8b, *blue*) does not provide detailed information about the envelope. This is because the fast variations in the instantaneous firing rate caused by the stimulus waveform average out over the envelope timescale, thereby making the neuronal firing rate approximately

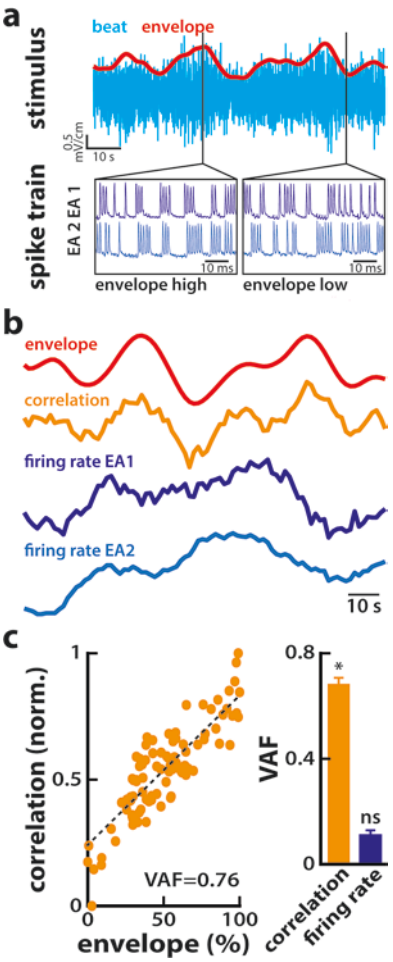

Fig. 9.8 **a:** Time-varying beat, its envelope, and the spiking responses from an EA pair to stimulus segments characterized by high and low envelopes. **b:** Time-varying envelope and the correlation coefficient from a pair of EAs as well as the corresponding firing rates of the two individual EAs. **c:** Correlation coefficient (*left*) as a function of envelope, indicating a strong linear relationship as characterized by a high variance accounted-for (VAF). Population-averaged VAF values (*right*) for the correlation coefficient as well as for single neuron activity. As indicated by low VAF values, the single neuron activity is a poor predictor for the envelope. ns, Not significant

constant and thus independent of the envelope. Consequently, single-neuron firing rates cannot reliably predict the envelope as quantified by a negligible VAF value (Fig. 9.8c, *right*).

Interestingly, similar results were seen in the vestibular system (Metzen et al. 2015), which is also part of the octavolateralis system. Indeed, correlations between vestibular afferents strongly covaried with the envelope. Remarkably, such coding is best for nonzero levels of neural variability (Metzen et al. 2015). It has been proposed that downstream neurons can effectively extract the envelope by performing a nonlinear operation on the summed input from peripheral sensory neurons. Such a circuit effectively functions as a physiologically realistic decoding algorithm because downstream neurons nonlinearly integrate input from convergent afferent axons in both the electrosensory and vestibular systems (Berman and Maler 1999; Massot et al. 2012).

9.4.2 *Electrosensory Lateral Line Lobe*

9.4.2.1 Responses of Electrosensory Lateral Line Lobe Pyramidal Cells to Sinusoidal Movement Envelopes

The responses of ON- and OFF-type ELL pyramidal cells to movement envelopes have been investigated using the same stimuli as for EAs, thereby allowing a direct comparison of the results. Overall, both ON- and OFF-type ELL pyramidal cells respond to movement envelopes through increases in firing rate (Fig. 9.9a). Interestingly, both ON- and OFF-type pyramidal cells will respond similarly to

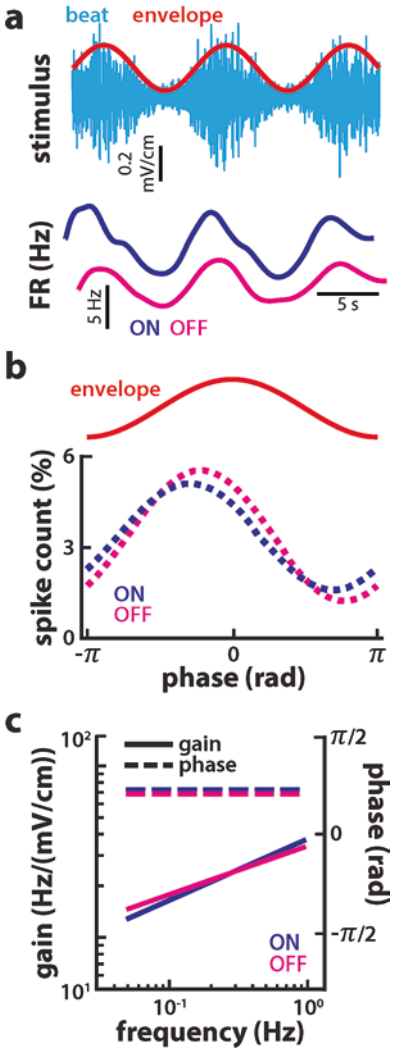

Fig. 9.9 **a:** ON- and OFF-type ELL pyramidal cells respond similarly to envelopes. Top: stimulus consisting of a noisy beat whose envelope is modulated sinusoidally; bottom: time-varying firing-rate (FR) responses for an ON- and OFF-type pyramidal cell. **b:** Best fits of the histograms for an ON- and OFF-type pyramidal cell in response to the envelope showing the similar phase relationship of both types to the envelope. **c:** Gain and phase for ON- and OFF-type pyramidal cells as a function of envelope frequency

envelopes in that both cell types display a similar phase preference (Huang and Chacron 2016; Huang et al. 2016); however, the cause is unknown. Moreover, ELL pyramidal cells are thought to respond to envelopes through nonlinear integration of EA input (Middleton et al. 2006) but has otherwise not been thoroughly investigated. There are other notable differences between ELL pyramidal cell and EA responses to envelopes, the first of which is that ELL pyramidal cell responses tend to lead the envelope (Fig. 9.9b) and their sensitivity tends to increase with increasing envelope frequency (Fig. 9.9c). However, the tuning to envelopes considerably varies across pyramidal cell populations and ELL maps (Huang and Chacron 2016). Interestingly, the tuning properties of ELL pyramidal cells to envelopes are similar in *Apteronotus leptorhynchus* and *Apteronotus albifrons* (Huang et al. 2016; Martinez et al. 2016), which likely underlies similar behavioral tracking responses.

The tuning properties of ELL pyramidal cells within all three maps to sinusoidal movement envelopes strongly depend on the baseline firing rate. Superficial cells whose somata are located most superficially within the pyramidal cell layer tend to display the lowest firing rates, whereas deep cells whose somata are located most deeply within the pyramidal cell layer instead tend to display the highest firing rates (Bastian and Nguyenkim 2001).

In general, superficial pyramidal cells within the LS display the most high-pass tuning and the greatest phase leads, followed by superficial pyramidal cells within the CLS, whereas superficial pyramidal cells within the CMS display the least high-pass tuning and weakest phase leads (Fig. 9.10a). Within each segment, superficial

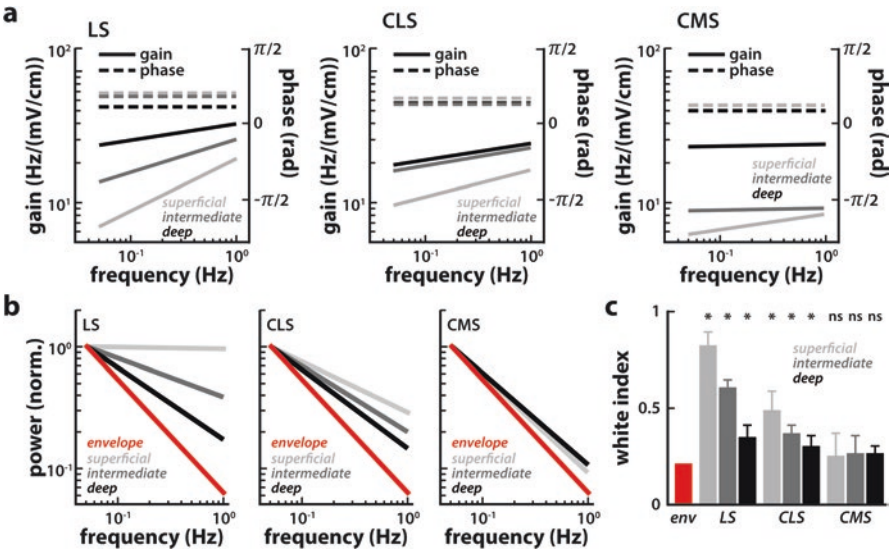

Fig. 9.10 **a:** Gain and phase for superficial, intermediate, and deep pyramidal cells in the three ELL maps. **b:** Response spectrum for superficial, intermediate, and deep pyramidal cells in the three ELL maps and the movement envelope. **c:** Whitening indices for the envelope (env) and for superficial, intermediate, and deep pyramidal cells in the three ELL maps. ns, Not significant

pyramidal cells display the most high-pass tuning and strongest phase lead, followed by intermediate pyramidal cells, whereas deep cells display the least high-pass tuning and weakest phase leads (Fig. 9.10a). Further theoretical work has shown that pyramidal cells effectively perform a mathematical operation known as fractional differentiation on envelopes (Huang et al. 2016), which is equivalent to displaying power-law adaptation in the temporal domain (Zhang and Chacron 2016). The implications of such fractional differentiation are explained in detail in Sect. 9.4.2.2. For now, the nature of the underlying mechanisms is briefly reviewed. Previous studies have shown that the small-conductance calcium-activated potassium (SK)-channel subtype SK1 is expressed abundantly in the ELL, with CMS cells showing little or no expression and LS cells showing the most (Ellis et al. 2007, 2008). Interestingly, superficial cells also display the most expression and deep cells the least. There is thus a strong correlation between SK-channel expression and high-pass tuning to envelopes (Huang and Chacron 2017). A more causal relationship between SK1-channel expression and envelope tuning was uncovered by applying SK1 antagonists and agonists within the LS of the ELL (Huang et al. 2016); application of SK-channel antagonists made the tuning of LS pyramidal cells less high pass (i.e., more similar to that of CMS cells), whereas application of SK-channel agonists instead made the tuning more high pass. Importantly, these changes in ELL pyramidal cell tuning had strong effects on the tracking behavior, thereby causing a mismatch between the behavioral sensitivity and natural envelope statistics (Huang et al. 2016).

9.4.2.2 Optimized Coding of Natural Movement Envelopes Through Temporal Whitening

What is the functional role of high-pass filtering of envelopes through fractional differentiation by ELL pyramidal cells? To answer this question, it is important to remember that the time course of movement envelopes under natural conditions is not sinusoidal. Interestingly, it was found that when using natural movement envelopes, the power spectrum of superficial LS pyramidal cells was independent of frequency (i.e., white; Fig. 9.10b). Further investigation revealed that this was due to a precise match between tuning and natural envelope statistics. Indeed, the strong high-pass tuning of LS superficial pyramidal cells effectively compensates the power-law decay in the spectrum of natural movement envelopes, such that the output power spectrum is white. This result is interesting because theoretical studies have posited that a constant output spectrum is associated with maximum information being transmitted (Rieke et al. 1996). As such, superficial LS pyramidal cells perform the most "whitening" as quantified by the white index (Fig. 9.10c) and other pyramidal cell types perform less whitening. The reasons underlying such diversity of envelope responses among cell classes and segments are largely unknown. However, it was proposed that the lack of envelope filtering by deep and CMS pyramidal cells could provide the necessary information to properly decode optimally transmitted information by LS superficial pyramidal cells in higher brain

areas (Huang et al. 2016). Interestingly, the similar tuning properties observed for LS pyramidal cells in *Apteronotus leptorhynchus* and *Apteronotus albifrons* predict that both species should experience similar natural movement envelope statistics. Further studies using the comparative approach are likely to greatly increase our understanding of envelope processing in the electrosensory system.

9.4.2.3 Mechanisms Underlying Electrosensory Lateral Line Lobe Pyramidal Cell Responses to Movement Envelopes

What are the mechanisms that enable ELL pyramidal cells to respond to movement envelopes? Whereas one would expect that nonlinear integration of afferent input would contribute to shaping their responses to envelopes, it was shown instead that envelope responses are mediated by descending feedback from higher brain centers (Fig. 9.11a). ELL pyramidal cells receive large amounts of descending feedback from higher brain centers (Berman and Maler 1999). Specifically, it was shown that descending input enables pyramidal cells to respond to envelopes through increases in firing rate, which, in turn, gives rise to behavioral responses (Fig. 9.11b; Metzen et al. 2018; Huang et al. 2018). Further studies are needed to understand how SK1 channels, which are located primarily on ELL pyramidal cell apical dendrites where feedback input terminates (Ellis et al. 2008) and which have been shown to be a key determinant of envelope tuning properties (Huang et al. 2016), mediate the envelope responses of ELL pyramidal cells.

9.4.2.4 Electrosensory Lateral Line Lobe Pyramidal Cell Responses to Social Envelopes and Underlying Mechanisms

ELL pyramidal cell responses to social envelopes have also been studied. In particular, it has been shown that ELL pyramidal cells, but not EAs, will respond to the envelope of narrowband noise (Middleton et al. 2006). It has been shown that a class of GABAergic interneurons, the ovoid cells, respond to envelopes, and the same study concluded that inhibitory inputs from these ovoid cells might be a mechanism mediating envelope responses in pyramidal cells. Each ovoid cell receives input from hundreds of EAs. Because the firing rates in ovoid cells are lower than those of EAs (Bastian et al.1993), the evoked excitatory postsynaptic potential of each individual EA in ovoid cells must be weak. It is therefore likely that ovoid cells extract the envelope due to synchronized EA input (Middleton et al. 2006, 2009). These responses are also most likely due to a threshold nonlinearity in ovoid cells (Middleton et al. 2006; Longtin et al. 2008), followed by low-pass filtering.

The effects of pyramidal cell heterogeneities on social envelope responses have also been investigated. Specifically, a negative correlation between response magnitude and baseline firing rate was observed. This implies that deep cells will respond the least to social envelopes, whereas superficial cells will respond the most. The underlying mechanisms likely include intrinsic nonlinearities such as spiking

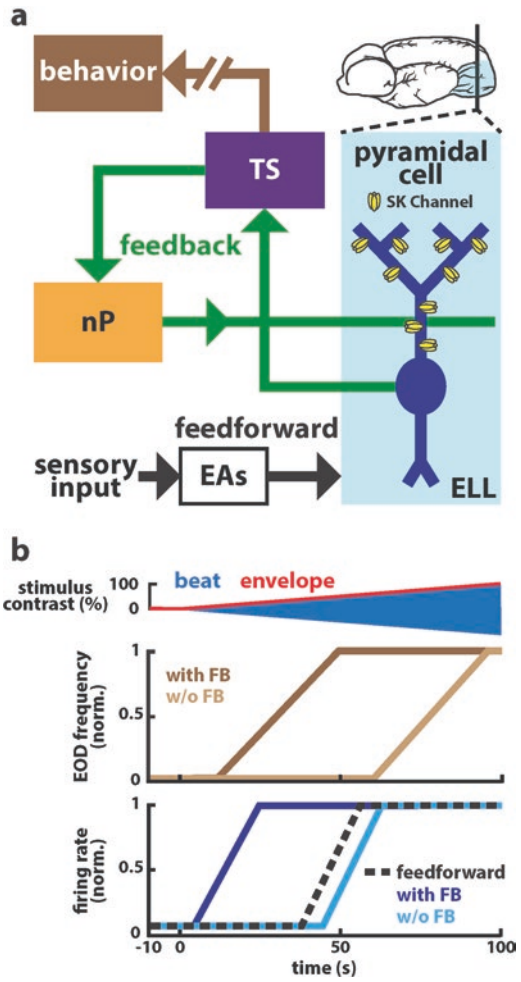

Fig. 9.11 **a:** Relevant circuitry showing ELL pyramidal cells receiving feedforward input from EAs and projecting to higher brain areas (i.e., midbrain TS). Neurons within the TS project back to the ELL via the nucleus praeeminentialis (nP), terminating on the apical dendrites of pyramidal cells that are equipped with small-conductance calcium-activated potassium (SK) channels. **b:** Relative contributions of feedback (FB) and feedforward inputs toward determining behavioral responses. For low contrasts, feedback is necessary to elicit changes in pyramidal cell firing rate (*bottom*) and behavior (*top*). Inactivating feedback reveals that feedforward input (*bottom*) is sufficient to alter pyramidal cell firing rate (*bottom*) and behavior (*top*) because both increase at similar contrasts for which EAs increased their mean firing rates

(McGillivray et al. 2012). However, the tuning properties of ELL pyramidal cells to social envelopes have not systematically been investigated to date and should be the focus of future studies.

9.4.2.5 Comparison with Other Systems

To summarize, a lot is known about how ELL pyramidal cells process envelopes. Overall, there are some similarities with other senses in that pyramidal cells tend to respond more strongly to envelopes than peripheral EAs. Indeed, in the auditory system, sensitivity to envelopes also increases in higher level areas (e.g., cochlear nuclei, inferior colliculus, and auditory cortex; Joris et al. 2004). As in the electrosensory system, the mechanisms by which sensitivity to envelopes is refined

centrally in the auditory system are poorly understood in general, although modeling suggests that the responses to envelopes of central neurons are the result of integration of afferent input from the periphery, as predicted from mathematical models (Hewitt and Meddis 1994; Wang and Sachs 1995).

9.4.3 Higher Electrosensory Pathways

This section reviews how envelopes are encoded by neurons within the midbrain torus semicircularis (TS) that receive afferent input from ELL neurons.

9.4.3.1 Responses of Torus Semicircularis Neurons to Social Envelopes

Anatomical studies have shown that the TS, which is homologous to the inferior colliculus of mammals, consists of 11 layers, comprises approximately 50 cell types, and receives sensory input from ELL pyramidal cells (Carr et al. 1981; Carr and Maler 1985). In general, the responses of TS neurons to stimuli are quite heterogeneous, with some neurons showing response profiles that are reminiscent of those seen in the ELL and others responding more selectively (Vonderschen and Chacron 2011; Sproule et al. 2015). The responses of TS neurons have only been characterized to date using social envelopes (McGillivray et al. 2012). Interestingly, although some TS neurons respond by phase locking to a given phase of the beat (Fig. 9.12a, *bottom purple*), others instead respond to two different phases (Fig. 9.12a, *bottom orange*). Such "ON-OFF" cells have been previously characterized and are thought to receive a balanced excitatory input from ON- and OFF-type ELL pyramidal cells (Partridge et al. 1981; Rose and Call 1993). Such different response profiles have been shown to strongly influence TS neural responses to social envelopes as described later within this section.

In general, TS neurons respond to social envelopes in one of three ways. First, some neurons will respond to both the envelope and the beat through phase locking in a manner that is similar to that seen for ELL pyramidal cells (Fig. 9.12b, *striped*). Second, other neurons will respond selectively to the beat and not to the envelope (Fig. 9.12b, *purple*). Third, other neurons will respond selectively to the envelope and not the carrier (Fig. 9.12b, *orange*). The emergence of envelope selectivity in the TS is thought to be important in order to process the envelope and beat in parallel to avoid ambiguity as required to generate appropriate behavioral responses. This is important because behavioral studies have shown that beat and envelope stimuli can overlap in their frequency ranges (Yu et al. 2012; Fotowat et al. 2013). The mechanisms that underlie response selectivity to the carrier and envelope in the TS have been investigated in detail. Interestingly, it was found that the TS neurons that respond selectively to envelopes tended to receive balanced input from ON- and OFF-type ELL pyramidal cells (i.e., classified as ON-OFF). These neurons will respond to both upstrokes and downstrokes in the stimulus, which strongly attenuates their overall

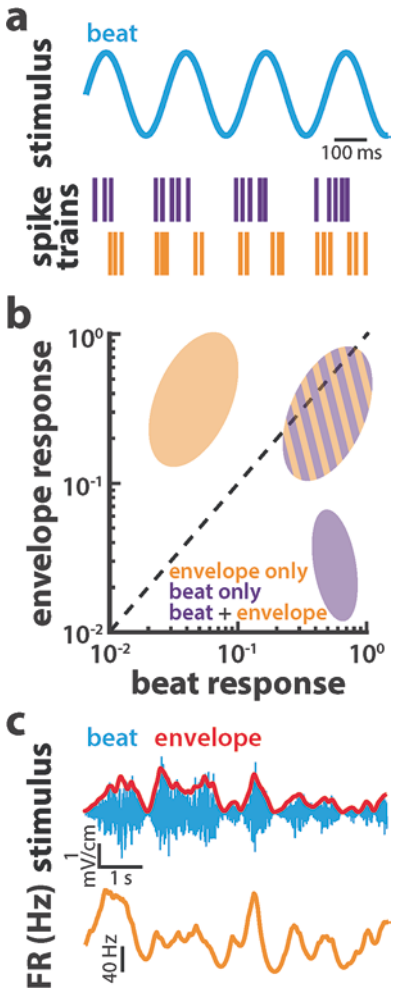

Fig. 9.12 **a:** Response of TS neurons to a sinusoidal beat (*top*) can be unimodal (*bottom, purple*) or bimodal (*bottom, orange*). **b:** Envelope response as a function of beat response from a population of TS neurons. In contrast with ELL pyramidal cells, three distinct clusters can be identified. Some TS neurons respond selectively to either the envelope or the beat and some TS neurons respond to both. **c:** stimulus (*top*) consisting of a noisy beat with a corresponding envelope. The time-varying FR of a TS neuron responded strongly to the envelope (*bottom*)

response to the beat and increases their selectivity in responding to the envelope (Fig. 9.12c). In contrast, TS neurons that respond to both the beat and envelope will respond either to carrier upstrokes or downstrokes, similar to ELL neurons. It is thought that the TS neurons that respond selectively to the beat receive input primarily from deep ELL pyramidal cells because these tend to display the weakest responses to envelopes (McGillivray et al. 2012).

9.4.3.2 Sparse Coding in Higher Brain Areas

In general, studies that have compared the response profiles of TS and ELL neurons have found that the former are on average more selective than the latter. This is sometimes referred to as "sparse coding" (Olshausen and Field 2004). Although the response profiles of TS neurons to movement envelopes have not been characterized

to date, it is expected that these will be more selective than those of ELL neurons. This implies that some TS neurons should respond selectively to movement envelopes, whereas others should respond selectively to social envelopes. Overall, the emergence of envelope selectivity in the TS is consistent with the results showing that neurons in higher brain areas (e.g., auditory cortex) will respond more selectively to envelopes (Joris et al. 2004). Moreover, studies in the visual system have shown that some neurons will respond selectively to the carrier, whereas others will respond selectively to the envelope (i.e., "linear" and "nonlinear" pathways; Baker 1999; Baker and Mareschal 2001). As such, studies focusing on how electrosensory neurons achieve response selectivity are likely to be applicable to other systems where less is known about the nature of the underlying mechanisms.

9.5 Summary

This chapter has reviewed our understanding of how envelopes are processed in the electrosensory system in order to lead to behavior. To summarize, weakly electric fish display robust and easily elicited behavioral responses to envelopes. The fact that these do not require movement because they can be elicited in immobilized animals greatly facilitates uncovering their neural basis. In general, peripheral EAs can respond to envelopes either at the single neuron level because of static nonlinearities (i.e., rectification and/or saturation) or at the population level by looking at correlations between their activities. ELL pyramidal cells receiving afferent input from EAs as well as feedback from higher brain areas display more robust, albeit more heterogeneous responses to envelopes. In particular, the tuning properties of some ELL pyramidal cells are matched to natural movement envelope statistics to maximize information transmission through whitening, which is mediated by SK1 channels and determines behavioral responses. Finally, neurons in higher order brain areas receiving input from ELL neurons tend to display more selective responses to envelopes, which is determined by the relative balance of input that they receive. In general, there are many commonalities between the envelope coding strategies used by the electrosensory system and other senses (e.g., audition, vision, vestibular), implying that sensory systems have evolved general coding strategies for these important features found ubiquitously in sensory input. In what follows, some important future avenues of research are highlighted.

First, it is well-known that sensory systems must constantly adapt to environments with changing statistics; this is known as sensory adaptation (Wark et al. 2007). This concept is likely to be applicable to envelopes in the electrosensory system. For example, the statistics of natural movement envelopes are likely to be different when fish are more active (e.g., at night) than when they are less active (e.g., during the day). Whether and how the electrosensory system adapts to such changes is presently unknown and should be the focus of future studies.

Second, although it is desirable for fishes to distinguish movement from social envelopes, comparing behavioral responses to these within the same species has not been the focus of many studies. Such studies, together with electrophysiological

recordings, are needed and will likely provide much needed insight as to how these different stimulus classes are processed in the electrosensory system. A comparative approach (i.e., comparing results obtained across multiple species) is also needed to distinguish mechanisms that are generally applicable from those that are species specific.

Last, more studies are also needed to understand what gives rise to envelope responses in ELL pyramidal cells and how downstream neurons process these. Such studies should not be limited to the TS and should also focus on higher order areas (e.g., the nucleus electrosensorius or the preglomerular complex) where it is expected that selectivity to envelopes is further refined and should determine behavior. In particular, further studies are needed to understand how the response profiles of ELL pyramidal cells are integrated downstream to give rise to the observed behavioral responses (e.g., envelope tracking).

Compliance with Ethics Requirements Michael G. Metzen declares that he has no conflict of interest.

Maurice J. Chacron declares that he has no conflict of interest.

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Chapter 10
Evolution of Submillisecond Temporal Coding in Vertebrate Electrosensory and Auditory Systems

Bruce A. Carlson

Abstract The ability to detect submillisecond differences in the arrival times of stimuli at different sensory receptors has evolved independently in multiple clades. Auditory and electrosensory systems across vertebrates provide well-studied examples of how specialized sensory pathways are able to achieve such extreme temporal sensitivity. These circuits share a remarkable number of similarities at the cellular and synaptic levels of organization despite serving different sensory modalities and despite arising from multiple independent evolutionary origins. This points to a degree of predictability in neural circuit evolution and to the power of natural selection in driving evolutionary change to neural circuits to solve a specific behavioral problem. However, these similar cellular and synaptic building blocks are used to construct different circuit solutions to this behavioral problem in different clades. These differences likely reflect some combination of chance, evolutionary history, and adaptation. Importantly, these differences also make it clear that discoveries in one organism cannot be extrapolated to other organisms, highlighting the importance of comparative approaches in addressing general problems in neuroscience.

Keywords Anticoincidence detection · Calyx · Coincidence detection · Convergent evolution · Delay line · Electric organ discharge · Electroreceptor · Interaural time difference · Jamming avoidance response · Jeffress model · Neural code · Phase locking · Sound localization

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10.1 Introduction

The environment is in constant flux. Making sense of the resulting sensory input and responding with appropriate behavior require that peripheral nervous systems accurately represent this temporal variation and that central nervous systems extract behaviorally relevant information from this peripheral input. Temporal processing by nervous systems varies across a wide range of timescales (Grothe and Klump 2000; Mauk and Buonomano 2004). This chapter focuses on the processing of submillisecond timing differences in select vertebrate sensory systems that have been thoroughly studied at cellular and circuit levels.

Differences in arrival times of sounds at the two ears, so-called interaural time differences (ITDs), are used by many tetrapods to determine where a sound originates in space. For example, humans are able to detect ITDs as small as about 10 μs (Klumpp and Eady 1956; Brughera et al. 2013). Similarly, weakly electric fish are able to detect submicrosecond time differences between electrosensory inputs at different locations on the body surface (Rose and Heiligenberg 1985; Kawasaki 1997). This extreme sensitivity may have evolved because the electric signals that these fish generate do not propagate as waves but exist as localized, nonpropagating electrostatic fields (Hopkins 1986b). This has an important consequence for temporal coding; whereas acoustic stimuli are degraded during propagation due to absorption, reflection, refraction, and reverberation, the fine temporal structure of electric signals is preserved, allowing information to be accurately transmitted through the environment at much shorter timescales.

Regardless of the temporal precision of sensory stimuli, it is remarkable to consider that certain neural circuits are able to extract information with submillisecond precision even though the duration of an action potential is approximately 1 ms, and synaptic potentials are typically even longer in duration. How can nervous systems extract information at a timescale that is faster than the signals neurons use to communicate with each other?

This question has been addressed in the auditory and electrosensory systems of a wide range of vertebrate species. We now know a great deal about cellular and synaptic mechanisms underlying the processing of submillisecond timing differences in circuits that have evolved independently as well as in homologous circuits that have diverged from a shared evolutionary origin. This provides a rich comparative resource for trying to understand how neural circuits have evolved to solve a specific computational problem. The brain of every species is unique, and we cannot simply extrapolate findings from one species to another (Carlson 2012; Brenowitz and Zakon 2015; Yartsev 2017). Which mechanisms are universal and which mechanisms are specific to particular lineages? Why have different nervous systems come up with different solutions to the same problem? Comparative approaches are necessary to answer such questions, and neuroscientists need these answers if we are to develop fundamental and predictive theories of brain function.

Research into the detection of submillisecond timing differences by vertebrate auditory and electrosensory systems provides an illustrative example of how challenging this problem is on the one hand and how informative a comparative approach can be on the other.

