

## **Bird brains and songs: neural mechanisms of birdsong perception and memory**

JOHAN J. BOLHUIS<sup>1,\*</sup>, HIROKO EDA-FUJIWARA<sup>2</sup>

<sup>1</sup> *Behavioural Biology, Utrecht University, Padualaan 14, PO Box 80086, 3508 TB Utrecht, The Netherlands*

<sup>2</sup> *Department of Chemical and Biological Sciences, Japan Women's University, Tokyo, Japan*

**Abstract**—The males of songbirds and parrots learn their songs from a tutor. Until recently it was thought that in songbirds, brain nuclei in the so-called ‘song system’ were involved in song learning, in addition to their role in song perception and production. Experiments involving measurement of the expression of immediate early genes (IEG) showed that exposure to song leads to activation of cells in brain regions outside the song system, notably the caudomedial neostriatum (NCM) and the caudomedial hyperstriatum ventrale (CMHV), suggesting that these regions are involved in auditory perception. In addition, neuronal activation in the NCM correlates with the number of song elements that a male has learned from its tutor, suggesting that NCM may be (part of) the neural substrate for stored tutor song. Songbird females do not usually sing, but nevertheless they can learn the characteristics of tutor song, and they can develop a perceptual preference for tutor song over novel song. When exposed to male song, female songbirds and parrots show increased IEG expression in NCM and CMHV that is related to song complexity. In addition, the IEG response in the NCM (and perhaps in the CMHV) of female songbirds may also be related to song learning.

*Keywords:* brain; Fos; memory; song; songbirds; ZENK.

### **AVIAN PARADIGMS IN THE SEARCH FOR THE ENGRAM**

An important question in the search for the neural mechanisms of learning and memory can be summarised in the following way: Is the neural substrate for information storage localised to particular regions of the brain? It has been argued that such a localisation of function is essential for a successful analysis of the neural mechanisms of learning and memory (e.g., Horn, 2000; King and Thompson, 2000). Roughly, the argument is that, in order to be able to analyse the neuronal correlates of learning and memory, one has to know where in the brain to look for

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\*Corresponding author. E-mail: [j.j.bolhuis@bio.uu.nl](mailto:j.j.bolhuis@bio.uu.nl)

these correlates. A well known attempt at localisation of function was that of Karl Lashley (1950), who was ‘in search of the engram’, the engram being the ‘mark’ or ‘trace’ left in the brain by the learning experience. Lashley conducted an extensive series of experiments involving lesions to and incisions of regions of the cerebral cortex of rats, testing the effects of these manipulations on maze learning. His main conclusion was that the effects of these lesions were not related to their location, but to their extent. Lashley famously concluded that “I sometimes feel, in reviewing the evidence on the localization of the memory trace, that the necessary conclusion is that learning just is not possible” (Lashley, 1950, pp. 477-478). Subsequent attempts to localise brain regions where memory is stored have been more successful, mainly because they have employed a number of different neurobiological techniques, most of which were not available in Lashley’s day.

Arguably the most advanced avian paradigm in the search for the neural substrates of learning and memory is that of filial imprinting in the domestic chick (*Gallus gallus*), particularly the work of Gabriel Horn and his collaborators (Horn, 1985, 1998, 2000; Horn et al., 2001). Filial imprinting is the learning process through which social behaviour of the young animal becomes limited to a particular object or class of objects, as a result of exposure to that object (Bolhuis, 1991; Bolhuis and Honey, 1998). Horn and his collaborators have found that there is considerable localisation of the neural substrate of imprinting memory. In particular, they found that a restricted region of the chick forebrain (the intermediate and medial hyperstriatum ventrale, or IMHV) is crucially involved in imprinting, and a likely substrate for the memory of the imprinting stimulus. Neuronal activation in the IMHV correlates significantly with the strength of filial imprinting (McCabe and Horn, 1994). Also, imprinting leads to changes in synaptic morphology and to an increase in the number of post-synaptic NMDA glutamate receptors. Interestingly, a recent electrophysiological analysis of the chick forebrain during imprinting revealed that the changes in responsiveness of neurons in the IMHV are surprisingly non-linear (Horn et al., 2001). These results challenge conventional suggestions, derived from Hebb’s (1949) original proposal, that learning involves a monotonic increase in synaptic strength to a stable asymptote (see Horn, 2000, for further discussion).

More recently, the neural mechanisms of sexual imprinting were investigated in zebra finches (*Taeniopygia guttata castanotis*), by Bischof and his collaborators (Sadananda and Bischof, 2002; Bischof, 2003). Sexual imprinting involves the formation of mating preferences that are expressed when the animals are sexually mature (Bolhuis, 1991; Bischof, 1994). The results of the neural analysis of sexual imprinting suggest that a number of forebrain regions may be involved in the learning process, including medial and lateral parts of the hyperstriatum ventrale and of the neostriatum (see Bischof, 2003, for review).

Song learning in songbirds is another prominent paradigm for the study of the neural mechanisms of behaviour. Considerable advances were made with regard to the analysis of the neural substrates of the perception, production, and the

motor learning of song (e.g., Nottebohm et al., 1990; DeVoogd, 1994; Nottebohm, 2000). However, despite intensive research in this area, our knowledge of the neural substrate of tutor song memory is limited. Nottebohm, one of the leading investigators in this field, stated that “We do not know how and where learned sounds are stored” (Nottebohm et al., 1990, p. 121; cf. Nottebohm, 2000). Recent studies involving analysis of the expression of immediate early genes (IEG) in the zebra finch forebrain in response to song (reviewed in Clayton, 2000; Mello, 2002) have given new impetus to the study of the neural substrate of the perception and learning of birdsong. These recent developments are the subject of the remainder of this review.

