They're Playing Our Song: Gene Expression and Birdsong Perception

Minireview

Gregory F. Ball* and Timothy Q. Gentner
Department of Psychology
Behavioral Neuroendocrinology Group
Johns Hopkins University
Baltimore, Maryland 21218

Neuroethology, a hybrid discipline straddling the behavioral and neural sciences, grew out of the field of ethology, the biological study of animal behavior. Neuroethology has benefited from the application of new technical and conceptual breakthroughs derived from other subdisciplines of neuroscience; the use of these new approaches has, in turn, produced principles of general significance to the field of neuroscience. Recent studies of immediate-early gene (IEG) induction in relation to birdsong perception are an excellent illustration of this cross-fertilization process.

One of the important legacies of ethology to behavioral neuroscience is the experimental documentation of the selectivity of perception. Based on experiments with many taxa, it has become clear that animals attend to only the particular stimulus attributes needed to make adaptive behavioral responses. In a variety of sensory systems, one finds neurons with tuning characteristics directly related to the behavioral relevance of a signal (see Konishi, 1991, for a general review of these phenomena). For example, barn owls (*Tyto alba*) possess specialized sensory systems to detect and process the interaural time and intensity differences in acoustic signals for prey localization. Several species of electric fish (genus *Eigenmannia*) possess highly specialized neural circuits to process the phase and amplitude information in conspecific electric signals. Likewise, it is becoming clear that songbirds also possess specialized neural circuitry for the production and perception of birdsong. This minireview will focus on studies of IEG expression in relation to the elucidation of neural systems underlying birdsong perception.

Birdsong and the Song Control Circuit

Birdsongs are species-typical vocal signals that are generally more elaborate than calls and are used in the context of courtship and mating. The term birdsong is often limited to such vocalizations produced by species in the songbird order (order passeriformes). Unlike most vocalizations produced by nonhuman animals, these songs are learned based on auditory experiences early in ontogeny. Decades of work by ethologists have established that songs are powerful releasers of behavioral responses in conspecifics and that specific acoustic attributes of song can elicit aggressive challenges from males and sexual responses from females (Reviewed by Ball and Hulse, 1998).

Detailed studies of two commonly studied songbird species, zebra finches (*Taeniopygia guttata*) and canaries (*Serinus canaria*) have revealed a vocal control circuit underlying these behavioral responses. Many nuclei in

this circuit are unique to songbird species and thus represent part of the neural specialization that has evolved in association with the ability to learn, produce, and perceive these complex species-typical vocalizations. A key nucleus in this circuit is HVc (sometimes referred to as the "high vocal center"). This nucleus projects to another forebrain nucleus RA (the robust nucleus of the archistriatum) to form part of a motor pathway that lesion studies demonstrate is required for song production (Figure 1). Electrophysiological studies in the zebra finch brain provide evidence that these motor pathways are hierarchically organized: populations of neurons in HVc appear to code for song syllables (large units within songs), while RA neurons code for song notes (smaller units within song syllables; Margoliash, 1997). Still other brain areas are involved in song perception: auditory information is critical for song learning, needed for the maintenance of song, and obviously essential for complex song perception. As one would expect, auditory pathways in the songbird brain
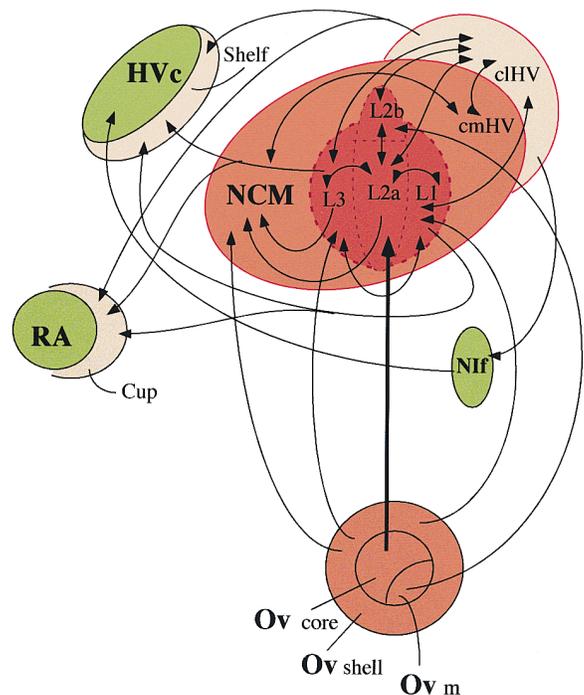

Figure 1. Primary Auditory Projection Pathways in the Songbird Forebrain

Auditory information ascends from nucleus ovoidalis (Ov) at the level of the thalamus to several sites in the telencephalon, including the major subdivisions of field L (the avian analog to primary auditory cortex in mammals) and the mediocaudal neostriatum (NCM). NCM receives projections from subdivisions of field L, and both NCM and field L possess reciprocal connections with the caudomedial and caudolateral regions of the ventral hyperstriatum (HV), respectively. Auditory information reaches the motor nucleus HVc via projections to the subjacent shelf region and via direct projection from the nucleus interfacialis (NIf). Similarly, the cup surrounding the motor nucleus (RA) receives projections from field L and clHV. The motor projection from HVc to RA is not shown.