10.2 Peripheral Coding of Submillisecond Timing Differences

10.2.1 Sound Localization Using Interaural Time Differences

Sounds propagate through air at about 330–360 m/s depending on humidity and temperature. For sounds that originate directly in front of the listener, the sound source is equidistant to both ears, causing the sound to arrive at both ears simultaneously (Fig. 10.1A). For sounds that come from the side, however, the sound will reach the near ear before reaching the far ear, creating an ITD. The exact value of the ITD depends on head size (how far apart the ears are), sound speed, and the azimuthal (horizontal) angle of the sound source with respect to the head. Because head size and sound speed are constants (or nearly so), the azimuth of the sound source can be determined using the ITD (Fig. 10.1A). ITDs are most useful at relatively low sound frequencies for which the sound wavelength is greater than the maximum ITD that can occur between the two ears (about 1500 Hz in humans). A wide diversity of terrestrial vertebrates use ITDs in the tens to hundreds of microseconds for azimuthal sound localization at low sound frequencies (Ashida and Carr 2011).

For an animal to detect such small ITDs, its peripheral nervous system must accurately and precisely encode the timing of sounds that reach each ear (Kiang et al. 1965; Carr 1993). Auditory nerve fibers exhibit a high degree of phase locking, meaning that their firing tends to occur at a specific phase of a periodic stimulus (Fig. 10.1B). Across a wide range of species, auditory nerve fibers respond with strong phase locking to sound frequencies well over 1 kHz, with some species, such as the barn owl, maintaining significant phase locking to frequencies as high as 10 kHz (Köppl 1997). At such high frequencies, individual afferent fibers do not spike during every cycle of the stimulus. Instead, the population of fibers collectively fires on each cycle, and they tend to do so at a specific phase of the stimulus, thereby providing a precise temporal representation of the timing of sound arrival at the two ears (Fig. 10.1B).

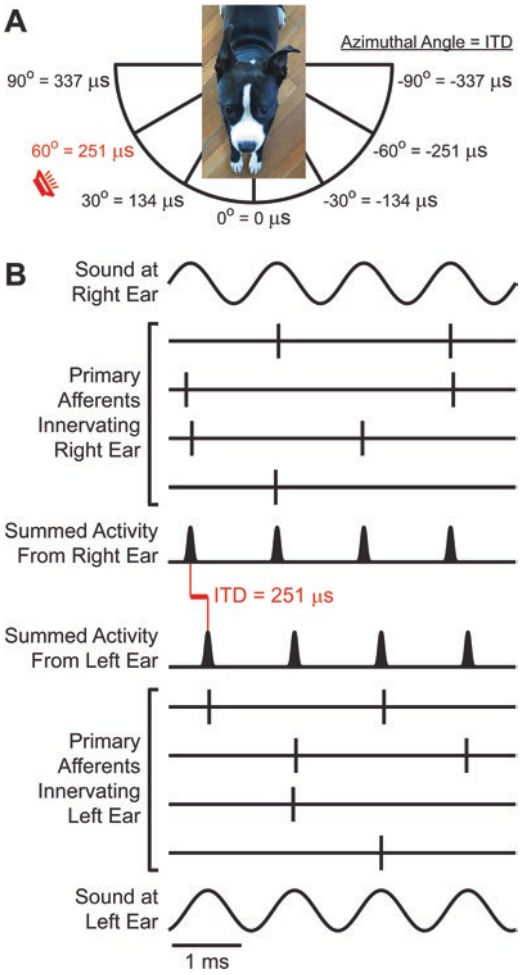

Fig. 10.1 Interaural time differences (ITDs) are used by many terrestrial vertebrates to determine the azimuthal (horizontal) location of a sound source. **A:** sounds coming from straight ahead reach both ears at the same time, whereas sounds coming from one side are closer to one ear, leading to a difference in arrival time. Approximate values for ITDs coming from different azimuthal angles relative to this subject's head were determined as ITD = $(r/c_o)*(\theta + \sin\theta)$, where r is the radius of the head (4.5 cm), c_o is the speed of sound (343 m/s in dry air at 20 °C), and θ is the angle (in radians) between the median plane of the head and the incident sound direction (Kuhn 1977). **B:** primary auditory afferents exhibit strong phase locking or a tendency to spike at a specific phase of the ongoing sound waveform, in this case an 800-Hz sine wave. Although individual afferents do not spike during each cycle of the sound, the summed activity across many afferents precisely represents the sound phase at each ear. This phase locking thereby relays the ITD to the central auditory pathway

10.2.2 Temporal Coding of Electric Communication Signals in Pulse-Type Mormyrid Fishes

Timing differences are also crucial for electrosensory processing in weakly electric fish. The electric organ discharge (EOD) of the pulse-type African mormyrids consists of discrete pulses of electricity with relatively long periods of silence between pulses (Fig. 10.2A). EOD waveform is highly stereotyped and communicates information about sender identity such as species, sex, age, reproductive status, and relative dominance status (Hopkins 1986a; Carlson 2002). Total EOD duration varies across species from <200 μs to >30 ms, and EOD waveforms can also differ in polarity, number of phases, and the time courses of voltage changes within each phase (see Markham, Chap. 5; Krahe, Chap. 7).

Electroreceptors and their associated primary sensory afferents exhibit a great deal of morphological and physiological diversity both within and across species (see Baker, Chap. 2; Leitch and Julius, Chap. 3). The electroreceptor organs that mediate electric communication behavior in mormyrids are called knollenorgans (Bennett 1965, 1971). In most species, knollenorgans are distributed broadly across the surface of the skin and generate all-or-none spikes in response to outside, positive changes in voltage or an inward current across the skin (Fig. 10.2A, B). An EOD generated by a neighboring fish leads to current flowing through the receiving fish such that there is inward current on one side of the body at the same time that there is outward current on the opposite side (Hopkins and Bass 1981). Thus, knollenorgans on opposite sides of the body receive EODs with opposite polarities, leading to differences in spike timing as a function of the stimulus waveform and location (Fig. 10.2B, C). Receptor spiking drives spiking of primary electrosensory afferents, which thereby relay these spike timing differences to the central nervous system. Thus, EOD waveform and sender location is represented by small differences in the timing of afferent input to the central electrosensory system (Baker et al. 2013), much like the representation of sound source azimuth by small differences in the timing of binaural input to central auditory systems.

10.2.3 Temporal Coding of Phase Modulations in Wave-Type Electric Fishes

In wave-type electric fishes, the interval between each EOD closely matches the duration of a single EOD, resulting in a continuous, quasi-sinusoidal waveform (Fig. 10.3A). Wave-type EODs are found in numerous species of South American Gymnotiformes as well as in a single known African species, *Gymnarchus niloticus* (see Markham, Chap. 5; Krahe, Chap. 7). These fish also utilize small timing differences in electroreceptor activation for their behavior. Objects located near the fish induce local modulations in the EOD, which the fish use to actively sense their environment (see Jung and Engelmann, Chap. 12). Discriminating purely resistive

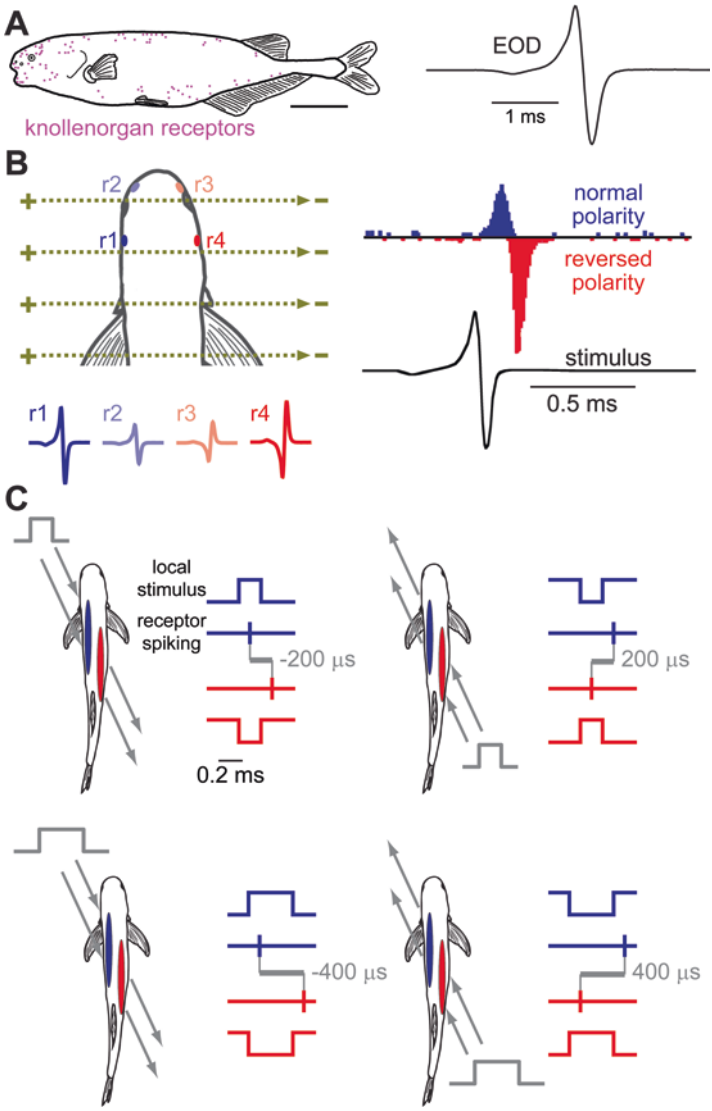

Fig. 10.2 Temporal coding of pulse-type electric organ discharges (EODs) by knollenorgan electroreceptors in mormyrids. **A:** in *Brienomyrus brachyistius*, as in most species of mormyrids, knollenorgans are distributed throughout the surface of the head, back, and belly (*purple dots*). The EOD consists of discrete pulses separated by longer periods of silence. Modified from Carlson et al. (2011). **B:** dorsal view of a fish's head (*left*). The EOD of a neighboring fish results in current flow through the body and different local stimuli at four knollenorgan receptors. Receptors oriented perpendicular to the current flow (r1 and r4) receive the strongest stimulus, whereas those at an oblique angle (r2 and r3) receive a weaker stimulus. Receptors on opposite sides of the body receive reversed polarity stimuli (r1 and r2 vs. r3 and r4). Knollenorgans respond to upward deflections in voltage. Therefore, these differences in amplitude and polarity result in small differences in spike times across the receptor population in response to an EOD stimulus, illustrated here as

objects from capacitive objects is necessary to distinguish between living and nonliving objects, and this depends on the ability to detect small changes in EOD phase (von der Emde 1998, 1999).

These fish also rely on modulations in the EOD phase for their communication behavior. In the presence of a neighboring fish generating its own EOD, the combination of two periodic signals at different frequencies leads to an ongoing pattern of constructive and destructive interference, resulting in a modulatory envelope or "beat" (Fig. 10.3B). The rate of amplitude modulation in this envelope is equal to the magnitude of the frequency difference between the two fish (see Metzen and Chacron, Chap. 9). Thus, the amplitude modulation rate is identical for frequency differences of equal magnitude but of opposite sign, and it cannot be used alone to distinguish which fish has the higher (or lower) EOD frequency. However, the phase of the combined EOD is also modulated at the same rate as the amplitude (see Stamper, Madhav, Cowan, and Fortune, Chap. 8). Importantly, the temporal relationship between amplitude modulation and phase modulation reverses when the sign of the frequency difference between the two fish is flipped (Fig. 10.3B).

Detecting small modulations in phase is therefore critical for performing the jamming avoidance response (JAR), in which two fish with similar EOD frequencies shift their frequencies away from each other so as to increase the frequency difference and minimize electrosensory interference (Heiligenberg 1991; Kawasaki 1993). One class of primary electrosensory afferent fires 1:1 with precise phase locking to each cycle of the stimulus, largely independent of EOD amplitude (Fig. 10.3C; Scheich et al. 1973; Bullock et al. 1975). The fish detect phase modulations by comparing the timing of afferent spikes arising from areas of the body that are strongly contaminated by a neighboring EOD with areas that are weakly contaminated and that are therefore subject to different degrees of phase modulation (Heiligenberg 1991). Thus, both EOD waveform recognition in pulse-type mormyrids and the JAR of wave-type electric fish depend on detecting small differences in spike timing between primary afferents.

10.3 Common Specializations for Precise Temporal Coding

Electromotor and electrosensory systems evolved independently in African and South American electric fishes (Lavoué et al. 2012). Likewise, tympanic ears capable of receiving airborne sounds evolved independently in the ancestors of modern

Fig. 10.2 (continued) compound poststimulus-time histograms (*right*). The upward histogram shows receptor responses to the normal stimulus polarity, whereas the downward histogram shows responses to the inverted waveform, representing the responses of receptors on opposite sides of the body. **C:** a monophasic square pulse is used as a simplified stimulus to precisely control the timing of receptor spiking responses. The sign of the spike timing difference (e.g., left before right vs. right before left) provides information about the location of the stimulus, whereas the magnitude of this difference provides information about the duration of the stimulus. Modified from Carlson and Gallant (2013)

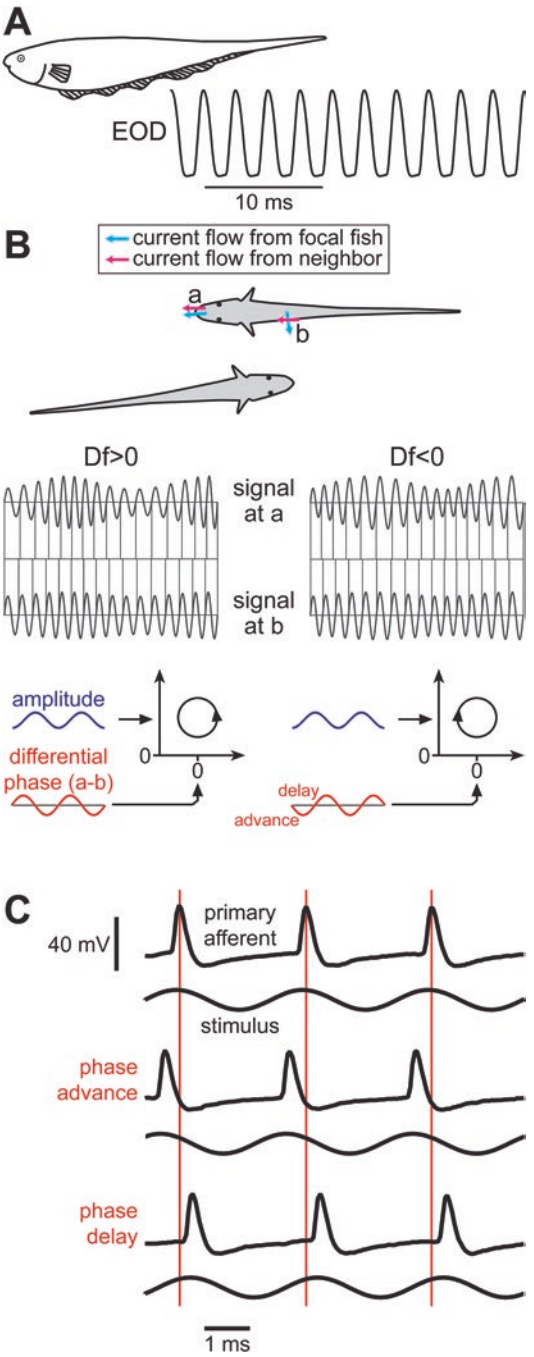

Fig. 10.3 Phase modulations of wave-type electric signals and their temporal coding by primary afferents. **A:** an EOD recorded from the South American gymnotiform fish *Eigenmannia virescens* approximates a sine wave. **B:** when two fish are in close proximity (*top*), their EODs interfere,

day sauropsids (reptiles and birds) and mammals (Clack 1997). Despite their independent evolutionary origins, in all of these sensory systems, precise timing information is encoded and processed in a dedicated central pathway, distinct from parallel sensory pathways that process other aspects of auditory or electrosensory stimuli.

In addition to ITDs, interaural intensity differences (IIDs) are used for sound localization in tetrapods. In mammals and sauropsids, individual primary auditory afferents encode both timing and intensity information. These primary afferents branch in the hindbrain to target distinct populations of postsynaptic neurons, leading to anatomically separate IID- and ITD-processing pathways (Takahashi et al. 1984; Irvine 1992).

In both African and South American wave-type electric fish, amplitude and timing information are encoded by separate classes of primary afferent fibers that give rise to distinct central circuits (Carr and Maler 1986). Pulse-type mormyrid fish have three different types of electroreceptors (Zakon 1986). In addition to the knollenorgan receptors that encode precise temporal information about EOD waveforms used in electric communication, mormyrids have two other types of electroreceptors that encode information used for passive and active electrolocation (ampullary and mormyromast receptors, respectively; see Baker, Chap. 2). Distinct primary afferents innervate the three receptor types, and these pathways remain segregated in the hindbrain (Bell 1986; Bell and Szabo 1986).

Despite the independent evolutionary origins of multiple auditory and electrosensory systems, their time-coding pathways share a number of distinctive anatomical and physiological features (Carr and Friedman 1999; Carr et al. 2001). These include large somas, minimal or nonexistent dendritic arbors, large-diameter axons with heavy myelination, convergence of multiple primary afferents onto hindbrain neurons, end bulb synapses that engulf large surface areas of the postsynaptic membrane, electrical or mixed chemical-electrical synapses, high concentrations of

Fig. 10.3 (continued) resulting in a beat characterized by amplitude modulation (AM; *blue*) and phase modulation (PM; *red*). To perform the jamming avoidance response (JAR), a fish detects the temporal relationship between AM and PM to determine the sign of the frequency difference (Df) between the two EODs (Df = neighbor's EOD frequency − fish's own EOD frequency; *center*). The fish's own EOD, generated internally, results in a current flow that is perpendicular to the skin surface at all points on the body surface. The direction of the current flow resulting from the neighboring fish's EOD depends on the relative orientation of the two fish, and it will maximally interfere with the fish's own EOD at locations on the body where it is also perpendicular to the skin surface. In this example, location "a" will experience greater interference than location "b." Thus, the fish can detect the PM by determining the difference in phase of the signal at location "a" relative to location "b." The waveforms show AM and PM for opposite signs of the Df. Lissajous plots show signal amplitude plotted against phase as a function that changes over time (*bottom*). For both signs of the Df, the resulting plots are circular, but the circles rotate in opposite directions for opposite signs of the Df. Modified from Carlson and Kawasaki (2007). **C:** an intracellular recording from a time-coding primary afferent reveals 1:1 spiking with the stimulus, with a high degree of phase locking. *Vertical red lines*, timing of primary afferent spikes when the phase is unmodulated, revealing that advances or delays in the local stimulus at the receptive field of an afferent are tracked precisely by afferent spiking. Modified from Carlson (2008)

calcium-binding proteins, and fast-acting receptors and ion channels. Such features help to maximize the reliability and temporal precision of synaptic transmission (Trussell 1997; Trussell 1999).

As somas and axons get larger, their input resistance decreases and their current-generating ability increases, making them less susceptible to noise caused by stray currents and ambient voltage fluctuations (Carr and Soares 2002). Increasing myelination increases resistance and decreases capacitance across axon membranes, whereas increasing axon diameter decreases intracellular resistance. These changes lead to an increase in the length (space) constant and a decrease in the time constant, thereby increasing action potential conduction velocity along the length of the axon (Waxman 1980). This helps to minimize the accumulation of timing noise (jitter) as action potentials propagate toward the presynaptic terminal. In addition, voltage-gated sodium and potassium channels with rapid activation and deactivation kinetics shorten both action potential duration and the refractory period, allowing for higher firing frequencies (Parameshwaran et al. 2001; Brown and Kaczmarek 2011).

Synapsing directly onto the soma rather than onto distal dendrites minimizes the conduction distance between the synapse and the spike initiation zone and thereby reduces both conduction delays and the loss of synaptic current (Carr and Soares 2002). Convergence of multiple afferents onto a single postsynaptic target helps to increase the temporal precision of phase locking (i.e., reduce jitter) at each stage of temporal processing (Carr and Soares 2002).

Electrical synapses are commonly found in the time-coding pathways of electric fish, and these minimize synaptic delays. For chemical transmission, large end bulb synapses dump large amounts of neurotransmitter in synchrony across many release sites, leading to large, rapid, and reliable postsynaptic currents that are sufficient to overcome the low input resistance of large somas and drive changes in voltage (Trussell 1999). At glutamatergic synapses, AMPA receptor splice variants with remarkably rapid kinetics and large conductances increase the speed and reliability of excitation (Trussell 1999; Brown and Kaczmarek 2011). High levels of calcium-binding protein may help shorten the time course of intracellular calcium increases and reduce the buildup of calcium in response to strong, repetitive synaptic activity (Friedman and Kawasaki 1997).

It's clear that independently evolved auditory and electrosensory systems have converged onto similar anatomical and physiological building blocks that help to optimize temporal processing. These traits are so distinctive that, were they identified in a newly discovered sensory pathway, one could predict with near certainty that the underlying neural circuit is involved in highly precise temporal coding. This points to a degree of predictability in neural circuit evolution and to the power of natural selection in driving evolutionary change to neural circuits to solve a specific behavioral problem. The specialized features of these circuits are metabolically costly (Laughlin 2001), underscoring the importance of timing for these circuits and the behaviors they mediate. Because of these costs, all of these circuits convert temporal codes into a new neural code that does not rely on such high timing precision. Despite these universal themes, however, evolution has taken these shared building blocks to construct a variety of neural circuits for detecting submillisecond

timing differences. These circuits solve this same basic problem using different mechanisms that establish different neural recoding schemes. This may point to a degree of unpredictability in neural circuit evolution, underscoring that there may be several neural solutions to the same behavioral problem.

10.4 Diverse Circuit Solutions to Detecting Submillisecond Timing Differences

10.4.1 Detection of Interaural Time Differences in Barn Owls

Following up on early psychophysical studies that revealed an important role for ITDs in sound localization in humans, Jeffress (1948) proposed a model for a neural circuit that could convert ITDs into a spatial map of sound source azimuth. The Jeffress model is composed of two key elements: delay lines and coincidence detectors. According to this model, the postsynaptic neurons act as coincidence detectors that are only depolarized above their spiking threshold when they receive synchronous excitatory input from both ears. For a postsynaptic cell to receive synchronous inputs from both ears, there must be some sort of internal delay in the circuit that exactly compensates for the acoustic delay between the two ears.

Jeffress proposed that afferent inputs from the two ears are arranged in an antiparallel fashion with their axons entering the circuit at opposite ends (Fig. 10.4A). In response to an auditory stimulus, a propagating action potential arising from one ear first excites a postsynaptic neuron at one end of the circuit. As this action potential traverses the length of the circuit, it reaches its postsynaptic targets at ever-increasing delays, thus establishing a systematic delay line. The antiparallel arrangement of axonal inputs from the two ears means that the two delay lines go in opposite directions. A sound coming from straight ahead will excite both ears at the same time. As a result, afferent input from the two ears will enter the opposite ends of the circuit at the same time, and the traveling action potentials will collide in the middle of the circuit (Fig. 10.4B). However, a sound coming from one side will excite the closer ear before the farther ear, giving afferent input from the closer ear a head start. Accordingly, the traveling action potentials will collide at a point that is offset from the middle of the circuit where the difference in axonal delays from the two ears compensates for the ITD (i.e., acoustic delay) between the two ears (Fig. 10.4B). As the sound source gets farther from the midline, this head start increases and the collision occurs even farther from the middle of the circuit. For a sound coming from the opposite side, everything is reversed: the other ear gets a head start and the point of collision occurs on the other side of the circuit. This combination of delay lines and coincidence detectors thereby converts a temporal code (ITDs) into a place code, wherein the location of a neuron within the circuit corresponds to the sound source azimuth that maximally excites it (Fig. 10.4C).

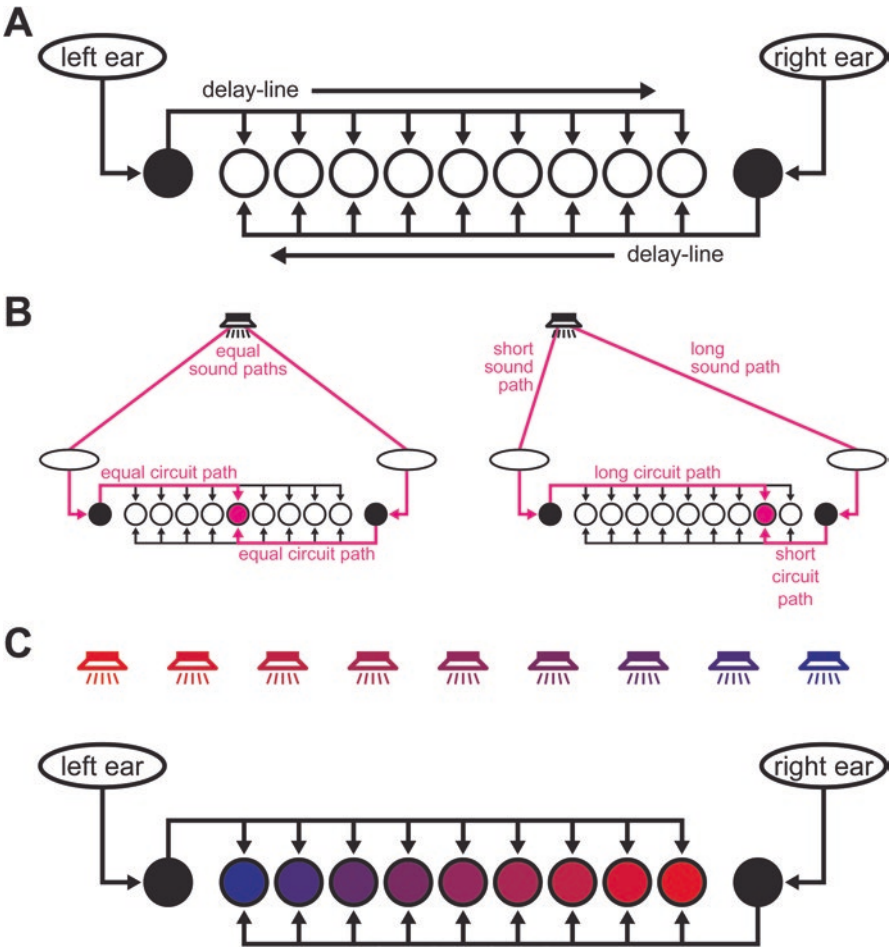

Fig. 10.4 The Jeffress model relies on delay-line coincidence detection to convert interaural time differences into a place code for sound source azimuth. **A:** axons relaying phase-locked auditory input from the left and right ears enter the circuit at opposite ends and traverse the length of the circuit, synapsing on postsynaptic targets throughout. This establishes delay lines running in opposite directions from the two ears, with cells at one end of the circuit receiving input from the left ear with a minimal delay and input from the right ear with a maximal delay and cells at the other end of the circuit receiving input from the left ear with a maximal delay and input from the right ear with a minimal delay. **B:** the postsynaptic neurons are coincidence detectors that respond maximally when they receive coincident excitatory input arising from the left and right ears. For a sound coming from straight ahead, the sound path to the two ears is equal in length and arrives at the two ears simultaneously (*left*). The inputs arising from the two ears will therefore be coincident in the middle of the circuit, where the two circuit paths are also equal in length. If a sound comes from the left, however, then there is a shorter path to the left ear (*right*). Therefore, the inputs arising from the two ears will be coincident at a location in the circuit where there is a shorter circuit path from the right ear compared with the left ear, at which differences in circuit path length compensate for differences in sound path length. **C:** this combination of antiparallel delay lines and coincidence detection leads to a spatial representation of the sound source azimuth, with sounds coming from one side exciting cells at one end of the circuit, sounds coming from the other side exciting cells at the other end of the circuit, and sounds coming from straight ahead exciting cells in the middle of the circuit

Over 40 years later, Carr and Konishi (1988, 1990) discovered that the auditory circuit responsible for ITD detection in barn owls was strikingly similar to the model proposed by Jeffress. Barn owls are nocturnal hunters that are able to locate pray relying solely on sound (Payne 1971). They can detect ITDs as small as about 10 μs, with a precision of around 1.5 μs (Knudsen et al. 1979; Moiseff and Konishi 1981). Primary auditory afferents innervate two distinct cochlear nuclei, with the magnocellular nucleus (NM) giving rise to an ITD-detecting circuit (Sullivan and Konishi 1984).

NM neurons are large with few dendrites (Carr and Boudreau 1993), and they receive input from primary afferents via large calyceal synapses (Carr and Boudreau 1991). They are able to fire at high frequencies with strong phase locking and little dependence on sound intensity (Sullivan and Konishi 1984; Köppl 1997). Their axons project bilaterally to terminate onto the somas and dendrites of neurons in the laminar nucleus (NL), which is the first site of binaural convergence in the ITD-processing pathway (Carr and Boudreau 1993).

Each NL neuron receives input from about 100 NM neurons, and this is associated with increased phase locking (Carr and Konishi 1990). Importantly, the ipsilateral and contralateral NM axons enter the NL on opposite sides (Fig. 10.5A). The ipsilateral axons enter the dorsal surface of the NL and continue down to the ventral end, whereas the contralateral axons enter the ventral surface of the NL and continue up to the dorsal end (Carr and Konishi 1988, 1990). Thus, there is an antiparallel arrangement of conduction delays for auditory input arising from the two ears, thereby establishing the delay lines proposed by Jeffress. The NL neurons act as coincidence detectors that respond maximally when they receive synchronous excitatory input from contralateral and ipsilateral NM neurons. Thus, sound source azimuths, represented initially by ITDs, get converted into a spatial map (Carr et al. 2015). Sounds coming from the ipsilateral side are represented by neurons at the ventral end of the NL and sounds coming from the contralateral side are represented by neurons at the dorsal end of the NL (Fig. 10.5B).