## BIRD SONG LEARNING

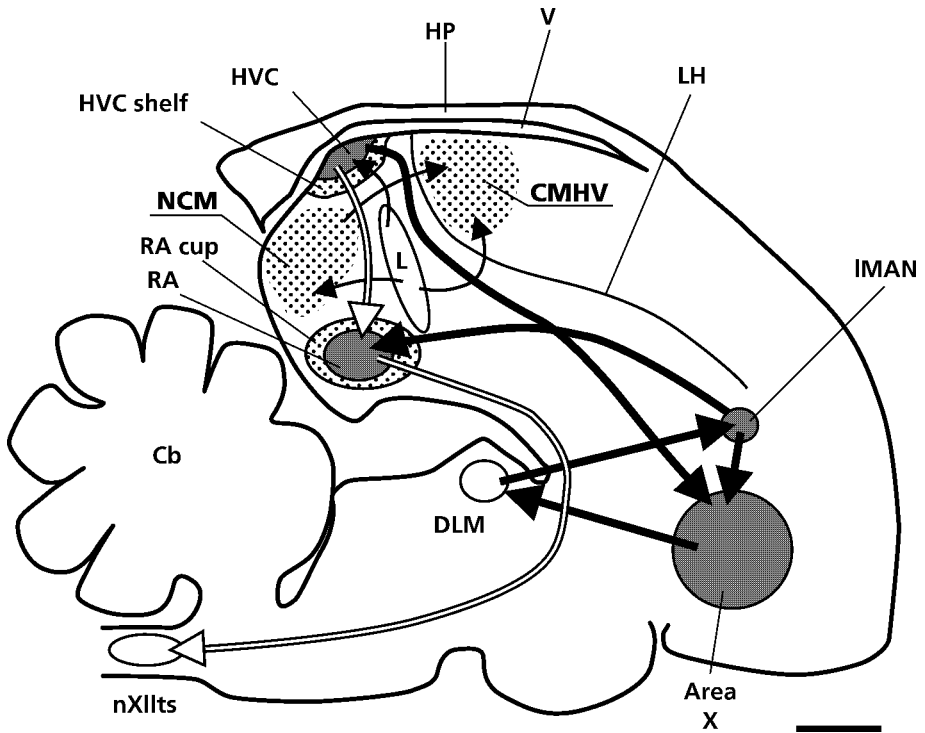
Of all avian species approximately half are songbirds (*Passeriformes: Oscines*). Usually, young songbird males learn their song from an adult tutor. Song learning is also found in two other avian taxa: parrots (*psittaciformes*, Gahr, 2000) and hummingbirds (*trochiliformes*, Jarvis et al., 2000). There are two phases in songbird song learning: a memory phase when the auditory information of the tutor song is stored (usually in a sensitive period), and a sensorimotor phase later in life, when the bird's own vocal output is compared with the stored information (Marler, 1987, 1991; Nottebohm et al., 1990; DeVoogd, 1994). A much-used metaphor for the mechanism underlying song acquisition is that of the template (Konishi, 1965; Marler, 1976), which is essentially the central representation of song. If young swamp sparrows (*Melospiza georgiana*) or song sparrows (*Melospiza melodia*) are exposed to equal numbers of taped songs from their own and from the other species, their adult song will consist mainly (song sparrow) or entirely (swamp sparrow) of copies of conspecific songs (Marler and Peters, 1989). Thus there appears to be some bias in what the birds will learn. This is thought to reflect the presence of a rough template in the animal, before it has heard song. The young bird is thought to learn songs that match its template and reject songs that do not. The template concept has been challenged, and it has become apparent that songbirds can also learn songs of other species, especially if they can interact socially with a tutor of the other species (Baptista and Petrinovich, 1984). Nevertheless, the template remains a useful metaphor. When the bird learns more songs the original rough template is refined. Later, when the bird starts to sing, its song output (known as sub-song) is not yet stereotyped and does not resemble the tutor song very well. During this sensorimotor phase the bird matches its own output with the refined template, and eventually produces crystallised adult song. Among songbirds a distinction can be made between ‘age-limited learners’ and ‘open-ended learners’ (Marler, 1987). The former (e.g., the zebra finch and white crowned sparrow, *Zonotrichia leucophrys*) do not alter their songs when they are adult. The latter (e.g., the canary, *Serinus canaria*), continue to alter their songs when adult, usually for every new breeding season.

## **SINGING AND THE BRAIN: IDENTIFICATION OF THE ‘SONG SYSTEM’**

Until recently it was thought that two forebrain pathways connecting a number of ‘song control nuclei’ (Nottebohm et al., 1991) comprise the neural substrate for bird song (see fig. 1). Together, the two pathways are dubbed the ‘song control system’ (Clayton, 2000) or simply the ‘song system’ (e.g., Nottebohm, 2000).

Evidence for the involvement of the ‘song system’ in song came from a series of neuroanatomical and lesion studies (Nottebohm et al., 1976, 1990; DeVoogd, 1994). The caudal pathway, including the High Vocal Centre (HVC) and the robust nucleus of the archistriatum (RA), was considered to be involved in the production of song. Lesions to nuclei in this pathway, or to any of its connections, result in immediate, profound and irreversible deficits in song in adult birds (Nottebohm et al., 1976; DeVoogd, 1994; Nottebohm, 2000). The rostral pathway, including HVC, the lateral part of the magnocellular nucleus of the neostriatum (IMAN) and Area X, was thought to play a role in song learning. This suggestion was supported by the finding that bilateral lesions to IMAN or Area X disrupt song acquisition, but have little effect on crystallised song in adults (Bottjer et al., 1984). In addition, lesion studies suggested that the HVC is involved in song preferences in female canaries (Brenowitz, 1991), reinforcing the suggestion of a role for this structure in auditory information storage. More recently, MacDougall-Shackleton et al. (1998) failed to find an effect of HVC lesions on female song preferences in a different species, the zebra finch. Lesions to the caudal hyperstriatum ventrale (CMHV, see fig. 1), originally included as control lesions, disrupted female preference for conspecific songs. The disruptive effects of lesions to a ‘song control nucleus’, even when these effects are limited to juveniles, do not establish the role of that nucleus in auditory learning and memory, as the lesions might have damaged efferent projections from that nucleus (Bolhuis and Macphail, 2001).

