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Neurophysiological and Behavioral Development in Birds: Song Learning as a Model System

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The avian song system is a particularly good model for studying the behavioral and physiological aspects of animal development. One seemingly trivial but very important reason for this is that the sound spectrograph enables sounds to be described, measured and analyzed objectively and in detail. Secondly, birdsong is one of the few behaviors which is performed by a separate chain of brain regions and is therefore relatively easy to investigate neurophysiologically. Work on song also provides a clear illustration of the subtle way in which birds are influenced by their internal and external environments during development.

The fact that some songbirds are capable of imitating the sounds of other individuals has been realized for hundreds of years and exploited by bird fanciers all over the globe [1, 2]. However, it was not until the late 1950's that biologists realized that most, if not all, songbirds learn their songs. Careful experimental study of singing behavior, starting with Thorpe's classic observations on chaffinches [3], have resulted in an extensive body of literature on the behavioral aspects of avian song learning. It was some years later that the first song control nuclei in the brain were identified [4]. Subsequent neuroanatomical studies have greatly advanced our knowledge of the neural and hormonal correlates of song learning. In this review we attempt to summarize the main findings to date and show how liason between neuroscience and ethology can lead to an increased understanding of how the nervous system works.

Sex Differences in Singing Ability and Brain Size

Of the 8500 living species of birds almost half are classified in the songbird suborder Oscines. In the majority of songbird species only males sing, to defend a territory and/or to attract females. This sex difference in singing ability provided the starting point for the neuroanatomical studies of song learning. The first studies were carried out by Nottebohm and his coworkers who identified a chain of song control nuclei, whose main components are the forebrain nuclei HVc and RA and the brainstem nucleus nXIIts which projects to the muscles of the syrinx where sound is produced: Lesions to these nuclei disrupted song [4]. Since then other components of the song control system have been discovered, e.g., MAN, NIF, UVA, and area X (Fig. 2) [5]. During their studies Nottebohm and Arnold [6] noticed that HVc and RA volumes were at least three times greater in male canaries which sing long, complex songs than in female canaries which sing short, simple songs. The sex difference in these song nuclei was not just one of volume: Males have larger numbers of neurons with more dendrites and more dendritic spines (e.g., [7]). These sex differences in the brain are not specific to canaries but seem to apply generally to those species in which the male does most, if not all, of the singing (e.g., [2]). In duetting species, where singing ability is shared more equally between the sexes, sexual dimorphism in the size of the song nuclei is greatly reduced. Nonetheless, in duetting species in which the female sings less complex songs than the male the number and volume of the song nuclei are more sexually dimorphic than in those in which the female's song is of comparable out-