Additional mechanisms beyond the axonal delay lines and coincidence detectors envisioned by Jeffress also likely contribute to the formation of this map. Differences in axon diameter and internodal distances between ipsilateral and contralateral axonal projections to the NL likely lead to differences in conduction velocity that add a delay (Seidl et al. 2010, 2014). In addition, relatively slow GABAergic inhibition can increase the reliability and temporal precision of excitatory coincidence detection through a number of different mechanisms operating at different points in the circuit (Burger et al. 2011).

10.4.2 Detection of Interaural Time Differences in Mammals

The Jeffress model was remarkably prescient in predicting the neural circuit mediating ITD detection in barn owls. Perhaps equally surprising was the subsequent discovery that mammals, which evolved tympanic hearing independently from birds,

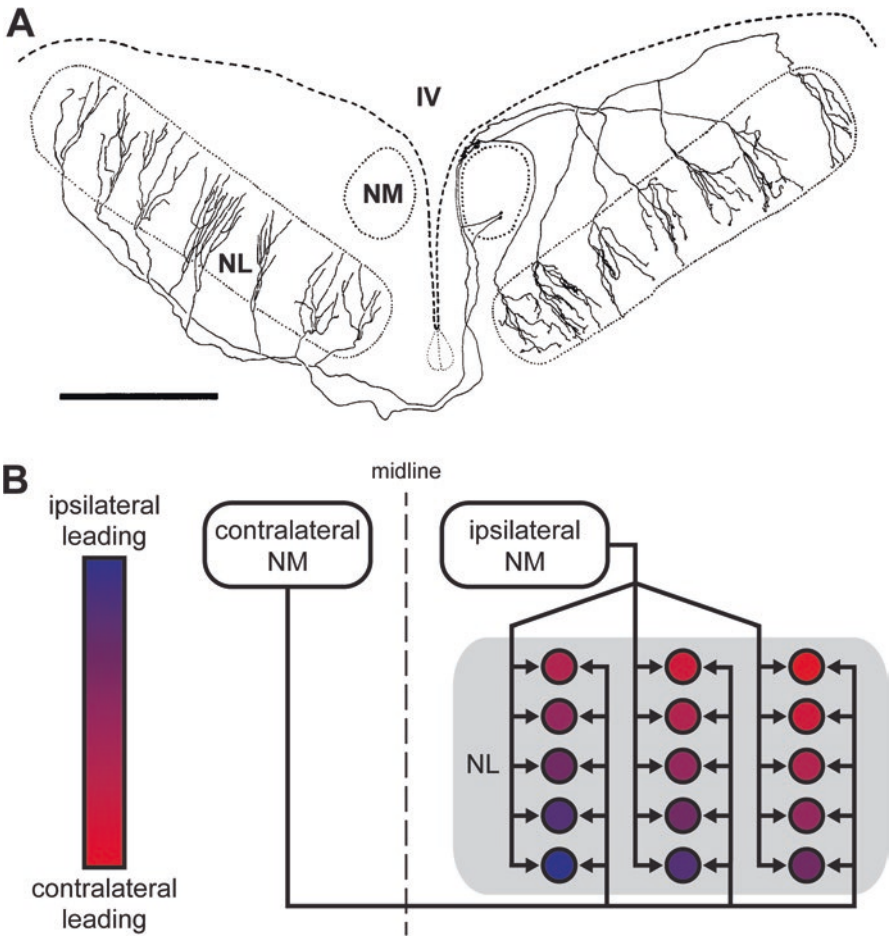

Fig. 10.5 The circuit for detecting interaural time differences (ITDs) in barn owls implements the Jeffress model to convert a temporal code into a place code for a sound source azimuth. **A:** drawing of a transverse section through the dorsal brainstem of a barn owl. NM, magnocellular nucleus; NL, laminar nucleus; IV, fourth ventricle. Two neurons in the NM were reconstructed after being labeled with horseradish peroxidase. Their axons project bilaterally, entering the ipsilateral NL at the dorsal surface and the contralateral NL at the ventral surface. This leads to a dorsal-to-ventral ipsilateral delay line and a ventral-to-dorsal contralateral delay line. From Carr and Konishi (1990). **B:** the antiparallel arrangement of delay lines originating from the ipsilateral and contralateral NM, combined with postsynaptic coincidence detection, establishes a topographic map of sound source azimuth. Neurons at the ventral edge of the NL respond to ipsilateral-leading ITDs and neurons at the dorsal edge of the NL respond to contralateral-leading ITDs. There is also a shift in ITD tuning along the mediolateral axis of the NL because of a medial-to-lateral delay line along contralateral NM axons before they enter the NL

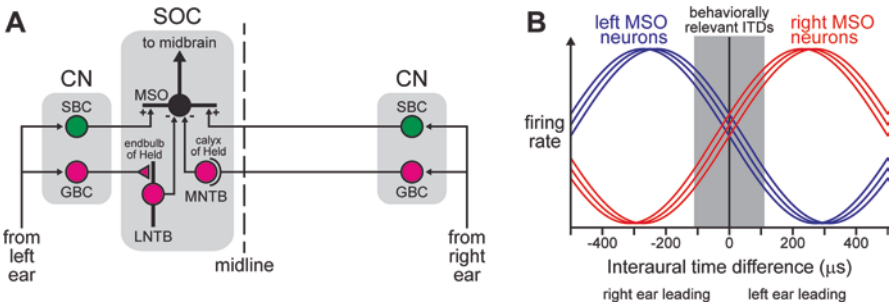

Fig. 10.6 The circuit for detecting ITDs in gerbils converts a temporal code to a hemispheric rate code for sound source azimuth. **A:** primary auditory afferents terminate on spherical bushy cells (SBCs; *green*) and globular bushy cells (GBCs; *magenta*) in the ipsilateral cochlear nucleus (CN). The SBCs provide excitatory glutamatergic input to neurons in the medial superior olive (MSO) within the superior olivary complex (SOC). Ipsilateral inputs from the SBCs terminate on the lateral dendrites, whereas contralateral inputs from SBCs terminate on the medial dendrites. The GBCs project ipsilaterally to terminate on neurons in the lateral nucleus of the trapezoid body (LNTB) via the end bulb of Held and contralaterally to terminate on neurons in the medial nucleus of the trapezoid body (MNTB) via the calyx of Held. Both LNTB and MNTB neurons terminate on the soma of MSO neurons to provide glycinergic inhibitory input. **B:** the firing rate of MSO neurons varies with sound source azimuth, with contralateral-leading ITDs eliciting stronger responses than ipsilateral-leading ITDs. Maximal responses are elicited by ITDs that are outside the behaviorally relevant range (i.e., the maximal ITDs that can be experienced given the distance between the ears; *gray box*). This suggests a two-channel, hemispheric rate code in which sound source azimuth is represented by the relative firing rates of neurons in the left versus the right MSO. For sounds coming from straight ahead, the left and right MSO firing rates are equal. For sounds coming from the right, the left MSO firing rate is greater than the right MSO firing rate and vice versa

seem to use a different mechanism. As in owls, ITDs and IIDs are processed in separate hindbrain circuits. The medial superior olive (MSO) is the first site of binaural convergence within the ITD pathway (Fig. 10.6A). Cats were the species of choice for early studies of the MSO circuit, but gerbils have emerged as the most commonly used species more recently. Anatomical studies have revealed axonal elongations that are suggestive of delay lines in the afferent inputs to the MSO (Smith et al. 1993; Beckius et al. 1999), and there is evidence that differences in axonal conduction velocities may also contribute to internal delays (Ford et al. 2015; Seidl and Rubel 2016). However, there does not appear to be a topographic map of ITDs in the MSO or a systematic antiparallel arrangement of delay lines from the two ears that could create such a map (Karino et al. 2011; Franken et al. 2015). Furthermore, MSO neurons (or their downstream targets) tend to respond most strongly to contralateral-leading ITDs that are greater than ITDs that could ever occur naturally given the distance between the two ears (Grothe et al. 2010).

Interestingly, the maximal slopes of the ITD tuning curves of MSO neurons do tend to fall within the behaviorally relevant range. Thus, as a sound source moves from right to left, increasing numbers of MSO neurons in the right hemisphere will be recruited and the overall firing rate of this neuronal population will increase

(Fig. 10.6B). The pattern is reversed for the left MSO. Thus, whereas the barn owl NL converts ITDs into a place code for sound source azimuth, the mammalian MSO appears to convert ITDs into a two-channel rate code in which sound source azimuth is represented by the relative amount of activity in the left and right MSOs (McAlpine et al. 2001). Others have challenged this coding scheme, however, noting that the preferred ITDs of MSO neurons can extend to smaller values that are within the behaviorally relevant range or even to ipsilateral-leading ITDs (Franken et al. 2015).

There has been a great deal of controversy regarding the mechanisms underlying ITD tuning in MSO neurons, in particular the source of internal delays that would compensate for acoustic delays and thereby establish a preference for a particular ITD. As in the barn owl NL, binaural coincidence detection of excitatory inputs from the two ears helps establish the ITD sensitivity of MSO neurons (van der Heijden et al. 2013; Plauška et al. 2016). Spherical bushy cells (SBCs) within the ventral cochlear nucleus relay phase-locked auditory input to the dendrites of MSO neurons, with ipsilateral inputs synapsing on the lateral dendrites and contralateral inputs synapsing on the medial dendrites (Fig. 10.6A). All else being equal, the longer axonal path length for contralateral excitatory inputs to reach the MSO neurons may contribute to a bias in ITD tuning toward contralateral-leading sounds.

Unlike the barn owl NL, however, there is also rapid, precisely timed, feedforward glycinergic inhibition (Brand et al. 2002; Grothe 2003). The dominant source of inhibition is from the medical nucleus of the trapezoid body (MNTB). MNTB cells receive input from globular bushy cells (GBCs) in the contralateral ventral cochlear nucleus (Fig. 10.6A). The GBC axons exhibit strong phase locking to sounds (Smith et al. 1991). They have the largest diameter of any axons in the auditory brainstem of mammals (Harrison and Warr 1962), along with additional anatomical specializations for increasing action potential conduction velocity (Ford et al. 2015). They also synapse onto MNTB cells through the calyx of Held, which is the largest, fastest, and most reliable synapse known in the mammalian brain (von Gersdorff and Borst 2002). As a result, MNTB neurons provide rapid, phase-locked inhibition to MSO neurons (Smith et al. 1998), in stark contrast to the relatively slow and diffuse GABAergic inhibitory input to the owl's NL. A secondary source of glycinergic inhibition to the MSO neurons comes from neurons in the lateral nucleus of the trapezoid body (LNTB), which receive excitatory input from ipsilateral GBCs via the end bulbs of Held. Both sources of glycinergic inhibition synapse onto the soma of MSO neurons (Fig. 10.6A).

A prominent model suggests that the faster and stronger inhibition arising from the contralateral MNTB compared with the ipsilateral LNTB establishes an internal delay (Pecka et al. 2008; Myoga et al. 2014). If the contralateral inhibitory input is faster than the contralateral excitatory input, this would effectively delay the depolarizing effect of contralateral excitation, thereby shifting the ITD tuning function in the contralateral-leading direction. This basic model can account for much of the experimental data obtained in vivo. Furthermore, it would be surprising if precisely timed inhibition did not play an important role in ITD tuning, given that the

predominant inhibitory input to the MSO neurons arises from a pathway that is clearly specialized for rapid and temporally precise synaptic transmission.

Nevertheless, recent studies have cast doubt on the relevance of this model to understanding ITD tuning in MSO neurons (Roberts et al. 2013; van der Heijden et al. 2013). A variety of additional mechanisms for generating internal delays have been proposed, including asymmetrical rise times for excitatory synaptic potentials arising from contralateral versus ipsilateral inputs (Jercog et al. 2010); differences in frequency tuning and, therefore, cochlear delays in the inputs coming from each ear (Joris et al. 2006; Plauška et al. 2017); an asymmetry in the anatomical location of the axon initial segment (Zhou et al. 2005); and an interaction between intrinsic membrane conductances and preceding synaptic activity (Roberts et al. 2013; Franken et al. 2015). Regardless of the mechanisms underlying ITD tuning in the MSO, it is clear that gerbils and barn owls solve this problem in fundamentally different ways.

10.4.3 Detection of Phase Modulations in Wave-Type Electric Fish

Weakly electric fishes offer yet more examples of neural circuit solutions to the problem of detecting submillisecond timing differences. The African Mormyroidea and South American Gymnotiformes evolved their electromotor and electrosensory systems independently (Lavoué et al. 2012). Wave-type fishes that perform the JAR have evolved in both groups (Bullock et al. 1975). Remarkably, these fishes use the same algorithm of comparing modulations in amplitude with modulations in phase to determine whether to raise or lower their EOD frequency, and they both have separate primary afferents dedicated to coding these two stimulus features (Kawasaki 1993). However, the neural circuitry underlying the detection of submillisecond timing differences between receptors is quite different between the two groups (Carr 2004; Kawasaki 2009).

In the Gymnotiformes, these timing comparisons are made by so-called small cells of the midbrain torus semicircularis, which have been studied most thoroughly in the glass knifefish *Eigenmannia virescens* (Fig. 10.7A). Large spherical cells in the hindbrain receive topographic input from primary time-coding afferents and project topographically to lamina 6 of the torus (Carr et al. 1986b). Spherical cell axons synapse onto the distal dendrites of small cells via mixed chemical-electrical synapses as well as onto the somas of giant cells via gap junctions (Carr et al. 1986b). Giant cells receive convergent input from multiple spherical cells representing a particular receptive field on the body surface, and this is associated with a reduction in timing jitter (Carr et al. 1986a). Giant cell axons project diffusely throughout lamina 6, synapsing onto numerous small cell somas (Carr et al. 1986b). Thus, a given small cell receives phase-locked input from two different receptive

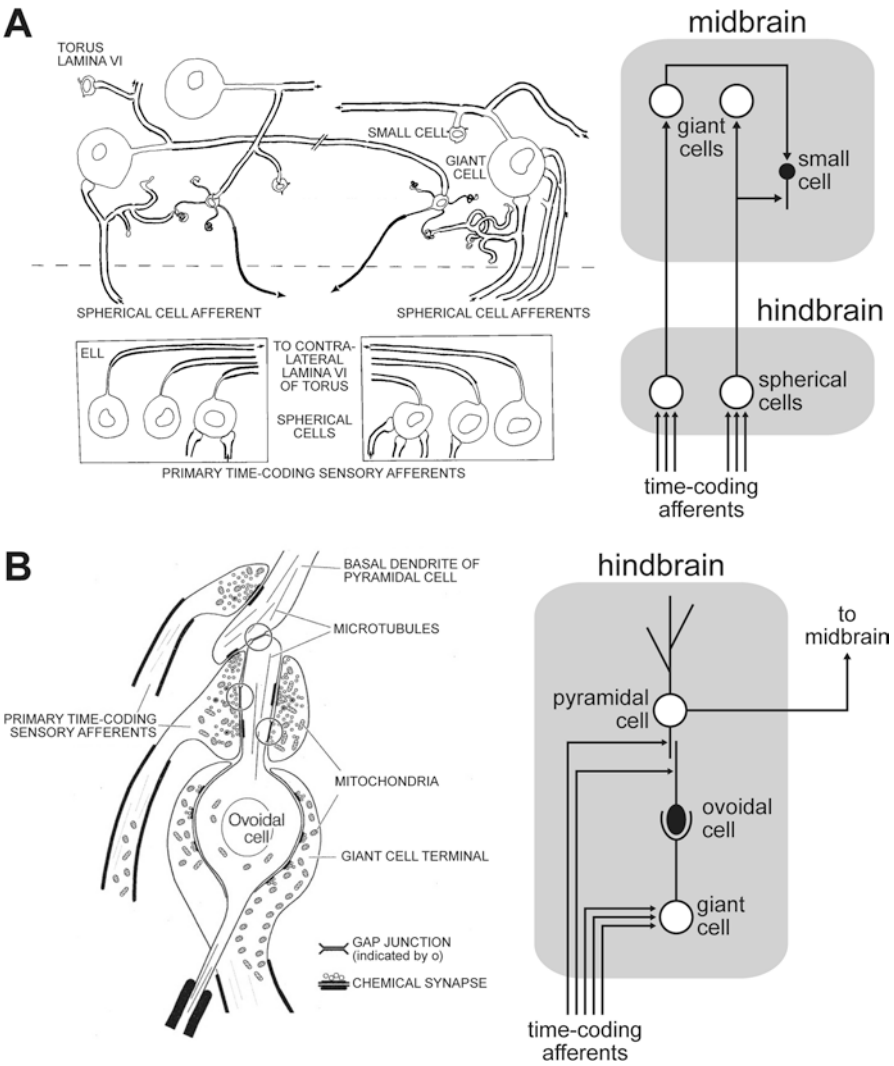

Fig. 10.7 Different circuits for detecting phase modulations of wave-type electric signals in the South American *Eigenmannia* and the African *Gymnarchus*. **A:** timing comparison circuitry in *Eigenmannia*. Time-coding primary afferents synapse onto the soma of spherical cells in the hindbrain electrosensory lateral line lobe (ELL). Spherical cells project to the contralateral lamina VI in the midbrain torus, where they target the dendrites of small cells and the somas of giant cells. Giant cells project widely throughout lamina VI, targeting the somas of small cells. Small cells exhibit sensitivity to timing differences between different electrosensory receptive fields. From Carr et al. (1986b). **B:** summary of the timing comparison circuitry in the hindbrain of *Gymnarchus*. Time-coding primary afferents synapse onto the soma of giant cells as well as onto the dendrites of both ovoidal cells and pyramidal cells in the hindbrain ELL. Giant cells synapse onto ovoidal cells with a giant terminal that embraces nearly the entire soma. Ovoidal cells and pyramidal cells connect with dendrodendritic synapses via gap junctions. Ovoidal cells have not been recorded from, but pyramidal cells exhibit sensitivity to timing differences between different electrosensory receptive fields. From Matsushita and Kawasaki (2004)

fields, an input from one part of the body surface onto its dendrite and an input from another part of the body surface onto its soma.

Small cells respond selectively to submillisecond advances or delays in electrosensory phase at one part of the body relative to another (Heiligenberg and Rose 1985). The underlying mechanisms for this remain unknown, but the anatomy of this circuit suggests one possibility (Carr et al. 1986b). A spherical cell synapse onto a small cell dendrite, as opposed to the soma, will introduce a conduction delay for a synaptic depolarization to reach the soma. If the somatic input from a giant cell is delayed by the same amount as this dendritic conduction delay, then the inputs from the giant cell and spherical cell will be coincident at the soma. As a result, the small cell will be maximally excited when the receptive field of the giant cell is stimulated with a small delay relative to the receptive field of the spherical cell. This conceptual model represents a different neuronal implementation of a delay-line coincidence-detection algorithm. In the Jeffress model, the delay is provided by axonal conduction, whereas here dendritic conduction provides the delay. In both cases, two excitatory inputs will be coincident on the soma of the postsynaptic neuron if the circuit delay perfectly compensates for the stimulus delay.

In the sole wave-type African mormyroid *Gymnarchus niloticus*, timing comparisons are made in the hindbrain rather than in the midbrain (Kawasaki and Guo 1996; Matsushita and Kawasaki 2004). The time-comparison neurons, called ovoidal cells, receive two excitatory inputs via mixed chemical-electrical synapses: a direct input from a single time-coding primary afferent axon onto their dendrite and an indirect input onto their soma from a single giant cell (Fig. 10.7B). The giant cells receive convergent input from many time-coding afferents. This wiring suggests that ovoidal cells compare local timing information provided by a single primary afferent with a global timing signal provided by a giant cell. Furthermore, the different synaptic locations of the two sources of input to ovoidal cells is suggestive of a circuit delay similar to that found in the small cells of gymnotiforms. Electrophysiological recordings have not yet been made from ovoidal cells, and their axonal targets remain unknown. However, the dendrites of ovoidal cells synapse with the basal dendrites of pyramidal cells via gap junctions. These pyramidal cells respond selectively to phase advances or delays at one part of the body surface relative to another, much like the small cells in the torus of gymnotiforms (Kawasaki and Guo 1996; Matsushita and Kawasaki 2005). Although the mechanisms for detecting these timing differences remain unknown, it seems likely that dendrodendritic excitation of pyramidal cells will be maximal when the excitatory input of the giant cell to the ovoidal cell soma slightly precedes that of the excitatory input of the primary afferent to the dendrite.

Despite the different circuitry for detecting timing differences in gymnotiforms and *Gymnarchus*, in both fishes, the temporal code in the periphery gets converted into a labeled-line code in which each individual neuron carries specific information about the stimulus, in this case information about phase advances or delays occurring at one point on the body surface relative to another. This is similar to the place code in the barn owl NL in which each neuron responds selectively to a particular ITD. However, there is no evidence for a spatial map among the timing disparity-

sensitive neurons in either group of fishes. In addition, the place code in the NL is a sparse code wherein a given ITD causes spiking in just the few neurons that are tuned to that ITD while other neurons remain silent. In contrast, the labeled-line code in wave-type fishes is a dense code in which large numbers of neurons fire because phase advances or delays occur between many points on the body surface simultaneously. Indeed, accurate performance of the JAR requires the detection of all these possible timing differences (Heiligenberg 1991).

10.4.4 Detection of Electric Signal Waveforms in Mormyrid Fishes

Pulse-type African mormyrid electric fish reveal yet another kind of circuit mechanism for detecting submillisecond timing differences (Xu-Friedman and Hopkins 1999; Baker et al. 2013). The primary afferents innervating knollenorgan electroreceptors project roughly somatotopically to the nucleus of the electrosensory lateral line lobe (nELL) where they terminate with large club endings onto large, adendritic spherical cells via mixed chemical-electrical synapses (Bell and Szabo 1986; Mugnaini and Maler 1987b). An estimated 3–4 afferents converge onto each nELL neuron. These neurons also receive GABAergic inhibition arising from the electric organ corollary discharge pathway (Bell and Grant 1989). Every time a fish generates an EOD, this inhibition briefly and completely silences these cells, thereby blocking responses to the fish's own EOD (see Perks and Sawtell, Chap. 11). This electrosensory pathway therefore functions solely in electric communication behavior.

The axons of nELL neurons project bilaterally to the torus semicircularis (Bell and Szabo 1986). The mormyrid torus has a nuclear organization, in contrast to the laminar organization found in gymnotiforms. The nELL axons terminate in two nuclei: the medioventral nucleus (MV) and the anterior exterolateral nucleus (ELa). The input to the ELa has all the hallmarks of a time-coding pathway: large axon diameters, heavy myelination, and large synapses (Xu-Friedman and Hopkins 1999). In contrast, the input to the MV is via thin collaterals that branch off from the main nELL axons, and these give rise to small bouton synapses. Very little is known about electrosensory processing in the MV, but the circuit in the ELa has been relatively well studied.

There are two cell types within the ELa, adendritic large cells and adendritic small cells (Szabo et al. 1983; Mugnaini and Maler 1987a). Shortly after entering the ELa, nELL axons synapse onto one to three large cells with large, mixed chemical-electrical synapses (Fig. 10.8A, C). The nELL axons then continue on a long and winding path through the nucleus while giving off several small *en passant* mixed chemical-electrical synapses onto small cells (Friedman and Hopkins 1998). After traveling several millimeters, these axons then branch extensively and synapse onto dozens of additional small cells. The large cells project to small cells

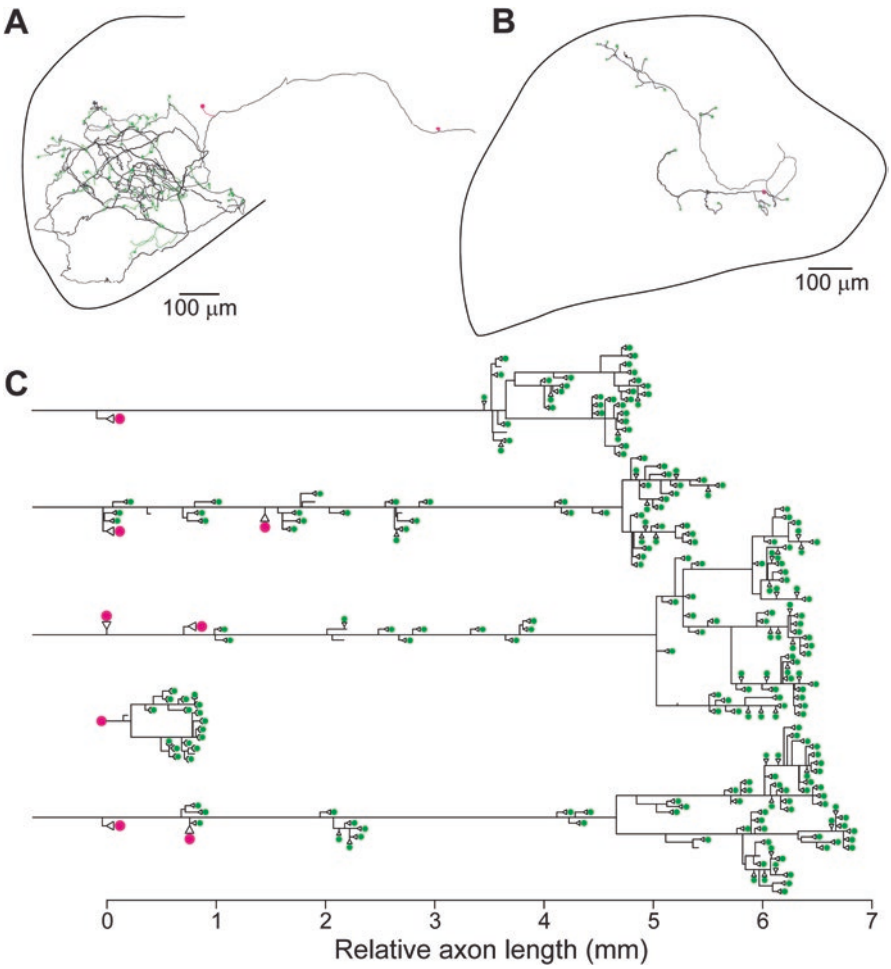

Fig. 10.8 Axonal delay lines and inhibition in the anterior exterolateral nucleus (ELa) of mormyrids. **A:** reconstruction of an axon from a biocytin-filled neuron in the hindbrain nucleus of the electrosensory lateral line lobe (nELL). The thickness of the axon represents its dorsal-to-ventral extent. Lateral is at *left*; anterior is *up*. *Green*, terminals onto small cells; *magenta*, terminals onto large cells. **B:** reconstruction of an ELa large cell. *Magenta*, soma; *green*, terminals onto small cells. **C:** linear reconstructions of four nELL axonal arborizations and one ELa large cell. From Xu-Friedman and Hopkins (1999)

through a more direct route (Fig. 10.8B, C), providing GABAergic inhibition via a large calyceal synapse that engulfs small cell somas (Mugnaini and Maler 1987a; Friedman and Hopkins 1998). Thus, small cells receive two inputs that arise from different receptive fields: a relatively direct inhibitory input and an excitatory input that is subject to an axonal conduction delay due to the elongated path of the nELL-to-small cell projection (Fig. 10.9A, B).

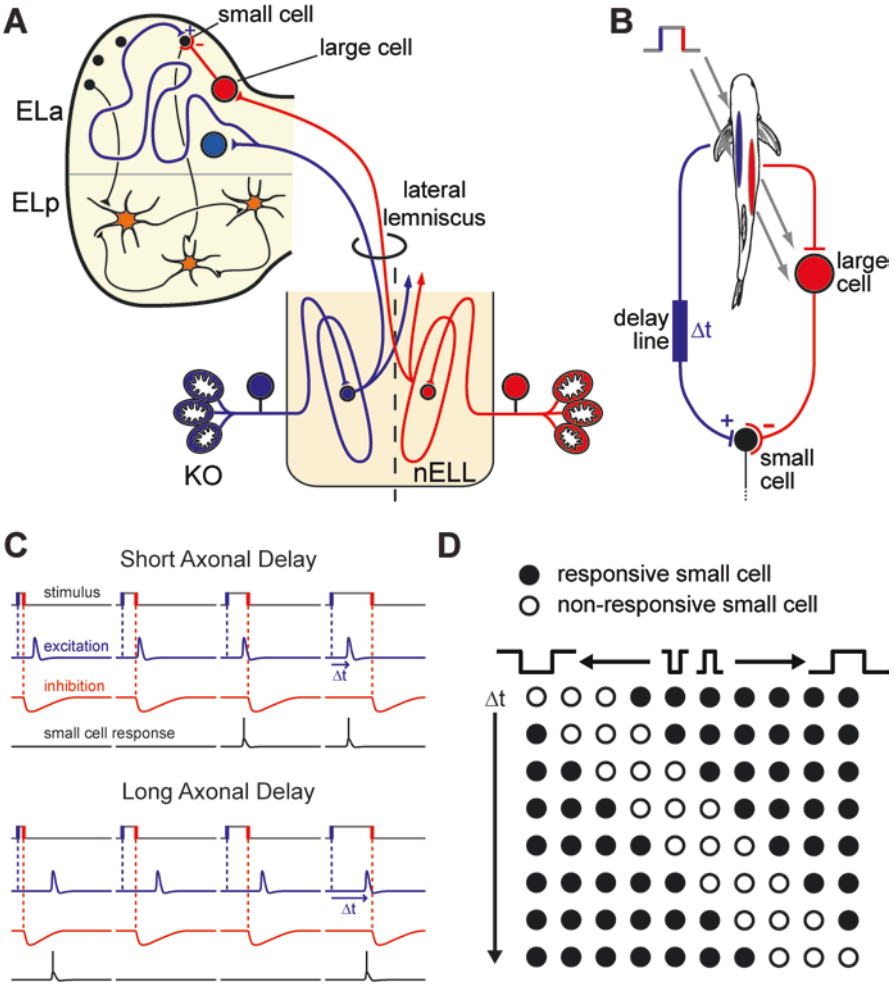

Fig. 10.9 Delay-line anticoincidence detection converts a temporal code into a population code for EOD waveform in mormyrid fishes. **A:** schematic of the knollenorgan (KO) pathway. Primary afferents from KOs project to the ipsilateral nELL. Neurons in the nELL project bilaterally to the ELa via the lateral lemniscus where they synapse onto large cells (*red* and *blue*) and, after an axonal delay, onto small cells (*black*). Small cells project ipsilaterally to the posterior exterolateral nucleus (ELp), where they synapse onto multipolar cells (*orange*). **B:** EODs can be approximated by a simplified square pulse. KOs on one side of the body surface (*blue*) respond to the upward edge of a square pulse, and this gives rise to an excitatory input to a small cell through an axonal delay line (Δt). KOs on the other side of the body surface (*red*) respond to the downward edge, and this gives rise to an inhibitory input to the small cell. **C:** the responses of small cells to square-pulse stimuli of different durations are determined by the length of the excitatory axonal delay. This delay is different for each small cell, and this leads to differences among the small cells in which stimulus durations result in coincident excitation and inhibition. *Dashed blue lines*, timing of stimulus onset; *dashed red lines*, timing of stimulus offset. **D:** there is a systematic relationship between the axonal delay (Δt) and the stimulus polarities/durations that elicit coincident excitation and inhibition. Each small cell responds to a wide range of stimuli, but the identity of the stimulus can be determined by assessing which cells across the population are responding and which cells are not. This represents a combinatorial code for the stimulus

There are two key differences between the circuit underlying timing comparisons in the mormyrid ELa and the Jeffress-like ITD-detecting circuit in the NL of barn owls. First, the nELL axonal delay lines do not have a systematic spatial arrangement. Instead, the tortuous path of these axons would seem to preclude any kind of topographic stimulus representation. Second, unlike the binaural excitation of NL neurons in the barn owl, small cells in the mormyrid ELa receive one excitatory input and one inhibitory input. Thus, if the circuit delay compensates for the stimulus delay between these two inputs, then excitation and inhibition will be coincident. This represents a delay-line *anti*-coincidence detection mechanism: the small cell will respond to any stimulus for which inhibition and delayed excitation are *not* coincident (Fig. 10.9C).

