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Research Report

Molecular characterization of the song control nucleus HVC in Bengalese finch brain

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ABSTRACT

Songbirds have a specialized neural substrate for learned vocalization, called the song circuit, which consists of several song nuclei in the brain. The song control nucleus HVC (a letter-based name) is the intersection point of the song learning and vocal motor pathways. Knowledge of the types of genes expressed in the HVC is essential in understanding the molecular aspects of the HVC. Gene expression in the HVC under silent conditions shows the competence necessary for singing. To investigate this, we compared the HVC with its adjacent tissues in searching for the molecular specificities of the song nucleus HVC using an in-house cDNA microarray of the Bengalese finch (*Lonchura striata* var. *domestica*). Our microarray analysis revealed that 70 genes were differentially expressed in the HVC compared with the adjacent tissue. We investigated 27 of the microarray-selected genes that were enriched or repressed in the HVC by in situ hybridization. We found that multiple calcium-binding proteins (e.g., *CAPS2*, parvalbumin and *ATH*) were enriched in the HVC. Meanwhile, the adult HVC showed low expression levels of plasticity-related genes (e.g., *CAMK2A* and *MAP2K1*) compared with the juvenile HVC. The HVC plays an important role during song learning, but our results suggest that the plasticity of this nucleus may be suppressed during adulthood. Our findings provide new information about the molecular features that characterize the HVC.

1. Introduction

Birdsong has been studied as a biological model of human language, because the process of song learning is similar to language development. Many studies have reported the neural mechanisms of birdsong using zebra finches. On the other hand, Bengalese finches have attracted attention as a model of human language from the song complexity and auditory feedback system (Okanoya and Yamaguchi, 1997; Sakata and Brainard, 2006; Katahira et al., 2007; Tumer and Brainard, 2007; Sakata et al., 2008). Vocalization and vocal learning depend on the structure of a set of neuronal nuclei in the songbird brain. Two forebrain pathways connecting a number of song control nuclei compose the birdsong neural

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Abbreviations: Area X, area X of the striatum; DLM, medial dorsolateral nucleus of the thalamus; HP, hippocampus; HVC, letter-based name, formerly the high vocal center; L, field L; lMAN, lateral MAN; MAN, magnocellular nucleus of the nidopallium; NC, caudal nidopallium; NCM, caudomedial nidopallium; RA, robust nucleus of the arcopallium

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substrate. The caudal pathway, including the HVC (a letter-based name) and the robust nucleus of the arcopallium (RA) is implicated in song production. The rostral pathway, including the HVC, the lateral part of the magnocellular nucleus of the neostriatum (lMAN) and Area X, has a role in song learning (Nottebohm et al., 1976, 1982; Fortune and Margoliash, 1995; Foster and Bottjer, 1998; Brainard and Doupe, 2000; see Fig. 1). Accordingly, the HVC consists of two types of projection neurons (i.e., those projecting into Area X of the rostral pathway and the RA of the caudal pathway) and fast-spiking interneurons (Hahnloser et al., 2002; Rauske et al., 2003; Mooney and Prather, 2005; Wild et al., 2005; Kozhevnikov and Fee, 2007). The songbird-specific nucleus HVC is in the caudal nidopallium (NC), a common avian structure. There is similar connectivity shared between the HVC and the dorsocaudal nidopallium ventral of the HVC, called the HVC shelf (Kelley and Nottebohm, 1979; Scheich, 1991; Wild et al., 1993; Margoliash et al., 1994; Vates et al., 1996; Fortune and Margoliash, 1995). The HVC shelf is equivalent to the dorsal NC for filial imprinting in the chick or pigeon by the connectivity from fields L1 and L3. Therefore, it was thought that the HVC and the shelf shared a common developmental and/or evolutionary origin. The neural substrate for birdsong has been investigated by histological and physiological analysis in many studies. In addition, investigating the molecular features of the HVC can lead to an understanding of the neural basis of learned vocalizations and genetic differences between species.