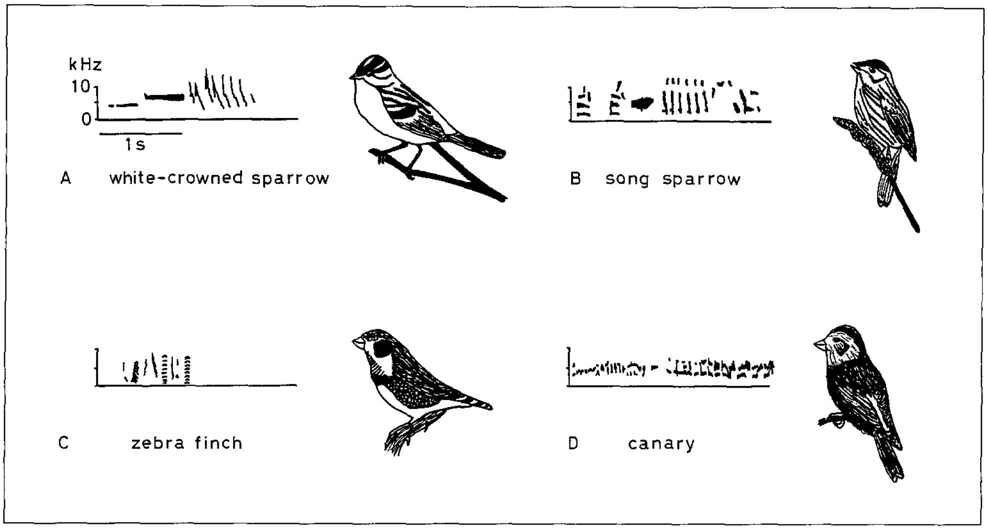

Fig. 1. Tracings of sonagrams of typical song phrases of A) white-crowned sparrow (*Zonotrichia leucophrys*), B) song sparrow (*Melospiza melodia*), C) zebra finch (*Taeniopygia guttata*), D) canary (*Serinus canaria*), showing how species differences in song can be objectively characterized and analyzed

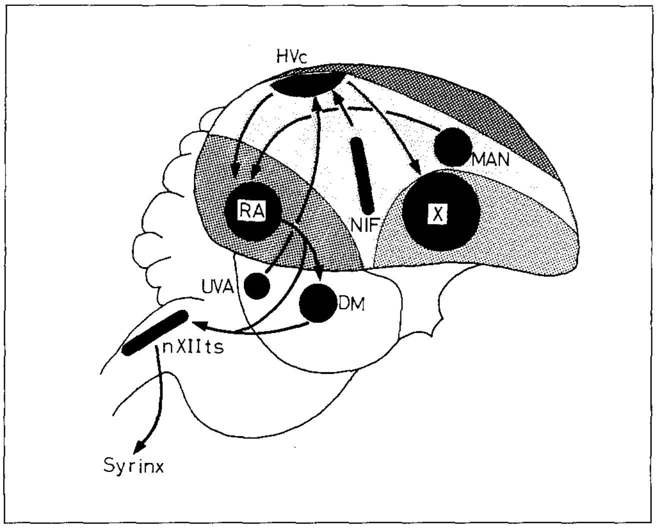

Fig. 2. A simplified diagram of the avian brain song system. The *arrows* show the connections between nuclei that are active during song production. *DM* nucleus dorsalis medialis, *HVc* nucleus hyperstriatum ventrale, pars caudale, *MAN* nucleus magnocellularis of the anterior neostriatum, *NIF* nucleus interfacialis, *RA* nucleus robustus archistriatalis, *UVA* nucleus uvaeformis, *X* area X, *nXIIts* nucleus hypoglossus, pars tracheosyringealis. The projection from nXIIts innervates the syrinx, the vocal organ. ▦ Hyperstriatum, ▨ archistriatum, ▒ lobus parolfactorius, ☐ neostriatum, DM + UVA: diencephalon, nXIIts: brainstem

put and complexity to that of the male [8]. A particularly clear example of the relationship between the size of the song nuclei and singing ability is seen by comparing eastern and western marshwrens: on the west coast of America marshwrens sing 2.6 to 2.7 times as many different songs as those in the east and, although they are 11 % lighter in body weight, their HVc and RA volumes are 41 and 21 % greater, respectively [9].

Further evidence for the importance of HVc and RA in determining singing ability comes from studying the effects of testosterone. Castration (i.e., the reduction of testosterone) reduces the amount of song output in zebra finches (review in [10]). Males have a larger percentage of androgen-accumulating neurons in these song nuclei than females [11]. In adult female canaries song can be induced by injecting testosterone, the song nuclei become enlarged and the structure of the neurons becomes more complex [4]. However, in adult zebra finch females testosterone has little, if any, effect [12].