Electrophysiological recordings from small cells, combined with pharmacological manipulation and computational modeling, provide support for this model and confirm that there is no spatial representation of peripheral timing differences (Lyons-Warren et al. 2013). Instead, each individual small cell responds to all stimuli that do not result in coincident excitation and inhibition. This window of inhibition varies across the small cell population because it is set by the axonal delay relative to the inhibitory synaptic delay, which is different for each small cell (Fig. 10.9C). This establishes a combinatorial code in which individual neurons respond to a wide range of stimuli, but the pattern of responsiveness across the population collectively represents specific stimuli (Fig. 10.9D).

10.5 Evolution of Neural Circuits for Detecting Submillisecond Timing Differences

Comparing neural circuits that have evolved independently between clades to solve similar behavioral problems can yield insight into both shared, fundamental mechanisms as well as the diversity of potential solutions. On the other hand, comparing neural circuits across species within clades that have evolved behavioral differences can give us insight into the process by which this diversity may have arose. In addition, comparisons across species that share homologous circuits but differences in behavior can point toward potential mechanisms for behavior.

10.5.1 Evolution of Interaural Time Difference Detection in Mammals

Not all mammals use ITDs for localizing sound sources. Mammals that have poor low-frequency hearing, such as mice, rats, bats, and opossums, do not appear to use ITDs for sound localization. Nevertheless, these animals have an MSO, but it differs in several respects from the MSO of ITD-sensitive mammals such as gerbils, guinea

pigs, cats, dogs, and humans (Grothe 2000, 2003). In mammals that use ITDs for sound localization, the bipolar MSO neurons are aligned tightly in a single sagittal plane so that their dendrites are in spatial register, which may ensure uniform timing of binaural excitatory synaptic inputs across the MSO population. However, this strict alignment is not found in mammals that do not use ITDs for sound localization (Kapfer et al. 2002; Fischl et al. 2016). In addition, the dendrites of MSO neurons in non-ITD-sensitive mammals are thinner and have greater branching, suggesting that the temporal precision of binaural integration is not as critical in mammals that do not use ITDs (Kapfer et al. 2002; Fischl et al. 2016).

Although glycinergic inhibitory synapses onto MSO neurons are confined to the soma in ITD-sensitive mammals, they are found throughout the soma and dendrites of non-ITD-sensitive mammals, suggesting differences in the temporal precision or effectiveness required of inhibition (Kapfer et al. 2002; Fischl et al. 2016). Furthermore, anatomical specializations that increase action potential conduction velocity in the GBC axons of gerbils are not found in mice (Ford et al. 2015; Stange-Marten et al. 2017). MSO neurons in non-ITD-sensitive mammals do exhibit sensitivity to temporal features of sounds that may play important roles in functions such as envelope coding (see Metzen and Chacron, Chap. 9), acoustic pattern recognition, or echo suppression (Grothe 2000; Fischl et al. 2016).

Both fossil evidence and phylogenetic reconstruction suggest that high-frequency hearing was the ancestral mammalian phenotype and that low-frequency hearing and ITD sensitivity are derived conditions that have evolved in several lineages (Grothe 2000; Grothe and Pecka 2014). Thus, it may be that an ancestral role for the MSO in processing temporal features of sound served as a preadaptation for the MSO to evolve ITD sensitivity in lineages that evolved low-frequency hearing. The shared specializations of MSO neurons found across these species would then be due to parallel evolution from similar ancestral circuits rather than homology.

10.5.2 Evolution of Increased Sensitivity to Interaural Time Differences in Archosaurs

Evolutionary differences in sound localization circuitry are also found among extant archosaurs (birds and crocodilians; Fig. 10.10). As in the barn owl, the NM-to-NL circuit in chickens and emus generally conforms to the Jeffress model (MacLeod et al. 2006; Köppl and Carr 2008). However, only the contralateral axonal input to the NL forms a delay line in chickens, with the ipsilateral NM giving rise to axons that provide relatively homogeneous input delays to NL neurons (Young and Rubel 1983; Seidl et al. 2010). Accordingly, ITD tuning in chickens goes from near zero at the medial edge of the NL to increasingly contralateral-leading ITDs at the lateral edge (Köppl and Carr 2008). The NL is enlarged in barn owls and, in addition to the medial-to-lateral contralateral delay line found in chickens, there are also antiparallel contralateral and ipsilateral delay lines within the NL (Fig. 10.10). These internal

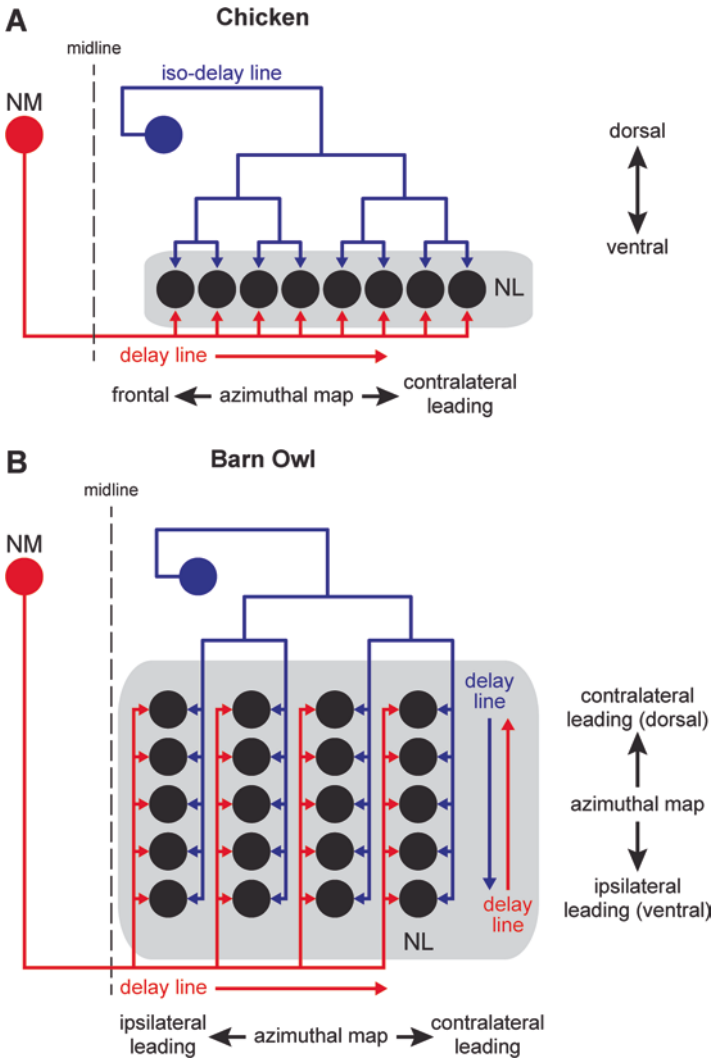

Fig. 10.10 Evolutionary change in the circuit mediating detection of ITDs in archosaurs (birds and crocodilians). **A:** in chickens, the contralateral projections from the NM to the NL follow a delay line that heads in a medial-to-lateral direction, whereas the ipsilateral inputs follow an iso-delay line in which all of the inputs are roughly equally delayed to their targets. This forms a topographic map of ITDs (sound source azimuth) along the mediolateral axis, with sound sources coming from directly in front represented by neurons at the medial edge of the map and sound coming from the far contralateral side represented by neurons at the lateral edge of the map. A similar circuit is found in emus and alligators. **B:** the NL of barn owls is enlarged and contains additional delay lines within the NL. Entering ipsilateral NM axons give rise to dorsal-to-ventral delay lines and entering contralateral NM axons give rise to ventral-to-dorsal delay lines. This forms a topographic map of ITDs along both the mediolateral and dorsoventral axes (see Fig. 10.5B). This expanded representation of ITDs is associated with greater ITD acuity in barn owls compared with that in chickens

delay lines establish an additional dorsoventral axis to the topographic map of ITDs in barn owls and extend the range of ITD sensitivity from ipsilateral-leading ITDs to contralateral-leading ITDs (Carr et al. 2015). Alligators are generally similar to chickens and emus in both the anatomy of their NM-to-NL circuitry as well as the mapping of ITD tuning by NL neurons (Carr et al. 2009). This suggests that the circuit found in chickens, emus, and alligators represents the ancestral state for archosaurs and that there was an evolutionary enlargement of the owl NL as an adaptation for both high-frequency hearing and greater acuity in ITD detection (Köppl and Carr 2008).

10.5.3 Evolution of Electric Signal Waveform Sensitivity in Mormyrid Fishes

Pulse-type African mormyrid fishes provide an especially striking example of evolutionary change in time-coding circuitry. In contrast to the broadly distributed receptors found in most mormyrid species, the receptors in nearly all known species of the subfamily Petrocephalinae are, instead, limited to three clusters on the head called rosettes (Harder 1968; Carlson et al. 2011). Rather than spiking, these rosette receptors actively oscillate at frequencies ranging from 1–3 kHz (Fig. 10.11A). An electrosensory stimulus causes an increase in oscillation amplitude and a reset of the oscillation phase (Baker et al. 2015). Inward current drives a reset to the positive phase of the oscillation cycle, whereas outward current causes a reset to the negative phase of the oscillation cycle. Thus, receptors on opposite sides of the body, which experience stimuli with opposite polarities, respond with phase resets that differ by 180°. However, unlike the spiking receptors of other mormyrid species (Fig. 10.11B), these phase resets do not vary with the stimulus waveform (Fig. 10.11C). Primary electrosensory afferents have not yet been recorded from in species with oscillating receptors, but it is likely that the relatively high oscillation amplitude of receptors immediately following a stimulus drives afferent spiking. This assumption is supported by recordings from the central electrosensory system, which reveal large, short-latency evoked potentials following an electrosensory stimulus (Vélez and Carlson 2016).

Although spiking receptors encode information about stimulus location into the sign of the spike timing difference and the stimulus waveform into the magnitude of the spike timing difference (Figs. 10.2C and 10.11B), oscillatory receptors only encode information about stimulus location into the sign of the timing difference (Fig. 10.11C). Indeed, behavioral studies suggest that species with oscillatory receptors cannot detect variation in EOD waveform, although species with spiking receptors can (Carlson et al. 2011). Furthermore, phylogenetic reconstruction suggests that oscillating receptors are the ancestral state from which spiking receptors evolved. This raises a fundamental question: How do central sensory circuits evolve to process new information coming from the periphery? More specifically, How did

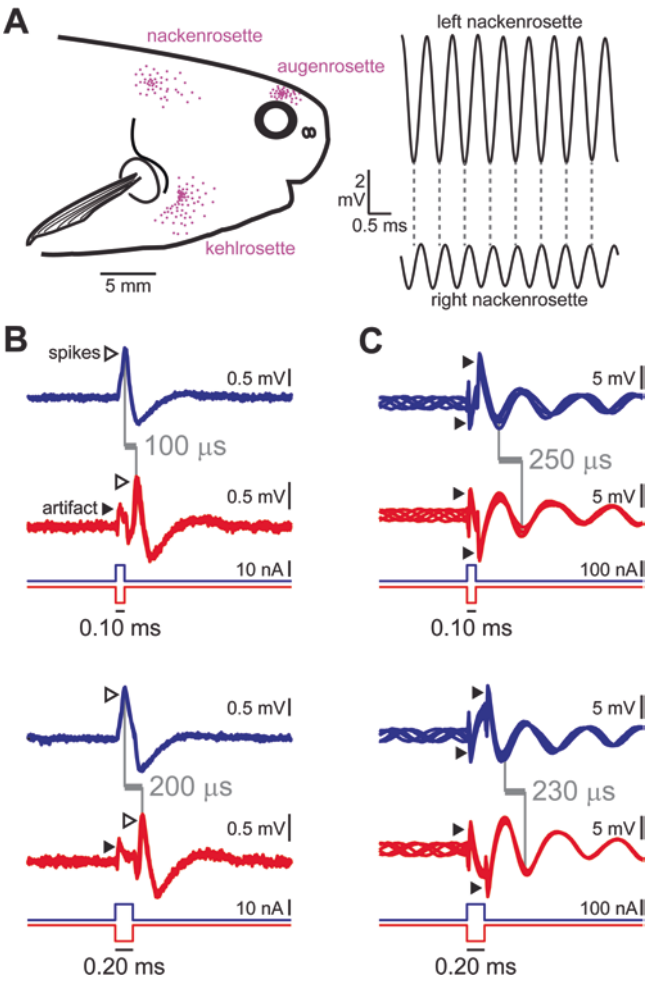

Fig. 10.11 Evolutionary change in the temporal coding of EODs in mormyrid fishes. **A:** in some species of mormyrids, such as *Petrocephalus tenuicauda* (*left*), the distribution of KOs is limited to three distinct clusters on the head called rosettes (*purple dots*), originally described in German as the nacken- (neck), augen- (eye), and kehl- (throat) rosettes. Unlike the spiking receptors of other mormyrids, these receptors generate spontaneous oscillations at frequencies ranging from around 1 to 3 kHz. The oscillations of different receptors are independent of each other, as illustrated in a continuous recording from two receptors located in different rosettes (*right*). **B:** extracellular recordings from a spiking KO receptor reveal that spike timing reflects pulse polarity and duration. Responses to 10 repetitions of each stimulus are shown superimposed. The receptor spikes in response to the onset of positive polarity pulses and to the offset of negative polarity pulses. These stimuli represent the local stimuli received by KOs on opposite sides of the body in response to a global stimulus, revealing that the difference in spike timing encodes the duration of the stimulus pulse. **C:** extracellular recordings from an oscillating KO receptor reveal that the oscillatory phase reflects pulse polarity but not duration. In response to a stimulus pulse, the ongoing spontaneous oscillations exhibit a phase reset and an increase in amplitude. Reversing the polarity of the stimulus shifts the phase reset by 180°, but the phase reset does not vary with pulse duration. Modified from Baker et al. (2015)

the central electrosensory pathway of mormyrids evolve the ability to detect the magnitude of timing differences from an ancestral pathway that could only detect the sign of timing differences?

There is a dramatic difference in the gross anatomy of the relevant nuclei within the torus semicircularis between the two types of mormyrids (Carlson et al. 2011). The exterolateral nucleus (EL) is about twice as large in species with spiking receptors compared to species with oscillating receptors. In addition, the EL is clearly divided into separate anterior and posterior nuclei (ELa and ELp, respectively) in species with spiking receptors, whereas such a division is not apparent in the gross anatomy of species with oscillating receptors. Surprisingly, however, the basic wiring of the underlying circuit appears identical between the two groups at the cellular and synaptic levels (Fig. 10.12; Vélez et al. 2017). Incoming axons from the hindbrain nELL synapse onto adendritic small cells and adendritic large cells in both groups. The large cells provide GABAergic inhibition to the small cells in both groups. And the small cells output to multipolar cells in both groups. In species with spiking receptors, the small cells and large cells are restricted to the ELa, whereas multipolar cells are restricted to the ELp. In species with oscillating receptors, this anatomical segregation is not as complete, which may account for the lack of an apparent ELa/ELp division in the gross anatomy. This presents a conundrum: How can a neural circuit evolve to perform new computations without adding new parts?

In the sole species with oscillating receptors that has been studied in detail, the axons of nELL neurons do not appear to follow the long, tortuous, and branching paths that are seen in species with spiking receptors (Fig. 10.12; Vélez et al. 2017).

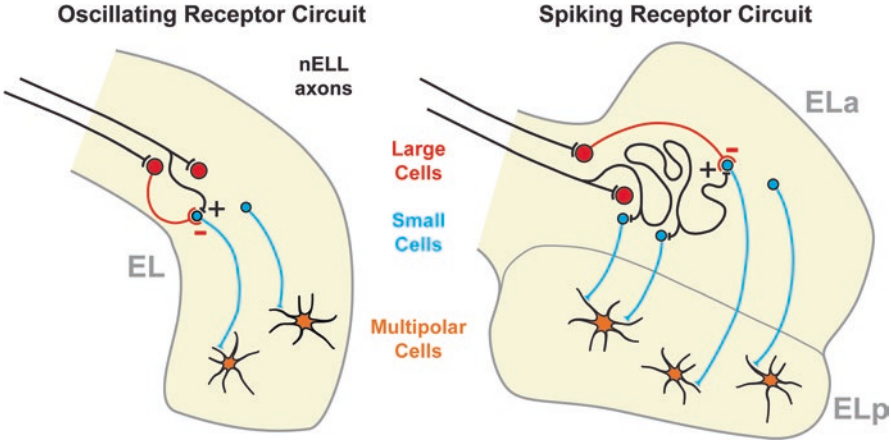

Fig. 10.12 Addition of axonal delay lines established behavioral sensitivity to EOD waveform in mormyrid fishes. In species with oscillating receptors, the exterolateral nucleus (EL) is small and undifferentiated, whereas in species with spiking receptors, the EL is divided into separate anterior and posterior nuclei. Nevertheless, the same three basic cell types are found in both circuits, and they are wired together in the same way. The evolution of EOD waveform sensitivity in species with spiking receptors is associated with an increase in cell numbers as well as an elaboration of the afferent axons projecting to small cells that establishes a delay line

Electrophysiological comparisons suggest that this anatomical difference does indeed establish a delay line in species with spiking receptors that is lacking in species with oscillating receptors (Vélez et al. 2017). The ancestral large cell-to-small cell inhibitory microcircuit may play a role in signal localization by establishing sensitivity to the sign of the peripheral timing difference (Vélez et al. 2017). The addition of delay lines through axonal elongation in species with spiking receptors would then have expanded on this directional sensitivity to establish sensitivity to small timing differences that code for EOD waveform. Finally, these elongated axons along with the addition of more small cells and multipolar cells to handle the wider range of timing information would have caused an enlargement of the ELa/ELp in species with spiking receptors as compared with the EL of species with oscillating receptors.

10.6 Summary

Despite their independent evolutionary origins, circuits that are specialized for precise temporal coding share several distinctive traits at the cellular, synaptic, and molecular levels of organization. Although the ways in which these different building blocks are assembled into functional circuits vary widely, these different circuit mechanisms also reveal common themes. Coincidence detection (or anticoincidence detection in the case of mormyrids) of two precisely timed synaptic inputs seems to play a critical role in each circuit. And in each circuit, coincidence occurs at a specific stimulus delay due to a compensatory delay within the circuit. These similarities have arisen through convergent evolution driving solutions to similar behavioral problems. But why have these circuits come up with different mechanisms of establishing internal delays? And why do some circuits rely on integrating excitation and inhibition, whereas others rely primarily on integrating multiple excitatory inputs? And why are precise temporal codes converted into so many different coding schemes?

One obvious answer lies in the independent evolutionary origins of each circuit. The electrosensory systems of African mormyroids and South American gymnotiforms evolved independently from mechanosensory lateral line systems (Lavoué et al. 2012). Likewise, tympanic middle ears that receive airborne sound appear to have evolved independently in mammals and sauropsids (Clack 1997; Christensen-Dalsgaard and Carr 2008). It may simply be that these various mechanisms and neural coding schemes all work sufficiently well at solving the behavioral problem, and chance has dictated which solution evolved in each circuit. However, each of these circuits originated from a different, preexisting ancestral neural circuit. This may have constrained the possible circuit solutions or made certain solutions more or less likely to occur. Examples of this can be seen in the evolution of ITD sensitivity from more general temporal processing within the MSO of mammals (Grothe 2000) and in the evolution of EOD waveform sensitivity from spatial processing within the ELa of mormyrid fishes (Vélez et al. 2017). In both cases, an existing

circuit was modified to establish highly precise temporal processing rather than building a whole new circuit from scratch.

Might there be more to this diversity than different ancestral starting points combined with chance? A modeling study suggested that the different ITD coding schemes used by the MSO of small mammals and the NL of barn owls represented optimal strategies from an information theoretic perspective for animals with different head sizes that detect ITDs across different sound frequency ranges (Harper and McAlpine 2004). However, it was soon discovered that chickens use the same coding scheme as owls despite being more similar to gerbils in both head size and in having relatively low-frequency ITD sensitivity (Köppl and Carr 2008). Nevertheless, this functional perspective may partially explain the evolution of different mechanisms for ITD processing in mammals and archosaurs (Grothe 2000; Grothe and Pecka 2014). Several lines of evidence suggest that the earliest mammals had small heads and heard relatively high-frequency sounds, whereas the earliest archosaurs had large heads and heard relatively low-frequency sounds. Thus, ITD sensitivity likely appeared early in the evolution of archosaurs, when a Jeffress-style solution to ITD processing would have been the optimal coding scheme. In contrast, the earliest mammals most likely relied solely on IIDs for azimuthal sound localization. ITD sensitivity in mammals would then be derived from an ancestral IID-processing system, arising only in those lineages that evolved low-frequency hearing. Because IID processing relies on integrating excitation and inhibition to establish a rate code for sound intensity, this may explain why the MSO does likewise in processing ITDs.

A functional perspective may also be useful in considering the various coding schemes used by electric fishes. The different auditory and electrosensory circuits discussed in this chapter all share the basic problem of detecting submillisecond timing differences. However, the number of peripheral inputs that must be compared differ greatly. For sound localization, a single timing comparison must be made between two receptive fields, the left and right ears. A continuous distribution of ITDs can be translated directly into neuron locations or relative firing rates. Electric fishes, however, have electroreceptors distributed widely across the body surface. They need to detect all timing differences throughout this array because the locations on the body surface that are subject to timing differences depend on the relative orientation of sender and receiver. As the number of receptive fields (n) increases, the number of pairwise comparisons (c) that must be made to detect all possible timing differences among those receptive fields increases as $c = n(n - 1)/2$. For sound localization, $n = 2$ and $c = 1$. Assuming that an electric fish has 20 electroreceptors (an underestimate), then $n = 20$ and $c = 190$. Thus, although azimuthal sound localization involves the coding of a single stimulus feature (ITD between the left and right ears), timing difference detection in electric fishes involves the coding of a much larger number of stimulus features (timing differences between all receptor pairs). Population codes, in which information is distributed across large numbers of neurons, are able to represent multiple stimulus features simultaneously with far fewer neurons than would be required of a place code or two-channel rate code (Rolls 1997; Averbeck et al. 2006).

In addition, for wave-type fishes to perform the JAR, they simply need to detect phase advances or delays at different points on the body surface. In principle, a labeled-line code could represent this information using just two neurons per comparison: one that detects advances and one that detects delays. In contrast, to identify the EOD waveform, pulse-type mormyrids need to determine not just whether the signal is advanced or delayed at one part of the body surface relative to another but also the precise magnitude of the timing difference. In addition, identifying a multiphasic EOD requires multiple timing comparisons. This behavior therefore requires much greater information-processing capability. A combinatorial code that is distributed across many neurons is likely to be a more efficient solution for this than rate, place, or labeled-line codes (Rolls 1997).

Most likely, the variety of circuit solutions to the problem of detecting submillisecond timing differences represents some combination of evolutionary history, chance, and adaptation. Regardless of the ultimate evolutionary causes for these differences, this diversity serves as a cautionary tale about the dangers of relying on a small number of "model systems" for addressing general questions in neuroscience (Katz 2016). Findings in one species simply cannot be extrapolated to another. In addition to studying species that are amenable to investigation, comparative approaches are needed to identify those features that are shared across species and those that are unique to particular lineages (Carlson 2012; Brenowitz and Zakon 2015; Yartsev 2017).

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Chapter 11
Influences of Motor Systems on Electrosensory Processing

Krista Perks and Nathaniel B. Sawtell

Abstract The first central stage of electrosensory processing in fish has proven to be a particularly useful model system for examining the general issue of how motor systems and behavior influence sensory processing. This chapter reviews this literature, focusing on a substantial body of work elucidating the synaptic, cellular, and circuit mechanisms for predicting and canceling self-generated sensory inputs. Some additional functions of motor corollary discharge signals in weakly electric mormyrid fish are also discussed along with the implications of studies on electrosensory systems for other sensory modalities and brain structures, including the auditory system and the cerebellum.

Keywords Cerebellum · Corollary discharge · Dorsal cochlear nucleus · Electric fish · Electrosensory internal model · Negative image · Proprioception · Reafference · Synaptic plasticity

11.1 Introduction

Laboratory studies of sensory processing typically focus on characterizing neural responses evoked by sensory stimuli delivered to passive subjects (Churchland et al. 1994). However, under most natural circumstances, sensory information is acquired actively through movement and exploration. Movements allow animals to acquire more and better information about the world but also pose a fundamental challenge for the nervous system. Self-generated sensory inputs could interfere with the detection and processing of behaviorally relevant stimuli or trigger inappropriate

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motor behaviors. The fact that such difficulties seldom arise raises the question of how sensory-processing structures in the brain distinguish between patterns of sensory-receptor activation due to external events and those due to the animal's own behavior. This so-called "reafference problem" has long been recognized and affects most, if not all, sensory systems (von Holst and Mittelstaedt 1950; Crapse and Sommer 2008).

Studies of electrosensory systems in fish have provided a detailed illustration of how this fundamental problem in neurobiology is solved. Neurons in the first stage of electrosensory processing generate specific predictions about the electrosensory consequences of the animal's own behavior. Combined experimental and theoretical studies of electrosensory systems have provided an account of how these predictions, termed negative images, are formed at the level of synaptic plasticity, cells, and circuits. By canceling out the effects of self-generated inputs, negative images enhance detection and behavioral responses to external stimuli. This chapter reviews these studies and discusses their implications for other sensory systems and brain structures, including the mammalian auditory system and the cerebellum. This chapter also reviews some additional functions that have been identified for the prominent and well-studied electric organ corollary discharge (EOCD) system of weakly electric mormyrid fish.

11.2 Electrosensory Systems and the Problem of Reafference

Studies of both passive and active electrosensory systems have demonstrated that reafference, defined as sensory stimulation related to an animal's own behavior, drives responses in electroreceptors. In elasmobranchs (the group that includes sharks, skates, and rays), ventilatory movements of the gills modulate the fish's own standing bioelectric field, which, in turn, modulates the firing of afferents innervating exquisitely sensitive ampullary electroreceptors that serve passive electrolocation in these fish (Montgomery and Bodznick 1999). In weakly electric mormyrid fish, the electric organ discharge (EOD) pulses (serving active electrolocation) have been shown to strongly affect ampullary electroreceptors involved in passive electrolocation (Bell and Russell 1978). Studies of the passive electrosensory system of mormyrid fish provided the first evidence for negative images in an electrosensory system and is discussed in detail in Sect. 11.4. The EOD of weakly electric fish sets up a self-generated electric field that is modulated by objects in the environment and drives responses in afferents innervating mormyromast electroreceptors for active electrolocation. In these fish, swimming movements alter the position of the electric organ relative to electroreceptors on the skin, causing modulations in the fish's self-generated electrical field as large or larger than those due to objects in the environment (Sawtell and Williams 2008; Fotowat et al. 2013). Some species of mormyrid fish have highly mobile chin appendages used for foraging (Amey-Ozel et al. 2015). Because this appendage is densely covered with electroreceptors,

its rapid movement during foraging behavior is also likely to be a major source of reafference (Engelmann et al. 2009).

