Chapter 10

Mirror neurons in the songbird brain: A neural interface for learned vocal communication

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Introduction

Since mirror neurons were first discovered in the monkey cortex almost a quarter of a century ago, they have fascinated neuroscientists because they appear ideally suited to provide a cellular interface between perception and action, one which is necessary for communication. Mirror neurons are activated both when the animal executes a specific movement and when the animal observes a similar movement executed by others and thus could efficiently encode sensory and motor representations of communicative behaviors, including speech and language (Rizzolatti et al. 2014). Specifically, mirror neurons operating at the auditory–vocal motor interface could efficiently mediate call and response behaviors that are at the foundation of "conversations" conducted by a wide range of animals, including humans. Moreover, by providing an inverse model necessary to translate sounds into vocal actions, auditory–vocal mirror neurons could be engines of speech learning. Although mirror neurons have been examined as a possible substrate for communication and social cognition (Heyes 2010), their engagement in communicative behaviors in primates has remained speculative (di Pellegrino et al. 1992; reviewed in Kilner and Lemon 2013).

The utility of mirror neurons for communication emerges from the specificity with which they represent specific behavioral gestures. As our understanding of these cells has grown, researchers have identified mirror neurons that are active in association with the range of gestures and other actions that are active in association with very specific behaviors, and these differences are relevant when considering how mirror neurons may facilitate communication and learning. In the earliest accounts of mirror neurons, approximately half of the neurons were described as "broadly congruent," meaning that they were active in association with a range of actions in either the sensory or motor domain, and that the behaviors represented by each cell were similar but not identical in the sensory and motor domains (di Pellegrino et al. 1992; Gallese et al. 1996; Rizzolatti et al. 1996). Further investigation revealed a subset of mirror neurons that were "strictly congruent," meaning that they were active in association with only a single action that was identical in each domain (Gallese et al. 1996). In the context of communication, broadly congruent cells

may support a more generalized sensorimotor correspondence necessary for early stages of learning and also for more flexible communication strategies, whereas the precise sensorimotor correspondence exhibited by strictly congruent cells could enable highly precise forms of communication and mimicry. Interestingly, early studies of mirror neurons were focused primarily on manual and orofacial behaviors, both of which can be recruited for communication, and subsequent work identified audiomotor mirror neurons, albeit not in the context of vocal communication (Keysers et al. 2003). In summary, a range of mirror neuron types has been described in the monkey cortex, although explicit links to communicative behaviors, and especially vocal communication, have not been established.

Spoken language appears to be a uniquely human trait, and thus critical questions are whether mirror neurons are present in human brains and whether they operate across the perceptual–motor space that undergirds speech (reviewed in Rizzolatti et al. 2014 but see also Rogalsky et al. 2011). It has been challenging to identify mirror neurons in humans because current neuroimaging techniques do not provide sufficient spatial and temporal resolution to enable researchers to characterize the sensory and motor properties of individual neurons. In neurosurgical settings, however, individual cortical neurons have been recorded as a human patient engages in sensory and motor tasks. Although these recordings have primarily sampled from the medial frontal and temporal cortical lobes, they have confirmed the presence of both strictly congruent and broadly congruent mirror neurons in the human brain (Mukamel et al. 2010). In addition, imaging studies have also provided considerable evidence of a mirroring system elsewhere in the human brain (reviewed in Rizzolatti et al. 2014).

The discovery of mirror neurons in the human brain has led to speculation regarding their possible function. Building on motor theories of perception that predate the discovery of mirror neurons (Liberman et al. 1967), a "direct matching hypothesis" posits that they enable us to reliably interpret the actions of others by comparing those actions against our own action repertoire; such a process seems especially well suited to receptive aspects of communication (reviewed in Rizzolatti et al. 2014). Mirror neurons have also been advanced as an efficient means of imitative learning, because mirror neurons that are activated while monitoring the actions of another could provide a template for the observer to generate similar actions. Presently, the role of mirror neurons in these processes remains largely speculative, and moving beyond speculation will require an understanding of the cellular and circuit mechanisms through which the underlying sensorimotor correspondence arises. This review considers integrated physiological and behavioral studies that have identified auditory–vocal mirror neurons in songbirds, which resemble humans in that they learn by imitation to produce a complex vocal repertoire essential for social communication.

Why search for mirror neurons in songbirds?