Several genes are reported to be highly expressed in the song nuclei (Clayton, 1997, 2004; Holzenberger et al., 1997; Jarvis and Nottebohm, 1997; Kimpo and Doupe, 1997; Akutagawa and Konishi, 1998; Rasika et al., 1999; Li et al., 2000; Scharff and White, 2004; Scott et al., 2004; Wada et al., 2004; Agate et al., 2007; Velho et al., 2007). Microarray technology has recently permitted high-throughput gene expression profiling (Schena et al., 1995). Lovell et al. (2008) reported screening for HVC markers using cDNA microarrays in the zebra finch. Zebra finches and Bengalese finches are closely related species but Bengalese finches are highly dependent on auditory feedback for vocalization and their songs have a highly complex syntax. These differences may derive from genetic differences because cross-fostering experiments have revealed that learned vocalization was restricted by the innate ability to learn. We generated a Bengalese finch cDNA microarray to profile the gene expression in the HVC under the same conditions as in Lovell's study (Lovell et al., 2008) (Fig. 2). Here, we compared the gene expression profiles between the HVC and, as a control, the region of the dorsocaudal nidopallium ventral of the HVC shelf region, in adult male Bengalese finches using two-color competitive microarray hybridization. We found that 70 genes were differentially expressed between the HVC and the shelf.

2. Results

2.1. *Construction of the cDNA library and microarray*

We constructed full-length enriched cDNA libraries from eight tissues of the Bengalese finch (Fig. 2A). We used multiple tissues to reduce redundancy among the clones we analyzed, considering the tissue-specific differences in gene expression. These libraries contained inserts that averaged approximately 1.6 kb. We deemed it acceptable to use target DNA for cDNA microarray analysis, because the probes were prepared by oligo dT-based reverse transcription and linear amplification of RNA; both reactions enrich the 3′-regions of the cDNA. We obtained 4361 clusters of sequences that closely matched (E-value less than $1e^{-30}$) sequences from other species in the nucleotide databases at the National Center for Biotechnology Information. We chose to examine 5′-sequences because the 3′-end of cDNAs is usually untranslated region, which is less

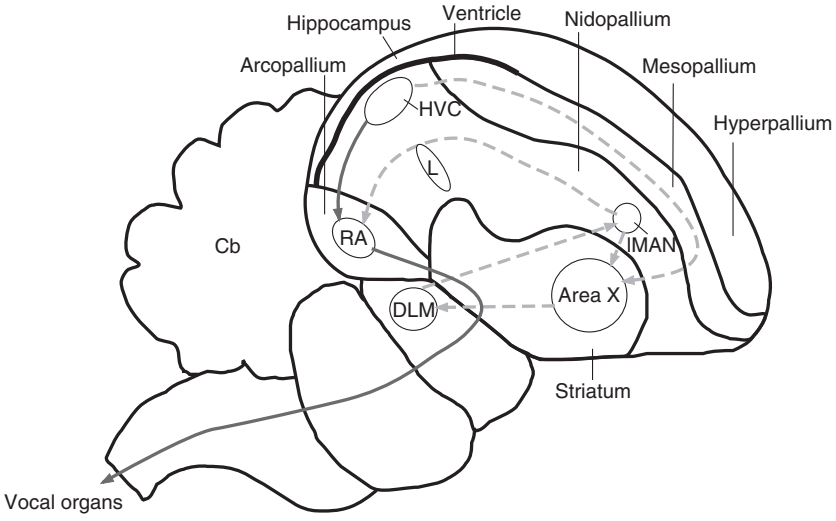

Fig. 1 – Schematic diagram of the male songbird brain. The rostral pathway is the song learning pathway consisting of the HVC (a letter-based name), the lateral magnocellular nucleus of the neostriatum (lMAN), the medial dorsolateral nucleus of the thalamus (DLM), and Area X (light gray dotted arrows). The caudal pathway is the motor (vocal) pathway consisting of the HVC and the robust nucleus of the arcopallium (RA) (dark gray arrows). Cb, cerebellum. L, field L.