*To whom correspondence should be addressed.

are interconnected with the song control circuit (Figure 1) and also show evidence of hierarchical organization. For example, the sensitivity to local temporal structures in the perception of conspecific song increases substantially between the primary auditory projection region in the telencephalon (field L) and the song control nucleus HVc (see Figure 1) where neurons are tuned exclusively or preferentially to a bird's own song (Margoliash, 1997).

Immediate-Early Gene Expression in Relation to Song Perception

Based on the work of David Clayton and colleagues, starting in the 1980s, a number of genes expressed as messenger RNA in the songbird forebrain were cloned (reviewed by Clayton, 1997). Although no genes were found that were specific to the song control system, several recognized classes of IEG transcription factors were identified in songbirds (Clayton, 1997). One IEG in particular, named *ZENK* (Mello et al., 1992) because it is homologous to genes cloned in other species (*ZENK* is an acronym for *zif-268*, *egr-1*, *NGFI-A*, and *krox-24*), has been found to be regulated specifically in response to conspecific song in the songbird brain.

The initial observation was that the *ZENK* mRNA and protein is expressed at high levels relatively rapidly (i.e., in less than 1 hr) in the auditory telencephalon in response to conspecific song (Mello et al., 1992; Mello and Ribeiro, 1998). Expression is especially high in areas of the auditory telencephalon such as NCM and cHV, regions not previously identified as specifically involved in song perception. *ZENK* expression is also high in other areas such as the HVc "shelf" and the RA "cup," areas that were previously thought to receive auditory inputs specific to song (see Figure 1). Further studies showed that the *ZENK* response is specifically tuned to novel conspecific song. *ZENK* expression is basal in response to simple tones, but it is twice as high in response to conspecific as opposed to heterospecific song (Mello et al., 1992). Furthermore, repeated presentations of the same conspecific song lead to a diminution of the *ZENK* response (i.e., habituation), while the response to a different conspecific song is still observed (Mello et al., 1995). The *ZENK* response to song is dependent on early experience; zebra finches raised in social isolation do not exhibit this response (Jin and Clayton, 1997). It is also important to note that the *ZENK* response is not limited to the laboratory. The playback of song to free-living, wild song sparrows (*Melospiza melodia*) results in the induction of the *ZENK* mRNA with a magnitude and distribution in the brain similar to that observed in laboratory-housed zebra finches and canaries (Jarvis et al., 1997).

IEG expression is known to be affected by changes in neuronal activity, although the exact relationship between IEG expression and neural activity is not well understood. Many neurons in the song circuit (i.e., areas essential for the learning and production of birdsong) exhibit increased electrophysiological activity in response to song (Margoliash, 1997). It might therefore be expected that *ZENK* expression would localize to the song circuit. Surprisingly, however, none of the studies of song perception identified *ZENK* expression in brain regions considered to be part of the song system per se. Therefore, the *ZENK* response, although clearly influenced by neuronal activation, does not simply map onto brain areas that exhibit enhanced physiological responses to song. However, it should be noted that IEG expression is high in areas within the song control circuit during song production. Two IEGs, *fos* as well as *ZENK*, have been found to be induced at high levels in HVc and RA, two nuclei essential for motor production, while male zebra finches are producing song (Jarvis and Nottebohm, 1997; Jin and Clayton, 1997; Kimpo and Doupe, 1997). This high induction is specifically tied to motor production, given that it even occurs when deaf birds sing. Thus, specific IEG induction can be observed in songbird forebrain in association with both the perception and the production of song.

