Silurian, by placoid scales of primitive sharks, also without cell spaces in the basal tissue and none in the dentine (*28*), and by scales of acanthodians, in which some bone has cell lacunae and the dentine also has spaces interpreted as those for odontocytes, and a network of fine tubules for interconnecting cell processes [mesodentine, (*28*)]. New discoveries of early vertebrate tissues are needed before the relationships of the Ordovician vertebrates to the other groups can be resolved.

The developmental basis for the evolution and diversification of the early vertebrate skeletal tissues is discussed by Smith and Hall (*6*), who look at the role of neural crest cells and the conservation of causal developmental sequences in odontogenesis and skeletogenesis throughout the groups. It is considered to be highly significant that the tissues described here, cellular bone and cellular dentine, are in juxtaposition within the denticle, of what is considered to be an early example of a primitively micromeric skeleton. These observations accord with the hypothesis that the first exoskeletal tissues of both the cranium and the trunk evolved as products of neural crest–derived cells (*6*).

The following proposals arise from the data reported here: (i) bone tissue with cell lacunae similar to that reported in Early Silurian osteostracans and gnathostomes occurs in the early Late Ordovician, much earlier than has been generally acknowledged; (ii) it is just as probable that the direction of evolutionary change in the exoskeleton was from cellularity to acellularity, as from the acellular tissue to the cellular—the latter has recently been the more accepted view; (iii) the presence in the same denticle, of mesodentine with odontocyte lacunae and attachment bone with osteocyte lacunae, suggests that the cells derived from neural crest gave rise to both dentine with cells and bone with cells, as the first vertebrate exoskeletal tissues. It follows that neither dentine nor bone gave rise to the other in development or evolution. Developmental data, on the neural crest–derived tooth tissues, shows that dentine and attachment bone develop in close association, and the two processes may be so tightly linked that one did not evolve without the other (*6*).

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# Altered Perception of Species-Specific Song by Female Birds After Lesions of a Forebrain Nucleus

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**Female birds that do not normally sing possess brain nuclei associated with song production in males. To determine whether one song nucleus, the caudal nucleus of the ventral hyperstriatum (HVc), acts in conspecific song perception, courtship responses of female canaries to canary and white-crowned sparrow songs were compared before and after HVc lesions. Bilateral lesions of a portion of the HVc resulted in copulation solicitations to heterospecific song as well as conspecific song. Control females continued to respond only to conspecific song. This suggests that the HVc is critical for conspecific song perception in females. Because female canaries do not normally sing, neurons in female HVc must develop response selectivity by a mechanism different from that proposed for male birds in the motor theory of song perception.**

SUCCESSFUL REPRODUCTION IN SEXUAL animals depends on mating with members of the same species. In many species, females are able to recognize conspecific males on the basis of their species-specific courtship signals. Numerous studies have documented the selectivity of neurons in sensory brain regions for conspecific signal features (*1*). The role played by brain nuclei in the behavioral recognition of conspecific mating signals by females, however, has received scant attention.

Song production in male songbirds is controlled by a network of brain nuclei (*2*). In many species females possess the same network of song nuclei as conspecific males, albeit they are of smaller size (*3*). The presence of these nuclei in the brains of females that do not normally sing is puzzling. Why should these females possess such song nuclei at all? No function for these song nuclei in females has yet been demonstrated empirically.

One possibility is that these song nuclei play a role in song perception in females (*4*). In males of several songbird species, neurons in several of these nuclei receive and respond to input from the auditory system (*5*). Neurons in male song nuclei respond more selectively to conspecific song stimuli than do neurons in purely auditory nuclei. For example, the caudal nucleus of the ventral hyperstriatum (HVc) of males is critical for song production. The HVc receives auditory input from field L, the primary forebrain auditory nucleus. Multineuron clusters in HVc respond preferentially to an individual bird's own song (*6*). Neurons in field L do not respond selectively to an individual's song, but show more generalized response properties (*6*).

Previous studies have not shown whether HVc or other song nuclei play a role in conspecific song perception in female birds, as they do in males. To examine this hypothesis, I tested the behavioral responses of female roller canaries (*Serinus canarius*) to playback of conspecific and heterospecific songs, before and after selective lesions of HVc. I found that lesions of part of HVc eliminated the discrimination between these

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signals without disrupting behavioral responses to song. The results indicate that HVc does act in song perception in female birds that do not normally sing (*7*).

