

Birdsong's clockwork

Fernando Nottebohm

Recordings from song premotor circuits in singing birds show how a population of neurons may form an explicit representation of time in a motor sequence.

The behavior of animals can be as characteristic of each species as the hard body parts, and sometimes even more so. This is particularly true for birdsong, which announces territorial ownership, breeding readiness and marital status. Yet there are three groups of birds—oscine songbirds, hummingbirds and parrots—in which the genetic constraints on song are loose enough for these birds to acquire their song by a process of vocal imitation. These birds have attracted much attention from those who would like to understand the mechanism and origins of vocal learning, a behavior so central to our own identity. A study by Hahnloser *et al.*¹ from Bell Laboratories, just published in *Nature*, brings this goal one step closer.

Zebra finches breed readily in captivity and achieve sexual maturity at three months of age, by which time their adult song motif is in place. For these reasons, they are often chosen for studies of how the brain acquires and produces learned song. **Figure 1** shows the caudal pathway responsible for production of learned song. Nucleus RA, which is part of this pathway, resembles in its connections layer 5 of motor cortex in mammals². Also shown is a rostral pathway necessary for the acquisition but not for the production of learned song^{3,4}. Area X, which is part of this latter pathway, is thought to be homologous to part of the mammalian basal ganglia⁵. The High Vocal Center (HVC) is shared by the rostral and caudal pathways, and this dual role is reflected in its cellular composition—cells that project to RA, cells that project to Area X and interneurons⁶. HVC, in turn, receives inputs from the ascending auditory pathway^{7,8} and from three other discrete nuclei, one of which, the nucleus interface (Nif), shows song-related activity earlier than HVC⁹ and therefore may be important in determining when and what the bird sings.

It was known from earlier work that the neuronal activity in HVC and RA during song is highly patterned, and that its onset

and termination are closely associated with the production of song¹⁰. Interestingly, similar activity occurs during brief episodes while the bird sleeps, and it has been suggested that these events constitute a silent form of song 'rehearsal'¹¹.

Using technology developed by senior author Michale Fee, the present study¹ analyzes the exact time relation between the firing of neurons in HVC and RA and its relation to song production. It shows that these temporal relations occur in a similar manner when the bird is awake and singing and when it is asleep and 'rehearsing' song. The authors suggest that the latter circumstance is propitious for deciphering how the brain 'reads' the song score, because the bird is silent and presumably not 'attending' to its behavior, so that the number of events to be explained is simpler. Moreover, it is possible to do more sophisticated experiments in the head-fixed, sleeping bird than in awake, freely behaving animals.

Hahnloser *et al.*¹ show that, typically, an RA-projecting HVC neuron, abbreviated here as HVC(RA), fires a single, 6-ms burst at exactly the same time during each rendering of a male zebra finch's song motif and that different HVC(RA) neurons fire at different times (**Fig. 2**). The burst of such a neuron does not bear an obvious timing relationship to the onset of song syllables, but defines a unique moment in the rendering of the song motif. This stands in contrast to the firing of individual RA neurons, which occurs repeatedly throughout song and is, in each occasion, driven by a different HVC(RA) neuron. Apparently, each HVC(RA) neuron acts as an 'overseer' (my term), responsible, by itself or jointly with a few other HVC(RA) neurons, for the firing of a 'gang' of RA neurons during a unique 6 ms instant in time. Taken together, the data suggest that RA neurons act as journeymen available for hire, and that each time they fire during a same song motif they are instructed to do so by a different HVC overseer. This arrangement makes sense because vocal tract muscles are known to be represented in RA¹², and so RA neurons are likely to come in different muscle flavors. Whereas each

instant of sound may require a different combination of airflow and vocal tract configuration, most muscles determining these matters are probably active, to various extents, at all times of sound production.

Male zebra finches have, on average, some 20,000 HVC(RA) neurons in each HVC, and the song motif lasts roughly 1,500 ms. The observations of Hahnloser *et al.* suggest that whereas a minor subset of this population is active only during the production of learned calls, the remainder is active during song. This would mean that somewhere between 40 and 80 HVC(RA) neurons fire during any one 6-ms interval. If this is so when the bird tries to imitate a previously heard song model, then, as it perceives an error in the corresponding auditory feedback, it must adjust the firing of some or all of these 100–200 cells or of twice as many if it does not know which HVC—left or right—made the 'mistake'.

The insights from Hahnloser *et al.* add much-needed detail to earlier accounts that attributed to HVC a leading role in the patterning of learned song^{2,10,13}. The new details, which are central to understanding how song is produced, were made possible by the techniques used, which required that antidromic stimulation from RA be confirmed before a cell was considered an HVC(RA) neuron. Earlier evaluations¹⁰ of HVC's role in song patterning had been based on the firing of unidentified HVC neurons, and consequently the HVC cells that fired most frequently when the bird sang (the interneurons)¹ provided the bulk of the material for analysis. That is why, Hahnloser *et al.* explain, previous studies missed the special role of the HVC(RA) 'overseers'.

