



Review

Birdsong memory and the brain: In search of the template

Johan J. Bolhuis^{a,b,*}, Sanne Moorman^{a,c}^a Cognitive Neurobiology and Helmholtz Institute, Departments of Psychology and Biology, Utrecht University, Utrecht, The Netherlands^b Department of Zoology and Sidney Sussex College, University of Cambridge, UK^c Department of Biology, Boston University, Boston, MA, USA

ARTICLE INFO

Article history:

Received 25 August 2014

Received in revised form 7 November 2014

Accepted 21 November 2014

Available online 29 November 2014

Keywords:

Learning
 Birdsong
 Songbirds
 Birdsong learning
 Filial imprinting
 Engram
 NCM
 CMM
 HVC
 Speech
 Language

ABSTRACT

In his pioneering research on the neural mechanisms of filial imprinting, Gabriel Horn has gone a long way to fulfilling Karl Lashley's dream of finding the 'engram' or memory trace in the brain. Here we review recent research into the engram(s) of song learning in songbirds, particularly zebra finches. When juvenile songbirds learn their songs from a tutor, they form and alter a central representation of the tutor song, known as the 'template'. Secondary auditory regions in the caudal medial pallium are likely to contain the neural substrate for the representation of tutor song, but the roles of the different regions remain to be elucidated. Female zebra finches do not sing, but nevertheless form an auditory memory of their father's song, for which the neural substrate is located in the caudomedial pallium. In males that are learning their songs, there is continual interaction between the secondary auditory regions and sensorimotor regions, similar to the interaction between Broca's and Wernicke's areas in human infants acquiring speech and language.

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* Corresponding author at: Cognitive Neurobiology and Helmholtz Institute, Departments of Psychology and Biology, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands. Tel.: +31 302535404.

E-mail address: j.j.bolhuis@uu.nl (J.J. Bolhuis).

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1. Introduction

Among Gabriel Horn's greatest scientific achievements is that in his analysis of imprinting (Bolhuis, 1991) he has realised Karl Lashley's dream of finding the 'engram' or memory trace in the brain (Bolhuis and Johnson, 2012; Brown, 2013). The way in which he achieved this is remarkable and pioneering (Bateson, in this issue; McCabe and Solomonia, in this issue; Moorman and Nicol, in this issue). Essentially, Horn followed what may be called a 'bottom-up' approach, without any presuppositions as to the locus of the memory trace or the neural mechanism of its formation (Horn, 1985, 2004; Bolhuis, 2000). Perhaps the only presupposition that Horn used was that memory formation would involve some kind of plastic change in the brain, at the level of the synapse; a hypothesis that he had developed (Horn, 1952) in parallel with Hebb (1949) and Konorski (1948). Horn argued that such plastic neural changes were likely to involve protein synthesis (Horn, 1985, 2004). In a series of elegant studies, Horn and collaborators employed a number of different techniques, including lesions, electrophysiology, autoradiography, and immunocytochemistry of immediate early gene products, enabling them to localise the neural substrate of imprinting memory to a restricted region of the chick forebrain, the intermediate and medial mesopallium or IMM (Horn, 1985, 2004). Among other things, Horn et al. discovered that memory-related neural activation is lateralised (Horn, 2004; Moorman and Nicol, in this issue), that regions outside the IMM are also involved (Horn, 1985, 2004; Bateson, in this issue), and that sleep plays an important role in learning and memory (Jackson et al., 2008; Vorster and Born, in this issue). The classic work of Horn and his collaborators has inspired our own research into the neural mechanisms of birdsong memory (Bolhuis and Gahr, 2006; Bolhuis et al., 2010; Moorman and Nicol, in this issue). Imprinting and birdsong acquisition are the two most prominent avian paradigms for the study of the neural mechanisms of learning and memory (Horn, 2004; Bolhuis and Gahr, 2006). In both cases learning occurs predominantly during a sensitive period early in development. In the course of our research we found a number of parallels between the neural mechanisms of imprinting and birdsong learning, including lateralisation and the role of sleep (Gobes et al., 2010; Moorman et al., 2012; Moorman and Nicol, in this issue). In addition, one of the regions that is involved in tutor song memory in male and female songbirds, the caudomedial mesopallium or CMM, overlaps anatomically with the IMM. In this paper we will review current evidence on the neural substrate of birdsong memory. First, we will provide a brief overview of the neural and behavioural mechanisms of birdsong learning, and its parallels with speech and language acquisition in human infants.

2. Birdsong learning

2.1. Template-guided learning

Songbirds learn their songs from an adult conspecific 'tutor' through a process that has parallels with human speech acquisition (Bolhuis et al., 2010; Bolhuis and Everaert, 2013; Moorman and Bolhuis, 2013). In many songbird species, song learning has two phases (Fig. 1). In the memorisation phase, auditory–vocal information contained in the tutor song is stored in long-term memory, while in the sensorimotor phase the bird's own vocal output is 'matched' with the information stored in memory. When

a juvenile songbird is reared in auditory isolation, the crude template is not altered by experience with adult song. When the bird is adult, it will sing a highly abnormal song that is based only on the information contained in the crude template, and feedback from the bird's own vocalisations (Fig. 1). This so-called isolate song (Marler, 1970; Fehér et al., 2009) does have some recognisable species-specific features, but it has a relatively simple structure and does not sound like the songs of socially raised birds. In socially reared birds, during auditory learning an internal representation of the tutor song is formed that has been called a 'template' (Konishi, 1965). It should be noted that 'template' is essentially a metaphor for the internal representation of the memory of the tutor song (Bolhuis and Gahr, 2006), and not identical with the neural substrate or 'engram' of this memory. During the sensorimotor phase the young bird starts to vocalise, and it is thought that its song output is matched with the template that was formed in the memorisation phase. When birds are surgically deafened at this stage, they cannot match their own vocal output to the template, and eventually they will sing a rudimentary song that bears little resemblance to that of the tutor (Konishi, 1965; Fig. 1). Birds that are reared normally will eventually sing a crystallised song (Fig. 1) that, in the case of age-limited learners such as the zebra finch, does not change substantially during adulthood (Catchpole and Slater, 2008; Moorman and Bolhuis, 2013).

2.2. Parallels with human speech acquisition

Behaviourally, there are at least three ways in which song learning in songbirds and speech acquisition in human infants are similar (Bolhuis et al., 2010; Moorman and Bolhuis, 2013; Bolhuis and Everaert, 2013). First, both human infants and songbirds acquire their speech and song repertoire, respectively, through a combination of predispositions and learning. Second, in both songbirds and humans there is a sensitive period early in development, during which auditory–vocal learning is optimal. Third, in both human infants and juvenile songbirds there is a transitional phase at the start of vocal production, when the vocalisations are quite different from those of adult conspecifics. During this transitional phase, which is called 'babbling' in human infants and 'subsong' in songbirds, the young individual's vocalisations gradually come to resemble the adult form.

2.3. Linguistic parallels between songbirds and humans?

Birdsong and human spoken language both involve complex, patterned vocalizations (Berwick et al., 2011, 2012; Moorman and Bolhuis, 2013; Bolhuis and Everaert, 2013). An essential aspect of human language is syntax, defined by Berwick et al. (2011) as "the rules for arranging items (sounds, words, word parts or phrases) into their possible permissible combinations in a language". It is thought that human language can be distinguished from animal vocalisations by its syntactic complexity, where hierarchies can be assembled by combining words and word parts into higher-order phrases and entire sentences (Berwick et al., 2011, 2013). There is no a priori reason why a version of the combinatorial computational system that is characteristic of human language could not have evolved in non-human animals, either through common descent (e.g. in apes) or through convergent evolution (e.g. in songbirds or parrots) (Berwick et al., 2013; Bolhuis et al., 2014). Although the auditory–vocal domain is just one possible external

