# 10 • Integrating breathing and singing: forebrain and brainstem mechanisms

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## INTRODUCTION

Song production in birds offers a tremendous opportunity for studying the underlying neural bases of complex, experience-dependent motor sequence generation. In many species of birds, song is made up of many different stereotyped motor gestures, known as notes or syllables, which are produced in precise sequential order (see Williams, this volume). The production of song motor sequences therefore requires not only the generation of motor commands that specify the features of each element (e.g. spectral content, duration, amplitude, etc.) but also the order, or sequence, with which these elements are produced. In the zebra finch, the subject of choice for many song researchers, both the features of individual syllables as well as the sequence with which they are produced are highly stereotyped across song renditions (Sossinka and Böhner, 1980; Cardin *et al.*, 2005) (Figure 10.1). This species is therefore ideally suited for deciphering the neural mechanisms underlying the specification of the features that make up syllables. It also offers the opportunity for understanding the neural basis of motor sequence generation and how these different control mechanisms (feature specification and sequence generation) might be coordinated.

The avian song control system is made up of a discrete interconnected network of brain structures that together act to control the syrinx (the avian vocal organ), the muscles of respiration, and a number of secondary structures that might also affect acoustic quality (Nottebohm *et al.*, 1976, 1982; Wild *et al.*, 2000). At the output end, this network is made up of distinct brainstem nuclei that directly control muscles of the syrinx (hypoglossal nucleus; nXIIts) and indirectly control muscles of expiration (nucleus retroambigualis; RAm) and inspiration (nucleus parambigualis; PAm) (Wild, 1993a, 2004a, and this volume). These brainstem nuclei in turn receive motor commands from the forebrain nucleus RA (nucleus robustus arcopallialis), which is itself innervated by nucleus HVC (used as proper name) (Figure 10.2).

It has generally been assumed that HVC and RA specify the combination of respiratory and syringeal motor commands necessary to produce the acoustic features of individual song elements. However, the existence of several anatomical pathways that link the brainstem vocal respiratory network (VRN, which is made up of nuclei PAm, RAm and DM, see Figure 10.2) back to song control nuclei, such as HVC, in the forebrain (Vates *et al.*, 1997; Reinke and Wild, 1998; Striedter and Vu, 1998) suggests that the song control system is not organized as a simple hierarchical descending pathway (Ashmore *et al.*, 2005). It appears, instead to be organized as a recurrent pathway with no identifiable single structure at the top of a motor hierarchy.

In this chapter, we first review the functional organization of the descending motor pathway, then present anatomical and physiological evidence linking the brainstem back to forebrain vocal control nuclei. We then describe an integrative model for song production that views song motor control as distributed along a recursive pathway where the brainstem vocal respiratory network plays a central role in determining key features of the song's temporal structure. Finally, given the paucity of available evidence of bottom-up influences on motor control (Wurtz *et al.*, 2005), we discuss how the avian song motor control system might serve as a powerful model system for understanding general principles of brainstem to forebrain interactions in the context of motor control of complex learned behaviors.

## FUNCTIONAL ORGANIZATION OF THE DESCENDING VOCAL MOTOR PATHWAY

### Peripheral control of vocal production

Birds produce a wide range of vocalizations that include song as well as shorter vocalizations known as calls. In

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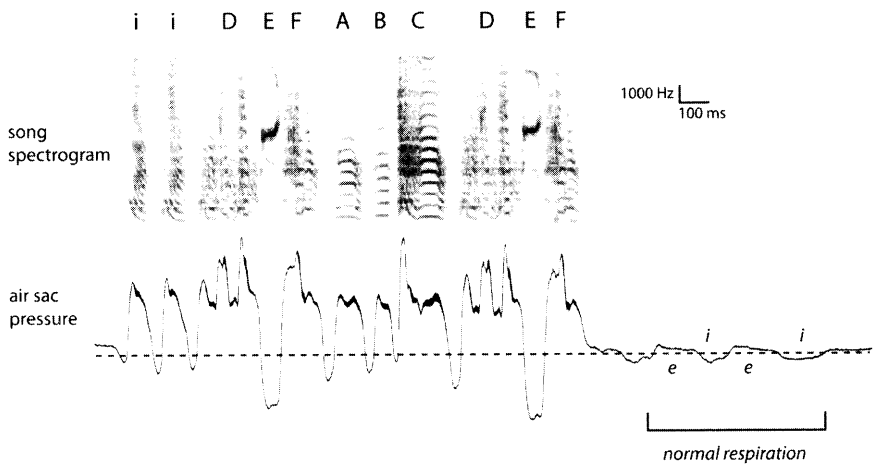


Figure 10.1 Respiratory profile of a male zebra finch song. This figure illustrates the acoustic features of a typical adult zebra finch song and its associated changes in air sac pressure. The top trace represents the acoustic sonogram of the song and the bottom trace the air sac trace. This particular song is only two motifs long and therefore allows a comparison of the fluctuations in air sac pressure during the song and those produced during normal respiration. In this example, normal respiration is shown following song offset. The majority of syllables in this song are produced during expiration (syllables A, B, C, D and F) except for syllable "E" which is produced during the inspiratory phase, a notable exception to the general rule that syllables are always produced during expiration (Goller and Daley, 2001). Note that individual expiratory (e) and inspiratory (i) pulses during normal respiration are significantly smaller than those produced during singing. The dotted line in the air sac pressure trace denotes ambient atmospheric pressure.

laboratory settings, male zebra finches usually produce two primary call types, known as contact (or distance) calls and shorter "tet'" calls (Zann, 1996; Vicario *et al.*, 2001). During normal respiration birds typically alternate between inspiratory and expiratory phases approximately 2–3 times per second. Once the bird initiates a vocalization, the pressure amplitude of individual respiratory pulses increases approximately 6–20 fold (Goller and Cooper, 2004, and this volume). During vocal production, expiratory and inspiratory pulses decrease significantly in length compared with normal respiration with expiratory pulses typically lasting longer (70–300 ms) than inspiratory pulses (30–100 ms) (Figure 10.1). Syllables are usually only produced during expiration, although there are a few exceptions in the zebra finch song where some syllable types are produced during inspiration (Goller and Daley, 2001). Inspiratory pulses, or minibreaths, serve to replenish the air supply between vocal expiratory pulses produced during a song (Goller and Cooper, 2004).

Singing is associated with a dramatic shift in respiratory control which is reflected in the marked changes in the amplitude and temporal pattern of respiratory activity (Suthers *et al.*, 1999). In many cases, expiratory pulses contain temporal fluctuations in their amplitude envelope which are sufficiently stereotyped to allow individual syllable identification based simply on each syllable's pressure pattern (Franz and Goller, 2002). The exact source of these rapid modulations in air sac pressure remains unclear; they could be mediated either directly by respiratory muscles or caused by syringeal-mediated gating of airflow, or both (Goller and Cooper, 2004). Aside from the rapid modulation of air sac pressure, the overall temporal pattern of air sac pressure is a direct consequence of respiratory muscle activity which receives motor commands via the intermediary of nuclei in the brainstem VRN (Wild *et al.*, 1998; Suthers and Zollinger, 2004). Activity in these areas is therefore assumed to directly influence song acoustic features, by regulating air sac pressure via activation of expiratory and inspiratory muscles. The brainstem VRN, however, also directly contributes to the temporal features of song since it determines the length of expiratory pulses (i.e. syllables) and the duration of the intervening inspiratory minibreaths (i.e. silent intervals). In addition to the changes in respiratory pattern, song is also associated with changes in syringeal motor activity. During the production of sound, the syringeal lumen moves rostrally and syringeal muscles rotate the third bronchial