Several features motivate studies in songbirds that seek to identify auditory–vocal mirror neurons and explore their role in learned vocal communication. Most importantly, like

human speech but unlike most other animal vocalizations, birdsong is a culturally transmitted behavior. Juvenile songbirds learn to sing in a process that is strikingly similar at genetic, neural, and behavioral levels to the development of speech learning in humans (reviewed in Brainard and Doupe 2013). In the first few months of their lives, juvenile songbirds listen to and memorize one or more tutor songs, and these memories can be stored for many weeks or even months before being recalled to guide imitative vocal motor learning (Marler and Peters 1981; Funabiki and Konishi 2003). Then, in a process that depends on auditory feedback and trial-and-error motor learning and that occurs in the absence of any additional instruction from a tutor, young birds match their own songs to the memorized tutor song, eventually producing a close copy of the original song model (reviewed in Mooney et al. 2008). Even after this process of vocal copying is complete, many songbird species continue to rely on auditory feedback to maintain their songs as adults, much like adult humans rely on their hearing to maintain stable speech (Konishi 1965; Nordeen and Nordeen 1992; Woolley and Rubel 1997). Finally, songbirds use their learned songs for social communication, including territorial defense and mate attraction (reviewed in Catchpole and Slater 2008). Notably, what a bird learns to sing influences his ability to recognize the songs of other birds (Balaban 1988; Searcy et al. 2003), much as natal speech learning exerts a lifelong effect on speech perception in humans (reviewed in Diehl et al. 2004). Thus, auditory perception and vocal motor performance are intimately linked throughout a songbird's life, providing a rich context in which to search for mirror neurons that could operate at the auditory–vocal interface.

Searching for mirror neurons in the songbird HVC, an auditory–vocal interface

In addition to its many behavioral advantages, the songbird brain contains a network of interconnected nuclei that are specialized for song production, perception, and learning. This "song system" contains cortical, striatal, thalamic, and brainstem components that are analogous, and in many cases homologous, to the network of structures in the human brain that are important for expressive and receptive aspects of speech (reviewed in Brainard and Doupe 2013). Therefore, mechanistic insights gained by studying the songbird brain, including the properties and functions of auditory–vocal mirror neurons, are likely to be especially informative to understanding the neural basis of human speech.

A critical site in the song system to search for auditory–vocal mirror neurons is the telencephalic sensorimotor nucleus HVC, which plays an essential role in singing and is implicated in song perception. Consistent with a sensorimotor structure, HVC contains some neurons that display premotor activity and others that display complex auditory responses that are highly tuned to the bird's song. Suggestive of a broad role in vocal communication, HVC receives afferents from auditory regions analogous to the secondary auditory cortex and also is the origin of two different anatomical pathways that serve distinct roles in song production, learning and perception (Fig. 10.1; reviewed in Prather 2013). One type of HVC projection neuron (PN; i.e., RA-projecting or HVC_{RA} cells) extends
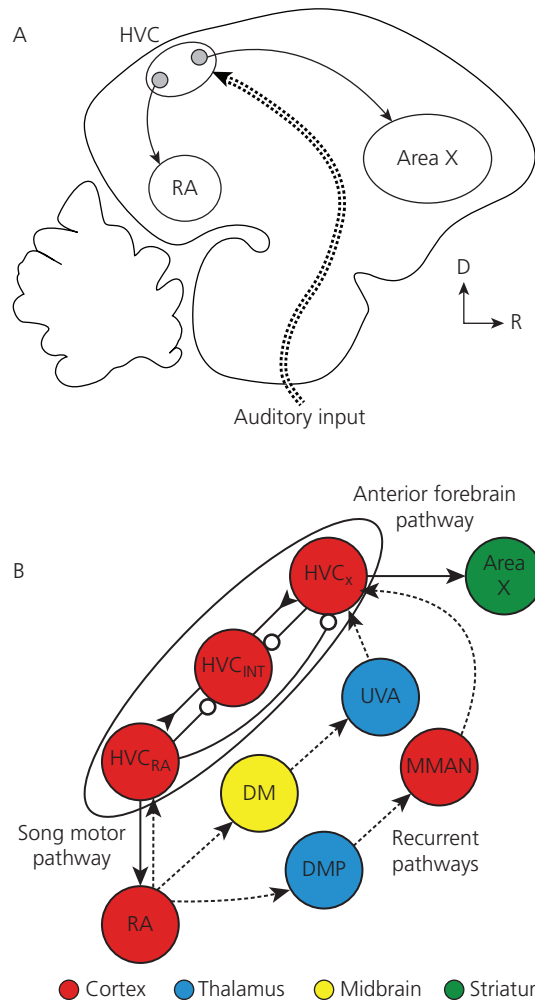
Fig. 10.1 The songbird brain contains a network of nuclei that are specialized for song learning, performance, and perception. (A) Within that network, a cortical structure called HVC (represented by an oval) gives rise to two pathways associated with different aspects of auditory perception and performance. The vocal motor pathway projects directly to the vocal motor cortex (the robust nucleus of the arcopallium [RA]), and the anterior forebrain pathway projects to the striatum (Area X) and through additional connections that eventually converge onto the vocal motor cortex. The pathways through which auditory input (indicated as dashed lines) reaches cells that project into the vocal motor pathway (HVC_{RA}), the mirror neurons that project into the anterior forebrain pathway (HVC_X), and the HVC interneurons (HVC_{INT}) have been well described (reviewed in Prather 2013; connections not shown); D, dorsal; R, rostral. (B) The vocal motor pathway and anterior forebrain pathway interact through local connections within HVC and through cortico-thalamo-cortical recurrent loops, which provide a path through which motor-related activity can also reach HVC_X mirror neurons; DM, dorsomedial nucleus of the intercollicular region; DMP, dorsomedial nucleus of the posterior thalamus; MMAN, medial magnocellular nucleus of the anterior nidopallium; UVA, thalamic nucleus uvaeformis.