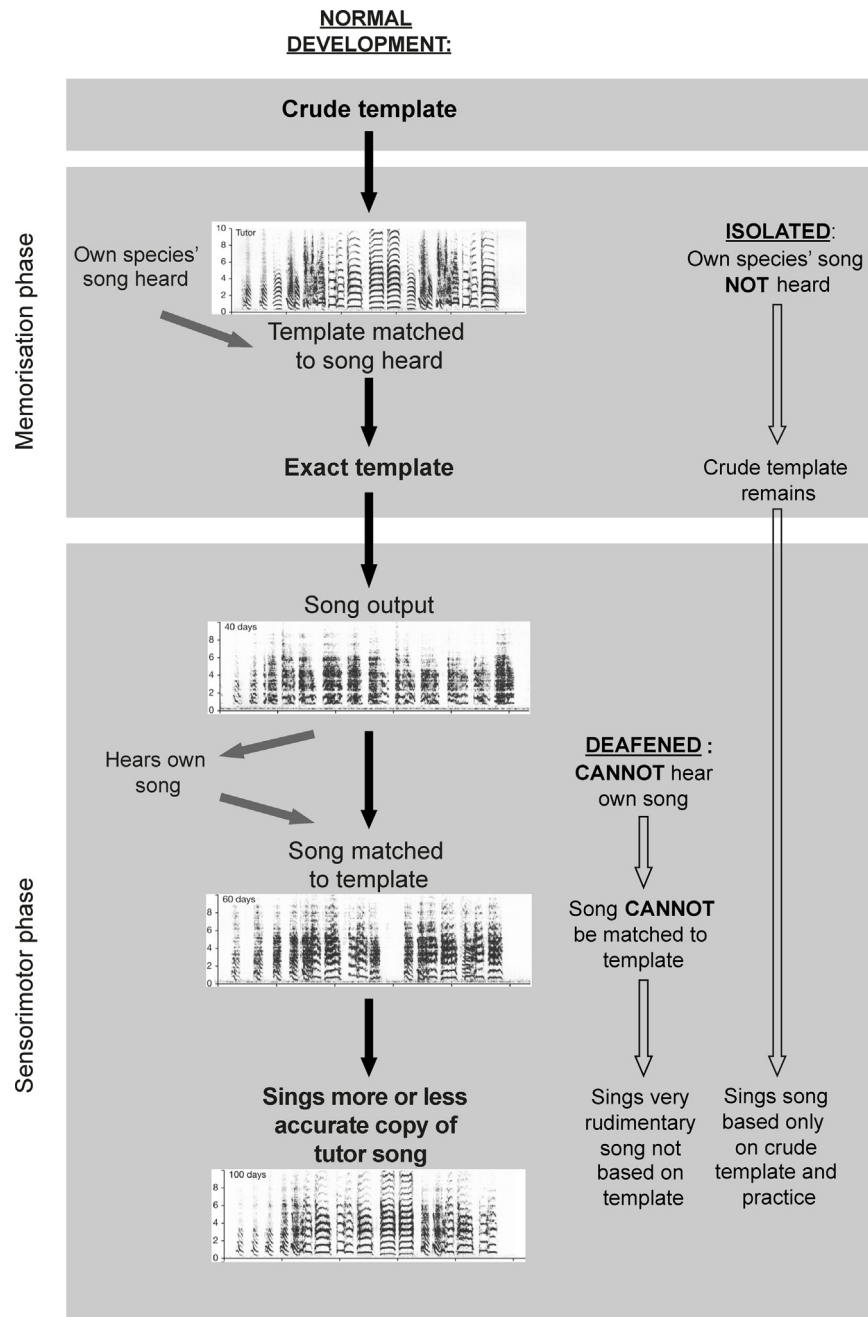


Fig. 1. Template-guided song learning. Stages in song development in some songbird species, and their presumed underlying mechanisms. Diagrams are sonograms (frequency over time) of the song of a zebra finch at different stages of development.

After Bolhuis (2005), adapted from Slater (1983) and Bolhuis and Gahr (2006), with permission.

interface for language (with signing being another), it could be argued that the strongest animal candidates for human-like syntax are songbirds and parrots. The songs of songbirds also consist of discrete acoustic elements that occur in a certain temporal order. Individual notes can be combined as particular sequences into syllables, syllables into ‘motifs’, and motifs into complete song ‘bouts’ (Moorman and Bolhuis, 2013). In principle, variable song element sequences may be governed by sequential syntactic rules, as shown e.g. in the work of Okanoya (2004). Marler (1977) has called this ‘phonological syntax’. Recent claims that songbirds may have the ability to acquire context-free syntactic rules (Gentner et al., 2006; Abe and Watanabe, 2011) have been challenged (Beckers et al., 2012; Everaert and Huybrechts, 2013), and Beckers et al. (2012) concluded that, although there is clearly evolutionary

convergence between humans and songbirds regarding processes of auditory–vocal learning, birdsong is not a credible model for the study of the mechanisms of human language syntax. Extant evidence shows that birdsong does not have the combinatorial complexity that is characteristic of human language syntax (Berwick et al., 2011, 2013; Bolhuis et al., 2014).

3. Birdsong and the brain

3.1. Songbird neuroanatomy

The songbird brain contains a number of brain regions that are involved in song perception, production and learning (see Fig. 2). Essentially, birdsong involves three interconnected neural

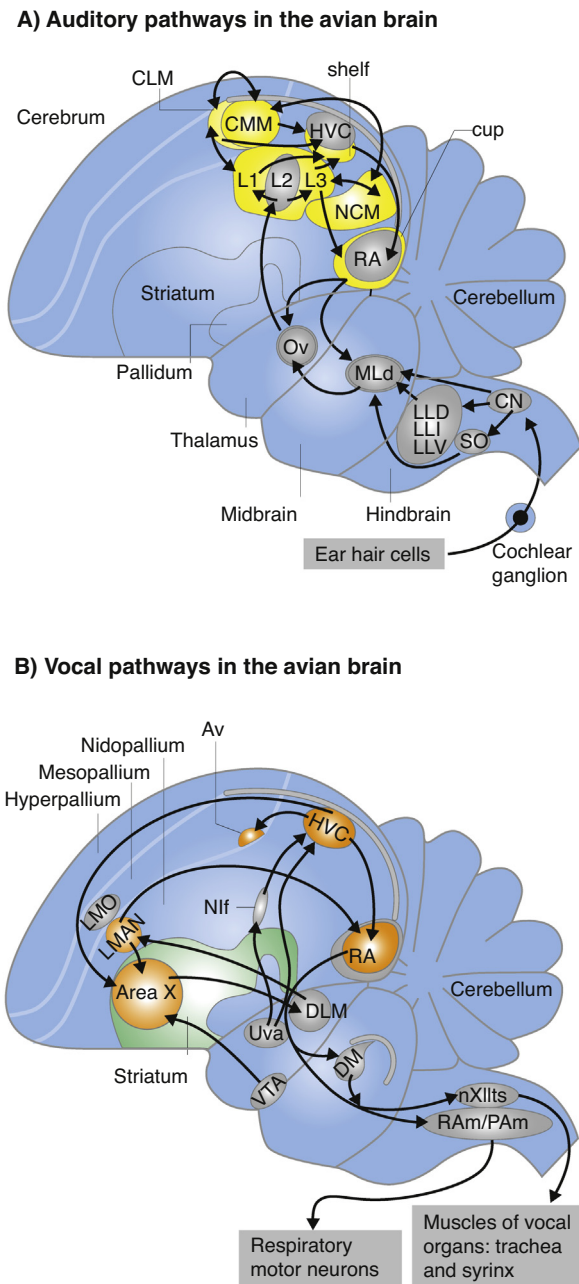


Fig. 2. Schematic views of the songbird brain. Two parasagittal sections show auditory (A) and vocal motor (B) pathways, indicating positions of brain regions and nuclei. (A) Brain regions depicted in yellow show increased neuronal activation when the bird hears song. The NCM and CMM regions are assumed to contain the neural substrate for tutor song memory. (B) The orange nuclei show increased neuronal activation when the bird is singing. Depicted are connections between the nuclei, of both the SMP and the anterior forebrain pathway (AFP). Area X, Area X of the striatum; Av, avalanche; CLM, caudolateral mesopallium; CN, cochlear nucleus; DLM, medial subdivision of the dorsolateral nucleus of the anterior thalamus; DM, dorsomedial subdivision of nucleus intercollicularis of the mesencephalon; HVC, a letter-based name; L1, L2 and L3 are subdivisions of Field L; LLD, lateral lemniscus, dorsal nucleus; LLI, lateral lemniscus, intermediate nucleus; LLV, lateral lemniscus, ventral nucleus; LMAN, lateral magnocellular nucleus of the anterior nidopallium; LMO, lateral oval nucleus of the mesopallium; MLd, dorsal part of the lateral nucleus of the mesencephalon; Nif, interfacial nucleus of the nidopallium; nXIIIts, tracheosyringeal portion of the nucleus hypoglossus (nucleus XII); Ov, nucleus ovoidalis; PAm, nucleus paraambiguous medullaris; RA, robust nucleus of the arcopallium; RA/PA/Am, nucleus retroambiguous medullaris; SO, superior olive; Uva, nucleus uvaeformis; VTA, ventral tegmental area.

Reproduced, with permission, from Moorman et al. (2011).

networks (Bolhuis and Gahr, 2006; Bolhuis et al., 2010; Moorman et al., 2011). First, secondary auditory regions, including the caudomedial nidopallium (NCM) and caudomedial mesopallium (CMM; Fig. 2A), are involved in song perception and are important for the recognition of tutor song (Bolhuis and Gahr, 2006). Second, the anterior forebrain pathway (AFP) is essential for sensorimotor learning and adult song plasticity (Doupe et al., 2005). The AFP is an anterior cortical–basal ganglia–thalamic loop that originates in HVC (acronym used as a proper name) and passes through Area X (part of the avian basal ganglia, Doupe et al., 2005), the thalamic nucleus dorsolateral anterior, pars medialis (DLM) and the lateral magnocellular nucleus of the anterior nidopallium (LMAN), and eventually connects with the motor pathway at the nucleus RA (Fig. 2B). Third, the song motor pathway (SMP) is involved in song production and certain aspects of song learning (Fig. 2B; Brainard and Doupe, 2000; Mooney, 2009). The SMP is a posterior motor pathway connecting the HVC, the robust nucleus of the arcopallium (RA) and the tracheosyringeal portion of the nucleus hypoglossus (nXIIIts). Together, the SMP and AFP pathways are commonly called the ‘song system’.

