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# S01-5 Evolution of brain structures for vocal learning in birds: a synopsis

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**Abstract** Vocal learning, the ability of animals to reproduce vocalizations, is found in only three mammalian groups (humans, cetaceans and bats) and three avian groups (parrots, hummingbirds and songbirds). Surprisingly, learners in each of the mammalian or avian groups are not closely related, and have close non-learning vocal relatives, suggesting that vocal learning evolved independently in each. Yet, behaviorally driven gene expression and other studies have identified in all vocal-learning birds seven remarkably similar telencephalic brain structures that are not found in vocal non-learners. These findings suggest that within the past 65 million years, three out of *c*. 30 avian orders evolved seven similar brain structures for a complex behavior, and that the evolution of such brain structures is under strong epigenetic constraints. Alternatively, vocal learning and the associated brain structures might have been present in a common ancestor, followed by major independent losses, again suggesting strong epigenetic constraints affecting this complex function. Here I review findings on the evolution of vocal learning and associated brain structures in birds, and consider their potential relevance to understanding vocal learning in general.

**Key words** Songbird, Parrot, Hummingbird, Neurobiology, Language, Constraints

## 1 Introduction

Vocal learning, the ability to imitate or improvise upon sounds, is rare, found in only six animal groups: three of the some 30 avian orders (parrots, hummingbirds, and songbirds) and three of the 28 or so mammalian orders (humans, bats, and cetaceans) (Nottebohm, 1972; Jarvis et al., 2000). Auditory learning, upon which vocal learning depends (Konishi, 1965), is merely the ability to form memories of the sounds heard and is present in all vertebrates that have been tested. For example, pet animals can acquire the meaning of sounds "sit" (in English) or "sientese" (in Spanish) through auditory learning even though knowledge of human words is not in their innate auditory repertoire. However, a pet cannot imitate and produce these sounds, which humans, parrots, and some songbirds can. Most vocal learners, nevertheless, imitate sounds of only their own species.

Nottebohm (1972) proposed that songbirds, hummingbirds, and parrots evolved their vocal learning abilities independently, because the closest relatives of each were vocal non-learners like more distantly related orders, such as chickens (Fig. 1A). Species of non-learning orders produce only innate vocalizations, presumably the ancestral condition. A similar case can be made for independent evolution of vocal learning in mammals. Nottebohm and colleagues also discovered that songbirds and parrots have cerebral brain nuclei that control vocal learning, and that some of these cerebral nuclei are similar to one another (Nottebohm et al., 1976; Paton et al., 1981). Vocal non-learners possess only midbrain vocal nuclei, which are involved in the production of innate vocalizations (Kroodsma and Konishi, 1991).

Accordingly, Brenowitz (1991) proposed that, similar to the behavior they control, the cerebral vocal nuclei of songbirds and parrots evolved independently though with some shared properties. Jarvis and colleagues used behavioral-molecular approaches to reveal an entire set of cerebral vocal structures in hummingbirds (Jarvis and Mello, 2000), as well as additional structures in songbirds and parrots (Jarvis and Nottebohm, 1997; Jarvis et al., 1998; Jarvis et al., 2000). These findings led to the novel conclusion that all three vocal-learning bird groups have a similar set of seven cerebral vocal brain nuclei (Fig. 1B), and produced hypotheses for how such similarities could have evolved (Jarvis and Mello, 2000). Herein I present (1) a comparative synopsis of cerebral vocal nuclei in the three vocal-learning bird groups, and (2) three alternative hypotheses to explain the evolution of vocal learning and associated cerebral vocal nuclei.

## 2 Cerebral vocal nuclei of vocal-learning birds

Most information on vocal cerebral nuclei comes from songbirds and the least from hummingbirds. The main techniques used to identify and study these nuclei are lesions, electrophysiological activity, and behaviorally-driven gene expression. Only behaviorally-driven gene expression has been used effectively in all three vocal-learning bird groups; and accordingly I focus on results from this approach.

### 2.1 Behaviorally-driven gene expression

This approach, otherwise known as behavioral molecular mapping, is a brain-imaging like procedure for animals performing a particular behavior or processing a particular stimulus. Brain electrical activities processing these behavior(s) activate gene expression. Products of that expression accumulate in brain areas responsible for the behavior and can be imaged in brain sections. The most useful gene examined in vocal-learning birds has been ZENK (Mello and Clayton, 1994; Jarvis and Nottebohm, 1997), a protein that binds to the DNA regulatory regions of select genes. Its molecular function enhances or represses transcription of mRNA. Hence, ZENK is a transcription factor. The method used to detect mRNA synthesis is called in-situ hybridization. The half-lives of the mRNA and protein are short, thus the accumulation and presence of the gene product is short-lived at less than 30 minutes. ZENK expression, however, does not always relate to neuron electrical activity. In many areas of the thalamus and in primary sensory neurons of the cerebrum (avian auditory L2), ZENK is not synthesized, regardless of brain activity.

