# Neural mechanisms of birdsong memory

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Abstract | The process through which young male songbirds learn the characteristics of the songs of an adult male of their own species has strong similarities with speech acquisition in human infants. Both involve two phases: a period of auditory memorization followed by a period during which the individual develops its own vocalizations. The avian 'song system', a network of brain nuclei, is the probable neural substrate for the second phase of sensorimotor learning. By contrast, the neural representation of song memory acquired in the first phase is localized outside the song system, in different regions of the avian equivalent of the human auditory association cortex.

#### Song system

A network of forebrain nuclei that is involved in the perception, acquisition and production of song.

#### Neuroecology

The study of the neural mechanisms of behaviour and cognition, using functional or evolutionary considerations. In a neuroecological analysis of memory, functional differences are related to neuromorphological differences.

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Memory is arguably the most important human cognitive capacity. In the search for the neural mechanisms of memory, mammals are usually preferred to birds as model systems, because of their closer evolutionary relatedness to humans. However, the recent article by the Avian Brain Nomenclature Consortium<sup>1</sup> has highlighted the homologies and analogies between the avian and mammalian brain. The bird brain is not a primitive form of the mammalian brain, but a complex, and in many ways similar, structure. In addition, it is becoming increasingly clear that birds have cognitive capacities that were thought to be the preserve of primates<sup>2-5</sup>. Therefore, comparisons of the neural mechanisms of learning and memory of birds with those of mammals will provide vital evidence about the evolution of the brain and cognition. Of the two major avian memory paradigms birdsong learning and imprinting — only the latter has so far revealed localization of the neural substrate of memory, which, in turn, has allowed a detailed study of its underlying neuronal and molecular mechanisms6.

Songbirds, parrots and hummingbirds share with humans and some other mammals the capacity for vocal learning. For successful song acquisition, young songbirds need to be exposed to the song of an adult conspecific (a 'tutor'), after which they progressively form their own song through a sensorimotor process of matching their own vocal output with the stored memory of the tutor song (BOX 1; FIG 1). Birdsong learning is considered to be the closest animal equivalent to human speech acquisition<sup>7-10</sup>. Analogous to the acquisition of human speech, songbirds form long-term memories of tutor song<sup>11</sup>. In contrast to avian imprinting, relatively little is known of the neuronal mechanisms of birdsong memory. This is because the neural substrate of tutor song learning and memory has not been localized. Most previous work in this field has been concerned with the brain regions involved in learning how to sing a song, whereas there has been little investigation of the neural substrate of the memory of the tutor song. In this article, we review contemporary research on the neural substrate of song memorization and sensorimotor learning in songbirds, and show that recent findings have cast new light on these issues.

## The song system in song learning and memory

In songbirds, an extensive network of interconnected brain nuclei has been identified as being involved in perception, learning and the production of song (BOX 2). This network is known as the song system<sup>12</sup>. Research into the neural mechanisms of song learning initially followed — often implicitly — a neuroecological approach<sup>13-16</sup>, in which functional differences were related to neural differences (BOX 3). The neuroecological approach to song learning is evident in four ways. First, songbirds (who need to learn their songs, BOX 1) have a song system that is lacking in non-songbirds (who do not learn such vocalizations)17 (FIG. 2). Second, in many species of songbird, some nuclei of the song system are larger in males (who sing learned vocalizations) than in females (who do not)18,19. Third, the volume of some nuclei in the song system of some species varies with season: they are larger in spring (the mating season) than in autumn and winter<sup>20,21</sup> (when the birds are likely to sing less, or not at all, or songs without sexual quality). Fourth, the number of learned song units (the song or syllable repertoire) is thought to correlate with the size of

## Box 1 | How songbirds learn their songs

Approximately half of all bird species belong to the suborder oscines of the order passeriformes, or songbirds. In many songbird species, only males sing songs, with females only producing calls<sup>132</sup>. Usually, male songbirds learn their song from an adult 'tutor' male (often their father) when they are young. There are two



phases of songbird song learning: a memorization phase, in which the vocal information of the tutor song is stored in long-term memory<sup>11</sup> (usually during a sensitive period), and a sensorimotor phase later in life, when the bird's own vocal output is compared with the memorized information. A much used metaphor for the mechanism underlying song memorization is that of the template<sup>62,133</sup>, which is essentially the central representation of song. If young songbirds are exposed to equal numbers of taped songs from their own and another species, their adult song will consist mainly or entirely of copies of conspecific songs, although they will be physically able to sing the heterospecific songs<sup>134</sup>. Thus, there is a bias in what the birds will learn, suggesting the presence of a rough template in the animal, before it has heard song. The young bird will memorize songs that match features of its template and reject songs that do not. Later, when the bird starts to sing itself, its song output (known as subsong, see FIG. 1) is not yet stereotyped and does not resemble the tutor song particularly well. During this sensorimotor phase, the bird matches its own output with the refined template, and eventually produces crystallized adult song. A distinction can be made between 'agelimited learners' and 'open-ended learners'. The former (for example, the zebra finch, see panel) do not alter their songs in adult life. The latter (for example, the canary), continue to alter their songs as adults, usually for every new breeding season. Image courtesy of E. Etman.

song nuclei<sup>22–25</sup>. However, with the exception of the first argument, there are several studies that suggest either a disassociation between song system morphology and song learning, or a species-specific relationship between the two, or no relationship at  $all^{26-32}$  (BOX 3; for a detailed discussion, see REFS 13,15,33).

