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The Zebra Finch, *Taeniopygia guttata*: An Avian Model for Investigating the Neurobiological Basis of Vocal Learning

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Songbirds are capable of learning their vocalizations by copying a singing adult. This vocal learning ability requires juveniles to hear and memorize the sound of the adult song, and later to imitate it through a process involving sensorimotor integration. Vocal learning is a trait that songbirds share with humans, where it forms the basis of spoken language acquisition, with other avian groups (parrots and hummingbirds), and with a few other mammals (cetaceans, bats). It is however absent in traditional model organisms such as rodents and nonhuman primates. Zebra finches, a songbird species from Australia, are popular pets and are easy to breed. They also sing a relatively simple and stereotyped song that is amenable to quantitative analysis. Zebra finches have thus emerged as a choice model organism for investigating the neurobiological basis of vocal learning. A number of tools and methodologies have been developed to characterize the bioacoustics properties of their song, analyze the degree of accurate copying during vocal learning, map the brain circuits that control singing and song learning, and investigate the physiology of these circuits. Such studies have led to a large base of knowledge on song production and learning, and their underlying neural substrate. Several molecular resources have recently become available, including brain cDNA/EST databases, microarrays, BAC libraries, a molecular brain atlas, a complete genome assembly, and the ability to perform transgenesis. The recent availability of many other avian genomes provides unique opportunities for comparative analysis in the search for features unique to vocal learning organisms.

BACKGROUND

The Australian zebra finch (*Taeniopygia guttata castanotis*) is a representative perching songbird (order Passeriformes, suborder oscine) of the *Estrildidae* family (subfamily *Estrildinae*), a group of granivorous passerines found throughout tropical and subtropical parts of Africa, Southeast Asia, and Australia. Zebra finches are highly social, living in large flocks in a diversity of habitats and conditions (Zann 1996). They show marked sexual dimorphism in size, weight, feather and beak coloration, and behavior (Fig. 1). Adult males possess dark parallel stripes that run horizontally over their throat and upper chest and give the species its name.

Since their domestication ∼150 yr ago, zebra finches have become popular birds, owing to their bright and attractive plumage patterns, highly social behavior, and the ease with which they can be bred in captivity. They are kept as pets throughout the world, and are represented by a large number of strains (∼30) and color morphs (>40 combinations). Since the use of zebra finches in experimental biology was introduced (Moris 1954; Immelmann 1969), they have become a choice experimental

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FIGURE 1. Photograph depicting an adult pair (male on *right*, female on *left*) of zebra finches (*Taeniopygia guttata*). Images courtesy of Chris Olson.

model organism, most prominently in the neurobiology of learned vocalizations (Zeigler and Marler 2004, 2008). Juvenile males learn to sing by imitating an adult male tutor, usually the father (Immelmann 1969; Slater et al. 1988). Exposure to song, as well as vocal practice, normally take place during a critical period (Eales 1985) approximately coinciding with pubertal maturation, marked by increased gonadal hormone secretion and the development of dimorphic plumage and behavioral traits (Zann 1996).

SOURCES AND HUSBANDRY

Zebra finches can no longer be obtained from their natural environment (Zann 1996), but they can be readily purchased from bird farms and local breeders at low cost. A potential issue, however, is that it is difficult to know the exact conditions under which the birds were raised, including what sounds and other environmental factors they were exposed to before adulthood. When controlling such factors is crucial, or when a developmental time-series is desired, breeding the birds is advisable.

There is considerable evidence of breeding seasonality for zebra finches in the wild, but also ample evidence of flexibility and opportunistic breeding (Zann 1996). The major factor seems to be food abundance, largely dictated by the availability of rain in most parts of Australia. In contrast, photoperiodicity, of utmost importance for seasonal breeders, does not seem to play a major role in the zebra finch life cycle. In captivity, zebra finches will breed easily, as long as nesting materials and food are supplied, and light, temperature, and humidity are in an adequate range (Zann 1996; see also Bateson and Feenders 2010; Schmidt 2010; and **Proper Care, Husbandry, and Breeding Guidelines for the Zebra Finch, *Taeniopygia guttata*** [Olson et al. 2014]). Breeding can be performed in indoor aviaries or single cages. The former is more cost-effective in terms of the number of birds generated, but it is generally not possible to control for parenthood and/or the source of tutor song during learning. Breeding in single cages is preferable when such factors need to be controlled.

