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Motor Circuits Help Encode Auditory Memories of Vocal Models used to Guide Vocal Learning

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Introduction

The sensations we experience early in life can exert lifelong effects on our perception and behavior. For example, early binocular experience is critical to the development of stereopsis and early auditory experience of speech sounds is critical to the development of both speech comprehension and production (Fromkin, Krashen et al. 1974; Lane 1976; Braddick, Atkinson et al. 1980; Fox, Aslin et al. 1980; Petrig, Julesz et al. 1981; Stoel-Gammon and Otomo 1986; Oller and Eilers 1988). These lasting effects on perception and behavior indicate that early experience leaves an indelible imprint on the brain. Actually, sensory manipulations during early postnatal life, including monocular or monaural deprivation, can permanently alter the structure and function of primary visual and auditory cortices (Hubel, Wiesel et al. 1977; Shatz and Stryker 1978; LeVay, Wiesel et al. 1980; Popescu and Polley 2010; Barkat, Polley et al. 2011), respectively.

Whether early sensory experience immediately involves motor regions of the brain is less clear, because the behavioral consequences of early sensory experience may only manifest months or even years later. Therefore, although early auditory experience of speech sounds has been shown to rapidly alter an infant's capacity for vocal perception, the vocal fluency that also depends on this early auditory experience only emerges after a period of vocal practice spanning several years. Similarly, the path an aspiring musician takes from first listening to a composition to successfully reproducing it can be arduous, involving months or years of intensive practice. This long delay between early auditory experience and vocal or instrumental mastery suggests that the motor regions of the brain that control the appropriate vocal or manual gestures do not participate in the initial encoding of auditory experience, and instead only contribute in later stages of motor learning. In contrast to this "sequential" model, here we review evidence from studies in songbirds that indicate that premotor regions important to the planning and execution of learned vocal sequences also help to encode the auditory experience of other birds' songs on which these learned vocalizations are modeled.

Songbirds are the preeminent organisms for studying the detailed neurobiological mechanisms of vocal learning. Song learning in oscine birds displays many remarkable parallels to human speech learning: both are sensitive period-limited forms of imitative vocal learning that depend on auditory experience of an appropriate vocal model as well as auditory feedback (Doupe and Kuhl 1999; Mooney, Prather et al. 2008). During an initial

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stage of sensory learning, young songbirds listen to and memorize the song of an adult tutor, which they then emulate during an ensuing period of sensorimotor learning that depends on vocalization-related auditory feedback. Sensory learning of the tutor song can occur rapidly (~minutes of total auditory experience), whereas sensorimotor learning is a prolonged and apparently laborious process during which the pupil may utter its song tens or hundreds of thousands of times before achieving a close match to the tutor song (Tchernichovski, Lints et al. 1999; Tchernichovski, Mitra et al. 2001) (Figure 1a). Moreover, a gap of weeks or even many months may separate the end of sensory learning from the onset of sensorimotor learning. This delay indicates that a long-lasting memory, or engram, of the tutor song is formed during sensory learning (Funabiki and Konishi 2003), and suggests that sensorimotor learning depends on a neural comparator that evaluates sing-related auditory feedback in relation to this memory (Konishi 2004).

The nature of this tutor song memory, including how and where it might be encoded in the songbird's brain, remains a matter of central importance and great debate (Basham, Nordeen et al. 1996; Bolhuis, Zijlstra et al. 2000; Terpstra, Bolhuis et al. 2004; Bolhuis and Gahr 2006; London and Clayton 2008; Roberts, Gobes et al. 2012). Resolving the locus and nature of this memory is critical to deciphering neural mechanisms for vocal learning and also can inform more general issues of how sensory experience acts on the developing brain to form long-lasting memories that guide perceptual and behavioral development. Moreover, birdsong provides a powerful system for examining not only those brain mechanisms important to learning, but also neural mechanisms for communication, courtship, and the control of complex motor sequences. Of these various issues, the current review focuses more exclusively on studies that have sought to identify the pathways and mechanisms that operate in the pupil's brain to encode a memory of the tutor's song.

Introduction to the Song System: Historical Perspectives

The modern appreciation of birdsong learning stems from the detection of song dialects in the chaffinch by Thorpe in England (Thorpe 1954), and the extension of these findings to North America sparrow species by Thorpe's student, Peter Marler, and his colleagues (Marler and Tamura 1964; Konishi 1965) [however, see (Barington 1773) for what might be the earliest published scientific account of song learning in birds using cross-fostering methods and acoustic isolation from a vocal model]. The notion that birdsong is a culturally transmitted vocal behavior gained more direct support from cross-fostering experiments: juvenile songbirds raised by a foster species developed songs resembling those of their foster parents, rather than of their own species (Immelmann 1969; Baptista and Petrinovich 1984; Baptista and Petrinovich 1986), whereas juveniles deprived altogether of tutor song experience develop acoustically impoverished "isolate" songs (Thorpe 1954; Thorpe 1958; Immelmann 1969; Marler 1970; Price 1979). Furthermore, either tutored or isolate juveniles that are deafened prior to sensorimotor learning fail to develop copies of the tutor song, underscoring the importance of auditory feedback to the sensorimotor learning process (Konishi 1965).

