Comparisons of Different Methods to Train a Young Zebra Finch (Taeniopygia Guttata) to Learn a Song

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1   **Abstract**

2   Like humans, oscine songbirds exhibit vocal learning. They learn their song by imitating

3   conspecifics, mainly adults. Among them, the zebra finch (*Taeniopygia guttata*) has been

4   widely used as a model species to study the behavioural, cellular and molecular substrates

5   of vocal learning. Various methods using taped song playback have been used in the

6   laboratory to train young male finches to learn a song. Since different protocols have

7   been applied by different research groups, the efficiency of the studies cannot be directly

8   compared. The purpose of our study was to address this problem. Young finches were

9   raised by their mother alone from day post hatching (dph) 10 and singly isolated from

10   dph 35. One week later, exposure to a song model began, either using a live tutor or taped

11   playback (passive or self-elicited). At dph 100, the birds were transferred to a common

12   aviary. We observed that one-to-one live tutoring is the best method to get a fairly

13   complete imitation. Using self-elicited playback we observed high inter-individual

14   variability; while some finches learned well (including good copying of the song model),

15   others exhibited poor copying. Passive playback resulted in poor imitation of the model.

16   We also observed that finches exhibited vocal changes after dph 100 and that the range of

17   these changes was negatively related to their imitation of the song model. Taken together,

18   these results suggest that social aspects are predominant in the success outcome of song

19   learning in the zebra finch.

20

1. Introduction

Local dialects or geographical variation provided an early clue that birdsong might be learned. But it was when birds were raised in controlled conditions in the laboratory and their vocalizations were analyzed with the spectrograph that it was clearly demonstrated (Catchpole and Slater, 2008; Zeigler and Marler, 2008). Thorpe (1954) was the first to demonstrate that young chaffinches (*Fringilla coelebs*) raised in social isolation failed to produce the normal song characteristic of their species. Since Thorpe's pioneering works in the fifties, young oscine songbirds of several dozens of species have been brought into the laboratory and reared in strictly controlled auditory environments to examine how their experience affected their songs. In nearly all cases, young songbirds deprived of song models develop abnormal songs, although some species-specific song features may be preserved (Brainard and Doupe, 2002). It was discovered that some species learn song only during a sensitive phase of early life whereas others have the possibility to transform their songs during their whole life (Brainard and Doupe, 2002; Catchpole and Slater, 2008). Songbird species differ also in their readiness to copy songs from tape-recordings and from live tutors (for a review, see Baptista and Gaunt, 1997). Some bird species learn conspecific songs from a loudspeaker easily (canaries *Serinus canaria*: Mundinger, 1995; chaffinches *Fringilla coelebs*: Thorpe, 1958; white-crowned sparrows *Zonotrichia leucophrys*: Marler, 1970; nightingales *Luscinia megarhynchos*: Todt et al., 1979; marsh wrens *Cistothorus palustris*: Brenowitz et al., 1995; swamp sparrows *Melospiza Georgiana*: Marler and Peters, 1988; song sparrows *Melospiza melodia*: Marler and Peters, 1987; red-winged blackbirds *Agelaius phoeniceus*: Marler et

1 al., 1972). In other species (Treecreepers *Certhia* spp.: Thielke, 1970; Bullfinches

2 *Pyrrhula pyrrhula*: Nicolai, 1959), tutoring by tape-recording appears to be virtually

3 ineffective. Passive playback of tape-recordings has several advantages: one can control

4 the timing of sound exposure, the quality and the amount of songs that are broadcast to

5 the young bird (Derégnaucourt, 2011). This method was useful to show that some bird

6 species do learn songs during particular time windows known as sensory phases

7 (Kroodsma and Pickert, 1980). Nevertheless, it has been shown that the timing of song

8 acquisition in birds trained by passive playback could be extended beyond the limits if

9 the young bird is subsequently exposed to live tutors (Baptista and Petrinovitch, 1984).

10 Several studies show that mere proximity, that is, spatial association, is necessary for

11 song sharing (Hausberger et al., 1995) and social learning (Mann and Slater, 1995). Song

12 acquisition processes also depend on the biology of each species. For example, it is likely

13 that learning from tapes is possible in species that learn their song from their neighbours

14 under natural conditions, with whom they do not interact physically and/or visually

15 (Derégnaucourt, 2011).

