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Sound Localization in the Alligator

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Abstract

In early tetrapods, it is assumed that the tympana were acoustically coupled through the pharynx and therefore inherently directional, acting as pressure difference receivers. The later closure of the middle ear cavity in turtles, archosaurs, and mammals is a derived condition, and would have changed the ear by decoupling the tympana. Isolation of the middle ears would then have led to selection for structural and neural strategies to compute sound source localization in both archosaurs and mammalian ancestors. In the archosaurs (birds and crocodilians) the presence of air spaces in the skull provided connections between the ears that have been exploited to improve directional hearing, while neural circuits mediating sound localization are well developed. In this review, we will focus primarily on directional hearing in crocodilians, where vocalization and sound localization are thought to be ecologically important, and indicate important issues still awaiting resolution.

Keywords

archosaur; auditory periphery; pressure-difference receiver; behaving alligator; skull anatomy; brainstem physiology

1. Introduction

Despite their dissimilar body plans, crocodilians and birds are both archosaurs, and are each other's closest living relatives (Hedges and Poling, 1999; Iwabe et al., 2005). The lineages that led to modern crocodilians and birds diverged during the middle Triassic, when archosaurs had a “nearly cosmopolitan biographic distribution” (Nesbitt, 2011).

Comparative studies of archosaur hearing should therefore inform discussions of hearing in all archosaurs, especially non-avian dinosaurs. In this review, we will focus primarily on sound localization in crocodilians, because the literature on avian hearing and sound localization has been recently reviewed (Köppl et al., 2014). Thinking beyond archosaurs, this comparative work should serve as a model for highlighting conserved principles of

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sound localization. This review will also discuss the on-going debate regarding differences in neural coding mechanisms for sound localization in mammals and birds, and the role of evolutionary and ecological constraints versus optimal coding models (Carr et al., 2009; Harper and McAlpine, 2004; Schnupp and Carr, 2009). Modeling studies (Harper and McAlpine, 2004) suggest that the neural coding strategy for ITDs may be determined by the physiological range of ITDs, which may be highly affected by the periphery, an area in which crocodilians have some specialization.

The ancestors of tetrapods moved onto land in the Devonian, and paleontologists estimate that tympanic hearing emerged about 100 million years later, after the major tetrapod lineages emerged (Clack, 2002). Tympanic hearing appears to have developed independently in the major tetrapod lineages, and would have increased the sensitivity of hearing (Christensen-Dalsgaard and Carr, 2008; Clack, 2002). Furthermore, it has been hypothesized that the tympana were not isolated by a closed middle ear cavity in these early tetrapods, but acoustically coupled through the pharynx and therefore inherently directional, acting as pressure difference receivers (Christensen-Dalsgaard, 2005). Throughout this review pharynx and mouth cavity are used interchangeably. Previous work (e.g. Christensen-Dalsgaard, 2005) has discussed coupling as occurring via the mouth cavity, though anatomically, bilateral Eustachian tubes open into the pharynx. The later closure of the middle ear cavity, to varying degrees, in turtles, archosaurs, and mammals is a derived condition, and would have changed the operation of the ear by decoupling the tympana (Christensen-Dalsgaard and Manley, 2013; Manley, 2010; Willis et al., 2013).

Since isolated middle ears would no longer be directional, there would have been selection for neural computation of sound source localization in archosaurs and mammalian ancestors (Manley, 2010). In archosaurs, however, extensive skull pneumaticity, or the presence of air spaces, provided connections between the ears that could have been exploited to improve directional hearing. This would have been especially relevant in smaller dinosaurs, where directional cues are smaller. Wilczynski (1984) has observed that peripheral changes generally underlie changes in the auditory brainstem, and we propose that the elaboration of the neural circuits used for sound localization in the birds and crocodilians emerged from exploiting these peripheral directional cues. For this chapter, we will first review the evidence for directional hearing in crocodilians, the organization of the crocodilian external and middle ears, and then the organization of the neural pathways associated with sound localization.

2. Evidence for Directional Hearing

Crocodilians are the most vocal of the non-avian reptiles, and their repertoire of vocal communication makes them comparable to birds (Vergne et al., 2009). The marsh-dwelling American alligator is considered especially chatty, and vocal communication is thought to be particularly useful in their low visibility habitat (Garrick and Lang, 1977). Behavioral observations show vocal communication during mating, defensive, group cohesion, and maternal care behaviors (Burghardt, 1977; Garrick and Lang, 1977; Hedges and Poling, 1999; Hunt and Watanabe, 1982; Iwabe et al., 2005; Passek and Gillingham, 1999; Young et al., 2014). The 1 KHz peak in the alligator audiogram (Higgs et al., 2002; Nesbitt, 2011)

coincides with a major component in the juvenile contact call (Köppel et al., 2014; Vergne et al., 2009). The physiological evidence (reviewed below) corroborates these behavioral observations, while additional behavioral data (reviewed below) supports sound localization utilization.