The manner in which the brain solves the reafference problem likely depends both on the nature of the self-generated signals and how they compare with the external signals that the system has evolved to process. If the animal's own behavior results in patterns of receptor activation that are very different from those due to external events, invariant spatial or temporal filtering strategies may contribute to removing reafference. Along these lines, a "common-mode rejection" mechanism for suppressing spatially uniform ventilatory reafference has been described in elasmobranchs (Montgomery 1984). Whereas electroreceptor afferents are strongly and uniformly modulated by ventilatory reafference, second-order neurons in the hindbrain show much weaker responses (Montgomery and Bodznick 1999). This difference is due, in part, to a commissural GABAergic inhibitory pathway that suppresses activity patterns that are shared by electroreceptors located on the opposite side of the body (Duman and Bodznick 1996). In many cases, however, the characteristics of reafference are similar to those of behaviorally relevant signals, necessitating more complex solutions.

Sperry (1950) and von Holst and Mittelstaedt (1950) performed a pioneering series of behavioral experiments in fish and flies that suggested that ambiguity in the origin of sensory stimulation could be resolved at central processing stages by integrating sensory information with additional signals related to the animal's own movements and behavior such as motor corollary discharge. A challenge for subsequent neurophysiological and neuroanatomical studies was to pinpoint such signals in the brain. In the case of vision, where the question of how visual perceptual stability is maintained in the face of rapid eye movements has been extensively studied, these were termed extraretinal signals (Grusser 1986). Roles for both motor corollary discharge signals related to eye movements and ocular proprioception have been identified in maintaining stable and accurate visual perception in primates (Sun and Goldberg 2016). However, due to the complexity of the neocortical structures involved, circuit-level questions regarding how visual and extraretinal signals are integrated have been difficult to address. The convergence of peripheral sensory input with multiple streams of information related to movements and behavior, including both corollary discharge and proprioception, is a prominent feature of the first central stage of electrosensory processing in the brains of fish. The relative simplicity of these circuits and their close proximity to the sensory periphery has made it possible to gain a detailed mechanistic understanding of how these circuits solve the reafference problem.

Finally, it should be noted that changes in sensory input due to behavior can also convey useful information (Gibson 1979). This is particularly clear in active sensory systems, such as the active electrosensory systems of weakly electric fish in which the animal generates signals used for sensing. A brief example of how motor corollary discharge signals in mormyrid fish may aid in the processing of information contained in the fish's own EOD is given in Sect. 11.5.2. Although not reviewed in depth here, electric fish are also a useful model system to address the general

question of how an animal's motor behavior may enhance sensory processing and perception. For example, when encountering a novel object, weakly electric fish engage in stereotyped patterns of movement termed probing motor acts (Toerring and Moller 1984). It has also been suggested that mormyrids use self-motion-derived electrosensory cues (analogous to optic flow in the visual system) to judge the distance of objects (Hofmann et al. 2017). Clearly, some components of sensory reafference are not canceled out and may, in fact, play critical roles in perception.

11.3 Convergence of Electrosensory and Behavior-Related Signals in Cerebellum-Like Structures

The first central stage of electrosensory processing in the brains of fish occurs in hindbrain structures that share numerous similarities with the cerebellum in terms of their evolution, development, patterns of gene expression, and circuitry (Bell 2002; Bell et al. 2008). The so-called cerebellum-like electrosensory processing structures discussed in this review are the dorsal octavolateral nucleus (DON) in elasmobranchs and the electrosensory lobe (ELL) of weakly electric mormyrid and gymnotiform fish (Bell and Maler 2005). Although strikingly similar in numerous respects, the electrosensory systems of these three groups of fish appear to have evolved independently (Finger et al. 1986). Cerebellum-like sensory structures are also found in other vertebrate sensory systems and include the dorsal cochlear nucleus (DCN) in the mammalian auditory system, the medial octavolateral nucleus (MON) in the mechanosensory lateral line system of fish, and the optic tectum in the visual system of teleost fish (Fig. 11.1).

Primary afferent fibers from electroreceptors terminate in the deep layers of the DON and ELL where they form a map of the sensory surface. Principal cells of these structures have basilar dendrites that are affected either directly by electroreceptor afferents or indirectly via interneurons. The spiny apical dendrites of principal cells receive numerous excitatory inputs from the thin, unmyelinated axons of granule cells that course long distances through a molecular layer, like the parallel fibers in the molecular layer of the cerebellar cortex. The molecular layer also contains GABAergic interneurons that receive parallel fiber input and provide feedforward inhibitory input to principal cells, similar to the molecular layer interneurons of the cerebellum.

In the elasmobranch DON and gymnotiform ELL, the main site of integration of electroreceptor and parallel fiber inputs are glutamatergic output neurons that project to higher stages of electrosensory processing in the midbrain. In the mormyrid ELL, such integration occurs in both glutamatergic output neurons as well as in a more numerous class of GABAergic neurons known as medium ganglion (MG) cells (Meek et al. 1996, 1999). MG cells inhibit the output cells and hence occupy a position in the circuitry of cerebellum-like structures that is similar to that of the Purkinje cells in the cerebellum. This similarity is particularly clear for the teleost cerebellum where cerebellar output neurons are located adjacent to the Purkinje

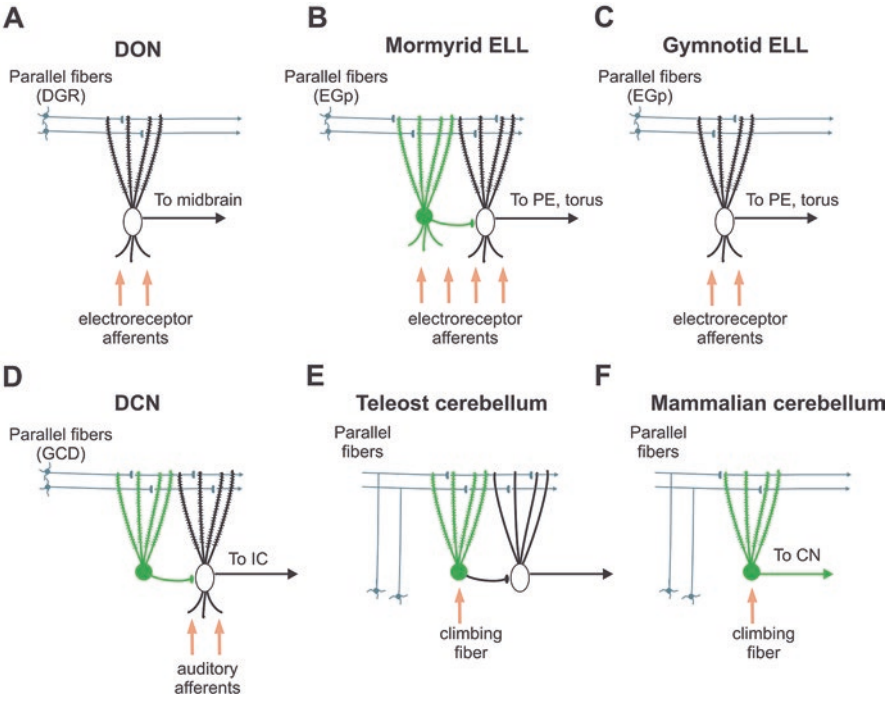

Fig. 11.1 Local circuits of some cerebellum-like structures (**A–D**), the teleost cerebellum (**E**), and the mammalian cerebellum (**F**). All cerebellum-like structures and the cerebellum receive input from a granule cell-parallel fiber system in a molecular layer (*blue*). Cerebellum-like structures receive a separate input from peripheral sensory receptors (*orange*) and the cerebellum receives a separate climbing fiber input from the inferior olive in the brainstem (*orange*). For all of the circuits shown, parallel fibers convey signals, such as motor corollary discharge and proprioception, relevant for predicting components of the peripheral sensory or climbing fiber input that are self-generated. *Green*, GABAergic Purkinje-like cells of the mormyrid electrosensory lobe (ELL) and mammalian dorsal cochlear nucleus (DCN) as well as the Purkinje cells of the teleost and mammalian cerebellums; *White*, excitatory efferent cells; CN, cerebellar nucleus; DGR, dorsal granular ridge; DON, dorsal octavolateral nucleus; EGp, eminentia granularis posterior; GCD, granule cell domain; IC, inferior colliculus; PE, preeminential nucleus

cells (as in the mormyrid ELL) instead of in a separate deep cerebellar nucleus (as in the cerebellum of most other vertebrates).

Instead of being located in a layer beneath the Purkinje cells, as in most vertebrate cerebella, granule cells in cerebellum-like structures are typically found in external granule cell masses. The granule cells themselves are similar in size and morphology to cerebellar granule cells. These granule cell masses are similar to the cerebellar granular layer in that they contain large GABAergic Golgi cells and, in some cases, a specialized class of glutamatergic interneuron known as the unipolar brush cell (UBC; Campbell et al. 2007; Borges-Merjane and Trussell 2015). Mossy fiber inputs to granule cells arise from numerous brain regions and convey a variety of signals including motor corollary discharge signals related to the EOD and

ventilation and swimming movements; proprioceptive signals related to the movements and position of the tail, trunk, and fins; electrosensory input from higher processing stages; and input from other sensory modalities such as the mechanosensory lateral line, (see Bell 2002; Bell et al. 2008 for original references).

11.4 Mechanisms for Predicting and Canceling Self-Generated Sensory Input

In vitro and in vivo electrophysiological studies and computational modeling of the DON, the mormyrid ELL, and the gymnotiform ELL all point to a common functional logic for this organization (Bell et al. 1997a; Bell 2001). Namely, the granule cell-parallel fiber system conveys signals that are used to cancel out predictable components of the electrosensory input to principal cells, including those due to the animal's own movements and behavior.

11.4.1 Negative Images: Neural Correlates for Sensory Predictions

Direct evidence for the generation and subtraction of predictions of electrosensory input patterns has been obtained from in vivo recordings of principal cells in the DON of elasmobranchs and the ELL of both mormyrid and gymnotiform fish (see Bell et al. 2008 for original references). In each case, pairing artificial electrosensory stimuli with central predictive signals (a proprioceptive or motor corollary discharge signal in the case of the mormyrid ELL; a proprioceptive or electrosensory signal in the case of the gymnotiform ELL; and a proprioceptive, electrosensory, or motor corollary discharge signal in the case of the elasmobranch DON) results in a marked decline in the response to the paired stimulus over a timescale of ~5–10 min (Fig. 11.2). Such changes cannot be explained by adaptation of peripheral receptors or fatigue of postsynaptic responses because they are not observed when the same electrosensory stimuli are delivered unpaired to central signals. Strikingly, these experiments also reveal changes in the response to the predictive signals alone (after turning the stimulus off) that resemble a negative image of the response to the previously paired (and now predicted) stimulus. The negative images develop over the same timescale as the decline in the paired response and are specific to the sign as well as to the spatial and temporal patterns of principal cell activity evoked by the stimulus.

Further evidence that negative images reflect a memory-based process comes from studies of the passive electrosensory system of the mormyrid ELL. In the mormyrid ELL, negative images are induced by pairing the motor command to produce the EOD with an electrosensory stimulus; the emission of the actual EOD is blocked

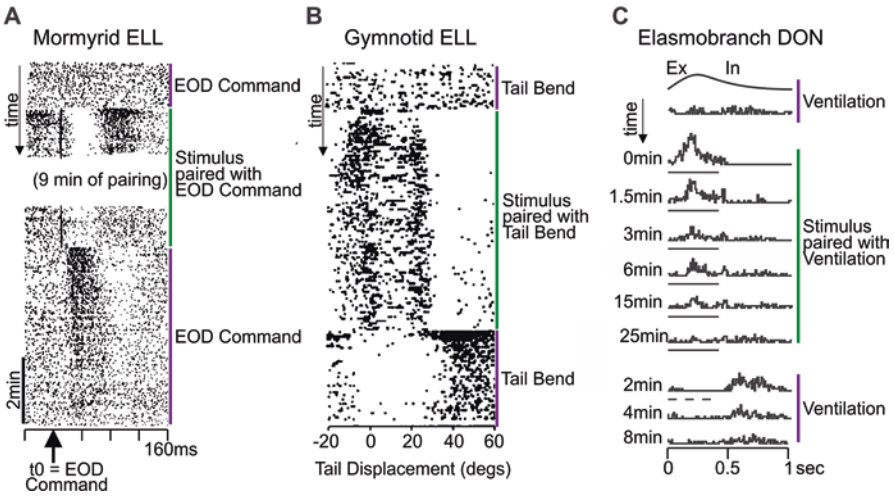

Fig. 11.2 Formation of negative images of predicted sensory responses in three different cerebellum-like structures. **A:** raster display of the responses of a cell in the ampullary region of the mormyrid ELL. Each dot represents an action potential, and each row shows the spiking activity time-locked to each electric organ discharge (EOD) command (*t*0). At the beginning of the experiment (*top*), the command alone has no effect on the cell's spiking activity. An electrosensory stimulus is then time-locked to the EOD command (*vertical black line* indicates onset), which evokes a pause-burst spiking response. After several minutes of pairing, the stimulus is turned off, and a response to command alone that was not present before the pairing and that is a negative image of the previously paired sensory response is revealed. By the end of the experiment (*bottom*), the cell no longer responds to command alone (as in the beginning of the experiment). From Bell (1986). **B:** raster display of spiking responses of a cell in the gymnotiform ELL. The tail is moved back and forth passively. Each row of dots shows the response to one cycle of movement. Initially, the tail bend has no effect on the cell. An electrosensory stimulus that evokes a burst-pause response is then delivered in phase with the movement. The electrosensory stimulus is turned off after several minutes of pairing, revealing a response to tail bending alone that was not present before the pairing and that is a negative image of the previously paired sensory response. From Bastian (1995). **C:** spiking responses of a cell in the elasmobranch DON. Each histogram shows the average response to one cycle of ventilation. Initially, the cell does not respond to the exhalation (Ex)-inhalation (In) ventilatory cycle of the fish (*top* histogram). An electrosensory stimulus that evokes a burst-pause is then delivered in phase with the ventilatory cycle. The response to ventilation plus the electrosensory stimulus decreases during 25 min of pairing. Turning off the electrosensory stimulus after pairing reveals the presence of a response to ventilation alone that was not present before and that is a negative image of the previously paired sensory response. From Montgomery and Bodznick (1994)

in these experiments by a paralytic agent. After turning the stimulus off, negative images decay on roughly the same timescale over which they are formed (5–10 min). However, if the EOD motor command is blocked by injection of the action potential blocker lidocaine into the EOD command nucleus, then negative images persist for at least 30 min. This finding suggests that the decay of negative images normally observed after pairing is not simply a passive "forgetting" but rather that negative images may be due to a persistent form of synaptic plasticity. Related experiments

in the elasmobranch DON paired an electrosensory stimulus with brief bouts of passive fin movements, separated by longer periods of rest (Zhang and Bodznick 2008). Negative images formed under these conditions persisted after rest periods of up to 3 hours but were rapidly extinguished when passive movements were delivered without a stimulus. These experiments demonstrate how negative images could function in the context of episodic behaviors such as swimming.

The function of negative images is not restricted to canceling out self-generated electrosensory inputs. In the mormyrid and gymnotiform ELL, granule cells receive input from a higher stage of electrosensory processing, the midbrain preeminential nucleus (Bastian and Bratton 1990; von der Emde and Bell 1996). Such inputs allow for negative images to be formed based on electrosensory information. Experiments in both the gymnotiform ELL and the elasmobranch DON have shown that negative images are formed when strong, focal activation of principal cells is paired with a spatially diffuse electrosensory stimulus (Bodznick et al. 1999; Bastian et al. 2004). Studies of gymnotiform fish have suggested that such negative images serve to cancel out interference due to the EODs of other fish. More generally, negative images based on electrosensory information could serve to remove any persistent spatial or temporal correlations in electrosensory input.

The discovery of negative images in electrosensory systems provided a striking confirmation of the longstanding ideas of von Holst, Mittelstaedt, and others that central signals could be used to predict and cancel out reafference. These findings were of obvious functional importance because they provided a mechanism for selectively removing the effects of self-generated stimuli while maintaining sensitivity to external stimuli. As described in Sect. 11.4.2, a number of advantageous features of electrosensory systems, including their accessibility to detailed electrophysiological studies, have allowed for significant progress in understanding the mechanisms of negative image formation.

11.4.2 Sites and Synaptic Mechanisms of Negative Image Formation

Several lines of evidence indicate that the formation of negative images is due to plastic changes occurring within the cerebellum-like structures themselves. Pairing predictive signals with intracellular current injections in vivo results in the formation of negative images in principal cells in all three groups of fish, indicating that synaptic inputs to the recorded cell are plastic (Bell et al. 2008). Given the diversity of signals involved in negative image formation, synapses between parallel fibers and principal cells are the most natural candidate for the site of the plastic changes. Immunohistochemical studies have shown that *N*-methyl-D-aspartate (NMDA)-type glutamate receptors are present in the apical dendrites of principal cells in the DON of elasmobranchs and the ELL of both mormyrid and gymnotiform fish (Bell et al. 2008, Zhang and Bodznick 2010). NMDA receptors are known to play central roles

in the induction of associative synaptic plasticity in many brain regions, including the hippocampus and cerebral cortex. Direct physiological evidence for plasticity at parallel fiber synapses with principal cells has been obtained in all three classes of fish (Bell et al. 2008; Harvey-Girard et al. 2010). In principal cells of the DON, pairing electrical stimulation of parallel fibers with an electrosensory stimulus results in depression of the response to parallel fiber stimulation alone. Pharmacological blockade of NMDA receptors disrupts negative image formation in vivo in both the elasmobranch DON and the mormyrid ELL (Zhang and Bodznick 2010; Enikolopov et al. 2018).

In vitro studies of the mormryid ELL provided evidence for the plasticity of parallel fiber synapses onto one class of principal cells, the MG cells. MG cells fire two types of action potentials, known as narrow and broad spikes (Grant et al. 1998). The narrow spikes occur at high rates and originate in the axon, whereas the broad spikes are infrequent, originate in the proximal dendrites, and back-propagate into the molecular layer. Hence, the two spike types in MG cells are similar in some respects to simple spikes and complex spikes in Purkinje cells (Sawtell et al. 2007). Repeated pairing of broad (but not narrow) spikes with electrical stimulation of parallel fibers results in persistent changes in the strength of parallel fiber-evoked excitatory postsynaptic potentials (EPSPs; Bell et al. 1997b). Critically, the polarity of the changes depends on the relative timing between the EPSP onset and the postsynaptic spike. EPSPs that preceded postsynaptic broad spikes by 50 ms or less are depressed, whereas those occurring at other delays are potentiated. The depression is dependent on postsynaptic calcium- and NMDA-type glutamate-receptor activation (Han et al. 2000). This was one of the first demonstrations of spike timing-dependent synaptic plasticity in the vertebrate brain (Markram et al. 2011).

A distinctive feature of the plasticity rule described in the mormyrid ELL is that presynaptic inputs that shortly precede, and hence could contribute to evoking a postsynaptic spike, are weakened. In more common Hebbian forms of plasticity, including those found in the hippocampus or neocortex, presynaptic inputs that precede a postsynaptic spike are strengthened. For this reason, plasticity in the ELL was referred to as anti-Hebbian. A similar form of anti-Hebbian plasticity occurs at parallel fiber synapses onto principal cells in the gymnotiform ELL (Harvey-Girard et al. 2010). In this case, brief bursts of pre- and postsynaptic spikes are required to induce synaptic depression and no potentiation is observed. It was immediately realized that such anti-Hebbian plasticity could potentially explain the negative image formation observed in principal cells in vivo. This intuition was formalized by computational models that showed how anti-Hebbian plasticity rules of the type demonstrated experimentally provide a simple and powerful mechanism for canceling principal cell responses that are predictable based on parallel fiber inputs (Nelson and Paulin 1995; Roberts and Bell 2000). Increases in principal cell firing that occur together with (i.e., can be predicted by) parallel fiber input are opposed by the weakening of parallel fiber synapses. Conversely, predictable decreases in principal cell firing are opposed by increases in parallel fiber synaptic strength.

11.4.3 Granule Cells Provide a Basis for Negative Image Formation

Modeling studies have highlighted the critical role of granule cells in providing the raw material out of which negative images are sculpted via parallel fiber synaptic plasticity. In the region of the mormyrid ELL involved in passive electrolocation, the ventrolateral zone (VLZ), negative images serve to cancel out responses evoked by the fish's own EOD (Fig. 11.3). Although the EOD pulse itself is extremely brief (<0.5 ms), it evokes a bi- or triphasic pattern of activation in ampullary electroreceptors that lasts ~200 ms (Bell and Russell 1978). To cancel out the effects of the EOD, negative images in the VLZ must be temporally specific in relation to the

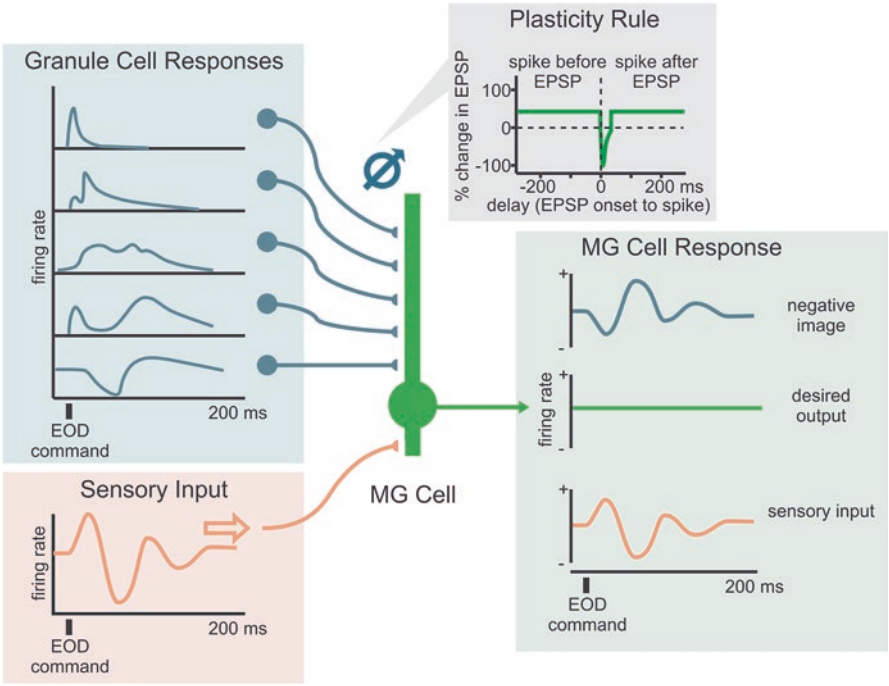

Fig. 11.3 Negative image formation in the mormyrid ELL. Medium ganglion (MG) cells (*center*, *green*) receive sensory input via electroreceptors (*orange lines*) along with motor corollary discharge input via granule cells (*blue lines*). To encode behaviorally relevant external events, the MG cell must cancel the sensory input due to the fish's EOD (*bottom left orange box*). EOD command, time of the motor command that drives the EOD. Previous results have shown that this is accomplished by the generation and subtraction of a temporally specific negative image of the self-generated electrosensory input (*bottom right green box*). An experimentally observed form of spike timing-dependent plasticity (*top right gray box*) at synapses between granule cells and MG cells can explain the negative images if granule cells exhibit a diversity of temporal responses that span the 200 ms over which negative images can be formed (*top left blue box*). EPSP, excitatory postsynaptic potential

fish's EOD motor command. Numerous studies, including experiments in which an artificial electrosensory stimulus is delivered at different delays after the EOD command, have confirmed that this is indeed the case (Bell 1982; Bell et al. 1993). Negative images observed in such experiments are specific to the paired delay up to ~200 ms after the EOD command. Modeling studies explained these paradoxical findings based on anti-Hebbian plasticity acting on a set of granule cells, each of which is active at a different delay after the EOD command, forming a temporal delay line (Roberts and Bell 2000). In the model, these temporally specific responses provided a baseline of excitation out of which negative images could be sculpted via associative synaptic depression and nonassociative potentiation. This model raised the important questions of whether temporal representations actually exist in granule cells and, if so, how they are generated. Because the motor command to discharge the electric organ is a brief spike burst lasting just a few milliseconds, some cellular or circuit mechanism(s) would seemingly be required to generate the diversity of temporal response patterns required by the model.

In vivo whole cell recordings from granule cells as well as additional cellular elements of the granule layer in mormyrid fish shed light on both of these questions (Kennedy et al. 2014). The recordings showed, as expected based on past results, that EOCD inputs to granule cells were highly stereotyped bursts restricted to short delays after the EOD command. In contrast, granule cell responses were more temporally diverse and delayed. A particular class of interneuron providing excitatory synaptic input to granule cells, the UBCs, appeared to be the source of the diverse and delayed responses. This is consistent with in vitro studies of UBCs in the mammalian cerebellum and cochlear nucleus that have found that UBCs possess a variety of synaptic and intrinsic properties capable of transforming brief synaptic inputs into more temporally diverse, sustained, or delayed output (Mugnaini et al. 2011). Importantly, granule cells do not form a perfect delay line like the one assumed in models. Instead, the majority show activity restricted to short delays, with a minority responding at longer delays. However, theoretical modeling shows that the anti-Hebbian synaptic plasticity rule measured in vitro acting on the granule cell responses recorded in vivo is indeed sufficient to explain the formation of temporally specific negative images to EOD-driven ampullary responses and can also account for previously unexplained features of negative images observed in in vivo recordings (Roberts 1999; Roberts and Bell 2000).

Recordings in the mormyrid ELL have also suggested that granule cells provide a higher dimensional recoding of their mossy fiber inputs, consistent with theories of cerebellar granule cell function (Litwin-Kumar et al. 2017). In addition to EOCD inputs described above, proprioceptive and skeletomotor corollary discharges reach the granule cell domain of ELL via mossy fibers originating in the spinal cord (Requarth and Sawtell 2014), similar to spinocerebellar pathways described in other vertebrates. Although each granule cell receives just a few (2–4) excitatory inputs from mossy fibers or UBCs, it was shown that these inputs may be of different types, e.g., a proprioceptive signal and an EOCD signal (Sawtell 2010). Such integration may allow granule cells to selectively encode specific combinations of events, such as a particular tail position and a particular time after the EOD

command. Collectively, such granule cell representations may provide the raw material for forming more complex negative images, such as those that would be required to predict and cancel the electrosensory consequences of the rapid and intricate probing motor acts made by mormyrid fish when exploring a novel object (Toerring and Moller 1984).

11.4.4 Behavioral Significance of Negative Images

Although it was suggested at the time of their discovery that negative images serve to enhance the detection and processing of behaviorally relevant stimuli, an experimental demonstration of this was not provided until many years later (Enikolopov et al. 2018). Recordings of neural responses of ELL neurons to prey-like stimuli before, during, and after negative image formation directly demonstrated the time course and extent of improvements in the neural encoding of prey-like stimuli due to negative images. Weakly electric mormyrid fish increase their EOD rate when they detect a stimulus (Post and von der Emde 1999). This simple unconditioned behavior, known as the electromotor novelty response, was used to demonstrate improvements in the behavioral detection of prey during negative image formation. The time course of improvements in neural coding and behavioral detection both matched the time course of negative image formation. Finally, pharmacological manipulations of synaptic plasticity in ELL were shown to disrupt both the neural and behavioral detection of prey-like stimuli, providing a causal link between the mechanisms of negative image formation and behavioral function.

11.4.5 Questions for Future Research

There is much still to be learned about the mechanisms of negative image formation and sensory cancellation in electrosensory systems. Most previous experimental and theoretical work has focused on understanding how negative images and sensory cancellation can be explained by bidirectional plasticity of excitatory synapses between granule cells and principal cells. However, such single-neuron models are likely to be a gross oversimplification in that plasticity distributed across many neurons at multiple sites in the network are likely to underlie sensory cancellation. In this regard, a major remaining puzzle about the mormyrid ELL is the functional importance of the two different classes of principal cells on which electrosensory input and parallel fiber input converge: the Purkinje-like MG cells and the glutamatergic output cells. Although both cell types exhibit anti-Hebbian plasticity at their parallel fiber synapses, the functional logic of such an arrangement remains unclear. If anti-Hebbian plasticity at synapses between parallel fibers and MG cells cancels the effects of the fish's own EOD, it would seem that the MG cells could play no role in canceling the effects of the EOD in the output cells that are their main

synaptic targets. Interestingly, analogs of MG cells have not been described in the DON or the gymnotiform ELL but are present in the mammalian DCN, where they are known as cartwheel cells (Berrebi et al. 1990). The respective roles of plasticity of parallel fiber synapses onto cartwheel versus DCN output cells are similarly unknown (see Sect. 11.6.1).

Another question for future work relates to how negative images operate under more naturalistic circumstances in which electrosensory reafference is more varied and complex than in the limited experimental settings studied in the past, i.e., immobilized preparations. Under natural conditions, electrosensory reafference depends on potentially complex interactions between electromotor behavior (e.g., EOD pulse rate), the movements of the fish, and the nearby environment. For example, the same change in tail position relative to electroreceptors on the skin is expected to have different effects on the fish's electrical field depending on whether the fish is in open water or hiding in a crevice (a nonconducting boundary; Pereira et al. 2005). In the simplest view, negative images could represent a prediction of the electrosensory consequences of behavior averaged over some relatively long timescale. Although compatible with what is known about ELL, such "average" predictions might have limited utility if the characteristics of reafference are highly dependent on behavioral context on much shorter timescales. The fact that granule cells receive many different streams of information, including extensive feedback from higher stages of electrosensory processing, suggests the possibility that negative images may possess specificity for certain contexts and/or the capacity to generalize appropriately from one context to another. Recordings from freely swimming fish may allow such questions to be addressed experimentally (Fotowat et al. 2013).

