Long-time storage of song types in birds: evidence from interactive playbacks

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In studies of birdsong learning, imitation-based assays of stimulus memorization do not take into account that tutored song types may have been stored, but were not retrieved from memory. Such a 'silent' reservoir of song material could be used later in the bird's life, e.g. during vocal interactions. We examined this possibility in hand-reared nightingales during their second year. The males had been exposed to songs, both as fledglings and later, during their first full song period in an interactive playback design. Our design allowed us to compare the performance of imitations from the following categories: (i) songs only experienced during the early tutoring; (ii) songs experienced both during early tutoring and interactive playbacks; and (iii) novel songs experienced only during the simulated interactions. In their second year, birds imitated song types from each category, including those from categories (i) and (ii) which they had failed to imitate before. In addition, the performance of these song types was different (category (ii) > category (i)) and more pronounced than for category (iii) songs. Our results demonstrate 'silent' song storage in nightingales and point to a graded influence of the time and the social context of experience on subsequent vocal imitation.

Keywords: nightingales; song type memories; silent storage; vocal interaction; bird song

1. INTRODUCTION

Hand-reared songbirds that had been trained during a song-learning experiment may not imitate every song type to which they had been exposed during the tutoring. Although the production of imitated songs is suitable to be used as a learning assay (but see, for example, Hughes *et al.* 2002), it might be misleading to conclude that a song that is not produced has not been memorized. This issue has been raised by several authors (e.g. Brenowitz *et al.* 1997; Nelson *et al.* 1997; Doupe & Kuhl 1999) and has recently been demonstrated for hand-reared nightingales (*Luscinia megarhynchos*) by using vocal matching as a behavioural tool (Geberzahn *et al.* 2002). Vocal matching is a widespread pattern-specific response that songbirds use in territorial counter-singing (review in Todt & Naguib 2000). In the study by Geberzahn *et al.* (2002), subjects were exposed to interactive playbacks (Todt 1981; Dabelsteen 1992) during their first singing season and could thereby be persuaded to produce some of those song types that they had not imitated before as a vocal matching response (song type matching). However, not all song types could be elicited in that way and the problem of judging whether memorization took place during the early sensitive phase remained open in these song types.

In this follow-up study, we focused on song types that were not imitated from the tutoring and at the same time could not be elicited by the playback experiments conducted in the first full song season. We investigated whether these song types were nevertheless stored to memory during the early sensitive phase. Our approach was to analyse the subjects singing in their second season for imitations of those song types. However, the production of these imitations alone would not be conclusive, because in

nightingales new memorization later in life has been demonstrated (Hultsch 1991a; Todt & Geberzahn 2003) and thus *de novo* acquisition of song types could have occurred during the interactive playbacks. Hence, we also checked for imitations of novel song types that the birds had not experienced before, and that were used as control stimuli during the interactive playbacks. We compared the proportion of novel song types acquired in the second season with the proportion of unsung song types that were tutored early but were not imitated in the first season (ETNI, early tutored but not imitated) and could not be elicited in the first season.

Nightingales show individually distinct profiles in the frequency of performing their different song types (Todt & Hultsch 1996). This was also found in studies on other songbirds, and is probably typical for species with large vocal repertoires (Catchpole & Slater 1995). Factors involved in determining how often a song type is used have been identified in the social domain, e.g. the singing routines of territorial neighbours (see Hultsch 1980; Payne 1981; Sorjonen 1987). We therefore compared the performance frequencies of ETNI song types that could not be elicited with the performance frequencies of novel song types. Thereby we hoped to obtain an additional cue on the strength of the underlying memories.

In addition, we analysed the performance of song types from the early tutor programme that were not used as stimuli during the interactive playbacks ('not repeated song types'). The emergence of 'not repeated song types' in the second season, which were not imitated in the first season (ETNI), would give us a strong cue that memorization had occurred in the early sensitive phase, and would conclusively demonstrate the striking ability of birds to store and retrieve songs without rehearsal over a long time-period, i.e. from the fledgling age to their second singing season.