axons that terminate in the song motor nucleus RA (the robust nucleus of the arcopallium) (Fig. 10.1). Similar to ways in which Broca's area and speech motor cortex are necessary for speech production, both HVC and RA form a song motor pathway (SMP) that is essential for singing (Nottebohm et al. 1976; reviewed in Mooney et al. 2008). A second HVC PN type (HVC$_X$ cells) projects to the striatopallidal structure area X (Fig. 10.1) and eventually converges onto RA through an anterior forebrain pathway (AFP) that closely resembles corticobasal ganglia loops in mammals. In contrast to the SMP, the AFP is not essential for the production of adult song; however, the AFP plays essential roles in the production of juvenile subsong, in the induction of experience-dependent changes in song structure, and in song perception and plasticity (Bottjer et al. 1984; Scharff et al. 1998; Kao et al. 2005; Aronov et al. 2008; Prather et al. 2009; Charlesworth et al. 2012). The SMP and AFP interact through local microcircuits within HVC and through extrinsic recurrent loops that convey motor- and auditory-related information to HVC (Mooney and Prather 2005; T. Roberts et al. 2008; Hamaguchi and Mooney 2012; reviewed in Schmidt et al. 2004). Therefore, the songbird forebrain nucleus HVC is a critical node onto which different types of information important to vocal communication converge and from where auditory and motor information about song can be widely distributed across the brain to facilitate action, perception, and learning.

A wide variety of evidence indicates that HVC is near the apex of a sensorimotor hierarchy for song. Lesion studies indicate an essential role for HVC in production of adult song (Nottebohm et al. 1976; Aronov et al. 2008), and a combination of electrophysiological recordings, focal microstimulation, and cooling studies in singing birds support a model where HVC$_{RA}$ cells are the source of a precise timing signal that controls temporal patterns of birdsong and where HVC$_X$ cells provide an corollary discharge of this timing signal to the AFP (Hahnloser et al. 2002; Ashmore et al. 2005; Kozhevnikov and Fee 2007; Long and Fee 2008; Prather et al. 2008). Notably, HVC lesions also can impair a bird's ability to recognize conspecific songs (Brenowitz 1991; Gentner et al. 2000), and the earliest electrophysiological recordings of HVC activity, which were made in anesthetized birds, revealed that HVC encodes specific information about the bird's song (Margoliash and Fortune 1992; Lewicki and Konishi 1995; Lewicki and Arthur 1996). Indeed, some HVC neurons respond to auditory presentation of specific syllables or combinations of syllables, making them some of the most highly selective auditory neurons yet to be described. These observations indicate that HVC is a site where song motor control circuitry interfaces with highly specific auditory representations of song, and thus is a potential site for auditory–vocal mirroring.

Evidence for auditory–vocal mirroring in HVC$_X$ neurons

Consistent with this idea, pioneering recordings made from HVC neurons in awake and freely behaving birds revealed that HVC neurons are active both when the bird sings and when it quietly listens to its own songs played from a nearby speaker (Katz and Gurney 1981; McCasland and Konishi 1981). Those population recordings did not distinguish between different cell types within HVC, but they revealed that both auditory and motor

representations of song coexist within HVC, but they did not resolve whether single neurons display both auditory and motor properties and the nature of any sensorimotor correspondence they exhibit. Resolving these problems presents significant challenges, because most songbirds weigh less than 25 g, limiting the mass of suitable recording devices to approximately 1 g, and because the various cell types in HVC are spatially intermingled and difficult to identify without antidromic stimulation methods.

To overcome these challenges, we adapted miniaturized technology to record extracellularly from antidromically identified HVC_X and HVC_{RA} cells in a variety of songbird species, including swamp sparrows, which use song for territorial defense and mate attraction, and from both zebra finches and Bengalese finches, which sing solely to attract mates. Swamp sparrows are especially advantageous for exploring the nature of any sensorimotor correspondence because they sing a small repertoire of acoustically distinct song types, each of which comprises a monosyllabic trill, and because they often sing in response to hearing a song, a natural territorial behavior known as countersinging. Because countersinging can be elicited from a bird by auditory presentation of one of its own songs, and because swamp sparrows have multiple songs, electrophysiological recordings made during the countersinging behavior allowed us to rapidly assess whether individual HVC neurons were active during singing and listening, and whether any sensorimotor correspondence they exhibited was broadly or strictly congruent.

