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Review

The opportunities and challenges of large-scale molecular approaches to songbird neurobiology

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ABSTRACT

High-throughput methods for analyzing genome structure and function are having a large impact in songbird neurobiology. Methods include genome sequencing and annotation, comparative genomics, DNA microarrays and transcriptomics, and the development of a brain atlas of gene expression. Key emerging findings include the identification of complex transcriptional programs active during singing, the robust brain expression of non-coding RNAs, evidence of profound variations in gene expression across brain regions, and the identification of molecular specializations within song production and learning circuits. Current challenges include the statistical analysis of large datasets, effective genome curations, the efficient localization of gene expression changes to specific neuronal circuits and cells, and the dissection of behavioral and environmental factors that influence brain gene expression. The field requires efficient methods for comparisons with organisms like chicken, which offer important anatomical, functional and behavioral contrasts. As sequencing costs plummet, opportunities emerge for comparative approaches that may help reveal evolutionary transitions contributing to vocal learning, social behavior and other properties that make songbirds such compelling research subjects.

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1. Introduction

Deciphering the molecular and genetic basis of learned behaviors is one of the central challenges in neurobiology. Through the early pioneering efforts of neurobiologists like Gabriel Horn, birds were shown to be highly informative model organisms with regards to uncovering plastic changes in the brain that may underlie learning and memory, especially in the context of visual imprinting (e.g., Horn et al., 2001; reviewed in Horn, 2004). These early studies in birds contributed substantially to the broadly accepted notion that the laying down of long-lasting memories requires specific and localized biochemical and molecular changes in the brain. Avian studies have particularly benefitted from a relatively simpler brain organization than in mammals, with telencephalic pallial areas having a nuclear and thus less heterogeneous spatial distribution than the mammalian cortex (Reiner et al., 2004a; Jarvis et al., 2005). This structural organization considerably facilitates anatomical, molecular and physiological studies of the avian brain. Nonetheless, the avian telencephalon shares with the mammalian brain the occurrence of specialized areas involved in sensory and

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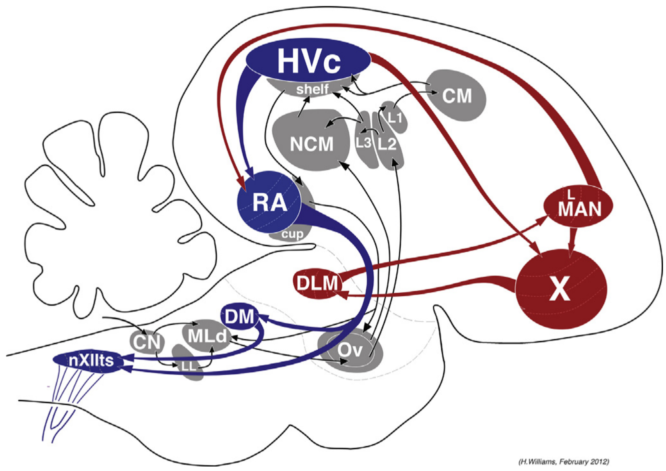

(H.Williams, February 2012)

Fig. 1. Simplified schematic diagram showing major elements of the song control system (sagittal view). Auditory input pathways are shown in gray: the experience of hearing song activates gene expression in many of these areas. The primary motor output pathway is in blue: the act of singing activates gene expression in these areas. The anterior forebrain pathway (AFP) is shown in red: this pathway is necessary for song learning and plasticity, and gene activity in its component nuclei varies with context of singing. Some additional elements (e.g., respiratory control pathways) are not shown but may be seen in a more comprehensive version of this figure produced and maintained online by Heather Williams (http://web.williams.edu/Biology/Faculty_Staff/hwilliams/Finches/circuits.html). Common abbreviations for the individual nuclei are used here; a full account of the circuitry is given in Reiner et al. (2004a). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

perceptual processing, motor control, multi-sensory and sensorimotor integration, and various aspects of learning and cognition (Reiner et al., 2004a,b; Jarvis et al., 2005, 2013). The analogs of thalamo-recipient cortical layers, long descending projections to sub-cortical targets, and intricate cortical-like microcircuitry with abundant inhibitory interneurons have also been identified in birds. These similarities point to conserved aspects of the functional brain organization of birds and mammals, and support the notion that insights on brain function and behavior gained from avian studies can be highly informative with regards to mammals, including humans.