Electrophysiological investigations revealed that there are neurons in nuclei in the song system (in particular, IMAN, Area X, HVC and RA) that respond selectively to conspecific song (Solis et al., 2000). Neurons in these regions respond better to the bird’s own song (BOS) than to the tutor song (Margoliash and Konishi, 1985; Solis et al., 2000) or to the song of another conspecific (Margoliash, 1986). Some neurons in IMAN and Area X respond equally well to BOS and tutor song, whilst a small proportion respond more to the tutor song (Solis et al., 2000). The findings of Solis et al. (2000) are consistent with there being a neural representation of the tutor song in these nuclei. Neurons in the song system nuclei HVC, IMAN, Area X and RA are activated when the bird is singing (Margoliash, 1986; DeVoogd, 1994; Solis et al., 2000; Brainard and Doupe, 2002). In addition, there is increased expression of immediate early genes (IEGs, see below) in these nuclei when the bird is singing, but not when it hears song, including tutor song (Jarvis and Nottebohm, 1997; Bolhuis et al., 2000). The results suggest that these brain nuclei are either involved in song production only, or in the auditory feedback of songs that occurs during the sensorimotor phase of song learning, and that can also occur in adult songbirds (Solis et al., 2000). Despite the obvious involvement of these forebrain pathways



**Figure 1.** A schematic diagram of a composite of parasagittal sections of the zebra finch brain. Drawing gives approximate positions of nuclei and brain regions. A series of lesion studies in adult and young songbirds led to the distinction between a caudal pathway (thick black arrows), considered to be involved in the production of song, and a rostral pathway (open white arrows), thought to play a role in song acquisition (e.g., Nottebohm et al., 1990; DeVoogd, 1994). Area X, DLM and IMAN are also part of the anterior forebrain pathway (AFP; e.g., Solis et al., 2000; Brainard and Doupe, 2002). Thin black arrows indicate known connections between Field L, an auditory projection region, and some other forebrain regions. The dark grey shaded nuclei show significantly enhanced expression of immediate early genes (IEGs) when the bird is singing (e.g., Jarvis and Nottebohm, 1997; Mello and Ribeiro, 1998). Stippled areas represent brain regions that show increased IEG expression when the bird hears song, including tutor song (e.g., Mello et al., 1992; Jarvis and Nottebohm, 1997; Bolhuis et al., 2000). Abbreviations: HVC, High Vocal Centre; NCM, caudal medial neostriatum; CMHV, caudal medial hyperstriatum ventrale; L, Field L; IMAN, lateral part of the magnocellular nucleus of the neostriatum; RA, robust nucleus of the archistriatum; HP, Hippocampus; LH, lamina hyperstriatica; Cb, Cerebellum; DLM, medial part of the dorsolateral thalamic nucleus; nXIIts, tracheosyringeal portion of the nucleus hypoglossus; V, ventricle. (Scale bar = 1 mm.) (Based on Jarvis and Nottebohm, 1997; Brainard and Doupe, 2000, 2002; Clayton, 2000; Bolhuis and Macphail, 2001; Mello, 2002.)

in song, the conclusion by Nottebohm (2000, p. 75) that “We do not know, yet, where in [the ascending auditory pathway] reside the auditory memories that are eventually imitated” is still appropriate (see also Bolhuis et al., 2000; Solis et al., 2000; Bolhuis and Macphail, 2001).

## SONG PERCEPTION AND IMMEDIATE EARLY GENE EXPRESSION IN MALES AND FEMALES

In recent studies, the expression of certain immediate early genes (IEGs) was analysed in the forebrain of male songbirds that had been exposed to conspecific song (Mello et al., 1992; Mello and Clayton, 1994; Jarvis and Nottebohm, 1997; Bolhuis et al., 2000, 2001a). Expression of these IEGs or their protein products is thought to be a reflection of neuronal activation (Sagar et al., 1988). Surprisingly, it was found that exposure to song does not lead to neuronal activation in nuclei in the traditional 'song system', but in different forebrain regions, particularly the caudal part of the medial neostriatum (NCM) and the caudal part of the medial hyperstriatum ventrale (CMHV, see fig. 1). Song production by itself does lead to IEG expression in nuclei in the 'song system' (Jarvis and Nottebohm, 1997; Kimpo and Doupe, 1997). Thus there is a dissociation between forebrain regions that are activated when the bird hears song, and when it is singing itself (Jarvis and Nottebohm, 1997).

The songs of male birds play an important role in attracting females and in stimulating female reproductive behaviour and physiology. In many species, females are able to discriminate conspecific from heterospecific song, and also between song variations within their own species (Searcy, 1992a). Females are important receivers in song communication, as mistakes in song recognition lead to suboptimal mate choice. However, the brain regions involved in song perception in females have only recently begun to be investigated.

In one of the first studies, Brenowitz (1991) tested whether nuclei in the song system play a role in song perception in females. Female songbirds have a song system, even though normally they do not sing. In female canaries, lesions of the HVC result in the loss of their ability to discriminate conspecific from heterospecific song. This result was confirmed in later studies (Del Negro et al., 1998; Halle et al., 2002), which also showed that lesioning the HVC of female canaries results in their failure to discriminate between different conspecific songs. A subsequent electrophysiological study of the HVC in female canaries showed that sexually stimulating songs of male canaries evoke different neuronal responses from non-sexually stimulating songs (Del Negro et al., 2000). Furthermore, the volume of HVC in female canaries is positively correlated with both the amount of sexual display in response to male song playback and the ability to discriminate between male songs of different quality (Leitner and Catchpole, 2002). These canary studies suggest that the HVC is involved in female perception of male song. In species other than the canary, there is preliminary evidence that the nucleus IMAN may be involved in female perception of song. DeVoogd et al. (1996) measured the volume of IMAN in several species of European *Acrocephalus* warblers and found that IMAN volume in females is correlated with conspecific male song complexity. In a within-species comparison of female cowbirds (*Molothrus ater*), the volume of IMAN was positively correlated with selectivity of sexual displays to male song playback (Hamilton et al., 1997).

The results of studies in the zebra finch, on the other hand, suggest a role in song perception of females for brain regions outside the conventional song system. Zebra finch songs include sound units that consist of two sub-units. The first sub-unit has a harmonic-like structure. Some neurons in male IMAN are sensitive to particular combinations of harmonics, while others respond only to a combination of the first and the second sub-units of the conspecific song (Maekawa and Uno, 1996). In contrast, IMAN neurons in female zebra finches respond less selectively to auditory stimuli (Uno, 1997). Uno (1997) suggested that such selective neurons as those found in males occur in other brain areas of females, because females are quite capable of making subtle behavioural discrimination between male songs (Cynx et al., 1990). Consistent with this suggestion, in female zebra finches, lesions of the CMHV, but not of the HVC, disrupt their ability to discriminate conspecific from heterospecific song (MacDougall-Shackleton et al., 1998). This lesion study in female zebra finches suggests a role in song perception for the CMHV, a brain region outside the 'song system'.