The results of electrophysiological research have shown that neurons in nuclei of the song system - in particular, those of the lateral magnocellular nucleus (IMAN), Area X, HVC (a letter based name, formerly known as High Vocal Centre) and robust nucleus of the arcopallium (RA) (FIG. 3) - are responsive to song, especially conspecific song<sup>34</sup>. In adult males, neurons in these regions respond more to the bird's own song (BOS) than to the tutor song<sup>34,35</sup>, or to the song of another conspecific36. Some neurons in IMAN and Area X respond equally well to the BOS and tutor song, whereas a small proportion respond more to the tutor song<sup>34</sup>. The whitecrowned sparrow (Zonotrichia leucophrys) is a species in which there is no overlap between the memorization and sensorimotor phases. In the memorization phase, neurons in the HVC of juvenile males showed no preference for songs with which they were tutored<sup>37</sup>. Later, in the sensorimotor phase, when the males started to sing themselves, HVC neurons responded preferentially to the BOS37. These findings suggest the presence of a neural representation of the tutor song in these nuclei

that becomes manifest only after the young birds start to sing, in the sensorimotor phase. The results of a recent electrophysiological analysis involving developing zebra finches (*Taeniopygia guttata*) suggest that neurons in the HVC show transient preferential responding to the song of their tutor<sup>38</sup>. In the early sensorimotor period (35–69 days after hatching), responsiveness of HVC neurons was greater to tutor song than to the BOS, new song, heterospecific song or white noise; in some cases this preference was significant. Later in development (>70 days after hatching) the preference of HVC neurons for tutor song switched to a preference for the BOS. Responsiveness of HVC neurons during the early sensorimotor period did not correlate with the similarity of the BOS to the tutor song<sup>38</sup>.

A series of studies on the possible role of NMDA (*N*-methyl-D-aspartate) receptors in birdsong learning have led to the suggestion that the song system might contain the neural representation of tutor song memory<sup>39-44</sup>. NMDA receptor binding in the song system nucleus IMAN in zebra finches peaks at 30 days after hatching and subsequently declines (a decline that also occurs in socially isolated males)<sup>39</sup>. An early hypothesis that NMDA receptor levels might limit the sensitive period for song learning was not confirmed<sup>39-42</sup>. Experimentally delayed song learning in the zebra finch did not result in delayed decline of NMDA receptor gene expression in the IMAN<sup>40</sup>. However, a hypothesis that the IMAN contains the neural substrate for tutor song memory was fuelled by two studies involving NMDA receptor blockers. Systemic injection of the non-competitive NMDA receptor blocker MK-801 impaired song learning in zebra finches, but only when the injections occurred on days on which the birds were exposed to tutor song<sup>43</sup>. A subsequent study involving infusions of the competitive NMDA receptor blocker AP5 into the IMAN yielded similar results44. In zebra finches, there is overlap between the memorization and the sensorimotor phases, and, as we have seen, during the latter, neurons in the song system become tuned to the BOS. Although it is possible that vocal output was affected by infusion of the NMDA receptor blocker into the IMAN, the site of its action is not clear, and we do not know whether these effects were on sensorimotor integration rather than on the formation of auditory memory. Finally, in these invasive studies only the IMAN was targeted, whereas other nuclei in the song system and regions outside it were not. These studies were performed on an assumption derived from analyses of the synaptic mechanisms of long-term potentiation (LTP). That is, NMDA receptor-dependent synaptic plasticity underlies memory formation<sup>45</sup>. In particular, it has been proposed that the same NMDA receptor-dependent synaptic mechanism underlies LTP and spatial learning in rats<sup>45,46</sup>. This hypothesis has been disproved in a number of studies<sup>46-50</sup>. Therefore, the involvement of NMDA receptors in memory-related neural plasticity is not clear.

While describing seasonal variation in the volume of some nuclei in the song system, Nottebohm<sup>20</sup> noted that "song 'learning' and song 'forgetting' are used here to refer not so much to the acquisition and loss of an auditory memory, but rather to the conversion of that

### Subsong

The first songs produced by young songbirds. These songs are relatively simple and may not resemble the song of the tutor. Subsong may still change and will eventually become crystallized song, which in many songbird species is the definitive song for that particular individual.







memory into a motor program, with the consequent matching of an auditory model". Many authors make this distinction — explicitly or implicitly — between a representation of the auditory memory of the tutor song and the motor memory of the BOS (see, for example, REFS 8,12,20,34,38,51). These two aspects of song may be associated with the memorization phase and the sensorimotor phase, respectively (BOX 1). By making this distinction, it becomes clear that most authors investigating the neural mechanisms of birdsong are addressing the motor memory component, which is reflected in the expression 'vocal learning'. Presumably, it is this 'vocal learning' that is thought to be subserved by the rostral pathway of the song system<sup>20,52,53</sup> (FIG. 3).

