

Inspiring song: The role of respiratory circuitry in the evolution of vertebrate vocal behavior

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Funding information

NIH, Grant/Award Number: NS091977;
NSF, Grant/Award Number: 1755423

Abstract

Vocalization is a common means of communication across vertebrates, but the evolutionary origins of the neural circuits controlling these behaviors are not clear. Peripheral mechanisms of sound production vary widely: fish produce sounds with a swimbladder or pectoral fins; amphibians, reptiles, and mammals vocalize using a larynx; birds vocalize with a syrinx. Despite the diversity of vocal effectors across taxa, there are many similarities in the neural circuits underlying the control of these organs. Do similarities in vocal circuit structure and function indicate that vocal behaviors first arose in a single common ancestor, or have similar neural circuits arisen independently multiple times during evolution? In this review, we describe the hindbrain circuits that are involved in vocal production across vertebrates. Given that vocalization depends on respiration in most tetrapods, it is not surprising that vocal and respiratory hindbrain circuits across distantly related species are anatomically intermingled and functionally linked. Such vocal-respiratory circuit integration supports the hypothesis that vocal evolution involved the expansion and functional diversification of breathing circuits. Recent phylogenetic analyses, however, suggest vocal behaviors arose independently in all major tetrapod clades, indicating that similarities in vocal control circuits are the result of repeated co-options of respiratory circuits in each lineage. It is currently unknown whether vocal circuits across taxa are made up of homologous neurons, or whether vocal neurons in each lineage arose from developmentally and evolutionarily distinct progenitors. Integrative comparative studies of vocal neurons across brain regions and taxa will be required to distinguish between these two scenarios.

KEYWORDS

central pattern generator, CPG, evolution, hindbrain, parabrachial, vocal

1 | INTRODUCTION

Acoustic communication is widespread across animal taxa, functioning to coordinate social behaviors among conspecifics as well as interspecies interactions. Species in all major clades of vertebrates—fish, amphibians, reptiles, birds and mammals—use acoustic signals to send and receive information between individuals. Vertebrate vocalizations are

controlled by rhythm-generating neural circuits located in the hindbrain and spinal cord (Bass, 2014). While the neural mechanisms and anatomical substrates underlying vocalization have been studied in several species, the evolutionary relationships between vocal circuits across vertebrates have not been explored in-depth. Specifically, the degree to which vocal behaviors and neural circuits have shared evolutionary origins or have evolved independently is poorly described.

A variety of evidence from the past several years supports widespread independent evolution of vocal behaviors and the circuits controlling them. First, phylogenetic analysis of tetrapods shows that vocalization likely arose independently across most tetrapod groups >100 mya. (Chen & Wiens, 2020). Second, several unique vocal organs that evolved independently exist across vertebrate taxa, such as the swimbladder of fish, the larynx of frogs, reptiles, and mammals, and the syrinx of birds (Senter, 2008). These vocal organs are all controlled by motor neurons located in the hindbrain and spinal cord. A third finding supporting multiple origins of vocal circuits is the discovery that the motor neurons that control the distinct vocal organs in fish and birds have unique developmental origins from those that control vocalization in frogs and mammals (Albersheim-Carter et al., 2016).

Premotor pattern generating circuits across tetrapods and fish are located in overlapping regions of the hindbrain and spinal cord (Bass, 2014; Bass, Gilland, & Baker, 2008) and homologous relationships between various vocal nuclei have been hypothesized, such as between the parabrachial complex in frogs and mammals and between the nucleus reticulospinalis (nRA) in mammals and retroambiguus in birds (discussed below). Given the recent evidence supporting multiple origins of vocal behavior during evolution, what explains the similarities between vocal hindbrain circuits across distantly related species?

In tetrapods, vocal production requires coordination with respiratory movements and the circuits controlling both behaviors are anatomically intermingled and functionally linked. (Jürgens, 2002, 2009; Wild, 1993, 1997; Wild, Kubke, & Mooney, 2009; Zornik & Kelley, 2007). Does this vocal-respiratory connectivity support the possibility that vocal circuits arose from exaptation of respiratory circuits? If so, known similarities between vocal circuits across taxa may not be due to a single origin of vocal behaviors, but instead to independent co-options of respiratory circuits across taxa. Across vertebrates, respiratory rhythms are generated by central pattern generators (CPGs), neuronal circuits that can autonomously generate rhythmic activity without rhythmic input (Cinelli et al., 2013; Del Negro, Funk, & Feldman, 2018), and these rhythm-generating circuits may have been expanded and functionally modified during vocal evolution (Bass & Baker, 1997). We thus propose potential evolutionary trajectories, in which respiratory circuits may have been repeatedly co-opted independently across taxa.