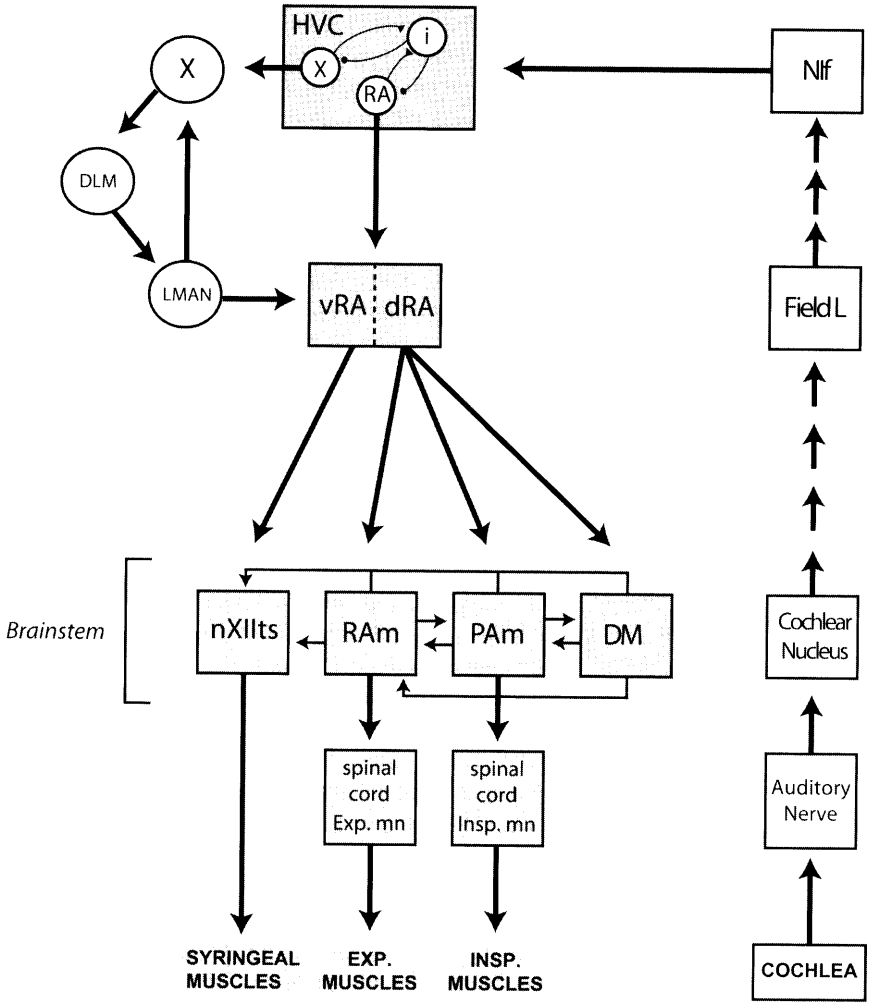


Figure 10.2 Simplified top-down representation of the avian song control system. Vocal control, and song control in particular, is driven by a specialized circuit referred to in the text as the "descending vocal motor pathway" (shown here in gray). As is typically depicted in most representations of this circuit, the forebrain nucleus HVC (used as proper name) lies at the top of this pathway and projects to the forebrain vocal control nucleus RA (robust nucleus of the arcopallium). This nucleus is functionally divided into two parts. The ventral portion (vRA) projects to the tracheosyringeal portion of the hypoglossal nucleus (nXII) in the brainstem. This nucleus contains the motoneurons that innervate the syrinx. The dorsal portion of RA (dRA) projects to nuclei in the brainstem that form part of what is referred in the text as the vocal-respiratory network (VRN). This network is highlighted by the light gray box and includes nuclei RAm, PAm and DM. RAm projects to expiratory motoneurons in the lower thoracic and upper lumbar regions of the spinal cord whereas PAm projects to inspiratory motoneurons in the lower brachial and upper thoracic regions of the spinal cord. In addition to forming part of the descending motor pathway, HVC, which is made up of at least three distinct cell types (X, I and RA), also projects to the anterior forebrain pathway and receives auditory inputs from nucleus NIf. The anterior pathway, which is made up of Area X, DLM, and LMAN is not necessary for song production but plays an important role in song learning and maintenance. Note that while HVC is only 3 to 4 synapses away from output musculature, it is at least 8 or 9 synapses (depicted by each arrow in the ascending auditory stream) away from the cochlea. Anatomical names: DLM, medial part of the dorsolateral thalamic nucleus; LMAN, lateral magnocellular nucleus of the anterior nidopallium; Field L is the primary auditory forebrain structure in birds; Area X, Area X of the medial striatum; NIf, nucleus interfacialis of the nidopallium; RAm, nucleus retroambigualis; PAm, nucleus parambigualis; DM, dorsomedial nucleus of the intercollicular complex.

cartilages, causing the medial and lateral labia to move into the air stream, where the positive pressure in the air sac causes expiratory airflow to induce labial vibrations and produce sound (Goller and Larsen, 1997a; Larsen and Goller,1999, 2002; Suthers and Zollinger, 2004 and this volume).

The avian syrinx is a bipartite structure and muscles in both halves of the syrinx often show differential activation patterns, especially during sound production (Suthers, 1990, 1997; Wild *et al.*, 1998; Suthers and Zollinger, 2004). Specific acoustic features, for example, are therefore often determined by the differential activation of both halves of the syrinx in a highly coordinated manner (Goller and Suthers, 1995; Floody and Arnold, 1997; Suthers and Zollinger, this volume). This allows for a range of phonic configurations: from both sides producing the same sound to both sides producing different sounds. Sound can even be produced exclusively unilaterally by allowing air to flow through only one half of the syrinx and not the other (Goller and Suthers, 1995). During the production of song bouts, sound production can therefore result from rapidly alternating airflow in both halves of the syrinx (Goller and Suthers, 1996a, 1996b; Suthers, 1997). Each syringeal half is controlled by six different muscle groups (see Suthers and Zollinger, this volume, for details) and recordings from these different muscles in a number of songbird species are beginning to reveal predictive relationships between specific muscle groups and the types of acoustic elements that result from their activation (Goller and Suthers, 1996b; Suthers and Zollinger, this volume).

Given the large number of different ways sound can be produced, an important requirement for the production of acoustically precise elements is that there is exquisite coordination between the syringeal and respiratory control systems (Sturdy *et al.*, 2003; Kubke *et al.*, 2005; Wild, this volume) Such coordination is not necessarily trivial given that syringeal and respiratory motor commands originating from nucleus RA in the forebrain are primarily ipsilateral in nature (Wild, 1993a, 1997a; Wild *et al.*, 2000). Because mismatches in syringeal motor activation would likely result in profound distortions of the acoustic signal, the rapid switching between sides without acoustic distortions suggests the existence of highly sophisticated neural mechanisms for coordinating the motor commands that reach each half of the syrinx.

## Telencephalic control of vocal production

Syringeal and respiratory motor areas in the brainstem are strongly innervated by nucleus RA, a telencephalic structure that forms part of the descending vocal motor pathway (Wild, 1993a, 1997a; Vates *et al.*, 1997). RA is itself innervated by nucleus HVC, a higher order vocal structure, often assumed to lie at the top of a hierarchically organized descending vocal motor pathway (Yu and Margoliash, 1996; Margoliash, 1997; Fiete *et al.*, 2004a; Solis and Perkel, 2005). It is also the first nucleus in the motor pathway to receive auditory inputs (Cardin and Schmidt, 2004a; Coleman and Mooney, 2004; Cardin *et al.*, 2005). HVC is itself made up of three general classes of neurons which are typically defined by their projection pattern (see Figure 10.2) (Nixdorf *et al.*, 1989; Dutar *et al.*, 1998; Mooney, 2000). These include (1) HVC interneurons ($HVC_I$), whose axon terminals are primarily restricted to the nucleus, (2) RA-projecting neurons ($HVC_{RA}$), which can be thought of as motor command neurons since they are the only neurons projecting directly to the descending motor pathway and (3) Area-X projecting neurons ($HVC_X$), which project to a part of the basal ganglia (Area X) that plays an essential role in song maintenance and learning (Bottjer *et al.*, 1984; Brainard and Doupe, 2000b; Brainard, this volume).

Although the concept of a hierarchically organized descending pathway is, in its most simplistic form, likely to be incorrect, there is nevertheless a wealth of data to suggest that HVC and RA play a major role in the production of learned vocalizations (Margoliash, 1997; Hahnloser *et al.*, 2002; Suthers and Margoliash, 2002; Ashmore *et al.*, 2005a). Here we critically review evidence linking HVC and RA to song production.

### *Lesions of the descending motor pathway prevent the production of learned vocalizations*

Like peripheral control mechanisms, the telencephalic neural pathway for song control is organized in a bilaterally symmetrical fashion with identical vocal control nuclei present in both hemispheres. Bilateral lesions of HVC or RA will completely abolish the production of song in male songbirds (Nottebohm and Arnold, 1976; Simpson and Vicario, 1990; Williams *et al.*, 1992). These lesions will also remove the learned component of the normally stereotyped male contact call, transforming it into a female-like call, which is acoustically

simpler and highly variable in duration (Simpson and Vicario, 1990). Interestingly, these lesions do not appear to affect the production of nonlearned short "tet" calls even though robust premotor activity is observed in HVC and RA during the production of these calls (see next section). Studies in which $HVC_{RA}$ projection neurons are selectively ablated using laser targeting, have shown similar song deficits to those elicited by HVC lesions (Scharff *et al.*, 2000). Deficits in the production of contact calls are also observed and are generally consistent with a feminization of the normally stereotyped male contact calls (C. Scharff, pers. comm., September 2006). Interestingly, lesions of telencephalic vocal control nuclei that abolish song production do not appear to eliminate secondary motor behavior associated with singing. Several studies have noted, for example, that birds still assume normal singing posture and beak movement despite the lack of phonation (Nottebohm *et al.*, 1976).

While the effects of bilateral lesions on vocal production are unambiguous, the observed effects of a unilateral lesion on vocal behavior are much more variable, ranging from subtle distortion of song output (Nottebohm *et al.*, 1976; Williams *et al.*, 1992) to complete elimination of song (Ashmore *et al.*, 2008). Results obtained from unilateral HVC or RA lesions are often difficult to assess because the intact contralateral nucleus is potentially able to compensate for the lesion. Our own studies in the zebra finch suggest that unilateral removal of RA in adult birds completely eliminates the ability to produce normal song (Ashmore *et al.*, 2008). Lesioned birds attempt to sing but only produce long strings of stuttered introductory notes or short contact calls. Other reports show that unilateral lesions of HVC only cause partial deficits to song. These results have been interpreted as evidence that different hemispheres contribute differentially to song output (Williams *et al.*, 1992). A primary difficulty with such interpretations, however, is that song is often assessed many days after the lesion. Given the great capacity for functional recovery following perturbation (Cardin *et al.*, 2005), this extended recovery period might mask direct effects caused by the lesion. This is particularly true for HVC, which has the capacity for regeneration of its $HVC_{RA}$ projection neurons (Kirn and DeVoogd, 1989; Alvarez-Buylla *et al.*, 1990a; Scharff *et al.*, 2000) and may therefore be able to functionally reorganize following lesions that do not completely eliminate all of HVC. Because neuronal replacement has not been shown to occur in RA, this structure is less likely to be subject to functional reorganization.

While lesion studies suggest that both HVC and RA are necessary for song production, it should be noted that the presence of song nuclei in the forebrain is not a necessary requirement for the production of complex song. A number of passerine birds of the suborder suboscine, such as the eastern phoebe (*Sayornis phoebe*) for example, are able to produce complex songs but lack the discrete telencephalic nuclei that make up the song system (Kroodsma and Konishi, 1991). Because the primary distinction between oscine and suboscine song is that oscines learn their song while suboscine birds do not (Kroodsma, 1984; Kroodsma and Konishi, 1991), a possible interpretation of these findings is that forebrain song nuclei are only necessary for the production of learned vocalizations. The existence of HVC and RA might therefore serve the purpose of integrating auditory feedback, which is critical for song learning and maintenance in oscine songbirds, with the motor commands that control song output.

### *HVC population activity during learned and nonlearned vocalizations*

Because zebra finches chronically implanted with electrodes in song control nuclei will produce a normal vocal repertoire (both spontaneously and elicited by the presence of a conspecific bird), this technique can be used to record neural activity patterns during the production of their various vocalizations (McCasland, 1987; Yu and Margoliash, 1996; Hessler and Doupe, 1999a; Hahnloser *et al.*, 2002; Schmidt, 2003). In addition, these electrodes can also be used to apply brief electrical stimuli to temporarily perturb neural activity in these structures and test the effect such perturbations have on vocal output (Vu *et al.*, 1998; Ashmore *et al.*, 2005a).

If premotor activity in HVC is necessary for the production of learned vocalizations, then perturbing activity in this structure should cause distortions to the vocal output. Several studies have tested this hypothesis by applying brief electrical stimuli to HVC (or RA) during song (Vu *et al.*, 1994, 1998; Ashmore *et al.*, 2008). Such perturbations cause changes in the song temporal pattern as well as short latency perturbations in both the acoustic and the respiratory pattern of the ongoing song. These results suggest that activity

in HVC and RA can directly influence the vocal output by perturbing both the syringeal and the respiratory system. This conclusion is supported by experiments showing that stimulation in HVC or RA during periods of normal respiration cause short latency activation of syringeal muscles (Goller and Cooper, 2004) as well as changes in air sac pressure (Ashmore *et al.*, 2005).

