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Tadpole Bioacoustics: Sound Processing Across Metamorphosis

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Many species of anuran amphibians (frogs and toads) undergo metamorphosis, a developmental process during which external and internal body morphologies transform dramatically as the animal transitions to a new ecosystem (from aquatic to terrestrial) and develops new behavior patterns (from filter-feeding to active pursuit of moving prey; from mostly mute to highly vocal). All sensory systems transform to some extent during metamorphosis, even in those "primitive" anuran species that remain fully aquatic in adult life. In this article, I review what is known about the development of the auditory system in anuran tadpoles. I identify crucial developmental windows for major maturational events in the ear and brainstem that showcase the structural and physiological reorganization of the substrates for hearing airborne sounds as the animal navigates the metamorphic transition. I argue that auditory development is dynamic and nonlinear, and I point out areas for future investigation. Understanding metamorphosis can shed light on how organisms adapt to major environmental challenges.

Keywords: bullfrog, hearing, larva, sound production, tadpole

Of the approximately 7,000 known species of anuran amphibians (frogs and toads), about three quarters undergo metamorphosis, a developmental process during which aquatic free-living limbless larvae (tadpoles) transform into terrestrial or amphibious limbed froglets. Even in those anurans that remain fully aquatic after the completion of metamorphosis, developing tadpoles are subject to dramatic alterations in their body plan, most obviously illustrated by the emergence of limbs and the loss of the tail (Gosner, 1960; Nieuwkoop & Faber, 1994). The central nervous system reorganizes to control these new limbs and to accommodate new inputs from growing sensory organs. In those "advanced" species that become fully or partly terrestrial after metamorphosis, the lateral line system, crucial for the tadpole's ability to orient underwater, degenerates and an ear adapted for processing airborne sounds develops (Fritzsch, Wahnschaffe, & Bartsch, 1988; Simmons & Horowitz, 2007). These morphological and anatomical changes are reflected in and guide changes in behaviors such as locomotion, orientation, feeding, and vocalization (Hoff, Blaustein, McDiarmid, & Altig, 1999). The duration and extent of metamorphosis vary considerably across different anuran species, reflecting the impact of ecological and environmental variables such as habitat, food supply, predation, population density, ambient temperature, and pollutants (McDiarmid & Altig, 1999). Because of this wide diversity, the study of metamorphosis provides a unique window into the biological and ecological factors affecting development and central nervous system plasticity in nonfetal vertebrate animals.

This review focuses on the metamorphic development of the auditory system in anuran tadpoles. The metamorphic transition from aquatic tadpole to amphibious frog impacts heavily the kind and processing of auditory input available to the organism, because of the different acoustic properties of underwater and terrestrial environments (Simmons, 2010). In their shallow water habitats, tadpoles are exposed to both the near-field or particle motion component of sound (low-frequency vibratory disturbances of the milieu, limited to a distance of about one wavelength) and to the far-field component of sound (pressure waves traveling through the air–water interface or through the water at greater distances). These sound sources emanate from predators hunting underwater (fish, turtles, adult frogs) and from the air (bats, birds, humans). Not only the movements but also the vocalizations of these predators propagate underwater (bullfrogs: Boatright-Horowitz, Cheney, & Simmons, 1999; túngara frog, *Physalaemus pustulosus:* Halfwerk, Jones, Taylor, Ryan, & Page, 2014) and would be available for detection by tadpoles. Moreover, anthropogenic noise such as machinery is present in many tadpole habitats. Detection of these sources would be biologically advantageous. After the completion of metamorphosis, the froglet is now exposed to airborne in addition to the underwater sounds experienced by tadpoles. To prepare for this new soundscape, the functional maturation of the substrates for hearing airborne sounds should occur prior to the final emergence to land.

I begin this review with a discussion of the morphological development of the peripheral (inner and middle ears, auditory/vestibular nerve) and then central auditory system; developmental

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changes limited to the vestibular system are not covered. I then turn to functional changes in the auditory system and in auditory behaviors over development and into the metamorphic transition to early postmetamorphic life. Most species of tadpoles do not vocalize, and adult frogs gain the ability to emit species-specific advertisement calls at sexual maturity. The limited information available on the development of vocal production abilities in tadpoles and postmetamorphic froglets is briefly discussed. I conclude with a summary of what is known about metamorphic changes in the operation of the lateral line system, a sensory system whose modifications over metamorphosis impact brain space for processing of sounds.

Even though approximately 5,000 species of metamorphosing anurans have been identified, experimental work on auditory, lateral line, or vocal development has been limited to only a few. Anatomical, neurophysiological, and behavioral data are available for the American bullfrog (*Rana catesbeiana*), an "advanced" species that undergoes extensive metamorphosis from a totally aquatic larva to a partly terrestrial, amphibious frog. Anatomical and behavioral data are available for the African clawed frog (*Xenopus laevis*), a "primitive" pipid frog that remains fully aquatic after the completion of metamorphosis. Data from other advanced (some ranids, hylids, bufonids, and leptodactylids) and primitive species are more limited but are included as available.

A Note on Staging

The sequence of developmental events over metamorphosis is described by standardized staging tables based on changes in gross cellular configurations (in embryos) and in body morphology (in postembryonic larvae). One caveat for any staging system is that changes in external morphology may not correspond precisely to changes in internal anatomy or in function. Thus, for ease, I will classify tadpoles into broad categories of hatchlings, early larval, late larval, and metamorphic climax stages (see Figure 1), based on the Gosner (1960) staging system. The Gosner system is the most widely used to classify developmental changes in advanced species. The Nieuwkoop-Faber (NF) system (Nieuwkoop & Faber, 1994) is the standard system used for staging development of *Xenopus* and other pipid frogs. McDiarmid and Altig (1999) list approximations between these two staging systems and others that are less commonly used.

The Gosner system extends from Stage 1 (fertilization) to Stage 46 (the end of metamorphic climax), while the NF system extends from Stage 1 (fertilization) to Stage 66 (the end of metamorphic climax). Hatchling stages (Gosner Stages 20–25; NF Stages 32–46) are the time of transition from an immobile embryo (Stages 1–19 or 1–31, respectively) to an active, limbless, and feeding larva in which external gills atrophy and the oral disk forms. During early larval stages (Gosner Stages 26–30; NF Stages 46–52), hindlimb buds begin to differentiate and grow, and lungs develop. During late larval stages (Gosner Stages 31–41, NF Stages 53–58), hindlimbs develop and toes differentiate. In metamorphic climax (Gosner Stages 42–46; NF Stages 58–66), forelimbs emerge and differentiate, the tail resorbs, adult jaws and tongue begin to form as the narrow oral disk transforms into the wide frog mouth, the skull ossifies, and the eyes migrate more dorsally and medially on the head (McDiarmid & Altig, 1999). One goal of this review is to add to these stage groupings information about the development of the sense of hearing. As will be discussed below, in bullfrog tadpoles, the end of the late larval period (Gosner Stages 38–41) is a particularly crucial time for auditory system development. After the end of metamorphic climax, postmetamorphic frogs are classified as froglets (see Figure 1) or juveniles, subadults, and adults, based on measures such as days since metamorphosis, width of the external tympanum, and body size (Boatright-Horowitz & Simmons, 1995). An interesting developmental trend during early postmetamorphic life is when sex differences in auditory morphology and behavior first appear.

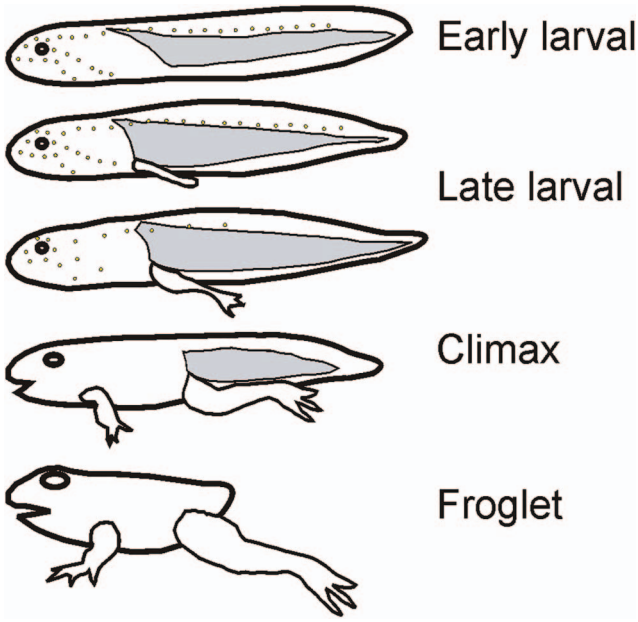


Figure 1. Schematics of stages of metamorphic development of bullfrog tadpoles from early larval stages to early postmetamorphic (froglet) life. Drawings are not to scale. Stage groups are identified according to McDiarmid and Altig (1999), as based on Gosner (1960) stages. Hatchlings, the transition from an immobile embryo to a free-swimming tadpole, are not illustrated. During the early larval tadpoles (Gosner Stages 25–30), hindlimb buds begin to form. The late larval period (Goner Stages 31–41) features progressive growth and differentiation of the hindlimbs and toes. The small dots along the head and side of the body in early larval and late larval stages show the approximate position of the lateral line neuromasts. Metamorphic climax (Gosner Stages 42–46) features development of forelimbs and toes, appearance of the wide frog mouth, and resorption of the tail. Froglet stages are those immediately subsequent to metamorphic climax, when the animal finally transitions to life on land. See the online article for the color version of this figure.