Recordings made during bouts of countersinging revealed that HVC_X neurons could be highly active during both listening and singing phases of the behavior, as predicted of auditory–vocal mirror neurons. During either phase, individual HVC_X neurons were active in association with a single song type in the bird's repertoire, firing at precisely the same times during each syllable in the effective trill (Fig. 10.2A–C). This precise correspondence between auditory and singing-related activity is reminiscent of strictly congruent mirror neurons, and the fact that HVC_X cells can be active during periods of quiet wakefulness suggests that they could function to facilitate song perception. In line with this view, individual HVC_X neurons also responded briskly to acoustically similar songs from the repertoire of another bird (Prather et al. 2008); this result indicates that these neurons can serve more than a self-monitoring function. In contrast, HVC_{RA} neurons did not express auditory responses in the awake bird, although they are highly active during singing (Hahnloser et al. 2002; Prather et al. 2008; Hamaguchi et al. 2014). These studies reveal remarkably local heterogeneity in the functional properties of HVC neurons and establish HVC_X cells as candidate auditory–vocal mirror neurons.

Although similar patterns of activity during listening and singing are consistent with a mirroring phenomenon, the singing-related activity could simply be driven by auditory feedback, rather than reflecting motor activity. To distinguish between these possibilities, we used the bird's singing to trigger auditory presentation of the effective syllable at slight temporal delays, distorting the singing-related auditory feedback that the bird experienced. This manipulation had no effect on the singing-related activity of HVC_X cells, as has been shown for this cell type in a variety of songbird species (Kozhevnikov and Fee 2007; Prather et al. 2008; Hamaguchi et al. 2014). However, presenting the effective
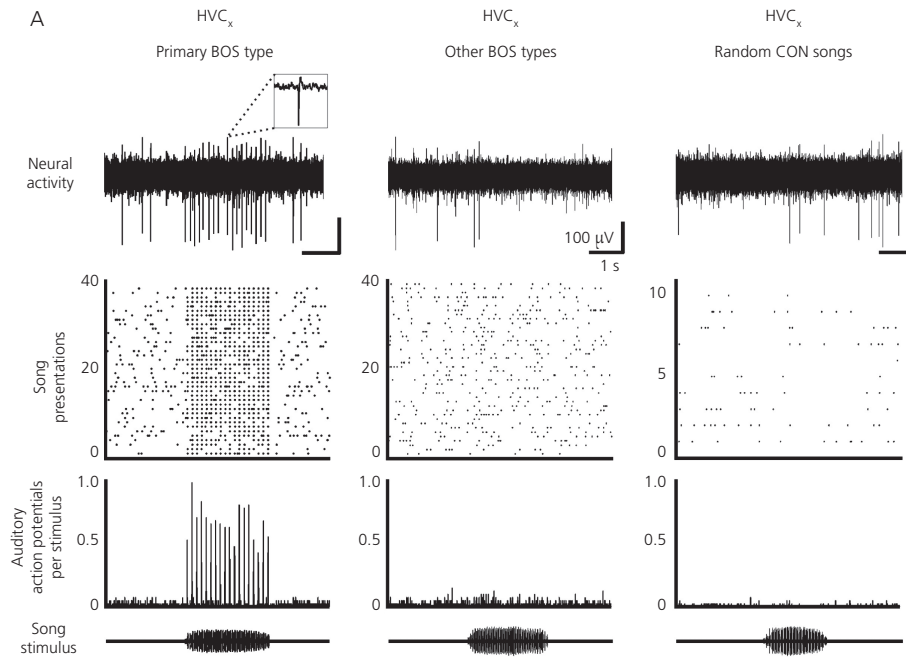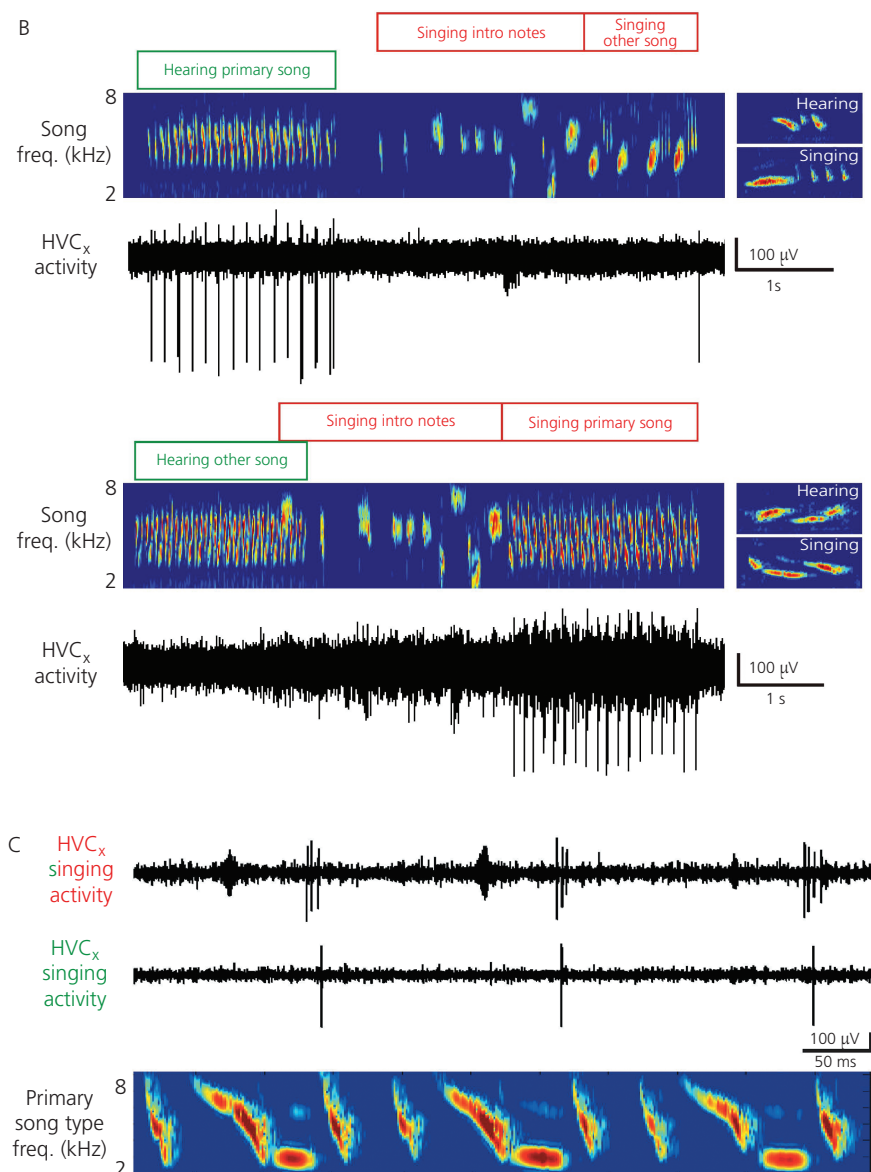