In male songbirds, as mentioned above, IEG studies revealed that the NCM and the CMHV are involved in song perception. Similarly, IEG studies of female songbirds suggest a role in song perception for brain regions outside the conventional song system; the NCM in starlings (*Sturnus vulgaris*; Duffy et al., 1999; Gentner et al., 2001) and canaries (Ribeiro et al., 1998), and the NCM and the CMHV in zebra finches (Bolhuis et al., 2001b; Bailey et al., 2002) and white-crowned sparrows (Maney et al., 2003). The NCM is conserved widely among bird species, at least among the three taxonomic groups in which males show song learning: songbirds, parrots and hummingbirds (Jarvis et al., 2000). Eda-Fujiwara et al. (2003) reported that IEG expression varied with male song complexity in the NCM (and to a lesser extent in the CMHV) of female budgerigars (*Melopsittacus undulatus*, a parrot), suggesting that the NCM (and perhaps the CMHV) is implicated in song perceptual processes such as complexity discrimination in female budgerigars. Thus, the NCM may play a role in song perception of females among a wide range of bird species.

## SONG COMPLEXITY AND NEURONAL ACTIVATION IN FEMALES

Birdsong is among the most acoustically complex of all non-human vocal communication signals, and a considerable number of studies have shown that females respond preferentially to more complex song (Kroodsma, 1976; Catchpole and Slater, 1995; Lampe and Saetre, 1995; Hasselquist et al., 1996; Mountjoy and Lemon, 1996; Searcy and Yasukawa, 1996; Gentner and Hulse, 2000). This is particularly important in the context of sexual selection by female choice, where females select males with more complex songs and drive the evolution of song complexity (Catchpole and Slater, 1995; Searcy and Yasukawa, 1996; Okanoya, 2000). One way to measure the complexity of birdsong is to determine what is known as syllable repertoire size, where the number of different syllables (discrete elements) of each male's song is counted. Using this method in a comparative study, DeVogel

et al. (1996) found that the volume of the IMAN in female European warbler species correlates with male song complexity as measured by syllable repertoire size. This might mean that IMAN is involved in some way in complexity discrimination in females.

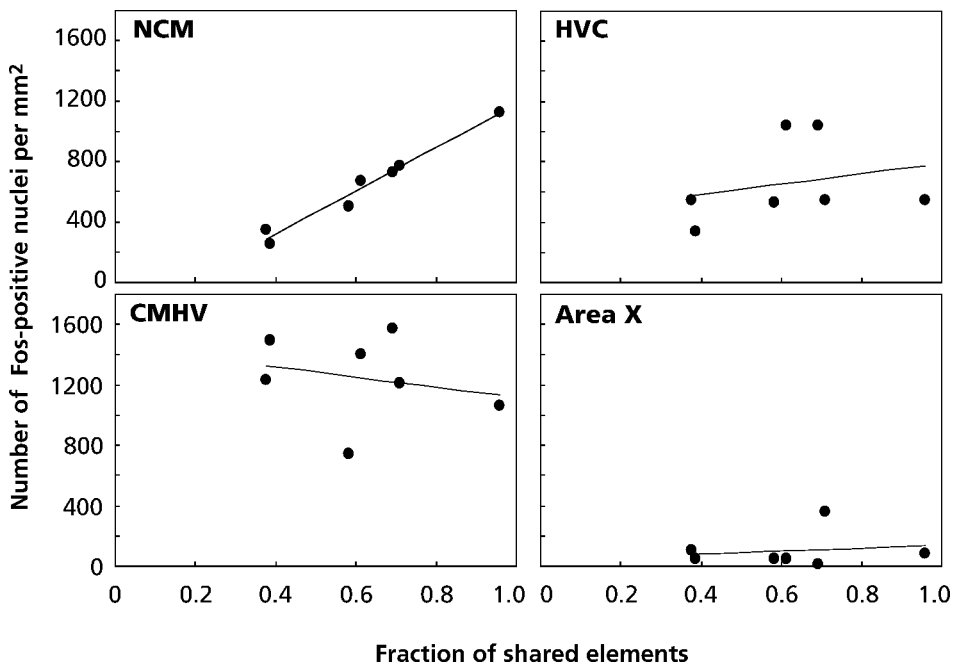
In several species of birds, females readily habituate to the repeated presentation of a single song (Searcy, 1992b). Thus, Searcy (1992b) proposed a habituation mechanism as the neural basis of female preference for song complexity. It has been speculated that a monotonous signal with a small repertoire size leads to habituation of the neuronal response to the signal in the central nervous system of females. In line with this argument, the neuronal responses to male song with a larger repertoire size would be expected to be less susceptible to habituation in the central nervous system of females. Mello et al. (1995) investigated mRNA expression of the IEG known as *ZENK* in the NCM of male zebra finches. These authors showed that *ZENK* mRNA expression in the NCM increases with repetition of a single song over the first 30 min of presentation. The level of expression then returns to baseline levels despite continued stimulation with the same song. Consistent with Searcy's (1992b) hypothesis, Eda-Fujiwara et al. (2003) showed that Zenk protein expression in the NCM (and to a lesser extent in the CMHV) correlated significantly and positively with the number of different syllables to which female budgerigars were exposed (fig. 2). Such correlations were not found for the hippocampus. These results suggest that the NCM is involved in the perception of song complexity in female birds.

Female starlings show greater Zenk expression in the NCM when they are exposed to 'long song', compared to 'short song' (Gentner et al., 2001). Song length is closely correlated with another acoustic feature, motif repertoire size and, in fact, motif repertoire size for the long songs used in the study of Gentner et al. (2001) was substantially larger than that for the short songs. Thus, it may be that longer songs are more effective than shorter songs because they are more complex in acoustic structure and, consequently are less likely to lead to neuronal habituation. This interpretation, as in the case of Eda-Fujiwara et al. (2003), is consistent with Searcy's (1992b) hypothesis. Recently, Sockman et al. (2002) exposed female starlings to 1 week of long-bout or short-bout song experience and then examined IEG response of the NCM and the CMHV of the females to a novel long-bout or novel short-bout song stimulus. Consistent with their previous study (Gentner et al., 2001) these authors found that, overall, long-bout songs elicited a significantly greater Zenk response than short-bout songs, in both the NCM and the CMHV. In addition, Zenk expression in response to long-bout songs was greater in females with long-bout experience than in females with short-bout experience. Sockman et al. (2002) reasoned that the experience-dependent NCM and CMHV response to mate-choice cues was a consequence not merely of habituation but of "cognitive processes beyond simple habituation". Although these results could still be explained in part as being the neural consequence of habituation to repeated exposure to song stimuli and dishabituation to novel song stimuli, we agree with



representation of tutor song is present somewhere in the brain” and that “it seems (...) plausible that such a representation lies elsewhere in the brain, perhaps in the earlier high-level auditory areas that also process songs of conspecifics” (Solis et al., 2000, p. 11839). These authors refer to the same regions that Nottebohm (2000) suggested were involved. Soon after the discovery of the IEG response to song in NCM and CMHV (Mello et al., 1992), Bolhuis (1994, p. 20) stated that “(...) there is an intriguing suggestion that the same brain region [i.e. intermediate and caudal parts of the medial hyperstriatum ventrale] may be involved in the neural substrate for the perceptual mechanisms involved in visual imprinting and in song learning”, and “Furthermore, it is important to establish whether lesions to the medial hyperstriatum (and the medial caudal neostriatum) in songbirds affect the learning and/or production of song”.