3.2. In search of the birdsong engram

3.2.1. The song system and tutor song memory

For some time after it had first been identified, the song system (Fig. 2B) was thought to contain the neural substrate for song memory, including memory of the tutor song (reviewed in Bolhuis and Gahr, 2006). This hypothesis was based on the results of experiments using electrolytic lesions. Lesions to nuclei in the anterior forebrain pathway (AFP), including LMAN and Area X, do not affect adult song, but cause abnormal song development in young zebra finches (Bottjer et al., 1984; Sohrabji et al., 1990; Scharff and Nottebohm, 1991). Juvenile male songbirds with lesions in Area X do not develop crystallised songs, while juveniles with lesions in LMAN produce aberrant but stable songs. These findings suggested that the AFP may contain the neural substrate for auditory memory (Basham et al., 1996; Nordeen and Nordeen, 2004). It is difficult to estimate how much birds that received permanent lesions to the AFP have memorised from their tutor because they never develop normal adult song. The impaired strength of song learning found in these birds could thus be the result of deficits in auditory learning and/or sensorimotor learning (Bolhuis and Gahr, 2006).

To circumvent the problems attached to permanent lesions, the song system nucleus LMAN was temporarily and reversibly inactivated in a series of studies concerning the role of *N*-methyl-D-aspartate (NMDA) receptors in birdsong (Aamodt et al., 1995, 1996; Basham et al., 1996; Heinrich et al., 2003; see Nordeen and Nordeen, 2004, for a detailed discussion of this work). In zebra finches, NMDA receptor binding in LMAN peaks at 30 days after hatching and subsequently declines – a decline that also occurs in socially isolated males (Aamodt et al., 1995). Basham et al. (1996) inactivated LMAN by injections with an NMDA receptor blocker on days that birds were exposed to tutor song in the sensorimotor phase of song learning, but not in between tutoring sessions. Therefore, normal sensorimotor learning in between sessions should have been unaltered while only auditory learning during tutoring sessions should have been affected. Song learning was significantly impaired in birds that received the experimental treatment, consistent with the suggestion that the AFP is involved in memorisation of tutor song. However, in zebra finches, there is overlap between the memorisation phase and the sensorimotor learning phase. Thus, Bolhuis and Gahr (2006) have argued that, because the experiments by Basham and colleagues were conducted in the sensorimotor phase, it is not clear whether NMDA receptor blocker infusion affected sensorimotor integration or the formation of auditory memory. In addition, in these studies only the LMAN was targeted, not other nuclei within

the song system or regions outside it. Thus, the exact role of the AFP in song learning and possibly memorisation of tutor song remains unclear.

Further support for the suggestion that the song system would contain the neural substrate of tutor song memory came from a series of electrophysiological studies. In songbirds, neurons in nuclei of the song system – in particular, LMAN, Area X, HVC and RA (see Fig. 2B) – are responsive to song, particularly to conspecific song (Solis et al., 2000). In adult males, neurons in these regions respond more 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). In LMAN and Area X, some neurons respond equally well to the BOS and tutor song, while a small proportion respond more to the tutor song (Solis et al., 2000). Volman (1993) found that in juvenile white-crowned sparrow males (*Zonotrichia leucophrys*) – a species in which the memorisation and sensorimotor phases are separated in time – neurons in the HVC in the memorisation phase showed no preference for songs with which the birds were tutored. Later, in the sensorimotor phase when the males started to sing themselves, HVC neurons showed preferential responding to the BOS. It is important to realise that these early electrophysiological studies used anaesthetised birds. Theunissen et al. (2004) review evidence that shows that song system neurons are considerably less responsive to song in awake zebra finches (e.g. Schmidt and Konishi, 1998; Dave et al., 1998).

An electrophysiological analysis involving unanaesthetized juvenile zebra finches suggests that neurons in the HVC show transient preferential responding to the song of their tutor (Nick and Konishi, 2005a). In the early sensorimotor period (35–69 days after hatching), responsiveness of HVC neurons was greater to tutor song than to the BOS, novel song, heterospecific song or white noise. During the late sensorimotor phase, more than 70 days after hatching, and into adulthood, the preference of HVC neurons for tutor song had 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. Taken together, these electrophysiological findings suggest that nuclei in the song system are responsive to song, particularly BOS. While juvenile zebra finch males may show preferential responding of HVC neurons to tutor song early in the sensorimotor period, these findings do not support the presence of a neural representation of the tutor song in the song system of adult birds.

With regard to the role of the song system in song memory, Bolhuis and Gahr (2006) suggested that a distinction be made between a representation of the auditory memory of the tutor song and the motor memory of the bird's own song, as many authors – explicitly or implicitly – have done (e.g., Doupe and Kuhl, 1999; Nottebohm, 1981, 2000; Solis et al., 2000; Marler and Doupe, 2000; Nick and Konishi, 2005a,b). Bolhuis and Gahr (2006) have argued that these two aspects of song acquisition may be associated with the memorisation phase and the sensorimotor phase, respectively. In addition, these authors suggested that the neural representation of motor memory involves nuclei in the song system, while regions in the caudomedial pallium contain the neural substrate of tutor song memory. It then becomes important to make explicit which of these putative memory mechanisms is being investigated. With regard to the role of the song system, evidence to support Bolhuis and Gahr's (2006) hypothesis was obtained in a recent study by Bolhuis et al. (2012). These authors exposed adult zebra finch males to either tutor song, to their own song, or to novel conspecific song. Neuronal activation (measured as IEG expression) was measured in the song system nuclei HVC, RA, and Area X (Fig. 2B). There were no significant differences in overall Zenk expression between the three experimental groups. However, IEG expression in the HVC was significantly positively correlated with the strength of song learning

only in the group that was exposed to the bird's own song, not in the other two groups. These findings, combined with the results of a number of electrophysiological studies, discussed above, led Bolhuis et al. (2012) to suggest that HVC may contain a neural representation of a memory of the bird's own song, that may be formed during juvenile song learning and guide the bird's vocal output.

Electrophysiological studies have shown that neurons in the song system nuclei HVC, LMAN, Area X and RA are activated when the bird is singing (Margoliash, 1986; Solis et al., 2000). In addition, there is increased expression of immediate early genes (IEGs, used as markers for neuronal activation; Mello and Clayton, in this issue) in these nuclei when the bird is singing (Jarvis and Nottebohm, 1997), but not when it hears song (Mello et al., 1992; Jarvis and Nottebohm, 1997), including tutor song (Bolhuis et al., 2000, 2012). These findings, combined with the absence of learning-related IEG expression in the song system after exposure to tutor song (Bolhuis et al., 2000, 2012) and the electrophysiological findings by Nick and Konishi (2005a), are not consistent with the hypothesis that the song system of adult songbirds contains the neural substrate of tutor song memory. Rather, the results suggest that these brain nuclei are either involved in song production only, or, in addition, in processing the auditory feedback of songs that occurs during the sensorimotor phase of song learning, which can also occur in adult songbirds (Brainard and Doupe, 2000, 2002). In that 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 caudomedial pallium, as discussed in the following section.

3.2.2. The caudomedial pallium and tutor song memory: the NCM

A large and growing body of evidence, much of it based upon gene expression studies (see Mello and Clayton, in this issue) suggests that not the song system, but rather regions in the caudomedial forebrain may contain the neural substrate for tutor song memory. Exposure to conspecific song in zebra finches or canaries led to increased IEG expression in the NCM and the CMM (see Fig. 2A; Mello et al., 1992; Mello and Clayton, 1994; Bolhuis et al., 2000). IEG expression was greatest when birds were exposed to conspecific song, compared to heterospecific song or pure tones (Mello et al., 1992). The fact that such activation was not seen in song system structures (as described earlier; Jarvis and Nottebohm, 1997; Bolhuis et al., 2000) suggests a dissociation between forebrain regions that are activated when the bird hears song, and those that are activated when the bird is singing itself. 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 (Bolhuis, 1994; Solis et al., 2000; Nottebohm, 2000).

Initial evidence suggesting a role for the NCM as (part of) the neural substrate for song memory was provided by studies that investigated habituation-like processes. Repeated exposure to the same song leads to a decrease in expression of the immediate early gene *ZENK* (Mello et al., 1995) and to decreased electrophysiological responsiveness in the NCM (Chew et al., 1996). This habituation is specific for the learned stimulus and its maintenance depends on gene expression induced locally in the NCM by song stimulation, based on the inhibitory effect of RNA or protein synthesis blockers within specific time windows after song presentation (Chew et al., 1995). Chew et al. (1996) concluded that it is likely that “the NCM is specialised for remembering the calls and songs of many individual conspecifics”.

The hypothesis that, more specifically, the NCM contains (part of) the neural substrate for tutor song memory has received support from a number of studies from our laboratory. In a series of experiments, we demonstrated a significant correlation between neuronal activation in the NCM of adult male zebra finches and the strength of song learning, measured as the number of song

elements that had been copied from the tutor song (Bolhuis et al., 2000, 2001; Terpstra et al., 2004; see Fig. 3A). In one study, we 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 (Bolhuis et al., 2000). 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 CMM, but not in two nuclei in the 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 from the tutor song and the expression of both Fos and Zenk protein in the NCM, but not in the CMM. The positive correlation between the strength of song learning and IEG expression in the NCM of adult zebra finches has been replicated twice (Bolhuis et al., 2001; Terpstra et al., 2004).