Investigation of the neural mechanisms of song learning is further complicated because learning in both the

memorization phase and the sensorimotor phase are dependent on auditory input, from the tutor or from the bird itself, respectively. In addition, these two phases overlap in many songbird species. These problems might be avoided in species in which the memorization phase and the sensorimotor phase are separated in time<sup>54</sup>. For example, swamp sparrows (Melospiza georgiana) reared in the laboratory could memorize songs that they heard from tapes between 22 and 62 days after hatching, but did not start to sing themselves until ~275 days after hatching55. The volume of the nuclei HVC and RA (FIG. 3), and the number of neurons in the HVC increased significantly during the memorization phase, whereas there was no such change during the sensorimotor phase. The problem with developmental correlations between phases of song learning and neural changes is that they need not be associated with learning (as was the case in the NMDA receptor studies)40-42. In addition, brain regions outside the 'song system' were not sampled in the swamp sparrow study55.

Neurons in the song system nuclei HVC, IMAN, Area X and RA are activated when the bird is singing<sup>34,36,53</sup>. Moreover, there is increased neuronal activation (measured as the expression of immediate early genes, IEGs) in these nuclei when the bird is singing<sup>56</sup>, but not when it hears song, including tutor song<sup>56-58</sup>. These findings, combined with the absence of learning-related neuronal activation in the song system<sup>58,59</sup> (see next section) and the electrophysiological studies discussed earlier<sup>37,38</sup>, render it unlikely that nuclei in the song system of adult songbirds contain the neural substrate of tutor song memory — although at this stage we cannot exclude that possibility. Rather, 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, which can also occur in adult songbirds<sup>34,60,61</sup>. In the latter case, it may be that there is a representation of the BOS in the song system that is being updated through continual interaction with regions in the caudal nidopallium and mesopallium, as discussed in the following section.

## Neuronal activation outside the song system

A consequence of concentrating on sensorimotor learning of song is that little is known of the neural substrate of the memory of the tutor song, or the song template<sup>62</sup> (BOX 1) that is the result of auditory learning<sup>12,34,61,63</sup>. Recent research involving analysis of the expression of IEGs has shed fresh light on this issue. Expression of IEGs or their protein products is thought to be an indication of neuronal activation<sup>64</sup>. Exposure of zebra finches or canaries (Serinus canaria) to conspecific song led to increased neuronal activation not in nuclei of the song system, but in different forebrain regions, particularly the caudal part of the medial nidopallium (NCM) and the caudal part of the medial mesopallium (CMM)<sup>57,58,65</sup> (FIG. 3). IEG expression was greatest when birds were exposed to conspecific song, compared with heterospecific song or pure tones<sup>57</sup>. Song production by itself does lead to IEG expression in nuclei in the song system<sup>56</sup>. Therefore, there is a dissociation between forebrain regions that are

#### Immediate early genes

(IEGs). Genes that can respond rapidly (within minutes) to stimulation of a cell such as a neuron. The protein products of such genes return to the cell nucleus where they affect the transcription of other, 'late response' genes. Expression of these genes (such as c-fos or ZENK) or their protein products (Fos and Zenk, respectively) signifies that the cell is activated. Therefore, IEG expression is used as a marker for neuronal activation. The genes can be stained by means of an in situ hybridization procedure, whereas the protein products can be made visible through immunocytochemistry.

#### Template

A metaphor for the central representation of song. Conventionally, it has been suggested that songbirds are born with a crude template that has species-specific characteristics. Auditory experience with the song of an adult conspecific male will then mould the template into a more precise representation of the tutor song.

# Box 2 | Singing and the brain

Until recently, it was thought that two forebrain pathways connecting a number of 'song control nuclei' comprise the neural substrate for birdsong (FIG. 3). Together, the two pathways are called the 'song control system'<sup>135</sup> or simply the 'song system'<sup>12</sup>. Early evidence for the involvement of the song system in song came from a series of neuroanatomical and lesion studies<sup>52,53</sup>. The caudal pathway, including the HVC (a letter based name) and the robust nucleus of the arcopallium (RA), is 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 production in adult birds<sup>12,52,53</sup>. The rostral pathway, including the HVC, the lateral part of the magnocellular nucleus of the neostriatum (IMAN) and Area X, was thought to have 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 crystallized song in adults<sup>136</sup>. However, such lesions might indirectly affect the development of premotor regions of the song system<sup>137,138</sup>. Recent studies involving the expression of immediate early genes (IEGs) showed that exposure to song does not lead to neuronal activation in nuclei in the song system. Such exposure does, however, lead to neuronal activation in other brain regions, particularly the caudal part of the medial nidopallium (NCM) and the caudal part of the medial mesopallium (CMM)<sup>56-58,65</sup> (FIG. 3). Song production by itself does lead to IEG expression in song system nuclei<sup>56</sup>.

activated when the bird hears song, and those that are activated when the bird is singing itself<sup>56</sup>. These findings led to the hypothesis that the regions in the caudal forebrain that are activated when the bird is exposed to conspecific song might be (part of) the neural substrate for memory of the tutor song<sup>12,34,66</sup>. This hypothesis has received support from studies showing a significant correlation between neuronal activation in the NCM of adult male zebra finches and the strength of song learning<sup>58,59,67</sup> (FIG. 4a).