2.1 Auditory brainstem response

Crocodylians have excellent hearing focused at relatively low frequencies (Carr et al., 2009; Harper and McAlpine, 2004; Higgs et al., 2002; Manley, 1970a; Schnupp and Carr, 2009; Wever, 1978a), and previous work in the American alligator has demonstrated their hearing ability in both air and water (Dinets, 2013; Harper and McAlpine, 2004; Higgs et al., 2002). Auditory brainstem response (ABR) audiograms show that the hearing range for these animals is best between 300 and 2000 Hz (Figure 1C). The ABR is a gross measurement of neurophysiological activity, with sensitivity thresholds consistently higher than those from behavioral or auditory nerve recordings. Nevertheless, in birds, audiograms based on scalp-recorded ABRs are similar in shape to behavioral audiograms, although they generally underestimate behavioral thresholds by about 30 dB (Brittan-Powell et al., 2002). ABR strength depends both upon synchrony and upon the number of fibers activated. At low frequencies, ABR thresholds were about 40 dB less sensitive than auditory units responding to similar frequencies, while at higher frequencies, differences were closer to 10–20 dB. These differences may reflect both differences in synchrony and the number of fibers activated. Discrepancies between ABR audiograms is likely attributable to the methods of stimulus presentation. Higgs et al. (2002) utilized traditional 10 ms tone stimuli, which produce distorted tones at low frequencies, and thus distorted low frequency threshold measurements. Current data utilized a masking paradigm (Bierman et al., 2014; Brandt et al., 2009; Christensen-Dalsgaard and Carr, 2008) (Figure 1B), which measures the difference between the response to broadband click stimuli in the presence and absence of a longer duration test frequency. This paradigm improves measurement of responses to low frequency tones. Regardless of the methods, these audiogram show that, in comparison to most birds, alligator hearing is limited to lower frequencies (Christensen-Dalsgaard and Manley, 2013; Fay, 1988; Higgs et al., 2002; Konishi, 1970; Manley, 2010; Willis et al., 2013).

A directional ABR, created by adjusting the relative placement of the speakers generating test frequency (masker tone) and click stimulus (Figure 1A), demonstrated that there is directional information encoded in the auditory nerve. Using a test frequency of 1 kHz, a ~10 dB difference in threshold was measured between ipsilateral and contralateral placement of the speakers (Bierman et al., 2014; Figure 1D). Additional experiments in three anesthetized animals showed similar directional differences using a 600 Hz tone (personal observation). Acoustical measurements further demonstrated that the cues for directional information were not a consequence of external head anatomy and thus support an interaural pressure difference receiver mechanism (Bierman et al., 2014).

2.2 Behavioral evidence for sound localization

Direct behavioral experiments on crocodylians are challenging. Dinets (2013) demonstrated that alligators show a directional response to underwater sound, although limitations of

design do not allow us to conclude that these responses were mediated by the auditory system, as opposed to the dome-pressure receptors. Beach (1944) described an incomplete (his label) data set in which a vocalization and postural change is elicited in an animal by the “plucking” of a steel rod. The author observed that the animal frequently moved toward the steel rod. However, similar concerns to Dinets (2013) apply, and auditory cues may not be the singular or most prominent information source.

In an effort to build upon previous work, we successfully trained and tested two juvenile alligators in a sound localization task in our laboratory. Animals were obtained as hatchlings from Rockefeller Wildlife Refuge (Lake Charles, LA) and were ~3 years old at time of data collection. For testing, a 5 ft diameter tank was equipped with portable computer speakers (Insignia, Richfield, MN) and customized vibratory fish feeders (Dynamic Aqua-Supply Ltd., Surrey, BC, Canada) filled with AquaMax trout chow (Purina). The tank was filled with ~5 inches of water and speakers were placed 1 inch above the water line. In order to eliminate external cues, behaviors were video monitored, and an experimenter isolated by a floor to ceiling curtain controlled feeders and speakers remotely. Early training utilized a moveable pole with attached speaker so that the alligators learned to associate a 1 s, 1 kHz sound with food. For later training and data collection the tone was played randomly from one of two speakers, placed on either side of the tank. A third food dispenser centered in the tank was used to encourage the animals to go to the center at the beginning of each session. Prior to the tone, animals were rewarded for going to the center of the tank. Once a tone played, animals were only rewarded for arriving at the correct speaker. Sessions started with the animals in the center of the tank. Animals very rarely reached the speaker after a single playing of the tone, usually stopping after several seconds of movement. Tones were repeated after the animal stopped moving (Figure 2A). Each tone was considered an independent trial. Across tank locations, the tone level ranged from 95 – 105 dB SPL. With training, animals reliably initiated movement within a couple seconds of the tone being played (Figure 2B) and, proceeded toward the sound source with no underlying preference between sides (Figure 2C).