In this review, we consider phylogenetic, anatomical, and physiological evidence across taxa to explore (a) whether vocal behavior and the underlying circuitry evolved multiple times throughout vertebrate evolution rather than in a single common ancestor, and (b) whether tetrapod vocalization has arisen by repeated co-option of respiratory circuits.

2 | DIVERSE MECHANISMS REFLECT MULTIPLE ORIGINS OF VOCAL BEHAVIOR

2.1 | Vertebrate vocal phylogeny

A recent phylogenetic analysis investigated the number of times acoustic communication evolved in tetrapods. Chen and Wiens (2020) explored existing publications and databases to sample the presence of vocalization across a diverse set of ~1,800 tetrapod species. Their maximum-likelihood ancestral-state reconstruction analyses revealed that vocal behavior likely has separate origins in amphibians, non-avian reptiles, birds, and mammals (Figure 1). Below, we describe a wide range of peripheral sound production mechanisms across tetrapods and fish, which further reflects independent evolution of vocal behavior.

2.2 | Vertebrate vocal organ diversity

2.2.1 | Larynx

Vertebrate acoustic communication employs a variety of sound-producing organs (Senter, 2008). In most tetrapods, vocalizations depend on modulated respiratory movements and a specialized vocal organ. In frogs, non-avian reptiles and mammals, vocal signals are generated by the larynx. The larynx is a valve in the trachea first evolved to prevent food or drink from entering the lungs (Negus, 1949). While its original function was, therefore, related to respiration, it has been modified in many taxa to allow air movement (usually expiratory) to produce sound by vibrating vocal folds. The larynx is a structure connected to the vocal cords and made up of several conserved cartilages, including the cricoid and arytenoid cartilages, as well as the thyroid cartilage in mammals (Negus, 1949). Depending on species, varying numbers of intrinsic muscles connect the cartilages together while extrinsic muscles connect the cartilages to surrounding structures. These muscles are homologous across mammals, reptiles, and frogs (Kingsley et al., 2018), suggesting that the larynx is a highly conserved structure.

2.2.2 | Syrinx

While also possessing a larynx that regulates respiration, birds evolved a dedicated vocal organ, the syrinx, that acts as a valve to regulate airflow and vocalization (Clarke et al., 2016; Kingsley et al., 2018; Riede & Goller, 2010). The syrinx is located at the junction between the trachea and bronchi inside the interclavicular air sac (Kingsley et al., 2018) with sound produced by vibrations of paired