Fig. 10.2 HVC_X cells are auditory–vocal mirror neurons (adapted from Prather et al. 2008). (A) In freely behaving swamp sparrows, HVC_X neurons are selectively responsive to one song type. Among this bird's repertoire of bird's own songs (BOS), this cell responds to only one song type (the primary BOS type for this cell), and conspecific (CON) songs and other randomly selected songs are not effective in driving auditory activity (top: raw data recorded during a single stimulus presentation; second row: rasters of responses to multiple stimulus presentations; third row: peristimulus time histograms of responses; bottom row: oscillograms of song stimulus). (B) HVC_X neurons display a precise sensorimotor correspondence. HVC_X cells are active in association with the primary song type that was defined in the auditory domain, but cells are inactive when the bird hears (top) or sings (bottom) other song types. (C) HVC_X sensorimotor correspondence is precise not only in the selective representation of only one behavior but also in the timing of the action potentials associated with that behavior in the singing and auditory domains. Together, these data reveal that HVC_X neurons are highly selective and are active in association with one and the same behavior in both the sensory and motor domains; thus, they are reminiscent of the strictly congruent mirror neurons described in the mammalian frontal cortex.

syllable with a superimposed and slightly offset copy of that syllable abolished HVC_X auditory responses that could normally be elicited during the listening phase (Prather et al. 2008). Therefore, the singing-related activity of HVC_X neurons is truly a motor-related signal, and HVC_X cells express a precise sensorimotor correspondence in the representation of signals used in learned vocal communication. Beyond establishing the existence of auditory–vocal mirror neurons, these studies also reveal a remarkably precise sensorimotor correspondence in the time domain; these results suggest that HVC's role in encoding temporal information about song extends across both sensory and motor space.