16   Among songbirds, song acquisition in the zebra finch (*Taeniopygia guttata*) has

17 received a lot of interest. This tiny bird is easy to breed in captivity. Its song is short (1-

18 1.5 sec) and very stereotyped. Young male zebra finches learn their song during a

19 sensitive period of early life and it is believed that once the bird is about 90 days old, its

20 song crystallizes and will not change for the remainder of its life (Zann, 1996). Early

21 results suggested that young zebra finches would not learn from passive playback of tape

22 recordings (Immelmann, 1969; Eales, 1989). However, significant learning was observed

1 in subsequent studies despite high inter-individual variability (Bolhuis et al., 1999; Houx

2 and ten Cate, 1999a, b; Houx et al., 2000; Funabiki and Funabiki, 2009).

3 Since Immelman's pioneering study in 1969, it has been assumed that the most

4 important factor in the selection of a zebra finch song model is the close interaction

5 between a young male and the male that rears him, generally his father (Price, 1979;

6 Böhner, 1983). Indeed, this bond will even override species-specificity since zebra

7 finches will learn the song of a Bengalese finch (*Lonchura striata*) foster father, even if

8 conspecifics share their breeding accommodation. Nevertheless, in controlled conditions,

9 a young male can also learn the song of an adult male with whom he does not have

10 physical contact or social attachment such as what develops between youngsters and the

11 male that is feeding them (Eales, 1985). Still, both visual and vocal interaction between

12 young zebra finches and their tutors are important for the accuracy of song learning

13 (Eales, 1989).

14 If fairly complete song imitation in the zebra finch has never been obtained with

15 passive exposure to a song model, such results have been obtained using operant

16 conditioning with song as a reward (Adret, 1993; Tchernichovski et al., 2001;

17 Derégnaucourt et al., 2005).

18 There might be several reasons why passive playback failed so far to induce

19 significant song learning in the zebra finch. First, the song playback sessions might be not

20 done at the appropriate moment for the birds. Second, the playback quota might not be

21 sufficient to capture the bird's attention. On several occasions investigators have

22 emphasized that very good imitation is possible even when a model was presented a

23 small number of times (Petrinovitch, 1985; Hultsch and Todt, 1989; Peters et al., 1992).

1   In zebra finches, in an operant conditioning context when the bird has to peck a key to

2   trigger song playback, 40 playbacks of the song motif per day, lasting a total of 30

3   seconds, result in significant learning. More exposure leads to less complete imitation

4   (Tchernichovski et al., 1999).

5   In our study we decided to test these hypotheses. Accordingly, we decided to play

6   back song at moments that are predictable for the birds, namely, just after the lights

7   switch on in the morning and just before the lights switch off in the evening. Also, we

8   restricted the quota of daily song exposure. We will compare these results with those of

9   birds trained with a procedure of operant conditioning, and also those of live-tutored

10   birds.

11   Hormonal status is closely correlated to the end of the sensitive period for song

12   learning and the crystallization of a stereotyped song in both open-ended (Marler et al.,

13   1987) and age-limited song learners like the zebra finch (Pröve, 1983). At dph 101, we

14   measured testosterone levels and calculated correlations with song imitation, song

15   stereotypy and singing activity. Early castration (Arnold, 1975a; Bottjer and Hewer,

16   1992) and exogenous testosterone (Korsia and Bottjer, 1991) both disrupt song learning

17   in the zebra finch. In the same species, several studies suggest that high testosterone

18   concentrations reduce the potential for vocal plasticity (Korsia and Bottjer, 1991;

19   Williams et al., 2003). We therefore expected that birds with low levels of testosterone

20   would be less likely to have a stereotyped song. Also, castration of male finches

21   decreases song rates, but normal singing activity is re-established after the castrated birds

22   have been injected with testosterone (Arnold, 1975b; Pröve, 1974). In our study, we

1   checked whether there could be a link between testosterone levels and singing activity at

2   dph 100.