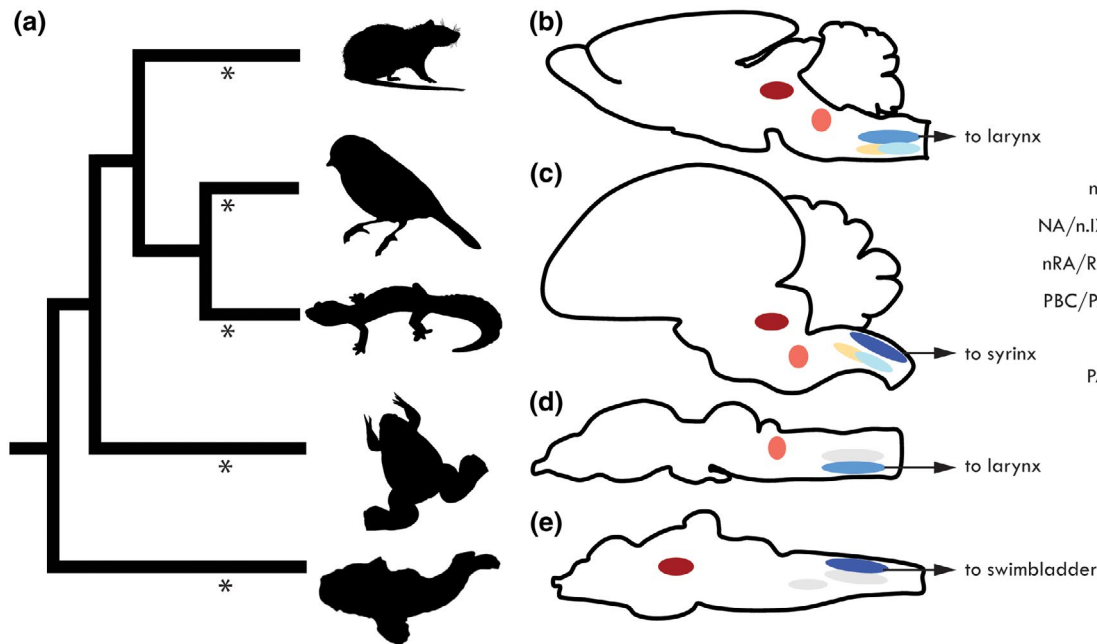


FIGURE 1 Evolution and diversity of vocal mechanisms in vertebrates. (a) Vertebrate phylogenetic tree depicting relationships between mammals, birds, non-avian reptiles, amphibians and bony fish (from top to bottom). Phylogenetic evidence suggests that vocal behaviors evolved independently at least once in each of these clades (indicated by asterisks). (b–e) Proposed homologous vocal nuclei in mammals (b), birds (c), amphibians (d) and fish (e). Sagittal view brain diagrams (anterior is left, dorsal is up) illustrating motor and premotor brain regions involved in vocal pattern generation. Gray ovals in (d) and (e) represent vocal regions in frogs (inferior reticular formation, Ri) and fish (pacemaker and prepacemaker nuclei) with uncertain relations to bird and mammalian nuclei. Abbreviations: n.XII, hypoglossal nucleus; NA, nucleus ambiguus; n.IX–X, cranial motor nucleus IX–X; nRA, nucleus retroambiguus; RAm, nucleus retroambigualis; PBC, pre-Bötzinger complex; PAm, nucleus parambigualis; PB, parabrachial complex; PAG, periaqueductal gray

labial structures located at the terminus of each bronchial tube (reviewed in Suthers & Zollinger, 2004). Cartilaginous tracheal rings and paired bronchial half-rings that ossify with age make up the structure of the syrinx and are connected by soft connective tissues that vibrate to produce sounds (reviewed in Düring & Elemans, 2016). This novel sound-producing organ may have evolved to take advantage of the resonating capacity of the longer trachea of birds compared to other tetrapods (Riede, Thomson, Titze, & Goller, 2019).

The syrinx is controlled by intrinsic and extrinsic muscles that vary across bird species—most birds possess two pairs of extrinsic muscles; basal birds often have no or few intrinsic muscles while songbirds have up to eight bilateral pairs of intrinsic muscles (reviewed in Düring & Elemans, 2016). While the developmental origins of the syrinx are distinct from those of the larynx, the syrinx shares many functional features such as gating and regulating airflow to the upper respiratory tract during vocalization. The muscles of the larynx and syrinx may share a developmental origin because they are both derived from occipital somites (Bass et al., 2008; Huang, Zhi, Izpisua-Belmonte, Christ, & Patel, 1999; Noden & Francis-West, 2006). However, only two muscles that control the syrinx have homologs in non-avian species, and these

muscles control the tongue and pectoral fins, suggesting that there is not homology between the syrinx and larynx (Kingsley et al., 2018).

2.2.3 | Swimbladder

Do fish vocalize as well? Bass et al. (2008) expanded the definition of vocalization to encompass sound-producing organs in bony fish. Bony fish have diverse sound production mechanisms, including pectoral fin vibrations, head-dependent mechanisms, and swimbladder vibrations (Ladich & Winkler, 2017). Swimbladder vibrations in toadfish are the most extensively studied of these mechanisms. In toadfishes, acoustic signals are generated by vibrations of their gas-filled swimbladder, an organ originally used for buoyancy (Alexander, 1966) and controlled by contraction of a single pair of intrinsic muscles. These vocalizations do not involve respiratory movements (Ladich & Fine, 2006).

While the sound-generating swimbladders evolved independently of the larynx and syrinx, intrinsic muscles of all three organs derive from occipital somites (Tracy, 1959). Thus, the larynx, syrinx, and swimbladder in tetrapods and fish are innervated by motor neurons that originate in the caudal hindbrain (Bass et al., 2008). We next explore the

similarities and differences of motor pools that control these independently evolved vertebrate vocal organs.

2.3 | Vertebrate vocal motor neuron diversity

Although vocal behaviors arose independently across vertebrate taxa, the motor pools controlling intrinsic muscles of most vertebrate vocal organs (including swimbladder, larynx and syrinx) originate in the same compartment of the embryonic hindbrain, rhombomere 8 (Bass, 2014; Bass & Baker, 1997; Bass et al., 2008). In mammals, motor neurons innervating the larynx reside in nucleus ambiguus (NA; Jürgens, 2009). Because hindbrain cranial nerves and motor nuclei are highly conserved across tetrapods, it has long been assumed that laryngeal motor pools in cranial nerve nucleus (n.) IX-X in frogs and reptiles comprise an NA homolog. In birds, syringeal motor neurons reside in a possible homolog of the mammalian hypoglossal nucleus (n.XII). Due to their dorsal midline location in the caudal brainstem, vocal motor neurons innervating the toadfish swimbladder via the occipital nerve are also potential homologs of n.XII. While ample anatomical evidence indicated homology of cranial motor nuclei across vertebrates, molecular evidence had been lacking.