RELATED SPECIES

Although zebra finches are particularly useful for studies on the physiological basis of vocal learning, other species have helped elucidate a range of variables that affect song and singing behavior. For example, the Bengalese finch (*Lonchura striata domestica*) has a complex and variable song that is suited for exploring the biological basis of syntactic organization (Okanoya 2004), and the canary (*Serinus canaria*, in particular the Waterslager strain), European starling (*Sturnus vulgaris*), and

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song sparrow (*Melospiza melodia*) present seasonal fluctuations in singing and in the properties of the song control circuitry (Brenowitz 2008). As their vocal learning is not restricted to a critical period in juveniles, comparisons with the zebra finch offer unique opportunities for uncovering the neuronal basis of seasonal plasticity. Vocal learning also occurs in Psittaciformes (parrots, budgerigar) and Apodiformes (hummingbirds) (Nottebohm 1972; Baptista and Schuchmann 1990). Comparative analysis across these groups provides opportunities for identifying independently evolved vocal learning mechanisms in multiple avian groups. In contrast, Passeriformes of the Suboscine suborder (e.g., flycatchers) are thought to lack vocal learning and associated brain structures (Kroodsma and Konishi 1991, but see Saranathan et al. 2007; Liu et al. 2013), providing opportunities to define neural and genomic features that are unique to vocal learning Passeriformes.

USES OF ZEBRA FINCHES AS A MODEL ORGANISM

Zebra finches sing readily in captivity, and their song is stereotyped and relatively simple to quantify in terms of vocal imitation. They can be considered aseasonal as their singing and breeding behaviors, as well as the anatomical organization of their song circuitry, remain largely unchanged throughout the year. As such, zebra finches offer distinct advantages over other songbirds for studying the neurobiology of song. A wealth of knowledge has accumulated on the bioacoustics properties and ontogenesis of finch song, as well as the anatomical, physiological, and neurochemical properties of the brain circuits for song production and learning (for reviews, see Zeigler and Marler 2004, 2008), including the following highlights.

Vocal Learning

Songbirds learn to vocalize by imitating an adult male tutor, usually their father. Songbirds share the vocal learning trait with humans, where it provides the basis for speech and language acquisition. Among mammals, there is evidence of vocal learning in cetaceans, bats (Boughman 1998; Deecke et al. 2000; Knornschild et al. 2010), and possibly elephants (Poole et al. 2005), but no substantiated reports of vocal learning in other mammals, including rodents (Mahrt et al. 2013), making avian species the only tractable organisms for studying mechanisms of vocal learning.

There are marked similarities between birdsong and human speech acquisition (Doupe and Kuhl 1999), including the presence of "babbling"-like immature vocalizations, crucial periods for learning, sensorimotor vocal development that is dependent on intact hearing and auditory feedback, and individual vocal variability and dialects. As in humans, vocal learning in zebra finches is influenced by social cues, requiring a live tutor or conditions that closely resemble the natural setting. This offers opportunities for understanding how social factors affect vocal learning.

Brain Activity Underlying Singing Behavior

Songbirds and humans share the involvement of cortical- and basal-ganglia-like circuitry in the acquisition and production of learned vocalizations (Jarvis 2004). However, because of the nuclear organization of the avian forebrain, areas that control birdsong are distinct (Nottebohm et al. 1976), making it possible to study connectivity and to map the locations of individual projection neurons and interneurons within the song system. This organization has also facilitated the development of methods for obtaining in vivo electrophysiological recordings from individual neurons in awake birds during singing behavior, advancing our understanding of how individual cells that are embedded in a complex circuit participate in the encoding of learned behaviors.

Brain Gene Expression

Activity-inducible immediate-early genes are rapidly induced in different areas when songbirds hear song or actively sing (Mello 1993; Jarvis et al. 1998). Thus, the expression of a natural behavior can be

linked to the activation of gene-regulatory programs in specific circuits. Recently, high-throughput methods have shown that several hundred genes are regulated during singing (Wada et al. 2006; Hilliard et al. 2012) and have identified a large number of molecular specializations of song nuclei (Lombardino et al. 2006; Li et al. 2007; Lovell et al. 2008). These advances have facilitated attempts to define the similarities in molecular organization of the avian brain in comparison with that of mammals.