The studies that led to the identification of the NCM as a possible locus of memory storage were conducted in adult zebra finches. Presumably, the neural representation of tutor song memory is being formed when the animals are exposed to tutor song as juveniles, during the memorisation phase. In addition, during the sensorimotor phase, when the young bird's vocal output is thought to be matched with the stored representation of tutor song or template (Konishi, 1965), development of the bird's own song may involve access to this representation. Gobes et al. (2010) tested this hypothesis. They found that in juvenile zebra finches that are in the middle of the sensorimotor learning period, IEG expression in the NCM was greater after exposure to tutor song than after exposure to novel song. The juveniles' songs in this study already resembled the tutor songs to some degree, and thus this finding is consistent with the suggestion that the NCM is involved in tutor song recognition memory (Bolhuis and Gahr, 2006). Interestingly, IEG expression measured during sleep at night correlated with the strength of song learning (Gobes et al., 2010; see Fig. 3C). The latter finding suggests that sleep may be important for the formation of tutor song memory (Gobes and Bolhuis, 2008; cf. Vorster and Born, in this issue; see Section 3.3).

As we have seen, expression of the IEG *ZENK* in the NCM is regulated by song at the time of acquisition of the auditory memory of the tutor song (Jin and Clayton, 1997; Stripling et al., 2001; Gobes et al., 2010). Upon cellular depolarization, several intracellular signalling pathways are activated that could result in *ZENK* induction. For example, the extracellular-signal-regulated kinase (ERK) – also known as mitogen-activated protein kinase (MEK) – is activated in the caudal pallium when birds are exposed to song playbacks (Cheng and Clayton, 2004). This pathway is required for *ZENK* induction, since local injections of an inhibitor that blocks both MEK and (downstream) mitogen activated protein kinases (MAPKs) activation can downregulate the responses of *ZENK* to song (Cheng and Clayton, 2004; Velho et al., 2005). London and Clayton (2008) provided juvenile birds with bilateral cannulae into the auditory lobule (comprising NCM, CMM and field L; Fig. 2) that could administer ERK-inhibitor drug U0126 at separate, specific time intervals. With some variation in the precise location, most cannulae were implanted in the NCM. The juvenile zebra finches were raised in the absence of a tutor, and exposed to song playbacks from tape for one-and-a-half hours per day, for ten days during the sensorimotor learning phase. Using this method, the control birds learned to sing a good copy of the tutor song. Birds that received U0126 infusions during tutor song playbacks, however, did not imitate the tutor song (Fig. 4C). To test whether this could be due to a general disruptive effect of U0126 on NCM functioning, the authors also housed birds with the tutor for ten days. Each day, they gave a drug injection, similar to the experimental birds, but these control birds could also hear the tutor song after the drug washed out. As expected, they did produce a good imitation of the tutor song

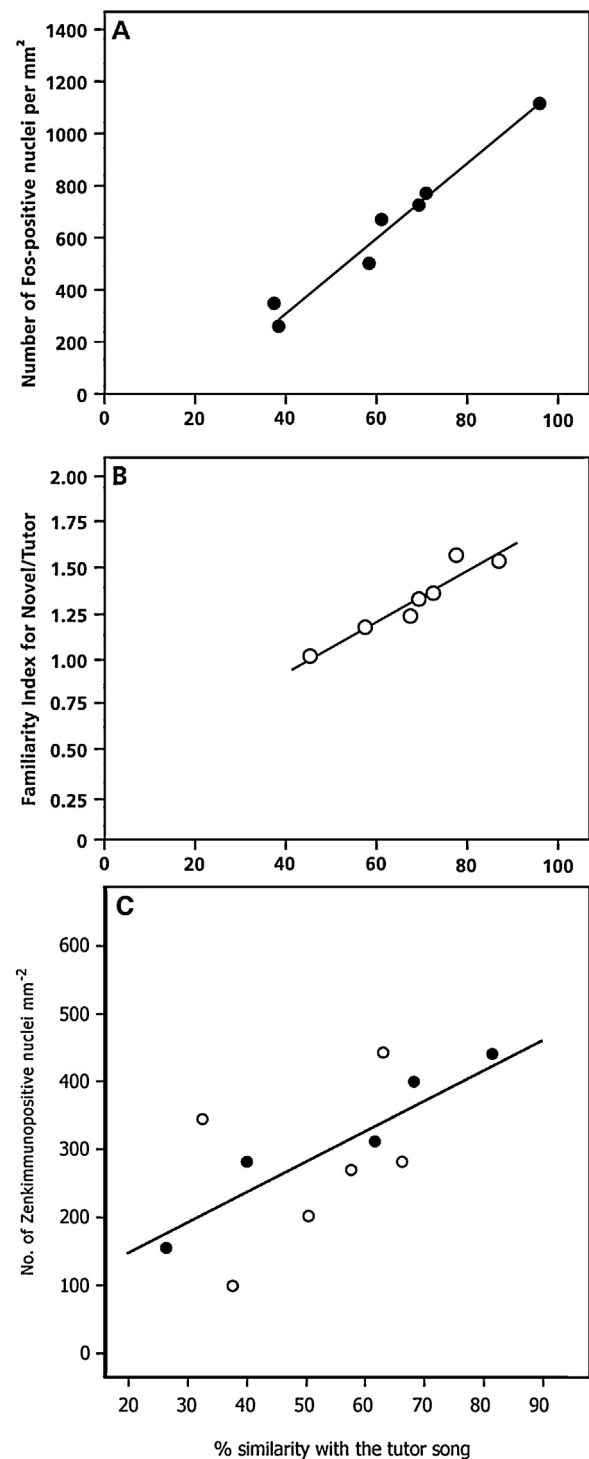


Fig. 3. Relationship between neuronal activation in the NCM and the strength of song learning (measured as percentage of song elements copied from the tutor song) in zebra finch males. (A) Relationship between the number of cells immunopositive for Fos (the protein product of the immediate early gene *c-fos*) and the strength of song learning, in zebra finch males that had been re-exposed to the tutor song. Adapted from Bolhuis et al. (2001), with permission. (B) Relationship between the familiarity index (FI) and the strength of song learning in male zebra finches. The FI is a measure of the relative habituation rate of neurons in the NCM. Adapted from Phan et al. (2006), with permission. (C) Correlation between the number of cells immunopositive for Zenk and the strength of song learning, in juvenile zebra finch males that were asleep. Open circles represent birds that heard novel song during the day (before sleep), filled circles represent birds that did not hear any stimulus during the day. The trend line is for the correlation of the whole sample. Adapted from Gobes et al. (2010), with permission.

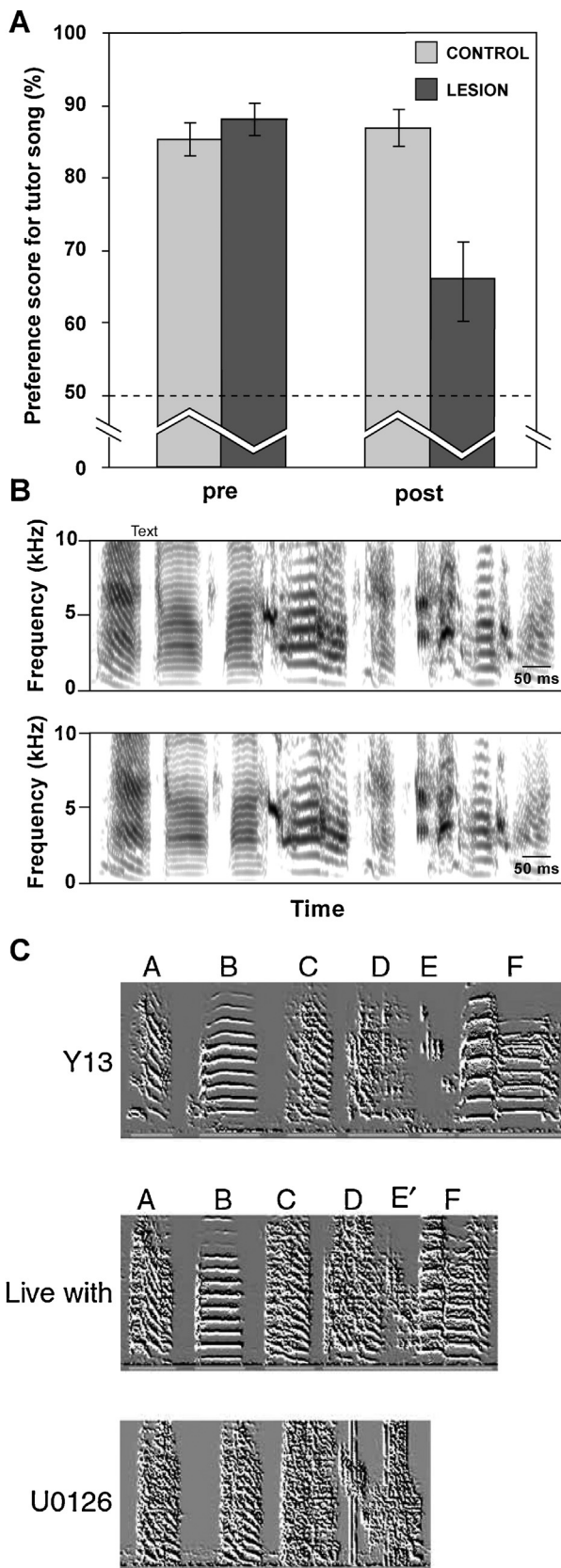


Fig. 4. Neural dissociation between birdsong recognition and production in the zebra finch. (A) Lesions to the NCM impair song recognition in zebra finch males. Song preference scores (expressed as a percentage) were measured by calculating the amount of time spent near a speaker that broadcast the song of the bird's tutor compared to a speaker that broadcast a novel zebra finch song. Before surgery ("pre"), birds in both groups showed a strong preference for the song of the tutor

(Fig. 4C). Also, U0126 did not inhibit general auditory processing, as was confirmed in an operant discrimination task. In conclusion, the authors showed that when ERK signalling is inhibited in the NCM during song learning in juvenile birds, tutor song imitation is impaired (London and Clayton, 2008).