Adult female canaries ($n = 17$) were brought into breeding condition with silastic implants of estradiol placed over the pectoral muscle (*8*). Each bird was housed individually in a sound-isolated chamber equipped with a loudspeaker. Starting 1 week after hormone implant, conspecific (roller canary) and heterospecific (white-crowned sparrow, *Zonotrichia leucophrys*) songs were played to the females (Fig. 1, A and B) (*9*). As a behavioral measure of song perception, I scored the number of copulation solicitation displays performed by the focal female during a 5-min test period. In this stereotyped display, performed in nature just before copulation, a female responds to conspecific song by thrusting her breast forward, raising her tail sharply, and holding her wings out to the side while vibrating them rapidly. This display has been used to test female responses to song in several species (*10*).

Playback tests were repeated every 2 days until the first day that a female canary indicated sexual receptivity by giving at least five solicitation displays during the 5-min presentation of canary song. When she attained this response level, she was anesthetized with Equithesin and received bilateral electrolytic lesions directed at the forebrain vocal nucleus HVc ($n = 13$ birds) (*11*). Starting 2 days after the lesion was made, she was again tested for responses to canary and white-crowned sparrow song. Testing continued at 3-day intervals for at least two more weeks. The remaining four birds received sham lesions (*12*). The observer was blind as to whether the subject was lesioned or sham-operated.

Before lesion of the HVc, female canaries discriminated between conspecific and heterospecific song. No female gave more than one solicitation display to white-crowned sparrow song per 5-min test period; this always occurred within the first 10 s of the onset of the playback. Playback of male canary song, however, evoked strong responses (that is, $\geq 5$ displays/5 min) from female canaries (Fig. 1, C and D) (*13*).

Histological analysis of brains indicated that in five females the lesions eliminated the medial and caudal portions of HVc on both sides (Fig. 2). These birds altered their behavior dramatically; they responded strongly to white-crowned sparrow song (Fig. 1C; pre- versus post-lesion responses to sparrow song, $P < 0.02$, paired $t$ test, two-tailed). After lesions, the number of solicitation displays to canary song and to sparrow song did not differ ($P > 0.05$).

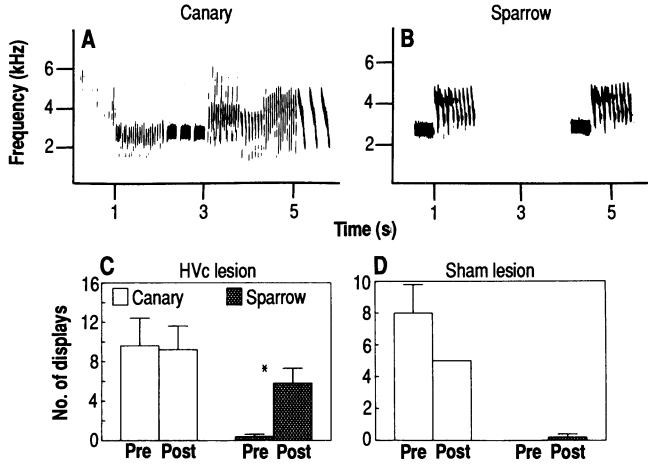

**Fig. 1.** Sonagrams of (**A**) canary song (segment) and (**B**) white-crowned sparrow song used as playback stimuli. (**C**) Median number of copulation solicitation displays (group X + 1 SEM) given by five female canaries to playback of canary and sparrow songs pre- and post-lesion of HVc. (*$P < 0.02$, paired $t$ test on median number of displays.) (**D**) Displays by four female canaries to canary and sparrow songs before and after sham lesions.

Responses to canary song before and after lesions showed no statistically significant difference ($P > 0.05$) (*14*).

In the remaining eight lesioned birds, the medial and caudal portions of HVc remained intact on one or both sides (Fig. 2). These females continued to respond only to canary song, even if the lateral and rostral portions of HVc were lesioned bilaterally.

Birds that received sham lesions also continued to respond strongly only to conspecific song (Fig. 1D; post-lesion responses to canary song versus white-crowned sparrow song, $P < .001$).

Three of the five birds that altered their behavior after HVc lesions were observed over relatively long time periods post-lesion (20 days, 51 days, and about 180 days). They continued to respond to sparrow song throughout these periods. None of the sham-lesioned or ineffectively lesioned birds ever altered their responses to heterospecific song. Comparison of these latter two groups with the former group thus indicates that lesions of HVc had a pronounced and fundamental effect on species-specific song responses (*15*). The prolonged duration of altered response in effectively lesioned birds shows that the change in song discrimination was not due to transient, nonspecific effects such as post-surgical trauma.