3   After dph 101, the birds were placed in a common aviary with conspecifics.

4   Several weeks later, their song was recorded again to quantify eventual vocal changes.

5

6   **2. Materials and methods**

7

8   *2.1. Study subjects*

9

10   62 male zebra finches from 16 different breeding pairs of our colony were used

11   for this study. Breeding pairs were maintained in separate cages (100 x 50 x 50cm) and

12   nest control was made daily. The chicks were raised by both parents until dph 10 when

13   the father was removed. The mother and the chicks were moved to another room in our

14   institute, to avoid any imprinting of the young on male song. The breeding cages were

15   maintained on LD 12:12 until the chicks fledged.

16

17   *2.2. Experimental groups*

18

19   On day 35, each chick was marked and the young males were isolated in an

20   individual sound proof chamber (LD 12:12; lights on from 8am to 8pm). Food and water

21   were provided *ad libitum*. Each bird stayed in social isolation until day 100 post-

22   hatching.

23   Birds in group 1 to 8 started training from day 43 (Table 1).

1 Birds in group 1 to 4 were exposed to a passive playback of 20 song models.

2 Birds in group 5 to 7 were trained with an operant conditioning procedure, with a daily

3 exposure to 20 song models.

4 For groups 1 and 5, there were two daily playback sessions: one in the morning (starting

5 at 8am) and one in the afternoon (starting at 2pm). During each playback session birds in

6 group 1 were exposed to a passive playback of 10 songs, and only the first 10 key pecks

7 were reinforced with song playback for group 5.

8 For groups 2 and 6, there was only one playback session in the morning, starting at 'lights

9 on.' Birds in group 2 were exposed to a passive playback of 20 songs and birds in group

10 6 could peck the key only until 2pm.

11 For groups 3 (passive playback) and 7 (operant conditioning), there was only one

12 playback session in the afternoon of 20 songs, starting at 2pm.

13 Group 4 was exposed to the passive playback of 20 songs that ended one minute before

14 'lights off.'

15

16   Some birds that trained using operant conditioning were subsequently used as

17 song tutors for the live tutoring group (group 8). On day 43 of the pupil's life, one of

18 these adult males was introduced to the sound-proof chamber and stayed with the pupil

19 until dph 100.

20

21   After dph 100, birds were moved to a common aviary where they could freely

22 interact with other males from the different experimental groups.

1   Several weeks to several months after the end of training, the birds were moved to sound-

2   proof chamber again for song recordings. After a week, they were moved back to their

3   aviary.

4

5   *2.3. Song recording and analysis*

6

7   *2.3.1. Song recording*

8   Vocal activity of each individual bird was recorded using the Sound Analysis Pro

9   software (SAP 2.0; Tchernichovski et al., 2004). The program was run on a PC equipped

10 with an Edirol UA1000 sound card (16 bits, sampling frequency: 44.1 kHz), connected to

11 multidirectional Earthworks TC20 microphones (one per sound box) placed above the

12 cage. Each bird's vocalizations were monitored continuously. To limit the recording of

13 cage noises, we adjusted the settings of the software (amplitude and duration thresholds)

14 such that mainly songs would be automatically identified and recorded.

15 Song files were then scanned using Sound Explorer (sound analysis software developed

16 by René Jansen, University of Amsterdam) and only files containing song bouts were

17 used for subsequent analysis.

18

19 *2.3.2. Singing activity*

20   Sound files recorded at dph 100 were visually scanned using Sound Explorer.

21 Only files containing song bouts were kept for the analysis. Using SAP we calculated the

22 duration of each of these song bouts to get an overall score of singing activity for the

23 whole day.

1 *2.3.3. Motif analysis*

2   We quantified how well pupils had copied their song model using a similarity

3 score procedure in SAP (Tchernichovski et al., 2000). The overall similarity score is a

4 product of 3 components: % similarity, mean accuracy and sequential match. Percentage

5 of similarity is the percentage of tutor's sounds included in final sections. Mean accuracy

6 is the average local similarity score across final sections. Sequential match is calculated

7 by sorting the final sections according to their temporal order with reference to the song

8 model, and then examining their corresponding order in the song motif.