The suggestion that NCM and CMHV may indeed be (part of) the neural substrate for the storage of the tutor song has received some support from recent studies demonstrating a significant correlation between neuronal activation in the NCM and the strength of song learning (Bolhuis et al., 2000, 2001a). In particular, Bolhuis et al. (2000) investigated the expression of two IEG protein products, Fos and Zenk, in the forebrain of zebra finch males that had been tape-tutored with an adult zebra finch song. When adult, the birds were re-exposed to their tutor song (or not re-exposed: controls). There was a significant increase in expression of both Fos and Zenk protein in the experimental birds compared to the control birds in the NCM and in the CMHV, but not in two nuclei in the conventional song system, HVC and Area X. Furthermore, in the experimental birds, but not in the controls, there was a significant positive correlation between the number of song elements copied and the expression of both Fos and Zenk protein in the NCM, but not in the CMHV. The males in this study were reared in social isolation and exposed to tape-recorded songs. It has been suggested (Marler and Doupe, 2000) that such social isolation may lead to a depression of IEG expression in the brain. Indeed, Jin and Clayton (1997) found that 30-40-day-old zebra finch males reared in social isolation showed no significant increase of *ZENK* gene expression in the NCM after exposure to adult song, in contrast to birds of the same age reared with their mother and siblings. To investigate the possible effect of isolation rearing on IEG expression, in a subsequent study, zebra finch males were reared with a live tutor during the sensitive period for song learning. When, as adults, they were re-exposed to the tutor song, the males showed increased expression of Fos in the NCM and the CMHV, compared to expression in two nuclei in the song system, HVC and Area X. As in the study by Bolhuis et al. (2000), the strength of the Fos response in the NCM (but not in the other three regions) correlated significantly and positively with the number of song elements that the birds had copied from the tutor song (fig. 3). Thus, the findings reported by Bolhuis et al. (2000) cannot be explained as an artefact of social isolation. Rather, like their tape-tutored counterparts, socially-tutored zebra finch males show localised neural activation in response to tutor song exposure that correlates with the strength of song learning.



**Figure 3.** Mean number of Fos-positive cell nuclei ( $\pm$ S.E.M.) per  $\text{mm}^2$  in the NCM, CMHV, HVC and Area X of live-tutored zebra finch males re-exposed to the tutor song. (From Bolhuis et al., 2001a, with permission.)

The suggestion that the NCM may be (part of) the neural substrate for learned tutor song (Bolhuis et al., 2000) receives some support from studies that investigated habituation-like processes involving this brain region. Mello et al. (1995) found that repeated exposure to a song leads to a decrease in the *ZENK* response to that song, suggesting that habituation occurs in the NCM. In addition, using multi-unit electrophysiological recording, Chew et al. (1996) reported decreased responding of units in the NCM after repeated exposure to the same song. Chew et al. (1996) conclude from their results that it is likely that “the NCM is specialized for remembering the calls and songs of many individual conspecifics”. As discussed above, Gentner et al. (2001) and Sockman et al. (2002) suggested that the variation in the IEG response in the NCM and the CMHV of female starlings, related to song complexity, could be interpreted in terms of neuronal habituation. Bolhuis et al. (2000) have argued that the waning of neural response in the NCM to repeated presentations of the same song is consistent with a role for this region in the detection of stimulus familiarity, which is an important aspect of recognition memory (Brown, 2000). As we have seen, the experience-dependent effects of exposure to song in the NCM of female starlings, as reported by Sockman et al. (2002), may require an explanation that goes beyond habituation. Given that *ZenK* expression in the study by Sockman et al. (2002) is not only experience-dependent, but also stimulus-dependent, information processing by the NCM appears to be

complex. Previously, Bolhuis et al. (2001a) reported experience-dependent IEG expression in the NCM of male zebra finches, whilst the CMHV of these birds showed stimulus-dependent neuronal activation in the form of a significant positive correlation between the length of the tutor song (i.e. the number of elements in the song) to which the animals were exposed and Fos expression in the CMHV. On the other hand, Eda-Fujiwara et al. (2003) found that Zenk expression in the NCM, and to a lesser extent in the CMHV, of female budgerigars was related to song complexity. It is not known whether IEG expression in either of these brain regions in the budgerigar is also related to song learning. The recent findings of Sockman et al. (2002), combined with other results, seem to indicate that the NCM and the CMHV are involved both in the processing of perceptual information concerning song complexity and in storage of song memory. This is what Sockman et al. (2002) seem to suggest when they state that the NCM and the CMHV form “a neural system that can adjust the criteria for stimulus relevance based on experience” (p. 2484).

It is important to establish the nature of the putative representation of auditory memory in the NCM (cf. Marler and Doupe, 2000). We do not yet know whether the findings by Bolhuis et al. (2000, 2001a) reflect a neuronal response to the tutor song, or to the bird's own song (BOS). Our results are insufficient to resolve this issue as the more a male has copied from the tutor song, the more this tutor song will resemble BOS. Thus, the increasing IEG expression with degree of song copying could also reflect a neuronal response to songs that increasingly resemble BOS. Kimpo and Doupe (1997) did not find Fos expression in NCM after exposure of zebra finch males to BOS. However, these authors did not find Fos expression in the NCM after exposure to conspecific song either. This negative result is inconsistent with similar experiments measuring IEG expression using *in situ* hybridisation or protein immunocytochemistry (Mello et al., 1992; Mello and Clayton, 1994; Jarvis and Nottebohm, 1997; Bolhuis et al., 2000, 2001a) and may have been due to the specific antibody used (see Bolhuis et al., 2001a, for discussion). In contrast, E.R. Jarvis (pers. comm.) has found that exposure to BOS induces levels of *ZENK* expression in NCM and CMHV that are similar to those after exposure to unfamiliar conspecific song, but it is not known how this relates to *ZENK* expression after exposure to tutor song. Chew et al. (1996) measured multi-unit responses in the NCM during exposure of awake zebra finches to different stimuli, including BOS, unfamiliar conspecific song or canary song. The response to BOS appears to have been greater than to canary song, and smaller (but not significantly so) than to conspecific song. However, in this study 28 female and only five male zebra finches were used. Of course, only males could be exposed to BOS, as females do not sing. As, according to Chew et al. (1996), responses to conspecific songs tended to be slightly larger in males than in females, it is not clear whether responsiveness to BOS in males was actually significantly smaller than to conspecific song. Unfortunately, the males in this study were not exposed to tutor song.