HVc lesions may have eliminated the ability of female canaries to perceive differences between conspecific and heterospecific songs. Alternatively, these lesions may simply have lowered the threshold for responding to acoustic stimuli in general. The latter explanation is unlikely, however, because lesions of HVc only increased the strength of response to heterospecific song. The results are more consistent with the conclusion that lesions disrupted the role of HVc in the perception of conspecific song in female canaries (*16*).

The response measure, copulation solicitation behavior, did not distinguish between two components of the perceptual process. Did effectively lesioned females actually fail to discriminate species differences in song, or did they just fail to respond to those differences? Psychophysical tests of song perception in HVc-lesioned birds can address these questions.

It will be interesting to examine the physiological response selectivity of single-units and multi-unit clusters in female HVc. I

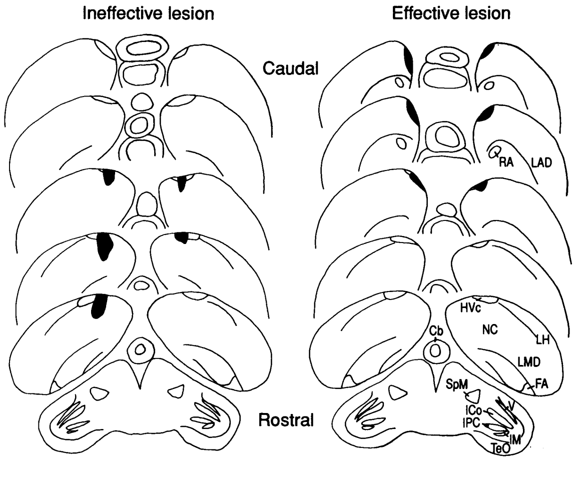

**Fig. 2.** Serial reconstructions of typical lesions that were ineffective or effective at evoking solicitation displays to white-crowned sparrow song by female canaries. Black indicates site of lesion. Cb, cerebellum; FA, fronto-archistriatal tract; HVc, caudal nucleus of the ventral hyperstriatum; ICo, intercollicular nucleus; IM, magnocellular nucleus of the isthmus; IPC, parvocellular nucleus of the isthmus; LAD, dorsal archistriatal lamina; LH, hyperstriatal lamina; LMD, dorsal medullary lamina; NC, caudal neostriatum; RA, robust nucleus of the archistriatum; SpM, medial spiriform nucleus; and TeO, optic tectum. Scale bar, 1 mm.

predict that female HVc neurons will respond to a broader array of conspecific song stimuli than is observed in male HVc, in which neurons respond specifically to the bird's own song.

A challenge remaining is to determine how song perception develops in female birds that do not normally sing. It has been proposed (*17*) that neurons in male HVc acquire their pronounced song selectivity through a mechanism similar to that presented in the motor theory of human speech perception (*18*). This theory posits that, to perceive a song syllable, a bird must first convert the sound it hears into the motor commands required to produce that sound. Male HVc neurons develop song selectivity during the sensorimotor phase of song learning (*6*). Song production and song perception are thus viewed as being functionally linked in males. Female birds that do not normally sing presumably do not develop the entire set of motor commands required in male HVc for the production of full adult song. However, females learn to perceive even subtle differences among the songs of conspecific males (*8, 19*). It thus seems unlikely that such perceptual learning in female birds can be explained fully by a motor theory of song perception, at least as proposed for males (*17*). Rather, the sexes may differ in the mechanisms by which neurons in song nuclei develop their selectivity to conspecific courtship signals.