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2. MATERIAL AND METHODS

(a) *Subjects*

Eight male nightingales taken from three nests in rural areas of Berlin, Germany, at the age of 3–7 days were hand-reared in the laboratory. They were housed as nest-groups until fledging (at age 15–20 days) and then caged individually (cage size: 120 cm × 50 cm × 40 cm high). At the age of three months (when subsong may begin), they were transferred to soundproof chambers (minimum size: 1 m × 1.50 m × 2.50 m) and after their second singing season to aviaries (minimum size 2 m × 3 m × 2.5 m). The light : dark (LD) regime was adjusted weekly to Central European time during the summer. From 21 September to 21 March, LD was set to 12 : 12 h.

(b) *Tutoring and playbacks*

We give only a short report about the tutoring and the interactive playbacks (for more details see Geberzahn *et al.* 2002): the tutoring began when subjects were between 21 and 28 days of age (June 1998) and continued until days 63–70. Each bird was exposed to 65 different song types recorded from free-ranging nightingales. Each song type was presented 30 times.

To record the birds' vocalizations automatically, we used a computer-controlled sound-activated registration device (R. Specht, Berlin) with Panasonic Hi-Fi F55 stereo video recorders and Sennheiser Me62 microphones, throughout this study. Recordings were analysed by sound spectrography using a Nicolet UA 500A spectrum analyser connected to a Tönnies film camera for hard-copy production of spectrograms. In addition, recordings were sampled and printed as spectrograms using the program AVISOFT-SASLAB PRO, v. 3.91 (R. Specht, Berlin).

First, the individual repertoire composition of each male was assessed for the period of vocal development (November 1998 to April 1999) and the first full song season (April/May 1999). We included the developmental phase of plastic singing (variable song containing precursors of imitations) to make sure that a song type considered as ETNI was not discarded from the repertoire during the process of repertoire constriction by the time of crystallization (Hultsch 1991b). In total, we analysed an average of $X \pm \text{s.d.} = 3.5 \pm 0.6$ h of plastic song for every male, distributed evenly over the period of song development (from week 34 onwards, criteria used for pattern identification; see Geberzahn *et al.* (2002)). For the first season full song, we analysed an average of $X \pm \text{s.d.} = 1095 \pm 265$ song patterns of each bird's crystallized singing (from week 47 onwards). A crystallized song pattern was accepted as an imitation of a presented song type when it contained its trill section plus at least two consecutive notes from the syllable complex preceding the trill (figure 1; Hultsch & Todt 1989).

According to the individual repertoire composition, a set of 30 different stimulus songs was chosen for each male to be used as stimuli during the interactive playbacks conducted at weeks 48–52. A given set contained song types from the following stimulus categories: (i) ETNI: they neither occurred during a bird's plastic singing nor in its full song in the first singing season; (ii) early tutored song types that had been identified as imitations in a bird's plastic singing, but not in his crystallized song ('discarded'); (iii) early tutored song types that were imitated from the tutoring and retained after crystallization ('crystallized'); (iv) song types which had not been included in the early tutoring ('novel').

We exposed a bird to a playback trial while he was singing spontaneously using a Sony MZ-R2 portable minidisc player connected to a Bose Room Mate speaker (for a justification of

the use of minidisc see Geberzahn *et al.* 2002). To simulate vocal interaction, stimulus songs were broadcast during the bird's silent intersong intervals (i.e. stimulus songs alternated with the bird's songs). A trial began as soon as a bird started singing and ended when he stopped singing. The set of 30 individually selected stimulus song types was presented to a given subject up to three times a day. We conducted playback experiments on 8–16 days and on 3 days in one subject (that stopped singing thereafter), respectively. Birds were exposed to the stimulus songs 17–35 times except for one male (exposure frequency: nine times). However, because in the second season the latter male performed all playback song types used as stimuli (including the novel song types), the ninefold presentation seemed to be effective and we did not exclude him from the dataset.