11.5 Additional Functions of Electric Organ Corollary Discharge in Mormyrid Fish

Corollary discharge pathways associated with the motor command to discharge the electric organ are particularly prominent and accessible to study in pulse-type mormyrid fish (Fig. 11.4A; Bell et al. 1983). The role of EOCD inputs in negative image formation and cancellation of reafference in ELL was discussed in Sect. 11.4. However, the role of EOCD signals are likely to be more diverse as evidenced by anatomical and electrophysiological data suggesting that they impact many regions of the mormyrid brain, including higher brain regions such as the telencephalon (Prechtl et al. 1998) and the hypertrophied cerebellum (Russell and Bell 1978). EOCD signals also likely impact the electromotor system that controls the rate and sequence of emission of the EOD (von der Emde et al. 2000; Carlson 2002). Unfortunately, very few studies have addressed these issues. As discussed in Sects. 11.5.1 and 11.5.2, additional functions of EOCD pathways have been identified in relation to the early processing stages of electrocommunication and active electrolocation in mormyrids.

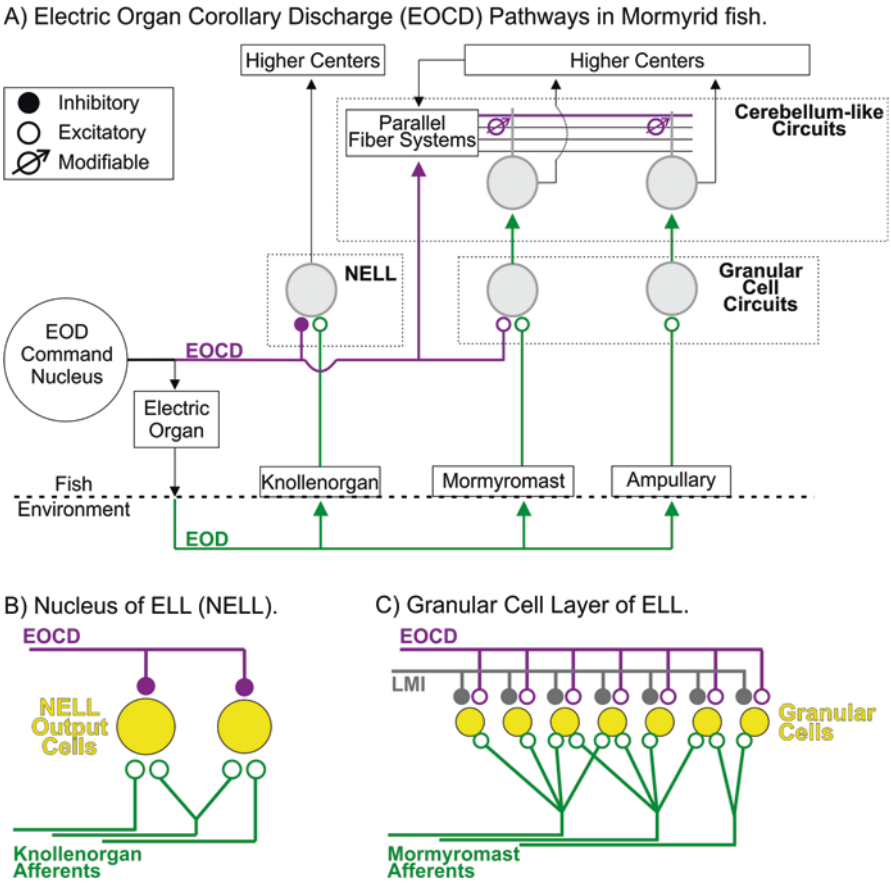

Fig. 11.4 Functions of electric organ corollary discharge (EOCD) in mormyrid fish. **A:** activation of the EOD command nucleus elicits an EOD. This reafferent stimulus evokes responses in afferents of all three electroreceptor types. Concurrently, activation of the EOD command nucleus (*green lines*) drives electric organ corollary discharge (EOCD) input (*purple lines*) to the first central processing stages associated with each type of electroreceptor. The role of EOCD input in cancellation (via modifiable synapses) of sensory reafference in the cerebellum-like circuits of mormyrid ELL is reviewed (along with examples from other cerebellum-like electrosensory structures) in Sect. 11.4. Section 11.5 reviews two additional functions accomplished by EOCD input in the nucleus of ELL (nELL) and the granular cell layer of ELL in mormyrid fish. Adapted from Bell (1989). **B:** knollenorgan afferents (*green*) form mixed chemical-electrical synapses on the output cells of nELL (*yellow*), which are inhibited by EOCD inputs precisely timed to gate out responses to the fish's own EOD in electrocommunication. **C:** mormyromast afferents form mixed chemical-electrical synapses on the granular cells of the ELL (*yellow*) and elicit inhibition via GABAergic large multipolar interneurons (LMI; *gray*). In addition to afferent input, granular cells receive a precisely timed EOCD-driven spike that seems to function in enhancing and recoding sensory responses to the fish's own EOD in active electrolocation

11.5.1 Inhibitory Gating of Self-Generated Input in the Electrocommunication System

In mormyrid fish, the detection and processing of EODs of other fish is mediated by a specialized class of electroreceptors known as knollenorgans, which terminate in a dedicated hindbrain nucleus, the nucleus of the ELL (nELL). The nELL is anatomically separate from the ELL, which is the first processing stage for active and passive electrolocation. Knollenorgans respond to the EODs of other fish by firing a single action potential. However, the fish's own EOD is far above the threshold of activation of knollenorgans, which must be sensitive enough to detect EODs of other fish at some distance to be useful for communication. How does the fish distinguish its own pulses from those of other fish? Knollenorgan afferents form mixed chemical-electrical synapses onto nELL output cells. nELL output cells also receive input from GABAergic neurons of the ventral lemniscus (Fig. 11.4B; Bell et al. 1981; Mugnaini and Maler 1987). Intracellular recordings from nELL output cells reveal two types of synaptic events: (1) EPSPs driven by stimulation of knollenorgan receptors and (2) inhibitory postsynaptic potentials time locked to the EOD motor command, which are driven by EOCD inputs to the nELL (Bell and Grant 1989). The EOCD-evoked inhibition is brief, precisely timed, and appears to be entirely fixed or nonplastic. Critically, knollenorgan responses to reafferent input arrive during the peak of EOCD-evoked inhibition and hence fail to evoke an action potential in nELL neurons. Different nELL output neurons receive knollenorgan afferent input at slightly different latencies (although response latencies are fixed for each afferent) due to variable axonal lengths for receptors located on different regions of the body surface. Remarkably, a corresponding variation in the timing of EOCD-evoked inhibition is observed, which ensures a tight matching of the timing of the EOCD inhibition relative to reafferent input. Although remarkably simple, this corollary discharge gating strategy is extremely effective in nELL because of the brevity and fixed latency of the reafference.

11.5.2 Roles for Corollary Discharge in Active Electrosensory Processing in Mormyrid Fish

In contrast to the case of the passive electrosensory and the electrocommunication systems, the fish's own EOD is the signal of interest for the active electrosensory system. Objects in the environment with conductivity different from the water alter patterns of EOD-induced current flowing through the fish's skin. These changes cast electrical images on the skin that are encoded by a specialized class of tuberous electroreceptors known as mormyromasts. Mormyromasts typically fire one to four action potentials following each EOD with a precise latency that is a function of EOD amplitude at the receptor. Increases in EOD amplitude (as would be caused by a conducting object such as prey) cause decreases in spike latency, whereas

decreases in EOD amplitude (as would be caused by a nonconducting object such as a rock) cause an increase in spike latency (Szabo and Hagiwara 1967). The timing of spikes is extremely precise such that submillisecond shifts in spike latency convey information about EOD amplitude (Sawtell and Williams 2008). Mormyromast afferents synapse onto a class of small, highly numerous interneurons in the deep layers of the ELL, known as granular cells (not to be confused with the granule cells that send parallel fibers to the principal cells discussed in Sect. 11.4.3; Fig. 11.4C; Meek et al. 1999; Zhang et al. 2007). Several lines of evidence indicate that the granular cells also receive precisely timed excitatory EOCD input (Bell 1990; Bell and von der Emde 1995). Rather than blocking reafferent input, as in the electrocommunication system, this input appears to enhance EOD-evoked afferent input. Evidence for this comes both from physiological studies showing that ELL principal cells respond much more strongly when stimuli are delivered around the time of the EOD command and from behavioral studies showing that fish more readily detect and respond to an electrosensory stimulus when it is delivered within ~12 ms of the EOD motor command (Hall et al. 1995). EOCD inputs to granular cells have also been hypothesized to play a role in "decoding" the tiny shifts in spike latency of mormyromast afferent input. Behavioral experiments have shown that fish can detect a 0.1-ms shift in the latency of an electrosensory stimulus (causing a shift in mormyromast spike latency) relative to the fish's own EOD motor command (Hall et al. 1995). Recordings from ELL output cells show that information about object-induced changes in EOD amplitude is coded by changes in spike number (as well as timing; Sawtell and Williams 2008). Such a transformation could be achieved, at least in part, by integrating precisely timed afferent spikes with a precisely timed excitatory EOCD input, although confirmation of this awaits direct in vivo recordings from granular cells.

11.6 Implications for Other Systems

The studies of reafference cancellation in electrosensory systems described in Sect. 11.4 are relevant to a number of general issues in neuroscience. Predicting sensory events is a critical function of the nervous system as a whole and likely involves a diverse set of cellular and circuit mechanisms distributed across many brain regions. Studies of cerebellum-like structures provide a useful example of how the mechanisms of such functions may be elucidated. Although sensory systems are typically studied in isolation from one another and also in isolation from motor systems, this reflects a methodological convenience more than a biological reality. Cerebellum-like structures in fish offer a system in which interactions between peripheral sensory input and signals from other sensory modalities and motor signals are both prominent and well characterized. Finally, forging links between synaptic plasticity, well-defined neural circuits, and systems-level function is a primary, but rarely achieved, goal of neuroscience. Cerebellum-like circuits in fish provide a foremost example of a vertebrate system in which it has been possible to make such links.

In addition to these broad implications, studies of cerebellum-like electrosensory structures in fish offer more specific insights into a number of similar structures including the cerebellum-like DCN of mammals and the cerebellum itself.

11.6.1 Reafference Cancellation in a Cerebellum-Like Circuit in the Auditory System

The DCN at the first stage of auditory processing in mammals is a cerebellum-like structure and shares many similarities with the cerebellum-like structures in fish discussed in Sects. 11.3 and 11.4 (Fig. 11.1D; Oertel and Young 2004; Bell et al. 2008). Fusiform cells are the major efferent cell type of the DCN (Cant 1992). Their basilar dendrites are contacted by primary afferent fibers from the cochlea, which form a tonotopic map in the deeper layers below the molecular layer. The fusiform cells extend their spine-covered apical dendrites up into the molecular layer where they are contacted by parallel fibers. The parallel fibers arise from granule cells located around the margins of the nucleus. The cartwheel cell is a second type of principal cell in the DCN. These cells are considered Purkinje-like in that they are GABAergic, have extensive spine-covered dendrites in the molecular layer, and share patterns of gene expression with Purkinje cells. Purkinje cells and cartwheel cells are similarly affected by genetic mutations in several mouse strains (Berrebi et al. 1990). Cartwheel cells inhibit the fusiform cells. Similarities between the local circuits of the mormyrid ELL and the teleost cerebellum were noted in Sect. 11.3. The DCN shares these similarities in that the parallel fibers of the DCN, like those of the mormyrid ELL and the teleost cerebellum, pass through and excite the dendrites of both efferent cells and Purkinje-like cells.

Movements of the animal's pinna, head, or body have predictable effects on how the cochlea responds to an external sound source, and orofacial behaviors such as chewing, licking, and vocalization will also have predictable consequences on auditory input. The granule cells of the DCN receive various types of input that provide information about such behaviors (see Bell 2002; Oertel and Young 2004 for original references). Thus, the signals conveyed by parallel fibers in the DCN molecular layer could generate predictions about changes in afferent activity from the cochlea, as in other cerebellum-like structures. In vitro studies have revealed parallel fiber synaptic plasticity mechanisms remarkably similar to those found in cerebellum-like structures in fish (Tzounopoulos et al. 2004, 2007). For example, in both the mormyrid ELL and the DCN, plasticity at parallel fiber synapses onto Purkinje-like cells (MG cells in the ELL and cartwheel cells in the DCN) is anti-Hebbian, NMDA receptor-dependent, presynaptically expressed, and reversed by a timing-independent form of parallel fiber synaptic potentiation.

The numerous striking similarities between the circuitry and synaptic plasticity of DCN and cerebellum-like structures in fish suggest that they may perform similar functions. Evidence for this hypothesis was provided by a study in mice showing

that self-generated sounds related to licking behavior drove much stronger responses in neurons of the ventral cochlear nucleus than in putative output neurons of the DCN despite both classes showing comparable sensitivity to external sounds, even during licking (Singla et al. 2017). Cancellation of reafference in this system depended, at least in part, on nonauditory signals conveyed by the parallel fiber system. Additionally, repeated pairing of an external sound at a fixed delay relative to licking led to a gradual reduction in the response to the paired sound, similar to cancellation of electrosensory stimuli paired with behavior-related signals in principal cells in fish. Although additional studies are needed, these results are consistent with a conserved reafference cancellation function for cerebellum-like structures in fish and mammals.

11.6.2 Implications for Cerebellar Function

The operation of cerebellum-like circuits associated with electrosensory processing in fish (and perhaps also those associated with mammalian auditory processing) appear similar in important respects to those in the cerebellum. Bidirectional plasticity at parallel fiber synapses has been linked to the formation of negative images of predicted sensory input in cerebellum-like structures and to motor learning in the mammalian cerebellum (Ito 1984). In both cases, plasticity acts to alter principal or Purkinje cell responses to parallel fiber input under the guidance of a separate nonplastic input. In the cerebellum, plasticity and learning are supervised by climbing fiber input from the inferior olive, whereas in cerebellum-like structures, the nonplastic signal is the peripheral sensory input itself. Anti-Hebbian plasticity of parallel fiber synapses in cerebellum-like structures generates negative images, which act within principal cells to oppose the effects of predictable electrosensory input. Plasticity of parallel fiber synapses onto Purkinje cells shapes what could be considered negative images of climbing fiber inputs. However, the main effect of such changes is not to directly cancel the effects of climbing fiber input within the Purkinje cells but instead to alter its simple spike firing patterns and thereby influence downstream neurons, i.e., those in the deep cerebellar or vestibular nuclei.

The similarities between cerebellar plasticity and negative image formation are nicely illustrated by the changes in Purkinje cell responses observed during delay eyelid conditioning. Delay eyelid conditioning is a classical conditioning paradigm in which a neutral stimulus, e.g., a tone, is paired with a periorbital shock or puff of air to the eye. Extensive past work has shown that information about the neutral stimulus, or conditioned stimulus (CS), is conveyed via the mossy fiber-granule cell system and information about the periorbital shock or puff of air, the unconditioned stimulus (US), is conveyed via climbing fibers (Medina et al. 2000b; Ohyama et al. 2003). After repeated CS-US pairings, animals blink their eyes in response to the CS alone, in anticipation of the US. The learning of this conditioned response is cerebellum dependent. Such pairing also leads to the emergence of pauses in

Purkinje cells, the timing of which is matched to the CS-US delay (Jirenhed and Hesslow 2011; Halverson et al. 2015). Such pauses are believed to drive the conditioned response by releasing cerebellar nucleus neurons from inhibition. Leading models of eyelid conditioning suggest that such Purkinje cell pauses emerge as a result of bidirectional plasticity at parallel fiber synapses such that synapses immediately preceding the US-evoked climbing fiber are weakened, whereas others are strengthened (Medina et al. 2000a; Medina and Mauk 2000; but see Johansson et al. 2014). Work in the mormyrid ELL provides a mechanism for how a brief signal (like the CS) can be recoded to generate a diversity of responses to the CS. Hence models that explain temporally specific learning and Purkinje cell responses in the context of eyelid conditioning closely resemble models of temporally specific negative image formation in mormyrid fish.

These similarities also extend to Marr-Albus and adaptive filter models of the cerebellum (Fujita 1982). In a proposal inspired in part by negative images and sensory cancellation in electrosensory systems, it was suggested that anti-Hebbian plasticity could improve vestibular ocular reflex performance by removing correlations between mossy fiber inputs signaling eye movement motor commands and sensory error signals, i.e., retinal slip, conveyed by climbing fibers (Dean et al. 2002).

The function of some regions of the cerebellum may also be similar to that described for cerebellum-like structures in fish. A role for the cerebellum in the cancellation of self-generated sensory inputs has been demonstrated in regions of the primate cerebellum involved in processing vestibular information. Whereas primary vestibular afferents respond identically to passive and active, i.e., self-generated head, movements, neurons in the rostral fastigial nucleus have been found that respond selectively to passive head movements (Brooks and Cullen 2013). When the relationship between the intended head movement and its vestibular consequences was abruptly altered, cerebellar neurons showed sensitivity to the now unexpected sensory consequences of self-generated movements (Brooks et al. 2015). This sensitivity declined as the animal adapted to the new relationship between motor commands and head movements. In addition to processing vestibular sensory input, many sensory areas of the brain are interconnected with the cerebellum (Baumann et al. 2015). However, the functional role of the cerebellum in sensory processing remains unclear in most cases. Based on studies of cerebellum-like structures in fish, cancellation of predictable sensory inputs could be suggested as one such function.

In the context of motor control, a leading idea is that the cerebellum is involved in generating so-called forward models (Ebner and Pasalar 2008; Machado et al. 2015). In a forward model, copies of a motor command are conveyed to the cerebellum together with information about the current state of the system, such as positions and velocities of the limbs. The cerebellum then generates a prediction about the sensory consequences of the commanded motor act in the current context. Taking into account all that is known about the current state of the system, a forward model that predicts the sensory consequences of a motor command allows fast,

coordinated movement sequences. Classical symptoms of cerebellar damage, such as decomposition of movement, slowness, and tremor, can all be understood as due to the absence of predictive forward models and reliance on peripheral feedback (Nixon and Passingham 2001; Bastian 2006). Quantitative effects of Purkinje cell degeneration in mice can be understood in terms of a failure of forward model predictions (Machado et al. 2015). Furthermore, electrophysiological studies in nonhuman primates suggest that the Purkinje cell output from large regions of the cerebellar hemispheres is indeed more tightly coupled to predictions about consequences of the movement than to the motor commands themselves (Pasalar et al. 2006). Although the forward model hypothesis seems plausible, what remains missing in the cerebellum is an understanding of how forward models are generated at the circuit level.

Examples of what are, in effect, forward models in the cerebellum-like structures of mormyrid and elasmobranch fish are described in Sect. 11.4. In these systems, corollary discharge signals come to elicit a prediction about the sensory input pattern that is expected to follow the motor command. As discussed, circuit mechanisms for generating such forward models are fairly well understood in cerebellum-like structures, the key ingredients being an appropriate plasticity rule acting on a sufficiently rich set of motor corollary discharge signals conveyed by granule cells. Hence, studies of electrosensory systems provide a proof of concept that forward models can indeed be generated within structures like the cerebellum.

11.7 Conclusion

Studies of cerebellum-like structures associated with electrosensory processing in fish have yielded unique insights into the fundamental question of how the nervous system distinguishes self-generated from external sensory input, including a relatively complete mechanistic account of how copies of motor commands are transformed into predictions of sensory events. These accounts are also notable in that they provide an understanding of how synaptic plasticity operating in a well-defined circuit performs a complex and behaviorally relevant computation. Insights from these studies are likely to extend to other cerebellum-like sensory structures, including those found in the mammalian auditory system, as well as to the cerebellum itself.

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Nathaniel B. Sawtell declares that he has no conflict of interest.

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Chapter 12
Active Electrolocation and Spatial Learning

Sarah Nicola Jung and Jacob Engelmann

Abstract This chapter presents an overview of the emerging research on spatial learning in weakly electric fish. In the first part, mechanisms by which active electrolocation can provide spatial information are summarized. This includes research on the intricate dynamics of sensorimotor behaviors that enable weakly electric fish to actively generate electrosensory flow. Starting from a summary of spatial learning mechanisms in nonelectric teleost fish, behavioral studies that have begun to investigate spatial learning in weakly electric fish are presented. The behavioral data are then connected with what is known about the neuronal substrate of spatial cognition in teleost fish in general, with a particular focus on the involvement of the dorsal telencephalon. Based on this, the final section summarizes the current data on the telencephalic networks of weakly electric fish. Comparative studies have led to partially novel and hypothetical views that posit similarities between forebrain networks of weakly electric fish and mammalian cortical and thalamocortical networks. Although being a newly emerging line of research, the sensory specialties of the active sensory system of weakly electric fish clearly offer a chance to widen research on the spatial cognition of teleosts by providing novel insights through comparative approaches.

Keywords Active electrolocation · Allocentric · Egocentric · Electric image · Electrosensory flow · Navigation · Path integration · Pattern completion · Pattern separation · Spatial learning · Telencephalic networks

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12.1 Introduction

An animal's behavior may be understood as a compromise between the costs of sensory information acquisition and the accuracy of the information obtained. In this view, sensing behaviors are the overt result of decision-making processes (Wolpert and Landy 2012). How animals optimize their motor behavior can reveal what kind of information they are seeking (Gordon et al. 2011; Hofmann et al. 2013b). Active sensory systems are particularly amenable to such studies because the process of sensory acquisition can be well quantified in time and space. This is especially true in weakly electric fish in which the near-range nature of electric sensing makes the analysis of what an animal is attending to comparatively easy. Furthermore, the energetic constraints of active electrolocation require weakly electric fish to finely regulate their sampling behavior (Salazar et al. 2013), and it has been suggested that this regulation is an overt expression of volition and attention (Jun et al. 2014).

Spatial memories can be regarded as one way to deal with sensory limitations. Instead of navigating based solely on sensory input, internalized information can replace elevated sensory sampling once animals are familiar with their environment (Monaco et al. 2014; Jun et al. 2016). This chapter addresses how weakly electric fish may form spatial memories using their electric sense to aid in navigation. The ease of measuring the sensory input and the animals' attention, connected with the comparatively simple structure of the teleost forebrain, make weakly electric fish a very interesting model in which to study general aspects of memory and learning in the future.

12.1.1 Electrolocation

Seminal studies in the 1950s provided evidence that weakly electric fish can detect and analyze nearby objects by means of their active electric sense (Lissmann 1951; Lissmann and Machin 1958). For this, they rely on information embedded in the modulation of the self-emitted electric signal. This signal is built up through the synchronous discharge of the electric organ (see Gallant, Chap. 4; Markham, Chap. 5) and is referred to as the electric organ discharge (EOD; see Table 12.1 for a complete list of abbreviations used in this chapter). The potential at the skin of the fish depends on this self-generated field, and objects in the environment can distort this basal electric field (Fig. 12.1A). This results in modulations of the potential at the skin, termed the electric image (EI) of an object (Fig. 12.1A, *right*). The electroreceptors embedded in the skin thus provide a two-dimensional representation of the three-dimensional world to the brain (see Leitch and Julius, Chap. 2). Because the amplitude of electric images decays steeply with distance (proportional to $1/d^4$; Chen et al. 2005), the working range of electrolocation is limited to a volume of about one body length (Lissmann 1958; Nelson and MacIver 1999). However, the three-dimensional electric field and the wide distribution of electroreceptors enable electric fish to sense in an omnidirectional manner (see Fig. 12.1B; Snyder et al. 2007).

Table 12.1 Abbreviations

CA3	Area 3 of the cornu ammonis
Dc	Central division of the dorsal telencephalon
Dd	Dorsal division of the dorsal telencephalon
Ddi	Intermediate division of the dorsal telencephalon
Ddmg	Magnocellular division of the dorsal telencephalon
DG	Dentate gyrus
Dl	Lateral division of the dorsal telencephalon
Dla	Anterior division of the dorsolateral telencephalon
Dld	Dorsal division of the dorsolateral telencephalon
Dlp	Posterior division of the dorsolateral telencephalon
Dlv	Ventral division of the dorsolateral telencephalon
Dm	Medial division of the dorsal telencephalon
Dm1	Rostral part of the dorsomedial telencephalon
Dm2	Caudal part of the dorsomedial telencephalon
Dp	Posterior division of the dorsal telencephalon
EI	Electric image
EOD	Electric organ discharge
Er	Endopeduncular nucleus
Hip	Hippocampus
Hy	Hypothalamus
IOR	Δimage-to-Δobject ratio
MC	Mossy cells
MEC	Medial enthorhinal cortex
M-L	Mediolateral
Ob	Olfactory bulb
PG	Preglomerular complex
R-C	Rostrocaudal
Vc	Central division of the ventral telencephalon
Vd	Dorsal division of the ventral telencephalon
Vl	Lateral division of the ventral telencephalon
Vv	Ventral division of the ventral telencephalon

Despite the physical limitations briefly outlined here, weakly electric fish can perform amazingly well based on their electric sense. While an in-depth review of their capabilities is beyond the scope of this chapter, it is important to note that they can determine the size, distance, resistance, capacitance, and shape of objects within their sensory volume (von der Emde et al. 2010). Here, we show that this depends on physical as well as anatomical and behavioral specializations.

Contrary to vision, EIs become wider and decrease in intensity with the increasing distance of an object (Fig. 12.1C; Rasnow 1996; Caputi and Budelli 2006). Two objects of different size and distance can have EIs of similar amplitude, causing a size-distance ambiguity (Fig. 12.1C, *left*). Nonetheless, weakly electric fish are able to estimate distances (Heiligenberg 1973a, b), for which they rely on the blurriness of the images (Rasnow 1996; von der Emde et al. 1998). Behavioral evidence for

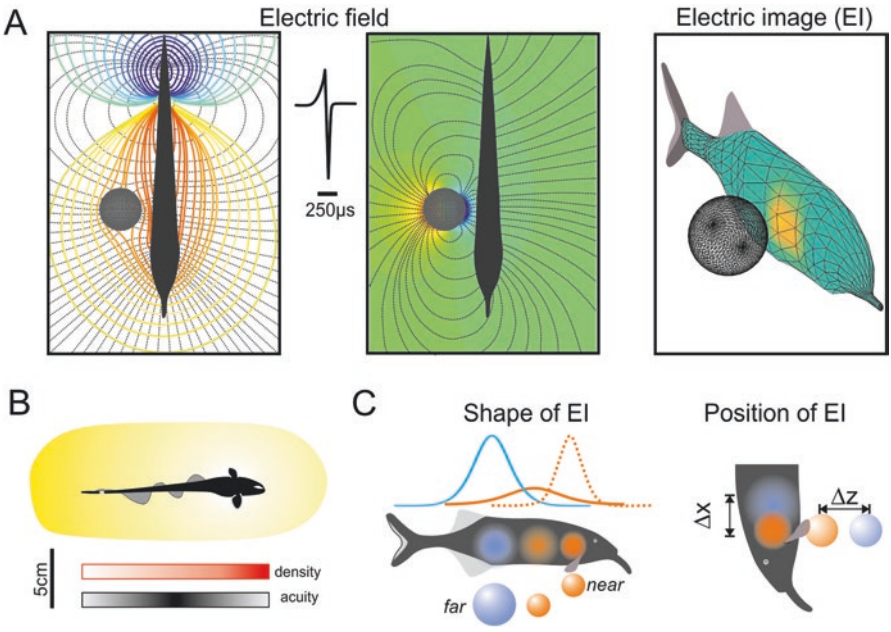

Fig. 12.1 The electric image (EI) of objects within the sensory volume depends on the distance and position. **A:** top view of the electric field modeled for the elephantnose fish (*Gnathonemus petersii*). *Left:* effect of a conductive sphere on the isopotential lines (*orange colors* show positive and *blue colors* show negative isopotential lines) and the electric field (*black lines*). The fields are shown for the positive peak of the electric organ discharge (EOD). *Center:* difference between the electric field with and without the object present is shown as a normalized color map (the equivalent perturbing field) with the field lines superimposed in black. *Right:* three-dimensional view showing the EI calculated based on the perturbing field. **B:** artistic representation of the sensory volume. Note that the electric sense, albeit limited in range, is omnidirectional. *Black bar*, theoretically predicted acuity for separating two objects; *red bar*, density of electroreceptors. **C:** schematic summarizing the effects of distance and size of objects on the EI. *Left*: effect of distance for a large (*blue*) and small (*orange*) conductive object. The calculated two-dimensional images are shown above the fish. Note the ambiguous effect of object distance and size. *Right:* effect of lateral distance of a conductive object on the position of the EI on the skin of the fish. Note that the shift of the EI depends on the lateral distance such that it moves more caudal with increasing distance. Data for **A** kindly provided by F. Pedraja; **B** modified from Snyder et al. (2007)

this was provided by von der Emde et al. (1998). These authors specifically proposed that fish estimate distance based on the maximum slope-to-amplitude ratio of the EI, a mechanism comparable to estimating the half-maximum width of EIs (Fig. 12.1C; Rasnow 1996; Lewis and Maler 2001).