Phan et al. (2006) made use of the neuronal habituation to song that had been demonstrated in the NCM (Chew et al., 1996). These authors found that neurons in the NCM of adult zebra finch males showed steeper rates of habituation to novel song than to the tutor song. 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 the familiarity index of NCM neurons and the strength of song learning (Fig. 3B).

Apart from songbirds, hummingbirds and parrots are the other avian taxa known to be vocal imitators (Moorman and Bolhuis, 2013). Eda-Fujiwara et al. (2012) investigated the neural mechanisms of auditory discrimination learning in the budgerigar (*Melopsittacus undulatus*), a parrot. Like other parrots, budgerigars can learn to produce human words. The authors investigated neuronal activation, measured as expression of the IEG *ZENK*, in relation to auditory discrimination learning of Japanese words by budgerigar males. The males successfully learned to discriminate two Japanese words produced by another male conspecific. Re-exposure to the two discriminanda led to increased neuronal activation in the caudomedial pallium (particularly the NCM and the CMM), but not in the hippocampus, compared to untrained birds that were exposed to the same words, or were not exposed to words. In addition, neuronal activation in the caudomedial pallium of the experimental birds correlated significantly and positively with the percentage of correct responses in the discrimination task. These results suggest that in a parrot, the NCM and the CMM are involved in auditory learning and memory, as they are in songbirds (Eda-Fujiwara et al., 2012).

If the NCM of zebra finch males were to contain (part of) the neural substrate of tutor song memory, lesions to this structure should impair recognition of the tutor song. This is precisely what Gobes and Bolhuis (2007) found when they had placed small neurotoxic lesions in the NCM of adult male zebra finches (Fig. 4). While sham-operated controls continued to show a strong behavioural preference for the tutor song over a novel song, this preference was significantly impaired in birds with lesions to the NCM. At the same time, song production was completely unimpaired in the lesioned animals as it was in the controls (Gobes and Bolhuis, 2007).

3.2.3. The caudomedial pallium and tutor song memory: the CMM

The CMM is also involved in song perception and discrimination in songbirds (Mello et al., 1992; Bolhuis et al., 2000; Avey et al., 2005; Bailey and Wade, 2005) as well as in parrots (Eda-Fujiwara et al., 2012; see Section 3.2.2). Although zebra finch females do not sing, they do form a memory representation of the tutor song, just like males. When males and females were exposed to tutor song and unfamiliar song in a preference test, both males and females preferred to hear the tutor song (Riebel et al., 2002). In adult female

over a novel song. After surgery ("post"), birds in the sham-operated group had maintained their preference, while this was significantly impaired in the group that received lesions to the NCM. (B) Representative spectrograms of the song of a zebra finch male before (top) and after (bottom) surgery show that song production was not altered by lesions to the NCM. (C) Spectrograms of a tutor song (top, bird Y13), a control bird that was raised with the tutor and imitated song well (middle) and a bird in which ERK signalling was blocked during tutoring with compound U0126, and did not imitate the tutor song (bottom).

A and B were adapted, with permission, from Gobes and Bolhuis (2007). C was adapted, with permission, from London and Clayton (2008).

zebra finches, not only the NCM is activated in response to tutor song, but also the CMM (Terpstra et al., 2006). Terpstra et al. (2006) investigated the neural substrate of tutor song memory in female zebra finches. Female zebra finches were reared with their fathers. When they were adult, the females showed a significant preference for the song of their fathers, indicating that they had learned the characteristics of this song. There was significantly increased neuronal activation (measured as IEG expression) in the CMM (but not the NCM or the hippocampus) when birds were re-exposed to their father's song, compared to females that were exposed to novel zebra finch song. These results suggest that in female zebra finches, the CMM may be (part of) the neural substrate for the memory of the father's song.

It has been suggested that tutor song memory in females functions as a reference frame or guidance for mate selection, perhaps as a way to recognise singers' reproductive quality or to recognise the local song when there is geographically based variation between conspecific songs (Hernandez et al., 2008; Riebel, 2003). Thus, it is expected that the CMM would play a role in mate selection. A lesion experiment suggested that this is indeed the case. Lesions in the CMM caused female zebra finches to perform courtship displays to heterospecific song, which they normally only do in response to conspecific song (MacDougall-Shackleton et al., 1998). In female canaries, zenk expression in the CMM was greater when the birds heard songs containing so-called 'sexy' syllables (canary syllables of which it is known that they are attractive to females), than when they heard 'non-sexy' syllables (Leitner et al., 2005). However, when comparing conspecific and heterospecific songs, there was neuronal activation in the CMM in response to both song types, while neurons in the NCM selectively responded to conspecific song (Bailey et al., 2002; Theunissen et al., 2004). In addition, in female starlings that were presented with attractive longer motifs or less attractive short ones, there was differential activation in the NCM, but not in the CMM (Gentner et al., 2001). Woolley and Doupe (2008) found significantly greater neuronal activation in the CMM of female zebra finches that heard directed song than undirected song. Directed song is the song that a male sings to a female; it is the same song that a male always sings (zebra finches have only one song), but it is sung faster and with less spectral variability than undirected song. Females preferred directed song over undirected song in a preference test (Woolley and Doupe, 2008). Female songbirds respond preferentially to more complex song (Searcy, 1992; Eda-Fujiwara et al., 2003; Bolhuis and Eda-Fujiwara, 2003, 2010). 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, 2008; Searcy and Yasukawa, 1996). Sockman et al. (2002) exposed female starlings to 1 week of long-bout or short-bout song experience and then examined IEG response in the caudomedial pallium to a novel long-bout or novel short-bout song stimulus. These authors found that, overall, long-bout songs elicited significantly greater IEG expression than short-bout songs, in both the NCM and the CMM. In addition, IEG expression in response to long-bout songs was greater in females with long-bout experience than in females with short-bout experience. Eda-Fujiwara et al. (2003) investigated the effect of song complexity on neuronal activation in the caudomedial pallium of female budgerigars. Neuronal activation in the NCM (and to a lesser extent in the CMM) correlated significantly and positively with the number of different elements to which the females were exposed. Taken together, these results suggest that the NCM and the CMM are involved in the perception of song complexity in female birds.

Kato et al. (2010) found that in female Bengalese finches (*Lonchura striata domestica*), both the NCM and the CMM showed significantly greater IEG expression to their father's song compared to novel conspecific song. In male Bengalese finches (Kato et al.,

2012) and male zebra finches (Terpstra et al., 2004; Bauer et al., 2008), the CMM was strongly responsive to BOS. Furthermore, neurons in the CMM were selectively activated by auditory perception of learned conspecific vocalisations in European starlings (Gentner and Margoliash, 2003; Gentner et al., 2004). In male European starlings that were trained in an auditory discrimination task, the CMM, but not the NCM, was activated during task performance. Interestingly, when the birds learned to discriminate two novel songs (same task, new stimuli), both the CMM and NCM were strongly activated (Gentner et al., 2004). The authors concluded that the CMM is important for long-term memory, while the NCM plays a different role, and is also involved in the perception of novel songs.

Overall, the results suggest that the NCM and the CMM are involved both in processing of perceptual information concerning song complexity and in storage of song memory in songbirds and parrots (for reviews see Bolhuis and Eda-Fujiwara, 2003, 2010; Bolhuis and Gahr, 2006; Bolhuis et al., 2010).

3.3. The role of sleep in birdsong learning and memory

3.3.1. Sleep is important for memory consolidation

It is well established that sleep plays an important role in learning and memory in humans and other mammals (for reviews, see Walker and Stickgold, 2004; Stickgold and Walker, 2005; Diekelmann and Born, 2010). More recently, it has become apparent that sleep is also important for early learning and memory formation in birds (e.g., Dave and Margoliash, 2000; Jackson et al., 2008; Gobes et al., 2010; see also Gobes and Bolhuis, 2008; Rattenborg et al., 2009; Vorster and Born, in this issue; Moorman and Nicol, in this issue). Gabriel Horn and colleagues have shown that in domestic chicks, a period of sleep immediately after visual imprinting training is necessary for memory consolidation to occur (Jackson et al., 2008). In zebra finches, Derégnaucourt et al. (2005) showed that sleep is important for song learning (see Section 3.3.2). Similarly, it was shown that sleep enhances consolidation of auditory memory in European starlings (Brawn et al., 2010, 2013).