The foundational behavioral research emphasized the importance of auditory experience of a song model and auditory feedback in song learning. In contrast, the pioneering neurobiological analysis of singing and song learning took a decidedly motor-oriented slant. Inspired by the observation that unilateral vocal (i.e., syringeal) nerve cuts in chaffinches could elicit markedly different song deficits depending on the side of the lesion (Nottebohm 1971), Fernando Nottebohm speculated that central circuits for birdsong may also be lateralized, similar to the lateralized language function in the human neocortex. To define the central circuits for birdsong, Nottebohm and his colleagues conducted pioneering neuroanatomical studies that mapped a multisynaptic pathway that originated in the

telencephalon and terminated on the syringeal motor neurons (Nottebohm, Stokes et al. 1976) (Figure 2a). They also confirmed that this pathway was essential to singing: bilateral lesions of the song premotor nucleus HVC, a component of this song motor pathway, rendered songbirds entirely mute for learned songs. Notably, although birds with HVC lesions no longer sing, they still can utter short notes and unlearned calls, much as a person with Broca's aphasia loses the capacity for fluent speech while retaining an ability to produce "emotional" vocalizations, such as laughter, cries, or groans.

In the several decades since Nottebohm's pioneering anatomical discoveries, tremendous progress has been made in mapping the connections and functional role of a variety of brain nuclei comprising a widely distributed network commonly referred to as the "song system." Prior to describing the song system in more detail, and considering how auditory experience of a tutor's song impinges on the song system to affect learning, it is worthwhile to emphasize that the history of discovery has influenced studies of song learning neurobiology in two important ways. First, the historical perspective has emphasized a circuit model in which the control of song is mostly "top-down," with less of an account given to recurrent architecture and other ascending pathways that provide input to HVC and other parts of the song system (Figure 2b–c). Indeed, a modern view of the song system, which includes a song motor pathway essential to singing and an anterior forebrain pathway essential to audition-dependent song plasticity, may tend to minimize the significance of the myriad ascending pathways that interact with these two component pathways of the song system, including the neuromodulatory pathways heavily implicated in experience-dependent plasticity in other vertebrates (Figure 2c). Second, the functional organization of the songbird's auditory system, and especially where and how it interfaces with the song system, is arguably less well understood than the organization of the pathways that influence song motor control. As noted recently by Hahnloser and Kotowicz (Hahnloser and Kotowicz 2010), a comprehensive account of the song system must encompass the auditory regions of the brain that process the two types of auditory experience critical to song learning and convey this information to the song system.

Functional Anatomy of the Song System

The 'song system' comprises two functionally distinct pathways: the song motor pathway (SMP) and the anterior forebrain pathway (AFP). The SMP is essential to song production, whereas the AFP is essential to auditory feedback-dependent vocal plasticity and is an important source of trial-to-trial variability in song performance (Fig. 2). The output nuclei of the SMP and AFP (HVC and LMAN, respectively) both supply input to the song motor nucleus RA, but the two pathways play dramatically different roles in singing and song learning. When LMAN, the output of the AFP, is either inactivated or destroyed, adult songbirds or juveniles in the midst of sensorimotor learning can still sing highly stereotyped renditions of their song, suggesting that the SMP is sufficient for song production (Bottjer, Miesner et al. 1984; Brainard and Doupe 2000; Kao, Doupe et al. 2005; Olveczky, Andalman et al. 2005). Moreover, electrophysiological recordings in singing birds and focal cooling experiments support a model in which HVC provides a precise timing signal that controls the temporal aspects of birdsong (Hahnloser, Kozhevnikov et al. 2002; Long and Fee 2008; Long, Jin et al. 2010). And although adult birds with HVC lesions no longer sing, they continue to produce short vocal elements similar to those found in 'subsong,' a vocal behavior displayed by juvenile songbirds prior to sensorimotor learning (Nottebohm, Stokes et al. 1976; Aronov, Andalman et al. 2008). A current view is that, during sensorimotor learning, a timing signal from HVC and a variability signal from LMAN both influence the singing-related activity of RA neurons, whereas the stereotyped (i.e., crystallized) song of the adult is more solely dependent on HVC, with LMAN exhibiting little or no influence on RA activity.