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7. Captive female canaries may produce a song-like vocalization if isolated for several months. This vocalization is much simpler and less stable in structure, and is produced less often, than normal male song [A. Pesch and H-R. Guttinger, *J. Ornithol.* **126**, 108 (1985); Nottebohm (*2*); personal observation]. There are no reports of song in wild female canaries.
8. All birds were kept in a short-day photoperiod (light:dark, 8:16 hours) for at least 2 months before the start of the experiment. They were then gradually shifted to a long-day photoperiod (light:dark, 14:10 hours) to bring them into breeding condition. When a female developed a vascularized brood patch, she received two subdermal implants of estradiol (inner diameter, 0.76 mm; outer diameter, 1.65 mm; length = 12 mm) to induce copulation solicitation behavior in response to conspecific song.
9. The conspecific stimulus was a 5-min sequence of male roller canary song containing 19 syllable types and consisting of bouts of 4.8 ± 2.3 phrases ($\bar{x}$ ± SD), lasting 5.4 ± 3.0 s, and separated by silent intervals of 1.8 ± 0.9 s. [See F. Nottebohm and M. E. Nottebohm, *Z. Tierpsychol.* **46**, 298 (1978)]. In preliminary studies, female canaries only responded to songs produced by males of the same strain. The heterospecific stimulus was a white-crowned sparrow song recorded from the *Z.l. nuttalli* dialect in northern California. [See L. Baptista, *Univ. Calif. Publ. Zool.* *105* (1975)]. White-crowned sparrow song was used because it contains frequency-modulated syllables as does canary song but has a very different syntactical structure. The single sparrow song lasted 1.55 s, repeated at intervals of 2.45 s for 5 min. Songs of both species were played at a maximum root-mean-square amplitude of 90 dB sound pressure level at 50 cm. The two songs were presented in random order each day, at 3-hour intervals to minimize facilitation or habituation effects.
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11. Lesions were made by passing 100 μamps of anodal direct current for 2 min through insulated tungsten wire. Sites of lesions were confirmed histologically.
12. For sham lesions, birds were anesthetized, and an electrode was lowered to the stereotactic coordinates of HVc, but no current was delivered to the electrode.
13. For statistical analysis I used the median number of each bird's displays obtained from her full set of pre-lesion and post-lesion playback sessions. There were 1 to 4 pre-lesion and 1 to 13 post-lesion playback sessions per bird.
14. Ranges for the median number of solicitation displays are as follows: (i) effectively lesioned birds, pre-lesion canary song, 5 to 20 displays; post-lesion canary song, 4 to 16.5 displays; pre-lesion sparrow song, 0 to 1 display; post-lesion sparrow song, 3 to 11 displays; (ii) sham-lesioned birds, pre-lesion canary song, 5 to 12 displays; post-lesion canary song, 5 displays; pre-lesion sparrow song, 0 display; post-lesion sparrow song, 0 to 1 display.
15. It is unlikely that lesions acted by disrupting fibers of passage between song nuclei other than HVc. Axons projecting from lateral MAN (magnocellular nucleus of the anterior neostriatum) to RA travel lateral and ventral to HVc [F. Nottebohm, D. Kelley, J. Paton, *J. Comp. Neurol.* **207**, 344 (1982)]. Only lesions of medial caudal HVc induced responses to sparrow song. Projections between other song nuclei do not pass adjacent to HVc.
16. To determine whether effective lesions of HVc eliminated all song discrimination, I played the songs of different bird species to one female canary 7 months post-lesion. She responded strongly (≥5 solicitation displays during 5 min of playback) to songs of the northern mockingbird (*Mimus polyglottos*), the bay wren (*Thryothorus nigricapillus*), and the buff-breasted wren (*T. leucotis*). These songs have different syntactical structures, but are similar to each other and to canary song in that each has syllables with pronounced frequency modulation. The same female did not respond to rufous-and-white wren (*T. rufalbus*) song, which contains little frequency modulation. These observations suggest that lesions of medial caudal HVc eliminated the species-specificity of responses and resulted in a more generalized pattern of signal discrimination. The altered pattern of response may be related functionally to reports that HVc neurons respond more selectively to species-specific song stimuli than do neurons in the afferent auditory nucleus, field L (*6*).
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# The Effect of Anti-Neoplastic Drugs on Murine Acquired Immunodeficiency Syndrome

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**The murine acquired immunodeficiency syndrome (MAIDS) is associated with proliferation of target cells that have been infected by a defective retrovirus. To control the growth of this primary neoplasia, virus-inoculated mice were treated with anti-neoplastic drugs. Paradoxically, cyclophosphamide, which is also immunosuppressive, was very effective in preventing the appearance and progression of the disease, in restoring a normal T cell function, and in depleting the number of infected target cells. This result suggests that the proliferating infected target cells were responsible for the immunodeficiency.**

THE DUPLAN STRAIN (*1*) OF MURINE leukemia virus (MuLV) induces a severe immunodeficiency syndrome in mice (*2–6*). The disease shows many similarities with human AIDS (*7, 8*), including abnormal T and B lymphocyte functions, polyclonal B-cell proliferation, lymphadenopathy, splenomegaly, hypergammaglobulinemia, enhanced susceptibility to infections, and late appearance of B cell lymphomas (*2–9*). The etiologic agent of this murine AIDS (MAIDS) has been identified as a defective retrovirus (*10, 11*).

The mechanism by which the immunodeficiency arises in these mice remains unknown, but viral replication does not seem to be required for the appearance of the

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