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

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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 sub-disciplines 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

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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 (J arvis 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 (J arvis and Nottbohm, 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 anatomic, 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., Yates 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 sys-
tems have adapted to attend to variation among conspe-
cific songs. Thus, while it is an important first step to
establish that the brains of songbirds respond differen-
tially to conspecific and heterospecific songs, our un-
derstanding 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 corre-
lated with relevant variation among conspecific vocal-
izations.

**Neuroethological Studies of Birdsong Perception**

Current neuroethological studies of song perception fo-
cus 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 demon-
strate the progress that contemporary neuroethologists
have made in addressing these questions. Gentner and
Hulse (1998) have demonstrated how behaviorally rele-
vant variation among the syllabic structure of conspe-
cific songs can be closely examined in the context of
individual vocal recognition, and work on ZENK expres-
sion in canaries by Ribeiro et al. (1998) reported in this
issue of Neuron demonstrates a novel method for quan-
titating 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 mainte-
nance of territorial boundaries and female mate selec-
tion (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 het-
erospecifics), while others, such as European starlings
(Sturnus vulgaris), rely on the perception of more com-
plex 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 tech-
niques, 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 ba-
is 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 (Gent-
ner 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 discov-
ery of neurons tuned to a bird’s own song (Margoliash,
1997). Contemporary research in this area has focused
on the ontogenetic development and response proper-
ties of these neurons. However, comparatively little is
known about the neural correlates that underlie the per-
ception 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 sophisti-
cated pattern analysis methods to investigate the col-
cective behavior of large populations of neurons that are
responsive to canary song syllables. Ribeiro et al. (1998)
have demonstrated that the spatial distribution and im-
munocytochemical labeling intensities of ZENK-express-
ing 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 docu-
mented 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 syl-
lables (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 neu-
rons in the auditory forebrain of birds that corresponds
to behaviorally relevant signals.

**Conclusion**

The recent findings in the neuroethology of song percep-
tion 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 discrimina-
tion 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
Nicolaides et al., 1997), could be used to examine electro-
physiological activity throughout NCM and other fore-
brain 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