Although sensory learning and sensorimotor learning can be separated by many months, the memory of the tutor song must ultimately be used to guide sensorimotor learning, and thus must be accessed by the song system. The interaction between HVC and LMAN inputs to RA is critical for sensorimotor learning and adult forms of auditory feedback-dependent vocal plasticity, making HVC and the AFP two sites where the tutor song memory could be accessed. For example, reversible inactivation of LMAN in juvenile birds engaged in sensorimotor learning blocks trial-to-trial song variability (Oliveczky, Andalman et al. 2005) and permanent lesions of LMAN prevent song copying in juveniles and deafening-induced song degradation in adults (Bottjer, Miesner et al. 1984; Brainard and Doupe 2000). Reversible inactivation of LMAN also can block adaptive shifts in syllable pitch driven by pitch-contingent disruption of auditory feedback (Andalman and Fee 2009; Warren, Tumer et al. 2011; Charlesworth, Warren et al. 2012). These short-term changes in song performance then undergo consolidation in a process that is thought to rely on changes in the efficacy of HVC to RA synapses. These observations indicate that during sensorimotor learning, inputs to RA from both HVC and LMAN are influenced by the memorized song model. Therefore, elucidating the neural circuit mechanisms for sensory learning requires a consideration of how memories of tutor song experience interact with HVC and the AFP.

Anatomy of the Auditory Forebrain and Auditory Inputs to the Song System

A current perspective is that this interaction is likely to occur immediately above those levels of the songbird's auditory system that are analogous to the primary and secondary regions of the mammalian auditory cortex (Bolhuis and Gahr 2006). In songbirds, auditory information is relayed from the thalamic nucleus ovoidalis (Ov; analogue of the medial geniculate body) to the auditory telencephalic region Field L (the analogue of primary auditory cortex). Some neurons in Field L project to adjacent secondary auditory forebrain regions, including the caudomedial neopallium (NCM), the caudal mesopallium (CM), and to an auditory region just ventral to HVC, known as the HVC shelf. As shown in Figure 2, interconnections between and among auditory forebrain regions are abundant, suggesting that these regions participate in higher order processing of complex auditory signals, including birdsong, an idea that is further supported by a variety of electrophysiological studies of NCM and CM neuronal activity in awake and anesthetized songbirds (Gentner and Margoliash 2003; Coleman and Mooney 2004; Nagel and Doupe 2006; Bauer, Coleman et al. 2008; Nagel and Doupe 2008).

A combination of anatomical and electrophysiological studies has also fleshed out some of the pathways by which song-related information from the auditory "cortex" could access the song system. One of these involves two small structures – the nucleus interface (Nif) and the nucleus avalanche (Av) – that are embedded in the Field L-CM complex and that provide synaptic input directly to HVC (Figure 2). These two reciprocally connected nuclei receive at least some of their auditory-related inputs from CM and also are innervated by the thalamic nucleus uvaeformis (Uva), which receives auditory-related information from the lateral lemniscus and respiratory-related motor and proprioceptive information from the medulla (Wild 1994; Akutagawa and Konishi 2005; Coleman, Roy et al. 2007; Akutagawa and Konishi 2010). Because Uva also directly innervates HVC, at least three channels of auditory information, arising from CM, Nif, and Uva, could enter the song system at the level of HVC. Moreover, HVC is the source of auditory-related responses that can be recorded downstream in the SMP and AFP, indicating that HVC serves as a critical interface between the auditory and song systems.

Nonetheless, another possibility is that auditory information important to song learning bypasses HVC altogether. More specifically, axons from the HVC shelf, NCM and CM make dense terminations in a region adjacent to RA, termed Aiv, which in turn projects to

the ventral tegmental area (VTA) (Vates, Broome et al. 1996; Gale, Person et al. 2008). This organization could enable auditory information to access the AFP through the VTA's extensive terminations in Area X. Thus, at least two routes link the songbird's complex auditory forebrain network to the song system, one of which involves HVC and the other of which involves VTA and Area X.

In addition to the bottom-up interactions between the auditory telencephalon and the song system, projections from the song system to the auditory system also have been detected and these "top-down" pathways could influence auditory processing important to song learning. Notably, a recent study has shown that some HVC neurons make axonal projections onto Av, raising the possibility that a song motor structure could directly influence the auditory system (Akutagawa and Konishi 2010). The convergence of recurrent respiratory-motor-related signals and auditory information in Uva also provides another pathway by which motor-related information could influence auditory processing. Although the functional significance of either of these motor – auditory pathways awaits elucidation, their existence challenges the idea that auditory – vocal interactions are merely a one-way street. Indeed, the recurrent architecture that links these two systems raises the possibility of bidirectional interactions between the auditory system and the song system during song learning.