Using these same electrodes, neural activity can be sampled from small populations of neurons (20–100 neurons) and activity patterns can be assessed in relationship to the production of different vocalizations. At typical HVC recording sites, neural activity is low during nonvocalizing periods but increases dramatically prior to song onset (Yu and Margoliash, 1996; Schmidt, 2003) and remains generally elevated during the entire duration of song (Figure 10.3a). Activity levels do fluctuate, however, and careful analysis reveals that activity, for the most part, decreases at the end of each syllable and increases again 40–50 ms prior to the onset of each syllable in the song (Figure 10.3b) (Yu and Margoliash, 1996; Schmidt, 2003). These fluctuations in spike rate are nearly identical at all recording sites in HVC, whether in the same hemisphere or in different hemispheres (Schmidt, 2003), suggesting that the overall firing rate pattern in left and right HVC is driven by a common input. These premotor activity patterns occur in both normal and deafened birds. Interestingly, the sustained increase in premotor neural activity and the tight temporal correlation with acoustic onset observed during song is also present during the production of contact calls and short "tet" calls (M. Schmidt, unpublished observations), even though neither of these vocalizations are abolished following complete lesions of HVC and RA (Simpson and Vicario, 1990). Because the contact call contains learned components that disappear following ablations of HVC and RA, the presence of premotor activity during the contact call suggests that HVC activity might be specifically associated with the production of the learned components of that call. The presence of activity during the "tet" call is more difficult to account for, however. Because "tet" calls are not learned and lesions of HVC and RA do not appear to affect their acoustic structure, production of these calls appears therefore to occur independently of any motor command that might be initiated in either of these nuclei.

Because multiunit activity in these studies reflects activation patterns for a large heterogeneous population of HVC neurons, it is not possible to distinguish between the different types of neurons that might be activated during vocal production. Recorded neural activity patterns, for example, could potentially be caused mostly by the activation of non-RA projecting neurons and therefore not reflect activity related to the motor commands that are sent out by the $HVC_{RA}$ projection neurons. In this context, one possible explanation for the finding described above is that multiunit "premotor-like" activity recorded in HVC during the production of "tet" calls might represent a copy of the motor commands (i.e. efference copy) generated in the vocal-respiratory brainstem (see next section) rather than the motor commands generated in HVC.

### *HVC motor output is represented by a sparse code that specifies each moment in time*

The evidence presented above suggests that HVC sends song motor commands to RA which, in turn, activates brainstem areas that control respiratory and syringeal muscle activity. By recording exclusively from $HVC_{RA}$ projection neurons, which represent the motor output of HVC, Fee and colleagues (Hahnloser *et al.*, 2002) have been able to gain direct insight into the nature of the motor command generated in HVC. While the number of neurons sampled in that study was small, the general trend suggests that individual $HVC_{RA}$ projection neurons exhibit only a single short burst of three to six action potentials lasting approximately 4–8 ms during each song motif (Hahnloser *et al.*, 2002). Any given $HVC_{RA}$ projection neuron is therefore only active for a short window of time associated with a specific syllable in the song. Multiple repetitions of that syllable, as would occur during the production of multiple motifs, result in that neuron firing a single burst for each repetition at exactly the same time in each motif. This firing pattern implies that any given $HVC_{RA}$ projection neuron only has a "sparse" representation during any given song motif.

Based on these findings, Fee and colleagues have proposed that each consecutive 10–15 ms time window in the song's motif is encoded by a distinct population ($\sim$200) of $HVC_{RA}$ projection neurons (Fiete *et al.*, 2004) and the entire duration of a song motif can therefore be broken down into discrete time windows each represented by a distinct population of $HVC_{RA}$ projection neurons. If each of these populations projects onto a distinct set of neurons in RA, each population of

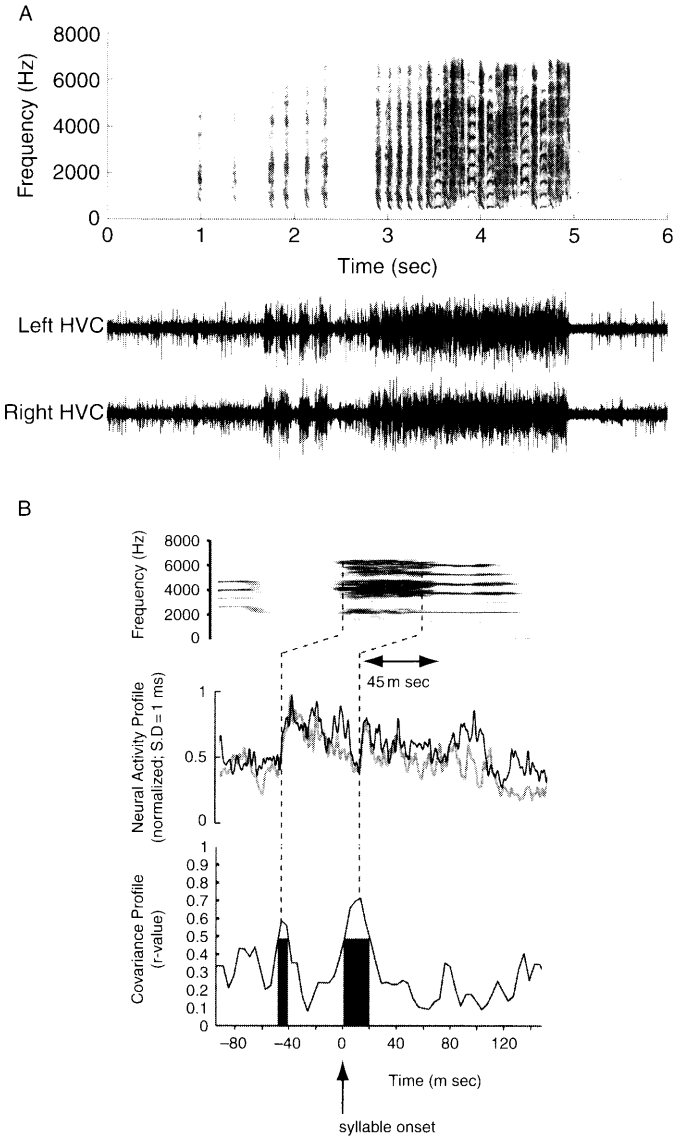


Figure 10.3 Multiunit neural activity in HVC during singing. (A) Simultaneous recording of premotor activity in left and right HVC during singing of an adult zebra finch. This example of raw neural traces reveals that premotor activity obtained from multiunit electrodes is generally elevated in both hemispheres during the entire song bout. Premotor bursts prior to the song can be observed during production of short calls and introductory notes. (B) Evidence for synchronization of premotor activity across hemispheres during short segments of the song trace. Simultaneous recording in left and right HVC during production of a two-note syllable (top panel). In this representative example, premotor activity (middle panel) in both left (gray) and right (black) HVC is elevated but highly modulated during the entire syllable. The onset of premotor activity occurs approximately 45 ms prior to the acoustic onset of the syllable. Using sliding window cross-covariance analysis (lower panel), premotor activity is shown to be highly correlated ($r > 0.5$) across hemispheres during two short periods of the motor trace. The black bars highlight these periods. When shifted by a 45 ms premotor delay time, the first period of correlated activity corresponds to the acoustic onset of the syllable and the second period to the transition between the first and second note of the syllable. (Reproduced with permission from Schmidt, 2003.)

$HVC_{RA}$ projection neurons could be in a position to specify the acoustic output associated with each time window (Figure 10.4).

### *Ensemble coding in RA driven by the sparse temporal code in HVC*

Based on the projection of its output neurons, RA can be functionally divided into a dorsal (dRA) and a ventral (vRA) part (Vicario and Nottebohm, 1988; Vicario, 1991a; Wild, 1993a; Vates *et al.*, 1997) (Figure 10.5). Neurons in dRA project to brainstem areas involved in vocal-respiratory control such as RAm, PAm and DM (Wild, 1993a, 1997a; Wild *et al.*, 2000 and this volume) Neurons in ventral RA project almost exclusively to the tracheosyringeal portion of the hypoglossal nucleus (nXIIts), and this part of RA is organized as a myotopic map of the different muscles that innervate the syrinx (Vicario and Nottebohm, 1988; Vicario, 1991a). Nucleus RA can therefore be viewed as containing two functional compartments, one more directly associated with respiratory control and one that is directly linked to syringeal muscle control (Ashmore *et al.*, 2005).

There is no evidence of functional segregation in the projections of HVC to RA and it is therefore currently assumed that neurons in both dRA and vRA are activated by sparse inputs from HVC (Hahnloser *et al.*, 2002). In contrast to HVC, single neurons in RA produce multiple bursts during each motif (Leonardo and Fee, 2005). The precision with which these bursts are produced is quite remarkable, with individual bursts occurring at nearly exactly (submillisecond accuracy) the same time in the motif from one rendition to the next (Chi and Margoliash, 2001). Thus in contrast to the sparse representation in HVC, the signal in RA becomes transformed into a temporally precise population representation where every neuron is active multiple times during the motif.

### *Unresolved issues for a simple HVC to RA sparse code model*

Although a "sparse" code for song representation in HVC suggests a novel and exciting way in which higher-level motor commands might be encoded, in its current form it raises a number of as yet unresolved issues.

#### Temporal scaling of song

While zebra finch song is often used as an example to illustrate the precision and reliability with which songs can be produced, careful analysis reveals that the speed of delivery, or tempo, of even so stereotyped a song can vary significantly. It is well known, for example, that the male song is produced at a higher tempo when singing toward a female (directed-song) than when singing alone (undirected song) (Sossinka and Böhner, 1980; Cooper and Goller, 2006; Kao and Brainard, 2006). Additionally, changes in tempo can also be observed within a given song when one compares the tempo of the first motif with the tempo of the third or fourth motif.

It is generally agreed that increasing song tempo is achieved by shortening or compressing the length of individual syllables (Cooper and Goller, 2006; Glaze and Troyer, 2006). Changing tempo therefore involves changing the length of the individual song elements within a motif. If each population of $HVC_{RA}$ projection neurons were to encode a fixed window of time, as implied by the simplified "sparse coding" model (Fiete *et al.*, 2004), changes in song tempo would have to scale equally across the entire motif. Several recent studies (Cooper and Goller, 2006; Glaze and Troyer, 2006) suggest, however, that scaling is not linear throughout the song. While the requirement for such nonlinear scaling is not inconsistent with a sparse code model per se, it is inconsistent with a model where the entire song sequence, syllables and gaps included, are scaled identically.