The issue of the nature of the representation in the NCM might be resolved by investigating neuronal activation in females in response to song. Females can learn the characteristics of male tutor songs, for which they show a preference in subsequent tests (Riebel, 2000). In fact, the mean song preference of zebra finch females measured in operant tests is not significantly different from that of their male counterparts (Riebel et al., 2002). Consistent with these results, Bolhuis et al. (2001b) found that tape-tutored female zebra finches showed a significantly greater preference for the tutor song in an operant task than untutored females. In addition, re-exposure to the tutor song led to a significant increase in IEG expression in the NCM and the CMHV, but not in a number of other forebrain regions. IEG expression in the NCM and the CMHV was greatest in the trained females. These preliminary results implicate the NCM and the CMHV in female song learning, and they are consistent with a role for the NCM in the neural representation of tutor song memory.

## CONCLUSION

Bird song learning has become a prominent model system for the study of the neural mechanisms of learning and memory. Although enormous progress has been made in the identification and analysis of brain regions that are involved in song, our knowledge of the neural substrate of tutor song memory is limited (Nottebohm, 2000). The identification of brain regions (NCM and CMHV) outside the conventional 'song system' that are activated when exposed to (tutor) song (Mello et al., 1992; Bolhuis et al., 2000; Clayton, 2000) offers a new perspective on this problem. Recent evidence suggests involvement of these regions in song perception in females as well as males, but further research is necessary to elucidate the exact role of these regions in auditory perception. In addition, future research efforts need to focus on a possible role for the NCM as (part of) the neural substrate for tutor song memory. The suggested role of the NCM in tutor song memory (Bolhuis et al., 2000, 2001a) needs to be confirmed using different experimental techniques. If this research confirms the role of NCM (and possibly CMHV) in song memory, research efforts can be directed at unravelling the cellular and molecular mechanisms underlying memory storage.

## REFERENCES

- Bailey, D.J., Rosebush, J.C. & Wade, J. (2002) The hippocampus and caudomedial neostriatum show selective responsiveness to conspecific song in the female zebra finch. *J. Neurobiol.*, *52*, 43-51.
- Baptista, L.F. & Petrinovich, L. (1984) Social interaction, sensitive phases and the song template hypothesis in the white-crowned sparrow. *Anim. Behav.*, *34*, 1359-1371.
- Bischof, H.-J. (1994) Sexual imprinting as a two-stage process. In: J.A. Hogan & J.J. Bolhuis (Eds.), *Causal Mechanisms of Behavioural Development*, pp. 82-97. Cambridge University Press, Cambridge.

- Bischof, H.-J. (2003) Neural mechanisms of sexual imprinting. *Anim. Biol.*, 53, 89-112.
- Bolhuis, J.J. (1991). Mechanisms of avian imprinting: A review. *Biol. Rev.*, 66, 303-345.
- Bolhuis, J.J. (1994) Neurobiological analyses of behavioural mechanisms in development. In: J.A. Hogan & J.J. Bolhuis (Eds.), *Causal Mechanisms of Behavioural Development*, pp. 16-46. Cambridge University Press, Cambridge.
- Bolhuis, J.J. & Honey, R.C. (1998) Imprinting, learning, and development: from behaviour to brain and back. *Trends Neurosci.*, 21, 306-311.
- Bolhuis, J.J., Zijlstra, G.G.O., Den Boer-Visser, A.M. & Van der Zee, E.A. (2000) Localized neuronal activation in the zebra finch brain is related to the strength of song learning. *Proc. Natl. Acad. Sci. USA*, 97, 2282-2285.
- Bolhuis, J.J., Hetebrij, E., Den Boer-Visser, A.M., De Groot, J.H. & Zijlstra, G.G.O. (2001a) Localized immediate early gene expression related to the strength of song learning in socially reared zebra finches. *Eur. J. Neurosci.*, 13, 2165-2170.
- Bolhuis, J.J. & Macphail, E.M. (2001) A critique of the neuroecology of learning and memory. *Trends Cogn. Sci.*, 5, 426-433.
- Bolhuis, J.J., Riebel, K. & Den Boer-Visser, A.M. (2001b) Song preference and localized immediate early gene expression in the forebrain of female zebra finches. *Behav. Pharmacol.*, 12 (Suppl. 1), S11.
- Bottjer, S.W., Miesner, E.A. & Arnold, A.P. (1984) Forebrain lesions disrupt development but not maintenance of song in passerine birds. *Science*, 224, 901-903.
- Brainard, M.S. & Doupe, A.J. (2000) Auditory feedback in learning and maintenance of vocal behaviour. *Nature Rev. Neurosci.*, 1, 31-40.
- Brainard, M.S. & Doupe, A.J. (2002) What songbirds teach us about learning. *Nature*, 417, 351-358.
- Brenowitz, E.A. (1991) Altered perception of species-specific song by female birds after lesions of a forebrain nucleus. *Science*, 251, 303-304.
- Brown, M.W. (2000) Neuronal correlates of recognition memory. In: J.J. Bolhuis (Ed.), *Brain, Perception, Memory. Advances in Cognitive Neuroscience*, pp. 185-208. Oxford University Press, Oxford.
- Catchpole, C.K. & Slater, P.J.B. (1995) *Bird Song: Biological Themes and Variations*. Cambridge University Press, Cambridge.
- Chew, S.J., Vicario, D.S. & Nottebohm, F. (1996) A large-capacity memory system that recognizes the calls and songs of individual birds. *Proc. Natl. Acad. Sci. USA*, 93, 1950-1955.
- Clayton, D.F. (2000) The neural basis of avian song learning and perception. In: J.J. Bolhuis (Ed.), *Brain, Perception, Memory. Advances in Cognitive Neuroscience*, pp. 113-125. Oxford University Press, Oxford.
- Cynx, J., Williams, H. & Nottebohm, F. (1990) Timbre discrimination in zebra finches (*Taeniopygia guttata*) song syllables. *J. Comp. Psychol.*, 104, 303-308.
- Del Negro, C., Gahr, M., Leboucher, G. & Kreutzer, M. (1998) The selectivity of sexual responses to song displays: effects of partial chemical lesion of the HVC in female canaries. *Behav. Brain Res.*, 96, 151-159.
- Del Negro, C., Kreutzer, M. & Gahr, M. (2000) Sexually stimulating signals of canary (*Serinus canaria*) songs: evidence for a female-specific auditory representation in the HVC nucleus during the breeding season. *Behav. Neurosci.*, 114, 526-542.
- DeVoogd, T.J. (1994) The neural basis for the acquisition and production of bird song. In: J.A. Hogan & J.J. Bolhuis (Eds.), *Causal Mechanisms of Behavioural Development*, pp. 49-81. Cambridge University Press, Cambridge.
- DeVoogd, T.J., Cardin, J.A., Szekely, T., Buki, J. & Newman, S.W. (1996) Relative volume of I-MAN in female warbler species varies with the number of songs produced by conspecific males. *Soc. Neurosci. Abstr.*, 22, 1401.
- Duffy, D.L., Bentley, G.E. & Ball, G.F. (1999) Does sex or photoperiodic condition influence ZENK induction in response to song in European starlings? *Brain Res.*, 844, 78-82.