To image ZENK expression and thus learned vocal behavior, individual birds were separated into three groups (Jarvis and Nottebohm, 1997): (1) Silent Controls — birds kept in quiet conditions without hearing or singing for a minimum of 60 minutes; (2) Hearing Only — birds exposed for ~30 minutes to conspecific singing either from live birds or taped recorded playbacks, but without singing themselves; and (3) Hearing and Vocalizing — birds singing for ~30 minutes and by default hearing themselves or other birds. The latter two treatments required at least 60 minutes of silence prior involving the subjects with song, in order to minimize effects from prior behaviors. Birds are sacrificed immediately after each session and their brains processed for detection of ZENK mRNA expression.

In songbirds, we identified a vocal nucleus not revealed by other methods, calling it "HVo-like" for its similarity to the HVo vocal nucleus of a parrot (Jarvis et al., 1998). In parrots, where other methods had showed only parts of vocal nuclei, we uncovered the complete morphology of those nuclei, as well as additional structures (Jarvis et al., 2000). Multi-taxon comparison then became a real possibility when we found that hummingbirds possessed a full set of hearing and vocal-active brain structures too (Jarvis and Mello, 2000) (Fig. 2).

In all three groups, hearing- and vocalizing-driven ZENK expression was found in separate discrete cerebral