#### Functional divisions within RA

Given the functional division of RA into respiratory-related and syringeal-related components, it is possible that each portion of RA might be responsible for different aspects of the song motor command (Vicario, 1991a; Ashmore *et al.*, 2005). It would follow that neurons forming part of the respiratory-directed motor outputs (dRA) would have different properties from those intended for syringeal muscles (vRA). Ignoring these functional subdivisions during recording in RA might directly impact the interpretation of the coding studies of HVC. If the projection of HVC neurons has some topographic relationship to the two divisions of RA – something currently unknown – then placement of the stimulating electrode in RA used to identify RA-projecting neurons could bias the sample of $HVC_{RA}$ projections neurons recorded in HVC. Unfortunately, in both the HVC and RA studies described above (Hahnloser *et al.*, 2002; Leonardo and Fee, 2005), the authors do not distinguish between the functional subdivisions of RA and so it is not clear

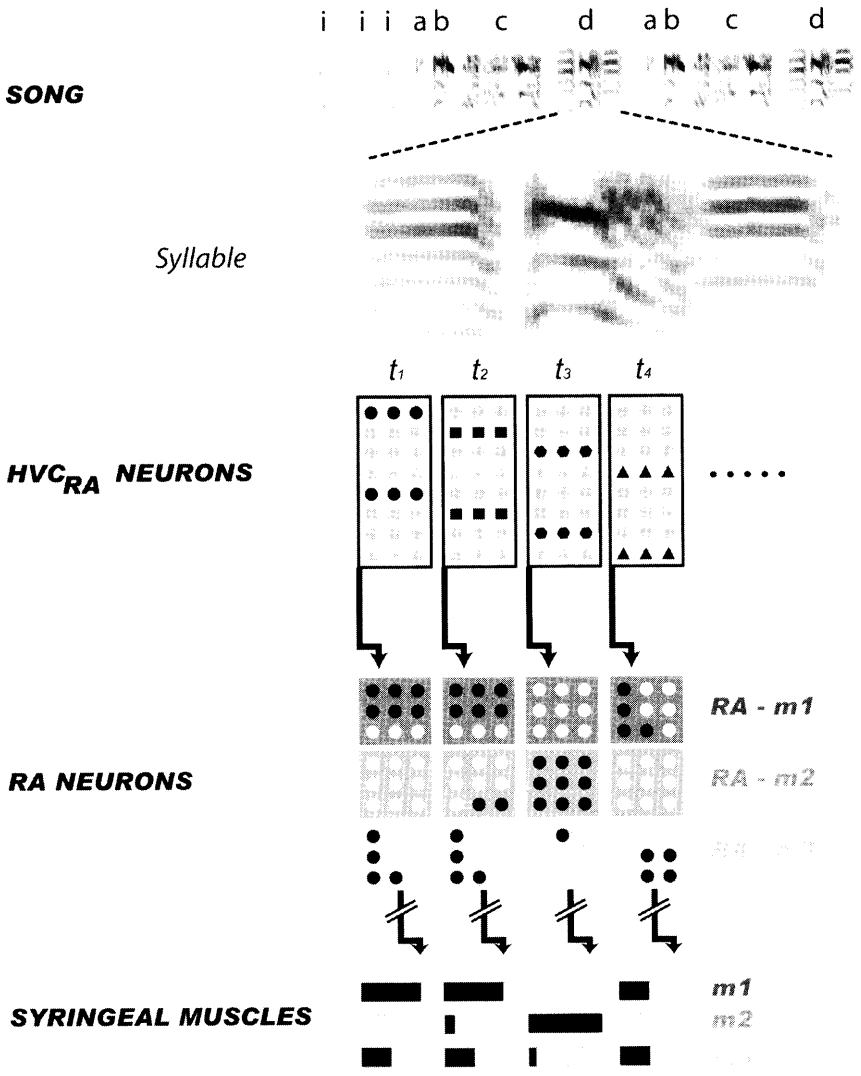


Figure 10.4 Conceptual representation of the "sparse code" model for song production. During the production of song in the zebra finch (top panel: "Song"), single unit recordings from RA-projecting neurons ($HVC_{RA}$ neurons) have shown that each neuron only fires a single burst during the entire duration of the motif and that this burst occurs at exactly the same acoustic transition from one motif to the next. Given the typical duration of a typical motif, the overall number of neurons in HVC and the duration of each burst (~10 ms), it has been hypothesized that about 200 neurons will simultaneously be active (i.e. produce a single burst) during each of these time windows (t) (Fiete *et al.*, 2004). The second panel ("$HVC_{RA}$ neurons") shows a schematic representation of this concept by dividing syllable "d" into discrete time windows ($t_1$, $t_2$, $t_3$, $t_4$ . . .) where different subpopulation of $HVC_{RA}$ neurons (round cells during $t_1$, square cells during $t_2$, etc.) are active during each time window. Each of these subpopulations of $HVC_{RA}$ neurons (e.g. square cells in window $t_2$) is thought to activate a discrete population of neurons in RA. This population of neurons is shown in the third panel ("RA neurons") by the black filled circles. To represent the known myotopic map in the ventral part of RA, which is known to project to nXIIts, RA in this figure is divided into three sections (RA-m1, RA-m2 and RA-m3) to schematically represent these functional subdivisions. For simplicity, the nXIIts layer has been omitted and only three out of the six muscle groups are represented (lowest panel "Syringeal muscles"). Activation of a specific subset of neurons in RA would therefore recruit various muscles groups and result in a distinct acoustic output for that specific window in time. In the case of window $t_1$, for example, activation of the round cell population in HVC leads to activation of a subset of neurons in RA which, in this example, activate muscle 1, a little bit of muscle 3 and not muscle 2.

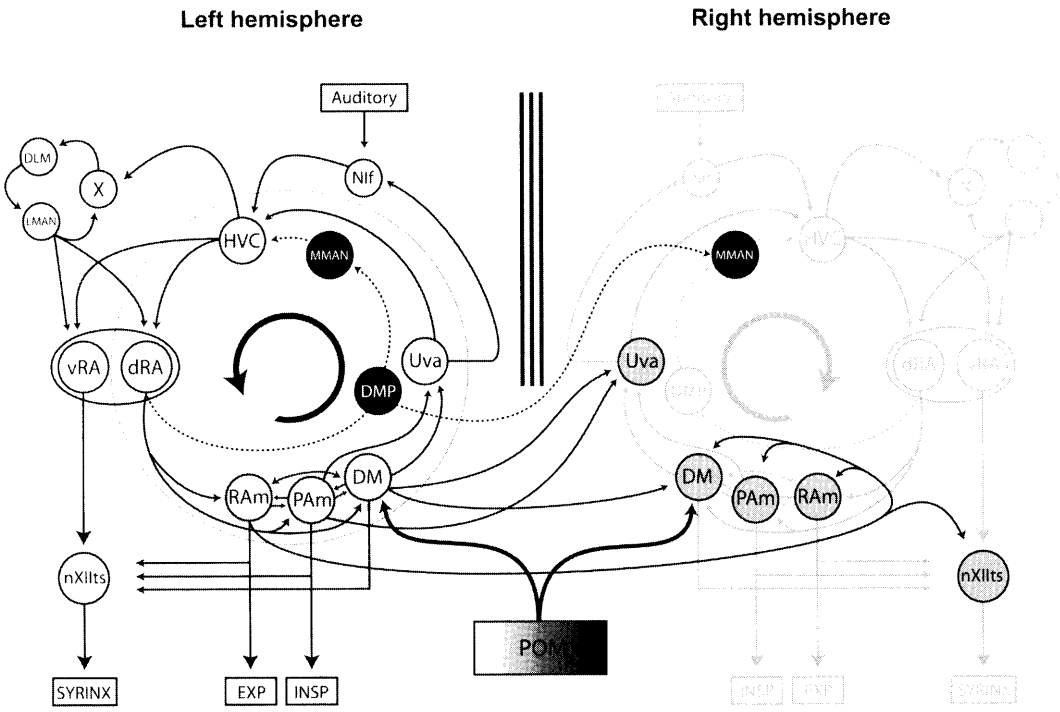


Figure 10.5 Diagram of the avian song system emphasizing its bilateral organization and the bilateral projections from the brainstem to the forebrain. Highlighted in gray is the portion of the song system that is thought to be involved in song pattern generation. This loop consists of the forebrain nucleus HVC, the dorsal portion of RA (dRA), the vocal-respiratory network (highlighted in darker gray), and the thalamic nucleus uvaformis (Uva). Nucleus DM of the vocal-respiratory network receives bilateral projections from the medial preoptic nucleus (POM) which might provide the "drive" into the system to initiate song. An additional loop consisting of the thalamic nucleus DMP and MMAN (filled black circles connected by a dotted line) has also been shown to play a role in song motor production (Foster and Bottjer, 2001; Coleman and Vu, 2005).The connection between vRA and nXIIts serves as an output pathway to the syrinx rather than as part of the song pattern generator. The three parallel lines between hemispheres illustrate the lack of commissural connections between forebrain song control nuclei. Many of the nuclei in the vocal motor system (DMP, RAm, PAm, and DM) project directly or indirectly to vocal motor nuclei in the contralateral half. These projections are bilateral but we only illustrate projections from the left to the right for simplicity. Nuclei receiving contralateral inputs are highlighted in dark gray. The anatomical connections shown here represent the major projections in the song system and have been compiled from different sources (Stokes *et al.*, 1974; Nottebohm *et al.*, 1982; Vates *et al.*, 1997; Reinke and Wild, 1998; Striedter and Vu, 1998; Sturdy *et al.*, 2003; Riters and Alger, 2004; Wild, 2004a). Weak projections have been left out. Anatomical names: DMP, dorsomedial posterior nucleus of the thalamus; MMAN, medial magnocellular nucleus of the anterior nidopallium; NIf, nucleus interfacialis of the nidopallium. INSP and EXP represent respectively the inspiratory and expiratory motor neurons. For other abbreviations, see Figure 10.2.

whether the sparse motor output observed in HVC can be generalized to all $HVC_{RA}$ projection neurons.