Development of the Inner and Middle Ears

Inner Ear

Anuran amphibians are unique among vertebrates in having three distinct auditory organs in their inner ear—the saccule, the amphibian papilla, and the basilar papilla. None of these organs contains a basilar membrane, and each is innervated by separate

branches of the auditory/vestibular (eighth cranial, nVIII) nerve (Simmons, Meenderink, & Vassilakis, 2007). The saccule is an otolith organ that in adults detects low-frequency (<300 Hz) sounds and vibrations. It is considered a mixed auditory/vestibular organ. The amphibian and basilar papillae both contain a flexible tectorial membrane overlying the hair cells, which are anchored on a nonflexible cartilaginous base. In adults, the amphibian papilla is tuned to sounds within the frequency range from about 100 to 1,800 Hz while the basilar papilla is tuned to higher frequencies within the species' vocal repertoire and thus operates as a matched filter (Gerhardt & Schwartz, 2001).

All inner ear organs (including the vestibular organs—the semicircular canals, the lagena, and the utricle) lie within the otic capsule. The tadpole's otic capsule becomes larger, thicker, and increasingly chrondrified over larval development (the spring peeper, *Pseudacris crucifer:* Hetherington, 1987; bullfrogs: Horowitz, Chapman, Kaya, & Simmons, 2001; Figure 2), but it does not develop the hard bony structure of the mammalian bulla. In several species of ranids, hylids, and bufonids, the oval window is already identifiable in the otic capsule by the end of hatchling and the beginning of early larval stages, but it is surrounded only by connective tissue (Hetherington, 1987, 1988; Horowitz et al., 2001). In bullfrog tadpoles, the oval window gradually increases in diameter from hatchling until metamorphic climax stages but at a rate slower than that of the otic capsule itself (Horowitz et al., 2001).

In all species of larval anurans studied to date, the inner ear organs develop prior to the middle ear. In this respect, the development of the ear can be described as following an inside-out or medial-to-lateral trajectory (Womack, Stynoski, Voyles, Coloma, & Hoke, 2018; Figure 3). The semicircular canals, which mediate balance, are the first organs to differentiate. In several species, including the bullfrog (Horowitz et al., 2001; Simmons & Alexander, 2014), the European common frog (*Rana temporaria;* Hertwig, 1987), and the African clawed frog (Bever, Jean, & Fekete, 2003; Paterson, 1949; Quick & Serrano, 2005), development of the auditory organs commences during hatchling stages with the formation of the saccule, followed in the early larval period by the separation of the lagena from the saccule (see Figure 2) and the differentiation of the amphibian and basilar papillae. All inner ear organs expand in volume and in numbers of hair cells throughout larval development but at different rates (bullfrogs: Li & Lewis, 1974; Simmons & Alexander, 2014; African clawed frogs: Díaz, Varela-Ramírez, & Serrano, 1995; Quick & Serrano, 2005). Bassó et al. (2016) showed that, in larvae of 13 neotropical anurans, the mass of the saccular otolith varies with species ecology. Nektonic species (those such as bullfrogs that live within the water column) have otoliths with larger masses than those of benthic species. Because larger mass is associated with better low-frequency hearing, this suggests that nektonic tadpoles have better sensitivity to low sound frequencies. Smirnov (1993) observed that in advanced species (some ranids, hylids, and bufonids), the amphibian papilla reaches its adult form prior to the end of metamorphic climax, while in pipids and other primitive species, the amphibian papilla continues to differentiate into the early postmetamorphic period. This again suggests species differences in hearing sensitivity. Past metamorphosis, numbers of hair cells in the saccule continue to increase concomitant with growth in body size (bullfrogs: Lewis & Li, 1973; cane toads [*Bufo marinus*]: Corwin, 1985), although at a slower rate than seen prior to metamorphosis (African clawed frogs: Díaz et al., 1995).

Middle Ear

Adult bullfrogs, like other terrestrial anurans, detect sounds through at least two separate middle ear transduction pathways (Mason, 2007). The opercularis pathway conveys sounds to the inner ear through the operculum, a cartilaginous structure located

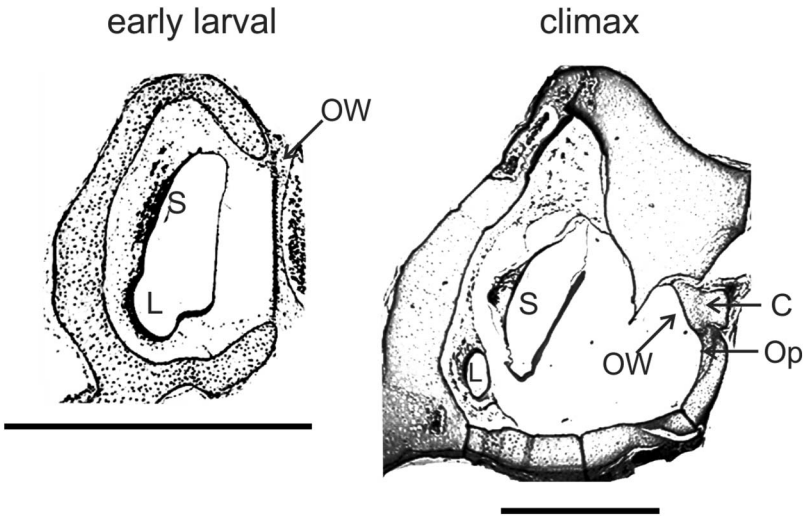


Figure 2. Horizontal images of the otic capsule of developing bullfrog tadpoles, taken at approximately the same rostral/caudal location through the head. In both images, lateral is to the right, medial is to the left, anterior is to the top, and posterior is to the bottom. Scale bar is 1,000 μm. Right image shows the otic capsule of an early larval (Stage 26) tadpole. Left image shows the otic capsule of a tadpole in metamorphic climax (Stage 44). Surrounding connective tissue has been digitally removed from both images. C = columella; L = lagena; Op = operculum; OW = oval window; S = saccule.

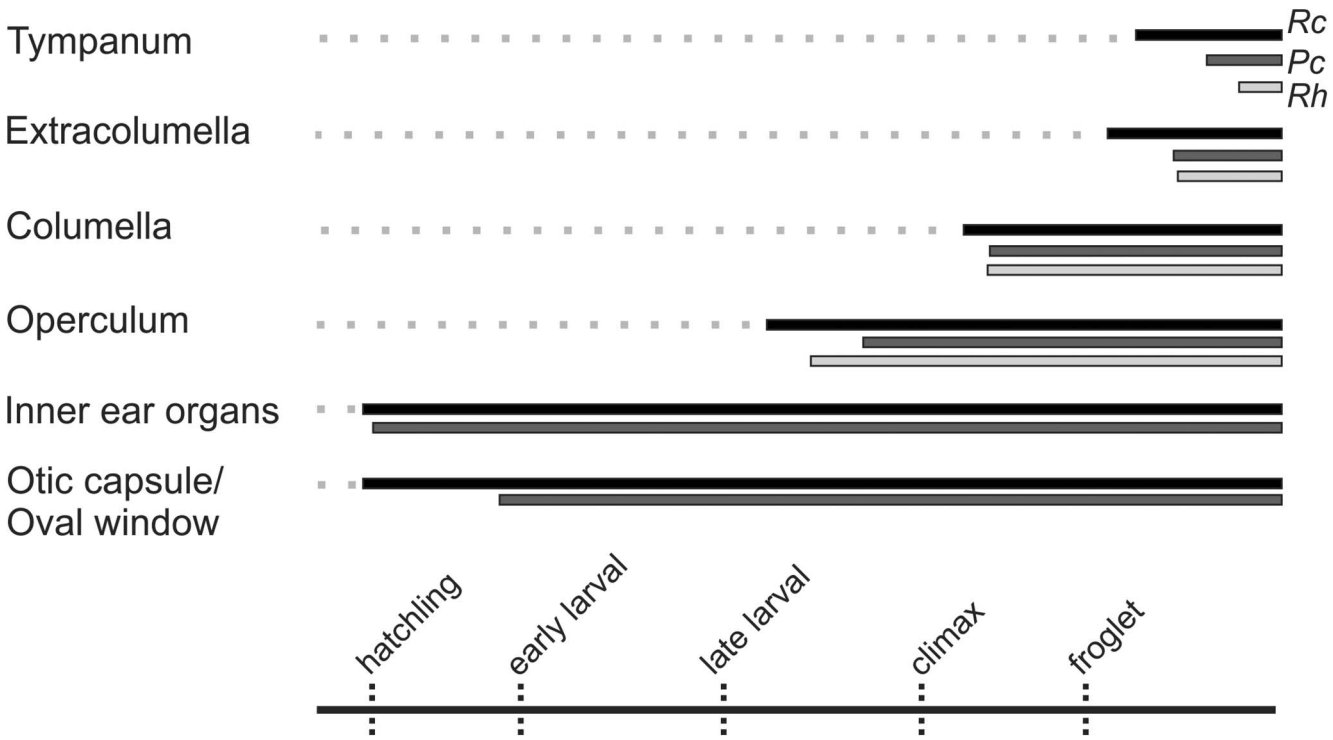


Figure 3. Summary timeline of the development of the ear of three species of terrestrial anurans. The lines depict the earliest appearance of the indicated structure for (in order, top to bottom for each structure) the bullfrog (*Rana catesbeiana,* Rc), the spring peeper (*Pseudacris crucifer,* Pc), and the giant toad (*Rhinella horribilis,* Rh). Data on otic capsule/oval window development and development of inner ear organs are not available for the giant toad. The solid line at the bottom indicates the progression of developmental stages from hatchlings (Gosner Stages 20–25) to early larval (Stages 26–30), late larval (Stages 31–41), and metamorphic climax (Stages 42–46) and then early froglet development. Data are replotted from Hetherington (1987), Horowitz et al. (2001), Simmons and Alexander (2014), and Womack et al. (2018).

at the caudal end of the oval window, and the opercularis muscle, which connects the operculum to the shoulder girdle. This pathway is particularly effective in transmitting low-frequency substrate vibrations to the saccule and the amphibian papilla. The tympanic pathway conveys sounds to the oval window in the inner ear through the external tympanum, the extracolumella (extrastapes), and the columella (stapes). Sounds entering the inner ear through this pathway will stimulate all three auditory organs. In addition, adult terrestrial frogs can detect airborne sounds via a transmission pathway from the lungs to the middle ear cavity (Mason, 2007).