3. Anatomical features of the crocodilian auditory system

3.1 External Structures

The crocodilian external ear seals out water by a muscular earlid (Shute and Bellairs, 1953; Wever, 1978b). The external auditory meatus is slit shaped, with a large earlid above, and small inferior earlid anterior and below. The anterior part of the meatus in living animals is usually open when the top of the head is out of water and closed when it is submerged. The posterior part of the meatus is nearly always closed, even when the head is above the surface. The earlid may protect the tympanic membrane from injury (Shute and Bellairs, 1953; Vergne et al., 2009). The presence or absence of the earlid does not affect localization cues within the animal’s auditory range (Bierman et al., 2014). The tympanum itself is large, and thin, and coupled to a columella (Figure 3A). Although crocodilians can hear underwater (Higgs et al., 2002; Young et al., 2014), their thin tympanum is more consistent with hearing in air.

The crocodilian tympanic membrane is a broad oval with a raised, slightly off-center point at the attachment of the extracolumella (Wever, 1978, Figure 3A). The cartilaginous extracolumella is composed of three processes that triangulate to hold the columellar shaft in place. Additionally, crocodilians, like many lizards, have an extracolumellar muscle attached to the tympanic membrane. Though the contributions of this muscle to signaling has not been studied, contraction likely affects sound conduction (Saunders et al., 2000). Saunders et al. further suggest that, since it is found in species with vocal communication, it may be activated reflexively during vocalizations.

3.2 Pneumacity of the Middle Ear

Examination of CT-based material has provided new insights into the organization of both brain and ear regions of archosaurs (Witmer and Ridgely, 2009). Witmer has pointed out that the study of living archosaurs can provide essential guides to the details of soft tissues and their associated bony elements, which can then be directly compared with the bony structures of the fossil specimens. They used the extant Australian freshwater crocodile, American alligators, ostriches and great horned owl for comparison with dinosaur fossils (Witmer et al., 2008; Witmer and Ridgely, 2008), to reveal that archosaurs have ears coupled by paratympanic sinuses (Figure 3 B, C). This coupling was later verified to ensure presence of thin membranes hadn't been overlooked during virtual sectioning (Bierman et al., 2014).

The presence of pneumatized tympanic sinuses among the archosaurs would have created a substrate for evolution of directional sensitivity based on the coupling of the middle ear cavities via the pneumatic spaces that surround the brain. Interestingly enough, comparative and developmental analyses reveal that the elaboration of middle-ear pneumaticity in the avian and crocodilian lineages was independent (Dufeu, 2011). “*The greatly elaborated pattern of pneumaticity observed in each of these groups is derived from diverticular expansions of the middle-ear sac, and the extent of the diverticular infiltrations into the braincase is unprecedented among amniotes. The functional implications of the high degree of middle-ear pneumaticity (such as acoustic function, or other biomechanical properties) have the potential to inform higher order questions regarding auditory performance, and style of communication behaviors for example*” (Dufeu, 2011, p. 3). Both Figure 3B, C and the reconstructions presented by (Witmer and Ridgely, 2008) show that the alligator skull has many large air-filled spaces. This is consistent with Archosauria being a highly pneumatic clade. Unlike mammals, archosaur sinuses are not enclosed within bone, but are more open (Witmer and Ridgely, 2008). High pneumaticity and the venting of the middle ear affects the transfer function of the middle ear, specifically increasing the efficiency and sensitivity of low frequency hearing (Guinan and Peake, 1967; Pickles, 1988).