Fig. 10.2 (continued)

Auditory–vocal mirroring has also been detected in HVC_X cells in Bengalese and zebra finches. This observation is consistent with the notion that the mirror neuron mechanism is a common feature of the songbird HVC. For example, recordings made by us and others have characterized auditory–vocal mirroring in a subset of HVC_X neurons in Bengalese finches (Prather et al. 2008; Fujimoto et al. 2011), a species that is distantly related to swamp sparrows and that performs songs with stark differences in phonology,

syntax, and repertoire size (Okanoya 2004). Furthermore, although HVC_X neurons in zebra finches are typically responsive to auditory stimuli only during states of sleep or anesthesia, Hamaguchi et al. (2014) conducted an especially challenging set of recordings across the wake–sleep boundary to determine that the highly phasic and selective auditory responses elicited in HVC_X cells during sleep closely mirrored their singing-related activity recorded during the daytime. Moreover, by using intracellular recording methods, this latter study also demonstrated that the precise correspondence of auditory and motor activity in individual HVC_X cells even manifested in the patterns of subthreshold synaptic activity recorded in these two states. Therefore, the sensory and motor phases of mirror neuron activity appear to engage the same synaptic and circuit mechanisms, and mirroring extends across several distantly related songbird species; such a result is indicative of a deeply congruent and general mechanism by which auditory and vocal representations are encoded at the level of individual neurons.

The ability to digitally edit and manipulate birdsongs has allowed further dissection of the acoustic features that drive the sensory responses of auditory–vocal mirror neurons in the songbird HVC. In swamp sparrows, this approach has revealed that specific note sequences in the effective syllable appear to be highly salient for driving auditory responses in HVC_X mirror neurons (Prather et al. 2008). In partial contrast, Fujimoto et al. (2011) found that individual HVC_X neurons can be active when Bengalese finches sang specific notes, regardless of whether the bird sang them alone or as part of specific note sequences (Fujimoto et al. 2011). Therefore, the vocal features represented by individual HVC_X neurons can span from single notes, the unitary building block of song, to combinations of notes (Prather et al. 2008; Fujimoto et al. 2011). Taken together, these features indicate that HVC_X mirror neurons are well suited to facilitate the encoding of phonological and syntactic features that are learned through imitation and used by birds to distinguish different songs and different individuals.

Auditory–vocal mirror neurons are closely linked to song perception

An attractive idea is that mirror neurons facilitate perception by enabling the sensory activity elicited by observing another individual's behavior to be directly compared to the activity generated in that same neuron when the observer executes a similar behavior. A corollary of this idea is that the sensory properties of mirror neurons should be tightly linked to the observer's perceptual performance. Swamp sparrows are especially useful for such studies, because sensitive behavioral tests can be applied to them to measure song perception and because prior studies have established that they perceive continuous changes in note duration in a categorical rather than a continuous manner (Nelson and Marler 1989; Prather et al. 2009). Notably, categorical perception is thought to preserve perceptual accuracy even in the face of subtle variance in stimulus properties or in noisy environments and also is a fundamental feature of how humans process speech (Beckers 2011).
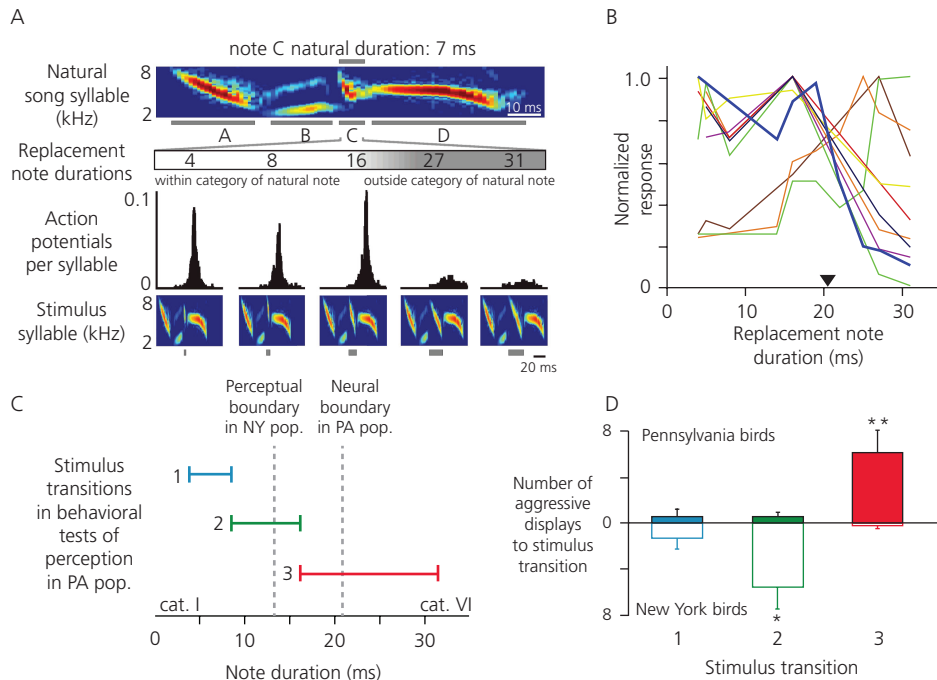
Fig. 10.3 The sensory activity of HVC$_X$ mirror neurons reflects the animal's perception. (A) When an individual note in each syllable of a swamp sparrow song (top: spectrogram, individual notes labeled) is replaced with a note from another bird's song that had similar spectral properties but a different duration (second row: duration of replacement notes), HVC$_X$ neurons express categorical responses to changes in note duration (third row: peristimulus time histograms of responses to each syllable; bottom row: spectrograms of song stimuli following insertion of replacement note [indicated by gray boxes]). (B) HVC$_X$ neurons express categorical responses, regardless of whether the note that was replaced is naturally of a short or long duration, and the location of the categorical boundary (black triangle at 21 ms) is consistent across neurons and across birds from the same population (each line represents the responses of one HVC$_X$ mirror neuron). (C) In behavioral tests of the bird's perception, some stimuli contained note transitions that crossed no putative categorical boundary (Group 1: 4–8 ms), other stimuli crossed a perceptual boundary reported for a population of swamp sparrows in New York (NY; Group 2: 8–16 ms; Nelson and Marler 1989), and other stimuli crossed the categorical boundary detected in our neurophysiological recordings from swamp sparrows in a population in Pennsylvania (PA; Group 3: 16–32 ms). (D) Behavioral testing revealed that our Pennsylvania birds (top, filled bars; mean and standard error shown) perceive strong differences when the note transition spans the boundary detected in recordings from HVC$_X$ neurons (Group 3) but not when the transition spans the New York boundary (Group 2) or when the transition spans no putative boundary (Group 3). In contrast, New York birds perceived strong differences when the note transition spanned the New York boundary but not the Pennsylvania boundary. These results reveal that there are population-specific differences and suggest that categorical neural responses are a learned feature of HVC$_X$ representation of specific vocal communication behaviors (bottom; open bars; adapted from Nelson and Marler 1989). A–C: Adapted by permission from Macmillan Publishers Ltd: *Nature Neuroscience*, 12 (2), Jonathan F Prather, Stephen Nowicki, Rindy C Anderson, Susan Peters, and Richard Mooney, Neural correlates of categorical perception in learned vocal communication, pp. 221–8, doi:10.1038/nn.2246, Copyright (2009), Macmillan Publishers Ltd. D: Reproduced from DA Nelson and P Marler, Categorical perception of a natural stimulus continuum: birdsong, *Science*, 244 (4907), pp. 976–978, doi:10.1126/science.272768 © 1989, American Association for the Advancement of Science.