Anatomical data from several advanced species concur that the opercularis pathway develops prior to the tympanic pathway (Figure 3; Hetherington, 1987, 1988; Horowitz et al., 2001; Womack, Christensen-Dalsgaard, & Hoke, 2016; Womack et al., 2018). The operculum begins to form in early larval stages, first appearing at the caudal end of the oval window. By the end of the late larval period (Gosner Stages 38–41), the operculum has spread rostrally, partially or even completely covering the oval window, and the opercularis muscle forms. Opercularis muscle fibers insert into the lateral surface of the operculum in the early stages of metamorphic climax, around the time of initial emergence of forelimbs (Hetherington, 1988). These morphological changes predict that the opercularis pathway will be functional during metamorphic climax stages but not before.

The tympanic pathway, in contrast, does not begin to form until metamorphic climax (see Figure 3) and is not functional until sometime in froglet life. In bullfrogs, both the columella and the extracolumella begin to differentiate during metamorphic climax (Horowitz et al., 2001). As the columella forms, it halts the rostral spread of the operculum over the oval window as it inserts into the more rostral area of the oval window (Hetherington, 1987, 1988). In other species of ranids, hylids, and bufonids, the extracolumella does not differentiate until later, after the completion of metamorphosis (Hetherington, 1987; Vorobyeva & Smirnov, 1987; Womack et al., 2016, 2018). The external tympanum develops still later, after the completion of climax but again with a time course that varies considerably across species (see Figure 3). Species differences in tympanic pathway development are related to body size—larger species develop an external tympanum sooner than smaller species, although both large and small animals show delayed tympanic pathway development relative to the development of the opercularis pathway. The external tympanum of developing bullfrogs is first visible on the side of the head approximately 24 hr after the completion of metamorphic climax (Boatright-Horowitz & Simmons, 1995), while in the smaller spring peepers, it is not visible until 60 days postmetamorphosis (Hetherington, 1987). In bullfrogs, the external tympanum continues to increase in diameter during postmetamorphic life, concom-

itant with increases in body and head size. Sex differences in tympanum width between male and female bullfrogs first appear at the end of the froglet period (snout-vent length of about 5.5 cm). After this point, the male's tympanum grows at a faster rate than that of the female's, even when animals are matched for body size (Boatright-Horowitz & Simmons, 1995). It is not known if this pattern of sex differences is similar in other advanced species.

Neither larval nor adult African clawed frogs have an external tympanum; instead, there is a cartilaginous tympanic disk that lies below the skin and fatty tissue of the head and in which is embedded an ossified extracolumella. The extracolumella and the tympanic disk begin to form during metamorphic climax stages, while the columella begins to form earlier, during the later stages of the late larval period (Nieuwkoop & Faber, 1994; Paterson, 1949). An opercularis pathway has not been identified in fully aquatic frogs (Mason, 2007). But because the inner ear organs differentiate prior to the middle ear, the developmental sequence in African clawed frogs is similar to the inside-out sequence identified in terrestrial and amphibious frogs.

Given the progressive development of the middle ear transduction pathways, how would sounds stimulate the inner ear organs during larval development? The opercularis pathway is mature during metamorphic climax and so could subserve neural responses to sound during this developmental period. Underwater sounds would produce movements of the operculum that could then stimulate the inner ear organs (Hetherington, 1987). The tympanic pathway is not fully differentiated until sometime in postmetamorphic life, when the external tympanum appears on the side of the head. From this point, both the opercularis and the tympanic pathways are operable, with the opercularis responsible for transmitting both seismic vibrations from the ground and particle motion from the water, and the tympanic pathway responsible for transmitting pressure stimulation to the inner ear. But the question remains, do early and late larval tadpoles without a functioning opercularis pathway detect sounds? Hetherington (1987, 1988) proposed that these young tadpoles could detect sounds through what he termed a "fenestral pathway," through which particle motion would be transmitted directly and with low attenuation through the body to the oval window and then to the hair cells in the inner ear. This pathway would be most efficient in stimulating the otolith organs, because these organs have greater mass than either the amphibian or the basilar papilla. If a fenestral pathway does operate in tadpoles, then this would be reflected in neural activation to sounds in the brains of tadpoles in early and late larval stages. Experiments testing this hypothesis are described below in the Functional Development section.

Development of Ascending Auditory Pathways

The frog's brain as a whole alters considerably in size and organization over metamorphosis (Horowitz & Simmons, 2007), reflecting developmental changes in both external (emergence of limbs, degeneration of the lateral line sensory organs in terrestrial frogs) and internal (progressive development of the inner and middle ears) morphology. These dynamic alterations in brain structure are manifested in changes in the size and spatial position of auditory nuclei, in the neurochemical organization of these nuclei, and in modifications of connectivity between these nuclei.

Eighth Cranial Nerve

Very little is known about the morphological development of nVIII in tadpoles. In bullfrog tadpoles, nVIII fibers can be seen extending from the otic capsule into the medulla in hatchling stages (Horowitz et al., 2001), but changes in fiber size or diameter over larval growth were not quantified in that study. In African clawed frogs, the numbers of myelinated nVIII fibers increase between early larval and early postmetamorphic stages but are comparable in froglets and in adults (López-Anaya, López-Maldonado, & Serrano, 1997). This suggests that larval development is a time of considerable growth of nVIII. Changes in fiber size follow a different trajectory, continuing to increase between early larval stages and adult frogs. This latter result suggests that conduction velocity is faster in adults than in tadpoles (López-Anaya et al., 1997), which in turn implies that transmission of auditory signals is temporally more precise in adults than in tadpoles. To date, however, there are no physiological data supporting this interpretation.

Medulla

In adult anurans, several nuclei in the dorsal medulla process sounds and vibrations. The dorsal medullary nucleus (DMN; sometimes termed the *dorsolateral nucleus*) receives input from the amphibian papilla, the basilar papilla, and the saccule. The saccule also projects to the lateral vestibular nucleus (LVN) and the medial vestibular nucleus (MVN), highlighting its operation as a mixed auditory/vestibular organ (Wilczynski & Endepols, 2007). Medullary termination areas of the inner ear organs have been described in bullfrog tadpoles (Horowitz, Tanyu, & Simmons, 2007; Jacoby & Rubinson, 1983), and those of the vestibular organs have been described in green frogs (*Rana clamitans:* Straka, Baker, & Gilland, 2001).

In hatchling bullfrog tadpoles, nVIII can be seen to project to a cluster of small, tightly packed cells located very laterally in the medulla, in an area identified as the developing DMN (Figure 4, left column, top). Throughout early and late larval development, this cell mass expands and is displaced medially as the brainstem itself expands and changes in shape (Kumaresan, Kang, & Simmons, 1998). By metamorphic climax, the DMN is located in a more medial and dorsal position in the medulla (Figure 4, left; Fritzsch, Nikundiwe, & Will, 1984; Jacoby & Rubinson, 1983), similar to its position in postmetamorphic froglets and adults (Wilczynski & Endepols, 2007). Numbers of cells in the DMN increase from hatchling stages up through metamorphic climax, then remain relatively stable through the first 90 days of postmetamorphic life (Figure 5, top). Contributing to these developmental changes in the DMN are migration of existing cells from lateral to medial, birth of new cells in the more medial position, and death of cells in the more lateral position (Chapman, Weinstein, & Simmons, 2006; Fritzsch et al., 1984). It is not known when during development nVIII projections to the DMN from the saccule, amphibian papilla, and basilar papilla first exhibit the tonotopic projection patterns seen in adult frogs (Wilczynski & Endepols, 2007).