(c) *Second season*

In the second season, we only considered those ETNI song types that could not be elicited by the interactive playbacks conducted in the first season. Therefore, we first determined which of the ETNI song types were performed as a vocal matching response during the playbacks, and excluded them from the dataset (five different types, 10.4% of ETNI stimuli). Because the novel song types did not elicit any song type matching during the playbacks, we included all of them. To reveal the number and performance frequency of song types for the different stimulus categories, we analysed a total of $X \pm \text{s.d.} = 888 \pm 177$ song patterns of each bird's crystallized song in the second season (weeks 97–98). The amount of song pattern analysed for the first and second season was sufficient to determine the complete repertoire as indicated by the plateau that is reached when the number of identified song types is plotted against the number of analysed songs (figure 2).

Two evaluators, one of which was blind to the experimental design, visually compared spectrograms made from the birds singing with spectrograms of master song types (see above for criteria used for pattern identification). To verify this assessment, we chose, at random, 118 song types: six from each of the categories crystallized, ETNI and novel, for each subject (if a bird had exactly or less than six song types in a category we chose all of them). We randomly determined one rendition for each song type. We asked five additional evaluators, blind to the experimental design, to make a statement on whether they agreed with the original assessment. Comparing the additional five evaluations with the original one revealed a percentage agreement of 94.4%.

(d) *Statistical analysis*

We compared the proportions of song types performed in the different stimulus categories (novel, ETNI, crystallized) and the relative performance frequencies of song types in these categories in the second-year crystallized singing. Therefore, we used a Friedman ANOVA by rank tests (two-tailed). In the case of a significant outcome, we used a Wilcoxon signed-ranks test (two-tailed) to specify which of the categories differed. To examine whether the interactive playbacks had an effect on both the proportion of song types performed and their performance frequency, we further compared song types that were used during the experiments in the first full song with those that were only presented during the early tutoring, and not repeated in the first full song with regard to the proportion of song types performed and their performance frequency. Therefore, we used a Wilcoxon signed-ranks test (two-tailed). We calculated exact p

Figure 1. Frequency spectrograms of four master song types (left) and their imitations performed by two subjects (WS and WB) in crystallized singing of the second season (right). (a) Novel song types only presented during the first full song season; (b) song types that were crystallized in the first full song season.

Figure 2. Number of song types encountered as a function of consecutive songs analysed. Exemplary curves are given for subject WS for the first season (plastic and full song) and the second season (full song).

values because the sample was too small for asymptotic tests (Siegel & Castellan 1988; Mundry & Fischer 1998).

3. RESULTS

(a) *Song types only presented during tutoring*

Considering those song types that were only presented during the early sensitive phase, up to 83.3% of the song

types that were not imitated in the first season (ETNI) were produced 1 year later (range: 0–83.3%; figure 3a, for more details see electronic Appendix A (available on The Royal Society's Publications Web site)). In all subjects, a sum of 28 imitations (of 23 different tutor song types) emerged in the second season for the first time. Because the birds had not experienced those tutor song types during the simulated interactions in their first full song period, they could only have memorized them as fledglings. The proportion of crystallized song types performed in the second year ranged from 73.9% to 100% (figure 3a). Only one subject kept all song types in the second full song season that he had crystallized the year before. The relative performance frequency in ETNI song types ranged from 0.5% to 1.3% and in crystallized song types from 0.9% to 2.2% (figure 4a).

(b) *Song types presented during interactive playbacks*

In the following section, we focus on those song types that were used as playback stimuli in the first singing season. The proportion of song types occurring in the full song repertoire of the second season differed significantly between the categories novel, ETNI and crystallized (Friedman test: $\chi^2 = 7.64$, d.f. = 2, $p = 0.02$; figure 3b,c). In the second season 50–100% of ETNI song types were performed. This proportion tended to be higher than that

Figure 3. Proportion of song types performed in the second singing season of the nightingales. (a) Song types presented only during early tutoring; (b) presented during early tutoring and the first full song season; (c) presented only during the first full song season. Categories: crystallized—songs produced after crystallization in the first singing season; ETNI—early tutored but not imitated in the first singing season; novel—not included in the early tutoring. The median of all subjects is given by the bold line, overlapping data points were offset horizontally. Note that n is reduced in one category because of the high acquisition rate in two subjects.