## Linking inspiration with HVC motor output

The sparse code in HVC is proposed to represent each time window in a song sequence. It is unclear, however, whether this output encodes only acoustic elements in the song or whether it encodes the entire song sequence, including the silent transitions between syllables as well as between consecutive motifs. Given that song occurs during the expiratory portion of the respiration cycle, it is possible that periods of inspiration could provide time markers for the occurrence of silent periods in the song. If so, one might expect some indication of this in the neural activity recorded from HVC during song. However, from the recordings of single unit activity shown in Hahnloser *et al.* (2002), there appears to be a lack of $HVC_{RA}$ projection neurons that burst during time windows associated with intersyllable silent intervals (Hahnloser *et al.*, 2002). While this might be a

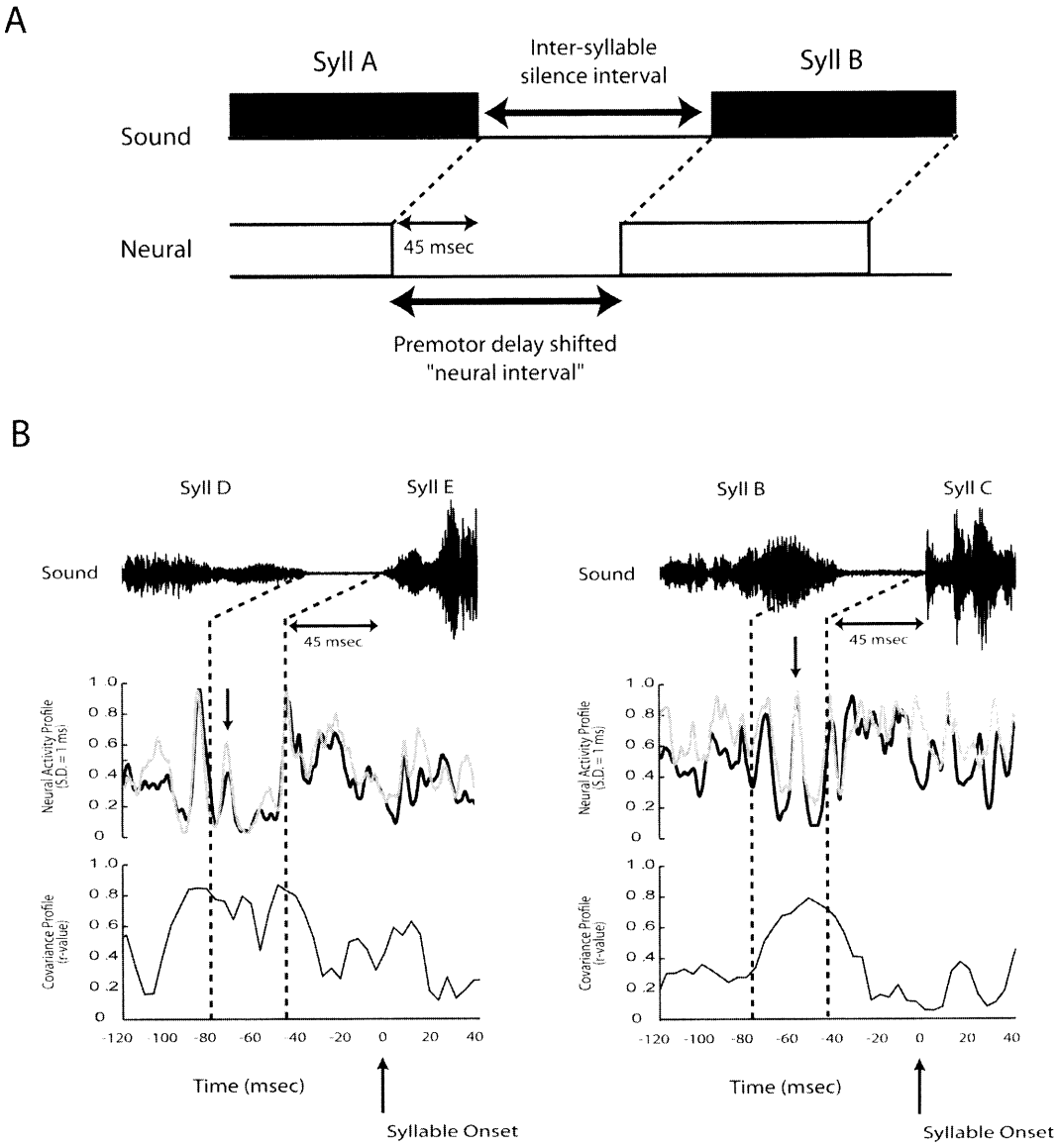


Figure 10.6 Synchronous bursting activity in left and right HVC during intersyllable silent intervals. (A) Schematic diagram illustrating the HVC neural equivalent ("neural interval") of the intersyllable silent interval. (B) Evidence that the HVC neural equivalent of the intersyllable silent interval contains stereotyped neural patterns that are highly correlated between hemispheres. Shown below are examples from two different birds showing the presence of one, or two, clear bursts of activity occurring coincidently in both hemispheres during the silent interval period. The dotted lines are aligned respectively to the syllable onset and offset and shifted by a 45 ms premotor delay. (Reproduced with permission from Schmidt, 2003.)

consequence of undersampling of these neurons, it contrasts markedly with the strong neural responses we observe in HVC during these silent periods using multi-unit electrode recording techniques (Schmidt, 2003). We show that approximately 80% of intersyllable silent intervals are associated with a robust, short-duration burst of activity in HVC (Figure 10.6) that is highly synchronized across recording sites, both in the same

HVC as well as across hemispheres (Schmidt, 2003). One interpretation is that rather than representing motor output, these synchronized bursts reflect the common synchronous input that drives activity in HVC in both hemispheres (see next section). This activity might therefore represent a timing command signal from the brainstem which functions to specify the inspiratory phase and, possibly, the onset of the next syllable.

## BRAINSTEM STRUCTURES AND THE INTEGRATION OF BREATHING AND SINGING: EVIDENCE FOR A RECURRENT VOCAL MOTOR CIRCUIT

### *Bottom-up projections from brainstem respiratory centers to forebrain vocal motor command centers*

The segregation of forebrain vocal control nuclei in each hemisphere and the simultaneous need for tight interhemispheric coordination requires mechanisms that ensure the tight coordination between the respiratory system and syringeal motor output in both halves of the syrinx (Williams, 1985; Schmidt *et al.*, 2004). Consistent with this notion, all three vocal respiratory nuclei in the brainstem (RAm, PAm and the dorsomedial nucleus of the intercollicular complex (DM)) are tightly interconnected ipsilaterally and two of these nuclei (RAm and DM) also have strong projections to their contralateral counterparts. All of the nuclei in this interconnected vocal respiratory network (VRN) project to the ipsilateral nXIIts and one of these nuclei (RAm) also projects to the contralateral nXIIts (Sturdy *et al.*, 2003; Kubke *et al.*, 2005) (Figure 10.5). Thus despite the segregation of vocal control nuclei in the forebrain, there is a significant amount of bilateral interconnectivity between the respiratory and syringeal motor system at the level of the brainstem. This connectivity could serve to coordinate respiratory and syringeal muscle activity on both sides of the syrinx.

In addition to this putative brainstem-level mechanism of coordination, there exists at least one additional way by which the respiratory system could coordinate vocal output. Specifically, two of the vocal respiratory brainstem nuclei (PAm and DM) project back to the forebrain nucleus HVC via the intermediary of the thalamic "relay" nucleus uvaeformis (Uva) (Vates *et al.*, 1997; Reinke and Wild, 1998; Striedter and Vu, 1998; Ashmore *et al.*, 2005). Because these connections are bilateral in nature they may serve to coordinate premotor activity in both hemispheres according to the respiratory demands of the system. Moreover, the connections between the vocal respiratory brainstem and the forebrain vocal control nuclei also close an anatomical loop. Thus the vocal motor control system, rather than being represented as a simple top-down motor pathway, can be represented instead as a recurrent circuit where no single structure stands at the top of a motor hierarchy (Ashmore *et al.*, 2005) (Figure 10.5). This view differs from most current models of song motor control where HVC is given a prominent and autonomous role in the production of the song motor sequence and where HVC is proposed to act as the main clock driving song behavior (Vu *et al.*, 1994; Yu and Margoliash, 1996; Fee, *et al*, 2004). The idea of a recurrent song circuit is further strengthened by a second pathway linking motor outputs from dRA back up to HVC via the intermediary of the dorsomedial posterior nucleus of the thalamus (DMP) and the forebrain nucleus MMAN (medial magnocellular nucleus of the anterior nidopallium) (Vates *et al.*, 1997) (Figure 10.5). Each hemisphere therefore potentially receives a rich source of information concerning both the respiratory state of the system (from bilateral PAm and DM projections) and the state of motor commands produced in RA (from bilateral DMP projections).

### *Bottom-up synchronization of forebrain premotor activity in both hemispheres*

In addition to the anatomical evidence, recording and ablation studies have provided additional support for the idea of a recurrent song circuit. First, simultaneous recordings from the HVC of both hemispheres during singing reveal a high degree of synchronization of premotor activity during both song (Schmidt, 2003) and call production (M. Schmidt, unpublished observations). Not only is premotor activity highly correlated with the onset of individual syllables (Figure 10.3b), and in some cases with individual notes, but most silent intervals between syllables contain pronounced bursts of premotor activity that are highly synchronized across recording sites both within HVC as well as across hemispheres (Schmidt, 2003) (Figure 10.6). These results suggest that synchronization of premotor activity in HVC is provided by a common input. In the absence of any known midline-crossing projections between left and right HVC and any other forebrain

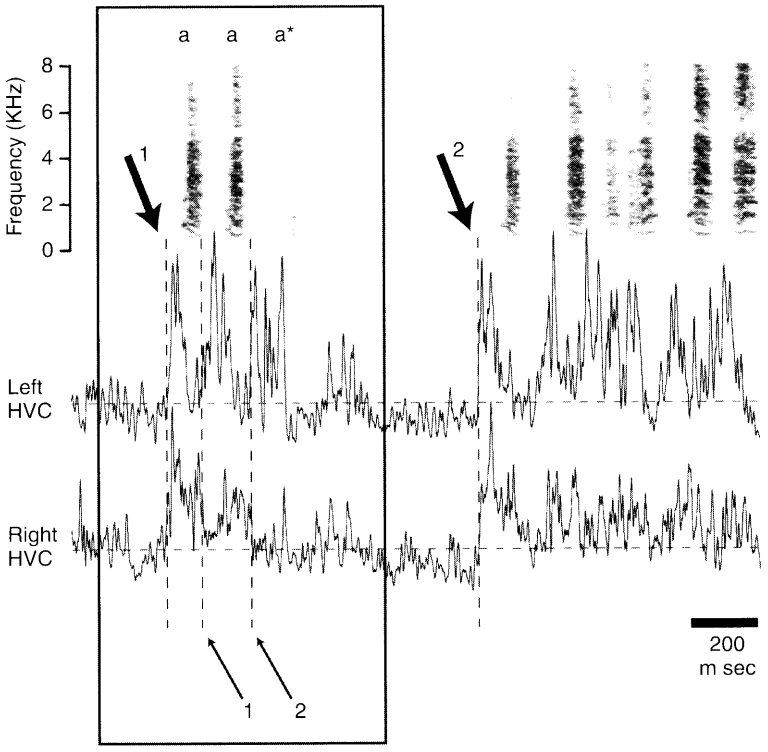


Figure 10.7 Partial loss of bilateral HVC synchronization following unilateral lesions of Uva. Premotor activity was recorded simultaneously in both left and right HVC while the bird attempted to sing (top trace). As is typical for birds recorded shortly after unilateral Uva lesion, singing attempts typically result in stuttered iterations of a few introductory notes or syllables. In the sequence of syllables highlighted by the box, the bird produces two identical song elements (a) followed by a truncated version of a third element (a*). In this example, where premotor activity was recorded 19 days after Uva was lesioned in the right hemisphere, HVC premotor activity in the intact left hemisphere shows distinct bursts of premotor activity that precede individual syllable elements by approximately 45 ms. HVC premotor activity on the side of the Uva lesion (right HVC) shows an initial premotor burst during production of the first syllable that is tightly correlated with the onset of the premotor burst in the contralateral hemisphere (thick arrow #1). A similar correlation is observed during a second attempt to sing (thick arrow #2). In contrast, however, the second burst (small arrow #1) is temporally delayed relative to the premotor pattern recorded on the intact contralateral side. The expected third burst (small arrow #2) on the lesioned side never occurs. This uncorrelated pattern of premotor activity across hemispheres is never observed in intact birds. In this example, neural activity has been rectified and smoothed with a wide Gaussian filter (S.D. = 10 ms) and normalized to the maximum firing rate. (Figure adapted from Coleman and Vu, 2000, and is identical to figure 6 in Schmidt *et al.*, 2004. Reproduced with permission from Schmidt *et al.*, 2004.)