The middle ear transfer function acts as a band-pass filter (Rosowski, 1994). Different parameters can be important for achieving the attenuation of low and high frequencies; low frequency attenuation can be related to elastic stiffness of the tympanic membrane and ossicle ligaments, and to compression and expansion of air in the middle ear cavity (Pickles, 1988; Saunders et al., 2000). When the tympanic membrane moves in and out, it compresses the air in the middle ear cavity, thus reducing the movement of the tympanic membrane. Given a constant sound pressure level, there is an inverse relationship between the

displacement of air particles and frequency; the displacement of air particles falls toward higher frequencies (Pickles, 1988, p. 21). If the middle ear cavity is vented, as in the case of the alligator, then transmission to low (which are associated with greater particle displacement), but not high, frequencies is increased (Guinan and Peake, 1967, Pickles, 1988). Generally, a larger middle ear cavity or tympanic membrane surface area, improves low-frequency sound conduction (Saunders et al., 2000).

In archosaur evolution, changes in middle-ear pneumaticity, occurring in parallel, would have provided cues from the auditory periphery that in turn could have been exploited to improve directional hearing. This would have been especially relevant in smaller dinosaurs. Furthermore, the efficacy of the coupling may be plastic. Birds may be able to adjust the connections between their middle ear cavities; Larsen (Larsen et al., 2006; 1997) observed that budgerigars develop a lower static air pressure in the middle ears when anaesthetized, which reduced eardrum vibrations. If their air spaces are vented, sound propagation through the interaural pathway increased directional hearing.

3.3 Structure of the interaural canals

In crocodilians, the middle ear cavities and other cranial sinuses are connected by passages both above and below the braincase (Bierman et al., 2014; Witmer et al., 2008; Witmer and Ridgely, 2008) (Figure 3B,C). Dorsally, the middle ear cavities are connected via the intertympanic recess. Ventrally, the middle ear cavities are connected through the quadrate sinus, the pharyngotympanic (Eustachian) recess, and the median pharyngeal recess. Owen (1843), first described “*the opening at the floor of the tympanic cavity, leads to a short canal,.. which curves towards its fellow from the opposite tympanum, but first swells into the rhomboid sinus*”. These large, patent anatomical connections support the hypothesis that alligator ears function as pressure-gradient receivers, which could contribute to the increased range of ITDs (Carr et al., 2009) and to the directional sensitivity observed with the ABR (Bierman et al., 2014).

Our laser vibrometry measurements showed that at least one of these pathways functions to couple the eardrums at sound frequencies in the range of alligator hearing (Bierman et al., 2014). Both ABR and vibrometry measurements demonstrated gains of ~10 dB around 1 kHz, while similar passive acoustical recordings at 1 kHz show about half that amount, with a maximum 4 dB ILD gain. Phase analyses of vibrometry data generated maximum ITD estimates of ~300 μ s, or a factor of three larger than the ITDs measured from head-related transfer function (HRTF) analysis, which were about 100 μ s. These additional delays are most likely caused by the sound transmission through the interaural canal. They clearly demonstrate an increase over the passive acoustic baseline, and support the role of internally coupled ears in increasing the magnitude of the binaural cues for localization.

3.4 Ear and auditory nerve targets

In crocodilians, the inner ear is large, with a long basilar membrane and unidirectional population of hair cells covered by a tectorial membrane (Wever, 1978a). Auditory nerve units are relatively sharply tuned (Manley, 1981, Klinke and Pause, 1981), and phase-locking is improved in comparison to that of lizards and tree frogs, where phase locking is

largely absent by 1,000 Hz (Christensen-Dalsgaard et al., 2011b; Hillery and Narins, 1987; Sams-Dodd and Capranica, 1994). Caiman auditory nerve phase locks to frequencies up to at least 1,500 Hz (Carr et al., 2009; Smolders and Klinke, 1986).

The auditory nerve projects into the medulla, the dorsal surface of which is largely made up of the cochlear nuclei (Figure 4A, B). The nerve projects to two cochlear nuclei, the nucleus magnocellularis and the nucleus angularis (Leake, 1974). The nucleus angularis lies anterior to the root of the auditory nerve, and bulges above the general surface of the brain (Manley, 1970a). It is composed of both large and small ovoid cells. Caudal to nucleus angularis, the nucleus magnocellularis lies medial to nucleus laminaris, then widens caudally. The nucleus magnocellularis contains characteristic large round principal cells (Leake, 1974), although its caudal region is capped by a small-celled component (Glatt, 1975). There is a similar small-celled region of the avian magnocellularis that receives low best frequency auditory nerve fibers (Köppl, 1994; Köppl and Carr, 1997). In general, the organization of the reptile auditory brainstem is similar among diverse groups (Carr and Code, 2000), although the brainstem nuclei in birds and crocodilians appear proportionally larger than those of lizards and turtles.