Albersheim-Carter and colleagues (2016) used molecular staining techniques to test if vocal motor neurons in tetrapods and fish have shared developmental origins. They found that expression patterns of transcription factor *Phox2b* were conserved in the hindbrain of frogs, mice, birds and fish. *Phox2b* is expressed in NA/n.IX-X in all species tested, but not in n.XII. These results support the homology of NA/n.IX-X across fish and tetrapods, as well as the homology of n.XII across species. What do their findings suggest about the shared origins of vocal motor neurons? Vocal motor neurons of frogs and mice are located in NA and express *Phox2b*; in contrast, vocal motor neurons in birds and fish are located in n.XII and lack *Phox2b* expression. These results indicate that the developmental, and therefore, evolutionary, origins of vocal motor neurons likely vary between some vertebrates (Figure 1). For example, motor neurons controlling vocal organs in mammals and birds are anatomically and developmentally distinct, supporting their independent origins. However, amphibian and mammalian motor neurons may share their development origins, though phylogenetic findings indicate these motor pools were co-opted for vocal purposes in separate evolutionary events.

3 | CENTRAL CONTROL OF VOCALIZATION: HINDBRAIN VOCAL CENTRAL PATTERN GENERATORS

The existing phylogenetic evidence of vocal behavior, diverse sound production organs, and distinct motor neuron

control in tetrapods and fish strongly support the hypothesis that vocal behavior evolved independently across different groups during vertebrate evolution. However, the premotor circuits controlling vocalization have many similarities. For instance, the motor neurons and many of the premotor neurons driving vocal motor output in toadfish, birds, frogs, reptiles and mammals originate in rhombomere 8 (Cambronero & Puelles, 2000; Kennedy, 1981; Kitamura, Okubo, Ogata, & Sakai, 1987; Straka, Baker, & Gilland, 2006). Considering this anatomical evidence, Bass and colleagues (2008) hypothesized that vocal circuitry across vertebrates originated in the same hindbrain compartment. They suggested that rhythm-generating circuits that arose in the protochordate-vertebrate transition, and served to drive newly evolved rhythmic behaviors like gill respiration, were later exapted to enable subsequent innovations including vocal behaviors. However, they did not directly explore whether vocal circuits were ancestral across all vertebrates or whether they arose multiple times.

Phylogenetic evidence now suggests these existing circuits were co-opted for vocal production independently across taxa. We propose that respiratory control nuclei were co-opted repeatedly as vocal behavior evolved independently in each group. What remains unclear is whether the same circuits were repeatedly co-opted, or whether different (but anatomically adjacent) circuits were harnessed during each vocal innovation. Below, we describe several vocal and respiratory nuclei with possible homologies across taxa.

3.1 | Integration of vocal and respiratory circuits in tetrapods

Because vocal production in tetrapods is primarily driven by respiratory movements, tetrapod vocal circuits are necessarily coupled with respiratory pattern generating neurons. Further, sounds produced by the syrinx and most larynges do not simply depend on normal respiratory-related air flow, but require increased air pressure to generate appropriate sounds (Gans, 1973; Riede et al., 2019). Therefore, vocal circuit activity must be precisely integrated with respiratory circuitry. The neuronal circuitry that generates respiratory rhythms is distributed along the full length of the hindbrain, including the ventral respiratory column (VRC) that extends from the caudal medulla to the pons (Del Negro et al., 2018).

Below, we first briefly summarize the vocal circuits of mammals, amphibians, and birds, and then, describe comparative anatomy, physiology and gene expression patterns of several vocal and respiratory regions located throughout the hindbrain.

3.2 | Tetrapod vocal and respiratory circuits

All vertebrate vocal motor neurons are located within the caudal hindbrain and/or rostral spinal cord. Frog and

mammalian laryngeal motor neurons are located in nucleus ambiguus (NA) and innervate the larynx via the vagus nerve (Figure 1). Hindbrain premotor circuits generate vocal patterns and temporally coordinate activation of the relevant muscles. In mammals, vocal motor neurons in NA receive descending inputs from several nuclei in the hindbrain, including the parabrachial-Kölliker-Fuse complex (PB), nucleus retroambiguus (nRA) and several nuclei in the reticular formation extending from the lateral pontine reticular formation to the lateral caudal medullary reticular formation (Jürgens, 2002, 2009). All of these nuclei receive descending inputs from the periaqueductal gray (PAG; Jürgens, 1994; Figure 1). In frogs, vocal motor neurons in NA receive direct inputs from a PB homolog, which in turn receives descending inputs from the extended amygdala (Figure 1; Hall, Ballagh, & Kelley, 2013; Schmidt, 1992; Yamaguchi, Barnes, & Appleby, 2017; Zornik & Kelley, 2007). To our knowledge, no existing evidence has identified a PAG homolog in frogs. Birds have vocal motor neurons in a specialized tracheosyringeal compartment of the hypoglossal nucleus n.XII (XIIIts) that innervate the syrinx via cranial nerve XII (Figure 1; Arnold, Nottebohm, & Pfaff, 1976). XIIIts receives inputs from respiratory centers in the medulla, such as parambigualis (PAm), which controls inspiration, and premotor nucleus retroambiguus (RAm), which controls expiration and is a putative homolog of the mammalian nucleus retroambiguus (Wild, 1993, 1997; Wild et al., 2009). In songbirds and non-songbirds alike, these vocal nuclei receive inputs from the medial nucleus of the intercollicular complex (DM), which is a likely homolog of the PAG (Kingsbury, Kelly, Schrock, & Goodson, 2011).