9 For the live tutoring procedure, overall similarity score was calculated from 100

10 asymmetric pairwise comparisons of the pupil's typical motif (10 exemplars per bird)

11 with the tutor motif (10 exemplars per bird). For the playback procedures, overall

12 similarity score was calculated from 10 asymmetric pairwise comparisons of the pupil's

13 typical motif to the song model broadcast. In asymmetric comparisons the most similar

14 sound elements of two motifs are compared, independent of their position within a motif.

15 The smallest unit of comparison is 9.26-ms–long sound interval (FFT windows). Each

16 interval is characterized by measures for five acoustic features: pitch, FM, amplitude

17 modulation (AM), Wiener entropy, and Pitch Goodness. SAP calculates the Euclidean

18 distance between all interval pairs from two songs, over the course of the motif, and

19 determines a p-value for each interval pair. This p-value is based on p-value estimates

20 derived from the cumulative distribution of Euclidean distances across 250,000 sound

21 interval pairs, obtained from 25 random pairs of zebra finch songs.

22 Neighboring intervals that pass the p-threshold value ($p = 0.1$ in this study) form larger

23 similarity segments (70 ms). The amount of sound from the song model that was included

1   into the similarity segments represents the similarity score; it thus reflects how much of

2   the material of the song model was found in the pupil's motif. To measure how

3   accurately pupils copied the sound elements of the song model, we used the accuracy

4   score from SAP. The accuracy score is computed locally, across short (9 ms) FFT

5   windows and indicates how well the sound matched to the sound in the song model. SAP

6   calculates an average accuracy value of the motif by averaging all accuracy values across

7   the similarity segments.

8

9   *2.3.4. Song stereotypy at dph 100*

10   We used two methods to measure song stereotypy: (1) a measure based on a

11   clustering procedure of sounds; (2): a measure of song syntax stereotypy based on

12   amplitude shapes of singing bouts.

13   Measure of clustered sounds: we used the 'clustering' procedure of the SAP software

14   (Tchernichovski et al., 2004). The original purpose of the 'clustering' procedure of SAP

15   is to detect syllable types and to automatically trace vocal changes during song

16   development. The nearest-neighbor hierarchal clustering method is implemented into an

17   extensive graphic user interface including a display of clusters in color code, assessment

18   of residuals, and an account of the number of members in each syllable type. In our

19   study, song bouts recorded on dph 100 were segmented into syllables based on a

20   threshold crossing of song power. We then ran the clustering procedure and took into

21   account the % of residuals, namely the proportion of sounds that could not be attributed

22   to any syllable type according to the clustering algorithm.

23

1 <u>Syntax analysis:</u> Measures of song syntax stereotypy have been designed for zebra finch

2 song (Scharff and Nottebohm, 1991). They require manual annotation of song sequences

3 of syllables following visual observation of spectrograms. This task is relatively easy for

4 adult birds with well-defined types of syllables, but is harder when song still contains

5 plastic and unclassifiable sounds, which was the case in our study. To measure syntax

6 stereotypy we selected for each bird ten song bouts and extracted 1 sec of sound starting

7 from the 1st syllable after the last introductory note. Then, following a modified version

8 of a method recently published (Kojima and Doupe, 2011), the continuous time course of

9 the amplitude envelope was compared among these 10 1-sec segments to obtain

10 correlation coefficients (CCs) using a custom-made program written in Matlab. Finally,

11 we took the mean of these 100 comparisons. We expected that the mean CCs among

12 sound segments would be higher in finches that produce stereotyped sequence of

13 syllables than in finches that produce more variable song sequences.

14

15 *2.4. Song playback*

16

17   We used three different song models (SM1, SM2 and SM3) for playback (Fig.

18 1A-C). Each song model consisted of two identical repetitions of a single song motif

19 recorded from an adult bird. The number of birds trained with each song model is

20 presented in table 1.

21 Three finches trained with SM1, 3 finches trained with SM2 and 4 finches trained with

22 SM3 were used as song tutors for the live tutor group (group 8).

23

1 *2.5. Hormone analysis*

2

3 The absence of adult males for finches trained with song playback and the

4 isolated housing conditions are both likely to have an effect on the circulating hormone

5 levels of the young developing males (Pröve, 1981; Livingston et al., 2000), which could

6 affect song learning and song stereotypy.