Physiological responses from the crocodilian auditory system are similar to those in birds and some lizards. Recordings from the cochlear nuclei in the caiman produced primary-like responses (Manley, 1974; 1970b). Like other animals, caiman auditory neurons exhibit varying thresholds. Manley (Manley, 1970a) reported neurons tended to be most sensitive in the 800–1,200 Hz range (Figure 4C). Crocodilian cochlear nuclei are tonotopically organized, in a similar fashion to birds (Konishi, 1970; Manley, 1970a). Manley's 1970 physiological determination of tonotopic organization in the auditory brainstem, re-evaluated in the light of more recent physiological and anatomical studies, revealed tonotopic axes in magnocellularis and laminaris that run from caudolateral low best frequency to rostromedial high best frequency regions, as is also the case in birds (for reviews, see Carr and Code, 2000; Grothe et al., 2004). Nucleus angularis extends a tail-like extension overlying nucleus laminaris and the rostral half of nucleus magnocellularis, which may manifest as a dorsal higher best frequency layer in Manley's tonotopic map (Manley, 1974; Marín and Puelles, 1995). Manley also notes a consistent vertical tonotopic organization of the nucleus angularis, like in birds (Konishi, 1973; Köppl, 2001).

4. Computation of interaural time differences

In both birds and crocodilians, the projection from the nucleus magnocellularis to the nucleus laminaris forms a circuit that conforms to the model proposed by Jeffress to explain sound localization by detection of interaural time differences (Ashida and Carr, 2011; Carr et al., 2009; Jeffress, 1948). The auditory nerve and nucleus magnocellularis encode the timing of the auditory stimulus by phase-locking in birds and crocodilians (Carr et al., 2009; Köppl, 1997; Smolders and Klinke, 1986). Nucleus magnocellularis projections to nucleus laminaris act as delay lines to create maps of interaural time difference that are tapped by coincidence detector neurons in laminaris. Internal delays, equal and opposite to the interaural delays, characterize both barn owls (Carr and Konishi, 1990; Pena et al., 2001), chickens (Funabiki et al., 1998; Köppl and Carr, 2008; Overholt et al., 1992) and alligators

(Carr et al., 2009) (Figure 5). The coupling between the alligator's middle ear cavities adds to the external delays created by the physical distance between the ears, to make a two component external delay and a functionally larger head, especially at the low sound frequencies to which alligators are sensitive (Bierman et al., 2014).

4.1 A wide range of ITD has been recorded in vivo

A key feature of the Jeffress model is the systematic representation of ITD, which creates a place code of azimuthal position. The place code may later be integrated with other sensory input to guide orientation and/or localization to stimuli. Place codes are found in archosaurs, but have not been found in mammals (for review, see Grothe et al., 2010). Maps of ITD have been found in the barn owl *in vivo* (Carr and Konishi, 1990; Carr et al., 2013) and in the chicken, both *in vitro* (Overholt et al., 1992) and *in vivo* (Köppl and Carr, 2008). Data from the alligator support a place code, because recording sites with best ITDs near 0 μ s were medial in laminaris and best ITDs in the contralateral hemifield were lateral in laminaris. One curious point, germane to the coupling of crocodilian ears, is that the range of recorded best ITDs was very large in the 2–3 year old alligators examined in (Carr et al., 2009), from 100 μ s ipsilateral ear leading to 1200 μ s contralateral leading, and median values of about 450 μ s, as compared to median values of about 90 μ s in chicken (Köppl and Carr, 2008) and 173 μ s in the gerbil (Pecka et al., 2008) (Figure 6). The alligators used for these measurements had ears that were about 3 cm apart (Carr et al., 2009), so many peaks of the ITD tuning curves should fall outside even the extended physiological range created through coupling of the ears (Figure 5A, B). Crocodilian head size increases throughout life to a head width of nearly 30 cm in adult alligators (Woodward et al., 1995), so larger animals may experience increased activation of laminaris neurons with best ITDs in far contralateral space. The younger, smaller head width, animals should experience a combination of slopes, or changes in firing rate with location, outside the physiological range, and peaks within range (Figure 5). These changes in available auditory cues may also be related to the different emphasis (and frequencies) in inter-specific acoustic communication in crocodilians at different ages. Humans and barn owls are able to discriminate artificially large ITDs (Sabeti et al., 2002), and they may be used to discriminate left from right. Animals may also use the information contained in the slopes of the ITD curves. Bala et al. (2003) showed that barn owls can use the information in the change in ITD slope to discriminate small changes in the location of sound sources, and it is likely that crocodilians also do so.