Gobes et al. (2010) investigated spontaneous neuronal activation (measured as IEG expression) in the forebrain of juvenile zebra finch males during sleep. In particular, these authors measured IEG expression in the NCM and CMM in the caudomedial pallium, and in the song system nuclei HVC and RA (Fig. 2). The males were in the middle of the sensorimotor song learning period. In males that had not been exposed to tutor song during the previous day, spontaneous neuronal activation during sleep correlated with the number of song elements that had been copied from the tutor song (Fig. 3C). In a subsequent study, Moorman et al. (submitted for publication; cf. Moorman and Nicol, in this issue) found that in the same animals, the degree of lateralisation of NCM neuronal activation was related to the strength of song learning. In addition, these authors found that, independent of the stimulus to which the animals were exposed, the left HVC was activated when the animals were awake during the day, whereas the right HVC was active when the animals were asleep at night.

The transient preferential responsiveness of HVC neurons to tutor song that was reported by Nick and Konishi (2005a; see Section 3.2.1) was measured in awake zebra finches. When the same birds were sampled when they were asleep, HVC neurons responded preferentially to BOS throughout. Thus, as the authors concluded, the responsiveness of neurons in HVC during the early sensorimotor period (up to 70 days after hatching) is state-dependent. That is, in these juvenile males, HVC neurons preferentially respond to tutor song during wakefulness and to BOS when they were asleep.

Taken together, the results of the studies by Moorman et al. (2014) and Nick and Konishi (2005a) suggest that during the (early) sensorimotor period, both the NCM and HVC exhibit different

patterns of activation during sleep and wakefulness. A possible scenario would be that during sleep, there is consolidation of tutor song memory in the NCM (measured as spontaneous learning-related neuronal activation), and through interaction with HVC a motor representation of the bird's own song might be formed. In contrast, during wakefulness, the tutor song memory representation in the NCM can only be addressed by exposure to the tutor song. Furthermore, apparently a transient representation of the tutor song is necessary in HVC in order to form a motor representation of the bird's own song.

3.3.2. Neural mechanisms of memory consolidation during sleep

An interesting phenomenon observed during sleep is neuronal replay: brain structures that were active during memory encoding are also activated during sleep, with similar temporal activation patterns (Pavlidis and Winson, 1989; Wilson and McNaughton, 1994; Peigneux et al., 2001; Dave and Margoliash, 2000). These activation patterns might be a reflection of the process of memory consolidation (e.g., Hennevin et al., 2007). Sleep might allow for what has been termed “off-line processing” (Margoliash and Schmidt, 2010), which might involve dynamic changes in synaptic connectivity. ‘Synapse consolidation’ could involve synaptic strengthening by reactivating neuronal networks, and/or a reduction of synapses through synaptic pruning (Vassalli and Dijk, 2009). Recent studies have focused on the possible synaptic processes that may occur during neural replay during sleep, but results are considerably divergent for different areas of the brain, different sleep phases, and different behavioural contexts (Tononi and Cirelli, 2006; Vassalli and Dijk, 2009; Diekelmann and Born, 2010; Stickgold, 2013).

Birdsong learning may be a suitable animal model to investigate this problem. Zebra finches have sleep characteristics very similar to humans (Low et al., 2008), and the relationship between brain activity and behaviour (song) is easily quantified (e.g., Mooney, 2009; Margoliash, 2010). A number of studies have demonstrated neuronal replay during sleep in songbirds, in the song system nuclei RA (Dave and Margoliash, 2000; Shank and Margoliash, 2009), Area X (Yanagihara and Hessler, 2012), HVC (Hahnloser et al., 2006), and Nif (Hahnloser and Fee, 2007). As we have seen, neuronal activation during sleep was also demonstrated in the NCM, where the level of activation correlated positively with the strength of song learning (Gobes et al., 2010; Moorman et al., 2014; see Moorman and Nicol, in this issue).

During song learning, new synaptic connections might be formed, or existing synaptic connections might be strengthened, to encode the tutor song. If some of those synapses were removed during sleep during a process of synaptic pruning, one might expect song deterioration to be the result, i.e., poor song imitation or performance after sleep. However, since the capacity to form new synaptic connections is also increased by pruning, there is ample opportunity to form new connections and improve song imitation again. If not all synaptic connections disappear during sleep this process should lead to gradual strengthening of the neuronal network and improvement of song imitation. Indeed, in a behavioural study in juvenile songbirds it was found that there was a cycle in song learning in which song imitation improved during the day, while song performance had deteriorated the next morning, after a night of sleep. However, during the day song performance increased again, and each day it reached a higher level of tutor song imitation than it had on the day before (Derégnaucourt et al., 2005). Birds with the strongest song deterioration in the morning eventually sang the best imitations of the tutor song (Derégnaucourt et al., 2005). These behavioural findings are consistent with a mechanism of synaptic pruning during sleep.

In visual imprinting in chicks, Jackson et al. (2008) showed that a period of sleep immediately after imprinting training is necessary

for memory consolidation (Vorster and Born, in this issue). In an earlier electrophysiological study, Horn and collaborators (Horn et al., 2001; Horn, 2004) enquired whether there was evidence for strengthening Hebbian ‘neural assemblies’ (Hebb, 1949) in the IMM during imprinting. Such neural assemblies would involve functional coupling between neurons that respond to the imprinting stimulus. To this end, the authors computed cross-correlograms between simultaneously recorded pairs of neurons in the IMM. It was found that, although the number of neurons that responded to the imprinted stimulus increased after training, there was no increase in functionally coupled neuron pairs (Horn et al., 2001; Horn, 2004). Horn (2004) proposed that a large set of uncoupled neurons is likely to have a larger storage capacity than a highly interconnected set of neurons would have. As not all neurons are intrinsically activated by the imprinted stimulus, uncoupled neurons can transmit their signal to those neurons instead of to each other, and thereby increase neuronal responsiveness to the imprinted stimulus. Horn (2004) suggested that the neurons that are responsive to the imprinting stimulus might form a set of parallel, largely uncoupled elements that would provide a larger storage capacity than a system with tightly coupled elements.

As we have seen, birdsong learning results in increased responsiveness of neurons in the NCM (and possibly the CMM), specifically to tutor song (see Bolhuis and Gahr, 2006; Bolhuis et al., 2010, for reviews). This process is similar to what occurs in the IMM during filial imprinting (Horn, 2004). In both cases, sleep appears to be crucial for memory consolidation. Further research is needed to establish the nature of the neural representation of song memory in the caudomedial pallium as well as the song system in songbirds.

3.4. Analogies and homologies with the human brain

In humans, the neural substrate of motor representations of speech is considered to involve Broca's area in the inferior frontal cortex, while perception and memory of speech is thought to involve Wernicke's area and surrounding regions in the superior temporal cortex (Bolhuis and Everaert, 2013; Berwick et al., 2013). It should be noted that Broca's and Wernicke's areas serve more complex roles than just production or perception of speech, respectively. For example, Broca's area is activated during perception of syntactically complex sentences (Friederici, 2011; Berwick et al., 2013). There are many analogies and homologies between the brains of birds and mammals that have prompted a complete revision of the nomenclature of the avian brain (Jarvis et al., 2005). Similarities in connectivity and function would suggest at least analogies between the human neocortex and the avian pallium (including the hyperpallium, mesopallium, nidopallium and arcopallium; Bolhuis and Gahr, 2006; Bolhuis et al., 2010). Bolhuis and Gahr (2006) suggested that the NCM and the CMM may be analogous with the mammalian auditory association cortex. Doupe et al. (2005) have argued that the AFP loop in the song system bears strong similarities in connectivity, neurochemistry and neuron types to the mammalian basal ganglia, while both LMAN and HVC have been tentatively suggested to correspond functionally to Broca's area (see Bolhuis et al., 2010, for further discussion).

Apart from these neuroanatomical parallels, there is evidence for a similar neural dissociation between auditory recognition and vocal production regions in the brains of songbirds and humans (Bolhuis and Gahr, 2006; Gobes and Bolhuis, 2007; Bolhuis et al., 2010). As we have argued above, regions in the songbird caudomedial pallium (including the NCM) contain the neural representation of tutor song memory that juveniles acquire (Bolhuis and Gahr, 2006; Gobes and Bolhuis, 2007; Bolhuis et al., 2010; Gobes et al., 2010), whereas nuclei in the song system are required for sensorimotor learning and song production (Brainard and Doupe, 2000). In the case of human speech there is a similar dissociation between

Wernicke's area in the superior temporal lobe – involved in auditory perception and memory – on the one hand, and Broca's area in the inferior frontal lobe – involved in vocal production – on the other.