Along with the expansion and change in the spatial position of the DMN, another dynamic reorganization of the dorsal medulla during larval life is seen in the degeneration of the lateral line nucleus (LLn) and its surrounding neuropil (LLnp; Fritzsch et al., 1988). The LLn receives input from series of

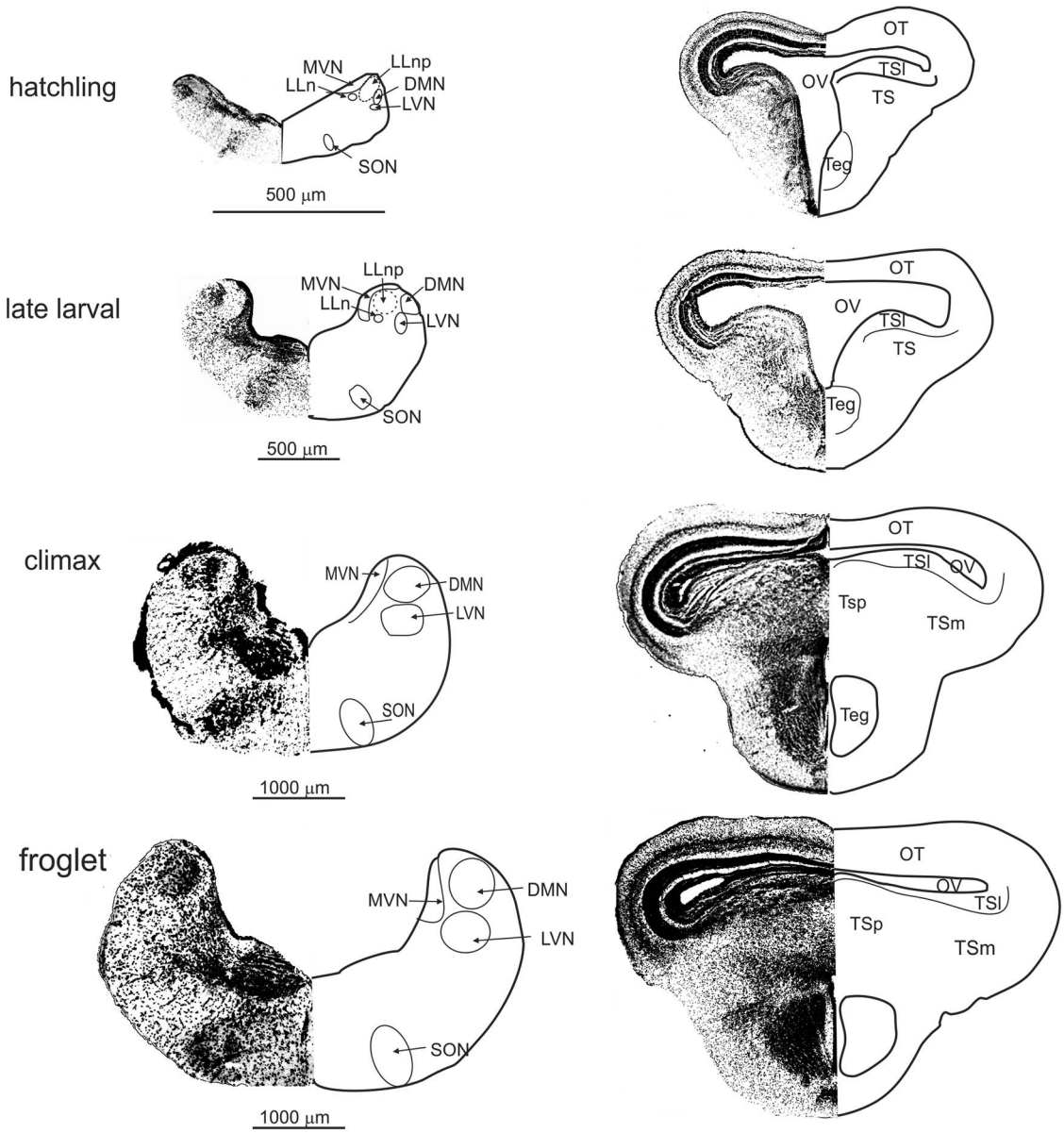
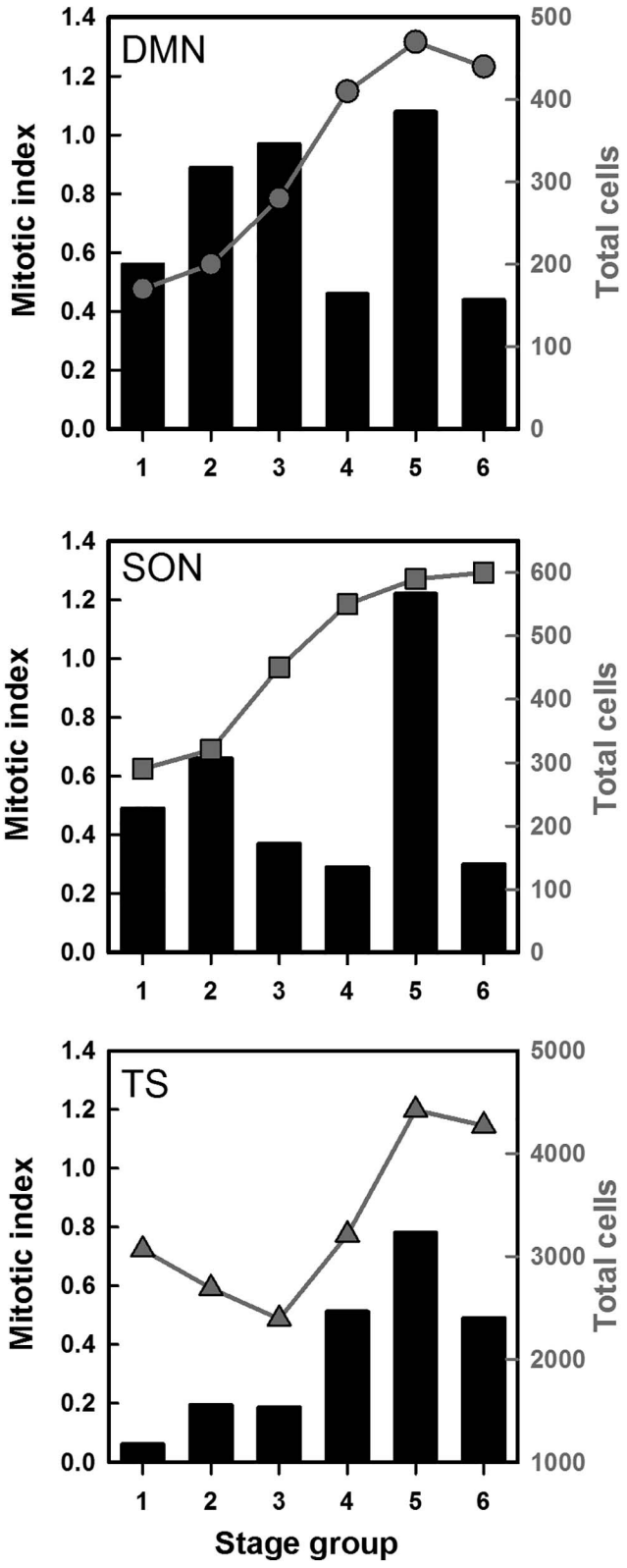


Figure 4. Schematics of sections through the medulla (left) and midbrain (right) of representative bullfrog tadpoles at four stages of development (hatchling, late larval, metamorphic climax, and froglet, from top to bottom). The left-hand side of each section shows cell bodies as visualized by a cresyl violet stain and the right-hand side shows corresponding labels of key nuclei. Sections are taken at a comparable rostral/caudal location in the brain and were chosen to illustrate the largest size of the DMN and TS, respectively. Hatchling sections are from a Gosner Stage 25 tadpole. Late larval sections are from Gosner Stage 36 (medulla) and Gosner Stage 40 (midbrain) tadpoles. Climax sections are from Gosner Stage 44 (medulla) and Gosner Stage 43 (midbrain) tadpoles. Scale bars as marked. Abbreviations for medulla sections: DMN = dorsal medullary nucleus; LLn = lateral line nucleus; LLnp = lateral line neuropil; LVN = lateral vestibular nucleus; MVN = medial vestibular nucleus; SON = superior olivary nucleus. Abbreviations for midbrain sections: OT = optic tectum; OV = optic ventricle; Teg = tegmentum; TSc = magnocellular nucleus of the torus semicircularis; TSl = laminar nucleus of the torus semicircularis; TSp = principal nucleus of the torus semicircularis; TS = torus semicircularis. Sections are derived from archival data.

sensors called neuromasts arranged along the sides of the tadpole's body. These neuromasts contain hair cells that respond to particle motion induced by water flow. In hatchlings and early stage tadpoles, the LLn is located medially and dorsally in the medulla, close to the area of the MVN (Figure 4, left column, top; Fritzsch et al., 1984; Jacoby & Rubinson, 1983). Up to the middle of the late larval period, separate inputs can be traced from the nVIII and from the lateral line nerve to the DMN and



LLn/LLnp, respectively (in nine advanced and one primitive species: Fritzsch et al., 1984; in bullfrogs: Horowitz et al., 2007). These separate projections indicate that the brains of tadpoles in these early developmental stages can process distinct auditory and lateral line cues. In bullfrogs and other advanced species, the LLn, LLnp, and the peripheral neuromasts all begin to degenerate by the end of the late larval period, and afferents from the inner ear organs begin to spread more medially and dorsally in the medulla, in effect taking over the now-empty brain space (Figure 4, left; Fritzsch et al., 1984; Horowitz, Chapman, & Simmons, 2007). Because the neuromasts do not degenerate in African clawed frogs and other pipids, the DMN and LLn in these species remain anatomically discrete and continue to receive different patterns of input throughout metamorphosis and into postmetamorphic life (Will, Luhede, & Görner, 1985).

The superior olivary nucleus (SON) in the ventral medulla is the first site of binaural convergence in the vertebrate brain. It is visible in the brains of hatchling bullfrog tadpoles in approximately the same spatial location as in metamorphic climax tadpoles (Figure 4, left column; Templin & Simmons, 2005) and adult frogs (Wilczynski & Endepols, 2007). The SON increases in cell number and cell density up through metamorphic climax and then remains stable through the first 90 days of the froglet period (Figure 5, middle; Chapman et al., 2006; Templin & Simmons, 2005). These increases in SON cell number prior to the final transition to a terrestrial existence may provide a substrate for maturation of the central substrate for binaural localization of airborne sounds.