song control nuclei (Wild, 2004a), such inputs are likely to originate from either of the pathways linking the vocal respiratory network to the forebrain (VRN/Uva pathway) or the DMP to MMAN pathway.

Support for a synchronizing role for the VRN/Uva pathway is suggested by the observation that unilateral lesions of Uva are followed by a temporary failure to produce normal song (Coleman and Vu, 2005). Following lesions, song attempts consist primarily of introductory notes followed by the first syllable of the first motif. These are then sometimes followed by several unformed syllables but mostly results in aborted song attempts. Interestingly, while normal song function recovers after several weeks, adding a unilateral lesion of MMAN prevents such recovery even though MMAN lesions alone have only minor effects on song (Foster and Bottjer, 2001; Vu and Coleman, 2001). Simultaneous neural recordings from left and right HVC suggest that the inability to produce syllables beyond the first syllable in Uva-lesioned birds (Figure 10.7) is caused by the lack

of synchronization of HVC activity patterns between hemispheres (Coleman and Vu, 2000). The idea that Uva plays an important role in song production and that it might serve to synchronize premotor activity in HVC is further supported by recordings showing bursts in Uva during singing that appear time-locked to the production of individual song syllables (Williams and Vicario, 1993).

One of the strongest arguments against a recurrent circuit for song control has been the evidence that brief electrical stimulation in HVC, but not its downstream target RA, could disrupt the song temporal pattern (Vu *et al.*, 1994). This suggested that HVC, possibly in combination with afferent inputs, was responsible for determining the temporal structure, including syllable sequencing, of song. In this scheme, RA simply followed commands initiated in HVC. More recent results from our laboratory, however, show clearly that stimulation in RA, as well as its downstream target PAm, causes a resetting of the song temporal pattern (Ashmore *et al.*, 2008). Importantly, stimulation in nXIIts, which is also a target of RA but does not form part of the loop that projects back to the forebrain, produces only temporary acoustic distortions without having any effect on the temporal pattern (Ashmore *et al.*, 2005). This suggests that disruption of neural activity within the recurrent loop of song control nuclei can influence premotor activity throughout the whole song system. This in turn implies that activity along the ascending pathway from the brainstem to the forebrain has the ability to influence premotor activity in both left and right HVC.

### *Bilateral bottom-up information flow between the respiratory system and forebrain song control nuclei*

The results from anatomical, lesion, and stimulation studies suggest that the VRN/Uva pathway transmits ascending information bilaterally into the forebrain song system during song production. This hypothesized model of song system network structure makes an important prediction, namely that neural transmission along the VRN–thalamus–forebrain pathway should be functionally robust. To evaluate whether the respiratory brainstem is capable of reliably relaying signals back to HVC in both hemispheres, we investigated whether brief electrical stimuli delivered to forebrain and brainstem vocal control nuclei in one hemisphere can transsynaptically evoke activity in forebrain vocal control nuclei of the contralateral hemisphere. Brief electrical stimuli (as few as one pulse) applied to either HVC or RA in one hemisphere cause short latency (20–30 ms) neural responses in the contralateral HVC or RA. The reliability of these responses is remarkable given that the shortest pathway between the HVC in one hemisphere and the RA in the other involves at least five synapses. The short latency of about 5 ms per synapse suggests that this motor pathway is dedicated for rapidly relaying signals across hemispheres. We have also shown that stimulation in either DM or PAm, two nuclei forming part of the VRN, can drive short latency transsynaptic responses (~15 ms) in the contralateral RA. These responses are completely abolished following contralateral lesions of Uva, confirming a role for this structure as a relay nucleus between the VRN and HVC (Figure 10.8).

The ability of brief electrical stimuli to elicit neural responses in contralateral song control nuclei suggests that spontaneous activity in the respiratory brainstem might also be able to influence baseline neural patterns in forebrain vocal control nuclei. While the majority of neurons in PAm are phase locked to the inspiratory phase of respiration, we have identified a class of neurons in this structure whose output is not tied to the respiratory pattern but rather to the bursting activity in the contralateral RA (Ashmore *et al.*, 2008). In fact, despite the existence of many PAm neurons that are precisely active during the inspiratory phase of respiration, and the ability of single stimulation pulses in PAm to elicit activity in the contralateral RA, RA never shows any sign of rhythmic activity time-locked to the bird's inspiratory pattern (Figure 10.9). If, as this observation suggests, activity linked directly to inspiration is prevented from flowing toward the forebrain, then PAm activity may influence forebrain vocal control structures via this distinct newly identified class of nonrespiratory neuron. Consistent with this view, Wild has recently identified a distinct class of neurons in PAm that projects directly upstream to Uva rather than downstream via the bulbospinal tract to inspiratory motorneurons in the spinal (Wild, this volume).

The data presented above are consistent with a major role for the VRN/Uva pathway in synchronizing HVC activity in both hemispheres. As mentioned previously, however, there exists an additional recurrent

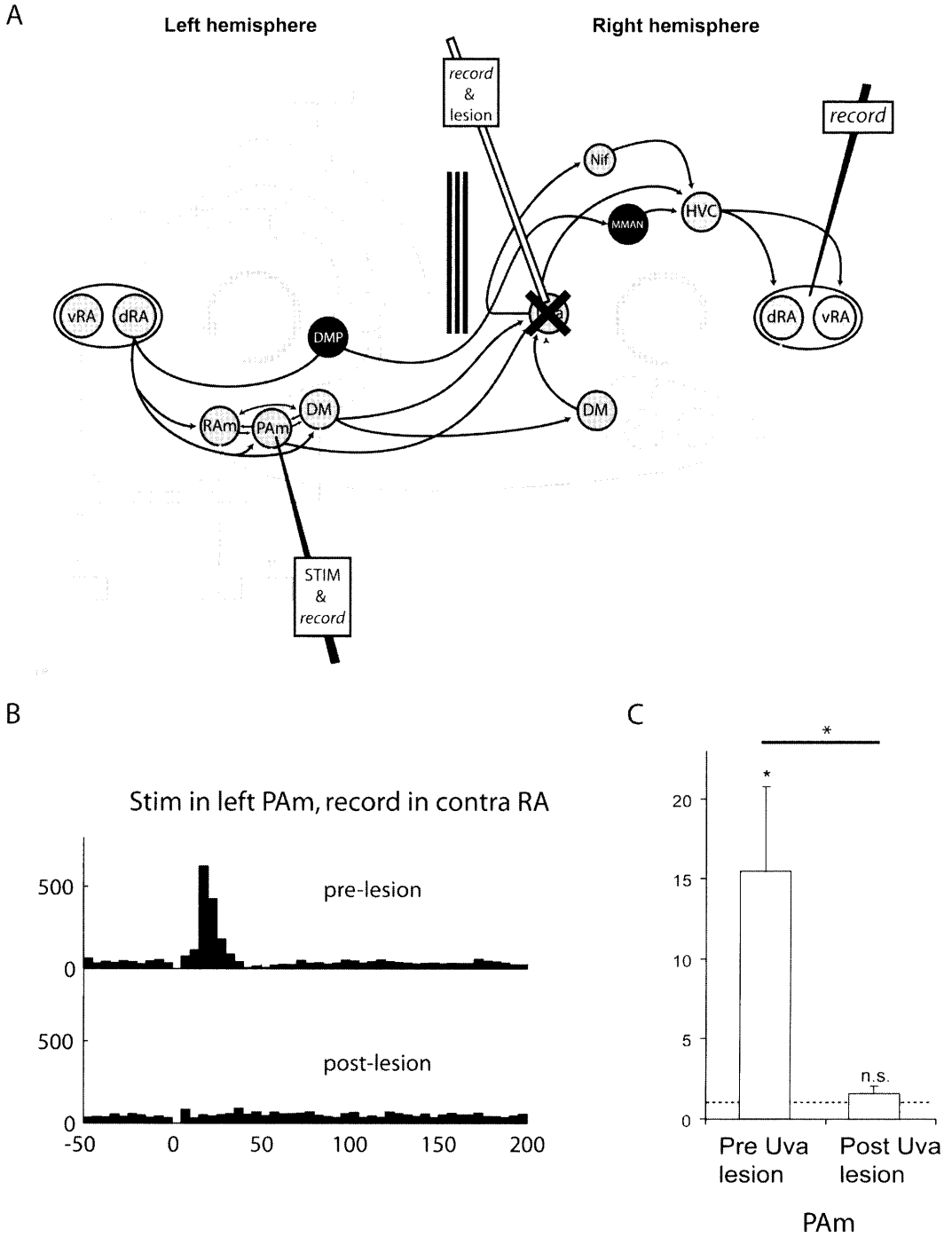


Figure 10.8 Bottom-up connectivity between respiratory brainstem and forebrain song control nuclei. (A) Diagram of the avian song system emphasizing the two known pathways crossing from one hemisphere to the other. The first is the connection from RA through the vocal respiratory network (DM and PAm) and Uva. The second, filled in black, is the pathway from RA to DMP to the contralateral MMAN. Both pathways converge on the contralateral HVC. Sites where either stimulation (RA, DM, and PAm), recording (Uva and