7 At dph 101, a blood sample (approx. 120 µl) was taken after venipuncture from

8 the wing vein and collected into heparinized capillaries. Plasma was immediately

9 separated by centrifugation and samples were then stored at -80˚C. Testosterone (T)

10 concentration was determined by direct radioimmunoassay (RIA; Goymann et al., 2006).

11

12 *2.6. Statistics*

13

14 We used a one-way ANOVA to compare learning success (similarity score with

15 the song model) between the three methods: live-tutoring, operant conditioning and

16 passive playback. The Tukey HSD test was used for post-hoc analysis. We used a two-

17 way ANOVA to test the effect of time and song model on learning success for birds

18 trained with passive playback and operant conditioning. The t-test was also used to

19 compare learning success between two training schedules for the same method. We used

20 linear regression to test correlations between different measures (similarity score at

21 100dph, similarity score after a 2nd recording, singing activity, T level and other acoustic

22 measures). General linear models were used to check whether there was a significant

23 difference among training procedures regarding these correlations. We used the

1 Kolmogorov-Smirnov test to check whether the data conformed to a normal distribution.

2 We conducted all analyses in SPSS version 17.0 (SPSS Inc., Chicago, IL, USA).

3

4 **3. Results**

5

6 *3.1. Recordings at dph 100*

7

8 *3.1.1. Song imitation*

9 We observed significant differences among tutoring regimes (Fig. 2A, one-way

10 ANOVA, $F_{(2,59)}= 15.691$, $P< 0.0001$). Birds trained with a live tutor learned better than

11 birds trained with operant conditioning (Tukey HSD, $P= 0.005$) and passive playback

12 ($P< 0.001$). Birds trained with operant conditioning did also learn better than birds

13 trained with passive playback ($P= 0.004$). Close-to-perfect imitation of the song model

14 was observed in finches trained with live tutoring and operant conditioning but not in

15 finches trained with passive playback (Fig. 1).

16

17 *3.1.2. Effect of song model and time of playback groups*

18 For both groups (passive playback and operant conditioning), we did not observe

19 significant differences among song models on the overall similarity score (song model:

20 $F_{(2,51)}= 0.877$, $P= 0.423$; interaction song model*method: $F_{(2,51)}= 1.423$, $P= 0.251$).

21

22 For both groups there was also no effect of time of playback on song learning.

23

1 For the operant training group, birds trained with two daily sessions learned better

2 than birds trained with one single daily session starting at lights on (Fig. 2B, t-test,

3 t(27.812)= -2.198, *P= 0.036*).

4

5 For the passive playback group birds trained with song playback at the beginning of the

6 light phase (one minute after lights on) did not learn better than birds trained at the end of

7 the light phase (one minute before lights off) (Fig. 2C, t-test, t(13)= 2.036, *P= 0.063*).

8

9 *3.1.3. Singing activity*

10 Regarding singing activity, we did not find a significant correlation with the

11 overall similarity score (Fig. 3A, linear regression, F(1,58)= 0.17, R= 0.054, *P= 0.682*),

12 meaning that birds that did learn well were not the ones singing more at dph 100.

13

14 *3.1.4. Testosterone levels*

15 Regarding T level, there was no significant correlation either with overall

16 similarity score (Fig. 3B, F(1,50)= 0.789, R= 0.125, *P= 0.379*) or singing activity (Fig.

17 3C, F(1,50)= 2.285, R= 0.209, *P= 0.137*).

18

19 There was no also significant correlation between T level respectively with mean

20 CCs (Fig. 3D, linear regression, F(1,50)= 0.414, R= 0.091, *P= 0.523*) and % of residuals

21 (unclustered sounds) (Fig. 3E, F(1,50)= 0.23, R= 0.068, *P= 0.634*). This suggests that

22 birds with low levels of T at the end of the experiment did not have more plastic songs

23 than birds with high levels of T.

1   *3.2. Recordings after transfer to the aviary*

2

3   We observed some changes in the acoustics of the songs produced by the finches

4   trained with the various methods. If these changes were subtle in some birds (Fig. 4A and

5   4B), some were more pronounced in others (Fig. 4C and 4D).