Further support for the NCM as (part of) the neural substrate for tutor song<sup>58</sup> comes from studies that investigated habituation-like processes involving this brain region. Repeated exposure to a song leads to a decrease in expression of the IEG *ZENK*<sup>68</sup> and to decreased electrophysiological responses of units in the NCM<sup>69</sup> to that song. Chew and colleagues concluded that it is likely that "the NCM is specialized for remembering the calls and songs of many individual conspecifics"<sup>69</sup>. A recent

electrophysiological study showed that neurons in the NCM of adult zebra finch males showed steeper rates of habituation to novel song than to the tutor song<sup>70</sup>. A familiarity index, based on relative habituation rates, was significantly greater in tutored males than in untutored males. In addition, there was a significant positive correlation between familiarity index of NCM neurons and the strength of song learning. The findings of a number of studies indicate that the NCM and CMM are involved both in processing of perceptual information concerning song complexity and in storage of song memory in songbirds and parrots<sup>71–73</sup>.

### Neural mechanisms of song memory in females

Recent investigations of the neural mechanisms of song memory have concentrated on female songbirds rather than males. In many songbird species, females do not sing, and only produce calls. Nevertheless, females of some species develop a preference for the song of their

## Box 3 | Neural plasticity in the song system

Nottebohm<sup>20</sup> first reported that seasonal variation in the volume of certain song system nuclei seemed to correlate with seasonal variations in the song of domesticated adult canaries. In particular, seasonal variation occurred in the volume of the song nuclei HVC (a letter based name) and the robust nucleus of the arcopallium (RA) (FIG. 3). Initially, it was suggested that the volume changes reflected increases in the size or number of dendrites or synapses<sup>20</sup>. Later, it was proposed that seasonal loss and addition of neurons might be the underlying mechanism<sup>139</sup>. However, further research suggested that there are permanent neuronal populations in the HVC and RA that show seasonal morphological changes<sup>32</sup>. The importance of either cellular mechanism for song learning is not clear<sup>13,32,140,141</sup>. In addition, the results of field studies<sup>30</sup> suggest that, under natural conditions, canaries change their song repertoires with the season, without significant changes in the volume or gross morphology of the HVC and RA (FIG. 5). In a longitudinal field study of individual, free-living wild canaries, it was suggested that ~25% of the syllables are sung seasonally, whereas the remainder are used throughout the year, despite seasonal changes in the temporal patterns of song<sup>30</sup>. During the breeding season, while testosterone levels are increased, males sing an increased number of fast frequency-modulated syllables, which are known to be sexually attractive to females. About 50% of the syllables that were lost after one breeding season reappeared in the following breeding season, which indicates that they were not forgotten. Furthermore, there are species, such as the rufous-sided towhee (Pipilo erythrophthalmus), in which seasonal changes in the volume of song system nuclei occur without overt song changes<sup>140</sup>. These conflicting observations might point to species or even population differences in the mechanisms of seasonal variation in singing, or to species differences in what is actually learned. Alternatively, there might not be a straightforward relationship between brain morphology and vocal learning.

Similarly, the significance of sex differences in song system morphology<sup>19</sup> for song learning is not clear<sup>13,15</sup>. Males and females of the African bush shrike (*Laniarius funebris*) sing songs of similar complexity, but there are significant sex differences in the volume of some of their song system nuclei<sup>29</sup>. Both sexes must learn their songs in order to perform their pair-specific duets, and there is no obvious relationship between song learning capacity and song system morphology in this species.



Figure 2 | **The brains of songbirds and non-songbirds differ.** These schematic diagrams of parasagittal views of the brains of a songbird (**a**) and a non-songbird (**b**) illustrate the dramatic differences between them. Songbirds have an elaborate network of interconnected forebrain nuclei that form an interface between auditory input (which converges on field L, the primary auditory projection region in the avian forebrain) and vocal output, which is produced in the syrinx, the avian vocal organ. Non-songbirds also have field L, and they can produce vocalizations in the syrinx, but they do not have the network of forebrain nuclei that songbirds have<sup>17,142</sup>. DLM, nucleus dorsolateralis anterior, pars medialis; DM, dorsomedial nucleus of the midbrain nucleus intercollicularis; HVC, a letter based name; IMAN, lateral magnocellular nucleus of the anterior nidopallium; mMAN, medial magnocellular nucleus of the anterior nidopallium; nXIIts, tracheosyringeal portion of the nucleus hypoglossus; RA, robust nucleus of the arcopallium; RAm, nucleus retroambigualis; rVRG, rostro–ventral respiratory group; X, Area X. Adapted, with permission, from REF. 17 © (1997) Elsevier Science.