In Search of Auditory Representations of the Tutor Song

Hearing a tutor song is essential to sensory learning, and thus the juvenile songbird's auditory system is undoubtedly necessary to the initial encoding of tutor song experience. Nonetheless, the auditory system could serve as the principle site for storing the tutor song memory, part of a more distributed network for memory storage that also involves the song system, or possibly even upstream of the site of memory storage (i.e., the memory is encoded entirely in the song system). Although neurophysiological recordings have found little evidence for tutor selective responses in Field L or CM (Amin, Grace et al. 2004), a series of recent studies suggest that song learning can exert a lasting influence on auditory representations in higher order auditory areas, including NCM. For example, playback of novel songs, the bird's own song, or the tutor's song can drive expression of the transcriptional regulator *egr-1* (also known by the acronym ZENK in the songbird literature) in the NCM of adult songbirds (Bolhuis, Zijlstra et al. 2000; Bolhuis, Hetebrij et al. 2001; Terpstra, Bolhuis et al. 2004). Furthermore, electrophysiological responses of NCM neurons to novel stimuli rapidly habituate in adult birds, suggesting that NCM or regions presynaptic to NCM may encode traces of auditory experience (Chew, Mello et al. 1995). Finally, although playback of the tutor's song and the bird's own song elicit similar levels of ZENK responses in NCM, the degree to which tutor song playback induces ZENK expression in an adult bird's NCM correlates positively with the amount of the tutor's song he copied during sensorimotor learning (Terpstra, Bolhuis et al. 2004). These findings are consistent with the idea that NCM can encode auditory experience in adult birds and have led to speculation that NCM could also help to store the tutor song memory.

Correlational analyses involving electrophysiological recordings or IEG expression patterns alone are insufficient to determine a brain structure's role in storing tutor song memories. To better understand the role of NCM in storing information about the tutor song, Gobes and Bolhuis lesioned NCM in adult birds and tested the birds' preferences for listening to their own songs or their tutors' songs (Gobes and Bolhuis 2007). They found that NCM lesions in adult birds did not affect their songs, indicating that adult birds do not rely on NCM, and any auditory memories that may be stored there, to maintain their stable songs. However, NCM lesions reduced the birds' preference for their tutors' songs over novel songs, suggesting that lesions of NCM may diminish a bird's ability to recognize its tutor's song. However,

because these experiments were performed in adult birds, it was unclear whether this information could be used by juvenile birds to guide sensorimotor learning.

Functional manipulations of NCM and adjacent regions of the auditory forebrain regions have attempted to more directly test this idea. To examine the role of NCM in sensory learning, London and Clayton infused a pharmacological inhibitor of the mitogen-activated protein kinase/ERK kinase (MEK), which has been implicated in forms of long-lasting synaptic plasticity, during controlled tutoring sessions in previously tutor-naïve juvenile male zebra finches (London and Clayton 2008). Birds that received the MEK inhibitor during tutoring sessions produced poor copies of the tutor song as adults. This was the first study to show that manipulating activity in the secondary auditory telencephalon can affect song learning, thus strengthening the idea that NCM could serve as an important site for encoding a tutor song memory. One potential caveat is that the drug treatment likely exerted relatively long-lasting effects in NCM (~hours), during which time the pupil may also have practiced its own song. Therefore, drug treated birds may exhibit impaired song copying because of interference with the encoding of a tutor song memory or disrupted processing of singing-related auditory feedback. Furthermore, pupils treated with the MEK inhibitor still learned elements of their tutors' songs, indicating that at least partial memory formation and storage had occurred. To resolve these issues, an important next step will be to disrupt activity in the juvenile's NCM only when the tutor is singing, an approach that may better delineate the role of this region in encoding tutor song memories.

Auditory Responses and Tutor Song Representations in the Song System

The earliest attempts to record singing-related activity in the song system, which involved chronic extracellular recordings in the HVC of freely behaving canaries, detected elevated activity not only during singing, as expected of a song premotor structure, but also when the bird's song was played back to it through a speaker (McCasland and Konishi 1981). Subsequent electrophysiological experiments, performed in anesthetized, sedated, sleeping, or awake birds, detected playback-evoked activity throughout the SMP and AFP, and even in the vocal nerve, and inactivation experiments point to HVC as a crucial conduit for conveying these responses to downstream components of these two pathways (Williams and Nottebohm 1985; Doupe 1997; Solis and Doupe 1997; Mooney 2000; Cardin and Schmidt 2003; Sturdy, Wild et al. 2003; Cardin and Schmidt 2004; Nick and Konishi 2005; Roy and Mooney 2007; Prather, Peters et al. 2008; Sakata and Brainard 2008; Prather, Nowicki et al. 2009) (Figure 3a). Although auditory activity in the SMP of some songbird species, including the zebra finch, is strongly state-dependent (see below), these various findings raise the possibility that HVC and its downstream targets could function in auditory processing of song in addition to serving a motor-related function. One idea is that the auditory responsiveness of song system neurons could enable them to help form and store tutor song memories. Indeed, embedding the tutor song memory in the song motor network could represent an especially efficient strategy for using these memories to later guide sensorimotor learning. Nonetheless, exactly what aspects of experience shape the auditory responses of song system neurons and more importantly, the functional significance of auditory responsive neurons in the song system, remain unclear.