4.2 The biophysics of ITD computation resembles that of the chicken

In addition to the similar circuits for encoding ITD described above, the biophysics of ITD computation may be similar in crocodilians and birds (Carr et al., 2003; Carr and Soares, 2002). Alligator, emu and chicken laminaris neurons share similar bitufted or bipolar morphology, with inputs from the ipsilateral and contralateral nucleus magnocellularis onto dorsal and ventral tufts, respectively (Carr and Soares, 2002; Jhaveri and Morest, 1982; MacLeod et al., 2006; Rubel et al., 1976) (Figure 6B). The crocodilians have a well-developed nucleus laminaris, which forms a sheet of bipolar spindle-shaped cells that is very similar to that seen in the basal land birds (Carr et al., 2009; Carr and Code, 2000) (Figure 6B). Like in chicken and emu, the lengths of the dendrites changed systematically along the

tonotopic axis, with neurons in the low frequency region having longer, more branched dendrites while those in the high frequency region were shorter and less branched (unpublished).

Brain slice experiments in alligator embryos revealed similar responses to bilateral stimulation as observed in chicken and emu slices (Figure 6C, Carr et al., 2009). When a suprathreshold depolarizing current was injected in the nucleus laminaris neurons, the cell generated a single action potential at the onset of membrane depolarization, as do similar neurons in birds and mammals (Gao and Lu, 2008; Reyes et al., 1996; Smith, 1995; Trussell, 2008). Coincidence detection in nucleus laminaris was simulated *in vitro* by electrically stimulating the ipsilateral and contralateral projection bundles from each nucleus magnocellularis (Figure 6A). Responses from laminaris neurons were evoked under two stimulating protocols: First, either ipsilateral or contralateral stimulation was used to generate subthreshold EPSPs. EPSP amplitude depended on the stimulus intensity. Stimulation amplitude was adjusted so that neither ipsi- nor contralateral stimulation generated an action potential. Then both ipsilateral and contralateral inputs were stimulated at varying delays (Figure 6C, D). Action potentials were generally generated by paired EPSPs at intervals less than about 3 ms (Figure 6D, 600 μ s interval). Maximum response probabilities were calculated from these experiments, and all cells showed high response probabilities when two EPSPs were generated in close coincidence (Figure 6C). The cells showed a wide range of interstimulus interval dependence, with response windows of half-widths of 1.78 ± 2.2 ms ($n=7$), similar to measurements in chicken (Funabiki et al., 1998; Kuba et al., 2002). These data allow comparison with chicken nucleus laminaris neurons.

To determine whether the mechanisms of crocodilian ITD detection were similar to those of birds, we developed a model based on a simulation of ITD detection in chicken and barn owl (Grau-Serrat et al., 2003), but with specific crocodilian modifications (Carr et al., 2009). Phase-locked inputs from auditory nerve and the nucleus magnocellularis were used to construct plausible input sequences for the model, and biophysical data were provided by *in vitro* studies of alligator embryos described above. Like in chicken, the model coincidence detectors fired well when their ipsilateral and contralateral inputs are in phase, the hallmark of a good ITD-sensitive neuron. Discrimination is good until about 1 kHz, consistent with the biophysical features observed in alligator *in vitro* recordings (Carr et al., 2009).

5. Comparison of Archosaurs with other Reptilia and Mammals

The transition from water to land would have been marked by changes associated with processing airborne sound. The ancestral atympanate tetrapods may have been only slightly sensitive to low frequency sound, similar to lungfish, which have good low-frequency vibration sensitivity and rudimentary sensitivity to air borne sound (Christensen-Dalsgaard et al., 2011a; Christensen et al., 2015). The subsequent invention of tympanic hearing occurred independently in the major tetrapod groups (Clack, 2002; Christensen-Dalsgaard and Carr, 2008; Clack, 2011), and would have increased the sensitivity of hearing. The ancestral tympanate condition appears to be sensitivity to low frequency sound, inferred principally from the size and weight of the columella (Gleich et al., 2005; Kundrát and Janáček, 2007; Starck, 1994; Witmer and Ridgely, 2008). The emergence of tympanic

hearing should also have led to changes in the central auditory system associated with increased sensitivity to higher frequency sound.