3.2.1 | Retroambiguus and retroambiguus

At the most caudal extent of the VRC is the nucleus retroambiguus (nRA) in mammals and retroambiguus (RAm) in birds (Wild et al., 2009). Anatomical and physiological studies revealed that nRA neurons receive inputs from a variety of respiratory and vocal nuclei, including the Bötzing and pre-Bötzing complexes, the PB complex, and the PAG. Neurons in nRA send direct projections to laryngeal and pharyngeal motor pools, and appear to serve as the “final common pathway” for vocal production in mammals (Holstege, 1989; Tschida et al., 2019). In cats, activating some parts of nRA induced vocalizations, but did not change inspiration; stimulation in other areas of nRA did not induce vocal output, but instead increased respiration rates by shortening both inspiratory and expiratory phases (Subramanian & Holstege, 2009). The authors interpret these results to indicate that subsets of premotor neurons in nRA control distinct behaviors, such as vocalization, respiration, and coughing. The apparent nRA homolog in birds, RAm, also appears to mediate expiration

and coordinate breathing and vocalization. Neurons in RAm receive inputs from upstream nuclei including DM, the functional equivalent to the mammalian PAG, and project to expiratory motor nuclei in the spinal cord to control air pressure needed for song, as well as XIIIts to coordinate vocalizations (Wild et al., 2009; Figure 1). A subset of the RAm neurons send bilateral inhibitory projections to motor neurons in XIIIts (Kubke, Yazaki-Sugiyama, Mooney, & Wild, 2005), while other RAm neurons provide excitatory inputs (Sturdy, Wild, & Mooney, 2003).

Thus, although birds and mammals have distinct vocal organs for generating vocalizations, they appear to share a critical premotor nucleus with related function, albeit via projections to distinct targets. Because birds and mammals likely evolved vocal behaviors independently (Chen & Wiens, 2020), these results suggest that a homologous hindbrain nucleus (nRA/RAm) was co-opted independently in each lineage.

3.2.2 | Parambigualis and pre-Bötzing complex

While RAm appears to regulate expiratory-dependent vocal output in birds, the region just anterior to it, nucleus parambigualis (PAm), controls inspiration (Ashmore, Renk, & Schmidt, 2008; Reinke & Wild, 1997, 1998). PAm neurons are active across all phases of the respiratory cycle, but are predominantly active immediately prior to and during inspiration (McLean, Bricault, & Schmidt, 2013). PAm neuron activity is likely to be tightly coordinated with vocal circuit activity to ensure proper timing of inspiratory phases during songs and calls (reviewed in Schmidt & Goller, 2016). Consistent with a role in vocal production, some PAm neurons that were not active during breathing were active during stimulation-induced calling in anesthetized birds (Ashmore et al., 2008). Interestingly, some PAm neurons project to a thalamic nucleus, Uva, which in turn sends feedback signals to the primary nucleus associated with song learning and production, HVC (Ashmore et al., 2008; Coleman & Eric, 2005; Nottebohm, Kelley, & Paton, 1982; Reinke & Wild, 1998; Schmidt, 2008). Thus, PAm may play a role in vocal production by virtue of its ability to modulate upstream cortical vocal circuits.

McLean et al. (2013) proposed that the rostral portion of PAm may be homologous to the pre-Bötzing complex (PBC), which is an essential component of the respiratory central pattern generator in mammals, with activity largely associated with inspiration. In both PBC and PAm, there is a large proportion of neurons associated with inspiration (Connelly, Dobbins, & Feldman, 1992; McLean et al., 2013; Schwarzacher, Smith, & Richter, 1995). Furthermore, the PBC and PAm both send projections to n.XII (Borgmann

et al., 2011; Reinke & Wild, 1998). Neurons in PBC also project throughout the ventral respiratory column, throughout the hindbrain, as well as to the pons, midbrain and thalamus (Yang & Feldman, 2018). Such widespread projections may serve many functions, including coordinating and facilitating the modulation of respiratory patterns. An intriguing possibility is that, like PAm, the PBC may also provide feedback information from the hindbrain to the cortex via thalamic relays. If so, we speculate that PBC and PAm may both play important roles in regulating the production of complex, learned vocalizations.