6

7   There was a significant positive correlation between overall similarity score at

8   dph 100 and self-similarity score (Fig. 5A, intra-individual comparison between song

9   motif produced at dph 100 and song motif produced later, linear regression, $F(1,40)=$

10   59.246, R= 0.773, *P< 0.001*). This indicates that finches that did not provide a good

11   imitation of the song model were more likely to change their song motif after dph 100

12   than birds that did provide a good imitation. There was no effect of training procedure on

13   this result (general linear model, $F(2,41) =1.687$, *P= 0.199*).

14   Moreover, we found a significant negative correlation between self-similarity

15   score and % of residuals after data clustering, indicating that birds that changed their

16   song more after training were also those that produced more variable sounds that could

17   not be clustered as distinct syllable types (Fig. 5B, linear regression, $F(1,40)= 19.315$, R=

18   0.571, *P0.001*). There was no significant difference between groups of birds trained with

19   different methods (general linear model, $F(2,41)= 0.614$, *P= 0.547*).

20

21   This could also be predicted at the end of the training phase since there was a

22   significant negative correlation between similarity scores at dph 100 and residuals after

23   clustering, meaning that birds that did provide a good imitation of the song model tended

1 to produce more distinct (clustered) syllables than worse learners (Fig. 5C, linear

2 regression, $F_{(1,58)}= 12.459$, R= 0.421, *p= 0.001*). There was no significant difference

3 between groups of birds trained with different methods (general linear model, $F_{(2,59)}=$

4 1.364, *P= 0.264*).

5

6 Regarding song syntax stereotypy, we observed a significant positive correlation

7 between self-similarity score (intra-individual comparison between song motif produced

8 at 100dph and song motif produced later) and the mean of CCs (Fig. 5D, linear

9 regression, $F_{(1,40)}= 33.946$, R= 0.678, *P< 0.0001*). This indicates that finches that did

10 not produce stereotyped sequences of song syllables at dph 100 were more likely to

11 change their song after training than birds that did provide a good imitation. There was no

12 effect of training procedure on this result (general linear model, $F_{(2,41)}= 0.690$, *P=*

13 *0.508*).

14

15 This could also be predicted at the end of the training phase since there was a

16 significant positive correlation between similarity score at dph 100 and mean CCs,

17 meaning that birds that did provide a good imitation of the song model tended to produce

18 more syntax-stereotyped songs than worse learners (Fig. 5E, linear regression, $F_{(1,58)}=$

19 20.869, R= 0.514, *P< 0.0001*). There was no significant difference between groups of

20 birds trained with different methods (general linear model, $F_{(2,59)}= 1.155$, *P= 0.323*).

21

22

23

4. Discussion

These results indicate that young male zebra finches learn poorly from passive tape playback even when timing and quota of song exposure are controlled. Better results are obtained when the bird can self-elicit tape playback using an operant conditioning procedure, despite a high inter-individual variability. One-to-one live tutoring is clearly the most effective method to get a close-to-perfect imitation of a song model. Moreover, the less the bird learned during the training period, the more likely it was to transform its song later when raised with conspecifics, highlighting again the importance of social factors on song development. Range of vocal changes could also be predicted at the end of the training period using different criteria to measure song stereotypy, but could not be predicted based solely on testosterone levels.

4.1. Vocal changes after training

The fact that live tutoring might extend sensitive periods beyond the limits defined by auditory stimulation alone is not new (Baptista and Petrinovitch, 1984, 1986) even in an age-limited learner such as the zebra finch (Morrison and Nottebohm, 1993; Jones et al., 1996).

We observed that birds that did provide worse imitation of the song model were those that produced more unclustered sounds and were more likely to change their song as adults. This suggests that bad learning is not a consequence of a lack of plasticity but rather by a lack of an adequate song model provided during the sensitive phase. It does

1   not necessarily mean that the method was inappropriate since even birds trained with a

2   live tutor were likely to change their song when imitation was not fairly complete. We

3   did not observe correlations between the different criteria of song stereotypy and

4   testosterone level. In a previous experiment, Livingston and colleagues (2000) showed

5   that isolated housing conditions delay testosterone increase during maturation, but that

6   differences with normally raised conspecifics disappear in adults. In our study, it would

7   have been interesting to look at the individual time course of testosterone levels.