Auditory Midbrain

The anuran auditory midbrain, the torus semicircularis (TS), is homologous to the mammalian inferior colliculus (Wilczynski & Endepols, 2007). In developing bullfrogs, the adult organization of the TS, particularly the identification of separate principal (medial) and magnocellular (lateral) nuclei, does not become apparent until metamorphic climax. In contrast, the laminar nucleus can be identified even in hatchlings (Figure 4, right column, top; Kumaresan et al., 1998). Developmental changes in TS organization are first illustrated by the anatomical continuity of the optic ventricle with the cerebral aqueduct. This anatomy is seen in hatchlings up to the end of the late larval period, at which time the optic ventricle and the cerebral aqueduct begin to be separated by a growing cell

Figure 5. Patterns of cell birth in the dorsal medullary nucleus (DMN), superior olivary nucleus (SON), and torus semicircularis (TS) across metamorphic development in bullfrog tadpoles, as determined from 5-bromo-2′-deoxyuridine (BrdU) expression. Data in each graph are plotted as mitotic index (left *y*-axis, bars) of BrDU-labeled cells; the right *y*-axis of each graph plots total numbers of cells (symbols and lines) determined from cresyl violet staining. Stage groups are as follows: 1 = hatchling; 2 = early larval; 3 = late larval (Gosner Stages 31–37); 4 = late larval (Gosner Stages 38–41); 5 = metamorphic climax; and 6 = froglets (up to 90 days postmetamorphosis). Data are replotted from Chapman et al. (2006) and Simmons et al. (2006). All three brain nuclei show a pronounced increase in mitotic index during metamorphic climax.

mass that will form the medial portions of the principal nucleus (Figure 4, right column). Numbers of cells in the TS peak during climax, as the ventricles close (Figure 5, bottom).

Cell Birth and Differentiation

Developmental changes in cell numbers in the brainstem of bullfrog tadpoles have been examined using the cell birth marker 5-bromo-2′-deoxyuridine (BrdU) and the expression of acetylcholinesterase (AChE; Chapman et al., 2006; Kumaresan et al., 1998; Simmons, Chapman, & Brown, 2006). Results of BrdU labeling experiments show that the DMN, SON, and TS all continue to add new cells throughout larval development, with a peak during metamorphic climax (see Figure 5). In these experiments, cell birth was quantified by the mitotic index, the numbers of BrdU-labeled cells as a proportion of the total numbers of cells available for labeling. In the DMN (Figure 5, top), the mitotic index increases from hatchling stages throughout the early stages of the late larval period (Gosner Stages 31–37). It decreases at the end of the late larval period (Gosner Stages 38–41) only to increase again during metamorphic climax. Mitotic index in the SON (Figure 5, middle) rises from hatching to early larval stages but then declines during the late larval period, before again increasing dramatically in metamorphic climax (Chapman et al., 2006). Mitotic index in the TS (Figure 5, bottom) is low up until Gosner Stages 38–41 and peaks during metamorphic climax (Simmons et al., 2006). These data show that metamorphic climax features a dramatic upregulation of mitosis in all three auditory brainstem nuclei, coincident in time with the maturation of the opercularis pathway and the initial differentiation of the tympanic transduction pathway.

The time course of differentiation of these newly born cells can be visualized by patterns of expression of AChE, the enzyme responsible for catalyzing the neurotransmitter acetylcholine. During early vertebrate development, AChE expression can be observed in brain areas undergoing cell differentiation, even if these areas do not serve a cholinergic function in adults (Massoulié, Pezzementi, Bon, Krejci, & Vallette, 1993). AChE expression also serves as a reliable histochemical marker for confirming nuclear borders (Puelles, Robles, Martínez-de-la-Torre, & Martínez, 1994). In developing bullfrogs, AChE expression in the DMN and SON is low in hatchlings and rises slowly throughout late larval and metamorphic climax stages. Expression peaks during the froglet period, temporally coincident with the final growth and maturation of the tympanic transduction pathway, and then remains stable in adults (Kumaresan et al., 1998). AChE expression in the TS increases from early to late larval stages, but in metamorphic climax, it begins to exhibit medial/lateral and dorsal/ventral gradations, which then continue throughout the froglet period and into adult life (Kumaresan et al., 1998). These results suggest that the neurochemical events underlying the structural and functional divisions of the adult TS (Wilczynski & Endepols, 2007) first emerge during metamorphic climax, prior to the transition to amphibious life.

In contrast to the time course of AChE expression in the DMN, that in the LVN reaches adult levels earlier, during the late larval period. Along with the earlier development of the semicircular canals (Simmons & Alexander, 2014), these data suggest that functional processing of vestibular and auditory cues undergoes different time courses of maturation. This may be related to the importance of vestibular compared to auditory sensation for developing tadpoles. One important vestibular function is the maintenance of balance as the tadpoles travel through the water column. Bullfrog tadpoles breathe using both internal gills and lungs. For adequate ventilation, particularly in hypoxic water, they swim to the surface to breathe air (Crowder, Nie, & Ultsch, 1998). These vertical movements would impose considerable demands on their ability to maintain balance. It is not known, however, if AChE expression patterns differ in LVN and DMN cells receiving projections from the saccule.

Gamma-aminobutyric acid (GABA) plays an important role in early brain development, separate from its role as an inhibitory neurotransmitter in the adult (Cherubini, Gaiarsa, & Ben-Ari, 1991). Its expression may signal ongoing processes of cell proliferation, migration, differentiation, and circuit building (Wu & Sun, 2015). In bullfrog tadpoles, GABA expression in the DMN, SON, and TS increases over larval development, with adult-like patterns of expression first emerging during metamorphic climax (Simmons & Chapman, 2002). This is coincident in time with the maturation of the opercularis transduction pathway and prior to that of the tympanic transduction pathway. Again, these results point to metamorphic climax as a critical developmental time for auditory system maturation.

Anatomical Connectivity Between Medulla and Midbrain

Neuroanatomical tracing experiments in developing bullfrogs show that some medullary-TS projections remain stable across metamorphic development, others undergo considerable modification, and new ones emerge (Boatright-Horowitz & Simmons, 1997; Horowitz et al., 2007). Projections between the DMN and the SON, as well as between the DMN and the TS, are observed throughout larval development as well as in froglets and adults (Simmons & Horowitz, 2007; Wilczynski & Endepols, 2007). In contrast, projections between the SON and the TS are not stable across development. The ipsilateral SON-TS pathway can be identified in early larval stages as well as in metamorphic climax. Remarkably, during the end of the late larval period (Gosner Stages 38–41), this pathway seems to disappear (Figure 6; Horowitz et al., 2007). Experiments based on reciprocal injections of several different tracers (horseradish peroxidase, *Phaseolus vulgaris* leucoagglutinin, cholera toxin B, 1,1′-dioctadecyl-5,5′-diphenyl-3,3,3′,3′-tetramethylindocarbocyanine chloride) show greatly reduced anterograde and retrograde label in the SON and TS specific to this time period (Horowitz et al., 2007). Moreover, this apparent loss of connectivity is unique to this pathway; DMN-SON and DMN-TS projections can still be traced using the same techniques during these developmental stages. These data indicate that the failure of SON-TS transport is not the result of technical error. Not only does the ipsilateral SON-TS pathway reemerge during metamorphic climax (see Figure 6), but also two new pathways can now be readily identified—one between the SON and the contralateral TS, and the other between the LVN and the ipsilateral SON (Horowitz et al., 2007). Both of these pathways are present in adult frogs (Wilczynski & Endepols, 2007).

The transient period of disconnection between the SON and the ipsilateral TS occurs within the same developmental time span—Gosner Stages 38–41—as the transient blockade of the oval win-

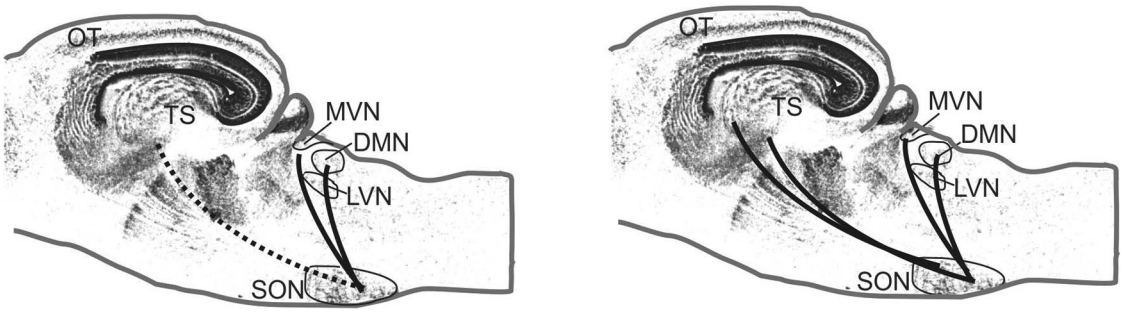


Figure 6. Schematics of sagittal sections through the brains of bullfrog tadpoles at Gosner Stage 39 (left) and Gosner Stage 45 (right). Abbreviations are as in Figure 4. The solid black lines illustrate the course of transport of tracers from injection sites in the SON to the dorsal medulla. Transport to the MVN and DMN is obvious at both developmental stages. At Gosner Stage 39, there is no or reduced transport to the TS from the ipsilateral SON, as shown by the dotted line; however, at Gosner Stage 45, clear transport to the TS is seen. Data from early larval stages are similar to those at Gosner Stage 45 (Horowitz et al., 2007).

dow by the formation of the operculum cartilage in the inner ear (Hetherington, 1987). On the basis of these convergent anatomical data, Boatright-Horowitz and Simmons (1997) labeled these developmental stages as a "deaf period." Functional data supporting this designation are presented in the next section.