Figure 4. Relative performance frequency of song types in the second singing season. For the discerned song type categories the median value is plotted for each subject. (a) Song types presented only during early tutoring; (b) presented during early tutoring and the first full song season; (c) presented only during the first full song season. Categories: crystallized—songs produced after crystallization in the first singing season; ETNI—early tutored but not imitated in the first singing season; novel—not included in the early tutoring. The median of all subjects is given by the bold line, overlapping data points were offset horizontally. Different values of n result from subjects failing to produce song types from the respective categories.

of novel song types (range novel: 0–100%; Wilcoxon signed-ranks test: $T^+ = 19.5$, $n = 6$ (two ties), $p = 0.09$). Remarkably, the proportion of ETNI song types was not lower than that of crystallized song types (range crys-

tallized: 90–100%; Wilcoxon signed-ranks test: $T^+ = 9$, $n = 4$ (four ties), $p = 0.25$; figure 3b). Thus, ETNI ‘caught up’ with crystallized in the second season, whereas the proportion of novel was significantly lower than that of

crystallized (Wilcoxon signed-ranks test: $T^+ = 21$, $n = 6$ (two ties), $p = 0.03$).

Song types from the different stimulus categories were performed differently often (Friedman test: $\chi^2 = 11.63$, d.f. = 2, $p = 0.001$; figure 4b,c). In 0.4–3.1% of cases an ETNI song type was uttered, whereas novel playback song types were only performed in 0.4–2% of cases. This difference was statistically significant (Wilcoxon signed-ranks test: $T^+ = 21$, $n = 7$ (one tie), $p = 0.03$; figure 4b,c). However, the relative performance frequency of ETNI song types tended to be lower than that of crystallized song types (range crystallized: 1.2–3%, Wilcoxon signed-ranks test: $T^+ = 32$, $n = 8$, $p = 0.06$; figure 4b).

(c) Effect of playback experiments

Whether subjects experienced a song type during the interactive playbacks or not had a clear effect in both ETNI and crystallized song types for the proportion of song types performed: in the second season, subjects performed more song types that were used as stimuli than song types that were not used (Wilcoxon signed-ranks test; ETNI: repeated during playbacks versus presented only early: $T^+ = 21$, $n = 6$, $p = 0.03$; crystallized: repeated during playbacks versus presented only early: $T^+ = 28$, $n = 7$ (one tie), $p = 0.02$; figure 3a,b). Remarkably, in one subject (WB) six of those crystallized song types that were not used as stimuli were absent in the second season.

The relative performance frequency of crystallized song types did not differ whether song types were experienced as adults or not (Wilcoxon signed-ranks test: $T^+ = 30$, $n = 8$, $p = 0.11$; figure 4a,b). Likewise, the relative performance frequency of ETNI song types did not differ (Wilcoxon signed-ranks test: $T^+ = 10$, $n = 4$ (one tie), $p = 0.13$; figure 4a,b); however, this could be due to the small sample size.

4. DISCUSSION

(a) Novel acquisition versus 'silent storage' of song types

Auditory song acquisition in nightingales is not constrained by an early sensitive phase during the fledgling age. This has been demonstrated in a recent study, showing that adult nightingales acquired new song types in their first singing season (Todt & Geberzahn 2003). Likewise, in the present study, the birds' repertoires in their second year contained imitations of models that they had not performed before, i.e. during their song ontogeny or as adult in their first spring. These new imitations were from two categories of model: (i) songs that had been tutored in the fledgling period and which birds had failed to imitate in their first year (ETNI); and (ii) novel songs that had been presented as playback stimuli in the birds' first full song period (novel). A comparison of acquisition rates from the two categories, however, revealed that birds tended to incorporate a higher proportion of ETNI than novel songs into their second year repertoire. Indeed the proportion of ETNI imitations in second year repertoires was similar to the proportion of song types that birds had already crystallized in their first year. It is suggestive to interpret the acquisition differences in ETNI and novel songs by postulating that ETNI songs were not acquired *de novo* when birds were re-exposed to them during the