Seasonal Singers

Most species, of which the zebra finch is one, are age-limited learners [13], i.e., they learn during a sensitive period early in life and their songs remain remarkably stable thereafter. Canaries are unusual in being non-age-limited learners which can modify their song repertoire each season [14]. In spring the testosterone level of adult male canaries is very high, they sing stereotyped songs and the HVc and RA volumes are about double the size of those in early autumn, when testosterone levels are low and their songs are as variable as those of juvenile males [15]. Within the song nuclei there are changes in both the number and spacing of the neurons which are under the joint control of hormonal and sensory stimulation: neuron spacing is affected by testosterone but for an increase in neuron number the bird must be able to hear song [16]. Clear evidence that the seasonal changes in song nuclei are not invariably associated with changes in hormone levels comes from studies on white-crowned sparrows. These birds breed seasonally (and are therefore subject to changes in levels of testosterone) but do not modify their songs each season and there are no seasonal changes in HVc and RA volume [17].

The plasticity of the song system in canaries, and probably in all species which alter their songs each season, is thought to be due to the production of new neurons throughout life which can replace older neurons, forming new neural circuits for song learning [18, 19]. Presumably, changes in the rate of neuron replacement could affect seasonal changes in song nuclei volume. Nottebohm suggests that the number of neurons in the brain might constrain the amount of song learning so that an updating of songs each season can only be achieved by those species in which the song neurons are seasonally replaced. In addition to seasonal changes in song, neurogenesis is thought to be required for song learning early in life. In zebra finches, for example, large numbers of new neurons are generated in juvenile males during the song learning phase but not in juvenile females which do not sing [20]. However, the exact role of neurogenesis is not fully resolved because some HVc neurons continue to be added to the brain of adult male zebra finches after song has fully crystallized [21].

Electrical Recordings from Song Control Neurons

In addition to the rather global correlations between the size of the song nuclei, hormones, and singing behavior, more direct evidence for the role of these song nuclei comes from electrical recordings. NIF is the first station where song-related patterns of neuronal firing can be observed [22], but most of the work has been done on HVc neurons. Katz and Gurney [23] were the first to unambiguously demonstrate auditory responses from HVc neurons. Some of these neurons are so finely tuned that they only fire in response to the bird's own song (see [24]). Some neurons in HVc, probably motor neurons, fire before or during song production whereas other, sensory neurons are inhibited while the bird is singing [25]. Margoliash [24] found that these song-specific neurons in HVc are more sensitive to frequency and amplitude modulation than to changes in length of autogenous song and suggests that they might be important in discriminating between the songs of neighbors and strange males during territory defense. In a closely related species, the white-throated sparrow, frequency is an important feature for individual recognition [26]. By experimentally altering certain features of song and playing back these tape-recorded songs to males at the edge of their territories, Brooks and Falls demonstrated that a 5 % decrease in frequency of the song elements or a 15 % decrease in frequency of the initial whistle is recognized by territorial males, whereas the same change in duration has no effect. One difficulty with Margoliash's hypothesis for individual recognition is in explaining how female zebra finches, and females of other species where HVc is very diminished, discriminate between different songs. Since females learn to discriminate between fathers, mates, and brothers on the basis of their songs (e.g., [27]), it seems likely that females process song differently from males. If this is the case, then the question becomes one of where in the brain these sex differences in song recognition lie. That these differences may not be restricted to HVc is suggested by Williams and Nottebohm's study [28]. They found that the syringeal motor neurons in male zebra finches fire in response to acoustic stimulation while those in females do not and suggest that this tracheosyringeal auditory response is important in song recognition.

Song Learning

For both age-limited and non-age-limited learners, song learning can be viewed as consisting of two parts: a sensory phase, during which sounds that are heard are stored; and a motor phase, when the young bird develops its own song by perfecting its motor output and matching this to sounds that it has heard previously. In some species such as the zebra finch the two phases appear to overlap. However, the two phases can occur at different times. For example, swamp sparrows produce songs consisting of sounds that they have heard several months earlier but have not practiced in the intervening period [29].

