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REPRESENTATION OF THE BIRD'S OWN SONG IN THE CANARY HVC: CONTRIBUTION OF BROADLY TUNED NEURONS

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Abstract—In songbirds, neurons in the song nucleus HVC exhibit a striking example of selective auditory response, firing more to playback of the bird's own song (BOS) than to conspecific songs. This song selectivity has been found in various songbird species, both those that sing a single individual-specific song as well as those, such as the canary, in which both song structure and individual-identity encoding in song is more complex. In the present study, we investigated how the BOS is represented in the HVC of anesthetized long-day canaries by using temporal and spectral variants of the BOS stimulus. We addressed the question of how selective HVC neurons were by quantifying the number of song elements, called phrases, that evoked auditory responses. Phrases that were individual-specific or that were frequently delivered in an individual's songs did not drive HVC neurons to a greater degree than others. Reordering phrases or altering their acoustic structure caused a decrease in the auditory responsiveness of HVC neurons. This sensitivity to the spectral and temporal features of the BOS involved neurons that failed to respond to BOS variants or were driven by a reduced number of phrases, as well as neurons whose auditory responsiveness extended beyond the features of the individual's song, responding to phrases that were not sung by the bird itself. Therefore, the neural strategy by which BOS structure is represented in the canary HVC may require something other than a strict representation of the repertoire of song components. We suggest that the individual's song could be coded, at least in part, by an ensemble of broadly tuned neurons.

Key words: auditory response properties, songbirds, HVC, song representation, order sensitivity.

Oscines songbirds are ideally suited to the study of the neural basis of learned forms of vocal communication. Their songs are elaborate vocalizations composed of various elements, often called syllables, organized according to sequential rules. There is variation between species in song structure. In some species, each male only has a single stereotyped song. In others, different song types are composed by reordering elements. Exploiting this diversity may help to determine whether the neural strategy used to represent vocalizations varies with birdsong structure.

In songbirds, the "song system" is a neural system that includes a network of brain nuclei specialized for singing and song learning. These nuclei are essential for learned vocal control, and are involved in both the sensory processing and the motor patterning of song (Nottebohm et al., 1976; McCasland and Konishi, 1981; Konishi, 1985; Vu et al., 1994; Doupe, 1997). Among these areas, the telencephalic nucleus HVC (acronym) serves as a major auditory-vocal interface and is thought to establish the correspondence between the auditory and motor representations of a vocalization (Margoliash, 1983; Yu and Margoliash, 1996; Mooney, 2000; Hahnloser et al., 2002; Prather et al., 2008). The HVC contains a type of neuron that displays similar patterns of auditory and singing-related activity (Prather et al., 2008). Regardless of cell type, HVC cells exhibit selective auditory properties, firing more to forward auditory playback of the bird's own song (BOS) than to reverse BOS or to songs of conspecifics (Margoliash, 1983; Lewicki and Konishi, 1995; Volman, 1996; Mooney, 2000). To date, this song selectivity has been found in various songbird species (Margoliash, 1983; Margoliash and Fortune, 1992; Mooney et al., 2001; Nakamura and Okanoya, 2004; Nealen and Schmidt, 2006). However, recent studies have reported interspecific differences in the auditory representation of the BOS in the HVC (George et al., 2005a; Nealen and Schmidt, 2006; Nishikawa et al., 2008).

Initially, studies of the auditory representation of the BOS in the HVC were performed in species that sing a single individual-specific song—the zebra finch (*Taenopygia guttata*) and the white-crowned sparrow (*Zonotrichia leucophrys*) (Margoliash, 1983, 1986; Margoliash and Fortune, 1992). These studies provided evidence that a proportion of HVC neurons are tuned to a single syllable (Margoliash and Fortune, 1992). Modifying the parameters of this syllable strikingly affects their auditory responsiveness, revealing the precision with which the temporal and spectral structure of the syllable needs to be preserved (Margoliash and Fortune, 1992). In these songbird species, others neurons are driven by a pair or a sequence of syllables of the BOS, and exhibit sensitivity to the temporal order of the syllables (Margoliash, 1983; Margoliash and Fortune, 1992; Lewicki and Konishi, 1995; Lewicki and Arthur, 1996). They respond less strongly when the syllable sequence of the BOS is reversed. More recently, a growing number of studies have investigated the auditory response properties of HVC neurons in species with a complex song structure, for example species that produce multiple song types or that produce songs with a less stereotyped ordering of syllables (Hausberger et al., 2000; Nealen and Schmidt, 2002, 2006; Nakamura and Okanoya, 2004). They suggest that auditory neurons in the HVC of these species may have broader response prop-

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Abbreviation: BOS, bird's own song.

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erties than HVC neurons in the zebra finch (George et al., 2005a; Nishikawa et al., 2008). Given the huge diversity in song structure among songbird species, the relationship between song structure and the manner in which songs are represented in the HVC thus needs to be further investigated.