Functional Development

Here, I describe our current understanding of the time course of functional maturation of the ascending auditory pathway from nVIII to the TS, based on experiments with bullfrog tadpoles. To date, there is no comparable work on other species of larval anuran.

Eighth Cranial Nerve

Responses of nVIII fibers to sounds have not been recorded from tadpoles of any species; instead, the only information available about functional properties of the auditory periphery come from studies of postmetamorphic bullfrogs. The best excitatory frequencies, that is, the tone frequency to which fibers respond best, extend up to higher frequencies (2,500 Hz) in froglets compared to adults (1,700 Hz; Shofner & Feng, 1981). These differences are consistent with differences in body size (27–46 mm in froglets; 152–178 mm in adults) and in the size of the external tympanum (smaller in froglets and so vibrates better at high frequencies; Boatright-Horowitz & Simmons, 1995). Note that these comparisons are for airborne sound; it is not known if nVIII responses to underwater particle motion also change in best excitatory frequency over postmetamorphic development.

Medulla

Underwater particle motion drives neural responses in the MVN and LVN of bullfrog tadpoles (Figure 7, top; Horowitz et al., 2007; Simmons & Flores, 2012). In these experiments, low-frequency (30–250 Hz) particle motion was produced by *z*-axis (vertical) stimulation of the water in which the anesthetized tadpoles were immersed by means of a specialized "shaker table." Neurons in both the MVN and LVN responded robustly and stably to *z*-axis stimulation; most sensitive frequency, the sound frequency evoking the best response at the lowest sound level (Figure 7, left top) and threshold at most sensitive frequency (Figure 7, right top) do not vary significantly between late larval and metamorphic climax stages, although there are more sites with high thresholds in late larval stages. Because in tadpoles the saccule, but not the amphibian papilla or basilar papilla, projects to both the MVN and LVN (Horowitz et al., 2007), these data suggest that stimulation of the saccule drives these medullary responses. In contrast, there is limited responsiveness in the DMN to *z*-axis stimulation, with measurable activity present in only 4 out of 53 tested tadpoles (Simmons & Flores, 2012). This limited responsiveness is expected given that the DMN receives projections from the amphibian and basilar papillae as well as from the saccule. There are no data in bullfrogs or in any other tadpole species that examine responses to *z*-axis stimulation in the SON; there are no data on sensitivity to *x*- or *y*-axis stimulation in any medullary nucleus.

Auditory Midbrain

Particle motion also drives neural activity in the bullfrog tadpole's TS but less robustly during metamorphic climax (Figure 7, middle; Simmons, 2015). Similar to the results seen in the MVN and LVN, most sensitive frequencies do not differ significantly between early, late, and metamorphic climax stages (Figure 7, middle left). Thresholds at most sensitive frequency (Figure 7, middle right) remain relatively stable from early larval stages through most of the late larval period; however, beginning around Gosner Stage 40, thresholds begin to increase, and many fewer TS sites responsive to particle motion can be identified (Figure 7, middle right). This is in contrast to the results observed in the MVN and LVN, where sensitivity to *z*-axis stimulation remains relatively consistent up through the end of metamorphic climax (Simmons, 2015).

Developmental and nonlinear changes in neural activity are also seen in TS responses to airborne sounds traveling through the air–water interface (Boatright-Horowitz & Simmons, 1997). Most sensitive frequencies to single tones decrease gradually from early larval to metamorphic climax stages (Figure 7, bottom left). Thresholds at most sensitive frequency are variable up to metamorphic climax stages, when they begin to decrease (Figure 7,

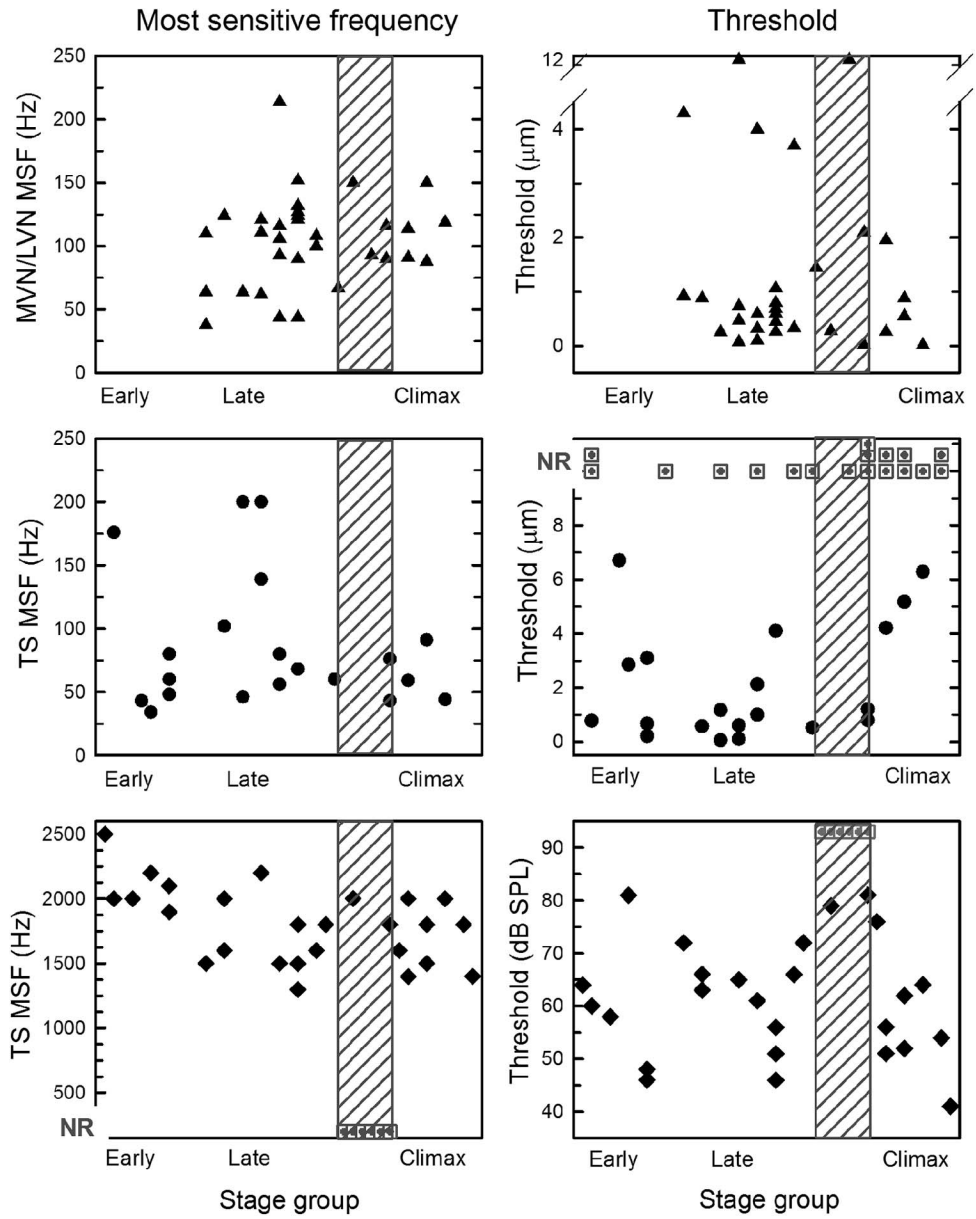


Figure 7. Most sensitive frequencies (MSF [Hz]; left column) and thresholds (microns displacement or decibels sound pressure level (dB SPL); right column) of central neural responses to acoustic stimulation in bullfrog tadpoles. Tadpoles are categorized by stage groups (*x*-axis). The dark gray cross-hatched bars delineate the "deaf period" during Gosner Stages 38–41. Top row: Neural activity in the medial vestibular nucleus (MVN) and lateral vestibular nucleus (LVN) in response to *z*-axis particle motion. Most sensitive frequencies and thresholds do not differ significantly between late larval and climax stages, although there are more sites with high thresholds in late larval stages. Early larval tadpoles were not tested. Middle row: Neural activity in the torus semicircularis (TS) in response to *z*-axis particle motion. Most sensitive frequencies are overall higher in early and late larval stages than in metamorphic climax, but thresholds are higher in climax, with more sites showing no response. Bottom row: Neural activity in the TS in response to airborne sounds. NR and the square/cross symbols = no response. Data are replotted from archival data, Simmons and Flores (2012), Simmons (2015), and Boatright-Horowitz and Simmons (1997). Some data points have been displaced slightly for clarity.

bottom right). During the end of the late larval period (Gosner Stages 38–41), neural responses to tones, as well as to noise bursts, are weak, evoked only by high levels of stimulation (>90 decibels sound pressure level [dB SPL] re: 20 μPa) or absent. This period of reduced TS responsiveness to airborne sounds is another indication, along with the apparent loss of SON-TS connectivity (see Figure 6), that the end of the late larval period may constitute a transient "deaf period" (Boatright-Horowitz & Simmons, 1997).

The "deaf period" may be a central consequence of the growth of the operculum over the oval window that occurs during this time period (Hetherington, 1987). As the operculum differentiates and then spreads over the oval window, it impedes the operation of the fenestral pathway, the primary means by which tadpoles younger than approximately Gosner Stage 37 would be able to detect sounds. If sound waves cannot be as effectively transmitted to the amphibian or basilar papillae, then thresholds to these sounds would increase. The opercularis muscle attachment and so the entire opercularis pathway are not fully operational until metamorphic climax. Thus, during climax, good neural responsiveness to sounds, including lower thresholds, should be seen, consistent with the data (Boatright-Horowitz & Simmons, 1997). Note, however, that tadpoles in the "deaf period" are not actually deaf. Some residual responsiveness to airborne sounds, albeit with high thresholds, can be still recorded in the TS during these stages (see Figure 7). Moreover, they can still detect underwater particle motion, as evidenced by neural activity in the MVN and LVN (see Figure 7), but with reduced sensitivity in the TS (Simmons, 2015; Simmons & Flores, 2012).