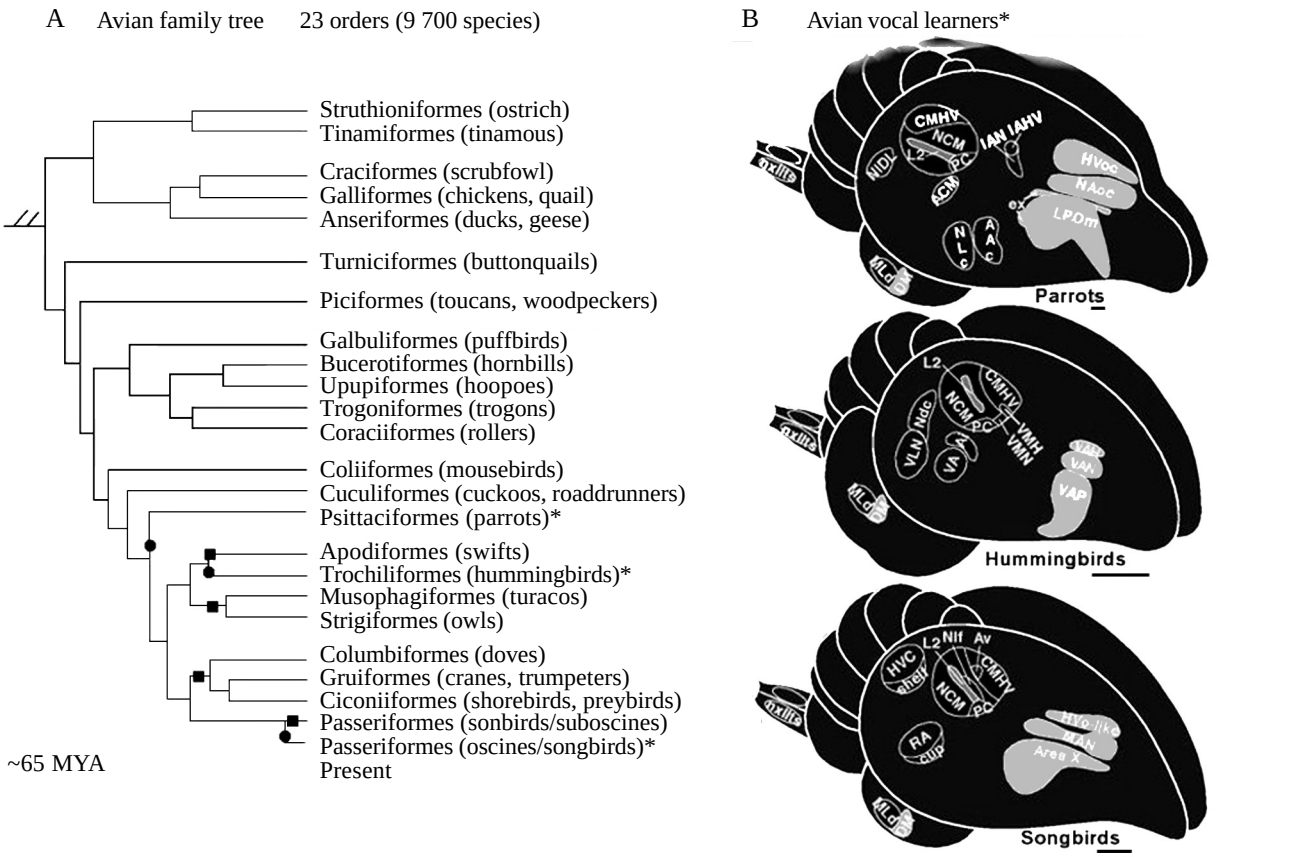


**Fig. 1 A: phylogenetic relationships of living birds, after Sibley and Ahlquist (1990), with vocal learners italicized; B: semi-3D view of auditory (black) and the seven cerebral vocal areas (light and dark gray) in vocal learning birds**

Modified from Jarvis et al. (2000). The three light gray-labeled cerebral vocal nuclei are in nearly identical locations in all three vocal learner groups and absent in vocal non-learners. The four dark gray cerebral vocal nuclei are in different locations across vocal learning orders, but in the same brain subdivisions relative to one another, and are also not found in vocal non-learners. All abbreviations are explained in Jarvis et al. (2000). Scale bars 1 mm.

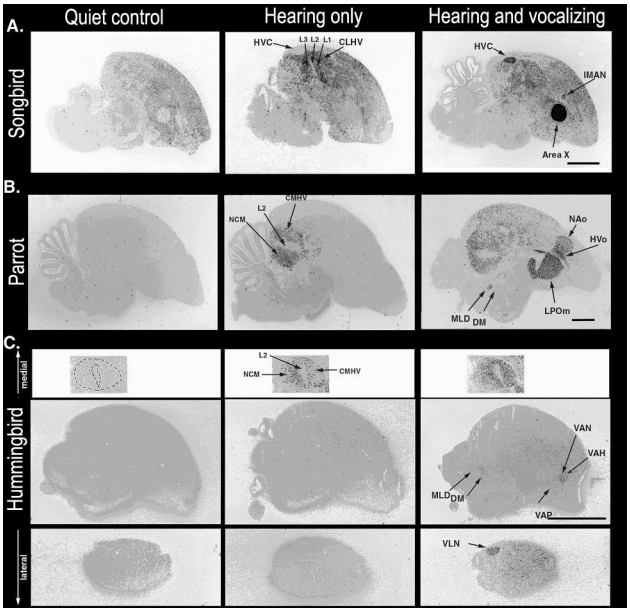


**Fig. 2 Hearing and vocalizing-driven ZENK expression in songbirds (canary), parrots (budgerigar) and hummingbirds (*Aphantochroa*)**

Shown are dark-field views of parasagittal brain sections hybridized to a radioactively-labeled canary ZENK riboprobe (black silver grains) and counterstained with cresyl violet (gray). Images are from one representative bird of each group. In the songbird brain, NCM, CMHV and the major part of HVo are not shown, as they are more medial. In the parrot brain, NLc, AAc, lAN, and lAHV are not shown, as they are located much more laterally. In the hummingbird brain, different medial-to-lateral planes are shown. For explanation of acronyms, see Jarvis et al. (2000). Scale bars 1 mm.

and non-cerebral brain areas (Figs. 1B, 2). The hearing-activated areas occurred in the posterior part of the cerebrum, and in almost the same position in all three vocal-learning groups. These brain areas form an auditory pathway found in both vocal-learning and vocal non-learning birds that begins in hair cells in the ear. They send their axons to cochlear nuclei in the brainstem, and from there to MLd of the midbrain, to Ov of the thalamus, to the primary auditory cells of the cerebrum (L2), and to secondary and tertiary auditory cells (L1, L3, NCM, CMHV, HVC shelf and RA cup) (Vates et al., 1996) (Fig. 1B, white structures; ZENK expression shown in Fig. 2).