Neuroanatomical Studies of Song Learning

Most of the neuroanatomical studies of song learning have used zebra finches as the study species. Herrmann and Bischof [30] found that the song nuclei HVc and RA show delayed development compared to other nuclei in the brain and their development is correlated to some extent with the motor development of song, confirming the results Nottebohm had previously found for canaries [4]. HVc increases in neuron number and RA increases in both neuron number and the spacing of the neurons. In contrast, the volume of MAN rapidly declines until day 40 (see [31]) and lesions to this song nucleus disrupt song if they are performed before day 40 but have no effect on adult birds. Bottjer and Arnold [31] suggest that MAN is important for song learning during the sensory phase but that the learnt information is later transferred to other song nuclei, presumably HVc and RA, at about day 40. Based on multicellular recordings in HVc, where specific groups of neurons were found to show a preference for autogenous song over song from the father and other males with whom young white-crowned sparrows had been housed during song development, Margoliash [24] suggested that the response properties of HVc neurons are established after the sensory phase. He further suggested that the HVc might be involved in the motor phase by selecting which song elements a young male should include in his own repertoire in adulthood.

Hormonal Studies of Song Learning

The neuroanatomical studies of song development have been concerned primarily with young males. In addition to the obvious genetic differences, differentiation into male and female patterns of song control nuclei is determined by the presence of sex hormones [32]. Testosterone injections in newly hatched female zebra finch chicks result in the development of a male-like song system, with enlarged song nuclei volumes, a larger complexity of dendrites, an enhanced number of spines, and a greater amount of androgen receptors [7, 33]. Since these effects can be created by both testosterone and the female hormone estradiol it is not clear whether testosterone is metabolized to estradiol in the brain or whether males do have high estradiol titers during early development. Pohl-Apel and Sossinka [34] demonstrated that newly hatched zebra finch females injected with the female hormone estradiol developed male-like song which they sung when treated with testosterone as adults. Their song complexity was correlated with the volume of RA [35]. Estradiol followed by testosterone treatment induces neither cell growth nor singing in adult females which indicates that the masculinizing effects of estradiol are confined to a specific period early in development [33]. These findings show that the song system of newly hatched chicks is in a plastic state and that its differentiation is sensitive to sex hormones. However, the extent to which these hormones are involved in the sensory phase of song learning is uncertain. There are some correlations between the time of song acquisition, the crystallization of adult song and hormone levels [10, 36] but castration of male zebra finches between 9 and 17 days of age has no effect on song learning [12].
What the neurophysiological work does suggest is that MAN might be important for the memorization phase of song learning, whereas HVc (and possibly RA) might play a role during motor development. The large seasonal changes in the HVc and RA of canaries, and the modification of their existing neural circuits accompanying these changes, go someway towards explaining why canaries can modify their songs in adulthood, whereas age-limited learners cannot normally do so. Moreover, that neurons are constantly being born in the canary brain, and that newborn neurons can replace older ones, suggests that song learning by both juvenile and adult birds may depend on the availability of young neurons that can be used to build novel circuits.

Back to Behavior

Although these recent results help us to understand the mechanism of song learning there are several behavioral attributes of song learning which have yet to be examined in terms of neurophysiology. One important ethological finding is that social interactions are of great importance for song learning. In many species birds require a visual stimulus in addition to song if they are to develop a normal song [37]. Interactions between an adult song tutor and a young pupil may govern what the pupil learns, or at least what he sings, as when young males learn from the father in preference to other males within earshot [38], or when young males choose tutors who are aggressive towards them [39], or those with high rank in the local community [40]. At present, virtually nothing is known about their neurophysiological mechanisms, other than that there are both visual and vocal projections to the HVc [41] and that there appear to be certain areas in the forebrain which are specifically activated in arousing situations and which are probably involved in song control [42]. Although merely speculative, these areas might play a role during song learning in determining which stimuli are likely to be memorized. Determining how motivation and arousal influence singing behavior and song learning at the neurophysiological level may be a very difficult task because many unknown components could be involved. Nevertheless, the song control system of birds may be the most promising starting point for such investigations. This is but one example of how birdsong can serve as a model system for understanding the interplay between behavior and neurophysiological events in the brain.