8   The nature of the vocal changes after training is not clear: the finches might have

9   learned from conspecifics but they might also have invented/improvised new sounds

10   based on those produced before or after dph 100. More experiments are required to

11   explore these aspects. Nevertheless, it has been observed in other studies that some of

12   these vocal changes were syllables learned from conspecifics (Morrison and Nottebohm,

13   1993; Jones et al., 1996).

14   Overall, vocal changes after the so-called sensitive period in birds trained with

15   tapes permitted Baptista and Petrinovitch (1984) to emphasize that live models can be

16   more effective than playbacks.

17

18   *4.2. Passive playback*

19

20   Finches trained with passive playback exhibited the worst learning scores. Passive

21   playback has long been thought to be inefficient in zebra finches but both timing and

22   quota of song exposure were not always controlled accurately. In particular, it was shown

23   that overexposure to taped playback using the operant conditioning procedure could

1 impair song learning (Tchernichovski et al., 1999). Our results suggest that the

2 inefficiency of passive playback is neither due to timing exposure, nor to sound quota.

3 Nevertheless, some learning occurred and there was also some inter-individual

4 variability. The fact that birds trained with playback just before the night exhibited song

5 learning indicate that immediate practice is not necessary in this species where the

6 sensory phase and the sensorimotor phase completely overlap (Roper and Zann, 1996). In

7 a recent experiment, Gehrold, Leitner, Laucht and Derégnaucourt (submitted) succeeded

8 in training young male zebra finches, kept in the same housing conditions as in this study,

9 to imitate short parts of a canary song bout. This suggests that some learning can also

10 occur when the young male zebra finch cannot interact with its environment either

11 through a reward obtained in self-eliciting song playback or through live interactions with

12 a conspecific.

13

14 *4.3. Innacuracies in song imitation*

15

16   Innacuracies in song imitation observed in the laboratory might have different

17 causes that are not mutually exclusive (Derégnaucourt, 2011): (1) the methods used to

18 train the bird to imitate a song may not be appropriate; (2) the bird might have

19 memorized the song model but will not produce it; (3) it does not make sense,

20 biologically speaking, to provide a fairly complete imitation. In the zebra finch (and other

21 oscine species), studies have confirmed hypotheses (1) and (2). For example, it has been

22 shown that both good learners and bad learners exhibit a preference for the song model

23 they have been exposed to during the sensitive period (Adret, 1993; Houx and ten Cate,

1   1999a). Tutor song preference can still be observed one year after the birds have been

2   separated from their tutor (Gobes and Bolhuis, 2007). Males could learn the complete

3   tutor song but not incorporate all of the tutor song elements into their own song (Nelson

4   and Marler, 1994; Geberzahn et al., 2002). This assumption was rejected by Terpstra and

5   colleagues (1994) since they observed a positive correlation between gene ZENK

6   expression in NCM (an auditory area) and quality of imitation of the tutor song following

7   exposure to the tutor song in adult finches. Also, inaccuracies in song imitation might

8   facilitate individual recognition (Böhner, 1983). Thus inaccuracies in song imitation

9   should not always be considered as errors or deficits since they could be adaptive and

10   even play a key role in the development of song cultures.

11

12   *4.4. Live tutoring*

13

14   In the starling (*Sturnus vulgaris*), passive playback enables vocal learning by

15   isolated birds whereas learning is weak or inefficient when they are housed with a

16   conspecific companion (Poirier et al., 2004). This might explain why passive playback

17   was initially thought to be inefficient in the zebra finch (Eales, 1989; Immelmann, 1969),

18   but our results show that accurate imitation of a song model cannot be obtained using

19   passive playback in singly isolated finches. Looking at the ultimate aspects of song

20   learning might help to understand why passive playback is not effective and why social

21   factors are so important in the zebra finch. In this species the strong resemblance between

22   tutor and pupil song suggests that for a young bird it is important to produce accurate

23   tutor learning rather than to obtain longer songs or larger repertoires (Gil et al., 2006).