father over new conspecific song74,75, which indicates that they are able to discriminate between different conspecific songs. For instance, in operant tests, both female and male zebra finches that were reared with their father showed a significant and similar preference for their father's song76. Therefore, although they do not sing, these females also learn the characteristics of the song of an adult male. In males, song output is being compared with this 'template' during the sensorimotor phase, eventually leading to crystallized song. In females, the 'template' of the father's song is presumably used in mate choice75,77. Whatever the function of song learning is in the two sexes, in both cases a memory is formed of the song of an adult male conspecific. It is possible, but not necessary, that the mechanisms and neural substrate of these two cases of memory formation are similar, or identical. An advantage of investigating these mechanisms in non-singing females is that learning the characteristics of the father's song is not intertwined with motor learning. Female songbirds — regardless of whether or not they sing - also have a 'song system', but with nuclei that are generally significantly smaller than those of their male counterparts<sup>19,29</sup>.

There are species differences with regard to the role of the song system nucleus HVC in female song perception and discrimination. In female canaries, bilateral lesions of the HVC resulted in the loss of their ability to discriminate between conspecific and heterospecific songs<sup>78</sup> and between different conspecific songs<sup>79,80</sup>. A subsequent electrophysiological study showed that sexually attractive songs of male canaries evoke different neuronal responses

in the HVC of females from songs lacking these attractive syllables<sup>81</sup>. Furthermore, the volume of the 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<sup>82</sup>. The results of these canary studies suggest that the HVC is involved in female perception of male song. 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<sup>83</sup>. (These two studies<sup>82,83</sup> are typical examples of neuroecological analyses<sup>13-16</sup>, therefore, the significance of changes in the volume of a brain nucleus is not clear). By contrast, the results of studies in the zebra finch suggest that brain regions outside the song system have a role in song perception by females. Electrolytic lesions of the CMM, but not lesions of the HVC, disrupted the ability of female zebra finches to discriminate conspecific from heterospecific song<sup>84</sup>. The discrepancy between the effects of lesions in canary and zebra finch females may be related to the fact that canary and zebra finch males are open-ended and age-limited learners, respectively (BOX 1). In the female canary, the HVC may be involved in the perception of new songs that are being learned by adult males. Furthermore, unlike zebra finch females, canary females sometimes sing, and may also learn to sing new songs, for which the HVC could be important. Finally, it is possible that the HVC in female canaries is involved in another motor act — the copulation solicitation display. The results of IEG expression studies of female songbirds



Figure 3 | The songbird brain. This schematic diagram of a composite view of parasagittal sections of a songbird brain gives approximate positions of nuclei and brain regions involved in birdsong. All structures are bilateral - for clarity only those on one side of the brain are depicted. Lesion studies in adult and young songbirds led to the distinction between a caudal pathway (red arrows), considered to be involved in the production of song, and a rostral pathway (thick black arrows), thought to have a role in song acquisition<sup>12,52,53,73</sup> (BOX 2). Thin black arrows indicate known connections between the field L complex, a primary auditory processing region, and some other forebrain regions. The yellow nuclei show significantly enhanced expression of immediate early genes (IEGs) when the bird is singing<sup>56</sup>. Dark green areas represent brain regions that show increased IEG expression when the bird hears song<sup>56-58</sup>, including tutor song<sup>58,59,67</sup>. Abbreviations: Cb, cerebellum; CLM, caudal lateral mesopallium; CMM, caudal medial mesopallium; DLM, nucleus dorsolateralis anterior, pars medialis; HP, hippocampus; HVC, a letter based name; L1, L2, L3, subdivisions of field L; LaM, lamina mesopallialis; IMAN, lateral magnocellular nucleus of the anterior nidopallium; NCM, caudal medial nidopallium; nXIIts, tracheosyringeal portion of the nucleus hypoglossus; RA, robust nucleus of the arcopallium; V, ventricle. Adapted, with permission, from REF. 15 © (2005) Koninklijke Brill NV, Leiden.

confirm a role in song perception and memory for brain regions outside the song system. These studies focused on the NCM in starlings (*Sturnus vulgaris*)<sup>85,86</sup> and canaries<sup>87</sup>, and on the NCM and CMM in zebra finches<sup>88,89</sup> and white-crowned sparrows<sup>90</sup>. IEG expression varies with male song complexity in the NCM (and to a lesser extent in the CMM) of female budgerigars (*Melopsittacus undulatus*, a parrot)<sup>71</sup>, indicating a role for the NCM (and perhaps the CMM) in song perceptual processes such as complexity discrimination in this species.