Fish must also determine the rostrocaudal (x, y) position of objects. This probably is based on the location of the peak of the EI on the skin (Rasnow 1996). This position should be readily available via somatotopically organized neuronal maps through the computation of a population vector (Lewis and Maler 2001). However, as first shown by Rasnow (1996), there is a systematic distance-dependent offset of the rostrocaudal position of the EI (Fig. 12.1C, *right*). As discussed in Sect. 12.1.2,

this effect can be used to obtain a dynamic distance cue. Despite the ambiguities inherent to the electric images, the precision of the prey-capture behavior reported in black ghost knifefish (*Apteronotus albifrons*; Postlethwaite et al. 2009) suggests that position (x, y, and z) as well as movement of the prey (or objects) are accurately tracked by the animals (MacIver et al. 2001).

Due to the superposition effect of EIs, the acuity of separating two nearby objects is moderate. At a 1-cm distance, two *Daphnia* have been modeled to be separable if the interprey spacing is about 10 mm (Babineau et al. 2007). Electroacuity thus is comparable to that of human touch, where the 2-point discrimination ability ranges from 5 to 50 mm (Rowin and Meriggioli 2007). The coherence of the electric field lines is highest at the midbody (Figs. 12.1A and 12.2A), suggesting that electroacuity should be best at the trunk. However, the density of electroreceptors is highest at the head (Fig. 12.1B), potentially resulting in a more balanced acuity.

Details of the electric field not only depend on the environment but also on the animals' body. Their low internal resistance funnels the current toward the head region. This is the source of the asymmetry of the dipole field seen in Fig. 12.1A. A direct consequence of this asymmetry is that the EI of an object depends on the rostrocaudal position of the object (see Fig. 12.2). Fish thus may be able to obtain different viewpoints of an object by changing their position with respect to an object (Hofmann et al. 2017; Pedraja et al. 2018) or by actively moving their electric organ (Sim and Kim 2011). Analyzing the electric scene from different viewpoints has also been proposed as a mechanism to deal with the superposition nature of EIs (Migliaro et al. 2005; Babineau et al. 2007). Although the asymmetry of the electric field may be used to actively extract spatial information, passive (prereceptor) mechanisms can further affect the properties of the EIs. For example, the epithelia containing the electroreceptors have a high resistivity that improves their sensitivity (von der Emde and Schwarz 2002; Migliaro et al. 2005). The density of the electroreceptors is typically highest at the head (Castelló et al. 2000; Bacelo et al. 2008), forming a short-range but high-resolution fovea, whereas the trunk may be preferentially used to detect objects with high sensitivity.

12.1.2 Sensory Flow and Electrolocation

The ability to localize minute prey items and the concomitant occurrence of stereotyped back-and-forth scanning motor patterns ("va-et-vient" movements) in prey-catching behavior have led to the hypothesis that spatiotemporal sensory patterns generated by these motor patterns can aid in electrolocation (for details on the tight relationship between electroreception and the motor system, see Perks and Sawtell, Chap. 11). Indeed, many of the well-known stereotyped probing motor acts may induce relative motion cues and hence are regarded as an active-sensing strategy to enhance electroreception (Hofmann et al. 2013b). Modeling the sensory input associated with the back-and-forth scanning movements revealed that in the absence of

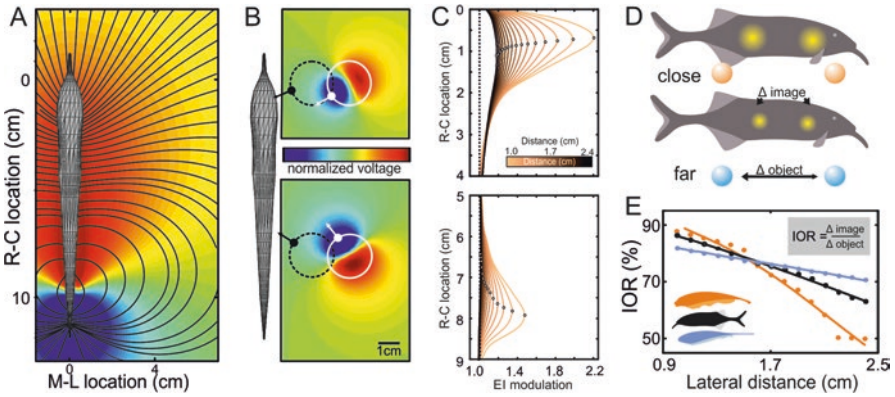

Fig. 12.2 **A:** top view of the calculated electric field of *Gnathonemus petersii*. *Red*, positive normalized voltage; *blue*, negative normalized voltage; *black lines*, electric field lines. R-C, rostrocaudal; M-L, mediolateral. **B:** perturbation of the field due to a metal sphere placed at two different rostrocaudal locations and a fixed lateral distance. The colors represent the voltage difference between the electric field with and without the object present: *red*, positive normalized voltage; *blue*, negative normalized voltage. *White circles*, position and size of the sphere; *white arrows*, polarization gradient of the electric field due to the object differs by almost 90° between the rostral and the caudal objects; *black circles*, spheres at a closer distance; *black arrows*, corresponding gradient. The angular difference of the polarization gradients is smaller for the closer object. **C:** modeled EIs for the object position shown in **B** for increasing lateral distances (for color coded distance, see color bar). Note that the maxima of the EIs (*black circles*) gradually shift to more medial positions on the animal's trunk for further distances. **D:** schematic of the EIs of a sphere moving at a fixed distance along the trunk. *Top*: schematic shows the sphere (*orange circle*) moving closer to the animal's skin; *bottom*: schematic shows the sphere (*blue circle*) moving at a further distance from the animal's skin. Although the object moves over an identical distance, the image speed (and hence distance) that the EI travels on the surface of the fish decreases with lateral distance. **E:** Δimage-to-Δobject ratio (IOR) decreases with distance in the three electric fish species that were tested (*orange*, *Apteronotus albifrons*; *black*, *Gnathonemus petersii*; *blue*, *Eigenmannia virescens*). Data modified from Pedraja et al. (2018)

movements, the superposition nature of EIs should make prey detection very difficult. However, relative movement was shown to result in time-varying local EIs that let the prey stand out (Babineau et al. 2007). Such time-varying signals do contain distance information that may be extracted, similar to the slope-to-amplitude ratio discussed in Sect. 12.1.2 (Hofmann et al. 2013a).

But are these motion-induced signals actually used? A behavioral study of the electroacuity of the elephantnose fish (*Gnathonemus petersii)* found that they are surprisingly good at discriminating gapped from solid objects (Fechler et al. 2012). This was suggested to indicate that the fish exploit the spatiotemporal sequence of different electric viewpoints. Importantly, the gap-detection experiments provide evidence for an enhancement of electroacuity in the presence of moving backgrounds, fitting to the predictions of Babineau (2007). This agrees well with a study on the same species that found that the natural dynamics of electrosensory

information (electrosensory flow) lead to the emergence of a highly reliable distance estimate (Hofmann et al. 2013a).

Are stereotyped motor patterns and, in particular, the back-and-forth scanning movements performed in a purposeful manner to shape the sensory flow? Such back-and-forth movements are suggestive of peering movements used by insects to exploit and generate a visual parallax for a visual depth estimation (Poteser and Kral 1995). Indeed, the asymmetrical electric field provides a similar depth cue through its nonlinear distortion of the field lines (Pedraja et al. 2018). The direction of the field lines (Fig. 12.2A) determine where an EI is created on the sensory surface of the fish. The dipole moment of the perturbation due to an object is parallel to this electric field as shown in Fig. 12.2B by the polarization gradient. Hence, as schematically shown in Fig. 12.2C, D, the EI of an object that recedes laterally from the fish not only becomes blurry but is also projected more toward the midbody. When the fish swims along a stationary object, the EI travels a fixed distance ($\triangle$image) over the skin. The $\triangle$image decreases with the lateral distance of the object, whereas the distance moved by the fish is constant ($\triangle$object; Fig. 12.2D). The apparent speed of the EI thus decreases with the distance of the object (image-to-object ratio; Fig. 12.2E). Relative motion between object and animal thus gives rise to a parallax-like distance cue that fish may exploit. This was tested using the well-established shelter-tracking behavior in which weakly electric fish maintain a centered position within a moving shelter. When the sides moved at different speeds, the fish shifted toward the slower side (Fig. 12.3A). As predicted by the motion parallax hypothesis, the magnitude of this shift increased with the speed difference (Fig. 12.3B). Sensory flow is exploited to estimate depth, strongly supporting the idea that back-and-forth scanning behavior is a strategy to actively acquire depth information (see Stamper, Madhav, Cowan, and Fortune, Chap. 8).

Significant progress toward understanding how weakly electric fish can encode the distance to moving objects has been made for looming and receding motions. Glass knifefish (*Eigenmannia virescens*) maintain a preferred distance when positioning between two swinging rods (Fig. 12.3C, *top*). This preferred distance is the distance where the ON- and OFF-cell populations of the electrosensory lateral line lobe (ELL) report the presence of a looming object optimally (Clarke et al. 2015). At this distance, these neurons transition from a tonic to a burst-firing mode (Fig. 12.3C, *bottom*) and the distance where this occurs is invariant of object size and speed (Clarke et al. 2015). This requires a dynamic control of the bursting activity that was shown to depend on sophisticated mid- and hindbrain feedback loops (Clarke and Maler 2017). Keeping bursting ON- and OFF-cells in balance will enable the animal to reliably track objects. Furthermore, a switch in the ratio of bursting ON-cells to OFF-cells provides information about the direction of the movement of the object (Fig. 12.3C, *right*). This is a compelling example that shows how neural coding and motor output together influence information transfer in active sensing.

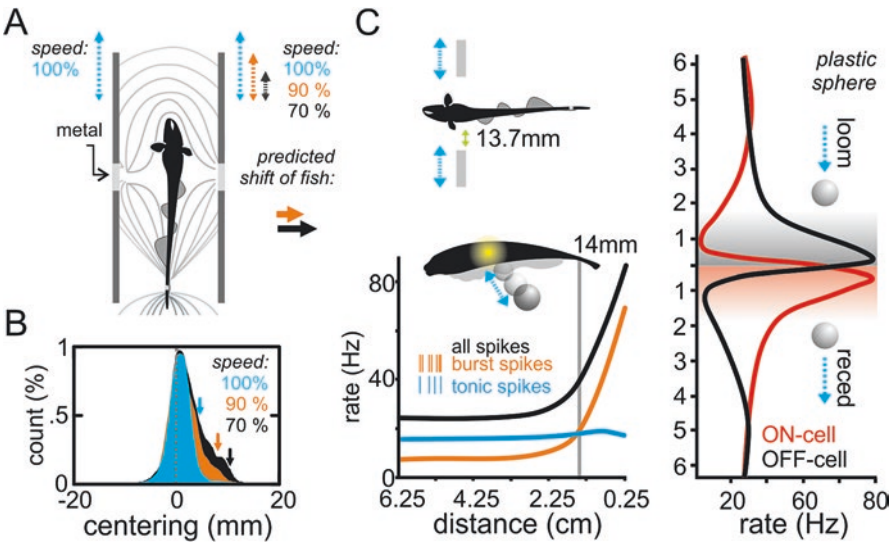

Fig. 12.3 **A:** top view of a fish positioned between moving shuttle walls (*gray*) that contain an embedded metal stripe (*black*). In the control condition, both walls move back and forth at 2 cm/s (*blue arrows*); in parallax conditions, one wall moves at 1.8 (90%; *orange*) or 1.4 (70%; *black*) cm/s. **B:** exemplary normalized distributions of a single fish's position during centering behavior. For illustrative purposes, the position data were adjusted to represent the parallax condition on the right while the experimental conditions were tested on either side at random. With a stronger parallax cue (black > orange > blue), the skewness of the distributions increased as quantified by the 90% quantiles (*arrows*). There was a significant shift toward the slower side, as predicted from the electric field study (Fig. 12.2). **C:** *top:* schematic of the paradigm used by Heiligenberg (1973b). When fish (*Eigenmannia virescens*) station between two rods (gray) that oscillate transversally (blue arrows), they maintain a preferred distance with respect to the closest rod of approximately 13.7 mm. *Bottom:* simulating this in a physiological experiment shows that this distance conveys optimal electrosensory information and is precisely the distance where ON- and OFF-cells of the electrosensory lateral line lobe (ELL) transition from tonic to burst-firing mode in brown ghost knifefish (*Apteronotus leptorhynchus*). Data is pooled for ON-and OFF-cell responses to looming metal and plastic spheres, respectively. *Right:* ON- and OFF-cell pair response to the looming and receding movement. Note that both types respond to the same change of local sensory contrast in a manner that suggests sensitivity to the temporal derivative of contrast. The colored background for both types shows regions of bursting. Encoding of motion requires combining ON- and OFF-population, whereas directionality is encoded in the balance between both populations. **A** and **B** modified from Pedraja et al. (2018); **C** modified from Clarke and Maler (2017)

12.1.3 Electrolocation and Multimodal Integration

The ability to integrate multimodal information is crucial to build up reliable sensory representations. Multimodal integration can lead to multisensory enhancement when stimuli of different modalities match temporally and spatially (Meredith et al. 1987; Meredith and Stein 1996). The magnitude of this multimodal enhancement is inversely proportional to the effectiveness of the single sensory component (Stanford et al. 2005), while the weighting of a single modality is inversely proportional to the variance of the stimulus (Ernst and Banks 2002).

Weakly electric fish also rely on different sensory modalities (von der Emde and Bleckmann 1998; Moller 2003). Synergistic effects between the active electric sense and vision (Rojas and Moller 2002) as well as passive electroreception and the mechanosensory lateral line (Pluta and Kawasaki 2008) have been found in electric fish, suggesting that multimodal enhancement may aid in electrolocation. Furthermore, studies on *Gnathonemus petersii* have shown that weakly electric fish are capable of spontaneous cross-modal object recognition (Schumacher et al. 2016, 2017). After being trained to discriminate between objects with either vision or the active electric sense only, fish were able to discriminate the objects correctly using the untrained modality alone. The different sensory modalities are weighted according to their reliability in a manner consistent with the notion of a division between far- and near-range sensory systems.

12.2 Spatial Learning

Aquatic environments are highly variable and offer ample potential cues that fish may use to orient. Two broad distinctions are commonly made: self- and world-centered orientation. The first is referred to as egocentric navigation, the latter as allocentric navigation.

Egocentric navigation is possible in the absence of any external sensory information, relying on self-generated (idiothetic) information from the proprioceptive and vestibular systems (Etienne 2004). As such, egocentric navigation depends on active movement (Mittelstaedt and Mittelstaedt 1982). Through integration of the current direction and velocity with knowledge of the positions visited, it theoretically is possible to determine the current position, and a vector to any memorized place along a trajectory can be calculated. Because this ability is frequently tested by forcing an animal to navigate to its home, this vector is called the homing vector. Behaviors in which the relationships between landmarks are used to navigate are called allocentric because the animal references its position in space with respect to external (allothetic) information. With allocentric input, animals can navigate to new locations, provided they have a cognitive representation of space. To form such a representation, however, path integration is initially required because it provides the knowledge of the distance and location of external cues. This may be seen as the conundrum of navigation: egocentric metrics must be acquired by active movements to establish an allocentric map (McNaughton et al. 2006; Buzsáki and Moser 2013). Cue learning can be considered as a special case in ego- and allocentric navigation, where a salient feature of the environment is used as a beacon to guide an animal's behavior.

12.2.1 Spatial Learning Strategies Used in Fish

Studies of spatial navigation and memory range from field observations to laboratory studies (Salas et al. 2006). A simple example illustrating the ability of fish to form spatial representations is the work on the frillfin goby (*Bathygobius soporator*;

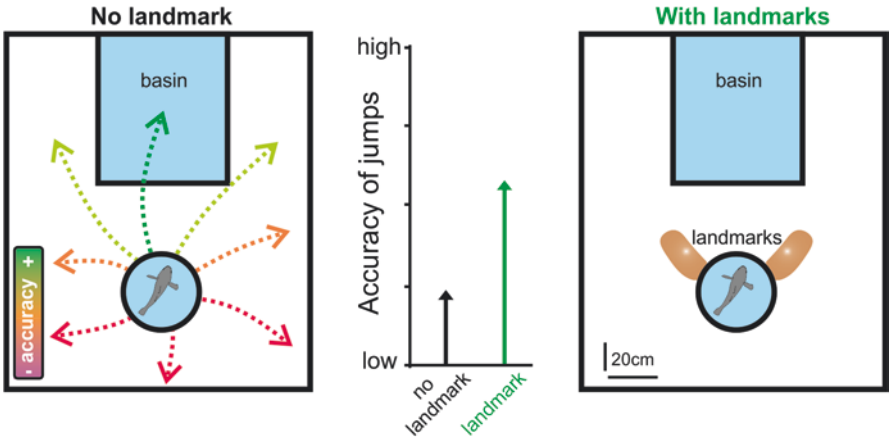

Fig. 12.4 Spatial learning in *Bathygobius soporator* depends on the availability of landmarks. The fish were put in an artificial tidal pool-like environment from where they try to escape by jumping toward the neighboring basin. This basin cannot be seen from the tidal pool. Precision of the evasive jumps was scored (*dashed-line arrows*) in presence (*right*) and absence (*left*) of visual landmarks. The behavior was significantly more accurate (directed) in the presence of the landmarks (*center*). Modified from Aronson (1971)

Aronson 1971). These fish live in tidal pools and accurately jump from their home pool to adjacent pools to evade predation. Testing this behavior in the laboratory, Aronson found that it depends on the presence of landmarks outside the pools (Fig. 12.4). On the one hand, this exemplifies the need for fish to deal with variable environments (tide); on the other hand, it shows that they can do so by formation of a spatial representation of their environments (landmarks). Note that the term cue is used when a single feature in the environment provides information about a goal, whereas the term landmark is used when the constellation of several cues is constant and therefore can be used in spatial learning.

As discussed here, exemplary studies on goldfish (*Carassius auratus*) are presented that bridge a behavioral account with the neuronal structures underlying navigation in fish (Odling-Smee et al. 2006; Rodriguez et al. 2006). In a seminal study, *Carassius auratus* were trained in a maze to either perform a fixed turning behavior or swim to a location defined through a set of stable visual landmarks external to the maze (Fig. 12.5; Rodriguez et al. 1994). In the first task, fish could rely on egocentric cues, whereas the second task required them to use external references. In test trials, fish relied on the previously trained strategy (Fig. 12.5). Note that the external landmarks were available to both groups, suggesting that the fish adjusted their strategies depending on requirements or context. Although experiments as the one discussed here focus on the navigation in two dimensions, work on pelagic fish has shown that fish also navigate in three dimensions (Burt de Perera et al. 2016).

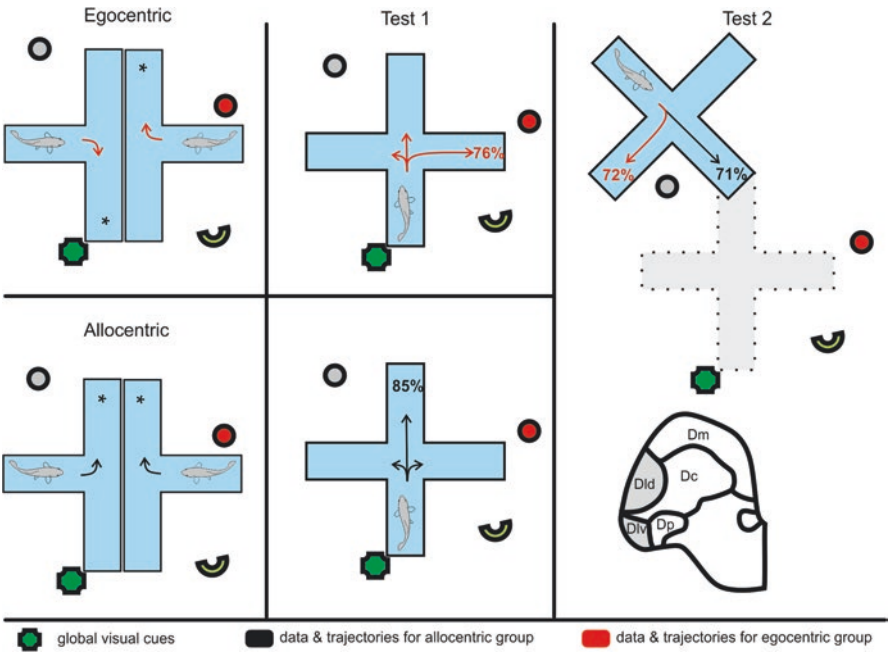

Fig. 12.5 Evidence for ego- and allocentric navigation strategies in *Carassius auratus*. *Left column:* in the egocentric task, *Carassius auratus* were required to perform a fixed turn strategy to receive a reward (*asterisks*), whereas they were required to navigate to a location in the maze that was only identifiable through the external visual landmarks (*symbols outside the maze*) in the allocentric group. Note that in this condition, the arm opposite to the starting position of the fish was closed. *Center column:* to test which strategy fish had learned to navigate the maze, fish were released from a new global (room centered) position. In these test trials, all arms of the maze were opened. Fish in the egocentric group preferred the turn strategy (*right*), whereas the allocentric group preferentially swam to the position corresponding to the place where the reward was presented in the learning trials. *Numbers*, percentage of trials that fish choose the arm. *Right column:* in a second test, the whole maze was rotated such that one arm of the maze was at the global position that was rewarded in the learning trials (*gray area* shows the previous location of the maze). Again, an egocentric strategy was preferred by fish trained for the turn strategy, whereas the allocentrically trained fish preferred to swim to the global position. Lesions to the ventral division (Dlv) and posterior division (Dld) of the dorsolateral telencephalon (*gray areas*) prevented fish from using an allocentric strategy (Salas et al. 1996a; Rodríguez et al. 2002). Dm, medial division of the dorsolateral telencephalon; Dc, central division of the dorsolateral telencephalon; Dp, posterior division of the dorsolateral telencephalon. Data redrawn from Rodriguez et al. (1994); schematic brain section redrawn from Wullimann and Mueller (2004)

12.2.2 The Role of Active Electroreception during Spatial Learning

Gymnotiform and mormyrid weakly electric fish perform seasonal migrations as well as foraging excursions in the dark (Corbet 1961; Moller et al. 1979). Their nocturnal lifestyle suggests that olfactory as well as electro- and mechanosensory

cues are essential in these behaviors. The near-range nature of these modalities suggests that local sensory input has to be stitched together to be transformed into an allocentric representation of space.

A study by Graff (2004) demonstrated that gymnotiform and mormyrid weakly electric fish are capable of electric-pattern recognition. In this study *Gnathonemus petersii* and *Sternopygus macrurus* (longtail knifefish) were trained to orient in a Y-maze according to the similarity of a local electric cue with a cue experienced at the start of a trial. The design of the object was such that the spatial patterns of the objects (Fig. 12.6A) were not globally available to the fish. Hence the fish had to sample the electrodes locally to generate a spatial representation of the object. Furthermore, the fish needed to compare the similarity of this object with the one in their home compartment. This demonstrates that weakly electric fish are able to attend to spatially segregated information in a manner similar to pattern separation and completion.

How these abilities are used in navigation was initially addressed in a paradigm where *Gnathonemus petersii* had to learn to shuttle between two compartments of an arena that were connected by an elevated opening in either the presence or absence of a local electric cue (Cain 1995; Cain and Malwal 2002). Acquisition of this task depended on the active electric sense, but silencing the electric organ after the fish had acquired the task did not impair their performance. This suggests that the fish used idiothetic information to follow a previously acquired route. In acquiring this route, the local electrosensory cue was also probably used as a beacon. This interpretation gains support from the finding that when the hydrostatic pressure in the tank was changed after the fish had acquired the task, they again began to attend to the local electric cue. Contrary to this, fish trained in the absence of the electric cue searched for the opening above the actual aperture (i.e., they changed their behavior in accordance with hydrostatic pressure).

The results show that local electrosensory information can be used to calibrate navigation but did not test for allo- and egocentric strategies. To do this, Walton and Moller (2010) trained *Mormyrus rume proboscirostris* to navigate a maze with and without visual input and with and without local electrosensory cues (Fig. 12.6B, *red lines* and *squares*, respectively). The electric cues were objects of different electrical properties but of identical visual appearance that were positioned at the turning points of the maze. Thus, they could be used to learn in which direction to turn and are thus referred to as local electrosensory cues. In the recall phase, the maze was removed and the fish were released from their original entry to the maze or from an entry not used during the training. Fish trained in the absence of local electric cues consistently headed in the direction opposite their release site (Fig. 12.6B, *center* and *right*), as would be expected if they relied on an egocentric behavior. Even fish trained in the presence of external visual landmarks showed this behavior, showing that the external visual landmarks were not used. When trained with both visual and electric cues, fish veered toward the position of the first local cue when released from their initial position during recall. This indicates that local electric cues are attended to during the learning phase. When these fish were released from a novel site in the absence of the local cues, no veering in the direction of the previous local cue occurred and the fish again swam straight (Fig. 12.6B, *right*). Hence, in the

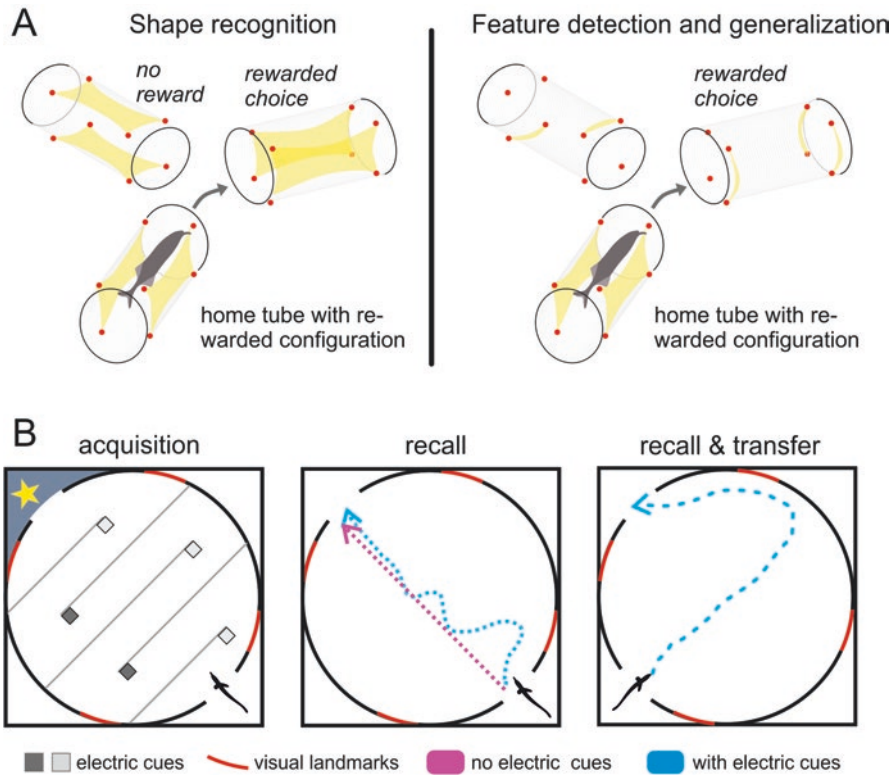

Fig. 12.6 **A:** summary of experiments addressing the ability of *Gnathonemus petersii* and *Sternopygus macrurus* to learn and generalize electric patterns like "vertical" and "horizontal" and to generalize these features when only partial sensory information is provided. *Yellow areas* are an artistic representation of locally increased conductivity that can be created through short circuiting between the connected electrodes (*red circles*). In these experiments, fish were able to correctly generalize. Due to the limited range of electroreception, the fish needed to sample the electric pattern sequentially and connect this locally acquired information to form a representation of the global pattern. To correctly solve the task, the test tube that had the same global configuration as the home tube had to be chosen (*left*) and generalization of the pattern was tested by presenting reduced patterns (*right*). Note that the pattern in the home tube against which fish had to compare the two patterns in either arm of the Y maze was turned off at the beginning of each trial. **B:** overview of an experiment in which *Mormyrus rume proboscirostris* were trained to navigate a maze in the presence of visual external landmarks (*red wall segments*). The *thin lines* in the circular arena indicate the partitions of the maze that were removed in probe trials after the fish had learned to successfully navigate through the maze. *Left*: during acquisition, the maze either contained no additional local electric landmarks or fish were trained with an "electrically enhanced" maze that contained conductive (*solid squares*) as well as nonconductive objects (*gray squares*). Test trials were performed in the absence of the maze (with/without local landmarks; *center*) and in the absence of the maze and local cues with fish starting from a novel release point (*right*). The main navigational strategies are summarized by the differently colored trajectories. Recall in the absence of local cues resulted in a targeted approach to the goal. The outbound trajectory of fish trained in presence of local cues and tested in presence of those was initially oriented toward the first cue, from where a more directed approach to the goal was found. This is consistent with an egocentric navigation strategy that relies on sensory-guided updating at a local landmark with path integration. If the fish were rotated during recall, they consistently showed a purely egocentric strategy. **A** modified from Graff et al. (2004); **B** modified from Walton and Moller (2010)

absence of local cues, weakly electric fish followed an egocentric strategy that they could combine with a cue-based strategy if local electric cues were present. As the first cue is outside the electrolocation range when the fish leave the start box, this may suggest that they had formed some internal representation of the location of this electric cue with regard to their start box and therefore headed in this direction during recall. However, this requires further research to be confirmed. Integrating the local cue as part of a learned motor routine can help in correcting for errors accumulated during path integration to the cue, a mechanism known from both mammals and ants (Etienne 2004; Knaden 2006). The notion that weakly electric fish seem to preferably follow egocentric strategies in recalling routes over short distances was further confirmed in two studies with *Gnathonemus petersii* (Schumacher et al. 2017; Jung et al. 2019).