- Eda-Fujiwara, H., Satoh, R., Bolhuis, J.J. & Kimura, T. (2003) Neuronal activation in female budgerigars is localized and related to male song complexity. *Eur. J. Neurosci.*, **17**, 149-154.
- Farabaugh, S.M., Brown, E.D. & Dooling, R.J. (1992) Analysis of warble song of the budgerigar *Melopsittacus undulatus*. *Bioacoustics*, **4**, 111-130.
- Gahr, M. (2000) Neural song control system of hummingbirds: comparison to swifts, vocal learning (songbirds) and nonlearning (suboscines) passerines, and vocal learning (budgerigars) and non-learning (dove, owl, gull, quail, chicken) nonpasserines. *J. Comp. Neurol.*, **426**, 182-196.
- Gentner, T.Q. & Hulse, S.H. (2000) Female European starling preference and choice for variation in conspecific male song. *Anim. Behav.*, **59**, 443-458.
- Gentner, T.Q., Hulse, S.H., Duffy, D. & Ball, G.F. (2001) Response biases in auditory forebrain regions of female songbirds following exposure to sexually relevant variation in male song. *J. Neurobiol.*, **46**, 48-58.
- Halle, F., Gahr, M., Pieneman, A.W. & Kreutzer, M. (2002) Recovery of song preferences after excitotoxic HVC lesion in female canaries. *J. Neurobiol.*, **52**, 1-13.
- Hamilton, K.S., King, A.P., Sengelau, D.R. & West, M.J. (1997) A brain of her own: a neural correlate of song assessment in a female songbird. *Neurobiol. Learn. Mem.*, **68**, 325-332.
- Hasselquist, D., Bensch, S. & von Schantz, T. (1996) Correlation between male song repertoire, extra-pair paternity and offspring survival in the great reed warbler. *Nature*, **381**, 229-232.
- Hebb, D.O. (1949) *The Organization of Behavior*. John Wiley & Sons, New York.
- Horn, G. (1985). *Memory, Imprinting, and the Brain*. Clarendon Press, Oxford.
- Horn, G. (1998) Visual imprinting and the neural mechanisms of recognition memory. *Trends Neurosci.*, **21**, 300-305.
- Horn, G. (2000) In memory. In: J.J. Bolhuis (Ed.), *Brain, Perception, Memory. Advances in Cognitive Neuroscience*, pp. 329-363. Oxford University Press, Oxford.
- Horn, G., Nicol, A.U. & Brown, M.W. (2001) Tracking memory's trace. *Proc. Natl. Acad. Sci. USA*, **98**, 5282-5287.
- Jarvis, E.D. & Nottebohm, F. (1997) Motor-driven gene expression. *Proc. Natl. Acad. Sci. USA*, **94**, 4097-4102.
- Jarvis, E.D., Ribeiro, S., da Silva, M.L., Ventura, D., Vielliard, J. & Mello, C.V. (2000) Behaviourally driven gene expression reveals song nuclei in hummingbird brain. *Nature*, **406**, 628-632.
- Jin, H. & Clayton, D.F. (1997) Localized changes in immediate-early gene regulation during sensory and motor learning in zebra finches. *Neuron*, **19**, 1049-1059.
- Kimpo, R.R. & Doupe, A.J. (1997) FOS is induced by singing in distinct neuronal populations in a motor network. *Neuron*, **18**, 315-325.
- King, D.A.T. & Thompson, R.F. (2000) Skill learning: the role of the cerebellum. In: J.J. Bolhuis (Ed.), *Brain, Perception, Memory. Advances in Cognitive Neuroscience*, pp. 215-231. Oxford University Press, Oxford.
- Konishi, M. (1965) The role of auditory feedback in the control of vocalization in the white-crowned sparrow. *Z. Tierpsychol.*, **22**, 770-783.
- Kroodsma, D.E. (1976) Reproductive development in a female songbird: differential stimulation by quality of male song. *Science*, **192**, 574-575.
- Lashley, K.S. (1950). In search of the engram. *Symp. Soc. Exp. Biol.*, **4**, 454-482.
- Lampe, H.M. & Saetre, G.-P. (1995) Female pied flycatchers prefer males with larger song repertoires. *Proc. R. Soc. Lond. B*, **262**, 163-167.
- Leitner, S. & Catchpole, C.K. (2002) Female canaries that respond and discriminate more between male songs of different quality have a larger song control nucleus (HVC) in the brain. *J. Neurobiol.*, **52**, 294-301.
- MacDougall-Shackleton, S.A., Hulse, S.H. & Ball, G.F. (1998) Neural bases of song preferences in female zebra finches (*Taeniopygia guttata*). *Neuroreport*, **9**, 3047-3052.
- Maekawa, M. & Uno, H. (1996) Difference in selectivity to song note properties between the vocal nuclei of the zebra finch. *Neurosci. Lett.*, **218**, 123-126.