Electrical recordings made in adult birds suggest that the bird's experience of its own song is the major influence on the auditory response properties of song system neurons: most neurons that are responsive to song playback are highly selective for the bird's own song, responding weakly or not at all to the songs of other birds (Margoliash 1983; Margoliash and Konish 1985; Margoliash and Fortune 1992; Mooney 2000; Rosen and Mooney 2003; Coleman and Mooney 2004). This pattern of self-responsiveness is even found in adult birds raised without a tutor (Kojima and Doupe 2007), indicating that self-experience is a critical

factor in shaping BOS-selectivity. Furthermore, selective auditory responses to the bird's own song in HVC and the AFP emerge as sensorimotor learning progresses (Volman 1993; Doupe 1997; Solis and Doupe 1997; Solis and Doupe 1999; Yazaki-Sugiyama and Mooney 2004; Nick and Konishi 2005). And when the song of an adult bird is distorted through peripheral manipulations of its vocal apparatus, many neurons in HVC and in LMAN slowly retune to the distorted song (Solis and Doupe 2000; Roy and Mooney 2007). Taken together, these observations suggest that self-experience, most likely in the form of singing-related auditory feedback, drives the development of song-selective auditory responses in the song system.

Song system neurons also can be excited by playback of the tutor's song. However, in an adult bird that has learned to produce an accurate copy of the tutor song, the tutor song could evoke responses because of its acoustic similarity to the bird's own song, rather than because these neurons encode a lingering trace of tutor song experience. Two observations suggest that auditory responses recorded in the song system are specifically affected by experience of the tutor song. First, some HVC recording sites in adult swamp sparrows, which memorize and copy many (~12) tutor songs as juveniles yet retain only a few of these copies in their adult repertoires, respond most vigorously to tutor songs that were copied only briefly or possibly not at all during sensorimotor learning (Prather, Peters et al. 2010) (Figure 3b–d). Second, juvenile zebra finches with vocal nerve lesions sing songs that are acoustically quite different from the tutor's song, but nevertheless contain some tutor song-selective AFP neurons (Solis and Doupe 1999; Solis and Doupe 2000). These observations raise the possibility that tutor song experience plays at least a limited role in shaping auditory responses in the song system.

One issue is how tutor song experience might access and affect song system neurons, because electrophysiological recordings made in freely behaving adult songbirds have sometimes failed to detect auditory-evoked activity in HVC or other parts of the song system (Leonardo 2004; Kozhevnikov and Fee 2007). Indeed, state-dependent gating of auditory responses is pronounced in the song system of adult zebra finches (Cardin and Schmidt 2003), and little or no auditory-evoked activity is detectable in the HVC or the AFP during periods of wakefulness. In contrast, song-evoked auditory responses have been detected in song system of awake sparrows, canaries, and Society finches (a close relative of the zebra finch), indicating that the “gate” between the auditory and song systems appears remain open in these species (McCasland and Konishi 1981; Prather, Peters et al. 2008; Sakata and Brainard 2008; Fujimoto, Hasegawa et al. 2011). Perhaps most importantly, playback experiments performed with awake juvenile zebra finches at early stages of sensorimotor learning have detected tutor song-selective responses in HVC, indicating that tutor song experience activates the song system at a stage of development when it could have consequences for learning (Nick and Konishi 2005). In this light, a crucial goal of future experiments will be to explore whether interactions with a live, singing tutor immediately affect the electrical activity of neurons in HVC or other parts of the song system in juvenile birds undergoing sensory learning.

Regardless of whether hearing a tutor song immediately activates song system neurons, hearing a tutor song rapidly alters the structural and functional properties of neurons in HVC, pointing to this sensorimotor structure as a potential component of a network for encoding the tutor song experience. Multiphoton *in vivo* imaging and electrophysiological studies show that when a juvenile zebra finch hears a tutor song for the first time, excitatory synapses on HVC projection neurons rapidly (within hours) stabilize and strengthen (Roberts, Tschida et al. 2010) (Figure 4). Furthermore, the amount of synaptic stabilization in the 24h following this first tutor experience correlates with the quality of song copying achieved many weeks later, consistent with a role for these synaptic changes in sensory

learning (Roberts, Tschida et al. 2010). Although the exact identity of the synapses that undergo strengthening remains unknown, intracellular recordings made from HVC neurons immediately before and after tutoring detect the emergence of complex, spontaneous bursts of synaptic activity, which are thought to be driven by inputs from Nif, HVC's major auditory afferent. Additionally, complex spontaneous action potential activity also emerges shortly after tutoring in RA (Shank and Margoliash 2009), presumably reflecting augmented drive from HVC, and the temporal profile of this bursting activity in RA correlates with temporal features of the tutor's song. Thus, the initial experience of the tutor song rapidly strengthens the functional properties of neurons and synapses in HVC and RA, which could point to substrate for encoding tutor song experience within the song motor pathway.