The seven vocal-activated areas in the three orders of vocal-learning birds fall into two positional groups: (1) three in nearly identical locations in the anterior cerebrum (Fig. 1B, light gray regions; Fig. 2), though each with different shapes; and (2) four in different locations of the posterior cerebrum, but still within the same brain subdivisions relative to one another and with similar shapes to some of them (Fig. 1B, dark gray regions). The position of the four posterior nuclei relative to the auditory areas differs in accord with the presumed age of each avian order. In parrots, presumably the oldest vocal-learning order, the posterior vocal nuclei are located away from the auditory areas; in the next oldest, hummingbirds, they are adjacent to auditory regions; and in songbirds, the most recent, they are embedded within the auditory areas.

### 2.2 Posterior vocal nuclei

In songbirds, the posterior vocal nuclei are part of a posterior vocal pathway that connects nucleus NIf→HVC→RA to the vocal nucleus of the midbrain (DM), as well as to sets of brainstem nuclei (nXIIts) that control syringeal muscles and respiratory muscles during vocalization. When the HVC and RA are bilaterally lesioned in a songbird, it is unable to produce learned song (Nottebohm et al., 1976, Simpson and Vicario, 1990). Innate vocalizations, however, are retained. After the NIf is lesioned, the bird can produce most learned syllables; but syntax, the ordering of learned vocalizations, is affected and becomes more variable (Hosino and Okanoya, 2000). The nuclei fire action potentials immediately before song output and during singing, and stop milliseconds before sound output ceases. Combined, these findings show that the posterior vocal pathway produces the learned vocalizations of songbirds.

Connectivity in the posterior nuclei of parrots is partly similar insofar as HVC-like (NLc) and RA-like (AAc) nuclei project to brainstem motor neurons involved in the production of sound (Durand et al., 1997). Lesion studies also implicate the posterior vocal nuclei in the production of learned vocalizations in parrots. For example, lesion of their HVC-like nucleus (NLc) revealed that it is required for the ability to speak English words (Lavenex, 2000).

Preliminary connectivity studies performed on hummingbirds (Gahr, 2000) indicate that their HVC-like nuclei (VAN), via their RA-like (VA) vocal nuclei, also project to vocal brainstem motor neurons. Neither lesioning nor electrophysiological studies have been conducted on the vocal nuclei of this order.

### 2.3 Anterior vocal nuclei

In songbirds, these nuclei are part of an anterior vocal pathway that forms a loop with projections from MAN→Area X→DLM→MAN (Luo et al., 2001). All connections of this pathway lie within the forebrain, consisting of the cerebrum and thalamus combined. When the main lateral portions of the anterior vocal nuclei are lesioned, as in lateral MAN (lMAN) or lateral Area X (lArea X), songbirds are still able to sing previously learned vocalizations but cannot learn new vocalizations (Scharff and Nottebohm, 1991). During non-imitative stages, which some adult songbirds undergo, anterior vocal pathway lesions have no obvious affect. However, as shown in gene expression results (Jarvis and Nottebohm, 1997), song-driven electrophysiological firing still takes place in anterior vocal nuclei in adults (Hessler and Doupe, 1999a). In adults though, lesions to Area X temporarily affect song syntax and song-driven gene expression; and song activity differs according to the social context in which the birds sing (Jarvis et al., 1998; Hessler and Doupe, 1999b; Kobayashi et al., 2001).

Hence in songbirds, the anterior vocal pathway may be responsible for vocal learning and some as yet undefined role in the social context of singing, as well as song syntax.

Connectivity studies in parrots have also shown that their anterior vocal nuclei are part of a forebrain loop (Durand et al., 1997). Lesions to the HVo-like (HVo) and MAN-like (NAo) vocal nuclei in parrots have shown that these, too, are required for vocal learning. No such studies have been conducted in hummingbirds.

Key differences between parrots and songbirds in known connectivity lie in the interactions between posterior and anterior vocal pathways (Durand et al., 1997; Jarvis and Mello, 2000). In songbirds, the posterior pathway sends input into the anterior pathway via a projection from HVC→Area X. The anterior pathway in turn sends output to the posterior pathway via a projection from lateral MAN→RA and medial MAN→HVC. In parrots, the posterior pathway sends input into the anterior pathway via a projection from RA-like (AAc) into all three anterior vocal nuclei: HVo-like (HVo), MAN-like (NAo), and Area X-like (LPO) sections. The anterior pathway uses the same region of the MAN-like nucleus (NAo) and projects to both its RA-like (AAc) and HVC-like (NLc) vocal nuclei. As connectivity for the HVo-like nucleus in songbirds has not been yet determined, it is not possible to make comparisons with parrots.