There is a similar human–avian parallel during speech and song acquisition. Human newborns show increased neural activity in the superior temporal lobe, but not in the inferior frontal cortex, in response to human speech (Imada et al., 2006). An fMRI study in 3-month old infants (which are in the 'cooing' stage of babbling in which syllables are not yet produced) showed activation in the superior temporal cortex (including Wernicke's area) as well as in Broca's area in response to hearing speech (Dehaene-Lambertz et al., 2006). Six- and twelve-month-old infants exhibited increased activation (compared to baseline) in both Wernicke's and Broca's areas when exposed to speech sounds (Imada et al., 2006). Taken together, these findings suggest that Wernicke's area is (part of) the neural substrate for speech perception in neonates and that Broca's area becomes active at a later stage, when infants start babbling (Bolhuis et al., 2010).

4. In search of the template: multiple memory systems?

We have reviewed evidence to suggest that regions in the caudomedial pallidum (in particular, the NCM and CMM) contain (part of) the neural substrate for tutor song memory (cf. Bolhuis and Gahr, 2006; Bolhuis et al., 2010). However, a number of recent studies suggest that nuclei in the song system may in fact contain the tutor song engram, either exclusively, or in addition to the caudomedial pallidum. Roberts et al. (2012) question whether the NCM is at all important for tutor song memory. When HVC activation was disrupted in juvenile zebra finches during tutoring, the birds were unable to copy the tutor song. This was due to disrupted HVC activation during tutoring, rather than missing time to practise their own song: the juveniles were exposed to tutor song during short sessions each day, and when HVC was disrupted outside the tutoring sessions, song imitation was unaffected. Disruption of Nif activation also impaired tutor song imitation. Nif is the major source of auditory information to the HVC, and therefore, the experiments indicated that auditory information concerning the tutor song is needed in the HVC, while the tutor is singing, for proper song imitation (Roberts et al., 2012). It was therefore suggested that the HVC is the locus of tutor song memory storage (Roberts and Mooney, 2013). Indeed, these results suggest that activation of the HVC during tutoring is important for song imitation, i.e., to produce a song that resembles tutor song. However, it is unclear whether or not these juveniles formed a memory representation of tutor song. To further test whether HVC is the neural substrate of long-term tutor song memory, recognition tests would have to show that the juveniles in which HVC signalling was disrupted also do not recognise tutor song in behavioural preference tests (Gobes and Bolhuis, 2007).

With regard to the role of HVC in song learning, in Section 3.2.1 we discussed electrophysiological evidence to suggest that in awake juvenile zebra finches early in the sensorimotor phase, many HVC neurons were tutor song-selective (Nick and Konishi, 2005a). When the birds were older (late sensorimotor phase or adult), the response to tutor song had decreased substantially, but there was a strong response to the bird's own song (Nick and Konishi, 2005a). As we discussed in Section 3.3, when Nick and Konishi (2005a,b) recorded while the birds were asleep, HVC neurons were preferentially responsive to BOS during the whole sensorimotor period as well as during adulthood. During sleep, HVC responsiveness to BOS in juveniles was strongest to the BOS as recorded on the same day, while an earlier version of BOS that was recorded when the bird was younger did not evoke strong responses (Nick

and Konishi, 2005b). These findings suggest that in the zebra finch HVC, neuronal responsiveness to tutor song in juveniles is transient, and is replaced by responsiveness to the BOS that is also continuously updated to the most current version. Thus, song learning may involve a transient representation of tutor song in HVC, the formation of which may have been disrupted in the study by Roberts et al. (2012).

Swamp sparrows (*Melospiza georgiana*) sing a large repertoire of songs, and juveniles do not imitate all of the tutor songs as adults. Neuronal responsiveness to tutor songs was tested in the HVC of adult swamp sparrows, both to songs that the bird had imitated from his tutor, but also to tutor songs that were not part of the bird's own repertoire. HVC neurons were responsive to many of the tutor songs, even those that were never imitated (Prather et al., 2010). This suggests that in swamp sparrows, HVC contains a representation of auditory tutor song memory. It may be that HVC plays different roles in swamp sparrows and zebra finches. Perhaps the difference between the two species lies in the fact that the swamp sparrow is an open-ended learner that can sing many different songs, while the zebra finch is an age-limited learner with only one single song in its repertoire. As described above, when zebra finches have learned their song, HVC neurons are not selective for the tutor song any longer, but have become responsive to the BOS (Nick and Konishi, 2005a,b). In swamp sparrows, that can learn new songs throughout their lives, a neural representation of the memory of the tutor song might be maintained in HVC.

Thus, HVC is important for song production and tutor song imitation, and there may be a transient representation or neuronal selectivity for tutor song in the HVC. However, future studies have to shed light on whether the HVC is part of the neural substrate of tutor song memory. In swamp sparrows there was long-term tutor responsiveness (Prather et al., 2010). It would be interesting to investigate whether in swamp sparrows, HVC is involved in behavioural tutor song recognition. It may be that HVC activation is driven by inputs from higher-order auditory regions. Indeed, it was shown that auditory input from CMM (Bauer et al., 2008) drives auditory responses in HVC. Furthermore, it was shown that HVC song selectivity declined rapidly when oestrogen production in the NCM was inhibited (Remage-Healey, 2012). It is not known whether HVC neurons are also tutor-song selective when input from these higher-order auditory regions would be blocked.

Tutor song-selective neurons have also been found in Area X (Solis et al., 2000) and LMAN (Achiro and Bottjer, 2013) in juvenile male zebra finches. Interestingly, Area X and LMAN of juveniles contain both cells that are tuned to tutor song and cells that are tuned to BOS. However, tutor-song selectivity was greatly reduced in adults (Solis and Doupe, 1997; Achiro and Bottjer, 2013), while adult songbirds can still recognise tutor song. The fact that tutor song selectivity in LMAN disappears after song learning suggests that neither Area X nor LMAN are the major locus of tutor song memory. Rather, because of its importance in auditory feedback, these regions might be involved in comparing BOS to tutor song in order to improve song imitation in juveniles. Further evidence that LMAN contains a transient tutor song representation comes from an experiment in which juveniles were tutored with two different adults consecutively. LMAN neurons responded to the current tutor song, but not to the earlier tutor song (Yazaki-Sugiyama and Mooney, 2004). Why would LMAN have a transient representation of tutor song? In juveniles, LMAN is important for sensorimotor learning and vocal exploration (Bottjer et al., 1984; Scharff and Nottebohm, 1991; Brainard and Doupe, 2000; Ölveczky et al., 2005). Indeed, transiently blocking neuronal activation in LMAN during tutoring impaired song learning (Basham et al., 1996). It is not known, however, whether the birds could not memorise tutor song, or whether song production was impaired. Current evidence suggests that LMAN is probably not the neural substrate for tutor song

memory, but rather it is a brain region that is important for integration of auditory feedback, BOS and a tutor song representation, to improve song imitation in juveniles.

Recently, it was shown that the NCM is important for song restoration in adult zebra finches (Canopoli et al., 2014). This study used a paradigm of negative feedback during specific parts of the song. When white noise is played when an adult bird sings, the animal alters its song in order to avoid the interference. When after a training period the interference is withheld, birds quickly change their song back to the original song (e.g., Charlesworth et al., 2011). Canopoli et al. (2014) made large bilateral neurotoxic lesions to the NCM in adult zebra finches that had altered their song under auditory feedback manipulations. Control birds that received sham lesions quickly changed their song back to the original song after the manipulations stopped, similar to what was demonstrated earlier. However, birds that had received lesions to the NCM showed severely impaired song recovery, demonstrating that the NCM is important for returning to the original song. This suggests that the tutor song representation in the NCM is a reference model for accurate song production in adults. Alternatively, the NCM could contain a memory representation of the BOS, or the NCM might be important in an auditory feedback pathway that allows the bird to detect aberrant song production and guides restoration of the song.

Miller-Sims and Bottjer (2014) recorded multi-unit activity from awake, restrained adult and juvenile male zebra finches, using single electrodes in one position in the NCM. They compared neuronal habituation (as in Phan et al., 2006) across the different ages. High rates of neuronal habituation (a steep decline from first to later response strength) have been found to reflect the novelty of the stimulus (Phan et al., 2006). In contrast to what was previously found in adults, the juveniles showed high habituation rates for tutor song, even though the song was already very familiar to them (Miller-Sims and Bottjer, 2014). Neurons in the NCM of juveniles were not selectively activated by tutor song, in contrast to what was found in adults. However, juveniles showed higher rates of neural habituation to tutor song than to other familiar conspecific songs, which suggests that the NCM has a special response for tutor song during song learning, in which the habituation rate does not decrease, even though the song is very familiar. For the special case of tutor song, memory formation might be more detailed and a stronger, long-term memory trace has to be formed. Therefore, the adult-typical reduction in habituation rate might only occur after the song has been fully and thoroughly memorised.