Several findings from pharmacological manipulations of song system activity during tutoring also suggest a role for this sensorimotor network in encoding tutor song experience. First, blocking NMDA receptors in either LMAN or HVC in juvenile zebra finches during tutoring severely impairs their ability to copy the tutor song (Basham, Nordeen et al. 1996; Roberts, Gobes et al. 2012). In contrast, NMDA receptor blockade at times when the tutor was absent had little effect on the subsequent quality of song copying. Second, blocking NMDA receptors in HVC also prevented tutoring-induced changes in dendritic spines, lending support to a model in which excitatory synapses on HVC neurons participate in the encoding of tutor song experience (Roberts, Gobes et al. 2012). Third, reversible inactivation or lesions of Nif made prior to tutoring also impaired subsequent song copying (Roberts, Gobes et al. 2012), even though Nif lesions made after tutoring exert no lasting effect on song (Cardin, Raksin et al. 2005; Roy and Mooney 2009). These findings are consistent with the idea that a distributed network including Nif, HVC, and the AFP helps encode the tutor song experience, even if they cannot confirm that these structures are where auditory memories of this experience are stored. For example, it remains plausible that non-auditory activity in this part of the brain is necessary to gate or calibrate auditory activity in the secondary regions of the auditory system that are situated downstream of HVC. Furthermore, as with drug treatments performed in NCM, the long half-life of these pharmacological manipulations complicates any interpretation, because juvenile zebra finches typically interleave periods of singing with listening to the tutor. Therefore, whether these drug treatments affect activity driven by auditory experience of the tutor song, singing-related auditory feedback, or song motor activity, remains uncertain.

A Necessary Role for Motor Circuits During Tutor Experience

More precisely controlled experimental approaches are necessary to circumvent concerns associated with the long-lasting effects of pharmacological manipulation and to better define the brain circuitry important to encoding and storing information about tutor song experience. Ideally, these new approaches should achieve several objectives. First, manipulation of neural activity in pupil's brain must be temporally precise, such that it is only disrupted when the tutor is singing but not when the pupil is singing or producing other vocalizations. Second, the manipulation of neural activity must be spatially precise, allowing selective disruption of activity within a specific brain region or defined population of neurons in the brain. Third, neural activity in the pupil's brain should be manipulated under remotely, so as not to interfere with the normal social interaction between the pupil and his tutor. A combination of methods that achieve these goals can better identify the neurons and circuits that encode tutor song experience and lead to the formation of tutor song memories.

Optogenetics (Boyden, Zhang et al. 2005) and voice recognition software (Tumer and Brainard 2007) provided the requisite combination of methods to clearly establish the role of HVC in encoding tutor song experience (Roberts, Gobes et al. 2012) (Figure 5a). Optogenetics, which relies on the transgenic expression of a light-activated cation channel

(Channelrhodopsin-2, or ChR2) in neurons and controlled illumination of these neurons using laser light and fiber optics, permits precise temporal control of neuronal excitation. In songbirds, viral vectors containing ChR2 can be used to focally infect a small brain region, such as HVC, further enhancing the spatial control achieved with this method. Furthermore, recent advances in on-line signal analysis enable rapid and reliable detection of an individual bird's song and even individual syllables within that song. Notably, Roberts et al combined voice recognition software to detect when the tutor was singing and concurrently trigger laser illumination of ChR2-expressing neurons in the pupil's HVC, enabling tutor song-contingent disruption of HVC activity (Roberts, Gobes et al. 2012) (Figure 5).

Juvenile male zebra finches subjected to tutor song-contingent optogenetic manipulation of HVC activity fail to copy from their tutors, indicating that normal patterns of activity in HVC (and possibly its downstream targets) are required to accurately encode tutor song experience. In fact, juveniles subjected to this treatment developed songs that in adulthood were highly similar to the songs of birds raised in complete isolation from a tutor, suggesting that they may have failed entirely to form a tutor song memory (Figure 5c). This was not a non-specific effect of repeatedly activating HVC, because pupils that experienced optogenetic disruption of HVC activity shortly after the tutor was removed each day ultimately displayed normal levels of tutor song copying. Moreover, Roberts et al were able to apply trains of electrical stimuli in a pupil's HVC only when the tutor sang one syllable in his polysyllabic song. In these experiments, the pupil's failed to copy the targeted syllable, but copied surrounding syllables, suggesting that HVC encodes tutor song experience in a temporally precise manner (Figure 5d-e).

As described previously, HVC receives a major source of auditory input from Nif, and also receives input from CM/avalanche (Coleman and Mooney 2004; Bauer, Coleman et al. 2008; Akutagawa and Konishi 2010). Notably, tutor song-contingent microstimulation Nif or reversible inactivation of Nif during short tutoring sessions also impaired tutor song copying, whereas tutor song-contingent microstimulation of Field L, part of the primary auditory forebrain, was without effect on the learning process (Roberts, Gobes et al. 2012). Because Field L is an indirect source of auditory input to CM/avalanche and Nif, these findings may better constrain the spatial extent of the circuitry that helps to encode tutor song experience.