playback. Rather, some memory from the early tutoring may have been retained. Thus, differently from the novel songs, the renewed exposure to the ETNI models may have activated the stored information which finally led to the higher acquisition rates for ETNI songs. Likewise, such information may also have consolidated the strength of the memory trace for unsung songs and, through a retrieval mechanism, subsequently facilitated their production in the second season (cf. Anderson 1995).

This assumption is supported by the evidence that the performance frequency of songs was different for the discerned categories. Subjects performed ETNI songs almost as frequently as those songs they had already crystallized in their first year. By contrast, the performance frequency of novel songs was clearly lower.

The strongest evidence for a long-term storage of ETNI songs comes from the fact that those unsung song types to which subjects were not exposed again as adults were imitated in the second season. This demonstrates that they had been memorized during the early tutoring and stored 'silently' until their performance in the second singing season. The possibility of a 'silent storage' over a long period has recently been raised by Gil *et al.* (2001). In a field study on willow warblers (*Phylloscopus trochilus*), the repertoire size grew to adult size only in the second year of the birds. However, as with field studies in general, it has to be taken into account that the warblers added newly learnt song types to their repertoire.

(b) Effects of interactive playbacks

The effects of stimulus repetition during the playbacks were also evident when the repertoire composition of second-year males was compared for the proportion of imitations of models reused during the playback experiments, and imitations of models presented only during the early tutoring: the birds' repertoires contained more imitations from the category 'repeated' (both ETNI and crystallized) than from the category 'early tutoring only'. From a functional point of view, the changes in repertoire composition from the first to the second year resulted in a higher amount of song-type sharing between a subject and the respective 'neighbour' simulated during the interactive playback. Learning from a neighbour may also account for the pattern of repertoire distribution in free-ranging nightingales. Like many other species, males share more song types with their neighbours than with more distant individuals (see Hultsch & Todt 1981) that, during territorial contests, may allow them to vocally address their rivals (e.g. by frequently exchanging matching responses; Todt & Naguib (2000)). In song sparrows (*Melospiza melodia*), song-type sharing has recently been demonstrated as an adaptive trait crucially involved in a male's territorial success (Beecher *et al.* 2000).

From a developmental perspective, the effects of the interactive playback on repertoire composition are reminiscent of the model of 'action-based learning' put forward by Marler & Nelson (1993). This model suggests that learning operates on a pre-existing repertoire as a function of experience and was originally developed to account for 'overproduced' song material during the plastic song phase that was discarded during the process of song crystallization (Nelson 1997). According to the model, social interactions during vocal development selectively reinforce

the performance of certain song types, whereas non-reinforced song types are discarded. If the repertoire composition of our birds was shaped by 'action-based learning', both the high acquisition and performance rates of the ETNI song category suggest that song production is not crucial for the mechanism to be effective. Rather, it would also operate on 'silently stored' song material.

The model of action-based learning was influential to the discussion of data on adult learning in songbirds. Song patterns newly emerging in adults would not necessarily result from late memorization, but might be traced back to memorization during the early sensitive phase and might just be incorporated into the repertoire later in life (e.g. Hough *et al.* 2000; Nelson 2000). As a consequence, Nelson (1997) questioned the ability of songbirds to learn as adults ('open-ended learning'). Our findings clarify this issue, at least for nightingales. Although a late-emerging pattern can result from early memorization (ETNI songs), the second year repertoire of our birds also contained novel song types heard during their first singing season. This clearly indicates adult song learning in nightingales.

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