Since ancestral tympanate ears were coupled across the mouth cavity, the auditory input to the ancestral central auditory system would also have been directional (Christensen-Dalsgaard et al., 2011b; Christensen-Dalsgaard and Manley, 2008). In lizards, the ears are strongly coupled; for frequencies between 2 and 5 kHz, the acoustic interaction of the eardrums in lizards produces up to a 40 dB difference in eardrum vibration in response to directional sound stimulation (Christensen-Dalsgaard and Manley, 2008; Vossen et al., 2010). This coupling of the middle ear cavities is achieved via the mouth cavity and large permanently open Eustachian tubes (Christensen-Dalsgaard, 2005; Christensen-Dalsgaard et al., 2011b).

The closure of the middle ear cavity in mammals and archosaurs is a derived condition, and would have profoundly changed the operation of the ear by decoupling the tympana and leading to a requirement for neural computation of directionality in the central nervous system (Christensen-Dalsgaard and Carr, 2008; Christensen-Dalsgaard and Manley, 2013; Christensen-Dalsgaard et al., 2014; Manley, 2010). In archosaurs, skull pneumatization would have re-created the connections between the middle ear cavities in both crocodilians and birds. Archosaur middle ears are not as well coupled as those of some lizards, but their interaural canals nevertheless generate significant directional cues (Bierman et al., 2014).

Increased sensitivity to direction may have led to increased neural processing of sound in the central auditory system. The organization of the crocodilian brainstem is anatomically and physiologically similar to that of the birds (for reviews, see Carr and Christensen-Dalsgaard, 2010; Carr and Code, 2000; Carr and Soares, 2006; Grothe et al., 2004). Furthermore, the archosaur neural circuit closely resembles that of the gecko (Tang et al., 2011). These similarities suggest that the ancestral reptile auditory nerve innervated two dorsally positioned first order nuclei, and that the most caudal of these structures, the nucleus magnocellularis, projected both ipsilaterally and contralaterally to the nucleus laminaris. The increases in size of these auditory nuclei in archosaurs may reflect the increased importance of neural processing of directional information.

6. Conclusion

Sound localization is evolutionarily important, although not all animals localize sound well. In mammals, threshold acuities range from about 1° for elephants and humans to more than 25° for gerbils and horses and a near absence of localization in some subterranean species (R. S. Heffner and H. E. Heffner, 1992a). A similarly wide range has been found for birds, with best acuities of about 3° in the barn owl (Bala et al., 2003). Heffner and Heffner (1992a) proposed that variation in acuity in mammals is best accounted for by the requirement for visual orientation to sound, in that species with broad fields of best vision require less accurate information than foveate species (R. S. Heffner and H. E. Heffner, 1992a; 1992b). This hypothesis has not been tested in birds or crocodilians, since fewer data are available for localization and gaze direction in birds (Klump, 2000; van der Willigen et al., 2002) and there are none for crocodilians.

In addition to mediating sound localization and the direction of gaze, the binaural system is also used to detect spatially separate signals. Waveforms at the two ears can differ in both ITD and interaural correlation (Coffey et al., 2006; Fitzpatrick et al., 2000; Joris and Yin, 2007; Louage et al., 2006; Shackleton et al., 2003). Signal detection is improved when signals are spatially separated from masking sounds (Saber et al., 2002), and psychophysical experiments show spatial release from masking in both birds and mammals (Blauert and Lindemann, 1986; Dent et al., 1997; Grantham and Wightman, 1978). Furthermore, there are binaural neurons sensitive to changes in interaural correlation in both owls (Albeck and Konishi, 1995; Konishi, 2007; Saber et al., 1999; 1998), and cats (Louage et al., 2006; Yin et al., 1987). The relative importance of the detection of interaural phase differences versus correlations is the subject of debate (Joris and Yin, 2007), but we assume that, like birds, alligators will show spatial release from masking.

For species that have inherently directional ears, we must ask how this initial directional information is handled during computations by auditory nuclei. Physiological and behavioral data demonstrate the utilization of such cues, but more detailed neuroanatomical and mapping studies are needed. Neuroanatomical studies should further compare biophysical cell type variation and the neuronal architecture of auditory nuclei with birds that have less directional information carried in the auditory nerve. Such information could then be combined with physiological experiments (described above) and head measurements to create a model for information flow. Incorporation of the head measurement, combined with recordings from larger animals, will be important for understanding how the computational circuitry accommodates, albeit gradually, to large changes in head size. Such a model would highlight conserved aspects of sound localization circuitry and how peripheral changes affect neural networks.