Female zebra finches that were reared with their fathers showed significantly increased neuronal activation (measured as IEG expression) in the CMM, but not in the NCM or the hippocampus, when they were re-exposed to their father's song compared with females that were exposed to novel zebra finch song<sup>89</sup> (FIG. 4c). Females in both groups showed a significant preference for the song of their fathers, indicating that they had learned the characteristics of this song. These results suggest that in female zebra finches, the CMM might be (part of) the neural substrate for the memory of the father's song. A recent study using female canaries showed a significantly

greater neuronal activation in the CMM, but not in the NCM, of female canaries that were exposed to songs they preferred compared with birds exposed to less attractive songs. In addition, male and female starlings trained in an operant task to recognize conspecific songs showed memory-related electrophysiological responsiveness in the CMM<sup>92</sup>. In the latter study, only the CMM was sampled.

# Specificity of song-related brain activation

A number of alternative explanations (other than a 'template' interpretation; BOX 1) have been put forward for the stimulus-related neuronal activation that was found in the NCM<sup>51,58</sup> and the CMM<sup>89</sup>. Neuronal activation in the NCM in response to tutor song is not an artefact of isolation rearing<sup>51</sup>, as a positive correlation between IEG expression and the strength of song learning was also found in the NCM of zebra finch males that were reared with a live tutor<sup>59,67</sup>. Does neural activation in the NCM reflect a neuronal response to the tutor song, or to the BOS? Our early results were insufficient to resolve this issue<sup>58,59</sup>: the more a male has copied from the tutor song, the more the BOS will resemble this tutor song. So, the correlations between IEG expression and the strength of song learning could also reflect a neuronal response to songs that are increasingly similar to the BOS. Subsequently, we found<sup>67</sup> that neuronal activation in both the NCM and the CMM does not differ in response to tutor song, the BOS or novel song (FIG. 4b). There was a significant positive correlation between neuronal activation in the NCM and the strength of song learning only in response to tutor song, not to the BOS or novel song<sup>67</sup>. The absence of such a correlation after exposure to the BOS is consistent with a role for the NCM in information processing for this song. However, the relatively low level of neuronal activation seen in the NCM in response to the BOS does not support this hypothesis. The differential responsiveness to the song of the father versus new song in female zebra finches<sup>89</sup> cannot be a reflection of a representation of the BOS, as females do not sing.

Are the NCM and CMM important for attentional mechanisms? It has been suggested51 that neuronal activation in the NCM58,59,67 might reflect a predisposition in 'good learners' to pay more attention to any song stimulus than 'poor learners'. This hypothesis was not confirmed. If good learners were predisposed to attend more to any song stimulus than poor learners, a positive correlation would also be expected between the strength of learning and neuronal activation in the NCM in groups exposed to the BOS or to novel song, which was not the case<sup>67</sup>. It may be that the differential neuronal activation found in the CMM of zebra finch females<sup>89</sup> is a result of the birds paying more attention to their father's song than to novel song. Such a high level of focused attention would be expected to lead to a more consistent response of the CMM of females exposed to their father's song than that in females exposed to novel song. In fact, variance in the former was significantly greater than in the latter, which suggests that an attentional explanation is also unlikely in females.





## Forebrain memory systems in birds and mammals

What are the roles of the NCM and the CMM in auditory memory in songbirds? The results of studies involving zebra finch males<sup>58,59,67,70</sup> suggest that the NCM might contain the neural substrate for tutor song memory. Moreover, studies involving male58,59,67,93 as well as female<sup>84-90</sup> songbirds show that the CMM is also important for song memory. Male starlings showed increased neuronal activation in the CMM, but not in the NCM, when they were exposed to familiar songs93. In zebra finches, males and females that were reared in the same way exhibited different patterns of neural activation in response to the same song stimuli67,89 (FIG. 4b,c). In females, learning-related neuronal activation occurred in the CMM, not the NCM. By contrast, in males re-exposed to their tutor's song, there was a significant correlation between the strength of song learning and neuronal activation in the NCM, but not the CMM<sup>58,59,67</sup>. The different functions of song learning in the two sexes might allow us to interpret these differential effects. Recognition of the father's song is important in both sexes (and the CMM might contain the neural substrate subserving memory of that song), whereas only males produce song, for which the NCM might contain the neural substrate<sup>67</sup>, perhaps serving as a parallel store to the CMM. There was a significant correlation between neuronal activation in the CMM and NCM of females that were exposed to their fathers' songs<sup>89</sup>, which suggests that the two brain regions might both comprise the neural substrate for the representation of tutor song, and function in parallel. Of course, there are avian species<sup>29</sup> in which females also sing and learn songs in the same way as males (BOX 3). Therefore, the existence of parallel stores is unlikely to be linked to sex. Rather, these data suggest that different sub-parts of the auditory forebrain of songbirds might be involved in different types of auditory memory.