One important contribution of the *ZENK* induction studies outlined above has been their inspiration for several anatomical, neurophysiological, and lesion studies that have, in turn, furthered our knowledge about the organization and function of the neural circuits mediating birdsong perception. Many of the connections between the auditory system and the song system illustrated in Figure 1 were only discovered after studies of *ZENK* induction made it clear that the connectivity of NCM and cHV is important for our understanding of the neural basis of song perception (e.g., Vates et al., 1996). Likewise, recent electrophysiological studies of NCM have placed further emphasis on the importance of this region for the processing of conspecific signals (Chew et al., 1995, 1996; Stripling et al., 1997). Neurons throughout NCM show a robust initial response to the presentation of conspecific vocalizations that quickly falls off upon subsequent presentations of the same song. Thereafter, although repeated presentation of a single song continues to elicit significant responses from single cells (relative to spontaneous firing rates), the initial modulation in the firing rate of these cells disappears and is observed again only upon presentation of a novel song. This link between the electrophysiological response properties of neurons in NCM and stimulus-specific variation among songs has led to the hypothesis that NCM is an important site for individual vocal recognition (Chew et al., 1996). Another area exhibiting high *ZENK* expression in response to conspecific song is cHV (see Figure 1). Recent studies of female zebra finches provide a possible behavioral correlate to this expression by suggesting that an intact cHV is required for the discrimination of conspecific from heterospecific song (MacDougall-Shackleton et al., 1998). Following electrolytic lesions to HVc, females maintained preferences (as measured by their sexual display rate) for conspecific as opposed to heterospecific song. However, following lesions of cHV, females performed courtship displays at a high rate in response to both conspecific and heterospecific song. Thus, an intact cHV, a nucleus involved in the processing of auditory information, but not HVc, appears necessary for female zebra finches to exhibit normal song preferences.

Studies of IEG expression in the forebrain of songbirds and the subsequent investigations inspired by them have greatly increased our knowledge of the structure and function of the song perception pathways, particularly with regard to species recognition. However, a wealth of field data demonstrates that birdsong functions primarily as a communication mechanism *within*

species, and so it follows that songbird perceptual systems have adapted to attend to variation among conspecific songs. Thus, while it is an important first step to establish that the brains of songbirds respond differentially to conspecific and heterospecific songs, our understanding of the selectivity of perception exhibited by birds and other species is contingent upon our ability to measure changes in brain and behavior that are correlated with relevant variation *among* conspecific vocalizations.

Neuroethological Studies of Birdsong Perception

Current neuroethological studies of song perception focus upon at least two critical questions. First, what are the physical parameters of song that elicit or allow for predictable changes in functionally relevant (adaptive) behavior? Second, how is the information provided by variation among these functional parameters represented in the nervous system? Two recent advances demonstrate the progress that contemporary neuroethologists have made in addressing these questions. Gentner and Hulse (1998) have demonstrated how behaviorally relevant variation among the syllabic structure of conspecific songs can be closely examined in the context of individual vocal recognition, and work on *ZENK* expression in canaries by Ribeiro et al. (1998) reported in this issue of *Neuron* demonstrates a novel method for quantitating the neuronal representations of song syllables.

Individual vocal recognition, or the ability to associate specific songs with specific singers, is an important behavioral adaptation for the manipulation and maintenance of territorial boundaries and female mate selection (see Kroodsma and Miller, 1996). Although nearly every species of songbird studied to date is capable of recognizing individual conspecifics by their song(s), the specific acoustic features of song that birds use for recognition are not yet well understood. Some species rely on relatively simple frequency cues to discriminate among the songs of neighboring conspecifics (and heterospecifics), while others, such as European starlings (*Sturnus vulgaris*), rely on the perception of more complex acoustic patterns. Male starlings present their songs in long elaborate bouts made up of sequentially patterned shorter syllables, and the syllables in one bout may or may not overlap with the syllables in other bouts from that same bird. Using operant conditioning techniques, Gentner and Hulse (1998) have demonstrated that both male and female starlings can readily learn to discriminate among the songs of several different males, and that these songs are categorized by starlings on the basis of individual identity. This recognition capability is robust and is maintained under a variety of stimulus conditions, even when the subjects are presented with novel song bouts from familiar singers. This ability for starlings to recognize individual conspecifics on the basis of their vocalizations is mediated primarily by the memorization of the specific syllables in an individual singer's repertoire and by the statistical patterning of the sequences in which those syllables are sung (Gentner and Hulse, 1998).

What is the neural basis for such discrimination? One of the most important recent advances in the study of the neural basis of song perception has been the discovery of neurons tuned to a bird's own song (Margoliash,