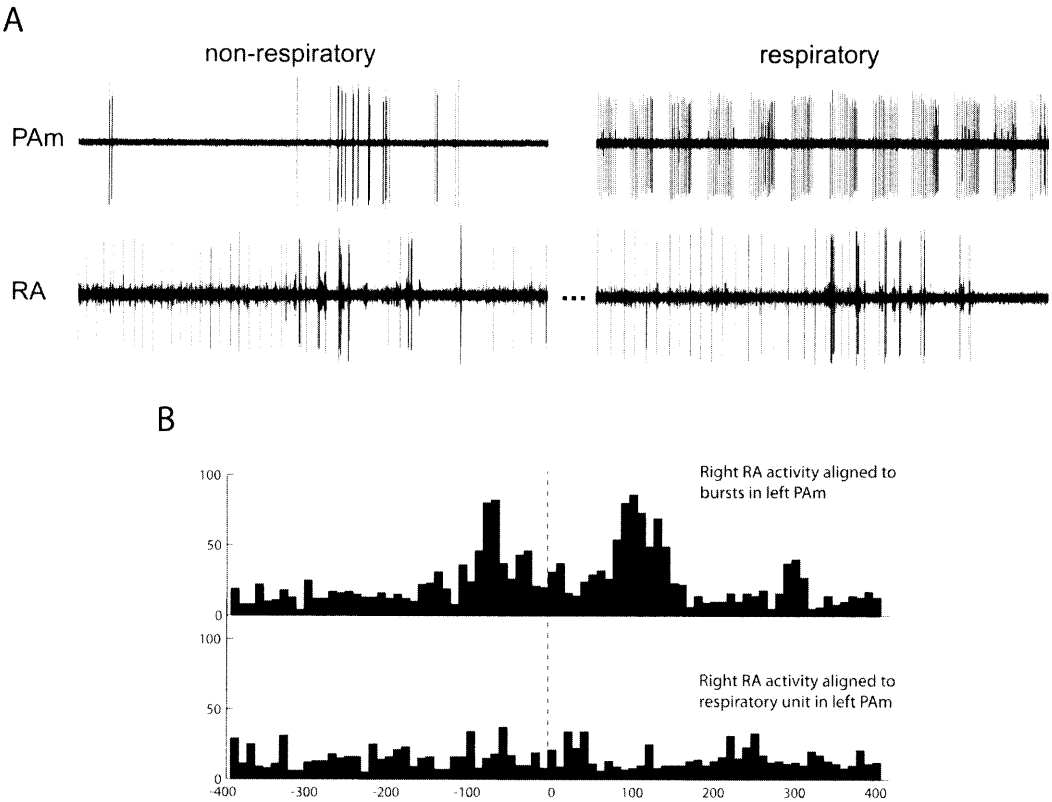


Figure 10.9 Intrinsic activity in a subset of paraambiguus (PAm) neurons is correlated with bursting in the contralateral robust nucleus of the arcopallium (RA). (A) Simultaneous recordings from PAm and the contralateral RA under anesthesia. Two separate units recorded in PAm are shown in the top panel. The neural trace to the left is from a nonrespiratory type neuron whereas the trace on the right is recorded from a respiratory-type neuron whose bursts are time locked to the inspiratory phase. Both neurons were recorded within close proximity to each other during the same penetration. The bottom trace represents neural activity recorded simultaneously from an RA neuron recorded in the contralateral hemisphere. Note that RA activity in both traces transition from a steady tonic rate to spontaneous bursting. Transitions typically observed in anesthetized RA recordings. Burst activity in the nonrespiratory PAm neuron is tightly coupled to the burst pattern in RA whereas no temporal relationship is observed between RA and the respiratory PAm neuron. (B) Two peri-event time histograms (PETHs) are shown, representing activity recorded in the right RA of one bird. In the top PETH, RA activity was aligned to bursts in nonrespiratory PAm units. In the bottom PETH, RA activity was aligned to the first spike of each rhythmically active burst. Peaks in the top histogram indicate that RA activity is high around the time of nonrespiratory bursts in PAm, but shows no correlation to respiratory activity. (Reproduced with permission from Ashmore *et al.*, 2008.)

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Caption for Figure 10.8 (cont.)

RA), or lesions (Uva) were performed are illustrated with an electrode. (B) Effects of lesions of the intermediate Uva between PAm and the contralateral RA. The PSTH shows activity following stimulation in the left PAm, recorded in the right RA, before and after electrolytic lesions of the contralateral Uva. In this example, lesioning Uva completely eliminates the evoked response in RA caused by brief electrical stimuli in PAm. (C) Quantification of evoked responses in the right RA from left PAm stimulation, before and after Uva lesions. The y-axis values indicate the mean peak-to-baseline ratio for each group (the value of the peak bin within 100 ms following stimulation divided by the mean of all bins in the 100 ms preceding stimulation, $n = 4$ birds). The dotted line represents a mean control peak-to-baseline value calculated from all birds prior to lesions (the peak bin prior to stimulation divided by the mean of all bins in a 100 ms window prior to stimulation), and the gray area represents one standard deviation from this mean. Error bars represent standard error. * indicates significance for the group by one-tailed paired *t*-tests, wherein each bird was paired with either its own control peak-to-baseline value, or paired between values obtained before and after Uva lesions. (Reproduced with permission from Ashmore *et al.*, 2008.)

circuit that might also play an important role in hemispheric coordination. This pathway consists of projections from ipsilateral RA to the thalamic nucleus DMP. DMP then projects to both the ipsilateral and contralateral telencephalic nucleus MMAN which then projects back to HVC. Several recent findings support an important coordinating role for this circuit. First, in the experiments where RA stimulation caused a short latency response in the contralateral RA, Uva lesions did not completely abolish this functional connection. This suggests that the alternate pathway through DMP and MMAN may contribute to the functional connectivity between left and right RA (R. A. Ashmore *et al.* unpublished data). Second, in birds with unilateral lesions of Uva, onset of premotor activity recorded in left and right HVC, while desynchronized for the second and third syllables in the motif, was nevertheless synchronized for the first introductory notes and the first syllable of the song (Figure 10.7; Coleman and Vu, 2000). This observation implies that synchronization of HVC activity across hemispheres is still intact for introductory notes and the first syllable even in cases where the VRN/Uva pathway is eliminated. If confirmed and extended, these findings suggest that the DLM/MMAN circuit forms an additional important bottom-up pathway in the control of song production.

## INTEGRATIVE MODEL OF SONG PRODUCTION

### *Functional relationship between brainstem and forebrain: a tale of two clocks*

At its most fundamental level, song is composed of acoustically distinct elements, known as notes, which are either produced in isolation or attached to one or more additional notes to produce a syllable (Sossinka and Böhner, 1980; Ho *et al.*, 1998; Tchernichovski *et al.*, 2000). Given the precision and general temporal and acoustic invariance of note production in species such as the zebra finch, it is easy to imagine that note elements are controlled by the execution of temporally precise motor commands delivered to both the respiratory and syringeal system (Chi and Margoliash, 2001; Leonardo and Fee, 2005). However, songs do not simply consist of isolated notes or syllables. Songs are made up of sequences of syllables that are separated by silent intervals. While song patterns vary greatly among species (Catchpole and Slater, 1995), even in birds with highly stereotyped songs like the zebra finch, the tempo and sometimes the order with which syllables are produced within these sequences can vary (Cardin *et al.*, 2005; Glaze and Troyer, 2006). This variability may depend on the social context in which songs are produced (Jarvis *et al.*, 1998; Castelino and Ball, 2005; Cooper and Goller, 2006) and the existence of such variability suggests that the temporal pattern with which syllable sequences are executed can be differentially modulated (Cooper and Goller, 2006; Glaze and Troyer, 2006).

The finding that HVC output to RA consists of discrete bursts of action potentials that are precisely time-locked to single acoustic events in the song provides a compelling model for HVC to act as a clock that regulates the temporal output of song (Hahnloser *et al.*, 2002; Fiete *et al.*, 2004). While recent evidence points to the possibility that inputs from LMAN to RA might be able to modulate the temporal output of RA (Kao and Brainard, 2006) and possibly even influence syllable sequencing (Olveczky *et al.*, 2005), it is unclear whether this sparse temporal output of the HVC to RA pathway is sufficient to explain all of the more global changes in song temporal patterning. The existence of bottom-up pathways that serve to synchronize song premotor activity in both hemispheres suggests the existence of a second clock system that might be responsible for setting overall song tempo (Figure 10.10) (Schmidt, 2003; Ashmore *et al.*, 2008). The observed pattern of synchronized premotor activity in HVC is certainly consistent with the hypothesis that HVC in both hemispheres receives precisely timed synchronizing inputs that act to specify the onset for each song syllable (Schmidt, 2003; Schmidt *et al.*, 2004). When the syllable is complex, such inputs might even specify the onset of the note components that make up these syllables. These timing signals would therefore serve the purpose of a second clock that would initiate the cascade of timed bursts in HVC which ultimately determine the specific acoustic features of syllables (Figure 10.4). By controlling syllable onset, such a bottom-up system could ultimately control the duration of inspiratory gaps between syllables and therefore control song tempo. Control of timing by two separate clocks, one for syllable/note duration (HVC clock) and one for overall song tempo (clock associated with bottom-up control system) might