3.2.3 | Pons and parabrachial area

While circuits in the caudal brainstem are essential for respiratory rhythmogenesis, anterior hindbrain areas have also been implicated in modulating respiratory output. For instance, the pontine parabrachial-Kölliker-Fuse complex (PB) is a central component of the respiratory circuitry. The region has long been implicated in respiratory phase switching based on studies in which PB lesions led to prolonged inspiration (Lumsden, 1923). Neuronal recordings in breathing rats revealed PB neurons that are active exclusively during expiration or inspiration, as well as neurons active during both respiratory phases (Song, Yu, & Poon, 2006). Stimulation studies in dogs showed that respiratory phase durations could be increased or decreased depending on the timing of stimulation within each respiratory cycle (Zuperku, Stucke, Hopp, & Stuth, 2017). PB also receives somatosensory feedback from the lungs and larynx via the nucleus of the solitary tract and other nuclei (e.g., Ezure, Tanaka, & Miyazaki, 1998; Farley, Barlow, & Netsell, 1992; Feldman & Gautier, 1976; Jürgens, 2002), which may contribute to its control of respiratory phase switching. Given the role of PB in regulating respiratory phases, and the dependence of tetrapod vocalization on airflow, it is a likely region to be co-opted during vocal evolution. PB does appear to play an essential role in vocalization in mammals.

PB projects to motor nuclei containing both respiratory and vocal neurons, including NA, and the trigeminal and hypoglossal cranial nerve nuclei (Song, Wang, Hui, & Poon, 2012). In monkeys, extracellular recordings in PB revealed neurons that are active during vocalizations, while stimulating areas near PB could induce species-typical calls (Kirzinger & Jürgens, 1991; Lütke, Häusler, & Jürgens, 2000). Other areas ventral and lateral to PB in the pontine brainstem have neurons with activity correlated with frequency-modulated calling (Hage & Jürgens, 2006). Cat vocalizations are also associated with single unit activity in PB (Farley et al., 1992). In horseshoe bats, the PB controls the timing of vocal patterns (Smotherman, Kobayasi, Ma, Zhang, & Metzner, 2006). Iontophoresis of GABA agonists increased

the duration of expiration and calls, while iontophoresis of GABA antagonists decreased expiration and call duration, and eliminated multisyllabic calls. These results suggest that inhibitory GABAergic transmission regulates PB-mediated respiratory phase switching during vocalization, perhaps by delaying the activity of neurons that terminate expiratory phases of vocal production (Smotherman et al., 2006). While electrophysiological recordings have not been made in bats, immunohistochemical staining of the immediate early gene cFos showed high PB expression in stationary, echolocating bats compared to silent and listening bats (Schwartz & Smotherman, 2011).

PB has a conserved role in controlling respiration and vocalization across taxa, and evidence supports its involvement in fish, frogs, and birds in addition to mammals, (Figure 1; Browaldh, Bautista, Dutschmann, & Berkowitz, 2016; Dick, Bellingham, & Richter, 1994; Forster et al., 2014; Murakami et al., 2004; Tomás-Roca, Corral-San-Miguel, Aroca, Puellas, & Marín, 2016; Wetzell, Haerter, & Kelley, 1985; Wetzell, Kelley, & Campbell, 1980; Wild, Arends, & Zeigler, 1990; Yokota, Oka, Tsumori, Nakamura, & Yasui, 2007; Zornik & Kelley, 2007; Zuperku et al., 2015). In the lamprey, a basal vertebrate that does not vocalize, respiration is driven by the paratrigeminal respiratory group (pTRG; Cinelli et al., 2013). This nucleus is a likely PB homolog because of its pontine location and its rhythmically active glutamatergic neurons that project to respiratory motor nuclei of the facial (VII), glossopharyngeal (IX) and vagal (X) cranial nerves (Gariépy et al., 2012). In birds, PB projects to both premotor (PAm and RAm) and motor (NA and XIIts) nuclei that coordinate breathing and vocalization (Reinke & Wild, 1997, 1998; Wild et al., 1990). The frog PB also plays a key role in regulating vocalization and respiration (Schmidt, 1992; Zornik & Kelley, 2011). In *Rana pipiens*, stimulating PB results in fictive calling, while lesions prevent calling (Schmidt, 1992).