The finding that swamp sparrows perceive continuous changes in note duration in a categorical manner allowed us to explore whether the auditory properties of HVC_X cells are tightly linked to perception, in which case their auditory responses should change in an all or none rather than graded fashion when note durations crossed the categorical perceptual boundary. Interestingly, we found that HVC_X neurons did express categorical responses to changes in note duration (Fig. 10.3A; Prather et al. 2009), but the categorical neural response boundary was different from the categorical perceptual boundary reported previously for swamp sparrows (Nelson and Marler 1989). One notable difference is that the birds used for neurophysiological recordings were collected from a population in western Pennsylvania, whereas the birds used in the original perceptual study were from a population in upstate New York. Because many songbird species, including swamp sparrows, express regional dialects that can vary in the spectral and temporal features of individual notes, one possible explanation is that the perceptual boundaries differed between these two populations (Krebs and Kroodsma 1980; Balaban 1988; Searcy et al. 2003). In line with this view, subsequent behavioral tests that we conducted established that the categorical perceptual boundaries differed between these two breeding populations and also revealed a close alignment between the perceptual and neural response boundaries of the Pennsylvania birds (Fig. 10.3B–D; Prather et al. 2009). Thus, the auditory response properties of auditory–vocal mirror neurons closely parallel perceptual performance; this result supports a role for these cells in song perception and recognition.

The link between perception and performance evident in the activity of HVC_X neurons is strikingly reminiscent of Rizzolatti's "direct matching hypothesis," which posits that mirror neurons enable communication by establishing an equivalent neural representation of actions performed by self and by others (Rizzolatti and Craighero 2004). The colocalization of neural representations of perception and motor performance has also been proposed as an excellent mechanism through which perception and performance may be compared in service of imitative learning (Iacoboni 2009). The discovery of a precise correspondence between auditory perception and vocal performance in such an experimentally tractable animal model provides a uniquely advantageous opportunity to test those ideas and to explore the synaptic, circuit, and experiential mechanisms that give rise to a precise auditory–vocal motor correspondence.

Forging causal links between mirror neurons, perception, and learning

An important goal of future studies will be to use the advantages inherent to studies of songbirds to address fundamental questions regarding the role of auditory–vocal mirror neurons in vocal communication and learning. One important issue to resolve is whether the activity of auditory–vocal mirror neurons is necessary to certain forms of song perception, particularly those that require the animal to distinguish its own song from other highly similar songs of other birds. A variety of studies suggest that HVC_X cells are a site where auditory responses that closely correspond to the bird's song repertoire emerge

from a diverse array of less selective auditory inputs. Testing a causal role for auditory–vocal mirror neurons in shaping the bird's perception of songs like its own can be tested using optogenetic techniques (e.g., T. Roberts et al. 2012) to transiently silence or activate HVC_X neurons during song discrimination tasks. Intersectional genetic methods can also be used to selectively ablate HVC_X cells to measure their role in song perception.