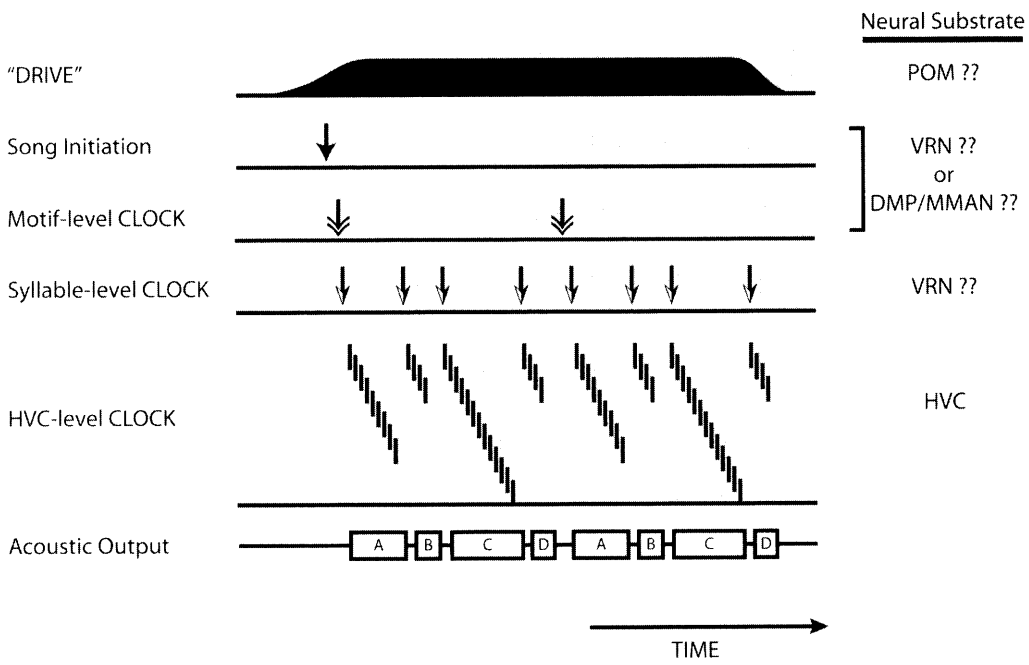


Figure 10.10 Conceptual model describing the various timescales involved in song production and their associated neural control systems. Zebra finch song (bottom row) can be described as a sequence of acoustically distinct syllables, known as motifs (schematized as boxes A–D), which are repeated multiple times to produce a song bout. Song can therefore be broken down into multiple timescales and it is therefore plausible that different aspects of song are under the control of different neural substrates within the song control system. In this scheme, song might be initiated by activation or "drive" of the song pattern generating circuit (top row) by structures such as the medial preoptic nucleus (POM). Such activation might then modify the brainstem vocal-respiratory network (VRN) to transition from a respiratory network into a song producing network. Timing of song onset (black arrow) and motif onset (double arrows) might therefore be under the control of either the VRN network or alternatively by the DMP/MMAN network, since DMP appears to be necessary for the synchronization of introductory notes and the first syllable in the song (see Figure 10.7). The onset of individual syllables (black/white arrows) in the motif is proposed to be determined by timing inputs generated in the VRN. These inputs then activate a cascade of "sparse" events (black tick marks) in both HVCs, which encode the acoustic features of each syllable on a 10 ms window scale.

more easily allow for the observed nonlinear scaling of song duration (Cooper and Goller, 2006; Glaze and Troyer, 2006).

### *PAm as a possible integrator of descending motor commands and respiration*

How the brainstem vocal-respiratory network, and the respiratory nucleus PAm in particular, might serve such clocking functions is unknown. Certainly respiration is key to all vocalization, and respiratory tempo is arguably key to vocalizations with complex temporal structure. However, the rate of respiration is often under the influence of multiple factors, both central and environmental. Low blood oxygen can increase respiratory rate (Lahiri *et al.*, 2006), as can behavioral states associated with excitement, agitation, or startle (Feldman and Del Negro, 2006). In addition, due to the physical structure of the respiratory apparatus in birds, converging signals governing expiratory and inspiratory cycles must be coordinated in order for efficient breathing to occur. Thus, nuclei governing respiration are ideally situated to integrate descending premotor signals with feedback from the periphery (Figure 10.11). Such integration might be crucial in song production, where commands imposed by the song system may make taxing demands of the periphery. If the peripheral musculature cannot meet those demands, or if they jeopardize oxygen homeostasis generally, then the demands of the song system may need to be tempered, or aborted. Preliminary evidence has suggested that

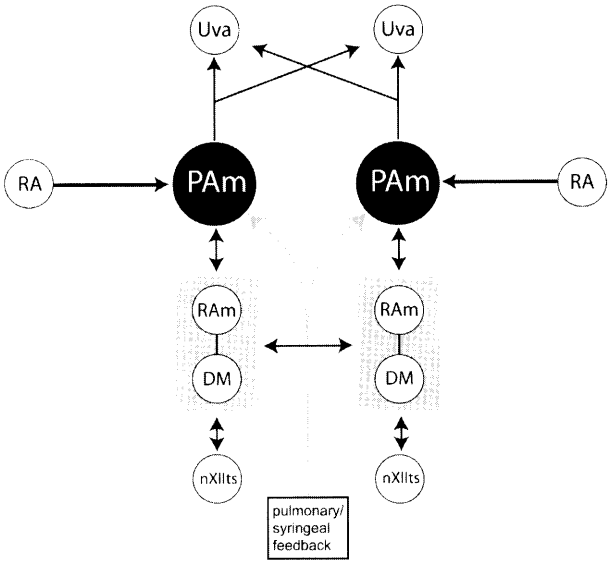


Figure 10.11 PAm as an integrator of vocal motor commands and respiratory feedback. Schematic showing a hypothesized model of signal integration in PAm. Four signal pathways are shown, based on existing anatomical and electrophysiological evidence. The first is the descending motor input from RA carrying premotor commands from the forebrain. The second group of pathways, projecting away from and also toward PAm, forms dense interconnections with other brainstem nuclei (RAm and DM; gray box), and signals along these may mediate peripheral coordination. The third pathway impinges on PAm from the periphery, carrying signals representing pulmonary or other somatosensory feedback (dotted gray). The fourth pathway is the ascending projection from each PAm into the thalamus (Uva), which carries timing input and signals necessary for interhemispheric coordination at the forebrain level. Not shown are PAm's bulbospinal projections onto respiratory neurons in the spinal cord. This model illustrates the potential of PAm to integrate premotor signals with peripheral feedback in order to generate coordinated signals for the vocal–respiratory network and forebrain vocal control centers in both hemispheres. Abbreviations as in Figure 10.2.

PAm receives peripheral feedback from the air sacs via the nucleus of the tractus solitarius (Wild, 2004b, and this volume), information that presumably reflects aspects of the current respiratory state. PAm thus lies at a vital intersection of descending motor commands and ascending respiratory feedback, two sources of information whose integration could provide useful input to both hemispheres of the song system. Since the rate of song production is an important variable in respiratory demand, it is perhaps not surprising that this hypothesized integration function of PAm might make this nucleus a critical component in determining the clocking rate of song production.

### *The vocal-respiratory brainstem as a possible integrator of motivational drive and song control*

Song length in zebra finches, and many other species, is related to the arousal level of the bird and is longer in instances where the bird is highly motivated to produce song (Jarvis *et al.*, 1998; Cooper and Goller, 2006). These longer song bouts, which in zebra finches translate into the production of more motifs, presumably serve the purpose of impressing a potential mate since female zebra finches (Neubauer, 1999) and starlings (Eens *et al.*, 1991b; Gentner and Hulse, 2000b; Gentner *et al.*, 2001; Sockman *et al.*, 2002) have demonstrated a preference for long song bouts over short song bouts. The neural correlate of the "drive" to sing in the presence of a sexually desirable individual has putatively been identified as the medial preoptic area (POM) (Riters and Ball, 1999; Riters and Alger, 2004; Riters *et al.*, 2004). The POM is involved in sexually motivated behaviors in a number of species and lesions to the POM in male starlings affect sexually related behaviors. Such lesions also result in little to no production of female directed song in these birds, while not affecting the level of undirected

song. Interestingly, recent anatomical evidence suggests that nucleus DM within the vocal respiratory network receives a strong projection from the POM (Figure 10.5) (Riters and Alger, 2004; see also Ball *et al.*, this volume). This nucleus might therefore provide the drive into the VRN (Figure 10.10) and, therefore, by controlling the number of motifs a bird sings, might also play a role in determining overall song bout duration.

## THE AVIAN SONG SYSTEM AS A MODEL FOR BRAINSTEM INSTRUCTION OF FOREBRAIN MOTOR ACTIVITY

With the exception of relatively simple rhythmic motor patterns such as breathing, chewing and locomotion (Marder and Calabrese, 1996; Marder and Bucher, 2001; Ramirez *et al.*, 2004; Feldman and Del Negro, 2006), models of motor control have often placed most of their emphasis on the forebrain (Tanji, 2001; Krauzlis, 2004). This has certainly been true for the production of learned vocal behaviors (Geschwind, 1970; Yu and Margoliash, 1996; Margoliash, 1997) where the brainstem has been primarily viewed simply as an output pathway for motor commands generated in the forebrain. In this article, we have presented evidence suggesting that the brainstem plays an active role in the generation of vocal motor commands. Rather than simply following instructions from the forebrain, the brainstem informs forebrain vocal centers about key temporal features of song.

Study of brainstem influences on motor nuclei of the forebrain is not new. A broad literature exists focusing on modulatory influences from dopaminergic, serotonergic, and noradrenergic brainstem centers such as the ventral tegmental area, raphe nucleus, and locus coeruleus (Schultz *et al.*, 1997; Berridge and Waterhouse, 2003; Aston-Jones and Cohen, 2005). These systems are increasingly viewed as shaping the details of motor production by regulating behavioral state, attentional focus, or selection from multiple possible actions. Likewise, many studies have examined the role of recurrent loops of connections in motor production, frequently involving projections from the forebrain (the mammalian cortex or the avian pallial regions) through the basal ganglia or cerebellum, through the thalamus, back to the forebrain (for mammalian/avian comparisons see Farries, 2004; Perkel, 2004). However, only a handful of studies to date have examined the role of brainstem nuclei in providing instructive input to motor nuclei of the forebrain.

The only well characterized example of brainstem to forebrain contribution to motor control is the system controlling eye saccade generation. In monkeys, there exist robust projections from the superior colliculus (SC) to the frontal eye fields (FEF), through the mediodorsal nucleus of the thalamus (MD) (Sommer and Wurtz, 2004a, b; Wurtz *et al.*, 2005). Recording and stimulation along this pathway has suggested that it conveys corollary discharge signals back to the forebrain, which potentially contribute to coordinating sequential saccades. There are obvious parallels with the account presented here in which brainstem nuclei close to the periphery transmit signals of impending action back to the forebrain, through the thalamus, in order to guide the production of sequential behavior in a temporally precise manner.

Vocal control systems in mammals are also tightly linked to the respiratory system (Jurgens, 2002; Smotherman *et al.*, 2006). One structure in particular, the parabrachial area, plays a key role in vocal control as it receives descending vocal motor commands and is known to significantly influence respiratory rhythm generation. Interestingly, recent work in the macaque has described ascending projections from the medial parabrachial area to cortical areas responsible for laryngeal control (Simonyan and Jürgens, 2005). Like the avian brainstem nuclei discussed in this chapter, the parabrachial area might therefore also form part of a possible recurrent network. Although functional characterization of these pathways has not been performed, these findings suggest the exciting possibility that motor circuits designed to integrate vocal production with respiratory control might share similar constraints in circuit architecture.

Our current work provides only indirect evidence for brainstem instructive mechanisms. It clearly demonstrates, however, that the birdsong system could serve as an important model for deciphering how the brainstem integrates multiple signals, and in turn influences forebrain motor output in a bottom-up fashion. Because a crucial component of vocal

production is the coordination between vocal output and respiration, the vocal respiratory brainstem might play an important role in instructing the forebrain in all vocalization-related behaviors including human speech.

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