Another interesting change in auditory processing over development is seen in the coding of the periodicity (envelope) of complex sound waveforms. In early larval stages, multiunit activity from the TS responds in a synchronous (phase-locked) manner to waveform periodicities as high as 250 Hz. During metamorphic climax and in young postmetamorphic froglets, synchronous activity is limited to an upper waveform periodicity of 100 Hz (Boatright-Horowitz, Garabedian, Odabashian, & Simmons, 1999). Synchronous activity is even more restricted in the TS of adult bullfrogs, being limited to waveform periodicities below 50 Hz (Simmons, Sanderson, & Garabedian, 2000). A broad range of synchronous activity has been proposed as a marker of neural plasticity (Wong, 1993). These data support the conclusion that the ascending auditory pathway is very plastic during larval development, coincident with the emergence of the opercularis transduction pathway and consistent with the high rates of cell birth and alterations in medulla-midbrain connectivity seen between early larval and metamorphic climax stages.

I propose that, throughout larval development, neural responses to underwater particle motion are mediated by the saccule. The saccule differentiates during hatchling stages, prior to the differentiation of the amphibian and basilar papillae. Because it is a large otolith organ, its ability to transduce acoustic stimulation via the fenestral pathway would not as be affected by the overgrowth of the operculum over the oval window during the "deaf period" as are those of the smaller papillae. The saccule projects to the DMN, MVN and LVN, which in turn project to the TS. These anatomical pathways are stable throughout larval development, with no evidence of any period of transient disconnection. The question then arises as to why TS activity to particle motion becomes more difficult to drive during metamorphic climax (Figure 7, middle right). The large increase in the volume of the TS (Kumaresan et al., 1998; Figure 4) and the spike in cell birth (Simmons et al., 2006; Figure 5) during climax both point to a reorganization of the TS during this developmental period. The less robust representation of particle motion in the TS may be a consequence of this reorganization, suggesting that sites responsive to particle motion may have been spatially displaced to another region of the TS not sampled in those experiments, or to another brain area. An increase in brain space for processing of airborne sounds would be adaptive to prepare the animal for the final transition to life on land. Moreover, it is possible that newly born TS cells receive their afferent innervation via the DMN and so become specialized to detect airborne sounds, rather than via the MVN or LVN, which would mediate responses to underwater particle motion.

Although the tympanic transduction pathway begins to differentiate during metamorphic climax, it is not fully functional until sometime in the froglet period (Hetherington, 1987, 1988; Horowitz et al., 2001; Figure 3). This implies that auditory processing in the froglet TS should differ from that in climax tadpoles. There are few experiments detailing such differences (Boatright-Horowitz et al., 1999). Multiunit audiograms recorded from the TS of bullfrog froglets are sharper and more sensitive than those from the TS of tadpoles (Boatright-Horowitz & Simmons, 1995), as expected given the appearance of the external tympanum after the completion of metamorphosis. Although it is known that the TS in adult frogs responds to underwater far-field sound (Lombard, Fay, & Werner, 1981), it is not known whether the TS of froglets shows similar sensitivity. Even in fully aquatic African clawed frogs, neurophysiological studies of auditory coding are restricted to adults (Elliott, Christensen-Dalsgaard, & Kelley, 2011).

Development of Auditory Behaviors

Physiological and anatomical experiments indicate that tadpoles can hear, if not in the same manner as postmetamorphic frogs. However, there are no studies of auditory behaviors in tadpoles. Behavioral experiments of sound discrimination in túngara frog froglets revealed the animals do not respond selectively to species-specific advertisement calls (presented through air) over no sounds (silence) until about 400 days after the completion of metamorphosis (Baugh & Ryan, 2010). It is unclear whether froglets younger than 400 days postmetamorphosis were unable to hear the calls, unable to discriminate them, unmotivated, or unable to generate the appropriate motor response. The time course of anatomical or functional development of the auditory system in túngara frogs is not known.

The time span between hatching and the completion of metamorphosis could provide a window for experience-dependent learning of species-specific vocalizations emitted by adults. However, the limited available data do not support the existence of active vocal learning in tadpoles. A study of túngara frogs found that the tadpole's acoustic rearing environment has no influence on the acoustic characteristics of the male's mature advertisement call (Dawson & Ryan, 2009). Nor did the acoustic rearing environment affect preferences of female túngara frogs for particular vocal signals; that is, in behavioral choice tests, adult females consistently preferred the species-specific call, even if they were raised as tadpoles in a soundscape where heterospecific calls were prevalent (Dawson & Ryan, 2009). These data suggest that the adult ability to recognize species-specific vocalizations is innate in túngara frogs. Similar experiments on other anuran species are needed to test the generalizability of this result.

Development of Vocal Production

Understanding tadpole bioacoustics requires understanding their vocal production mechanisms as well as their hearing abilities.

Even though tadpoles are exposed to sounds in their underwater biotopes, their ability to produce sounds is extremely limited. Most tadpole species have been described as mute, and vocal production, in the sense of voluntary emission of sounds rather than only passive expulsion of air from the lungs or the body, is unusual. Three carnivorous species—tadpoles of Bell's horned frog (*Ceratophrys ornata*), of Cranwell's horned frog (*Ceratophrys cranwelli*), and of Azzurra's canyon frog (*Gephyromantis azzurrae*)—produce "metallic-like" sounds or clicks during predator–prey interactions (Natale et al., 2011; Reeve et al., 2011; Salgado-Costa et al., 2014). Because vocal production has not been reported in noncarnivorous tadpoles, the evolution of vocal production in these species may be tied to their specific foraging strategy. However, the function of these sounds remains unclear. Tadpoles of Bell's horned frog have lungs, a glottis, and laryngeal muscles, and they produce sounds by moving air from the lungs through the glottis. But even in these species, the arytenoid and cricoid cartilages in the larynx that are necessary for full sound production in adults do not differentiate until the beginning of metamorphic climax, and the vocal sac itself does not differentiate until the completion of climax (Natale et al., 2011). These developmental changes mean that any sounds produced by these tadpole species will be simpler in form and more limited than the sounds produced by postmetamorphic frogs with a mature vocal tract. There are no behavioral or physiological data available on the hearing sensitivities of these vocal tadpole species.

Tadpoles of the spadefoot toad (*Pelobates fuscus*) typically eat plankton but can become cannibalistic if stressed or crowded. These tadpoles first produce "clicking" and then "high-pitched tonal" sounds during metamorphic climax stages; postmetamorphic froglets can emit three different types of calls (ten Hagen et al., 2016). The function of these calls is unclear but have been proposed to reflect general arousal in a feeding context. As is the case for Bell's horned frog, Cranwell's horned frog, and Azzurra's canyon frog, the time courses of development of the auditory and vocal systems are not known for spadefoot toads.

In sexually mature anurans, the larynx is larger in males than in females. Data collected from several species suggest that this sex difference emerges in postmetamorphic life but before the animals become reproductively active. For example, at the end of metamorphosis, the larynx of túngara frogs is structurally similar in males and in females (Guerra, Ryan, & Cannatella, 2014). There are no vocal cords and no fibrous mass, both of which are crucial for sound production in adult túngara frog males. The vocal sac, which radiates sound from the vocal tract into the external environment, does not emerge in these male frogs until the onset of sexual maturity (Guerra et al., 2014). In African clawed frogs, sex differences in size and numbers of laryngeal muscle fibers are first seen in postmetamorphic juveniles, between 11 and 24 months after the completion of metamorphosis (Zornik & Kelley, 2011). During this time span, juvenile females sometimes produce calls that are acoustically similar to the release calls made by adult sexually mature females, but they do not produce courtship calls. Juvenile males are more vocal than juvenile females, but they do not produce their species-specific advertisement calls until they reach sexual maturity (Zornik & Kelley, 2011). Bullfrog tadpoles do not make sounds. The arytenoid cartilage in the larynx becomes more elastic as males mature from froglets to adults, providing the substrate for vocal production in sexually mature adults (Laureano et al., 2015).

From the existing data, it appears that the maturation of vocal production abilities lags behind that of hearing abilities, although there are no data on hearing abilities in those tadpole species that produce sounds. More extensive research is required to ascertain the temporal relationship between vocal and auditory development on both anatomical and functional levels.

Flow Sensing and Lateral Line Function

I end this review with a discussion of the function of the tadpole's lateral line system, which senses disturbances in the water—in effect, particle motion—by action of the peripheral neuromasts and their central connections. Particle motion may be the most important stimulus available for tadpoles to sense their environment. A functioning lateral line system allows tadpoles to maintain their position in a body of water, to avoid being swept away by currents, and to detect changes in ambient water flow that may be generated by swimming or vocalizing predators.

The peripheral lateral line neuromasts contain hair cells with one long kinocilium and several smaller stereocilia. In African clawed frog tadpoles, the kinocilium extends beyond the surface of the skin and deflects in response to water flow along the body (Roberts, Feetham, Pajak, & Teare, 2009; Shelton, 1970). In this way, the neuromasts can sense net velocity of current. They are first visible in hatchling stages (NF Stage 32), where they appear on the head close to the eyes. Over the rest of metamorphic development, more and more neuromasts appear over the head and body and their innervation patterns become more complex (Mohr & Görner, 1996; Shelton, 1971). During metamorphic climax, they sink further into the skin and the cilia are now embedded in a gelatinous cupula that serves to amplify water movements. Not only do neuromasts become more numerous, but also they spatially redistribute along the body surface; in particular, more become concentrated around the eyes (Shelton, 1970; Simmons, Warnecke, Vu, & Smith, 2015). The lateral line system is maintained in adult African clawed frogs. These frogs are active stalkers of live moving prey and so the additional sensory input deriving from the neuromasts in the region around the eyes may help guide detection of moving prey in murky waters.