1   Even if song production is used during courtship displays to females (Riebel, 2009), there

2   is no evidence that female zebra finches have a preference for particular syllables, as

3   shown for example in canaries (Vallet and Kreutzer, 1995). Song is not used in a

4   territorial context (Zann, 1996). One possible function is that song could constitute a

5   group signature. Like zebra finches, starlings live in large social groups and also learn

6   better from a live tutor than from taped songs (Chaiken et al., 1993). In this species it was

7   clearly demonstrated that song sharing reflects social affinities (Eens et al., 1992;

8   Hausberger, 1997). These aspects need to be studied in zebra finches.

9

10   *4.5. Developing new methods to train young finches to learn a song*

11

12   Even if fairly complete imitation observed in the one-to-one tutoring is an artefact

13   of captivity, it constitutes a baseline for researchers that use birdsong as a biological

14   model to study the molecular, cellular and behavioural correlates of vocal learning. For

15   example, using this paradigm, Haesler and colleagues (2007) found evidence the role of

16   gene FoxP2 for song learning, establishing birdsong as an interesting model for speech

17   dyspraxia. The problem with live tutoring is that one cannot control the behaviour of the

18   tutor. The association of song with a stuffed model (Bolhuis et al., 1999; Houx and ten

19   Cate, 1999b) or a non-social visual stimulus (Hultsch et al., 1999) confirms the

20   importance of visual and/or auditory components that has been indicated in earlier studies

21   (Todt et al., 1979; Eales, 1989) and that could be involved in a process of associative

22   learning. Since social interactions also appear predominant in the song learning process

23   of the zebra finch, new methods should also take into account these aspects. There is a

1 growing interest for the use of robotics in animal behaviour research (Webb, 2008).

2 Developing a robot zebra finch could allow us to identify the respective salience of each

3 stimulus for song acquisition.

4

5 **5. References**

6

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17

8

9   **Legends of figures**

10

11   Fig. 1. Spectrograms of songs produced by adult male zebra finches. A to C: three song

12   motifs produced by three different males. These songs were used as song models for the

13   experiment. D: song of the best learner from the live tutor group. E: song of the best

14   learner from the operant training group. F: song of the best learner from the passive

15   playback group.

16

17   Fig. 2. Comparison of the different methods used to train a young zebra finch to learn a

18   song. A: similarity, accuracy and sequential match to the song model for finches trained

19   with the three different methods: live tutoring, operant conditioning and passive

20   playback. B: similarity, accuracy and sequential match to the song model for two groups

21   of birds trained with operant conditioning: one daily session of 20 key pecks (starting in

22   the morning) and two daily sessions of 10 key pecks each (one in the morning and one in

23   the afternoon). C: similarity, accuracy and sequential match to the song model for two

1 groups of birds trained with passive playback: one exposure of 20 song models in the

2 morning starting 1 min after lights on, and one exposure of 20 song models in the

3 evening ending 1 min before lights off.

4

5 Fig. 3. Singing activity, testosterone level and song stereotypy at the end of the training

6 phase. A: correlation between overall similarity scores and singing activity. B: correlation

7 between overall similarity score and testosterone level. C: correlation between singing

8 activity and testosterone level. D: correlation between song syntax stereotypy (mean

9 CCs) and testosterone level. E: correlation between unclustered sounds and testosterone

10 level. See the text for details about the acoustic measures.

11

12 Fig. 4. Spectrograms of songs produced by adult male zebra finches. A and B: two

13 exemplars of songs produced by the same male at dph 100 (A) and several months later

14 after transfer to aviary. In the case of this bird, changes in the acoustic structure of the

15 song were subtle. C and D: songs of the bird that exhibited the most changes in its song

16 following transfer to aviary after 100 dph. C: song at dph 100; D: song recorded several

17 months later.

18

19 Fig. 5. Changes in the acoustic structure of the song following transfer to aviary after 100

20 dph. A: correlation between overall similarity score at dph 100 and self-similarity score.

21 B: correlation between unclustered sounds at dph 100 and self-similarity score. C:

22 correlation between unclustered sounds at dph 100 and overall similarity score at 100

23 dph. D: correlation between mean CCs at dph 100 and self-similarity score. E: correlation

1   between mean CCs at dph 100 and self-similarity score. See the text for details about the

2   acoustic measures.

3