The NCM and CMM are widely conserved among bird species<sup>1,94-96</sup>. In filial imprinting in the domestic chick, memory of the visual imprinting stimulus is subserved by two parallel stores<sup>6</sup>. The neural substrate of one of these stores is located in the intermediate and medial mesopallium (IMM), a brain region that partially overlaps with the CMM<sup>6,97,98</sup>. In addition, the IMM is also involved in colour recognition in a passive avoidance task in domestic chicks<sup>99</sup>. Song preference learning in female songbirds has been compared to sexual imprinting<sup>77</sup>. In domestic chickens, the IMM is also involved in sexual imprinting<sup>100</sup>. Therefore, the medial part of the mesopallium might be (part of) a general recognition system in birds and contain representations of imprinted stimuli, conditioned stimuli and learned song<sup>6,66</sup>.

Parallels with the mammalian brain. In the revised interpretation of the avian brain<sup>1,95</sup> it is suggested that the pallium, which includes the hyperpallium, mesopallium, nidopallium and arcopallium, is homologous to the mammalian neocortex. After considering two theoretical proposals, the members of the Avian Brain Nomenclature Consortium<sup>1</sup> suggested that it would be premature to suggest one-to-one homologies between avian and mammalian pallial regions. In the avian forebrain, field L2 receives auditory connections from the thalamus, and, in turn, projects onto fields L1 and L3 (FIG. 3). These two regions project to the caudal mesopallium and caudal nidopallium, respectively. Therefore, it is plausible that the field L complex is homologous to the primary auditory cortex (in the mammalian superior temporal gyrus), which also consists of three 'core' regions that receive inputs from the thalamus<sup>101-103</sup>. In primates, the auditory association cortex consists of a



**b** Number of seasonally and annually identical syllables











Figure 5 | Seasonal changes in physiology and vocal repertoire of wild canaries on Madeira. The graphs in panel a represent syllable measures taken during a breeding season and the following non-breeding season of the same males, respectively. The three graphs in panels **b-d** represent syllable or physiological measures taken during breeding season 1, non-breeding season, and breeding season 2, respectively In this bird species, the size of the song syllable repertoire remained unchanged throughout the year (a). However, the composition of the syllable repertoire was markedly different during breeding seasons compared with during non-breeding seasons (b). This variation correlated with fluctuations in the plasma level of testosterone (c). It was inferred that many of the syllables that were seasonally lost were subsequently recovered, so the birds' total syllable repertoire was constant on an annual basis. The volume of the song system nuclei HVC (a letter-based name) and RA (robust nucleus of the arcopallium) also remained unchanged throughout the year. This suggests that seasonal variations in the composition of syllable repertoire are not related to changes in the volume of nuclei in the song system. Volumes of song nuclei represent medians and quartiles. Levels of plasma testosterone represent medians and quartiles of the entire breeding or non-breeding seasons. Connected dots in c indicate seasonal changes in T levels of the same individuals. Adapted, with permission, from REF. 30 © (2001) Academic Press.

complex network of brain regions, including the medial and lateral belt regions, the parabelt regions and projection regions in the prefrontal cortex<sup>101-104</sup>. Consequently, the projection regions of the field L complex (the NCM and CMM) might be homologous to the mammalian auditory association cortex, most likely with belt and parabelt regions. Compared with familiar sounds, new sounds evoke significantly greater neuronal activation (measured by expression of the IEG protein product Fos) in the rat auditory association cortex, but not in a number of other brain regions, including the primary auditory cortex, the hippocampus and the perirhinal cortex<sup>105</sup>. The authors suggest that the rat auditory association cortex is involved in auditory recognition memory. Similarly, in monkeys, species-specific calls evoke neural activation specific to the left superior temporal sulcus<sup>106</sup>. Lesions to the superior temporal cortex have been shown to impair auditory memory<sup>107,108</sup>, whereas lesions to the superior temporal gyrus or the temporal lobe, but not to the perirhinal cortex, impair performance in an auditory recognition task109.

In humans, regions traditionally associated with speech perception, which are centred around Wernicke's area in the superior temporal lobe, are distinguished from speech motor areas, including Broca's area, in the frontal lobe<sup>101</sup>. However, in humans, it has been suggested that speech perception affects speech production from birth onwards<sup>101,110,111</sup>. For instance, there is some evidence to suggest that speech perception modulates the excitability of tongue muscles<sup>101,112</sup>. Infants in their first months of life acquire sophisticated information about their native language simply by listening before they know the meaning of words<sup>110</sup>. This early experience affects not only their discrimination ability and listening preference but also alters subsequent perception and motor performance. The early experience is language-specific, such that speakers that learn a second language after puberty produce it with an accent typical of the primary language<sup>110</sup>. The results of a functional MRI (fMRI) study revealed that, similar to adults, 3-month-old babies who were exposed to speech had significant activity in brain regions in the left hemisphere, including the superior temporal gyrus<sup>113</sup>. These findings show that precursors of adult cortical language areas are already active in infants long before the onset of speech production. So, there is an interesting analogy between the mechanisms of human speech acquisition and song learning in songbirds. That is, brain regions involved in auditory learning in humans and birds are anatomically separate from those involved in sensorimotor learning, and vocal learning involves continual interactions between them.