In contrast, lateral line neuromasts are visible along the heads and bodies of bullfrog tadpoles only up to the end of late larval stages, when they begin degenerating (Schmidt, Knowles, & Simmons, 2011; Figure 1). The lateral line system is important for allowing bullfrog tadpoles to sense their environment during stages of development when neither the opercularis nor the tympanic sound transduction pathway is functional. In this species, the maturation of the opercularis pathway is correlated in time with the degeneration of the lateral line system (Horowitz et al., 2007).

There are considerable differences between tadpole species in the numbers and spatial distribution of neuromasts, related to the animal's ecology and life history (Brown & Simmons, 2016; Hoff et al., 1999; Lannoo, 1987; Quinzio & Fabrezi, 2014). These differences likely reflect the importance of sensory input from the lateral line in guiding behavior. In comparable experiments, African clawed frog and bullfrog tadpoles were tested in a standard assay of neuromast function—rheotaxis, the orientation toward the direction of a flow source. Responses of these two species in the same behavioral assay differ

considerably. African clawed frog tadpoles exhibit positive rheotaxis—swimming into and then orienting toward the direction of the current (Roberts et al., 2009; Simmons, Costa, & Gerstein, 2004; Simmons et al., 2015). Once the tadpoles adopt this position, typically within a short latency (<60 s), they maintain it for the duration of the flow, in a behavior called station-holding. Positive rheotaxis and station-holding are observed at all developmental stages, from NF Stage 37 to metamorphic climax, and at all flow speeds with which the tadpole is challenged. It is a stereotyped and robust response (Figure 8; Simmons et al., 2004, 2015). Conversely, bullfrog tadpoles tested under the same flow conditions are more randomly oriented, and their responses are more affected by the specific flow dynamics of the testing tank (Brown & Simmons, 2016; Schmidt et al., 2011). In some flow conditions, these tadpoles swim away from, rather than toward, the source of the current. Once downstream away from the current source, they then adopt orientations that are random with respect to the direction of flow. In other flow conditions, they still swim downstream but then turn and orient toward the current source. In all flow conditions, bullfrog tadpoles exhibit long response latencies (up to 300 s). They do not station hold but continue to swim, although within a more localized area, when flow is present, and the precision of their orientation response (the vector strength) is significantly less than in African clawed frogs (Brown & Simmons, 2016; Figure 7).

Some of these species differences may reflect differences in habitat. African clawed frog tadpoles are lentic (midwater suspension feeders) while bullfrog tadpoles inhabit both lentic (stationary) and lotic (flowing) waters and feed on a greater variety of food sources (McDiarmid & Altig, 1999). At comparable developmental stages, African clawed frog tadpoles have more neuromasts (22.8 per mm of head/trunk/tail length) than do bullfrog tadpoles (2.7 per mm body/tail length). They may exhibit more stereotyped rheotaxis because they have more sensors. The lateral line system of African clawed frogs may be overall more precise because it will be needed after metamorphosis, while the bullfrog loses this sensory system and already in larval life may privilege input from the developing auditory and vestibular systems.

Future Directions

About three quarters of the 7,000 or so known species of anurans undergo metamorphosis. Most of these metamorphosing species transform from fully aquatic tadpoles to terrestrial or amphibious adults, while others (the primitive species) remain aquatic throughout life. The other one quarter or so anurans are direct developers without a tadpole stage (Callery, Fang, & Elinson, 2001). It is well known from ecological and herpetological research that tadpoles of different species vary considerably in habitat, morphology, body plan, and behaviors (Hoff et al., 1999; McDiarmid & Altig, 1999). These variations are likely reflected in species differences in the timeline of auditory system maturation, but more research with a greater variety of species will be crucial for uncovering these divergent timelines. In particular, different habitats provide distinct underwater soundscapes that tadpoles can experience during development. The soundscape, in turn, may exert considerable influence on the maturational trajectory of the auditory and perhaps even the vocal systems. Delineating the range of sounds to which tadpoles are exposed and to which they respond in different habitats and during different metamorphic stages will be important for guiding further research on tadpole bioacoustics.

Males of most anuran species become highly vocal at sexual maturity and emit advertisement and aggressive calls to attract mates and maintain territorial boundaries. Adult female vocalizations are limited to distress and alarm calls, although females of some species make courtship calls (Wells, 1977). Our understanding of the development and maturation of vocal production abilities is very limited. Only recently have reports emerged of sound production in a few tadpole species, and the function of these vocalizations remains uncertain. Behavioral studies in one species suggest that tadpole rearing environment does not affect their auditory preferences in early postmetamorphic life, and so preferences for conspecific vocalizations are innate. But these studies need to be replicated with other species, most importantly those in which we have some understanding of the time course of auditory system maturation. Our limited understanding of how and when froglets and juvenile anurans begin to develop the kinds of selective behavioral responses to vocalizations seen in adults

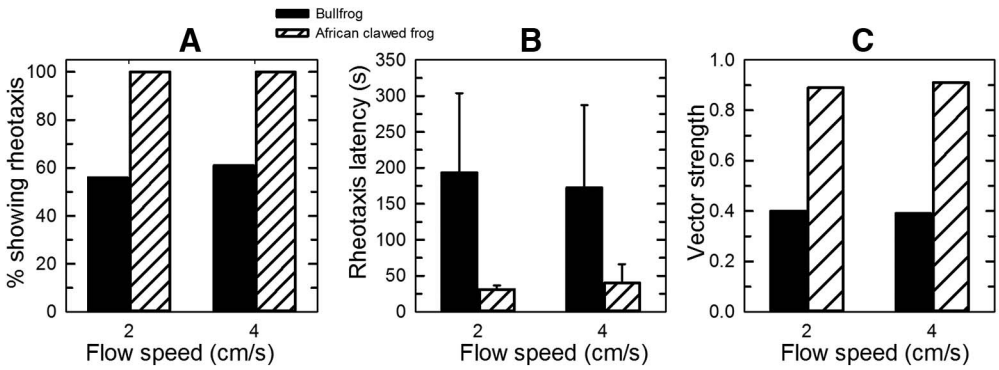


Figure 8. Bullfrog tadpoles and African clawed frog tadpoles exhibit different flow-sensing behaviors when tested in identical flow conditions (flow speeds, *x*-axis). (A) Percent tadpoles showing positive rheotaxis toward the source of the current. Bullfrog tadpoles are significantly less likely than African clawed frog tadpoles to show rheotaxis. (B) Latency to positive rheotaxis ($M \pm SD$). Bullfrog tadpoles have significantly longer latency to rheotaxis than do African clawed frog tadpoles. (C) Vector strength or precision of rheotaxis. Vector strength of 1 indicates perfect rheotaxis. African clawed frog tadpoles show greater precision of rheotaxis than do bullfrog tadpoles. Figure is modified from Brown and Simmons (2016).

in large part stems from the lack of reliable techniques to study auditory behaviors in tadpoles. New behavioral assays are critically needed to investigate the functional maturation of the auditory system and how this functional maturation might vary across species and habitats.

Metamorphosis is a dynamic process, with major changes in the auditory system occurring throughout larval life. One example is the sequential, inside-out pattern of development of the auditory periphery. This developmental trend has been verified in all of the few species examined so far, even though some details remain obscure. But this pattern raises the interesting question not only of the auditory world of tadpoles but also of the auditory world of froglets. Do froglets share an auditory world with metamorphic climax tadpoles, that is, are they limited to hearing through the opercularis system? What are the genetic and molecular triggers that turn on the final differentiation of the external tympanum? What is the adaptive significance of a prolonged time period of final development of the tympanic transduction pathway? Another dynamic aspect of metamorphosis is the progressive changes in key nuclei in the auditory brainstem, particularly as related to expansion of structure size, cell birth, addition of new pathways, loss of old pathways, and changes in histochemical expression. Metamorphic climax can now be identified as a crucial time period for these developmental events.

Metamorphic development is not only a dynamic process but also a nonlinear process. This is illustrated most clearly by auditory midbrain processing of airborne sounds. The TS of bullfrog tadpoles exhibits considerable modifiability in its responses to airborne sounds over development, as shown by poor sensitivity in the early larval period, better sensitivity throughout most late larval stages, the transient "deaf period" of greatly reduced sensitivity at the end of the late larval period, and increased sensitivity in metamorphic climax. These data all derive from studies of bullfrog tadpoles, and it is not known if similar effects would be found in other tadpole species.

Even in bullfrog tadpoles, many questions about functional coding in the auditory system remain. For example, it is not known when developmentally the adult tonotopic organization of the ascending auditory pathway emerges. Another ripe area for further research is in the coding of species-specific vocalizations. In adult anurans, central neural processing of vocalizations is consistent with a matched filter coding scheme, in which neurons in the auditory midbrain and thalamus are selectively tuned to the spectral frequencies in the male's advertisement calls (Gerhardt & Schwartz, 2001). We currently have no understanding as to when and how the substrates for this matched filtering strategy emerge in any tadpole species.

Metamorphosis is a fascinating and rich area of research that can contribute significantly to our understanding of plasticity of sensory processing as related to changing environmental demands.

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