A remarkable example is the study of songbirds, one of the few groups of organisms where juveniles are capable of learning their vocalizations by imitating a model, typically the song of an adult male, which is usually referred to as a tutor (Marler and Peters, 1977; Nottebohm, 1972). Songbird learning bears remarkable similarities to human speech learning, including the early production of immature babbling-like vocalizations (subsong), the requirement for auditory feedback during the sensorimotor vocal learning phase, and the occurrence of regional variations or dialects in the adult repertoire, characterizing a cultural transmission of vocal patterns (Doupe and Kuhl, 1999). Furthermore, vocal production and learning depend upon a set of discrete interconnected brain areas collectively known as the song control system (Nottebohm and Arnold, 1976), which includes nuclei in cortical-like areas, basal ganglia, and thalamus (Fig. 1). Different parts of this circuitry are involved in the production and/or learning of complex vocal patterns, as demonstrated very elegantly in zebra finches (authoritative reviews on the song system in Zeigler and Marler, 2004, 2008). Output projections from this circuitry allow for cortical-like areas to exert descending control over vocal and respiratory brainstem areas, a connectivity feature that has only been seen in animals that have evolved vocal learning (Jarvis, 2004). This vocal control circuitry operates in concert with auditory processing centers, thought to be important for the perceptual processing and auditory memorization of song (Chew et al., 1995; Mello and Clayton, 1994; Bolhuis et al., 2001), which is also an essential step in songbird learning (London and Clayton, 2008). Besides vocal learning per se, studies of the vocal control system in finches and other songbird species have contributed substantially to our understanding of brain sex dimorphisms, the effects of sex steroid on the brain and behavior, and neurogenesis and neuronal replacement in adulthood, among several other contributions (reviewed in Zeigler and Marler, 2004, 2008).

The detailed knowledge available on the anatomical and functional organization of the song system has set the stage for defining the molecular properties of its component auditory and vocal control pathways, and for identifying molecular and genetic correlates of learning. Early efforts benefitted greatly from the analysis of a few activity-inducible genes (a.k.a. immediate early genes, IEGs), including ZENK (a.k.a. zif-268, egr1, ngfia and krox-24) and c-fos (Mello and Clayton, 1994; Mello et al., 1992; Kimpo and Doupe, 1997 and reviewed in Clayton, 1997, 2000; Mello, 2002). More recently, new methods for broad scale high-throughput analyses have drastically changed the landscape of songbird research. Here we review the progression of this research from its early focus on IEGs to the current emphasis on high-throughput approaches. We consider both the insights gained and the challenges still to be met in future research.

2. Lessons/insights from single activity inducible genes

IEGs are rapidly and transiently induced in activated neuronal cells, and their study, which traditionally uses methods focusing on single genes, has been very useful for mapping brain activation (Mello et al., 1992; Kimpo and Doupe, 1997; Velho et al., 2005; Park and Clayton, 2002; Wada et al., 1521; Stripling et al., 2001; Chaudhuri and Cynader, 1993; Curran and Morgan, 1985; Morgan et al., 1987; McCabe and Horn, 1994; Bailey et al., 2002; Gentner et al., 2001; Phillmore et al., 2003). Importantly, animals are allowed to behave freely, thus minimizing issues related to stress or restraint. Furthermore, as one can map the entire brain through serial sections, this approach is unbiased, and can lead to novel and sometimes surprising findings. The method also allows for a direct assessment of activated cell populations, as well as the determination of their phenotype through multiple labeling or by combining gene expression with other approached such as tract-tracing. Importantly, IEGs like ZENK and c-fos encode transcription factors that can modify programs of gene expression in the activated cells (Clayton, 1997, 2000; Curran and Morgan, 1985; Morgan et al., 1987). Thus, mapping with IEGs like ZENK potentially reveals areas that are undergoing activity-induced neuroplasticity changes.

Due to a combination of all the features above, IEG expression analysis can contribute many important insights. In songbirds, analysis of the IEG ZENK has been instrumental for the identification and functional studies of brain structures that are activated in the context of vocal communication (Mello, 2002). Of particular interest, the act of hearing song was found to predominantly activate the caudomedial nidopallium (NCM) and mesopallium (CMM), structures subsequently shown to be part of the avian central auditory processing pathways (Mello and Clayton, 1994; Vates et al., 1996; Mello et al., 1998). In contrast, the act of singing activates IEG expression in the primary nuclei of the vocal control circuit (Kimpo and Doupe, 1997; Jarvis and Nottebohm, 1997; Jin and Clayton, 1997). This distinction, considered surprising at the time, clearly establishes distinct subsystems in the brain for perceiving vs. producing complex vocal signals. There is an interesting parallel in chicken, namely the demonstration that the IEG c-fos is induced in the intermediate medial mesopallium (IMM, named IMHV before implementation of the newer avian brain nomenclature; Reiner et al., 2004a) in the context of imprinting (McCabe and