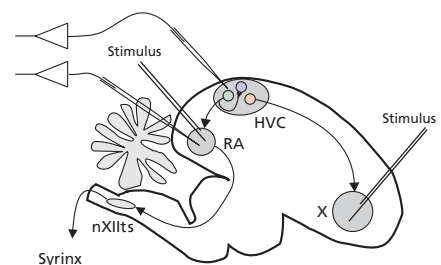


Fig. 1. A parasagittal section through the songbird brain showing how authors recorded from identified neurons in the forebrain song control regions HVC (high vocal center) and RA (robust nucleus of the archistriatum) during singing. RA neurons project to brainstem regions that control the syrinx, the vocal organ. The same song control regions and connecting pathways are found on the right and left sides of the brain, and both sides are involved in song production. (Reprinted with permission, Nature Publishing Group and M. Fee, ref. 1).

The author is at the Rockefeller University Field Research Center, 495 Tyrrel Road, Millbrook, New York 12545, USA.
e-mail: nottebo@mail.rockefeller.edu

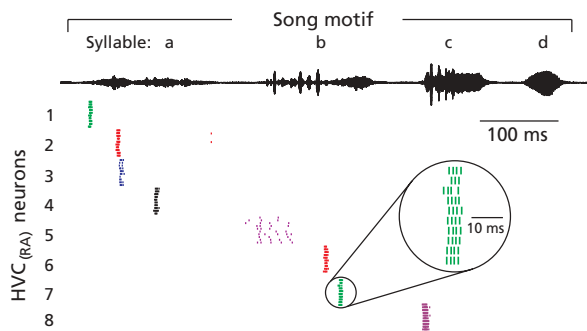


Fig. 2. RA-projecting HVC neurons fire in bursts at a single precise time in a song. Eight neurons recorded in one bird are shown. Each row of tick marks shows action potentials generated during one rendition of the song; roughly 10 renditions are shown for each neuron (neural activity is aligned by the acoustic onset of the nearest syllable). The amplitude spectrum of the song is shown at top (Reprinted with permission, Nature Publishing Group and M. Fee, ref. 1).

The above observations suggest to Hahnloser *et al.*¹ that “HVC(RA)neurons code for a unique time within the RA sequence.” This time code is credited with much of the patterning of song because at least 70% of the bursting seen in RA is driven by the highly programmed input from HVC. The authors make it clear that they do not know, yet, how the division of labor between HVC(RA) neurons comes about and what makes each of them fire during its 6-ms contribution to the bird’s song.

This story is of great interest and elegance because it is the first time that the cells and timing behind the control of a complex learned skill have been identified. In addition, the story is doubly intriguing because the HVC(RA) neurons that I have called ‘overseers’ are produced late in ontogeny as a male zebra finch first learns its song¹⁴, and then continue to be produced and replaced in adulthood for reasons that remain unknown¹⁵. Because

adult zebra finches do not modify their song, the new cells must assume, one supposes, roles much like those of the cells they replace.

The present report does not delve into how each HVC(RA) cell acquires control over the subset of RA cells it innervates. Does that control result from its birthdate and position within HVC? Is it shaped by competition with other cells of the same type? Is it affected by auditory and motor vocal experience? Once an HVC(RA) cell defines its control over a subset of RA cells, the sounds it ‘produces’ will depend on other circuit activity that precedes and accompanies its 6-ms firing.

Summing up, HVC(RA) overseers are part of a clockwork that tells them when to fire, but knowledge of the entire song must exist in the web of connections, including those that link the left and right HVC, that tell each overseer when to chime in with its precious 6 ms of the song motif. There is no decision maker, just decisions made. And so even difficult, learned and beautiful music can be reduced in the brain of a bird—and a

human?—to the tiny metaphorical clicks of neuronal cogwheels, which, as in a recorder, are fixed in time. So much to sing, so little freedom!

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The double life of netrin

Susana Cohen-Cory

Netrin-1 initially attracts and later repels retinal axons. A new paper shows that both intrinsic and extrinsic cues are involved in regulating this developmental switch in responsiveness.

During development, growing axons travel long distances to reach their targets and form specific connections. Along their journey, axon growth cones make choices as they navigate through diverse territories, encounter intermediate targets, and reach their targets. Many guidance molecules

have been characterized that can guide axons to their targets. Guidance cues steer axon growth cones by attraction or repulsion, and may have dual roles, attracting some axons while repelling others. Proper reading and integration of multiple attractive and repulsive guidance cues is therefore necessary for precise wiring of different parts of the brain. In this issue, Shewan and colleagues now provide evidence supporting a bifunctional role for one of these guidance cues, netrin-1, during retinal axon pathfinding *in vivo*¹. An

intrinsic program within the growth cone helps netrin-1 steer the growth cone through its journey to its target.

Netrin-1 is an evolutionarily conserved glycoprotein that can attract some axons and inhibit others². The particular response of a growth cone to netrins may reflect the complement of receptors it bears, as well as the presence of other cues at the choice point. Netrins attract axons through a mechanism requiring the ‘deleted in colorectal cancer’ (DCC) receptors, whereas they repel axons by binding the unc-5 receptor³. In addition, netrin can bind and activate the adenosine A2B receptor, which stimulates cAMP production⁴. The level of cytosolic cAMP present at the growth cone can also dictate the growth cone response to a guidance signal. For example, altering cAMP levels can reverse the growth cone turning response to guidance cues, including netrin-1 (refs. 5, 6). Axons are attract-

The author is at the Mental Retardation Research Center, Department of Psychiatry and Biobehavioral Sciences, University of California Los Angeles, Los Angeles, California 90095, USA. e-mail: scohen@ucla.edu