- Maney, D.L., MacDougall-Shackleton, E.A., MacDougall-Shackleton, S.A., Ball, G.F. & Hahn, T.P. (2003). Immediate early gene response to hearing song correlates with receptive behavior and depends on dialect in female white-crowned sparrows. *J. Comp. Physiol. A*, in press. DOI: [10.1007/S00359-003-0441-Z](https://doi.org/10.1007/S00359-003-0441-Z).
- Margoliash, D. (1986) Preference for autogenous song by auditory neurons in a song system nucleus of the white-crowned sparrow. *J. Neurosci.*, *6*, 1643-1661.
- Margoliash, D. & Konishi, M. (1985) Auditory representation of autogenous song in the song system of white-crowned sparrows. *Proc. Natl. Acad. Sci. USA*, *82*, 5997-6000.
- Marler, P. (1976) Sensory templates in species-specific behavior. In: J. Fentress (Ed.), *Simpler Networks and Behavior*, pp. 314-329. Sinauer, Sunderland, Mass.
- Marler, P. (1987) Sensitive periods and the roles of specific and general sensory stimulation in birdsong learning. In: J.P. Rauschecker & P. Marler (Eds.), *Imprinting and Cortical Plasticity. Comparative Aspects of Sensitive Periods*, pp. 99-135. John Wiley & Sons, New York.
- Marler, P. (1991) Song-learning behavior: the interface with neuroethology. *Trends Neurosci.*, *14*, 199-206.
- Marler, P. & Doupe, A. (2000) Singing in the brain. *Proc. Natl. Acad. Sci. USA*, *97*, 2965-2967.
- Marler, P. & Peters, S.S. (1989) Species differences in auditory responsiveness in early vocal learning. In: S. Hulse & R. Dooling (Eds.), *The Comparative Psychology of Audition: Perceiving Complex Sounds*, pp. 243-273. Lawrence Erlbaum, Hillsdale, NJ.
- McCabe, B.J. & Horn, G. (1994) Learning-related changes in Fos-like immunoreactivity in the chick forebrain after imprinting. *Proc. Natl. Acad. Sci. USA*, *91*, 417-421.
- Mello, C.V. (2002) Mapping vocal communication pathways in birds with inducible gene expression. *J. Comp. Physiol. A*, *188*, 943-959.
- Mello, C.V. & Ribeiro, S. (1998) ZENK protein regulation by song in the brain of songbirds. *J. Comp. Neurol.*, *393*, 426-438.
- Mello, C.V., Vicario, D.S. & Clayton, D.F. (1992) Song presentation induces gene-expression in the songbird forebrain. *Proc. Natl. Acad. Sci. USA*, *89*, 6818-6822.
- Mello, C.V. & Clayton, D.F. (1994) Song-induced ZENK gene expression in auditory pathways of songbird brain and its relation to the song control system. *J. Neurosci.*, *14*, 6652-6666.
- Mello, C., Nottebohm, F. & Clayton, D. (1995) Repeated exposure to one song leads to a rapid and persistent decline in an immediate early gene's response to that song in zebra finch telencephalon. *J. Neurosci.*, *15*, 6919-6925.
- Mountjoy, D.J. & Lemon, R.E. (1996) Female choice for complex song in the European starling: a field experiment. *Behav. Ecol. Sociobiol.*, *38*, 65-71.
- Nottebohm, F., Stokes, T. & Leonard, C.M. (1976) Central control of song in the canary. *J. Comp. Neurol.*, *165*, 457-486.
- Nottebohm, F., Alvarez-Buylla, A., Cynx, J., Kirn, J., Ling, C.-Y., Nottebohm, M., Suter, R., Tolles, A. & Williams, H. (1990) Song learning in birds: the relation between perception and production. *Phil. Trans. R. Soc. Lond. B*, *329*, 115-124.
- Nottebohm, F. (2000) The anatomy and timing of vocal learning in birds. In: M.D. Hauser & M. Konishi (Eds.), *The Design of Animal Communication*, pp. 63-110. MIT Press, Cambridge, Mass.
- Okanoya, K. (2000) Sexual selection for song complexity and modifications of brain structures in songbirds. *Jpn. J. Ornithol.*, *49*, 79-85.
- Ribeiro, S., Cecchi, G.A., Magnasco, M.O. & Mello, C.V. (1998) Toward a song code: evidence for a syllabic representation in the canary brain. *Neuron*, *21*, 359-371.
- Riebel, K. (2000) Early exposure leads to repeatable preferences for male song in female zebra finches. *Proc. R. Soc. Lond. B*, *267*, 2553-2558.
- Riebel, K., Smallegange, I.M., Terpstra, N.J. & Bolhuis, J.J. (2002) Sexual equality in zebra finch song preference: evidence for a dissociation between song recognition and production learning. *Proc. R. Soc. Lond. B*, *269*, 729-733.

- Sadananda, M. & Bischof, H.J. (2002) Enhanced fos expression in the zebra finch (*Taeniopygia guttata*) brain following first courtship. *J. Comp. Neurol.*, **448**, 150-164.
- Sagar, S.M., Sharp, F.R. & Curran, T. (1988) Expression of *c-fos* protein in brain: metabolic mapping at the cellular level. *Science*, **240**, 1328-1331.
- Searcy, W.A. (1992a) Measuring responses of female birds to male song. In: P.K. McGregor (Ed.), *Playback and Studies of Animal Communication*, pp. 175-189. Plenum Press, New York.
- Searcy, W.A. (1992b) Song repertoire and mate choice in birds. *Am. Zool.*, **32**, 71-80.
- Searcy, W.A. & Yasukawa, K. (1996) Song and Female Choice. In: D.E. Kroodsma & E.H. Miller (Eds.), *Ecology and Evolution of Acoustic Communication in Birds*, pp. 454-473. Cornell University Press, New York.
- Sockman, K.W., Gentner, T.Q. & Ball, G.F. (2002) Recent experience modulates forebrain gene-expression in response to mate-choice cues in European starlings. *Proc. R. Soc. Lond. B*, **269**, 2479-2485.
- Solis, M.M., Brainard, M.S., Hessler, N.A. & Doupe, A.J. (2000) Song selectivity and sensorimotor signals in vocal learning and production. *Proc. Natl. Acad. Sci. USA*, **97**, 11836-11842.
- Uno, H. (1997) Song perception in female birds: the contribution of the vocal system. *Biomed. Res.*, **18**, 45-51.