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List of Abbreviations

ABR	auditory brainstem response
EPSP	excitatory postsynaptic potential
HRTF	head-related transfer function
ILD	interaural level difference
ITD	interaural time difference

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Highlights

- Physiological and behavioral data support sound localization in crocodilians.
- Directional hearing is affected by skull pneumaticity and coupled eardrums.
- Evolutionary changes in middle-ear pneumaticity affect localization cues.
- Changes in auditory periphery may be reflected in neuroanatomical circuitry

positive values, for comparison with the eardrum directionality. A, B and D are from Bierman et al., 2014.

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Figure 2. Sound localization behavior

A. Tank set-up (Ai, schematic; Aii, video image) with overlaid trace of alligator movement during a single session. Animals started at feeder (red dot) in center of tank. Yellow (Ai) and black (Aii) dots represent moving position of snout tip through time. Blue dots represent time points at which a 1 s, 1 kHz stimulus, tone was played. All tones were played when animal was stationary.

B. Graph of presence of movement within 3 seconds of initiation of stimulus. Significance was tested using a single sample 2-tailed *t*-test. Alligators were tested independently (G1 and G2) and then as sum, in all cases $p < 0.0001$, $n = 406$ from 93 sessions.

C. Graph of the direction of movement for each trial in which movement occurred. Single sample 2-tailed *t*-test analysis showed that movements toward the sound source were strongly preferred ($p < 0.0001$).

Figure 3. Anatomy of the alligator auditory periphery

A. Tympanic membrane and columella dissected from a juvenile American alligator and photographed by D. Inouye; scale bar, 0.25 cm. Tracing from CT scan (B) and tissue section (C) show that the middle ear cavities are connected by passageways dorsal and ventral to the brain case (b).

B. Illustration based on CT data acquired by the University of Texas High-Resolution X-ray CT Facility. Data made available courtesy of Christopher Brochu and DigiMorph.org. The approximant placement of tympanic membranes (t) has been drawn for reference.

C. From Bierman et al., (2014), a single piece of brown suture has been threaded the contiguous airspaces of a thick coronal slice. Dorsal to the brain case connections run through the caudal tympanic recess (ctp) and intertympanic recess (itr). The ventral connection is through the quadrate sinus (qs), pharyngotympanic (Eustachian) recess (ptr) and median pharyngeal recess (mpr). For reference, the columella (c), and earlid (el) have been labeled unilaterally. The top of the images is dorsal. Scale bar, 1 mm.

Figure 4. Auditory nerve anatomy and physiology

A. Sagittal section showing nucleus angularis (NA) bulging into 4th ventricle, nucleus laminaris (NL) and the nucleus magnocellularis (NM).

B. Line drawing of brainstem auditory nuclei at the level of nucleus laminaris (NL) and caudal nucleus angularis (NA). A small portion of rostral NM lies medial to NL.

C. Sensitivities of auditory units as a function of their best frequencies in Caiman. Each dot is one unit. Redrawn from Manley, 1970a.

Figure 5. Physiological range of ITD

A. Interaural delay curves plot the response of nucleus laminaris (NL) neurons as a function of ITD. Four single unit recordings were selected to display a range of different responses, including best frequency and maximum peak-to-trough height. The physiological range of 300 μs measured from laser vibrometry is shown as a grey rectangle. Note one peak response (i.e. maximal firing rate) is inside this range, while others are on the edge, or outside. Redrawn from Carr et al. (2009).

B. Differences between left and right mean phase predicted best ITD, consistent with coincidence detection. Open squares units in left, open circles from right NL, filled symbols neurophonic recordings. From Carr et al. (2009).

Figure 6. Coincidence detection in the alligator embryo

A. Matching diagram and photomicrograph of a transverse section through nucleus laminaris (NL) showing the compact layer of bitufted neurons with input from the ipsilateral nucleus magnocellularis (NM) onto the dorsal dendrites, and input from the contralateral ear via the contralateral NM onto the ventral dendrites. The projection bundles from ipsilateral and contralateral NM were electrically stimulated with electrodes placed on each side of the dendritic layers (red and blue arrows). NA, nucleus angularis, CB, cerebellum. Bar = 500 μm. B. NL neuron labeled with Lucifer Yellow. Note bitufted dendrites. Scale bar, 20 μm.

C. Superimposed curves of response probabilities for 4 NL neurons as a function of the interstimulus interval in alligator embryo brain slice (see Carr et al., 2009). Following the protocol of Funabiki et al. (1998), the mean response probability was calculated by interpolations between individual data points, shown as symbols connected by continuous or dashed lines.

D. NL neuron EPSPs and spikes in response to bilateral stimulation at a delay of 600 μs.