Models for Tutor Song Memory

Is HVC a focal site for encoding auditory experience of the tutor song, part of a distributed network for this encoding, or simply an "AND-GATE" that modulates encoding in a downstream region (Figure 6)? Although the disruptive effects of optogenetic and electrical stimulation in HVC on sensorimotor learning strongly suggest that HVC helps to encode the tutor song experience, they do not clearly establish whether HVC is a site where long-lasting memories of this experience are stored. For example, HVC may rapidly encode and relay tutor song experience to one or more downstream memory storage sites (i.e., the AFP, avalanche, or RA), without actually serving as a permanent site for memory storage. Additionally, HVC may generate non-auditory activity that is necessary to gate or calibrate auditory activity in downstream sites, such as Av, without encoding or conveying any information about the tutor song experience. Nevertheless, several observations suggest that HVC functions as a critical site in a network for encoding memories of the tutor song. First, blocking NMDA receptors in HVC blocks tutor song copying and tutoring-dependent spine enlargement, a rapid structural change that correlates with the subsequent amount of tutor song copying. This link between synaptic structure and the quality of behavioral learning suggests that spines on HVC neurons that are strengthened during tutoring could form a long-lasting representation of this experience. Second, tutoring enhances spontaneous,

temporally prolonged bursts of synaptic activity that can be recorded in HVC and in RA, and these bursts of activity are likely to be driven by NIf, which also provides a major source of auditory input to HVC. These findings point to the NIf – HVC synapse as a critical site for registering the effects of tutor song experience, an idea that is further supported by the observation that transient manipulations of NIf activity during tutoring severely disrupt copying. Finally, responses to tutor song models can be evoked in HVC and the AFP even when the pupil's song repertoire differs sharply from those models, either because of developmental or experimental alterations in the sensorimotor learning trajectory. Such persistent representations might be expected if HVC functions as part of a repository for encoding tutor song memories formed during sensory learning, which is only partially overwritten by repeated auditory feedback experience of the bird's own song during sensorimotor learning.

An influential model advanced more than four decades ago by Konishi posits that sensory learning leads to the formation of an internal representation of the tutor song, or acquired template, against which the pupil subsequently evaluates his song performance during sensorimotor learning (Konishi 1965; Konishi 2010). Because sensory learning is driven by auditory experience of a tutor song and sensorimotor learning depends on auditory feedback, it has long been assumed that the template is an auditory representation of the tutor song residing within higher levels of the auditory system. The various lines of experimental evidence considered here support a model where sensory learning involves a more distributed network that extends into song premotor regions, including HVC. One potential consequence of this distributed representation is that tutor song memories could be encoded in sensorimotor or motor coordinates, rather than in a purely auditory framework. Perhaps sensory experience of the tutor song is mapped directly and rapidly onto the premotor network, altering the same circuitry that plays a critical role in determining the temporal pattern of the bird's song. This functional change may rapidly organize HVC activity to generate the temporal backbone of the bird's song, upon which the more precise refinements of sensorimotor learning could more slowly be made. In support of this view, the pupil's initial tutor song experience leads to the rapid emergence of complex, prolonged bursts of activity in both HVC and RA, and to the generation of songs with longer and more complex phrase-like structure.

Summary and future directions

1. Current evidence indicates that the song premotor nucleus HVC plays a role in sensory learning and may serve as part of distributed network for storing tutor song memories. Whether HVC encodes the auditory experience of the tutor song or provides a motor-related calibration or gating signal to downstream sites, including CM, remains uncertain. An important goal will be to better resolve the nature of circuit interactions between HVC and secondary regions of the auditory telencephalon to which it projects.
2. Higher levels of the auditory telencephalon including NCM and CM are also likely to be involved in the acute encoding of tutor song experience and possibly in the storage of tutor song memories. An important goal will be to employ tutor song-contingent optogenetic and electrical stimulation methods in NCM and CM to more directly test their role in sensory learning and memory storage.
3. A memory storage circuit should be sensitive to overwriting or erasure, as has been demonstrated for circuits important to olfactory imprinting and fear conditioning. Experiments using optogenetic or electrical stimulation methods should seek to test whether artificial song memories can be implanted in the juvenile's brain that he

will attempt to copy, and whether destabilization of synapses strengthened by tutoring is sufficient to prevent song copying or cause a copied song to degrade.

4. Information about the tutor song acquired during sensory learning must access the song system during sensorimotor learning. Current evidence suggests that error correction circuits involve the auditory telencephalon and the AFP, making the characterization of this circuitry and its relationship to HVC, of principle importance for near term research.

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Highlights

Imitative vocal learning requires auditory experience of a vocal model

In songbirds, vocal motor circuits help encode this auditory experience

Vocal motor circuits may encode memories of the vocal model

This strategy for auditory learning may generalize to other vocal learners, including humans

Figure 1.