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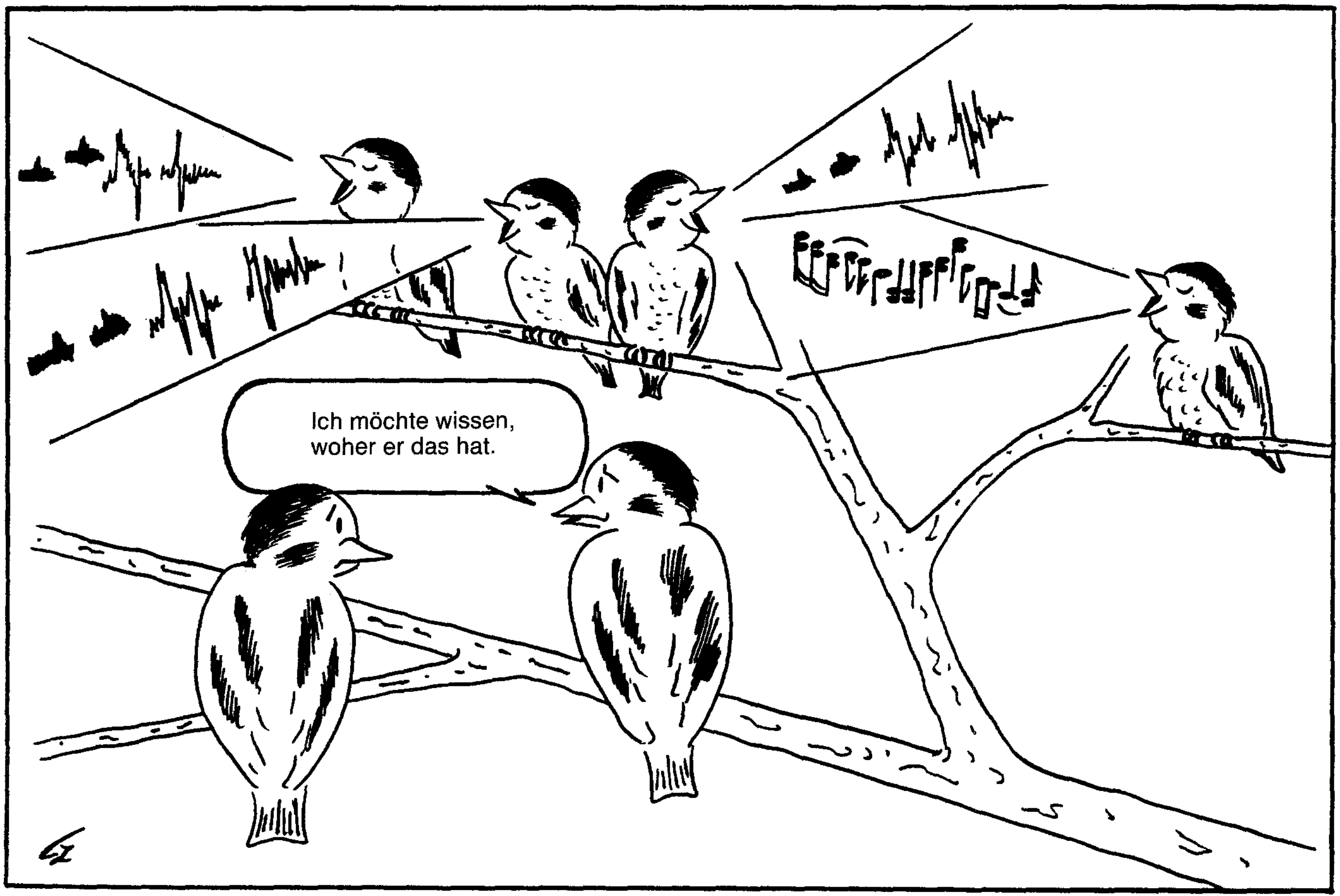
Mechanisms of avian song learning



J. Czichos