## 3 Hypotheses for the origin of vocal learning

It is established that the basic cellular arrangement for learned vocal communication in birds comprises an auditory pathway to acquire sounds, vocal nuclei in the cerebrum, and seven nuclei with one or more of each in each major cerebral subdivision except the *pallidum* (an area homologous to the mammalian *globus pallidus*). Because the auditory pathway also has one or more nuclei in each major cerebral subdivision, I suggest that this is a basic system of organization in the avian brain, comparable to the different systems in the mammalian brain which involve all six layers of the cortex and the basal ganglia below it.

For vocal learning, and perhaps motor learning in general, cerebral nuclei need to be divided into two pathways: a posterior vocal pathway that must project to lower vocal motor neurons for the production of learned vocalizations, and an anterior vocal pathway that must form a loop and control vocal learning and other aspects of learned vocalizing. The projections of the posterior pathway to motor neurons are akin to the motor cortex projections in the mammalian brain; the anterior loop, in contrast, is akin to cortical-basal ganglia-thalamic-cortical loops in mammals. Variation is permissible. The shapes of the vocal nuclei and their relative sizes, the location of the posterior vocal pathway relative to auditory and anterior vocal pathways, and their connectivity, may vary without impairing vocal learning. For example, the HVo and MAN-like vocal nuclei in parrots are, relatively, much larger than in songbirds and hummingbirds. Parrots display more vocal plasticity than songbirds or hummingbirds, and the enlargement of these nuclei could be implicated in its facilitation.

Although clear differences exist, the striking similarities beg the question as to how such similarities evolved. Modern birds supposedly evolved from a common ancestor around the cretaceous-tertiary boundary at the time of the extinction of dinosaurs, ~65 million years ago (Feduccia, 1995). How did seven similar brain structures appear in three distantly related vocal-learning birds in the ensuing time? To put these questions in perspective, the phylogenetic relationship between parrots and songbirds (Fig. 1A) is as far distant as that between humans and dolphins (Novacek, 1992). To explain these similarities, colleagues and I have put forward three hypotheses (Jarvis et al., 2000).

**Hypothesis 1**: Three out of the 30 or so avian orders evolved vocal learning independently in the past 65 million years (Fig. 1A, dots). For each event, seven similar brain structures for learned vocal communication evolved convergently. This would suggest that the evolution of brain structures for complex behavior is under strong epigenetic constraint. Such constraints may be in part outside the genes, i.e. imposed by environment and/or the morphology of the syringial/respiratory system. Such selection in the brain may have to adhere to some genetic constraints, with a requirement that an anterior part of the cerebrum, which normally controls motor learning, hooks up with a more posterior part that normally controls movement, so that all then hook up to muscles that control vocalizations. According to this hypothesis, if pigeons or some other group were to evolve vocal learning, one would predict that in another 50 million years or so, another seven similar brain regions in well-defined posterior and anterior locations would be present.

**Hypothesis 2**: An alternative hypothesis invokes a common ancestor for all modern birds that possessed vocal learning and the seven cerebral vocal nuclei. These traits were retained in only the three orders today, and lost independently at least four times in the interrelated vocal non-learning orders (Fig. 1A, squares). Such independent losses would suggest that maintenance of vocal learning and cerebral vocal structures is under strong epigenetic constraint too, and that considerable survival costs accrue to vocal learning or that many bird groups evolved in adaptive zones that did not require vocal learning. One constraint could be predation, in which context-learned vocalizations are more variable and thus less habituated to by predators (Jarvis and A Ferreira, in preparation). If such losses in vocal learning occurred independently, a similar scenario applies in theory to mammals. However, chimpanzees and other primates would have had to lose the trait recently and independently many times.

**Hypothesis 3**: The third hypothesis proposes that avian vocal non-learners have rudimentary cerebral vocal nuclei, previously overlooked, which have become enlarged

independently in the vocal learners. If true, this challenges the idea that cerebral vocal nuclei are unique to vocal learners. It would suggest that these brain structures are universal, even for mammals, with potential for vocal learning not yet widely expressed.

Because the auditory pathway is remarkably similar among all vocal learning and non-learning birds, I argue that the pathway in the vocal learners was inherited from a common avian ancestor with non-learners, or even from a common reptilian ancestor with mammals. Because the auditory pathway consists of cerebral nuclei in most vertebrate groups, I argue that this is why pets succeed at auditory learning. Whichever of the above hypotheses is true, singly or in combination, the answer for the evolution of cerebral vocal pathways in birds will be fascinating. Each hypothesis suggests that the evolution of brain pathways for complex behaviors is constrained by as yet unknown factors.

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