Another important goal of future studies will be to identify how vocal motor activity and auditory experience contribute to the precise sensorimotor correspondence expressed by HVC_X cells. One useful approach to determine the flexibility and dynamics of the auditory–vocal mapping mechanism that gives rise to auditory–vocal mirror neurons will be to manipulate the sensory experience of the animal, either by tutoring juvenile birds with artificial songs or by acute manipulation of singing-related auditory feedback. A thorough understanding of how a precise sensorimotor correspondence is generated in auditory–vocal mirror neurons will also depend on unraveling the neural circuitry through which auditory and motor-related signals converge onto HVC_X neurons. Although various pathways through which auditory information reaches HVC_X cells are fairly well understood (reviewed in Mooney et al. 2008), the sources of motor-related input to HVC_X neurons await identification. One likely source of motor-related input is HVC_{RA} cells, which generate a temporally precise premotor signal and provide direct and indirect synaptic input to HVC_X cells through the local HVC microcircuit (Mooney and Prather 2005). Presumably, HVC_X cells also receive motor-related signals from extrinsic sources, including from the thalamus and other telencephalic regions. Selective optogenetic manipulation of these various sources of motor-related input to HVC_X cells can be used to probe their relative contributions to generating the motor component of the mirror neuron behavior.

Another interesting possibility is that auditory–vocal mirror neurons do not simply relay a motor-related signal to downstream components of the AFP but also actively influence song motor activity, perhaps by signaling HVC_{RA} neurons through the HVC_X to HVC_{RA} local microcircuit or extrinsic forebrain circuits (Kao et al. 2005; Mooney and Prather 2005; Olveczky et al. 2005; Aronov et al. 2008; Hamaguchi and Mooney 2012). In such a scheme, HVC_X activity would play a causal role in shaping subsequent vocalizations, providing a potential mechanism through which the performance of one note could influence the phonology or the sequencing of subsequent notes in the song (Fee and Goldberg 2011; Fee 2014). In fact, singing-related activity of HVC_X cells correlates with syllables that immediately follow rather than precede that activity, consistent with a motor process and in contrast with their auditory responses, which must necessarily be driven by preceding syllables (Kozhevnikov and Fee 2007; Hamaguchi et al. 2014). Furthermore, activity in the AFP, which is implicated in song initiation and song complexity, also has been shown to influence activity in HVC through recurrent circuits (Hamaguchi and Mooney 2012). Finally, activity of some HVC_X neurons is associated with the performance of specific vocal sequences (Fujimoto et al. 2011) and performing notes in specific sequences results in coarticulated changes in phonological properties and associated neural activity (Wohlgemuth et al. 2010). These various findings support a model in which the singing-related activity of auditory–vocal mirror neurons plays an important role in the generative aspects of vocal communication.

Understanding the role of auditory–vocal mirror neurons in song learning will depend on monitoring and manipulating mirror neuron activity in juvenile birds as they memorize and vocally copy a tutor song. An emerging idea is that HVC_X cells serve as the source of singing-related corollary discharge that is passed into the basal ganglia, where it serves as "action-representation signal" that can be compared to a sensory feedback-dependent "outcome-representation signal" to facilitate reinforcement learning (Farries and Fairhall 2007; P. Roberts et al. 2008; Leblois et al. 2010; Murugan et al. 2013; Fee 2014). Manipulating the singing-related activity of HVC_X cells in juvenile birds should alter the action-representation signal, with the expectation that the song motor learning trajectory should also be affected. Moreover, auditory experience of the tutor song in juvenile songbirds exerts rapid effects on the structural and functional properties of HVC neurons, including HVC_X neurons, and this early experience exerts lasting effects on the auditory response properties of HVC neurons. Therefore, an intriguing idea is that auditory–vocal mirror neurons may at earlier stages of development contain information about the actions of others (i.e., the tutor song) prior to incorporating a representation of self-generated actions (i.e., the bird's own song). These mechanisms of behavioral learning and memory can be tested using newly developed technologies that permit individual neurons to be isolated and monitored either optically or electrophysiologically over many months (Guitchounts et al. 2013). Finally, the natural variation in song behavior among the thousands of songbird species can be exploited to identify themes and variations in the properties and functions of auditory–motor mirror neurons (Brenowitz 1997). Together, the many ethological advantages inherent to songbirds and the increasingly powerful array of tools to record or manipulate neural activity underscore the rich opportunity songbirds provide to explore the role of auditory–vocal mirror neurons in vocal learning and communication.

Acknowledgments

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