The canary (*Serinus canaria*) is an example of a species with a fairly complex song structure (Güttinger et al., 1978; Nottebohm and Nottebohm, 1978). In the nucleus HVC of breeding canaries, neurons respond preferentially to a given BOS over its reversed version or a conspecific song (Del Negro et al., 2005; Lehongre and Del Negro, 2009). This suggests that the song of an individual possesses distinctive features that allow it to be distinguished from the songs of other conspecifics. Concordantly, our recent acoustical analysis of canary song has shown that both the repertoire of song elements, called phrases, and the sequence of phrases within a song could potentially contribute to individual signature encoding (Lehongre et al., 2008, 2009). The repertoire of any individual contains a few phrases that are not sung by other individuals. Also, most sequences larger than two phrases within an individual's songs are specific to that individual. We investigated how BOS structure is represented in the HVC of the breeding canary. To this end, we collected single-unit responses driven by a selected BOS in anesthetized long-day male canaries, and identified the phrases of this sound stimulus that provided the auditory drive. Our aim was to determine whether certain neurons were tuned to particular song phrases, such as individual-specific phrases. We assessed the selectivity of neurons in the HVC of long-day canaries by presenting several acoustic variants of the BOS stimulus, including a variant in which the sequence of BOS phrases was manipulated.

EXPERIMENTAL PROCEDURES

Subjects

Twenty-four singing adult male canaries (*Serinus canaria*) were used for this experiment. All canaries were reared in the laboratory and were chosen from a pool of 1- to 3-year-old birds that had had previous sexual experience. They belonged to an outbred strain of the common canary, with a heterogeneous genetic background.

Prior to electrophysiological investigations, birds were exposed to a long-day photoperiod ("spring-like": 14/10 h light/dark cycle). They had previously been kept under a short-day photoperiod ("autumn-like": 10/14 h light/dark cycle) for at least 4 months before a progressive shift in photoperiod. Under these laboratory conditions, song patterns are typical of the breeding period (Storey and Nicholls, 1976; Del Negro et al., 2005).

Song recordings

To record song, each bird was placed in a sound-attenuation chamber equipped with a Marantz PMD 201 recorder (Osnabrück, Germany) and an MD46 cardioid Sennheiser microphone (Wedemark, Germany; frequency response: 40 Hz to 18 kHz). Recorded songs were digitized with Avisoft SASlab pro40 software (Avisoft Bioacoustics, Berlin, Germany) at a sampling rate of 44 kHz and a 16-bit amplitude resolution.

Song description

A canary song consists of a series of phrases, where each phrase is composed of the repetition of a syllable generally formed of 1 or 2 notes (see two examples of songs of a given bird in Fig. 1A). A male canary has a repertoire of around 20–30 distinct phrases (range 12–40). Only a part of the repertoire (on average, 8–10 phrases) is present in each song, and phrases may be recombined in different ways (Fig. 1D). There is thus no fixed repertoire of song types, as in some songbird species. However, some phrases, and also some sequences in a given number of phrases ("n," varying from 2 to 6) that occur in a fixed order, regularly recur in songs of the same individual (Del Negro et al. 2005; Lehongre et al., 2008; Fig. 1B, C). The succession of phrases in a song is probably constrained by some form of syntactic organization. The most frequently delivered sequence of n phrases (from 2 to 6) appears more often than might be expected based on the overall frequency of its constituent phrases (Lehongre et al., 2008).

Individually distinctive song features

In a previous study, we analyzed variations in song features within and between individuals, to determine how the identity of the singer could be encoded in song (Lehongre et al., 2008). In the phrase repertoire of each individual, only a few phrases (range: 2–6) are specific to the bird. The others are shared by two or more birds living in the same or in a different aviary (Lehongre et al., 2008, 2009). Also, two familiar birds share up to 70% of their repertoire, while this value is around 50% for two unfamiliar birds (Lehongre et al., 2009). In the phrase repertoire of each bird, phrases also differ in the frequency of their occurrence. Therefore, even if two birds share a large part of their repertoire, their songs differ in the manner of delivery of this repertoire. In addition, most sequences of phrases that are contained in the songs of an individual are not sung by other birds, regardless of sequence length (Lehongre et al., 2008). Consequently, all songs of an individual, except for very short ones, can be viewed as being individual-specific.

Song analysis

For each bird, a detailed analysis of the repertoire was carried out. Briefly, an adequate length of song, that is at least 350 s of song excluding pauses (about 40 songs), was recorded. Previous studies have reported that once 200–250 s of song have been analyzed, all new songs are composed of phrases that have been sung at least once before (Güttinger, 1985; Halle et al., 2003). Identifying different phrases of the bird's repertoire is straightforward: in spectrograms, syllables differ in spectral and temporal parameters and in their repetition rate. As these syllable features show considerable stereotypy, so the same phrase can be easily recognized in subsequent recordings.

As a first step, the phrase repertoire was analyzed individually (see Fig. 1 for an example). The different phrases were assigned a number (one or two digits; individual labeling) and the frequency of their occurrence in an individual's songs was calculated. Ranks were attributed to the phrases according to their frequency of occurrence, with the first rank being assigned to the most frequent phrase. As frequently occurring phrases were very often sung in the same order and formed fixed sequences, a Matlab software routine (The MathWorks, Natick, MA, USA) was written by K. Lehongre to list sequences of n phrases (from 2 to 6) and the frequency of delivery of each of these sequences.

In order to compare the repertoire of phrases and sequences between individuals, phrases were classified and labeled with respect to six parameters (common labeling): the number of elements, the type of frequency modulation characterizing the notes of the syllables (generally upwardly or downwardly sweeping fre-