With the exception of the studies by Cain (Cain 1995; Cain and Malwal 2002), which documented a tight link between familiarity of an environment and the EOD frequency, the electric sampling behavior during spatial learning has not been studied in the work discussed thus far. This is surprising because the electric sampling can be directly linked to motor planning and knowledge of an environment. Pulse-type weakly electric fish are known to show electromotor orienting responses to changes in their environment (Sokolov 1990), the so-called novelty response (Post and von der Emde 1999; Caputi et al. 2003). Novelty responses are considered an economic strategy to update the memory of the recent sensory past whenever it deviates from the current sensory state (Heiligenberg 1980; Caputi et al. 2003). A similar link exists between active sensing and motor planning in the banded knifefish (*Gymnotus* sp.). When *Gymnotus* initiate movement in the absence of an observable change of their sensory input, this is preceded by an increase in EOD frequency, a so-called up state (Jun et al. 2016). Up states as well as novelty responses are overt electrical behaviors that reflect top-down control. Consequently, the study on *Gymnotus* sp. (Jun et al. 2016) started to focus on sampling density (number of EODs per distance traveled) in the acquisition and recall of spatial memory tasks. In this study, the fish had to find food within a large circular arena. Because the experiments were performed in darkness, the fish had to rely on idiothetic cues and near-range sensory information, including the electric sense. Food was presented in a fixed spatial relationship to four different local electric landmarks (see Fig. 12.7A), and the ability to navigate to the food was investigated.

In agreement with the data of Cain et al. (1994), the local electric landmarks improved performance. Fish trained in the absence of landmarks followed a random search strategy, taking longer trajectories to locate the food than fish trained with stable landmarks. After having acquired the task, the fish spent significantly less time at the landmarks (Fig. 12.7B), sampled them less (Fig. 12.7B), and showed fewer stereotyped back-and-forth motor patterns at the landmarks (Fig. 12.7B; as introduced in Sect. 12.1, the back-and-forth movements generate sensory flow that produces a distance metric). In probe trials without food being present, the fish increased these motor patterns and the sampling density at the landmarks again (Fig. 12.7B).

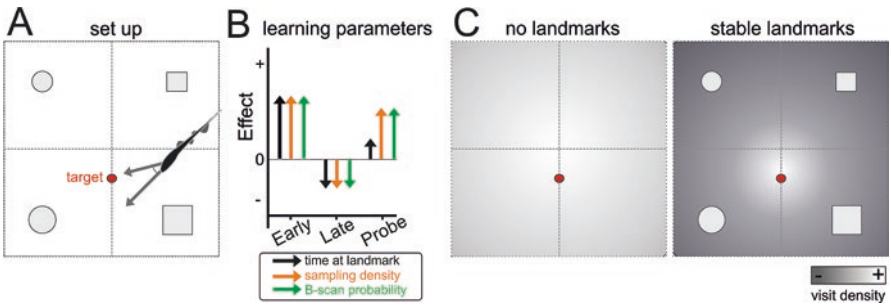

Fig. 12.7 Spatial learning and active sampling behaviors. **A:** overview of the arena in which *Gymnotus* sp. were tested with four stable landmarks (*gray squares*) and a food-baited goal location (*red circle*). **B:** overview of the change in parameters observed near the landmarks at the early and late phase of learning and the probe trials. Search time (*solid black lines and arrows*), sampling density (*solid orange lines and arrows*) and back-scan probability all decline while the fish acquired the task (*compare early and late data*). Sampling density and B-scans are again elevated in the probe trials (*green arrows*). **C:** schematic showing the visit density distribution in probe trials for fish trained in the absence and presence of landmarks. Note that the fish are more accurate and precise in targeting the goal if they were trained with landmarks. Redrawn after Jun et al. (2016)

The changes in sampling behavior observed by Jun et al. (2016) are consistent with the interpretation that on encountering a salient cue in unexplored terrain, electric fish perform place learning and associate this landmark with their idiothetic information of the cue's position. Afterward, the landmarks are sampled less and may be used to compensate for errors in path navigation. Once the fish encounter a change in the pattern of local landmarks (including the food location in these experiments), they resample them, probably with the aim to recalibrate. This is supported by the finding that the fish were able to reduce their heading error over the course of the probe trials. A second series of experiments add support to the importance of landmarks in which fish were trained either with stable landmarks or in the absence of landmarks (Fig. 12.7C). Only the stable group shifted their mean sampling density distribution toward the true goal (Fig. 12.7C, *right*) and thus had a higher accuracy and precision. This shows that fish acquired and used relational information they obtained electrically to guide their behavior. Hence, contrary to the experiments reported thus far, this shows that they are capable of forming some form of global representation based on the highly localized sensory input.

This suggest that weakly electric fish incorporate relational knowledge of electric landmarks acquired through local active electric sampling to support idiothetic navigation. How egocentric and sensory information can be transformed to relational allocentric representations of space is currently unknown. A study in *Apteronotus leptorhynchus* recorded from the preglomerular complex (PG), an analog of the mammalian thalamus that provides visual and electrosensory input to the dorsolateral pallium (see Sect. 12.3.1; Wallach et al. 2018). Neurons in the PG were found to transform the topographic visual information into temporal information about time between object encounters. When combined with information on swim speed, the distance between objects could be computed. In this way, sequential

exploration of different landmarks could provide egocentric distance measures from which allocentric spatial maps may later be computed.

In summary, all available data consistently demonstrate that weakly electric fish can use egocentric strategies in spatial learning. These strategies were shown to benefit from the presence of local cues and landmarks. With increasing familiarity, the electric sampling of these decreases. This suggests that fish tend to rely more on path integration mechanisms in familiar terrain, but active electric sampling of cues is increased when such internalized strategies fail. Relying on path integration whenever possible and updating the path integration mechanisms only by reorientation toward electrically detectable local landmarks seems to be an efficient strategy in the absence of far-range sensory information. Hence, weakly electric fish are highly suitable to study the contribution of active exploratory behaviors for the computation and acquisition of spatial relationships. In a broader context, active movements are the key element to disambiguate an animal's environment and offer a direct link between overt behaviors and internal processes like spatial learning and memory.

12.3 Neural Substrate of Spatial Learning in Teleost Fish

12.3.1 Primer on the Teleost Telencephalon

The telencephalon is crucial for spatial learning in fish, and hence a coarse anatomical overview is presented in this section (for a comprehensive overview, see Meek and Nieuwenhuys 1998). The telencephalon is separated into the dorsal pallium (area dorsalis) and the ventral subpallium (area ventralis). The relationship between the teleost and tetrapod dorsal pallia has stirred a veritable scientific debate (e.g., Mueller et al. 2011; Yamamoto et al. 2017). In part, the difficulty of establishing homologies is based on the different morphogenesis of the dorsal telencephalon, which in actinopterygian fish develops though eversion of the forebrain anlage, whereas in all nonactinopterygians, it is characterized by an evagination process (Fig. 12.8). The details of this process have not yet been resolved, but it is evident that evagination will lead to considerably different topologies of potentially homologous brain areas (for reviews summarizing the different interpretations, please refer to Northcutt 2008; Braford 2009; Nieuwenhuys 2009). Nieuwenhuys' (1963) topology-based nomenclature in describing the forebrain is used throughout the chapter. Accordingly, the dorsal telencephalon is separated into a medial division (Dm), dorsal division (Dd), central division (Dc), lateral division (Dl), and posterior division (Dp; see Fig. 12.8C). Except for two areas, no consensus on homologies has been reached. Hodological criteria, expression studies, and functional studies make it likely that the Dl is a homologue to the amniote hippocampus (medial pallium; Fig. 12.8), whereas similar arguments have led to the view that the Dm is the homologue of the amniote amygdala (ventral pallium; Fig. 12.8; Portavella et al. 2004; Vargas et al. 2009).

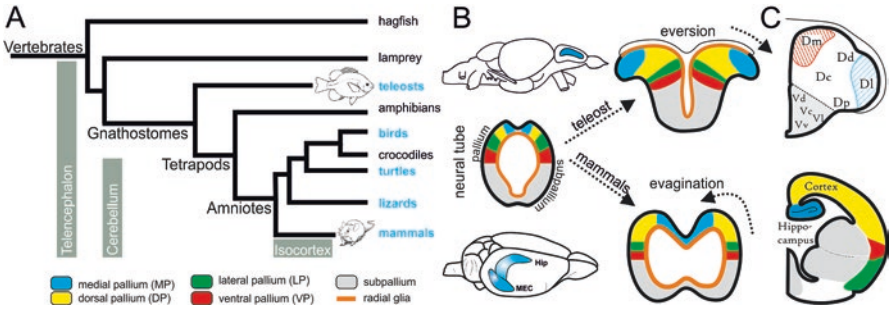

Fig. 12.8 **A:** vertebrate cladogram with key brain innovations (modified after Murray et al. 2017). Where lesions of the medial pallium have been tested (blue species), spatial navigation consistently was impaired. **B:** schematic comparing the development of the actinopterygian telencephalon and nonactinopterygian telencephalon. Assuming that four pallial zones are present in the early stage, the bottom row exemplifies the process of evagination resulting in the hippocampus being internalized by the growth of the isocortex. Because of this process, the four zones maintain their original order. The eversion characteristic for actinopterygian fish results in a rearrangement of the pallium. The details of this rearrangement are presently unresolved. The brain schematics show a zebrafish (*top*) and mouse brain (*bottom*) with the medial pallium areas implicated in navigation depicted in *blue*. MEC, medial enthorhinal cortex; Hip, hippocampus. **C:** schematic half-cross sections comparing teleost (*top*) and mammalian forebrain (*bottom*). Most authors see sufficient support to consider the Dm of teleosts (*red striped area*) as a homologue of the lateral pallium (LP, amygdala) and the lateral division of the dorsolateral pallium (Dl; *blue striped area*) as a homologue of the medial pallium (MP, hippocampal formation). Dd, dorsal division of the dorsolateral pallium; Vc, central division of the ventral pallium; Vd, dorsal division of the ventral pallium; Vl, lateral division of the ventral pallium; Vv, ventral division of the ventral pallium. **B** and **C** modified from Meek and Nieuwenhuys (1998), Mueller et al. (2011), and Yamamoto et al. (2017)

The ventral part of the telencephalon is divided into a dorsal division (Vd), central division (Vc), lateral division (Vl), and ventral division (Vv; see Fig. 12.8C). Again, no clear comparative pattern has emerged yet, but in toto the ventral pallium may be considered similar to the basal ganglia of nonacinopterygians (Mueller and Wullimann 2009).

12.3.2 Experiments Addressing Sites of Spatial Learning

To navigate, motor output needs to be calibrated. The mammalian cerebellum contributes to this in two ways. It computes idiothetic self-motion information (vestibular, proprioceptive, motor-command efference copy, and, potentially, optic flow information) that is integrated into an allocentric frame of reference in the hippocampus. The computation of self-motion cues is instrumental for path integration (Wallace et al. 2002). In return, the cerebellum uses this reference frame to coordinate actions (Rochefort et al. 2013).

In teleosts, evidence for a direct cerebellar input to the telencephalon is limited (Vonderschen et al. 2002; Ikenaga et al. 2006). In mormyrid fish, this includes a

direct cerebellotelencephalic projection from the valvula cerebelli to the Dd (Wullimann and Rooney 1990). This projection may provide a pathway for electrosensory information (for a review of the neuroanatomy in electric fish, see Bell and Maler 2005). Goldfish with lesions to the corpus of the cerebellum took longer to learn to navigate in a hole-board task (Fig. 12.9) and performed less well than sham-operated fish (Durán et al. 2014). All fish were impaired when landmarks were removed or rearranged. However, the selective effects of lesions were found when only the nearest landmark (i.e., a beacon) to the goal was removed (Fig. 12.9, *red arrows*). The effect was as strong as removing all proximal landmarks.

The teleost optic tectum (homologue of the superior colliculus) contains sensory topographic maps as well as a motor map. It thus provides a body-centered site for sensory-motor transformation and is the major motor output center of teleosts (Isa and Sasaki 2002; Torres et al. 2005). In weakly electric fish, this includes electromotor output (Wullimann and Northcutt 1990; Carlson 2002). However, no studies have addressed how the motor output of the tectum contributes to spatial navigation or learning.

Allocentric navigation in mammals depends on the place cell system of the hippocampal formation (O'Keefe and Dostrovsky 1971; O'Keefe and Nadel 1979), including the medial enthorhinal cortex (MEC) as a key element. It integrates idiothetic input and provides metrical relationships like head direction, speed of movement, or distance to the hippocampus. From this, an allocentric representation of space is created in the hippocampus (Hafting et al. 2005; Fyhn et al. 2007). The MEC is considered as the site where path integration occurs. Furthermore, it may

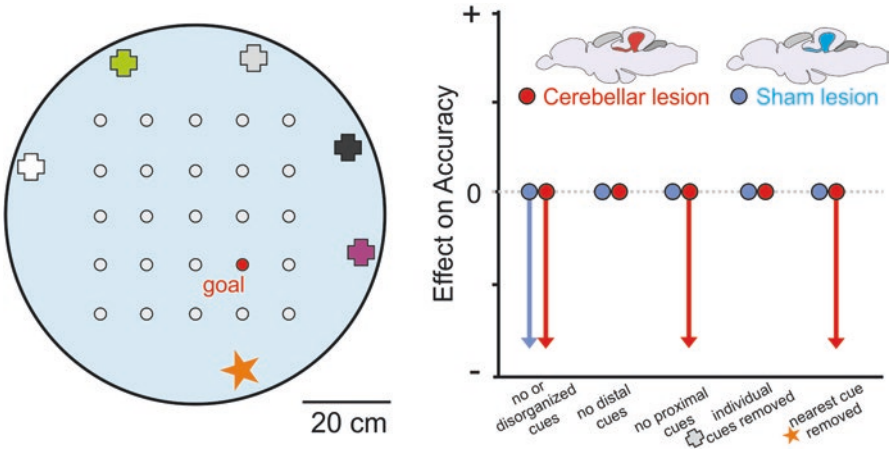

Fig. 12.9 Effect of cerebellar lesion on spatial learning. *Left:* schematic of the hole-board arena. *Circles*, the 25 holes in the bottom of the tank; *red circle*, the goal. Star and plusses, six landmarks (objects distributed in the arena). *Right:* effect on the accuracy of the localization of the goal for different conditions in recall. *Blue*, data from sham-operated fish; *red*, data from cerebellum-operated fish. Cerebellum-lesioned fish had significantly reduced performance in the absence of proximal cues, specifically the cue closest to the goal (*orange star*). Modified from Durán et al. (2014)

also be elemental in resetting the path integrator because head direction cells of the MEC were shown to change their tuning with respect to allocentric cues (Taube 2007).

Similarities of self-referenced egocentric navigation and episodic memory on the one hand and allocentric navigation and semantic memory on the other hand have led to the hypothesis that episodic and semantic memories have evolved from navigation circuits (Buzsáki 2005; Eichenbaum and Cohen 2014). This evolutionary perspective suggests that the fundamental principles of cortical memory formation and recall can be studied in much simpler brains, including the brains of teleost fish. There is general agreement that the medial pallium of early vertebrates likely evolved as a general memory navigation system (Murray and Wise 2004). However, homologies between forebrain structures of teleost fish and amniotes are currently not fully resolved (Fig. 12.8). This paragraph summarizes key findings, mainly in *Carassius auratus*, which led to the view that the dorsolateral pallium of teleosts is the homologue of the mammalian hippocampus. This view is largely based on lesion studies (Fig. 12.8) that revealed that allocentric strategies are selectively impaired by lesions of the dorsolateral telencephalon (ventral division [Dlv] and posterior division [Dld]; see Fig. 12.5; Rodríguez et al. 2002; Salas et al. 2003; Broglio et al. 2010). These lesions did not affect egocentric or cued learning (Salas et al. 1996b). The view that the Dl may be the homologue of the mammalian hippocampus is further supported through gene expression studies (see, e.g., Ganz et al. 2014), the report of place cells in the dorsolateral pallium of *Carassius auratus* (Canfield and Mizumori 2004), and the finding that neurons in the goldfish Dl have elevated neuronal activity during spatial learning (Broglio et al. 2010; Ocaña et al. 2017). In summary, the lateral pallium in teleosts is considered to be the homologue of the mammalian hippocampus and was shown to enable relational allocentric learning.

12.3.3 Forebrain Circuitry in Weakly Electric Fish and Their Implications in Spatial Learning

The following overview of the dorsal forebrain of weakly electric fish is based mainly on an excellent series of papers on the telencephalon of *Apteronotus leptorhynchus and Gymnotus* sp. Where available, information for the mormyrid *Gnathonemus petersii* is included. Rostral to the anterior commissure, the dorsal pallium of *Apteronotus leptorhynchus* consists of a large Dl and a distinct small Dlv. The Dlv as well as the Dp are the major recipients of olfactory input (Sas et al. 1993), which in *Gnathonemus petersii* is confined to the Dp (see Fig. 12.10B; Rooney et al. 1989). In both species, Dl is the major recipient for extratelencephalic input from PG (Fig. 12.11): In *Apteronotus leptorhynchus*, PG receives electrosensory input through the midbrain torus semicircularis and the optic tectum. In *Gnathonemus petersii*, Dl receives input via the ventral PG that obtains sensory input from the medial ventral nucleus of the torus semicircularis (von der Emde and Prechtl 1999). Additional electrosensory input is provided to the posterior division of Dl (Dlp) of mormyrids through a cerebellotelencephalic projection from the valvula (Wullimann and Rooney 1990).

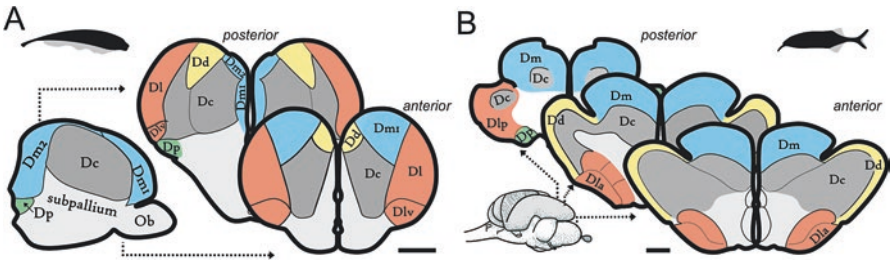

Fig. 12.10 Major dorsal forebrain areas of electric fish. **A:** transverse sections though the forebrain of *Apteronotus leptorhynchus* at a level rostrocaudal to the anterior commissure. *Bottom left*, schematic sagittal section of the forebrain. **B:** transverse sections of the forebrain of *Gnathonemus petersii*. *Bottom left*: side view of the brain. *Dotted-line arrows*, identified subareas of the four major dorsal divisions. Dla, anterior division of the Dl; DLp, posterior division of the Dl; Dm1 and Dm2, parts 1 and 2, respectively, of the Dm; Ob, olfactory bulb. Scale bars, 500 μm. **A** based on photomicrographs from Trinh et al. (2016) and Giassi et al. (2012b); **B** based on photomicrographs from Rooney et al. (1989) and Meek and Josten (1993)

The Dl borders on Dd. Dd can be identified in *Apteronotus leptorhynchus* through its lack of expression of CaMKIIα and extrinsic connectivity. Although Dd appears to be prominent in *Gnathonemus petersii*, no details are available yet. Notably, Dd may be absent in zebrafish (*Danio rerio*; Mueller et al. 2011). Dm borders on Dd and is divided into a rostral (Dm1) and a caudal (Dm2) part in *Apteronotus leptorhynchus*. No subdivisions of Dm have been reported for *Gnathonemus petersii,* where Dm is considerably larger, but modality-specific areas were reported based on physiological investigations (Prechtl et al. 1998). In both species, Dm receives input from the PG (von der Emde and Prechtl 1999; Giassi et al. 2012a). The large core of the pallium is formed by Dc. Dc is the major output region of the dorsal pallium (Fig. 12.11) and receives input from PG in *Apteronotus leptorhynchus* and *Gnathonemus petersii*.

As discussed in Sect. 12.3.2, Dl is considered as the teleost homologue of the hippocampus. Fitting to this view, the Dl of *Apteronotus leptorhynchus* was shown to express FoxO3, a hippocampal marker (Harvey-Girard et al. 2012). A study on *Danio rerio* (Ganz et al. 2014) reported selective expression of Prox1, a marker of the gyrus dentatus in mammals and birds, in the Dld. This has led to the hypothesis that this part of the Dl might be homologous to the gyrus dentatus (Ganz et al. 2014), a view supported by the pattern of neurogenesis and cell migration of interneurons (Grandel et al. 2006). However, the expression of Prox1 could not be confirmed in the Dl of *Apteronotus leptorhynchus.* In this species, it was shown that the Dl connects to the intermediate division of the Dd (Ddi; Fig. 12.11B), a highly recurrent area that projects to the magnocellular division of the Dd (Ddmg) and the subpallium. If the Dl, or parts of Dl, truly resembles the gyrus dentatus, this puts Ddi in a position comparable to area 3 of the cornu ammonis (CA3) field of the hippocampus (see Fig. 12.11B; Elliott et al. 2017). In their interpretation, Elliott et al. (2017) further consider the subpallial GABAergic cells of the entopeduncular

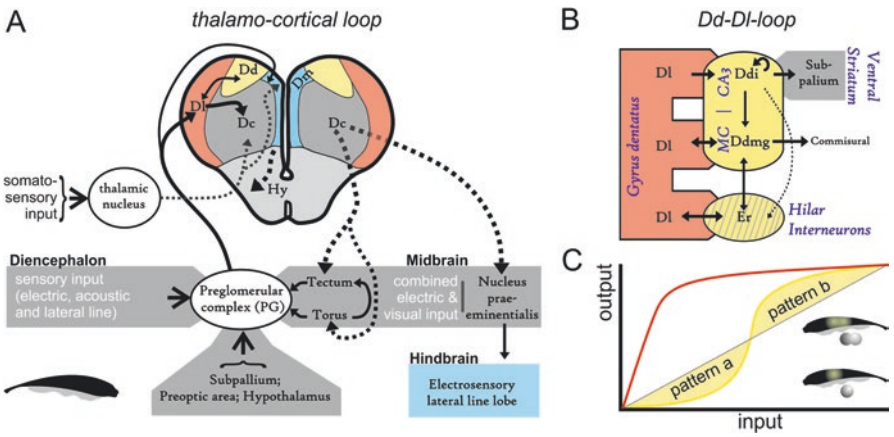

Fig. 12.11 **A:** reduced overview of the afferent and efferent connections of the dorsal telencephalon in weakly electric fish (*Gymnotus* sp. and *Apteronotus leptorhynchus*). The figure focuses on the hippocampus-like network and connections that are relevant in the context of spatial learning. Hy, hypothalamus. With the exception of the somatosensory input through the thalamic nucleus to Dc (Corrêa et al. 1998; Meek and Nieuwenhuys, 1998), the data are based on the work of Giassi et al. (2012a, b) and the references cited therein. **B:** schematic overview of the Dl-Dd network. Presumed functional mammalian homologues are indicated in blue font. Although Dl together with the rostral endopeduncular nucleus (Er) shares several aspects of the gyrus dentatus, the Dd and magnocellular division of the Dd (Ddmg) are akin to the CA3 region. The projection to the intermediate division of Dd (Ddi) is similar to the mossy fiber pathway. Dl is further reciprocally connected with Ddmg and Er. Ddi is strongly recursive and projects to Ddmg. This projection is similar to the back-projection from the CA3 region to the DG, suggesting a functional equivalence of Ddi and the CA3 region. Ddmg is comparable to the mossy cells (MC) of the gyrus dentatus with prominent commissural projections and connects with Er. This is equivalent to DG → hilar interneurons that, in turn, project diffusely to the gyrus dentatus, as does Er to Dl. The colors are used to bind functionally coherent divisions (Dl versus Dd and Er) together. Data based on Elliot et al. (2017). **C:** schematic representation of pattern separation and pattern completion in relation to the connectivity pattern of Dl → Dd → Dl. *Red line*, hypothetical response of neurons performing pattern separation; *yellow line*, hypothetical response of neurons performing pattern completion. Separation is characterized through strong nonlinearity that makes the output of the Dl more dissimilar from the input. Patterns a and b are two very similar electric patterns that a fish may be required to separate. Completion as found in mammalian CA3 neurons would, in turn, result in a unified output for diverging input (*yellow area* below the *dotted unity line*). The network hypothesis in **B** suggests that Dl and Dd perform pattern separation and completion, respectively. Modified from Yassa and Stark (2011)

nucleus (Er; Fig. 12.11B) to be comparable to pallial hilar GABAergic interneurons (the Er, at least functionally, forms a unit with the Dd despite being of subpallial origin; Fig. 12.11B, *gray lines in yellow area*). The Dl together with the Ddi, Ddmg, and Er may form a network comparable to the connections between the dentate gyrus (DG) and the CA3 (DG → CA3 pathway, including the back projection of the CA3 [Ddi] via mossy cells [Ddmg] to the Dl). This is intriguing because it suggests that canonical cortical circuits may exist and hence can be investigated in less complex teleost brains. However, this hypothesis requires more comparative work.

Results obtained in gymnotiform fish offer yet an alternative hypothesis that extends the thalamocortical loop hypothesis (visual input → thalamus → layer IV → layers V/VI → superior colliculus) of Ito and Yamamoto (2009) to include the sensory-recipient pallial areas. Being the major recipient of PG places Dl in similar positon as cortical layer IV (Ito and Yamamoto 2009), whereas PG resembles the thalamus (see Ishikawa et al. 2007). The Dl of *Apteronotus leptorhynchus* has a laminar and recurrent columnar organization, which, while not a defining feature of cortical layer IV, stresses the organizational resemblance of the preglomerular-telencephalic loop with the organization of mammalian sensory cortices (Trinh et al. 2016). The Dc is the major output of the dorsal telencephalon projecting to PG, the deep layer of the teleost optic tectum (homologue of the superior colliculus and a key motor output center of teleosts), and the hypothalamus as well as to other sensory midbrain areas (see Fig. 12.11A; Giassi et al. 2012a). Furthermore, most Dc neurons in *Apteronotus leptorhynchus* are spiny glutamatergic neurons (Giassi et al. 2012c) and express *FoxP2* and *OTX1*. Altogether, this makes the Dc comparable to sensory cortex layers V/VI in mammals (Harvey-Girard et al. 2012).

The reciprocal connectivity between the Dc and the optic tectum has been implicated in establishing the relevance of visually and/or electrically sensed features and the consecutive choice of an accurate behavior (Fig. 12.11; Giassi et al. 2012a). The saliency of behaviorally relevant electrosensory information (e.g., prey) typically is low, leading to the hypothesis that behavioral relevance of such signals should be determined through top-down processes that establish a weighted priority of attention for signals of low saliency. In mammals, this is achieved in the superior colliculus (Fecteau and Munoz 2006). The teleost homologue of the superior colliculus, the optic tectum, represents multimodal and abstracted electrosensory features (Bastian 1982) and is reciprocally connected with Dc.

Efficient learning, including spatial learning, requires storing different items in a manner that separates them well. Accordingly, representations of different items should be uncorrelated, a process considered to be accomplished through pattern separation in the hippocampus. Similarly, retrieving memories must be stable despite incomplete sensory input and therefore should rely on pattern completion. As initially shown by Marr (1969), recurrent networks with sparse representations can perform such tasks. Detailed theoretical work that is largely supported by physiology and anatomy indicates that the strongly recurrent CA3 region is the site where pattern completion is achieved, whereas pattern separation takes place in the gyrus dentatus (for a review, see Rolls 2016).

In the context of spatial learning in electric fish, pattern separation is required in the acquisition of information regarding local electrosensory cues. The ability to memorize and compare spatial electrosensory patterns requires sensory input that is gathered in temporal contiguity to be bound together and separated from information gathered at a different place and time (Sect. 12.2.2). The hypothesis that compares the Dl → Dd with the mammalian DG → CA3 network (Fig. 12.11B) posits that Dl and Dd are the key sites for pattern separation and completion, respectively. This leads to predictions that should be tested in future behavioral and neurophysiological research of these parts of the dorsal pallium of weakly electric fish.

12.4 Chapter Summary

Current research postulates that hippocampal-like circuits of navigation are a shared characteristic of vertebrates. Because hippocampal circuits also form the basis for declarative and episodic memories in mammals (Buzsáki and Moser 2013), understanding the core circuits of spatial navigation in teleosts is likely to reveal core circuits shared by vertebrates in general. However, to date, the neural basis of navigation in teleosts remains ill defined. Future research should provide anatomical, neurophysiological, and behavioral data to generate an evolutionary and holistic perspective on navigation as a specific form of cognition.

The work summarized here shows that weakly electric fish rely on spatial memories in a variety of behaviors and that dedicated neuronal networks of their dorsal telencephalon are involved in their spatial cognition capabilities. Where researchers have started to tackle the neuronal substrate of these networks, striking similarities to cortical and thalamocortical networks of mammals have emerged. Weakly electric fish are of particular interest because a wealth of anatomical, physiological, and behavioral data is available that should ease unraveling to what extent the telencephalon of fish can serve as a blueprint of the intricate cortical networks of cognition in mammals. The near-range sensing strategy of weakly electric fish, the ease at which this behavior can be measured and quantified, and the tight link between their overt electric sampling behavior and spatial attention and learning make them uniquely suited for the comparative study of neural mechanisms mediating navigation.

Compliance with Ethics Requirements Sarah Nicola Jung declares that she has no conflict of interest.

Jacob Engelmann declares that he has no conflict of interest.

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