Recently, Yazaki-Sugiyama and colleagues (personal communication) investigated the responsiveness of neurons in the NCM to tutor song and to a number of alternative stimuli. Flecke and Yazaki-Sugiyama (manuscript in preparation) performed multi-electrode electrophysiological recordings in the dorsomedial part of the NCM of anaesthetised zebra finch males. The juvenile males were isolated from their father before 12 days after hatching. Starting at 55 days after hatching, the males were tutored by a male zebra finch for five days, after which multi-unit recording started. The birds were exposed to tutor song, or a number of other song stimuli including BOS and unfamiliar zebra finch songs. In tutored birds, 7.6% of all units preferentially responded to tutor song, which was significantly greater than the biased responsiveness to any of the other song stimuli. In age-matched isolated controls that had not been exposed to tutor song, only 2.3% of all units preferentially responded to the same song, which was not significantly different from preferential responsiveness to any of the other stimuli. In a subsequent electrophysiological study in awake juvenile zebra finches, Yanagihara and Yazaki-Sugiyama (2014) recorded single-unit activity in the NCM of freely moving males before and after tutor song exposure. A number of auditory stimuli were presented during neural recording, including tutor song, BOS, unfamiliar conspecific song, heterospecific song, male calls or female calls. Before

tutoring, NCM neurons did not exhibit preferential responsiveness to any of the song stimuli that were presented. However, after 4–5 days or more of exposure to a live tutor, a group of 19 neurons (out of 246) showed highly selective responses to the tutor song. Another group of 12 neurons responded selectively to BOS, and 5 other neurons selectively responded to both tutor song and BOS. Taken together, these findings suggest that relatively brief exposure to tutor song shapes the response properties of a subset of neurons in the NCM of juvenile zebra finch males. Importantly, learned songs appear to be sparsely represented in the NCM, with about 15% of cells responsive to tutor song, BOS, or both. This may explain why Miller-Sims and Bottjer (2014) did not find preferential responsiveness to tutor song in the NCM of juvenile males in their study. The results from Yazaki-Sugiyama's laboratory are consistent with a role for the NCM in the representation of tutor song memory, as we have hypothesised (Bolhuis and Gahr, 2006; Bolhuis et al., 2010).

5. Conclusions and discussion

5.1. The 'bottom-up' approach to the study of the neural substrate of memory

The 'bottom-up' approach to the search for the engram, pioneered by Gabriel Horn, has proved to be successful when applied to birdsong – in particular the analysis of the neural substrate of tutor song memory. This approach has yielded strong evidence for the involvement of at least two higher auditory brain regions (the NCM and the CMM) in tutor song recognition memory. In particular, the NCM is crucially involved in tutor song recognition in male zebra finches (Bolhuis and Gahr, 2006; Bolhuis et al., 2010), while the CMM shows preferential neuronal activation in response to the song of the father in zebra finch females (Terpstra et al., 2006). We have reviewed evidence to suggest that both these brain structures may be involved in the representation of tutor song memory in both males and females. In addition, the neuronal substrate for tutor song memory could comprise a distributed network, where auditory regions are important for tutor song recognition, while song system nuclei also have transient representations of tutor song to guide vocal learning. Instead of one single neural substrate for tutor song memory, a distributed network might involve NCM, CMM, HVC shelf, RA cup and CLM, as all of these regions have been shown to be involved in auditory perception (Mello and Clayton, 1994; Mello and Ribeiro, 1998). However, the specific roles of HVC shelf, RA cup and CLM have not been investigated to date.

Application of the 'bottom-up' approach to birdsong, using multiple neurobiological methods, has yielded results that mirror those in the filial imprinting paradigm (Horn, 2004; cf. Bateson, in this issue; McCabe and Solomon, in this issue; Moorman and Nicol, in this issue). First, in both cases, multiple interacting brain regions are involved in the representation of memory. Second, memory-related brain activation is lateralised, with the hemispheres playing different roles in memory consolidation (Moorman and Nicol, in this issue). Third, sleep appears to be crucial for memory consolidation in both paradigms (Vorster and Born, in this issue).

5.2. Interacting networks in learning and memory

The evidence that we have reviewed here suggests that the two major neural networks involved in song learning – the caudomedial pallidum and the song system – do not function in isolation. Rather, there seems to be continual interaction between these two systems (Bolhuis and Gahr, 2006; Bolhuis et al., 2010), with the two brain hemispheres playing different roles. In addition, the nature of the interaction between different brain systems may depend on the

behavioural state (sleep or wakefulness) of the animal (Nick and Konishi, 2005a; Moorman et al., 2014; Moorman and Nicol, in this issue).

In Sections 3.2.2 and 3.2.3 we have discussed the roles of the two major regions in the caudomedial pallium, the NCM and the CMM. The CMM appears to be particularly important in female song recognition, but this structure is also involved in auditory learning and memory in males. Further research is needed to elucidate the roles of these two brain structures. Interaction between the two major networks is particularly apparent in the early sensorimotor period in zebra finches, when there is transient preferential responsiveness to tutor song in HVC (Nick and Konishi, 2005a,b). As we have seen, the transient tutor preference of neurons in HVC was only observed during wakefulness – during sleep, these same neurons preferentially responded to the bird's own song. In Section 3.3.1 we speculated on the possible significance of this phenomenon for song learning.

How do the song system and the caudomedial pallium interact? We know relatively little about the neuroanatomical connections between these two systems. The song system nucleus HVC receives input from a number of auditory regions: CM (Bauer et al., 2008), Nif (Janata and Margoliash, 1999; Cardin and Schmidt, 2004; Coleman and Mooney, 2004; Hahnloser and Fee, 2007) and Uva (Coleman and Vu, 2005; Coleman et al., 2007; Akutagawa and Konishi, 2005, 2010). In addition, as Fig. 2 shows, there seem to be 'enclaves' of auditory neurons in the motor system, and vice versa. We have already mentioned HVC shelf and RA cup (Kelley and Nottebohm, 1979; Vates et al., 1996; Mello and Clayton, 1994; Fig. 2A). These two regions each surround a song system nucleus, but are activated when the bird hears song, not when it sings (Mello and Clayton, 1994; Jarvis and Nottebohm, 1997). More recently, two auditory regions have been identified that are adjacent to RA, known as Ad (dorsal arcopallium; Bottjer and Altenau, 2010) and AIV (ventral intermediate arcopallium), respectively. The AIV receives auditory information from field L1, CM and HVC shelf, and projects to VTA (Fig. 2B), and was shown to be important for auditory feedback (Vates et al., 1996; Mandelblat-Cerf et al., 2014). In contrast, nucleus avalanche (Av) is a small song-related nucleus (Fig. 2B) within the auditory caudal mesopallium (of which the CMM is the more medially located part) that is activated when the bird sings, but not when it hears song (Nottebohm et al., 1982; Jarvis and Nottebohm, 1997), with the exception of BOS (Akutagawa and Konishi, 2010). Nucleus Av is connected to Nif and HVC (Akutagawa and Konishi, 2010). It is plausible that these neural 'enclaves' play a role in the exchange of information between the two major networks involved in song learning.

5.3. Looking to the future

Taken together, the evidence that we have reviewed here, combined with a number of intriguing new findings that we discussed in Section 5.2 suggest a number of promising avenues for research. As we have seen, zebra finch females form a memory of the song of their father, although they do not sing themselves. This behavioural difference between males and females could provide important clues as to the neural organisation of birdsong memory. Song memory in females could be seen as a 'pure' auditory memory, while in males, the memory of the tutor song is likely to be used as a template for motor song learning. Future research needs to elucidate the differential neural mechanisms involved.

We have made the case for the NCM as containing (part of) the neural substrate for tutor song memory. The NCM is not a uniform structure, but has subdivisions that are likely to play different roles in song memory (Ribeiro et al., 1998; Terpstra et al., 2004; Maney and Pinaud, 2011). An important step forward

will be to identify the neural networks in the NCM that are involved in the representation of tutor song memory. To investigate this problem, the most promising method would seem to be optogenetics, with which tutor-song activated neurons could be selectively inhibited (Roberts et al., 2012), to investigate whether song memory critically depends on their activation or, alternatively, a 'false memory' could be created artificially, without actual song exposure (Ramirez et al., 2013, 2014). In addition, the neurons involved in song memory need to be characterised, e.g., regarding the neurotransmitters involved, and whether they are interneurons.

As we discussed in the previous section, a picture is emerging of the intricate connections between the song system and the caudomedial pallium. It is important to further analyse the connectivity of the different nodes in this complex network, and to investigate how these networks interact during song learning. Related to this, we are only beginning to understand the role of lateralisation in song learning. It may be that lateralised brain activity is key to interaction between the motor and the auditory systems, as we have suggested (Moorman et al., 2014; Moorman and Nicol, in this issue). Furthermore, it has become apparent that sleep is crucial for memory consolidation in birdsong learning, as it is in imprinting (see also Vorster and Born, in this issue). In Section 3.3 we have sketched a possible scenario for the role of sleep in birdsong learning and memory. It will be important to further investigate the function of sleep in song learning, for instance through deprivation of sleep or particular sleep phases, as has been done by Gabriel Horn and collaborators in imprinting (Jackson et al., 2008; Nicol and McCabe, 2013; see Moorman and Nicol, in this issue).

Acknowledgements

This review is dedicated to the memory of Gabriel Horn. We are grateful to Dr. Yoko Yazaki-Sugiyama (Okinawa Institute of Science and Technology Graduate University, Okinawa, Japan) for providing us with unpublished results from her laboratory and to the Netherlands Organisation for Scientific Research (NWO) for financial support (NWO-Earth and Life Sciences Open Programme, NWO Gravity and Horizon Programmes).

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