Sexual Dimorphism and Neurosteroid Actions

The zebra finch song system is dimorphic, with nuclei conspicuous in males but small or absent in females (Nottebohm and Arnold 1976). This dimorphism is mirrored in behavior, as female finches produce unlearned calls, but do not sing (Vicario 2004). Studies on sex dimorphism and sex steroids have revealed that the male zebra finch brain is a source of estrogen (Schlinger and Arnold 1992). Recent technical advances have enabled in vivo measurements of real-time steroid fluctuations within discrete brain areas, leading to the realization that some neurosteroids are produced on a much more rapid timescale than previously thought (Remage-Healey et al. 2008).

Adult Neurogenesis

A fundamental contribution of songbirds has been the demonstration of adult neurogenesis within song control nuclei, conclusively establishing that an adult vertebrate brain can generate novel neuronal cells (Goldman and Nottebohm 1983; Paton and Nottebohm 1984; Alvarez-Buylla et al. 1988). Protocols for the analysis of adult neurogenesis in songbirds are well established and can be coupled to tract-tracing and marker analysis (Alvarez-Buylla et al. 1990), allowing further insights into the properties of the neuronal types that can be replaced in adults.

GENETICS, GENOMICS AND ASSOCIATED RESOURCES

Several resources have recently become available. Large-scale brain cDNA library sequencing efforts, by the Songbird Neurogenomics (SoNG) Consortium (Replogle et al. 2008) (involving 63,000 reads), the Songbird Brain Transcriptome Database (Wada et al. 2006) (19,220 ESTs) and the Nottebohm laboratory (Li et al. 2007) (9845 reads), have identified more than 17,214 transcripts, representing an estimated 11,500 to 15,000 genes. Approximately 92,000 ESTs have been deposited with the NCBI, and corresponding cDNAs can be obtained through the Clemson University Genomics Institute (CUGI). A systematic analysis of brain gene expression by in situ hybridization is currently under way, taking the form of a publically accessible molecular brain atlas (ZEBrA: http://www.zebrafinchatlas.org), prepared in registration with a histological atlas (http://zebrafinch.brainarchitecture.org/introduction/; Karten et al. 2013).

The complete sequence (~1.2 Gb) of the zebra finch genome, which includes 33 chromosomes and three linkage groups, has been assembled based on Sanger sequencing and bacterial artificial chromosome (BAC) fingerprinting (Warren et al. 2010); BAC clones and filter sets are available through CUGI. When combined with the large number of avian genomes (>40) that are being made available through the Avian Phylogenomics Consortium (Zhang et al. 2014), comparative-genomics analyses offer unprecedented opportunities to link broad aspects of vocal learning and the emergence of associated brain circuitry to unique features of avian genomes.

The ability to manipulate gene expression in live animals is essential for directly assessing the role of genes in vocal learning. Localized brain injections of lentiviral-vector-based knockdown constructs have been used to show that expression in basal ganglia of the forkhead box transcription factor *FOXP2,* a gene related to a familial form of vocal dyspraxia in humans, is required for song learning in zebra finches (Haesler et al. 2007). Lentiviral vectors have also been used for generating transgenic zebra finches (Agate et al. 2009), an important advance that begins to open the doors to in vivo genetic manipulations in songbirds.

TECHNICAL APPROACHES

Finally, for detailed related information, readers can consult the following accompanying protocols that describe **Proper Care, Husbandry, and Breeding Guidelines for the Zebra Finch,** *Taeniopygia guttata* (Olson et al. 2014), **An Optimized Protocol for High-Throughput In Situ Hybridization of Zebra Finch Brain** (Carleton et al. 2014), **A Method for Exploring Adult Neurogenesis in the Songbird Brain** (Asik et al. 2014), **In Vivo Detection of Fluctuating Brain Steroid Levels** (Ikeda et al. 2014), **In Vivo Recording of Single-Unit Activity During Singing in Zebra Finches** (Okubo et al. 2014), and **Generation of Transgenic Zebra Finches with Replication-Deficient Lentiviruses** (Velho and Lois 2014).

ACKNOWLEDGMENTS

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