In the PB of several *Xenopus* species, vocal premotor neurons burst rhythmically during fictive vocalization (Barkan, Kelley, & Zornik, 2018; Zornik & Yamaguchi, 2012) and send glutamatergic projections to respiratory and vocal motor neurons (Zornik & Kelley, 2008). The role of PB in *Xenopus* is interesting because unlike other frogs, they call underwater, and generate sounds without air movements (Kwong-Brown et al., 2019). However, *Xenopus* evolved from terrestrial frogs whose vocal mechanisms likely involved respiratory movements, suggesting that PB was first co-opted in frogs for air movement-based call production, and maintained its vocal role in *Xenopus* even though breathing and calling mechanisms became uncoupled (Zornik & Kelley, 2007, 2008).

Thus, PB may be an ancient nucleus whose role in respiration has been conserved across vertebrates. Although PB appears to also be involved in vocal production in all tetrapods studied so far, this is unlikely an ancestral trait

given that vocal behaviors appear to have evolved independently in mammals, birds, reptiles and amphibians (Chen & Wiens, 2020). Instead, it is possible that because of the close association of PB with respiratory circuits and projections to caudal hindbrain motor nuclei, it was well suited to be co-opted multiple times during parallel tetrapod vocal evolution.

In order to support any of these proposed hypotheses and homologies, additional physiology experiments to characterize shared function of vocal circuits paired with gene expression analyses to identify potentially homologous nuclei and neurons will need to be performed across a diverse set of vocal and non-vocal vertebrates.

4 | PROPOSED ORIGINS OF VOCAL CIRCUITS ACROSS FISH AND TETRAPODS

The primary goal of this Review was to explore the overlapping nature of premotor circuits controlling diverse vocal

motor systems across fish, amphibians, birds and mammals. Phylogenetic analysis of vocal and non-vocal tetrapods supports several independent, but ancient, origins of vocal behaviors (Chen & Wiens, 2020). This is further corroborated by the numerous unique vocal organs and peripheral sound-production mechanisms across vertebrates. Despite these independent evolutionary innovations, vocal circuits share many characteristics. Most motor neurons that control vertebrate vocal organs originate in the caudal hindbrain, though they do not all share the same genetically defined developmental origins across vertebrates (Albersheim-Carter et al., 2016; Bass et al., 2008). Motor neurons, in turn, receive inputs from nearby premotor circuits.

As described above, tetrapods share multiple regions of the medulla and pons that are associated with both respiration and vocalization. This overlap of central circuitry is not surprising given that most tetrapod vocal sound production mechanisms involve airflow-induced vibrations (Kingsley et al., 2018). Because it is likely that this similar pattern of overlap between respiratory and vocal circuits evolved multiple times, what forces drove this pattern of repeated parallel