The hippocampus has been implicated in memory in both mammals<sup>114-116</sup> and birds<sup>13-15,117</sup>. In recent analyses of the role of the temporal lobes in memory in mammals, emphasis has shifted from the hippocampus to the adjacent entorhinal and perirhinal cortices<sup>116,118-121</sup>. Claims that the hippocampus is important in spatial memory in food-storing birds<sup>16,117</sup> have been disputed<sup>13-15</sup>, and auditory–spatial memory in barn owls appears to involve the midbrain and the arcopallium of the forebrain<sup>122</sup>. In songbirds, the hippocampus contains neurons that are responsive to auditory stimuli (M.G., unpublished observations). However, in both male and female zebra finches there was no significant increase in neuronal activation in the hippocampus in response to tutor song<sup>67,89</sup>. Overall, the expression of IEGs was very low in the hippocampus of both males and females. Similarly, there was very little expression of IEGs in the hippocampus of female budgerigars<sup>71</sup> and male ring doves<sup>96</sup> (Streptopelia risoria) in response to conspecific vocalizations. Bailey and co-workers<sup>88,123</sup> reported increased expression of Fos, but not of ZENK, in response to conspecific song (compared with heterospecific song) in female zebra finches at 30 days after hatching and as adults. There were no such differential responses in females at 45 days after hatching124 or in males at 30 or 45 days after hatching<sup>123,124</sup>.

Taken together, these findings suggest that in both mammals and birds, auditory recognition memory involves auditory association regions, rather than conventional memory-related brain structures, such as the hippocampus and adjacent cortical regions.

## **Conclusions and future perspectives**

In summary, the evidence reviewed here suggests that the 'song system' is involved in birdsong, although its precise role is not clear. Evidence for plasticity of song system nuclei in relation to motor learning is inconsistent. In addition, neuronal activation in relation to tutor song memory (and perhaps also to memory of the BOS) occurs in brain regions outside the conventional song system. The avian brain regions that contain the putative memory substrate are homologous to the mammalian auditory (association) cortex<sup>1,95</sup>. One of these regions overlaps with a brain region that has been shown to contain a neural substrate for memory in visual imprinting<sup>6,97,98</sup>. Further experiments with male and female songbirds and non-songbirds are necessary to elucidate the roles of the nidopallium and mesopallium in the different aspects of song memory. These problems could be investigated further using newly developed fMRI techniques in songbirds<sup>125,126</sup>, as well as electrophysiology in awake birds using telemetry127. What is the role of IEGs in memory? Are they expressed during recognition, retrieval or during reconsolidation89,128,129? If the NCM and/or the CMM are (part of) the neural substrate for song memory, lesions to either of these regions should affect song learning. Lesions to the NCM were found to

impair song preferences without affecting song in male zebra finches<sup>130</sup>. An important new development in this field of research is the neural analysis of female songbirds. Male song is an important stimulus in sexual selection. Investigation of females has mainly been concerned with the neuronal responses to male songs of varying complexity. More recently, the neural mechanisms involved in female song memory have begun to be studied<sup>89</sup>.

At this stage, we cannot be certain whether the NCM and CMM contain the neural substrate for song memory, or whether these brain structures are 'relay stations' for a neural representation that is stored elsewhere in the brain. This important problem can only be tackled in a series of experiments using different techniques, as has been done for the imprinting paradigm6. If localization of the neural substrate of song memory in male and female songbirds can be confirmed and consolidated, this will open the way for detailed cellular and molecular analyses, similar to developments in imprinting research<sup>6</sup>. The role of the NCM and CMM in song acquisition during the memorization phase needs to be investigated in juveniles. In addition, localization of the neural substrate of tutor song memory is important for the analysis of the neural mechanisms of sensorimotor learning. How does information about the tutor song, which may be stored in the NCM and CMM, influence sensorimotor learning? Connections between the NCM, CMM and nuclei in the song system seem to be sparse. Detailed neuroanatomical analysis of forebrain connectivity will be necessary to investigate the existence of a closed-loop pathway involving the NCM, CMM and nuclei in the song system, which could serve as a 'comparator'38,131 for integrating song system output, auditory feedback and information in the template in sensorimotor learning. The strong and stimulus specific responsiveness of the NCM and CMM to tutor song suggests that these brain regions continue to be important in adult songbirds. A possible scenario is that the NCM and CMM are important for song acquisition in the memorization phase, whereas the song system is important during the sensorimotor phase and for song production - possibly through interactions with the NCM and CMM. Within the song system, it seems that the caudal pathway is important for song production, whereas the rostral pathway has a role in song learning and song modification in adults. Future research will be necessary to resolve these issues.

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#### Competing interests statement

The authors declare no competing financial interests.

## FURTHER INFORMATION

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