A) Schematic illustrating the developmental progression for song learning in zebra finches during the first 3–4 months post-hatching. **B)** Schematic illustrating central ideas for how tutor song is learned during sensory learning. In one standard model, a memory of the tutor song is stored in auditory forebrain areas during sensory learning and only later used to guide changes in motor circuits during sensorimotor learning. A second set of models, suggests that auditory experience of the tutor song can directly engage motor circuits during sensory learning, leading to a more distributed representation of tutor song memory in motor circuits (middle model) or distributed in auditory and motor circuits (lower model).

Figure 2.

Schematic parasagittal sections of the songbird brain illustrating the organization of the song motor pathway (blue) and anterior forebrain pathway (red), the ascending auditory pathways (green) and the auditory inputs to HVC (white). Note that the thalamic nucleus Uva receives ascending auditory information from the lateral lemniscus as well as respiratory and proprioceptive feedback from portions of the VRG and midbrain regions analogous to the mammalian periaqueductal grey (not illustrated). Av, nucleus avalanche; CM, caudal mesopallium; DLM, medial part of the dorsolateral thalamic nucleus; HVC, abbreviation used as proper name; LMAN, lateral magnocellular nucleus of the anterior nidopallium; NCM, caudomedial nidopallium; Nif, nucleus interface; OV, nucleus ovoidalis; RA, robust nucleus of the arcopallium; Uva, nucleus Uvaeformis; VRG, ventral respiratory group; nXIIts, tracheosyringeal division of the hypoglossal nucleus;.

Figure 3.

A) Electrophysiological recordings from HVC_X neurons in swamp sparrows show auditory (under green box) and singing (under red box) related activity when birds are engaged in “matched countersinging”. Figure adapted from Prather et al., 2008. **B–C)** Adult swamp sparrows crystallize only a small fraction of the song models they are exposed to during sensory learning. **B)** Sonograms of 7 model songs that a single swamp sparrow was exposed to during sensory learning and the 2 songs that the bird sang as an adult. Broken arrows indicate model songs that were at least partially copied during sensorimotor learning, but not retained in the adult repertoire and the question mark indicates a model song that did not appear to be copied at all during sensorimotor learning. **C)** Venn diagram illustrating the relationship between the number of song models juvenile swamp sparrows were exposed to and the fraction of those songs practiced during sensorimotor learning and those sung as part

of the adult repertoire. **D)** HVC neurons in adult swamp sparrows respond strongly to playback of their adult crystallized songs and to tutor songs that were not retained in their adult repertoire, but not to novel songs. Figures B–D were adapted from Prather et al., 2010.

Figure 4.

A) In vivo two-photon imaging of dendritic spines on HVC projection neurons permits tracking of the gain (blue arrowheads) and loss (yellow arrowhead) putative synaptic connections during song learning. **B)** Longitudinal in vivo two-photon imaging of dendritic spines in HVC shows a rapid and sustained decrease in dendritic spine turnover following tutor exposure in juvenile zebra finches. **C)** Repeated intracellular recordings of spontaneous neuronal activity in the HVC of a juvenile zebra finch reveals the rapid emergence of bursting activity following a single day of tutor experience. **D)** Cumulative frequency distribution of spontaneous synaptic activity showing the amplitude of depolarizing postsynaptic potentials recorded in HVC before and after tutoring (top) or recorded in age matched control birds raised with or without exposure to a tutor (bottom). Figures adapted from Roberts et al., 2010.

Figure 5.

A) Schematic illustrating the strategy and timeline for tutor song-contingent optogenetic disruption of HVC experiments. **B)** Sonograms of a tutor's song and the adult songs of two of his pupils, including a control and one that received optogenetic activation of HVC during tutoring; scale bar = 200ms; ordinate = 0 – 9 kHz. **C)** Optogenetic disruption of a juvenile finch's HVC only when its tutor is singing disrupts subsequent copying of the tutor's song (green-experimental, black-controls, green filled diamond—average for birds raised in isolation from a tutor ($n = 3$ birds), black filled diamond—average for birds raised with free access to the same tutor used for optogenetic experiments ($n = 3$ birds)). **D)** Sketch of the experimental design in which the pupil's HVC is microstimulated at 170Hz for 200ms while the tutor is singing syllable 'c'. **E)** Pupils fail to imitate the syllable paired with HVC microstimulation. **F)** Sonograms of the tutor's song and the adult song of one of his pupils that was microstimulated in HVC when the tutor sang syllable 'c'. Green bar under syllable 'c' and scale bar at lower right = 130ms; ordinate = 0 – 9 kHz. Figures were adapted from Roberts et al., 2012.

Figure 6.

Sketch of three potential circuits models for encoding a memory of the tutor's song, including a models wherein the tutor song memory is encoded in HVC (upper model), in HVC and downstream regions, including auditory regions downstream of HVC such as CM and NCM (middle model), and in which HVC activity serves as a gate or calibration signal for encoding a tutor song memory in regions downstream of HVC (lower model).