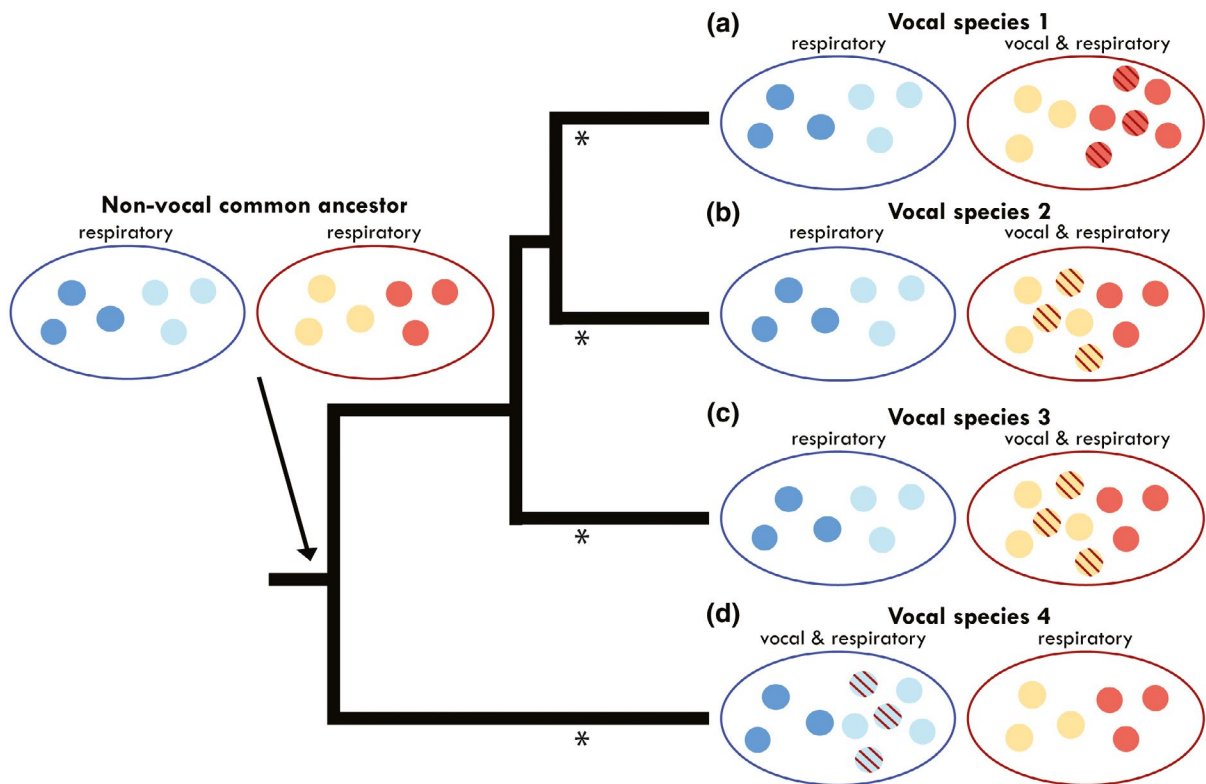


FIGURE 2 Hypothetical respiratory circuits co-opted for vocal production. The evolution of vocal circuits may have resulted from the expansion and functional diversification of respiratory circuitry present in a non-vocal common ancestor of all extant tetrapods; diagram illustrates two hypothetical respiratory nuclei (ovals), each containing two developmentally distinct neuronal subtypes identifiable via expression of distinct genetic markers (indicated by light and dark shaded circles). Vocal neurons may have arisen in the same nuclei independently in two species, albeit via elaboration and functional divergence of developmentally distinct cell types (e.g., vocal species 1 and 2 in (a) and (b); newly evolved vocal neurons with diagonal stripes). Alternatively, two species might independently co-opt the same neuronal populations (e.g., vocal species 2 and 3 in b and c). Finally, different species may co-opt neurons in distinct respiratory nuclei (vocal species 1–3 in a–c, versus vocal species 4 in d)

evolution? Air breathing was a necessary innovation to allow the transition to living on land, and it is possible that the respiratory airways served as the most convenient means of sound production across all tetrapods, allowing repeated evolution of air-driven sound production.


A reasonable hypothesis is that because of the inherent rhythmicity of respiratory networks, they were poised to be co-opted independently during vocal evolution in each taxon. In this evolutionary trajectory, multiple scenarios for circuit exaptation are plausible. One possibility is that the same respiratory nucleus may be co-opted for vocal production in two independent events across lineages, but the developmental origins of the newly evolved vocal neurons may differ (Figure 2a,b). Alternatively, developmentally homologous neurons may be co-opted independently, providing an example of convergent evolution (Figure 2b,c). Finally, two lineages may evolve vocal circuits by exapting distinct respiratory nuclei (Figure 2c,d). Because vocal and respiratory circuits are found along the length of the hindbrain, it is possible that some combination of each of these scenarios may be found for each cross-taxon comparison. Finally, while some vocal co-option events are ancient, others may have occurred more recently; identifying the exact trajectories will require investigations of many species within each clade of interest.

Identification of developmentally important genes, such as transcription factors, in vocal and respiratory neurons is required to distinguish between the scenarios described above. For example, determining whether the same subtypes of respiratory neuron homologs have been co-opted repeatedly, or whether distinct cell types were harnessed in each lineage will require rigorous description of the genetic expression of developmental markers of each vocal neuron subtype. Such approaches are regularly applied within single species to understand the origins of distinct cell types within or between different regions of the nervous system, such as identification of factors associated with the development of mouse spinal neurons (Sweeney et al., 2018). However, studies addressing the developmental origins of central pattern generator neurons across species are rare. Other promising techniques include single-cell mRNA sequencing, which has allowed the identification of putatively conserved forebrain cell types between reptiles and mammals (Tosches et al., 2018), and “slide-seq,” which maps RNA sequences with high resolution onto brain slices (Rodrigues et al., 2019). Hoke and colleagues (2019) have recently proposed that investigations of behavioral and neural circuit evolution will benefit from adopting the lens of evolutionary developmental biology (evo-devo) approaches and concepts. We propose that combining evo-devo concepts with gene expression, physiological and anatomical methods across vertebrate species and developmental stages will help identify homologous and non-homologous vocal circuits across taxa.

ACKNOWLEDGMENTS

E.Z. received funding from the National Institutes of Health (NS091977) and the National Science Foundation (1755423). We thank two anonymous reviewers for helpful comments on the manuscript.

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How to cite this article: Barkan CL, Zornik E.

Inspiring song: The role of respiratory circuitry in the evolution of vertebrate vocal behavior. *Develop Neurobiol.* 2020;00:1–11. <https://doi.org/10.1002/dneu.22752>