1997). Contemporary research in this area has focused on the ontogenetic development and response properties of these neurons. However, comparatively little is known about the neural correlates that underlie the perception of conspecific song in adults. Ribeiro et al. (1998) have made a significant advance in filling this gap in our knowledge by the discovery of an important step in the transformation of an acoustic signal into a functional neural representation. They have employed sophisticated pattern analysis methods to investigate the collective behavior of large populations of neurons that are responsive to canary song syllables. Ribeiro et al. (1998) have demonstrated that the spatial distribution and immunocytochemical labeling intensities of *ZENK*-expressing neurons in the brains of canaries hearing conspecific song are closely correlated with variation among the syllables in male canary songs. Moreover, in contrast to more "classical" organization schemes well documented for auditory forebrain regions in other species, where neurons are organized tonotopically and respond best to a specific fundamental frequency, the *ZENK* expression patterns elicited by various narrow band syllables (i.e., whistles) are not replicated by presenting pure tone stimuli that simply match the fundamental frequencies of these conspecific song syllables. Thus, the spatial organization of neuronal populations in NCM observed by Ribeiro et al. (1998) is not just another example of simple tonotopy, but instead appears to correlate primarily with variation in conspecific song. This is significant in that it provides compelling evidence of a salient organization underlying populations of neurons in the auditory forebrain of birds that corresponds to behaviorally relevant signals.

Conclusion

The recent findings in the neuroethology of song perception discussed here point to important ways in which approaching the question of neural representation in songbirds has led to advances in our understanding of both the neural and the behavioral mechanisms of perception. What remains as a significant challenge is the integration of these components, and one promising possibility in this regard is to examine the functional role of *ZENK* expression in the context of individual vocal recognition. *ZENK* expression may be directly related to the categorization of classes of songs on the basis of individual identity, or the consolidation of neuronal representations into long-term memory, as suggested by work in other systems (Clayton, 1997). By combining operant conditioning techniques—where the behavioral relevance of different songs can be tightly controlled—with *ZENK* immunocytochemistry, one could dissociate between IEG responses that are due to basic discrimination among different songs and/or those that are due to higher level cognitive processes such as learning. Finally, now that NCM has been identified as a site for population-level neuronal representations of song, it is important that we extend our knowledge about the real-time dynamics of this region using electrophysiology. Recent technical advances that allow for simultaneous recording from large numbers of neurons (reviewed by Nicolelis et al., 1997), could be used to examine electrophysiological activity throughout NCM and other forebrain regions, in response to behaviorally relevant songs.

For all these behavioral studies, the application of methodologies that could specifically block *ZENK* expression or the action of the ZENK protein would be especially welcome. The already important advances in the study of perceptual processes made by neuroethological studies will no doubt be augmented by the continued coordination and refinement of neural and behavioral methodologies.

Selected Reading

Ball, G.F., and Hulse, S.H. (1998). Am. Psychol. *53*, 37–58.

Chew, S.J., Mello, C., Nottebohm, F., Jarvis, E., and Vicario, D.S. (1995). Proc. Natl. Acad. Sci. USA *92*, 3406–3410.

Chew, S.J., Vicario, D.S., and Nottebohm, F. (1996). Proc. Natl. Acad. Sci. USA *93*, 1950–1955.

Clayton, D.F. (1997). J. Neurobiol. *33*, 549–571.

Gentner, T.Q., and Hulse, S.H. (1998). Anim. Behav., in press.

Jarvis, E.D., and Nottebohm, F. (1997). Proc. Natl. Acad. Sci. USA *94*, 4097–4102.

Jarvis, E.D., Schwabl, H., Ribeiro, S., and Mello, C.V. (1997). Neuroreport *8*, 2073–2077.

Jin, H., and Clayton, D.F. (1997). Neuron *19*, 1049–1059.

Kimpo, R.R., and Doupe, A.J. (1997). Neuron *18*, 315–325.

Konishi, M. (1991). Neural Comput. *3*, 1–18.

Kroodsma, D.E., and Miller, E.H., eds. (1996). Ecology and Evolution of Acoustic Communication in Birds (Ithaca, NY: Cornell University Press).

MacDougall-Shackleton, S.A., Hulse, S.H., and Ball, G.F. (1998). Neuroreport, in press.

Margoliash, D. (1997). Neuron *19*, 963–966.

Mello, C.V., and Ribeiro, S. (1998). J. Comp. Neurol. *393*, 426–438.

Mello, C.V., Vicario, D.S., and Clayton, D.F. (1992). Proc. Natl. Acad. Sci. USA *89*, 6818–6822.

Mello, C.V., Nottebohm, F., and Clayton, D.F. (1995). J. Neurosci. *15*, 6919–6925.

Nicolelis, M.A.L., Fanselow, E.E., and Ghazanfar, A.A. (1997). Neuron *19*, 219–221.

Ribeiro, S., Cecchi, G.A., Magnasco, M.O., and Mello, C.V. (1998). Neuron *21*, this issue, 359–371.

Stripling, R., Volman, S.F., and Clayton, D.F. (1997). J. Neurosci. *17*, 3883–3893.

Vates, G.E., Broome, B.M., Mello, C.V., and Nottebohm, F. (1996). J. Comp. Neurol. *366*, 613–642.